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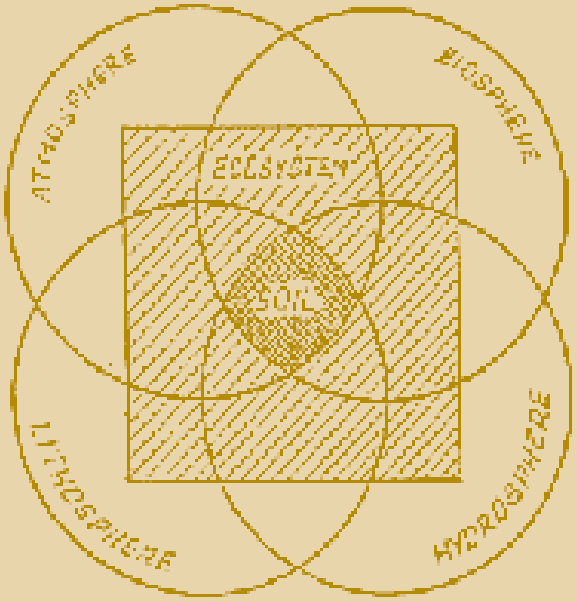

Second Edition, Revised and Expanded

edited by Mohammad Pessarakli

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edited by

Mohammad Pessarakli

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In the memory of my beloved parents, Fatima and Vahab,
who regretfully did not live to see this work,
which in no small part resulted from their gift of many years of love to me.

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Preface

The dynamic and expanding knowledge of environmental stresses and their effects on plants and crops has resulted in the compilation of a large volume of information since the first edition of the *Handbook of Plant and Crop Stress* was presented to scientists and professionals in the field of agriculture. This fact necessitated that this unique comprehensive source of information be revised and all the new findings in this field be included in the new expanded edition. Like the first edition, the new expanded edition is also a unique, comprehensive, and complete collection of the issues on stress imposed on plants/crops.

More than two-thirds of the material in this edition is new, and it has been included in this volume in 37 new chapters. The other one-third of the material (19 chapters) has been updated. Therefore, overall, about 80% of the book is new, and it seems that a totally new volume has emerged. This new edition contains 11 parts, four more than in the first edition.

Since the early 1900s, soil/plant scientists have observed that plant growth and crop yields decrease under salinity, drought, and/or other environmental stress conditions. Reduction in plant growth was reported as a result of modification in the physiological process and environmental conditions that control growth. Stresses imposed on plants by pollution or application of agrichemicals have recently attracted the attention of scientists, investigators, and environmentalists in the field of agriculture and related areas. The mechanisms by which salinity, drought, high/low temperatures or heat, high/low pH, high/low light, nutrient deficiency, pollution, agrichemicals, climatic changes, or any other stresses affect plant metabolism, thereby reducing growth and development, are still not completely understood. Among the plant physiological processes, the change in nutrient uptake and metabolism induced by salt, drought, and/or other stress factors is commonly accepted among scientists as one of the most important factors in abnormal plant metabolism, reduced growth, and decreased crop yield. The need for minimizing these stress effects as well as other environmental stresses on plant growth and crop yield is vital. Thus, a greater awareness of these stress factors and their related problems is essential to scientists, growers, and all others involved in the field of agriculture.

This handbook is a comprehensive, up-to-date reference book effectively addressing issues and concerns related to plant and crop stress. Although many reference books about soil salinity, sodicity, specific plant/crop salt and water stress, pollution, and other environmental stresses have been published, they all exist in relative isolation from one another, covering only one specific topic.

Efficiency and effectiveness in solving plant and crop stress problems are dependent on the accountability and coordination of all the factors and the interrelationships involved with plant/crop stress. Although previous authors have indeed competently covered the many areas separately, the areas are, nonetheless, interrelated and should be covered comprehensively in a single text. Thus, the purpose of this book is to fill this niche.

The updated and expanded edition of the *Handbook of Plant and Crop Stress* has been pre-

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pared by over 100 contributors, who are among the most competent and knowledgeable scientists, specialists, and researchers in agriculture from 25 countries. It is intended to serve as a resource for both lecture and independent purposes. Scientists, agriculture researchers, agriculture practitioners, and students will benefit from this unique comprehensive guide, which covers plant stress problems from the soil to the atmosphere.

As with other fields, accessibility of knowledge is among the most critical factors involved with crop stress problems. Without due consideration of all the elements contributing to a specific crop stress problem, it is unlikely that a permanent solution will be achieved. For this reason, as many of the factors as possible are included in this handbook. To further facilitate the accessibility of the desired information in the areas of stress covered in this collection, the volume has been divided into 11 parts: Soil Salinity, Sodicity, Low/High pH, and Soil Nutrient Deficiency Problems; Plants, Crops, and Stressful Conditions; Plant and Crop Responses Under Salt, Drought, Heat, Temperature, Light, and Other Stressful Conditions; Plant and Crop Responses Under Pollution Stress; Plant and Crop Responses Under Agrichemical Stress Conditions; Molecular Biology and Microbiological Aspects of Plant Responses Under Salt, Drought, and Other Environmental Stress Conditions; Genetic Factors and Plant/Crop Stress; Examples of Empirical Investigations of Specific Plants and Crops Grown Under Salt, Drought, and Other Environmental Stress Conditions; Future Promises: Plant and Crop Adaptation and Cultivation Under Stressful Conditions; Climatic Changes, Elevated Carbon Dioxide, and Plant/Crop Responses; and Beneficial Aspects of Stress. Each of these parts comprises one or more chapters that, independently, discuss as many aspects of stress as possible.

Numerous figures and tables appear in this technical guide to facilitate comprehension of the presented materials. The volume also includes a comprehensive index to increase further the reader's accessibility to the desired information.

I would like to express my appreciation for the secretarial assistance that I received from Elenor R. Loya, College of Agriculture, University of Arizona, for the completion of this work.

In addition, my sincere gratitude is extended to Russell Dekker (Chief Publishing Officer, Marcel Dekker, Inc.), who supported this project from its initiation to its completion. Certainly, this job would not have been completed as smoothly and rapidly without his most valuable support and sincere efforts. Also, the patience shown by the Production Editor, Rod Learmonth, and his careful and professional handling of the material in this volume is greatly appreciated. The remarkable work of the copyeditor, Kitty McCullough, during the course of completion of this book is sincerely acknowledged.

The invaluable efforts of each and every one of the contributors who responded to my request for contributions to this volume are deeply appreciated. Their proficiency and knowledge in their area of expertise made this significant task possible.

Also, I thank my wife, Vinca, for her support in the completion of this work. Last, but not least, I would like to thank my son, Mahdi, who had great patience and understanding and let me take the time to complete this project that otherwise would have been spent with him.

Mohammad Pessarakli

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1

Soil Salinity and Sodicity as Particular Plant/Crop Stress Factors

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INTRODUCTION

Salinity and sodicity problems are common in arid and semiarid regions, where rainfall is insufficient to leach salts and excess sodium ions out of the rhizosphere. In addition, these areas often have high evaporation rates, which can encourage an increase in salt concentration at the soil surface.

The presence of a cliche horizon and/or a cemented hardpan layer at varying depths plus insufficient precipitation for leaching often adds to the salt accumulation in these soils. Newly established irrigation projects, with improper planning and management practices, may also add salts to soils [1].

Soil salinity and sodicity problems are present in nearly every irrigated area of the world and also occur on nonirrigated croplands and rangelands. Thus, virtually no land is immune from salinization. Therefore, for sustaining life on earth, control of these problems and finding new ways to utilize these extensive saline and sodic soils and water resources, at least for agricultural purposes, are vital and urgent. Reclamation, or at least minimizing the effect of salinity and/or sodicity, is important and necessary. In this respect, proper utilization of water for both plant growth and soil salinity and sodicity control is probably of the greatest importance.

The main focus of this introductory chapter is to summarize general information on salt-affected (saline and sodic) soils and factors influencing their formation and reclamation.

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SIGNIFICANCE OF SOILS IN RESPECT OF CROP STRESS

As far as all the crops are grown on soils, soil properties have substantial influence on the life conditions of plants and crops. In nature, usually particular plant species grow on specific soils. Thus, specific relationships exist between a particular soil and the vegetation cover of that specific soil. For example, Kreeb et al. [2] investigated soil and vegetation relationships associated with alkaline-saline soil surfaces.

Plant development and successful crop production require proper soil conditions, including adequate water and nutrient supply. Unfavorable soil conditions (environmental stress [3–5], salinity and/or sodicity [6,7], inadequate nutrient supply [8,9]) have an adverse effect on the life of the plants, sometimes seriously hindering their effective production.

Based on the above facts, we can speak of stress factors originating in the soil; that is, such unfavorable soil conditions which cause, or contribute to, the stress factors that plants and crops are exposed to.

It is impossible to list all or most of such factors in a short introductory chapter. Therefore, we limit the range of this chapter to a general description of soil behavior and its function in nature and production as well as to an outline of one of the most serious factors originating in salt-affected soils. For more in-depth information regarding salt-affected soils, the readers are referred to the more comprehensive available sources [10–31].

PLACE AND ROLE OF THE SOIL IN NATURE

It is generally accepted that the soil is a substantial part of the environment, comprising different substances and forming a special kind of ecosystem inside the given ecosystem, with various properties and attributes. It is also accepted that the soil of the continents is of high diversity, which is dealt with by several branches of soil science; for example, taxonomy, classification, survey, and mapping.

The soil, or the pedosphere, which is an environmental synonym of the soils of a given territory, has a specific place in nature. It is a natural body, similar to rocks, waters, or biota in the sense that they too have their own materials, mass and energy fluxes, development, and regularities. This fact should be mentioned because, not only in newspapers but also in technical literature, soils are frequently treated either as living substances or as nonbiological substances. Neither of these approaches is correct, because one of the characteristics of the soil is its complexity, the fact that it contains both living and nonliving substances, forming as a result of both biotic and abiotic processes.

The soil as a natural body is inseparable from the rocks and the crust of weathering on the surface of the continents from which it has developed, on the one hand, and from the biological processes on the other hand. The main characteristics that distinguishes the soil from the rocks is the result of biological processes: the production of organic matters by the activities of microorganisms, plants, invertebrates and other animals, and finally human beings which transforms the rocks into soils capable of supplying plants and crops with nutrients and water.

The processes of soil formation started concurrently with the appearance of life on the continents and continued during the billions of years of interactions between living substances and rocks under the influence of climatic conditions, with particular regard to the action of water, geomorphological patterns, and the time factor. As a result of their interactions, specific mass and energy fluxes formed the different soil types in various environmental conditions.

With the appearance of the human race on the face of the earth, even changes in the environment became different. Owing to human activities, the natural processes affected by biotic and abiotic factors accelerated and several others which were unknown or minimal before developed.

The role of soils in nature is complex and many sided, including biospheric, hydrospheric, and lithospheric functions. Their interaction is illustrated in Figure 1 [11], which clearly shows that

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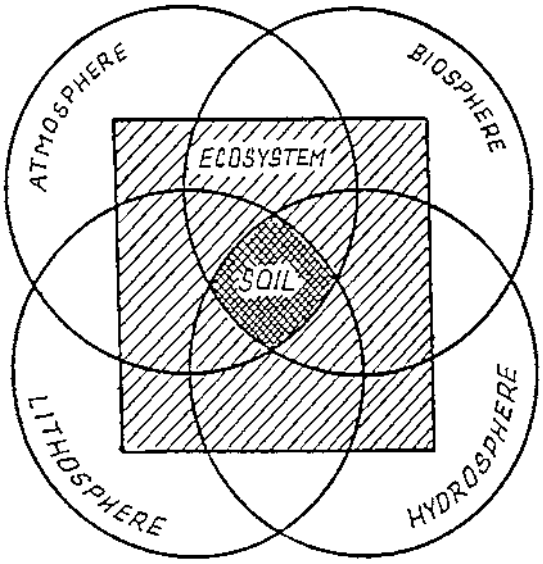

FIGURE 1 Schematic diagram of the interaction of lithosphere, atmosphere, biosphere, hydrosphere, ecosystems, and soils. (From Ref. 11.)

the soil is a specific body related to the ecosystem. Even the word *soil* is very often used as a synonym of ecosystem when characterizing the given ecological conditions in a certain place. If we want to be precise, we must agree that the ecosystem includes the pedon, in other words, the soils. However, the soil includes different phases (solid, liquid, gaseous), living and nonliving substances, plants, animals, and microbes and has its own energy and material fluxes. Therefore, it can be considered an ecosystem in itself. In this respect, when speaking of soils versus their plant cover, we can consider the soils of a given location as the basis, ladder, and foothold, for instance, in savannas, or in the tropical belt, a well-defined plant cover develops and very often the soil properties promote or limit the living conditions of certain plant species or associations.

Based on the above considerations, it can be accepted that certain soil types, when discussed as the habitat for certain plant associations, are often named as the ecosystem of the plant association concerned, as the pedon includes, apart from the plants, most of the components of the ecosystem.

Evidently the soil, as a specific natural entity, is far from being identical with the vegetation and, in spite of their close correlation, direct conversion between soil types and vegetation is hardly possible. Still there are soil types which, more or less, determine the ecological function for certain types of vegetation either by providing beneficial conditions for its development or by limiting the ecological conditions for other types of vegetation.

This is perhaps best demonstrated in the case of salt-affected soils where high electrolyte contents of extreme pH conditions limit the development of the majority of plants and serve as a habitat only for such species which can survive or tolerate the unfavorable conditions caused by the salinity and alkalinity of the soil. For example, the grass *Leptochloa fusca* that grows vigorously on the salt-affected soils can tolerate extremely saline and sodic (alkaline) conditions [25]. This species is also well adapted to the waterlogging encountered on saline and sodic (alkaline) soils. Other investigators [2,7,32,33] have also reported on the soil and vegetation relationships that specific plant types are adapted and growing on specific habitats. In such respects, salt-affected soils can be considered as habitat or ecosystems for halophytes and, if we agree on this, correlations can be found between the different types of salt-affected soils and their flora and fauna as components of the ecosystem.

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In order to cast light on both the theoretical and practical aspects of such considerations, it is necessary to describe briefly the properties and grouping of salt-affected soils with regard to the possibilities of the occurrence and distribution of halophytes and xerophytes developing on them.

EXTENSION AND GLOBAL DISTRIBUTION OF SALT-AFFECTED SOILS

Nearly 10% of the total land surface is covered with different types of salt-affected soils. Table 1 demonstrates the distribution of salt-affected soils in the world [34], and it shows that no continent on our planet is free from salt-affected soils. They are distributed not only in deserts and semidesert regions, but also frequently occur in fertile alluvial plains, river valleys, and coastal areas close to densely populated areas and irrigation systems [11–14,16,17,26].

Figure 2 shows the distribution of salt-affected soils throughout the world [12,17].

TABLE 1 Salt-Affected Soils on the Continents and Subcontinents

Continent	Area (millions ha)
North America	15.7
Mexico and Central America	2.0
South America	129.2
Africa	80.5
South Asia	87.6
North and Central Asia	211.7
South-East Asia	20.0
Australasia	357.3
Europe	50.8
Total	954.8

FIGURE 2 Global distribution of the salt-affected soils.

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DEVELOPMENT AND GROUPING OF SALT-AFFECTED SOILS

In spite of the fact that the properties and attributes of salt-affected soils have been well known for a long time, it is appropriate to give a brief definition of this group of soils right at the start, because the salinity and sodicity (alkalinity) as well as the acidity of soils are substantial stress factors seriously affecting the productivity of the land [3–9,12,17,29,35–38].

Salt-affected (i.e., saline, saline-sodic, and sodic) soils usually have low biological activity both because of osmotic and ionic effects of salts and due to limitation of carbonaceous substrates. Rao and Pathak [39] reported that microbial growth was depressed in sodic (alkali) soils due to, at least in part, limitation in carbon substrate (carbon stress) and in saline soils due to salt stress.

For detailed information on the formation of salt-affected soils, the readers are referred to Szabolcs [11,12] and Pessarakli [17].

Salt-affected soils can be characterized as soils formed under the dominant influence of different salts in their solid or liquid phases, which will then have a decisive influence on the development, characteristics, physical, chemical, and biological properties, and eventually the fertility of the soil. Whenever and wherever this phenomenon occurs, it produces specific formations of soils where the high electrolyte concentration and its consequences overshadow the former soil-forming processes or former soil properties and environmental conditions, often radically changing them.

High electrolyte concentration is the only common feature of all salt-affected soils. Their chemistry, morphology, pH, and many other properties may be different depending on the character of salinization and/or alkalization.

Salt-affected soils, in the broader sense, can be divided into the following groups:

1. Saline soils that develop under the influence of electrolytes of sodium salts with nearly neutral reaction (dominantly Na_2SO_4, NaCl, seldom $NaNO_3$). These soils occur mainly in arid and semiarid regions and form a major part of all the salt-affected soils of the world. High contents of soluble salts accumulated in these soils can significantly decrease their value and productivity.
2. Sodic (alkali) soils that develop under the influence of electrolytes capable of alkaline hydrolysis (mainly Na_2CO_3 and $NaHCO_3$ and seldom Na_2SiO_3 and $NaHSiO_3$). This group is well extended in practically all the climatic regions from the humid tropics to beyond the polar circles and their total salt content is usually lower than that of saline soils, sometimes even strongly sodic (alkaline). Virgin sodic (alkali) soils have a high pH and high exchangeable Na and are often barren. Sodic soils exhibit poor physical conditions that adversely influence water and air movement in the soils. Sodicity causes soil erodibility and impairs plant growth [27].
3. Salt-affected soils that mostly develop owing to the presence of $CaSO_4$ (gypsiferous soils) or, rarely, in the presence of $CaCl_2$. Gypsiferous soils can mainly be found in the arid and semiarid regions of North America, North Africa, the Near, Middle, and Far East, and also in Australia.
4. Salt-affected soils which develop under the influence of magnesium salts. This group occurs in arid, semiarid, and even semihumid regions and has a particular significance, especially those soils which have a heavy texture.
5. Acid-sulfate soils whose salt content is composed mainly of $Al_2(SO_4)_3$ and $Fe_2(SO_4)_3$. This type of salt-affected soils is broadly extended in the tidal marsh areas along the seashores of all the continents. These soils are particularly common in, for example, North Europe, the western and eastern coastlines of Africa, and along the coastline of Southeast India, and develop on sulfurous marine sediments.

Inland acid-sulfate soils can also be found in different areas of the world, such as the western territories of the United States, Asia Minor, and China. Such soils developed as a result of fluvial glacial processes and have had no connection with seashores in recent geological times.

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Evidently the different groups of salt-affected soils have diverse physicochemical and biological properties besides the one they have in common; that is, a comparatively high electrolyte content.

The grouping of the salt-affected soils and their properties causing plant and crop stress are presented in Table 2. The five groups in Table 2 represent the formations of different salt-affected soils described above, indicating their chemical types, the environmental conditions where they dominate or occur, the pattern of their main adverse effect on production, and the basic methods of their reclamation. For detailed information on formation and reclamation of salt-affected soils see Szabolcs [11,12] and Pessarakli [17].

In Table 2, the adverse properties of different salt-affected soils causing crop stress are also included. From these, it is clear that, in various groups, different properties are responsible for hindering the development of plants and crops by causing stress.

In saline soils, it is the high salt concentration in the solid and liquid phases which results in high osmotic pressure, hindering the normal development of plants. the stress factor is the salinity with all its disadvantageous consequences of plant life. Apart from this, some compounds of the salt content of these soils, for example, chlorides as toxic elements, also act as one of the stress factors.

In sodic (alkali) soils, as a rule, not the high salt concentration but the sodic (alkaline) pH value is the stress factor, particularly in cases where there is a high concentration of sodium carbonate in the solid and liquid phases of the soil. The high pH hinders the life function of crops and limits their development.

In another group of sodic (alkali) soils, which sometimes does not have very alkaline pH value (solonetz type), the comparatively low concentration of sodium salts capable of sodic (alkaline) hydrolysis constitutes a stress factor through its action, resulting in poor water physical properties in the soil. As a consequence of this phenomenon, the wilting point in the soil increases and the plants suffer from water deficiency, even in wet soils, owing to the swelling of clay saturated with sodium ions.

In magnesium soils, which have not been adequately studied, the combination of toxic effect, calcium deficiency, and poor soil physical properties are the stress factors.

In gypsiferous soils, the acidic pH, and sometimes the toxic effect of the high gypsum content, contribute to the appearance of stress factors for plant and crop life in areas with large extensions of intensively gypsiferous soils.

In acid-sulfate soils, the very high acidity, with a pH sometimes below 2, poses stress with all the adverse effects of extreme acidity. Furthermore, the high aluminum content of the soil solution has an intensive toxic effect. Apart from this, the temporary or permanent waterlogging in such soils acts as a stress factor hindering the normal air and nutrient regimen necessary for plant life in these soils.

Besides the salt-affected soils developing as a result of natural soil-forming processes, the so-called secondary salt-affected soils have an increasing importance that is both scientific and practical. Secondary salt-affected soils are those which have been salinized owing to manmade factors, mainly as a consequence of improper methods of irrigation. The extension of secondary salt-affected soils is rather sizeable, and this adverse process is as old as irrigated agriculture itself. Ancient civilizations in Mesopotamia, China, and Pre-Columbian America fell as a consequence of the salinization of irrigated land. The process is also advancing vigorously at present, and more than half of all the irrigated lands in the world are under the influence of secondary salinization and/or alkalization.

When speaking of the manmade factors of salinization, we also have to mention potential salt-affected soils which are not salt-affected at present, but in case of the extension of irrigation, deforestation, overgrazing, and other manmade measures, can and will be salinized unless the necessary preventive procedures are undertaken in due time. No global records are available of the size of potential salt-affected soils; however, the area that they cover is larger than that of existing salt-affected soils.

Secondary salt-affected soils can be divided into the following two categories: secondary

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TABLE 2 Grouping of Salt-Affected Soils and Their Properties Causing Plant and Crop Stress

Type of salt-affected soils	Electrolyte(s) causing salinity and/or alkalinity	Environment	Properties causing plant and crop stress	Methods for reclamation
Saline	Sodium chloride and sulfate (in extreme cases nitrate)	Arid and semiarid	High osmotic pressure of soil solution, toxic effect of chlorides	Removal of excess salt (leaching)
Alkali	Sodium ions capable of alkaline hydrolysis	Semiarid, semihumid, and humid	High (alkali) pH, poor water physical conditions	Lowering or neutralizing the high pH by chemical amendments
Magnesium	Magnesium ions	Semiarid and semihumid	Toxic effect, high osmotic pressure, Ca deficiency	Chemical amendments, leaching
Gypsiferous	Calcium ions (mainly $CaSO_4$)	Semiarid and arid	Low (acidic) pH toxic effect	Alkaline amendments
Acid sulphate	Ferric and aluminium ions (mainly sulfates)	Seashores and lagoons with heavy, sulfate-containing sediments, diluvial inland slopes and depressions	High acidity and the toxic effect of aluminium	Liming

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formation of salt-affected soils caused by irrigation and secondary formation of salt-affected soils caused by human activities other than irrigation.

Secondary Formation of Salt-Affected Soils Caused by Irrigation

In spite of the negative experiences, the salinization of irrigated and surrounding areas has not diminished. On the contrary, it is still on the increase.

According to the estimates of the Food and Agriculture Organization (FAO) and the United Nations Educational, Scientific, and Cultural Organization (UNESCO), as much as half of all the existing irrigation systems of the world are, more or less, under the influence of secondary salinization, alkalization, and waterlogging. This phenomenon is very common not only in old irrigation systems but also in areas where irrigation has only recently begun.

According to the estimates of the above-mentioned agencies, 10 million hectares of irrigated lands are abandoned yearly because of the adverse effects of salinity due to irrigation, mainly secondary salinization and alkalinization.

The mentioned losses and damages are not evenly distributed among the irrigating countries. In some of them, the damage may be relatively small, whereas in others, it actually constitutes the major problem in agriculture or even in the national economy of the country in question. In this respect, unfortunately, there are countless sad examples. In Pakistan, Ahmad [40] carried out statistical analyses in respect of secondary salinized land. According to his data, out of 35 million acres (approximately 16 million ha) of total irrigated territory, salinized areas account for 5.3 million acres (approximately 2.4 million ha) after a few years of irrigation. He indicated among the causes of secondary salinization in Pakistan the joint effect of irrigation and ground water. According to Zavaleta [10], practically all irrigated alluvial soils in Peru show the features of salinity and sodicity (alkalinity). It is known from FAO reports and the papers of Kovda [41] that more than 40% of irrigated soils in Iraq and Iran is affected by secondary salinization. In a country report on salinity in Syria, the FAO [42] estimated the adverse effects of salinity as follows:

1. In more than 20,000 ha, salinity developed to a level where these soils had to be taken out of cultivation, and the loss is estimated at a total of 30,000 tons of cotton per year.
2. In about 30,000 ha, the yield decreased by 50%, and the total loss is estimated at 20,000 tons of cotton per year.
3. In about 60,000 ha, the yield decreased by 20%, and the total loss is estimated at about 18,000 tons of cotton per year.

At present, no continent is free from the occurrence of this very serious phenomenon. In Argentina, 50% of the 40,000 ha of land irrigated in the 19th century are now salinized. In Australia, secondary salinization and alkalization take place in the valley of the River Murray, and in Northern Victoria, 80,000 ha have been affected. The same phenomena can be observed in Alberta, Canada, and similar processes have been recorded in the northern states of the United States, where irrigation was introduced much later than in the dry western states. It is noteworthy that these last examples, and many other irrigated regions, are far from being arid areas, and the majority of salt accumulating is associated with the sodium salts capable of sodic (alkaline) hydrolysis and not with the neutral sodium salts that we are familiar with in desert and semidesert areas.

Secondary Formation of Salt-Affected Soils Caused by Human Activities Other Than Irrigation

When speaking of secondary salinization, most people have irrigation and drainage in mind. However, there are also other anthropogenic factors causing these adverse phenomenon. It is true that the majority of secondary salt-affected soils develop as a result of improper methods of irrigation,

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but there are other human effects which more and more often trigger this process in many places both in arid and humid areas.

Some of these anthropogenic processes include, but are not limited to, the following:

Overgrazing

This process occurs mainly in arid and semiarid regions, where the natural soil cover is poor and scarcely satisfies the fodder requirement of rather extensive animal husbandry. If the natural vegetation is sparse or annihilated on account of overgrazing, progressive salinization develops and, step by step, the scarcity of the plant cover becomes increasingly pronounced. Sometimes the process ends in desertification, because even the poor pasture diminishes and no other fodder resources are available. According to Theunissen [43], the gradual decline in the ecological condition of natural pastures as a result of overgrazing and the application of insufficient management decisions, coupled with the detrimental effects of long-term drought, has left extensive areas of high-potential grazing land in southern Africa in urgent need of restoration. However, owing to the limited number of grasses currently available for rehabilitating and restoring the vast number of different habitats encountered, selecting indigenous grasses suitable for restoration of denuded areas in the arid and semiarid grasslands of southern Africa was initiated.

Deforestation in Semihumid and Semiarid Areas

Particularly in the past few decades, it has become evident in many tropical and subtropical countries that deforestation results in the salinization and alkalization of soils due to the effects of soil migration both in the upper and lower layers. In South East India, for example, vast territories of former forest land became intensely saline and sodic (alkaline) in a few years after the annihilation of the woods. Similar phenomena occurred in, for example, the forest steppe areas in Russia, Iran, East-Central Europe, and Latin America.

Salinization Caused by Contamination with Chemicals

In spite of the fact that the amount of chemicals applied in agriculture is practically negligible in comparison with the salt content of several soils, we have considered the fact that this kind of salinization more and more often occurs in modern intensive agricultural production, particularly in greenhouses and intensive farming systems. When production takes place in semiclosed systems (e.g., greenhouses), where the chemicals applied will not be removed regularly, the accumulation of salts or their components becomes possible in the upper layer of the soil, resulting in salinity and sodicity (alkalinity). In Japan, the Netherlands, and other countries with intensive agriculture, and particularly horticulture, such types of salinization more and more frequently appear, causing serious losses of crop yields.

Accumulation of Airborne or Waterborne Salts

Owing to the concentration of industrial plants, the emission of chemical compounds may accumulate in the soil and, if their concentration is high enough, they result in salt accumulation in the upper layer of the soil.

A similar phenomenon appears when, owing to water system regulations, sludge water disposal, and other hydrotechnical measures, water with considerable salt concentration contaminates the upper soil layer, causing salinization and/or alkalization.

RECLAMATION OF SALT-AFFECTED SOILS

Population growth and increasing demand for food and agricultural products necessitate using the salt-affected soils and marginal lands for food production. These soils are needed for the agricultural extension, and hence reclamation is required.

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Reclamation is needed on the millions of hectares of slowly permeable salt-affected (i.e., saline-sodic and sodic) soils throughout the world [44].

Different techniques have been used for reclamation of salt-affected soils. Saline soils are usually reclaimed by leaching the salts out of the soil through irrigation and drainage systems. Whereas, reclamation of sodic (alkaline) soils requires application of chemical amendments followed by the leaching process.

Present recommendations for reclamation of the salt-affected soils are usually based only on relatively simple and often empirical relations. Various amendments and management strategies have been used for reclamation of the salt-affected soils. To evaluate particular reclamation strategies, some specific considerations should be noted as follows:

1. Quantity of water needed
2. Quality of water needed
3. Quantity of amendments to be used
4. Type(s) of amendment(s) to be used
5. Time required for reclamation to be completed

Chemical reactions such as cation exchange, precipitation, and dissolution of solid phases (reclamation amendments) and the soil hydraulic properties and corresponding changes in the water flow and solute transport rates must be considered [31].

Among the various reclamation practices, a combination of added gypsum amendment and crop rotation usually has been proven to be the best.

Since reclamation of salt-affected (saline-sodic and sodic) soils by chemical amendments has become cost effective and requires high capital investment, cultivation of salinity and sodicity-tolerant plants ''saline agriculture'' may be another alternative.

Cultivation of different salinity and sodicity-tolerant plant types and species has been used by several investigators (i.e., grasses [7,25,43], agronomic crops [45], forest species [38,46–48]) for reclamation purposes. These plants can mobilize the native lime (calcium carbonate, $CaCO_3$) in these soils through root action, a substitute for the chemical approach. Qadir et al. [7], studying the combination of chemical amendments and biological (using plants) reclamation technique, reported that the soil treated with gypsum at a high rate (100% GR) removed the greatest amount of Na^+ from the soil columns and resulted in a marked decrease in soil salinity (EC, electrical conductivity) and sodicity, sodium absorption ratio (SAR). The performance of grass treatment in enhancing the leaching of Na^+ was between the gypsum treatments.

According to Kumar [25], the grass *Leptochloa fusca* is very useful and effective in the reclamation of salt-affected soils. This plant can tolerate extremely saline and sodic (alkaline) conditions. Since its growth is not affected by gypsum application, planting with *Leptochloa* is an alternative biological rather than a chemical method for the reclamation of sodic (alkaline) soils. This plant is also well adapted to the waterlogging encountered on saline and sodic (alkaline) soils. The plant improves the soil physical, chemical, and biological properties so that within 2 or 3 years many commercial and forage crops can be grown on the soil [25]. *Leptochloa* excretes salts through specialized glands and is, therefore, reasonably palatable to farm animals. It must be noted that because of its vigorous growth on sodic (alkaline) soils, *Leptochloa* does not allow satisfactory growth of companion plant species, especially in the initial years of soil reclamation.

Subramaniam and Babu [48] also used a forest shrub species for reclamation of sodic soils. According to these investigators [48], *Sophora mollis*, which grows as a shrub to a medium-sized tree and is used for both fodder and firewood, can be used in the reclamation of sodic (alkaline) soils.

Although slow but definite improvement is achieved in the physicochemical properties of the salt-affected soils by encouraging the vegetation growth on such lands. The tree species in general are effective in improving the soil properties as reflected by the changes in physicochemical characteristics of the soil such as bulk density (BD), water-holding capacity (WHC), hydraulic conductivity (HC), and pH, EC, OC (organic carbon), N (nitrogen), and exchangeable cations (Na^+ and Ca^{2+}) [46].

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Owing to the low biological activity and depressed microbial growth of salt-affected (i.e., saline, saline-sodic, and sodic) soils, there is a need for applying organic amendments (i.e., plant residue or manure) during sodic (alkali) soil reclamation. In reclamation of saline soils, organic amendments must be applied following the leaching process.

Kumar et al. [35] conducted a combination of biological and chemical reclamation studies on a highly sodic (alkaline) soil. These investigators [35] found that rice produced satisfactory yields in the first year of gypsum application, but sorghum and *Sesbania* yields were very poor. The yield of *Leptochloa* was not affected by gypsum application. In their crop rotation practice, Kumar et al. [35] reported that the green forage yield of sorghum was greatest when sorghum followed *Leptochloa* grown for 2 years and the harvested grass was left to be decomposed on the site.

In a biological reclamation study of saline soils, Helalia et al. [49] reported that amshot grass significantly reduced the soil salinity compared with either ponding or gypsum application, and this grass produced a higher fresh yield than clover cultivated in such soils.

The above findings indicate that biological reclamation with the salinity- or sodicity-tolerant plants (i.e., *Leptochloa*, grasses, shrubs, or trees) is a proper substitute for chemical reclamation with gypsum, and the former has an economic advantage over the latter.

Compost or any other organic materials is recommended to be used during the reclamation process of the salt-affected soils. The results of a field experiment conducted by Avnimelech et al. [24] verified that compost application improved both physical and chemical conditions of saline and sodic (alkaline) soils. Compost application to such soils is expected to release acids which would ultimately lead to the replacement of exchangeable sodium by calcium. In addition, compost application would stabilize soil structure and enhance plant growth. These investigators [24] found that the municipal solid-waste compost application was equivalent or even superior to the addition of gypsum, the most common amendment used to reclaim sodic (alkaline) soils. This was evident from the substantial increase in crop yields. The combined application of compost and gypsum raised yields to the levels equal to that of the commercial fields.

In a field experiment, Batra et al. [30] compared the microbiological and chemical amelioration of a highly deteriorated sodic (alkaline) soil using two reclamation technologies:

1. Growing Karnal grass (*Leptochloa fusca*) as a first crop with no chemical amendment (biological reclamation)
2. Gypsum application as a chemical amendment for different crop rotations

These investigators [30] reported that the microbiological properties changed more than the chemical properties of sodic (alkali) soil as the time period advanced.

In a biological reclamation study carried out on saline soils, Apte and Thomas [50] found that a brackish water, nitrogen-fixing cyanobacterium, *Anabaena torulosa*, could successfully grow and fix nitrogen on moderately saline soils (EC of 5.0–8.50 dS m^{-1}). These investigators [50] reported that cyanobacterium exhibited high rates of nitrogen fixation and substantially enriched the nitrogen status of saline soils. However, permanent removal of Na^+ from saline soils using cyanobacteria or any other microorganisms may not be possible, since Na^+ is released back into the soil subsequent to the death and decay of cyanobacteria or other microorganisms. Amelioration of soil salinity by simultaneous application of *Anabaena torulosa* during crop growth seems to be an attractive possibility for reclamation, especially since it can also supplement the nitrogen requirement of the crops growing on these soils.

Blue-green algae that tolerate excess Na and grow extensively on the soil surface in wet seasons was found to be effective in sodic soils reclamation [51]. However, a permanent reclamation of such soils by using only blue-green algae as a biological amendment to achieve sodic (alkali) soil reclamation is neither possible nor comparable with an effective chemical amendment such as gypsum.

In the reclamation process of the saline soils, De Villiers et al. [33] compared different annual and perennial species. Of the six species tested, the perennials seemed to be more effective and better suited for rehabilitation purposes under saline soil conditions.

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The type of chemical compound being used also influences the reclamation process of salt-affected soils. Sharma and Upadhyay [52] reported that, among the up-to-date known chemical compounds, cyclohexathiazenium chloride (S-6N-4)-2+Cl-2 is the best and the most suitable chemical to reclaim the sodic (alkaline) soil at any pH of the soil.

When good-quality water is not available for leaching the salts out of the soil, low-quality water can be used for the initial stages of reclamation. In this regard, Singh and Bajwa [53] studied the effects of gypsum and sodic irrigation on the precipitations of Ca^{2+} and removal of Na^{+} from a sodic soil reclaimed with different levels of gypsum and growth of rice in a greenhouse experiment. Dubey and Mondal [22] also used low-quality saline water in conjunction with organic and inorganic amendments for the initial stages of reclamation of sodic soils. Using low-quality water, Joshi and Dhir [54] evaluated the rehabilitation of degraded sodic soils using residual sodium carbonate water (low-quality water) combined with gypsum treatment and found that the combination treatment was effective in lowering the soil SAR and improved the water infiltration rate. In the first year of gypsum treatment, it was possible to establish the crop. In the second year, a moderate production of wheat (2610 kg ha^{-1}) and raga (*Brassica* sp) (2000 kg ha^{-1}) was obtained [54].

Using the most common technique, irrigation water and drainage system, for reclamation of the salt-affected soils, the results of an investigation carried out by Millette et al. [23] demonstrated the ability of fall irrigation to leach salts from the surface soil during a period of low consumptive use, which could lead to reclamation. Long-term monitoring would be required to determine whether a further and permanent decline in salinity could be achieved.

Concerning other reclamation materials and techniques, results of Jones et al. [55] indicate that acid whey is effective in reclaiming sodic soil by lowering ESP (exchangeable sodium percentage), SAR, and pH and by improving the infiltration rate. Rao and Leeds Harrison [56] used simulation models for desalinization of a drained two-layered saline soil using surface irrigation for different water management practices to increase leaching efficiency. Based on image elements and their correlation with the ground features, Rao et al. [57] suggested categorizing sodic soils in moderately and strongly sodic groups. The delineation thus made would help the execution of a reclamation program for sodic soils at the study sites. Abdel-Hamid et al. [58] monitored soil salinity in the northern Nile delta of Egypt by using data collected via landsat and the geographical information system (GIS). The collected data were used in making recommendations for reclamation of the saline soils of the Nile delta area.

The vast areas of salt-affected soils still remain a burden for the affected societies, particularly the developing countries, where the adequate resources needed to reclaim them with the available technology involve initial heavy investments. The process of degradation, which has been due to reckless destruction of vegetation, can be reversed by reestablishment of vegetative cover which results in slow but definite improvement in such soils. This phenomenon has been very much demonstrated by various parameters influencing the soil welfare in several investigations which show a positive sign of improvement both in terms of physical and chemical properties of the salt-affected soils. Such soils should, therefore, be brought under any type of vegetation (i.e., sod, shrub, tree) cover, if not found to be economical for regular farming and growing agronomic crops [46].

Even by the execution of the reclamation processes, nutrient status and their behavior in salt-affected soils (i.e., saline-sodic and sodic) during reclamation by crop rotation and chemical amendments requires a comprehensive assessment. This is usually because some soil nutrients are also lost and leached out of the soil during the leaching process of the soluble salts and the exchangeable sodium. In this regard, several investigators [8,9,36–38] have studied nutrient status and behavior during the reclamation processes. Swarup et al. [36] reported the effect of gypsum on the behavior of soil phosphorus during the reclamation of a sodic soil. According to Bhojvaid et al. [38], soil nutrient status under the tree plantation was higher than that of the nonsodic farm soil. This finding confirms that successful tree plantation may restore the productivity and fertility of highly degraded sodic soils.

Regardless of the techniques used in reclamation of salt-affected soils, postreclamation management practices, that is, proper choice of crops, crop rotation, method of irrigation, quality and

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quantity of water used for irrigation and reclamation, fertilization, and the economics of reclamation, must be taken into consideration and followed to achieve successful results.

CONCLUSIONS

In this chapter, information has been given on the important functions of the soil in relation to soil-originated stress factors for plant and crop development as well as a little more detailed information of particular problems related to salt-affected soils and their formation and reclamation.

The properties of the stress factors for plant and crop growth originating in soil are diverse and many sided. We know comparatively little about the status of salt-affected soils and, particularly, for finding methods to improve the situation of reclaiming these soils (salt-affected soils) and ensure better plant and crop development. Therefore, target-oriented studies of the different kinds of soil-originated stress factors for plant and crop growth are necessary so that the complex correlations and the actions in the soil-plant-water system can be understood for the purpose of a better characterization of stress factors on the one hand and improving the environmental and production conditions on the other hand.

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2

Influence of Sodium on Soils of Humid Regions

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INTRODUCTION

A salt-affected soil is defined as one that has been adversely affected to the extent that it is no longer suitable for the growth of most crops by the presence or action of soluble salts. This group of soils includes both saline and sodic soils. James et al. [1] defined a saline soil as one that contains a quantity of soluble salts sufficient to interfere with the growth of most crops. On the other hand, a sodic soil possesses enough exchangeable sodium (ExNa) also to have an adverse effect on the growth of most plants. A saline-sodic soil contains both soluble and exchangeable Na at levels that impose stress on plant growth.

Salt-affected soils are a common feature of arid and semiarid landscapes. In humid regions, soils may become salt affected when they are irrigated with brackish water or treated sewage effluent, intruded by sea water, or contaminated with oil well brines. Some differences exist between salt-affected soils found in arid and semiarid regions and those found in humid and tropical regions. Sodic soils found in arid and semiarid regions are usually associated with high pH and dominated by the 2:1–type clay minerals. Salt-affected soils in humid or tropical regions generally have low pH, and they are often, but not always, dominated by 1:1–type clay minerals.

The loss of plant productivity from the excess of salinity is a worldwide problem. Where salinity is a problem, an effective use of soil and water resources dictate the production of agricultural crops. Numerous laboratory and field experiments have been conducted in order to determine the plant growth and yield response to various levels of soil salinity. For example, Shalhevet et al. [2] found that the yield of peanuts grown in artificially salinized plots was reduced to 50% at EC_e (EC_e = specific electrical conductance of saturated extract) of 4.7 dS m^{-1} and by 20% at EC_e of 3.8 dS m^{-1}. Additionally, these investigators reported that salt tolerance was much higher during germina-

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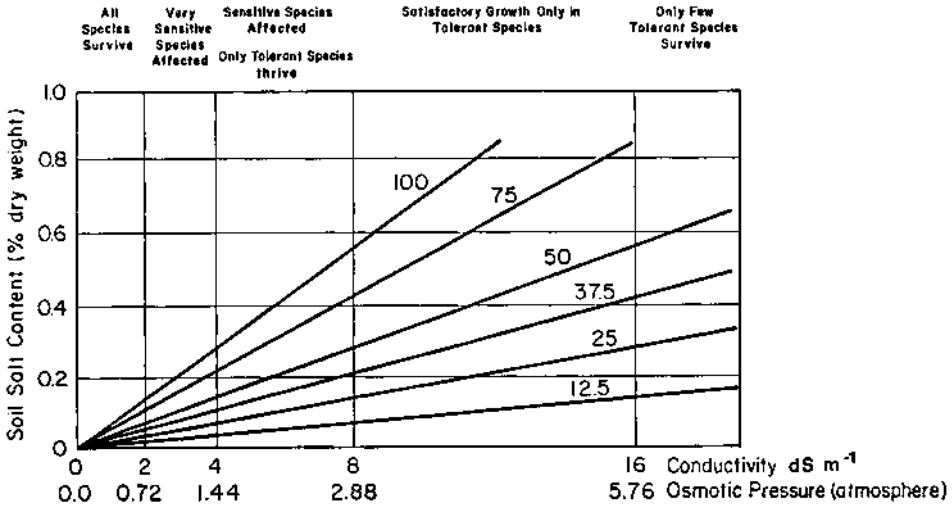

FIGURE 1 Relationship between electrical conductivity (EC) of soil solution and salt content. The numbers in the plot represent grams water needed to saturate 100 g soil. (It takes 12.5 g water to saturate 100 g sand, 100 g water to saturate 100 g clay, and about 50 g water to saturate 100 g of most Kentucky soils.) (From Ref. 6.)

tion than during subsequent growth. A 50% reduction in germination occurred at $EC_e = 13$ dS m^{-1}. Shalhevet and Yaron [3] reported a 10% yield reduction in tomatoes for every 1.5 dS^{-1} increase in EC_e above 2 dS m^{-1}. The adverse effects of soil salinity on plant growth and productivity vary with the type of plant being grown. A summary of the general response of plants to salinity is presented in Figure 1.

The presence of salinity in the soil solution resulting from either indigenous salt or that through irrigation can affect plant growth in three ways: (a) It can increase the osmotic potential and hence decrease water potential, thereby reducing water availability, the *osmotic effect*. (b) It can increase the concentration of certain ions that have an inhibitory effect on plant metabolism, a process known as the *specific-ion effect* [1]. (c) It can adversely affect soil structure such that water permeability and soil aeration are diminished [4], the *physicochemical effect*.

Osmotic Effect

The osmotic effect on plant growth is related to water availability or soil-water potential. Under normal field conditions, the soil-water potential, U_w, is determined by the osmotic potential, U_s, the matrix potential, U_m, and the gravitational potential, U_p. Mathematically, U_w is described by the equation

$$U_w = U_m + U_s + U_p \tag{1}$$

At any given matrix potential and a fixed gravitational potential, an increase in salinity is manifested by a reduction in U_w [1]. Bresler et al. [5] pointed out that physicochemically it can be shown that U_s of a solution is directly related to total dissolved solids (TDS). The relationship between U_s and TDS can be expressed by the following equation [6]:

$$U_s \text{ (bar)} = -5.6 \times 10^{-4} \times \text{TDS (ppm)} \tag{2}$$

Another way to express the above relationship is through the specific conductance of a soil's solution. The EC measurement is based on the principle that the amount of electrical current transmitted by a salt solution will increase as salt concentration in the solution increases. The U.S. Salinity Laboratory Staff [6] described the relationship as

$$U_s \text{ (bar)} = -0.36 \times \text{EC (dS m}^{-1}\text{)} \tag{3}$$

Specific Ion Effect

An excess of Na ions in the soil solution can be inhibitory to various plant physiological processes. Hausenbuiller [7] and Donahue et al. [8] reported that the sensitivity of plants to various Na levels in the soil solution and/or exchanger phase is highly dependent on plant species as well as the stage of plant development. Symptoms of Na toxicity can be easily seen when the leaves of sensitive plants contain approximately 0.25% Na on a dry-weight basis [1,5]. The Na^+ toxicity is characterized by leaf tip burn, necrotic spots, and limited leaf expansion, which in turn directly reduces plant photosynthesis and yield [9,10].

Obvious specific effects of Na^+ on plant physiological processes are observed when plants are grown in high-Na environments. High sodium concentrations have been shown to increase K^+ leakage and decrease root elongation [11]. Meire and Poljakoff-Mayber [12] reported that, when Na^+ is present in high concentration in the solution, transpiration rate of peas was reduced in proportion to salinity. Porath and Poljakoff-Mayber [13] found that Na^+ also affected the respiratory pathway of pea roots. High Na^+ in soil solution also has an antagonistic effect on Ca^{2+} and Mg^{2+} uptake [9]. Geraldson pointed out that salinity caused Ca deficiency symptoms in tomato, pepper, and celery plants. This is most likely caused by Na^+ displacing Ca^{2+} from membranes of root cells [15].

Physicochemical Effect

An excess of exchangeable Na is harmful to plants principally because it induces undesirable physical and chemical conditions in soils. The dispersion effect of exchangeable Na on clays is related to the highly hydrated nature of this ion. Soils disperse only when they are in equilibrium with an electrolyte solution under the ''flocculation value.'' The flocculation value depends on solution composition (sodium adsorption ratio, SAR), solution ionic strength, clay mineralogy [16–18], and pH [19,20]. For example, flocculation values for Na/Ca–montmorillonite are 3, 4, and 7 $mmol_c\ L^{-1}$ and 6, 10, and 18 $mmol_c$ L-1 for Na/Ca–illite with exchangeable sodium percentage (ESP) values of 5, 10, and 20, respectively [21].

Clay dispersion causes modification of soil pore distribution, which in turn affects soil hydraulic conductivity. An increase in Na levels in the soil solution or on the exchange phase (ESP) causes soil-saturated hydraulic conductivity to decrease [22–24].

The magnitude of ESP is related to the relative ratio of Na to Ca in the solution phase, also known as sodium adsorption ratio. An empirical relationship between ESP and SAR representing soils of the arid West was developed by the U.S. Salinity Laboratory Staff [6]. This ESP-SAR relationship is as follows:

$$\text{ESP} = \frac{100(-0.0126 + 0.014575\ \text{SAR})}{1 + (-0.0126 + 0.01475\ \text{SAR})} \tag{4}$$

where SAR in $(\text{mmol L}^{-1})^{1/2}$. When SAR is approximately in the range of 10–15, the ESP is also in the range of 10–15. In this ESP range, soils of the arid West will undergo dispersion. However, this relationship does not apply to all soils.

General Information on Saline-Sodic Soils

The removal of sodium from the soil profile of any given salt-affected soil is necessary, because Na is one of the most pronounced ions that influences plant growth and yield in salt-affected soils

[25]. Sodium is not only toxic to most plants because of its specific ion effect but it also influences certain soil properties. The major concern is the eventual deterioration of soil structure, resulting in decreased water infiltration and gas exchangeability [5].

Management and reclamation of salt-affected soils are necessary to maintain, or increase, their productivity. At least three processes take place during reclamation of a sodic soil: (a) the Na^+ on the exchange complex is replaced by Ca^{2+}; (b) the soil-saturated hydraulic conductivity is improved following Ca application; and (c) sodium salts are removed from the soil profile through leaching [1]. Thus, reclamation of salt-affected soils often requires the removal of excess soluble salts as well as reduction of the soil ESP (see Ref. 26 and references therein). The only proven method to reduce the soluble salt concentration in the root zone is through leaching. Reduction of the ESP is more difficult, because sodium ions adsorbed on exchange sites must first be replaced with divalent cations and then be leached from the root zone. According to Hoffman et al. [25], the amount of leaching required is dependent on the salt content of irrigation water, salt tolerance of the crops, climatic conditions, and soil and water management practices.

Imhoff Cone

In reclaiming salt-affected soils, the dispersion and hydraulic conductivity properties should be considered along with their exchange behavior. In this chapter, clay dispersion is based on the Imhoff cone technique. This Imhoff cone is commonly used by engineers to determine settleable solids. Settleable solids are the particles that settle in the bottom of an Imhoff cone during 1 h of settling [27].

In this chapter, the utility of Imhoff cone test results in predicting relative suppression in soil hydraulic conductivity is demonstrated. Such predictive potential–based Imhoff cone results (an engineering standardized test for evaluating settleable solids) will allow us to classify sodic soils with respect to their potential to undergo dispersion and/or restrict water movement. The results of this evaluation are presented in the latter portion of this chapter.

General Objectives

The effect of clay dispersion on saturated hydraulic conductivity in a soil is well established. However, there is a need for information on the influence of saturated hydraulic conductivity by various combinations of ionic strength, SAR, and pH for soils that have developed under temperate climatic regimes.

The U.S. Salinity Laboratory Staff [6] reported that an ESP of 15 is considered as the critical value above which most crop plants will not grow well because of adverse soil physicochemical effects induced by the presence of sodium salts. However, this threshold ESP value represents soils of the arid West and may not be universally applied to all soils. There is a great deal of information on the behavior of sodium chloride in soils and in soil solution suspensions [6,21]. However, most of this research pertains to soils of the arid West, which are often alkaline and consist mostly of 2:1 clay minerals. In the temperate regions of the United States, soils are often acid, their mineralogy is highly mixed (1:1 plus 2:1 clay minerals), and the 2:1 minerals are highly interlayered.

There is a need to understand sodicity and reclaimability of soils with a mixed type of charged site mineralogy (permanent plus variable charge), because (a) the ESP-SAR relationship has not been extensively investigated for these soils, which are present in the temperate region of the United States, and (b) brine discharges from oil wells have become a problem in the temperate regions of the United States.

Oil production often occurs in geographical locations where oil is not in abundant supply and environmental safeguards are not in place. Such oil wells, also known as ''stripper wells,'' are producing a large quantity of brine. This brine is often discharged into agricultural lands and/or into natural water supplies. In the state of Kentucky, it has been estimated that more than 375,000 L of brine per day is discharged onto land and surface waters. These brines contain approximately

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0.5 mol L^{-1} sodium chloride. Similar brine problems exist in many other southeastern and northeastern states. In the state of Ohio, for example, approximately 16 million L of brine per day is produced from such wells [28].

Information is needed on temperate region soils regarding their reactions with Na^+, the critical Na^+ loads under which these soils undergo dispersion, and to predict such critical Na^+ loads. This information is needed by farmers to reclaim brine-contaminated farmlands and/or irrigate lands with brackish water and is also needed by state, federal, and oil company personnel to develop guidelines for brine discharge management.

THERMODYNAMICS OF SODIUM-CALCIUM EXCHANGE IN SOILS

Soils are multicomponent systems consisting of solid (inorganic and organic components), liquid (soil solution), and gaseous phases. These three dynamic phases are to some extent in a constant state of flux, trying to maintain a state of equilibrium. The change in one phase will influence the other two phases until a new equilibrium state is approached. Cation exchange is one type of equilibrium interaction. This involves interexchange between cations in the solid phase with other cations in the solution phase.

Cation exchange reactions result primarily from the excess of negative charge of soil colloids. There are mainly two types of negative charge found in soil systems: permanent negative charge and variable or pH-dependent negative charge [29,30]. A permanent negative charge is generated because of isomorphic substitution of elements of smaller positive charge for those of higher positive charge in the crystal structure of clay minerals. Variable negative charge on mineral surfaces results from organic matter functional groups, such as carboxyls, and/or surface hydroxyls of inorganic minerals [31]. The magnitude of the variable negative charge is influenced by pH as well as ionic strength. An increase in pH and/or ionic strength is followed by an increase in negative charge [32]. In soil systems of temperate regions, these two types of negative charges are always present, but in some soils, one type of negative charge is more dominant than the other.

Because soils contain a mixture of various types of clay minerals and because more than two cations are present in such soil systems (Ca, Mg, K, Na, NH_4), a rigorous theoretical description of ionic distribution is difficult. Several theoretical approaches have been used in deriving binary exchange equations. Those most often mentioned in the literature are the thermodynamic and the double-layer approaches. The formal thermodynamic approach, based on the mass action principle, gives no direct information about the molecular mechanisms and the forces operating in such systems. On the other hand, the diffuse double-layer approach provides a description of Coulombic forces operating on ion exchange processes [21].

Bohn et al. [33] summarized the limitations of most cation exchange equations: (a) Binary cation exchange is frequently considered but rarely the simultaneous presence of additional cations is acknowledged even for highly acidic systems. (b) The cation exchanger is assumed to possess constant cation exchange capacity, but often cation exchange capacity varies with the nature of the exchanging ions, solution concentration, and pH. (c) Simple stoichiometric (1:1) ion exchange is generally assumed, but apparent deviations from 1:1 stoichiometry are usually explained in terms of simultaneous adsorption of molecules or in terms of the formation of complex ions. (d) Complete reversibility is usually taken for granted.

A large number of studies involving Na-Ca exchange have been conducted with respect to influence of ionic strength and solution composition. A few studies, however, have dealt with the role of pH on Na-Ca exchange reactions. This omission could be due to the fact that most salt-affected soils in the arid West exhibit pH values in the neutral range. Salt-affected soils in the temperate regions of the United States are often acid in nature and of mixed type of charge site mineralogy. That is, they are composed of minerals that contribute significant quantities of variable

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and permanent charge. It is not known how brine affects the Na-Ca exchange reactions in such soils.

Sodium-Calcium Exchange Theory: Mass Action

A binary exchange reaction at equilibrium involving Na^+ and Ca^{2+} on a soil system can be written as

$$\frac{1}{2}\,Ex_2Ca + Na^+ \Leftrightarrow ExNa + \frac{1}{2}\,Ca^{2+} \tag{5}$$

where Ex is an exchanger phase taken to have a charge of negative one (-1) and Na^+ and Ca^{2+} denote solution species. A criterion of chemical reaction equilibrium is [34]:

$$\sum \nu_i \mu_i = 0 \tag{6}$$

where ν_i is the stoichiometric coefficient in chemical reaction for species i and μ_i is the chemical potential for species i.

The chemical potential μ_i of species i in solution is identical to the partial molar Gibbs energy, G_i, and at constant T

$$d\mu_i = dG_i = RTd \ln f_i \tag{7}$$

relates these quantities to the fugacity, f_i, in solution. Integration of Equation (7) from the standard state of species i to a state of species i in solution gives

$$\mu_i - G_i^o = RT \ln\left(\frac{f_i}{f_i^o}\right) \tag{8}$$

where G_i^o is the molar Gibbs energy for species i, R is the gas constant, and T is the system temperature. The ratio f_i/f_i^o is defined as the activity, a_i, in solution. For a gas, the standard state, f_i^o, is the ideal gas state of pure i at a pressure of 1 bar (or 1 atm). Thus for gas phase reactions, $a_i = f_i/f_i^o = f_i$. For solids and liquids, the usual standard state is the pure solid or liquid at 1 bar (or 1 atm) and the system temperature.

From the preceding equations and definitions,

$$\mu_i = G_i^o + RT \ln a_i \tag{9}$$

and at thermodynamic equilibrium for a chemical reaction,

$$\sum \nu_i (G_i^o + RT \ln a_i) = 0 \tag{10}$$

from which it follows that

$$\Pi(a_i)_i^{\nu} = \exp\left(-\frac{\sum \nu_i G_i^o}{RT}\right) = K_{eq} \tag{11}$$

where Π signifies the product over all species i in the chemical reaction and K_{eq} is the equilibrium constant for the reaction. The pure component Gibbs energy, G_i^o, is a property of pure species i in its standard state and fixed pressure. It depends only on temperature. It follows from Equation (11) that K_{eq} is also only a function of temperature and ΔG^o is the standard Gibbs energy change of reaction. Furthermore, activities, a_i, are not completely defined without also defining the pure component reference states f_i^o and G_i^o.

The thermodynamic exchange equilibrium constant K_{eq} for reaction (5) at room temperature (22°C) and 1 atm pressure is thus represented by

$$K_{eq} = \frac{a_{Ca}^{1/2}\, a_{ExNa}}{a_{Na}\, a_{Ex_2Ca}^{1/2}} \tag{12}$$

where a_{Na} and a_{Ca} are the activities of solution phase Na^+ or Ca^{2+} and a_{ExNa} and a_{ExCa} are the activities of exchange phases Na^+ and Ca^{2+}. Activity, a_i, is defined by the equation

$$a_i = f_i \chi_i \tag{13}$$

where f_i = activity coefficient of species i and χ_i = concentration of species i. For mixed electrolyte solutions, the single ion activity concept introduced by Davies [35] is employed to estimate f_i [36].

The activity component of the adsorbed or solid phase is defined by employing the mole fraction concept introduced by Vanselow [37]. According to Vanselow [37], for a heterovalent binary exchange reaction such as Na^+-Ca^{2+}, assuming that the system obeys ideal solid-solution theory, the activity term (a_{Exi}) is defined by

$$a_{ExNa} \approx X_{Na} = \frac{ExNa}{ExNa + Ex_2Ca} \tag{14}$$

and

$$a_{Ex_2Ca} \approx X_{Ca} = \frac{Ex_2Ca}{ExNa + Ex_2Ca} \tag{15}$$

where X_{Na} and X_{Ca} are mole fractions of Na^+ or Ca^{2+} and Ex denotes exchange phase with a valence of -1. For a system where ideal solid-solution behavior is not obeyed,

$$a_{Exi} = f_i \chi_i \tag{16}$$

where f_i is the adsorbed ion activity coefficient. Note that in the mole fraction concept, the sum of exchangeable Na^+ (ExNa) and exchangeable Ca^{2+} (Ex_2Ca) is expressed in moles per kilogram soil. Because of this, the denominator of Equations (14) and (15) is not a constant even though the sum of exchangeable Na^+ and exchangeable Ca^{2+} when expressed in units of charge equivalents is a constant. Equivalent fractions E_i for Na^+ and Ca^{2+} are defined by

$$E_{Na} = \frac{ExNa}{ExNa + 2Ex_2Ca} \tag{17}$$

and

$$E_{Ca} = \frac{2Ex_2Ca}{ExNa + 2Ex_2Ca} \tag{18}$$

Equation (17) is used to estimate exchangeable sodium percentage simply by multiplying E_{Na} by 100. This above binary systems cation exchange capacity (CEC) of the soil is taken to be

$$CEC = ExNa + 2Ex_2Ca \tag{19}$$

Based on this concept, an equilibrium exchange expression for reaction (5) can be given as

$$K_v = \frac{X_{Na} a_{Ca}^{1/2}}{X_{Ca}^{1/2}\, a_{Na}} \tag{20}$$

where $a_{Na}/a_{Ca}^{1/2}$ is known as the sodium adsorption ratio and K_v is the Vanselow exchange selectivity coefficient.

Commonly, the magnitude of K_v is taken to represent relative affinity of Na^+ with respect to Ca^{2+} by the clay surface [38,39]. When K_v equals 1 at a given level of exchangeable Na^+, the

exchanger at that level of Na load shows no preference for either Na^+ or Ca^{2+}. On the other hand, a $K_v > 1$ at any given level of exchangeable Na^+ signifies exchanger preference for Na^+ and a $K_v < 1$ at any given level of exchangeable Na^+ signifies preference for Ca^{2+}.

Upon making the proper substitutions and rearranging Equation (20) to the form of a quadratic equation

$$(\text{ExNa})^2 = \frac{(K_v\ \text{SAR}\ \text{CEC})^2}{(K_v\ \text{SAR})^2 + 4} \tag{21}$$

If one takes the positive root of Equation (21) and redefines the left hand term of this equation as exchangeable sodium percentage (ESP)

$$\text{ESP} = \frac{\text{ExNa}}{\text{CEC}}\,100 = \frac{K_v\ \text{SAR}}{[4 + (K_v\ \text{SAR})^2]^{1/2}}\,100 \tag{22}$$

Taking limits of Equation (22) as SAR approaches zero and SAR approaches infinity,

$$\lim_{\text{SAR}\to 0}\ \text{ESP} = 0 \tag{23}$$

and

$$\lim_{\text{SAR}\to \infty}\ \text{ESP} = 100 \tag{24}$$

In Equation (22), it is assumed that CEC and K_v are constant for the entire N^+-Ca^{2+} exchange isotherm. The plot of this equation in terms of ESP versus SAR gives a curvilinear function asymptotically approaching 100 [40]. Note that the shape of this plot is K_v dependent.

A variable K_v with respect to the exchangeable Na^+ load on the soil can be transformed to the thermodynamic exchange constant K_{eq} as follows:

$$K_{eq} = K_v \frac{f_{Na}}{f_{Ca}^{1/2}} \tag{25}$$

where f_{Na} and f_{Ca} are adsorbed ion activity coefficient for Na^+ and Ca^{2+}. Argersinger et al. [41] noted that any variation in K_v with respect to exchange-phase composition is followed by a variation in the solid-phase activity coefficients f_i. Furthermore, any variation in E_{Na} must be compensated for by a variation in E_{Ca}.

Based on this, Argersinger et al. [41] generated two equations that give values for $\ln f_{Na}$ and $\ln f_{Ca}$ at any value of E_{Ca}:

$$\ln f_{Na} = (1 - E_{Na})\ln K_v - \int_{E_{Na}}^{1} \ln K_v dE_{Na} \tag{26}$$

and

$$\frac{1}{2}\ln f_{Ca} = -E_{Na}\ln K_v + \int_0^{E_{Na}} \ln K_v\, dE_{Na} \tag{27}$$

where E_{Na} is the equivalent charge fraction of adsorbed Na^+. The equation for E_{Na} is given by Equation (17). For a detailed discussion of Equations (26) and (27), refer to Evangelou and Phillips (42) and references therein.

A number of researchers have carried out various studies involving binary heterovalent exchange on various clay minerals. For example, Sposito and Mattigod [43] showed that for the exchange reactions of Na^+ with trace metal cations (Cd^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, and Zn^{2+}) on Camp Berteau montmorillonite, K_v was constant and independent of exchanger composition up to an equivalent fraction of trace metal cations of 0.70. These observations indicate that the cationic mixture on the

exchanger phase up to an equivalent fraction of trace metal cations of 0.70 behaves as an ideal mixture. However, Van Bladel et al. [44] studied Na^+-Ca^{2+} exchange reaction on the same kind of mineral and found that there is a more pronounced selectivity of clay for Ca^{2+} ions at the calcium-rich end of the isotherm. Levy and Hillel [45] reached the same conclusions studying Na^+-Ca^{2+} exchange on montmorillonitic soils.

Based on these Na^+-Ca^{2+} exchange studies, the magnitude of K_v is variable in nature and detailed and meticulous experiments are required in order to quantify it. In general, it can be said that the selectivity coefficient K_v of a binary exchange reaction depends primarily on the ionic strength and on two dimensionless parameters, one a measure of the proportion of cations in the soil absorbing complex and the other a measure of their proportions in the soil solution phase [39,46].

Sodium-Calcium Exchange Theory: Diffuse Double Layer

The diffuse double layer is the swarm of ions accumulating near a charged surface, balancing the charge of that surface. The distribution of ions in that swarm is assumed to follow a Boltzmann distribution (see Ref. 47 and references therein):

$$n_c = n_c^{\infty} \exp\left(\frac{-z_c e\varphi}{kT}\right)$$
$$n_a = n_a^{\infty} \exp\left(\frac{z_a e\varphi}{kT}\right) \tag{28}$$

where n and z are the electrolyte concentration and valence for the cation (c) and anion (a) as a function of distance from the surface, n_c^{∞} and n_a^{∞} are the cation and anion concentrations in the bulk solution, e is the electronic charge, φ is the electrical potential as a function of distance from the surface, k is Boltzmann's constant and T is temperature. At any point in the system, the local net charge density (ρ) is given by

$$\rho = [\Sigma(z_c e n_c) - \Sigma(z_a e n_a)] \tag{29}$$

For symmetrical electrolytes of the form NaCl or $CaSO_4$,

$$\rho = \left[z_c e n_c^{\infty} \exp\left(\frac{-z_c e\varphi}{kT}\right) - z_a e n_a^{\infty} \exp\left(\frac{z_a e\varphi}{kT}\right) \right] \tag{30}$$

where Equation (28) has been substituted into Equation (29). Given that

$$\sinh(x) = \frac{e^x - e^{-x}}{2} \tag{31}$$

and, recognizing that electroneutrality requires

$$n_c^{\infty} = n_a^{\infty} \tag{32}$$

Equation (30) can be written as

$$\rho = -2 e n_c^{\infty} \sinh\left(\frac{ze\varphi}{kT}\right) \tag{33}$$

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For mixtures of symmetric electrolytes of the form NaCl and $CaSO_4$, again recognizing that electroneutrality requires

$$n^{\infty}_{Na} = n^{\infty}_{Cl} \text{ and } n^{\infty}_{Ca} = n^{\infty}_{SO_4} \tag{34}$$

Equation (30) can be written as

$$\rho = -2e\left[n_1^{\infty} \sinh\left(\frac{e\varphi}{kT}\right) + 2n_2^{\infty} \sinh\left(\frac{2e\varphi}{kT}\right)\right] \tag{35}$$

where subscripts 1 and 2 refer to the univalent and divalent salt respectively and the ion valences have been included explicitly. Equations (33) and (35) then describe the net charge density, at any point (x) in the solution, as a function of the electrical potential at that point. When the point x is taken to be very far from the charged surface (x^{∞}), the electrical potential = 0 and hence $\rho = 0$. To describe the variation in electrical potential as a function of distance from the surface, one makes use of the Poisson equation, which in one dimension reads

$$\frac{d^2\varphi}{dx^2} = -\frac{\rho}{\varepsilon} \tag{36}$$

where ε is the dielectric constant. Substituting Equation (35) into Equation (36) yields

$$\frac{d^2\varphi}{dx^2} = \frac{2e\left[n_1^{\infty} \sinh\left(\frac{e\varphi}{kT}\right) + 2n_2^{\infty} \sinh\left(\frac{2e\varphi}{kT}\right)\right]}{\varepsilon} \tag{37}$$

It is customary to let

$$y = \frac{e\varphi}{kT} \tag{38}$$

so that Equation (37) can be simplified to

$$\frac{d^2y}{dx^2} = \frac{2ze}{\varepsilon kT}\left[n_1^{\infty} \sinh(y) + 2n_2^{\infty} \sinh(2y)\right] \tag{39}$$

Considering the interaction between two flat plates separated by a distance, 2d, Equation (39) can be integrated once [48], with the appropriate boundary conditions ($dy/dx = 0$ for $x = d$ when $y = y_d$) to yield,

$$\frac{dy}{dx} = -\frac{\sqrt{4e^2}}{\varepsilon kT}\left[n_1^{\infty}(\cosh y - \cosh y_d) + 2n_2^{\infty}(\cosh^2 y - \cosh^2 y_d)\right]^{0.5} \tag{40}$$

Letting

$$\beta = \frac{\sqrt{4e^2}}{\varepsilon kT} \tag{41}$$

Equation (40) reads

$$\frac{dy}{dx} = -\beta\left[n_1^{\infty}(\cosh y - \cosh y_d) + 2n_2^{\infty}(\cosh^2 y - \cosh^2 y_d)\right]^{0.5} \tag{42}$$

Erickson [48] made an elegant observation in that Equation (42) need not be further integrated

to determine the fraction of monovalent ion in the diffuse double layer. Electroneutrality requires that the charge on the particle be balanced by the charge in the diffuse double layer. Therefore, the particle surface charge density, σ_0, can be expressed as

$$\sigma_0 = -\int_0^d \rho dx = \varepsilon \int_0^d \frac{d^2\varphi}{dx^2} = -\varepsilon\left(\frac{d\varphi}{dx}\right)_{x=0} = -\frac{\varepsilon kT}{e}\left(\frac{dy}{dx}\right)_{x=0} \tag{43}$$

Substituting Equation (42) into Equation (43) yields

$$\sigma_0 = \left(\frac{\varepsilon kT}{e}\beta\right)[n_1^\infty(\cosh y - \cosh y_d) + 2n_2^\infty(\cosh^2 y - \cosh^2 y_d)] \tag{44}$$

In the same way, the concentration of monovalent ions in the diffuse double layer is given by

$$\sigma_1 = -2n_1^\infty e \int_0^d \sinh y \, dx \tag{45}$$

where Equation (33) was used. By substituting Equation (42) into Equation (45),

$$\sigma_1 = \frac{2n_1^\infty e}{\beta}\int_{Y_d}^{y_0} \frac{\sinh y \, dy}{[n_1^\infty(\cosh y - \cosh y_d) + 2n_2^\infty(\cosh^2 y - \cosh^2 y_d)]^{0.5}} \tag{46}$$

integrating Equation (45), the fraction of the surface charge neutralized by the monovalent ions (σ_1/σ_0) is given as [48,49]

$$\frac{\sigma_1}{\sigma_0} = \frac{n_1^\infty}{\sigma_0\sqrt{n_2^\infty}\sqrt{\beta}} \sinh^{-1}\frac{\sigma_0\sqrt{\beta}}{\frac{n_1^\infty}{\sqrt{n_1^\infty}} + 4u_d\sqrt{n_2^\infty}} \tag{47}$$

where

$$u_d = \cosh\frac{e\varphi_d}{kT} \tag{48}$$

and φ_d = potential in the plane midway between the clay plates. Since

$$SAR = \frac{n_1^\infty}{\sqrt{n_2^\infty}}\sqrt{1000} \tag{49}$$

where n_1^∞ = Na and n_2^∞ = Ca, Equation (20) can be written

$$\frac{\sigma_1}{\sigma_0} = \frac{SAR}{31.6\sigma_0\sqrt{\beta}} \sinh^{-1}\frac{31.6\sigma_0\sqrt{\beta}}{SAR + 126.4u_d\sqrt{Ca}} \tag{50}$$

In the above derivation, it was assumed that the surface charge is a constant. Although derived for symmetrical electrolytes, Equation (47) has been shown to work reasonably well for the Na-Ca-Cl system [49,39].

From Equations (47) and (50), and recognizing the shape of the function $y = \sinh^{-1}(x)$, the following observations can be made. First, increasing SAR increases σ_1/σ_0, although not linearly. Second, increasing ionic strength, which is accounted for by the $\sqrt{Ca}$ term, decreases σ_1/σ_0 [50]. And third, increasing CEC ($\sim \sigma_0$) increases σ_1 but decreases σ_1/σ_0 [50]. All of these observations are consistent with the results of Evangelou and Phillips [40]. Typically, it has been assumed that the soil particles were sufficiently far apart so that $\varphi_d = 0$ and $u_d = 1$ [49,51]. Shainberg et al. [39] have shown that in systems where tactoids are formed such that $\varphi_d \neq 0$, increasing ionic strength

increases σ_1 for the internal tactoid surfaces but decrease σ_1 for the external clay surfaces. This suggests that montmorillonitic soils will behave differently than soils with mixed mineralogy [39,52].

Note that if an approach similar to Erickson's [48] were used to calculate ion accumulation in the diffuse double layer for a homovalent exchange system, Equation (47) would read [51]

$$\frac{\sigma_1}{\sigma_0} = \frac{n_1^\infty}{n_1^\infty + n_2^\infty} \tag{51}$$

where here the subscripts 1 and 2 refer to two monovalent ions (e.g., 1 = Na^+ and 2 = K^+). That is, the unmodified diffuse double layer theory does not predict ion selectivity: the fractional concentration of ion 1 in the diffuse double layer is equal to the fractional concentration of ion 1 in the bulk solution. Bolt [53] has shown that the effects of dielectric saturation, ion polarization and ionic interactions are small if $\sigma_0 < 160$ $cmol_c$ kg^{-1}. Shainberg and Kemper [54] have shown that incorporating ion hydration energies into the Stern-modified diffuse double layer theory, the observed ion affinity sequence $K^+ > Na^+ > Li^+$ can be rationalized. Because the hydration energies of ions has implications for ion exchange reactions on soils and ion selectivity at the plasmalemma level, the results of Shainberg and Kemper [54] are reviewed here.

The Stern modifications to the diffuse double layer theory are (a) ions can get no closer to a surface than the radius of that ion and (b) some ions may specifically sorb to the surface (i.e., without the hydration waters). The diffuse double layer is then separated into two parts, a layer of specifically sorbed ions (Stern layer) and the ordinary diffuse double layer. The equations for the diffuse double layer are modified such that φ is the potential at the Stern layer instead of at the particle surface. Following this, Shainberg and Kemper [54] outline the following. The total surface charge density, σ_T, is divided into two components, one for the Stern layer (σ_s) and one for the diffuse double layer, σ_{ddl},

$$\sigma_T = \sigma_S + \sigma_{ddl} \tag{52}$$

The concentration of cations in the diffuse double layer, n_C^{ddl} is given, following Equation (28) as

$$n_C^{ddl} = n_C^\infty \exp\left(\frac{-ze\varphi_s}{kT}\right) \tag{53}$$

where φ_S is the electrical potential at the Stern layer. The concentration of cations in the Stern layer, n_C^S, is given as

$$n_C^S = n_C^{ddl} \exp\left(\frac{E_S - E_{ddl}}{kT}\right) \tag{54}$$

where E_S is the potential energy of an ion in the Stern layer and E_{ddl} is the potential energy of an ion in the diffuse double layer. The charge density of cations in the Stern layer is given as

$$\sigma_S = ze\delta\, n_C^S \tag{55}$$

where δ is the thickness of the Stern layer, or combining Equations (53), (54), and (55)

$$\sigma_S = ze\delta\, n_C^\infty \exp\left(\frac{-ze\varphi_s}{kT}\right)\exp\left(\frac{E_S - E_{ddl}}{kT}\right) \tag{56}$$

An approximate form of the surface charge density in the diffuse double layer [55] is given as

$$\sigma_{ddl} = \sqrt{\varepsilon kT\, n_C^\infty}\exp\left(\frac{-ze\varphi_S}{kT}\right) \tag{57}$$

Combining Equations (52), (56), and (57) and setting

$$B = \sqrt{\varepsilon k T n_C^{\infty}}$$

$$H = ze\delta n_C^{\infty} \exp\left(\frac{E_S - E_{ddl}}{kT}\right), \text{ and} \tag{58}$$

$$Y_S = \frac{-ze\varphi_S}{2kT}$$

yields

$$H \exp(2Y_S) + B \exp(Y_S) - \sigma_T = 0 \tag{59}$$

The positive root of Equation (59) is

$$\exp(Y_S) = \frac{-B + \sqrt{B^2 + 4H\sigma_T}}{2H} \tag{60}$$

That is, the Stern layer potential (Y_S) can be written as a function of the fundamental constants k, e, E_S and E_{ddl}, and the experimental variables ε, T, and n_C^{∞}. Shainberg and Kemper [54] provide the detail for determining E_S-E_{ddl}. Once Y_S is known, n_C^{ddl} can be determined from Equation (53) and σ_S and σ_{ddl} from Equations (56) and (52). The results for Li^+, Na^+, and K^+ are that 16% of the Li^+, 36% of the Na^+, and 49% of the K^+ is in the Stern layer (Fig. 2A) [54]. That is, when comparing monovalent ions, the higher the ion hydration enthalpy, the less likely it is that the ion will be located in the Stern layer.

The above equations have implications for ion uptake by plants as well as ion exchange reactions on soil surfaces. Because cell membranes are negatively charged, the presence of an aqueous solution establishes an electric double layer. In many, but not all, cases ion toxicity effects are more closely correlated with ion (concentrations) activities at the membrane surface that with bulk solution ion (concentrations) activities [56]. In these instances, it is possible to rationalize the effects of ion interactions on ion uptake without invoking the presence of specific ion carriers, multiple sites, or other metabolic explanations. For example, Maas [57] evaluated the effect of increasing concentrations of Li, Na, and K on the uptake of Li, Na, K, and Ca into excised maize roots (see Fig. 2B). The results (see Fig. 2B) are entirely consistent with the preceding equations. In Equation (47), increasing r (the relative monovalent ion concentration) increases σ_1/σ_0 and hence the monovalent ion concentration in the double layer. Accepting that for an ion to move into a cell it must first move to the surface of the cell membrane, increasing the relative monovalent ion concentration in the solution phase necessarily increases ion uptake. When comparing Li, Na, and K, the extent ion uptake increases should be proportional to the ability of the ion to move into the Stern layer. That is, increasing the solution phase Li concentration has a modest effect on Li uptake, whereas increasing the solution K concentration has a large effect on K uptake. The effects of monovalent ion concentration on Ca uptake can be explained similarly. To suppress Ca uptake, the monovalent ion has to compete at the Stern layer level. Therefore, from Shainberg and Kemper's analysis [54], one would predict that K would be more effective at suppressing Ca uptake than Li (see Fig. 2B).

SALT CONCENTRATION AND pH INFLUENCE ON SODIUM-CALCIUM EXCHANGE

In order to demonstrate the influence of salt concentration and soil pH on Na^+-Ca^{2+} exchange on soils representative of humid regions, data on two such soils are given here. These two soils are the Pembroke (fine silty, mixed, mesic, Mollic Paleudalf) from Hardin County, Kentucky and the Uniontown (fine silty, mixed, mesic, Typic Hapludalf) from Union County, Kentucky [36]. The

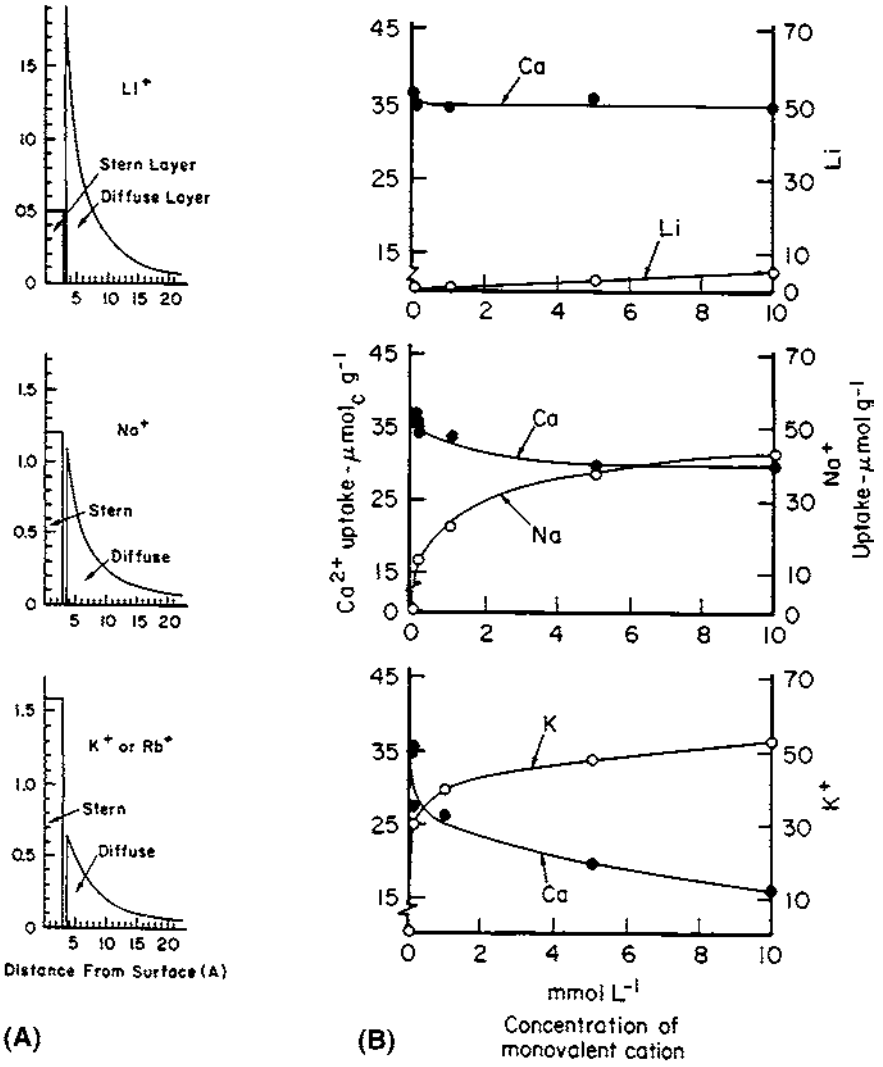


FIGURE 2 (A) The effect of increasing K^+, Na^+, and Li^+ concentration on the uptake of Ca^{2+}, K^+, Na^+, and Li^+ in 24 h. The concentration of Ca^{2+} was 10 $mmol_c\ L^{-1}$ and the pH was 6 (after Ref. 57). (B) Calculated cation concentrations near a charged surface (after Ref. 54).

Pembroke soil is much higher in clay content than the Uniontown soil. The Pembroke soil is dominated by kaolinite and to a lesser extent by mica, vermiculite, and hydroxy-interlayered vermiculite and smectite. The Uniontown soil is dominated by vermiculite and to a lesser extent by mica, kaolinite, and hydroxy-interlayered vermiculite and smectite. Another important difference is the much greater iron content of the Pembroke soil.

The data in Table 1 show the mean value of the summation of exchangeable Na^+ and exchangeable Ca^{2+} as a function of pH and chloride concentration for the Pembroke and the Uniontown soils, respectively. Each mean value reported is represented by 15 different ExNa or ExCa loads. The plus or minus value associated with each mean value represents the difference in metal adsorption when one of the metals (Na^+ or Ca^{2+}) on the exchange phase approaches zero. Thus, for any

TABLE 1 Mean-Sum *M* of Exchangeable Na and Ca of Pembroke and Uniontown Soils as a Function of pH and Chloride Concentration[a]

	Pembroke ($cmol_c\ kg^{-1}$)			Uniontown ($cmol_c\ kg^{-1}$)		
Cl ($mmol\ L^{-1}$)	pH 4.3	pH 6.1	pH 7.5	pH 4.3	pH 6.3	pH 7.7
5	7.4 ± 0.2	8.8 ± 0.3	9.7 ± 0.4	8.9 ± 0.4	9.5 ± 0.5	10.5 ± 0.6
50	11.2 ± 1.4	12.7 ± 1.3	13.6 ± 1.2	11.2 ± 1.4	13.4 ± 1.6	14.1 ± 1.5
200	27.7 ± 3.3	27.7 ± 5.5	28.9 ± 5.4	20.8 ± 2.1	23.2 ± 2.8	25.4 ± 2.9

[a] M + S = effective charge (EC_g) of Ca-loaded soil; M − S = effective charge (EC_g) of Na-loaded soil; S = deviation from the average.
Source: From Ref. 36.

mean value plus the deviation from the mean, the sum signifies the effective charge (EC_g) of the soil when the latter is loaded with Ca^{2+}, and for any mean value minus the deviation from the mean, the difference signifies the EC_g of the soil when the latter is loaded with Na^+. The data in Table 1 clearly demonstrate that the EC_g of these two soils is highly ionic strength dependent, specific ion dependent, and to a lesser degree pH dependent. The variation in effective soil charge as a function of the type of metal was previously reported by Fletcher et al. [58], Hutcheon [59], and Faucher and Thomas [60].

The ESP versus sodium adsorption ratio SAR plots of the Pembroke soil are presented in Figures 3 and 4. These two figures demonstrate that the ESP-SAR relationship of the Pembroke soil is independent of pH and ionic strength. The data also imply that the K_v for this soil should be independent of pH and ionic strength [40]. This is demonstrated in Figures 5 and 6. These two figures show that there are at least two classes of exchange sites with respect to Na^+-Ca^{2+} exchange on the Pembroke soil. These data also point out that at low ESP values (ESP $<$20) the soil exhibits a high affinity for Na^+, perhaps because of steric processes. At ESP $>$20, however, the magnitude of K_v remains constant, approximately 1, which suggests no ion preference [61]. Furthermore, it appears that the Pembroke soil behaves as an ideal exchanger between ESP of about 20 and 100. These observations are consistent with the information presented by van Bladel et al. [44] and Levy and Hillel [45].

The apparent lack of influence of pH and ionic strength on the K_v of such soils could be related to a number of processes that take place on a clay surface as pH and/or ionic strength increases. For example, Pratt et al. [62] have demonstrated on a number of soils that as pH decreases the exchange selectivity coefficient of Na^+-Ca^{2+} exchange increases. This increase signifies increase in affinity of the Na^+ by the clay surface through decreasing surface charge density. The Pratt group's [62] data tend to support this conclusion. Additionally, Shainberg et al. [39] have shown that for Na^+-Ca^{2+} exchange, as ionic strength increases the affinity for the Na^+ by the illite surface also increases. The latter observation, however, depends on whether one deals with an external surface or internal surface. For example, an increase in ionic strength on an external surface (low electrical potential surface) could increase the affinity for the Ca^{2+}. However, an increase in ionic strength on an internal surface (high electrical potential surface) could increase the affinity for the Na^+. Considering that mix mineralogy soils are made up of external and internal surfaces, a canceling effect on the magnitude of K_v due to an increase in ionic strength could be obtained.

The ESP versus SAR plots for the Uniontown soil are also shown in Figures 3 and 4. That pH and ionic strength have a strong influence on K_v is strongly supported by these figures. This is substantiated in Figures 5 and 6. The K_v data in Figure 5 show that as pH increases K_v also increases; consequently, Na^+ is preferred by the solid phase. Furthermore, as ESP increased K_v also increased. Stumm and Bilinski [63] showed that deprotonating clay edge surfaces have greater affinity for a monovalent cation than a divalent cation, because the former (monovalent cation) requires much less free energy to desolvate and thus come closer to the adsorbing surface. On the other hand,

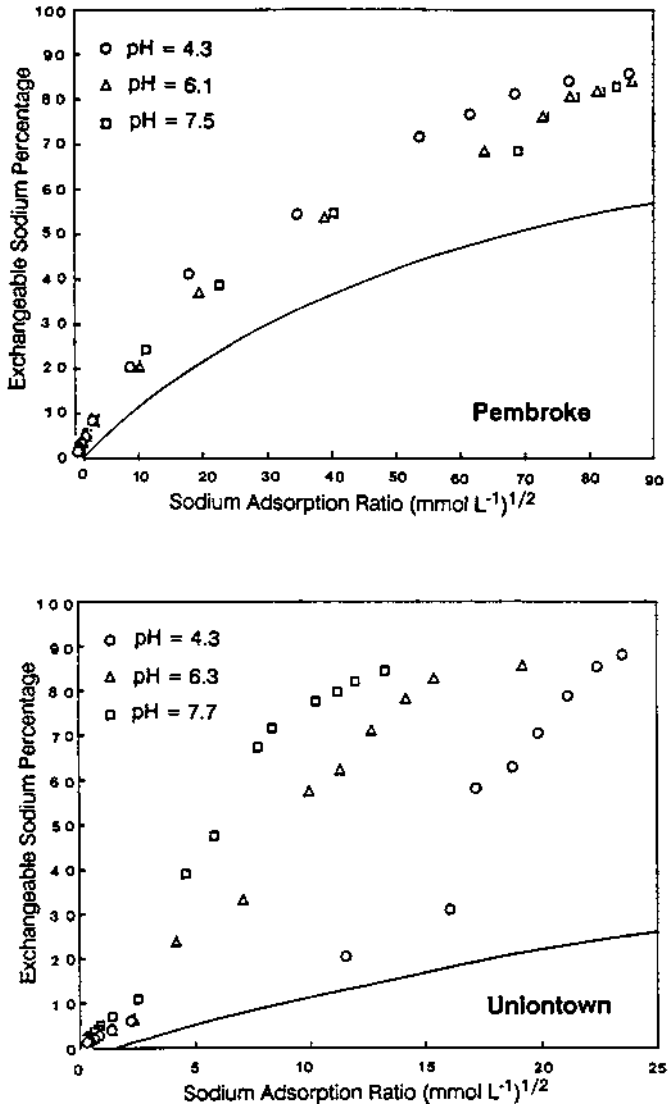


FIGURE 3 Relationship between exchangeable sodium percentage (ESP) and sodium adsorption ratio (SAR) at a chloride (Cl) concentration of 5 mmol L^{-1} of Pembroke and Uniontown soils at three pH values. The solid line without data represents most salt-affected soils in the western United States. (From Refs. 6 and 36.)

according to the data shown in Figure 6, as ionic strength increases, K_v decreases. This indicates that under high ionic strength the soil prefers Ca^{2+}. This also implies that under high ionic strength the divalent cations are most likely to carry out the soil deprotonation process. Finally, the data in Figures 3 and 4 clearly demonstrate that the two humid region soils exhibit a much higher affinity for Na^+ than the average salt-affected soil in the western United States. Note the difference in the Na^+ adsorption isotherms exhibited by the humid region soil and the western U.S. soils. This implies

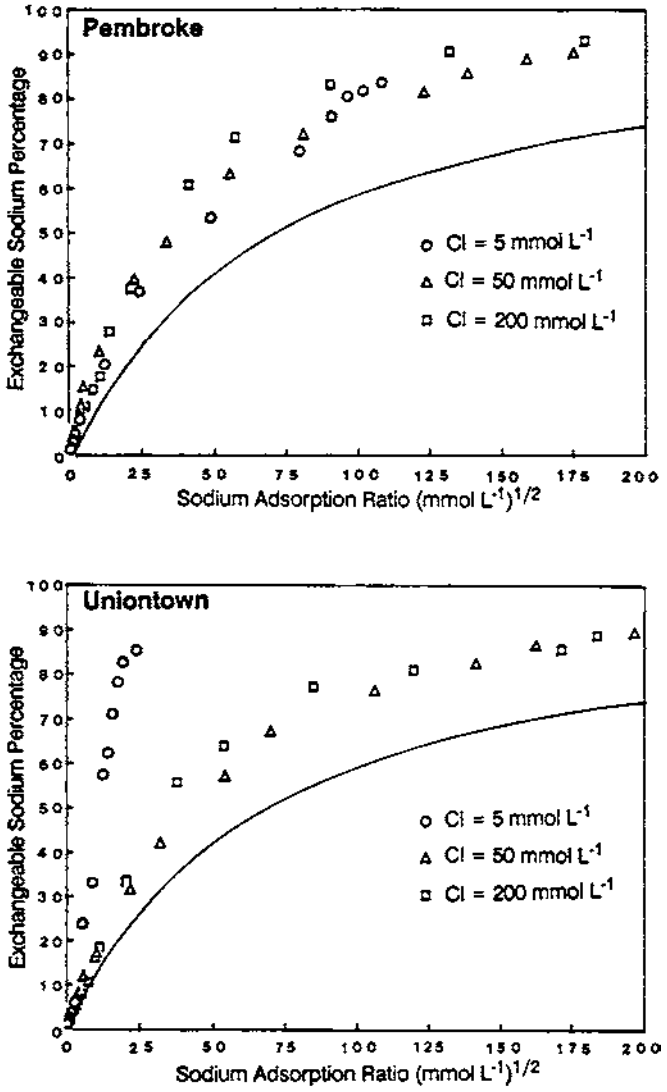


FIGURE 4 Relationship between exchangeable sodium percentage (ESP) and sodium adsorption ratio (SAR) at three chloride (Cl) concentrations of the Pembroke and Uniontown soils at pH 4.3. The solid line without data represents most salt-affected soils in the western United States. (From Refs. 6 and 36.)

that physical behavior and reclamation practices for these two groups of soils are expected to be different.

Values of f_{Na} and f_{Ca} for the Pembroke and Uniontown soils are plotted as a function of ESP in Figure 7. These data represent the two soils, at pH 6.1 for the Pembroke soil and 6.3 for the Uniontown soil, in all three chloride levels. These treatments were chosen because the two soils show the largest differences in K_v as a function of ionic strength as well as when the pH is near neutral. The data show that for the Pembroke soil Ca^{2+} is tightly bound to the charged surface (f_{Ca}

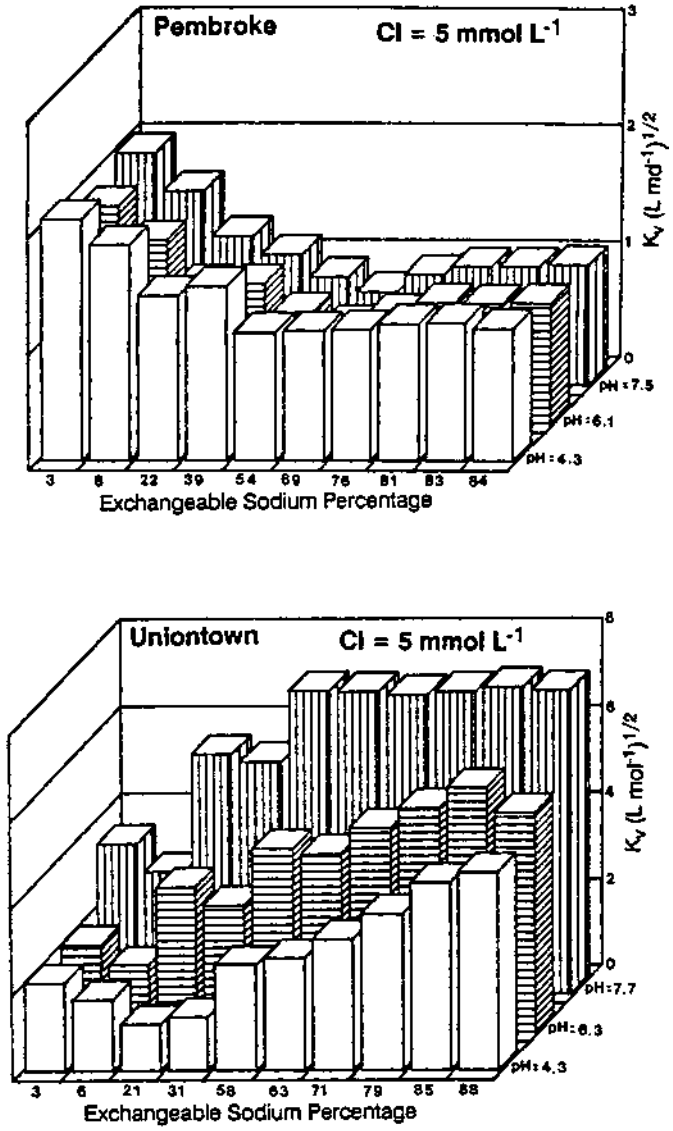


FIGURE 5 Influence of exchangeable sodium percentage (ESP) on the Vanselow exchange coefficient of Pembroke and Uniontown soils at a chloride (Cl) concentration of 5 mmol L^{-1} and at three pH values. (From Ref. 36.)

< 1). This adsorption strength increases as ESP increases. Also at low ESP values, f_{Na} is less than 1, which signifies that the Na ion is specifically interacting with the surface. Furthermore, as ESP increases, f_{Na} increases and becomes approximately 1. Ionic strength also appears to have influence on the magnitude of f_{Ca}. The data show that f_{Ca} at 200 mmol L^{-1} Cl concentration is larger than f_{Ca} at 5 and 50 mmol L^{-1} Cl. This could be because the Pembroke soil is dominated by external adsorption sites [42].

The findings demonstrated in Figure 7 for the Pembroke soil are not in full agreement with

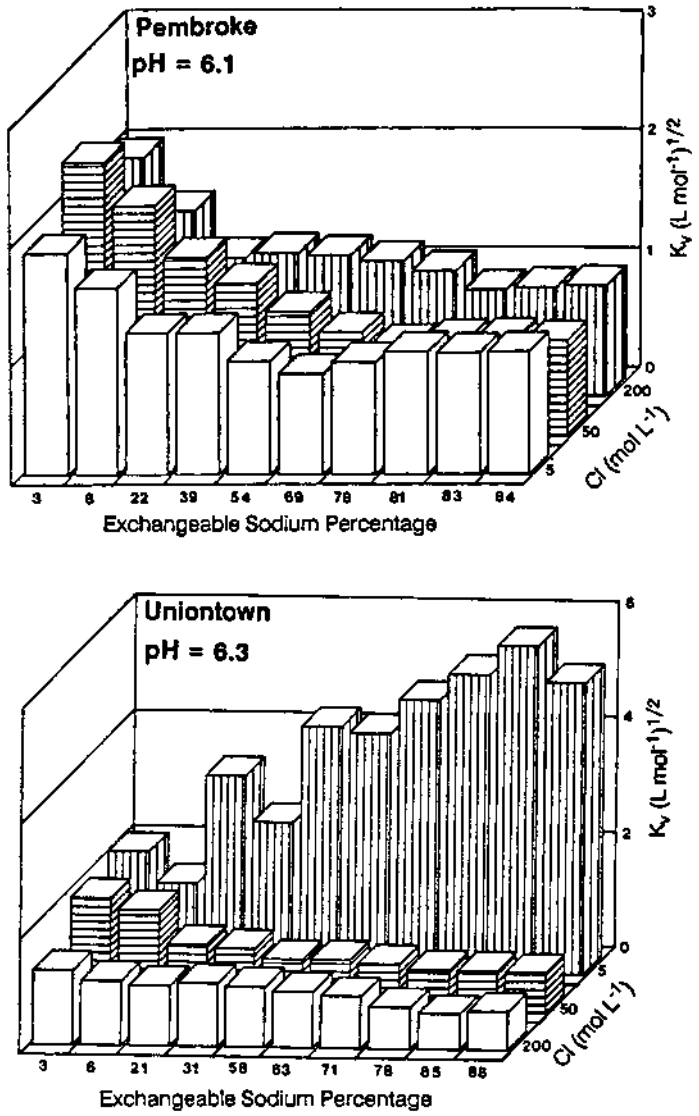
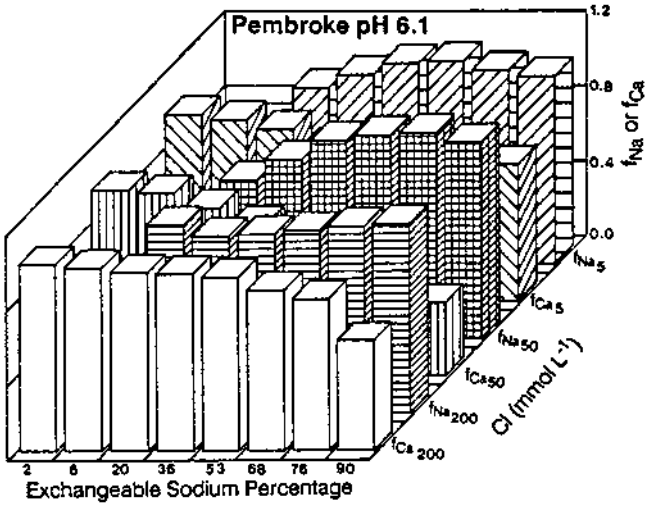


FIGURE 6 Influence of exchangeable sodium percentage (ESP) on the Vanselow exchange coefficient of the Pembroke and Uniontown soils near pH 6 and at three chloride (Cl) concentrations. (From Ref. 36.)

the findings also shown in Figure 7 for the Uniontown soil. This is especially true for f_{Na}. These differences could be attributed to the mineralogical differences of these two soils. The Pembroke soil, because of its high kaolinite content, is dominated by external surface area; therefore, it is expected to exhibit high specificity for Na^+ [42]. On the other hand, the Uniontown soil, because of high vermiculite content (large internal surface area), is expected to exhibit low specificity for the Na^+, especially at low ionic strength [42]. It is important to keep in mind that temperate region soils are of mixed mineralogy and much of the Na^+-Ca^{2+} exchange behavior is also subject to the interactions between fixed- and variable-charge components.



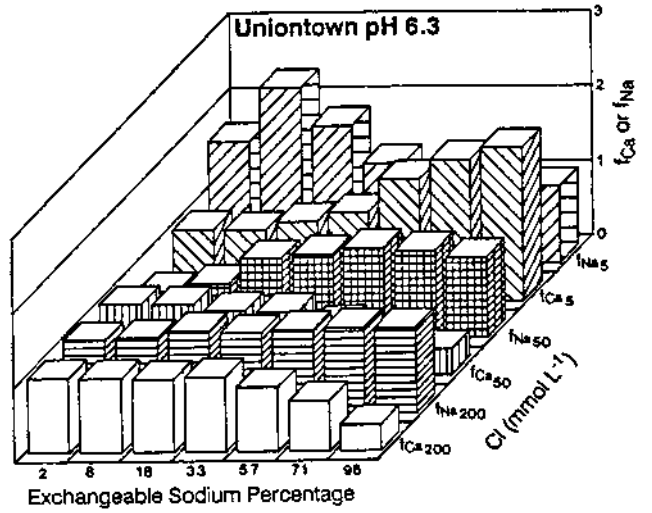


FIGURE 7 Influence of exchangeable sodium percentage (ESP) on the adsorbed ion activity coefficient of the Pembroke and Uniontown soils near pH 6 and at three chloride (Cl) concentrations. (From Ref. 36.)

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SODIUM INFLUENCE ON SOIL DISPERSION AND SATURATED HYDRAULIC CONDUCTIVITY

The classic theory of colloidal stability developed by Derjaguin and Landau [64] and Verwey and Overbeek [65] (DLVO theory) generally accounts for the influences of ion valence and concentration on suspended colloid interactions. According to the DLVO theory, the long-range repulsive potential (μ) resulting from diffuse double layers (DDLs) of like charged colloids retards the coagulation or flocculation rate of clay colloids.

Colloidal stability (maximum dispersion) depends on maximum Φ (Φ_{max}), which describes the maximum repulsive energy between two planar colloidal surfaces. Furthermore Φ_{max} is controlled by surface electric potential (Ψ) and ionic strength. The component, Ψ, is controlled by the pH of the colloidal suspension, assuming that the colloids involved exhibit pH-dependent charge [66]. Generally, in clay colloids on increasing pH, Ψ becomes more negative and thus Φ_{max} increases. Conversely, on decreasing pH, Ψ becomes less negative. When Ψ approaches zero, Φ_{max} approaches zero. This leads to colloid coagulation or flocculation [19,20,67,68]. Increasing I in a colloidal suspension decreases Φ_{max}, which enhances colloid flocculation rate [69].

In addition to these components (Ψ, I) controlling colloidal flocculation or stability [21,69,70], additional components in the case of clay colloids are also involved. These additional components include relative proportion of monovalent to divalent cations in the bulk solution [21], type of cations, shape of particles and initial particle concentration in suspension [71], type of clay minerals present, and relative proportion of clay minerals [47].

The above observations of the effect of clay mineral type and their relative proportion on dispersion and flocculation behavior suggest that certain interactions between the various colloids change their dispersive behavior or colloidal stability. Based on these observations, soils of mixed mineralogy and with various proportions of different clay minerals are expected to have unique dispersive properties.

Many processes and/or conditions in the soil environment are highly dependent on colloid dispersion or flocculation. Such processes and/or conditions include erosion, water suspension of solids, soil structure, and hydraulic conductivity, among many others. A number of studies involving sodic soils have been carried out in order to relate soil dispersive properties to saturated hydraulic conductivity. For example, Suarez et al. [72] was able to link soil dispersion in suspensions measured spectrophotometrically to saturated hydraulic conductivity. Other researchers measured the percentage of clay in suspension during a given settling period and then established relationships between percentage of clay in suspension (dispersion index) versus saturated hydraulic conductivity. The purpose in establishing clay dispersion–saturated hydraulic conductivity relationships is to develop rapid tests for predicting hydraulic conductivity of salt-affected soils and/or to evaluate mechanisms that are involved in regulating saturated hydraulic conductivity.

The data in Figure 8 show that the potential of the soils to undergo dispersion is related to ESP. This is true only at low ionic strengths. When ionic strength was adjusted to 200 mmol L^{-1}, there appeared to be no effect of ESP on soil dispersion due to suppression of the double-layer repulsive forces. These data are consistent with qualitative predictions of clay dispersion equations [73]. The data in Figure 9 also show that, even at pH 4.3, both soils exhibit dispersion at low ionic strength. This observation suggests that at pH 4.3 both of these soils will likely exhibit a net negative charge.

It can be summarized from Figures 8 and 9 that the Uniontown soil was more sensitive to dispersion under decreasing electrolyte concentration and increasing ESP but less sensitive to pH changes than the Pembroke soil. The data also demonstrate that for any given electrolyte concentration, pH and ESP, the dispersion index of the Uniontown soil was always greater than that of the Pembroke soil. This appeared to be in agreement with the thermodynamic exchange parameter of these soils. The magnitude of adsorbed ion activity coefficient f_{Na} [36] for the Uniontown soil is greater than 1. Considering that $f_{Na} > 1$ could signify that Na^+ ''reside'' in the diffuse layer, one expects the Uniontown soil to be highly dispersive. On the other hand, the magnitude of f_{Na} for the

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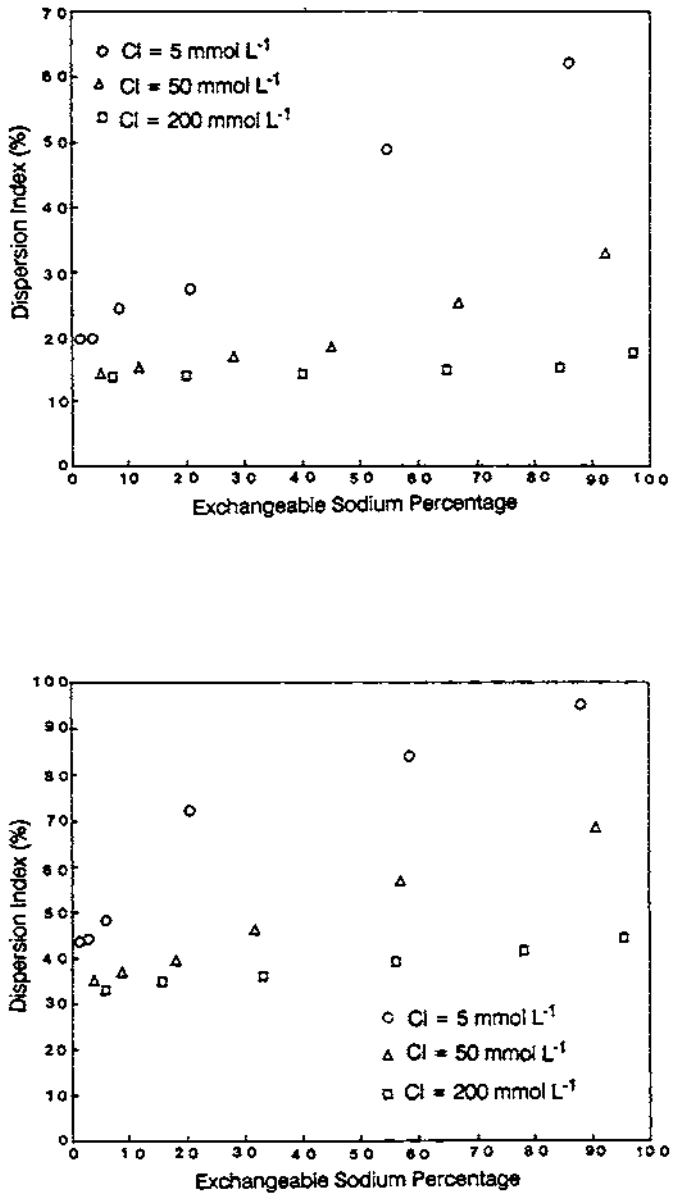

FIGURE 8 Influence of exchangeable sodium percentage (ESP) on the dispersion index (DI) of the Pembroke (top) and Uniontown (bottom) soils near pH 4 and at three chloride (Cl) concentrations. (From Ref. 73.)

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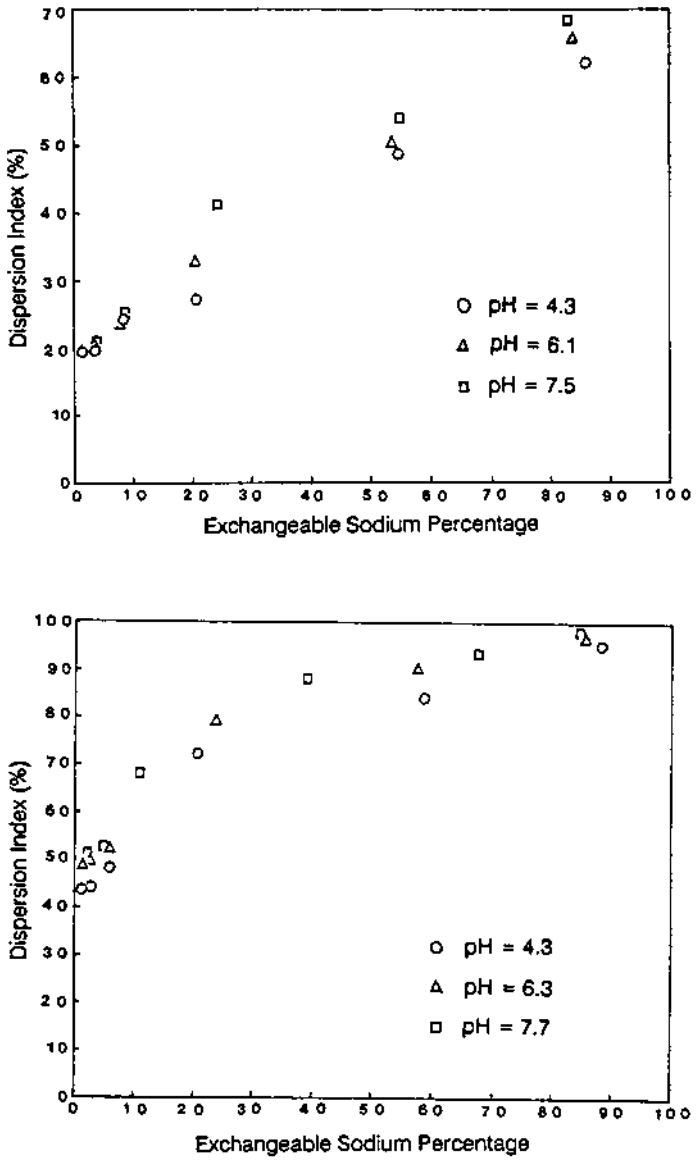

FIGURE 9 Influence of exchangeable sodium percentage (ESP) on the dispersion index (DI) of the Pembroke (top) and Uniontown (bottom) equilibrated with a solution of 5 mmol L^{-1} chloride (Cl) at three pH values. (From Ref. 73.)

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Pembroke soil is less than 1. Assuming this signifies that Na^+ forms outer-sphere complexes with the clay surfaces, this soil would be expected to be less dispersive than the Uniontown.

The presence of exchangeable Na^+ significantly decreases soil permeability [74–76]. The mechanism(s) responsible for decreasing soil permeability in the presence of Na^+ can be demonstrated by looking into the components controlling water or soil solution movement potential under saturating conditions.

Soil-saturated hydraulic conductivity is described by Lagerwerff et al. [77].

$$K = \frac{kg}{\eta} \tag{61}$$

where k = permeability of the soil
g = gravitational constant
η = kinematic viscosity or the ratio of solution viscosity to fluid density

For soil systems contaminated with brackish solutions, kinematic viscosity is not significantly affected [77] and thus the components controlling water flow velocity are the hydraulic gradient (H) and soil permeability (k). The latter component (k) is influenced by clay dispersion and migration and clay swelling. These processes may cause considerable alteration to soil matrix characteristics, such as porosity, pore-size distribution, tortuosity, and void shape [78]. Detailed description of the physicochemical mechanisms influencing clay dispersion and/or clay swelling are given in Marsi and Evangelou [73].

The deterioration of soil physical properties influencing k is accelerated directly or indirectly by the presence of high Na^+ on the soil's exchange complex and the electrolyte composition and concentration of the soil solution [74,79–82]. To improve soil physical properties of Na-affected soils, Ca^{2+} is usually added to replace Na^+ on the exchange sites. Calcium reduces clay swelling and enhances clay flocculation [83].

Additional components influencing the effect of Na^+ on saturated hydraulic conductivity of soil include clay mineralogy, clay content, soil bulk density, Fe and Al oxide content, organic matter content, salt concentration, and Na^+/Ca^{2+} ratio [78,84–90]. The hydraulic properties of soils dominated by 1:1–type clay mineralogy (i.e., kaolinite) and Fe or Al oxides are relatively insensitive to variation in soil solution composition and concentration in contrast to those dominated by 2:1–type clay minerals (i.e., montmorillonite). McNeal and Coleman [79] stated that each soil has a unique saturated hydraulic conductivity response threshold because of its unique properties.

Martin et al. [91] studied the importance of pH on saturated hydraulic conductivity (SHC) and found that the same total quantity of Na^+ on a soil will reduce SHC more effectively at a lower pH than at a higher pH. These investigators [91] concluded that the reduction in soil CEC as pH decreased was responsible for decreasing soil SHC, since the same amount of Na^+ represents a greater ESP at a lower soil pH. Suarez et al. [72] reported that for the same ESP or SAR value, the SHC decreased as pH increased. The pH effect on hydraulic conductivity is pronounced only when the soil contains a high quantity of variable-charge minerals and organic matter.

In contrast to the studies on the effect of the electrolyte concentration and composition on saturated hydraulic conductivity, fewer studies have examined the influence of pH, solution composition and salt concentration on SHC. It seems necessary to understand the influence of pH on soil hydraulic conductivity, because in humid region soils contaminated with oil well brine are often associated with low pH, either the pH drifts downward as extensive leaching is taking place or the pH rises when alkaline brines are discharged onto the soil.

Reductions in the relative saturated hydraulic conductivity (RSHC) as a function of pH and chloride concentration are summarized in Table 2. These data show ''threshold'' ESP or SAR values which are defined as 20% relative reduction in RSHC. It is clearly shown that the ESP-SAR critical threshold is highly dependent on pH and Cl concentrations. It varies from an SAR of approximately 0.30 to an SAR of approximately 90. These values strongly indicate that the critical SAR threshold reported by U.S. Salinity Laboratory Staff [6], in the range of 10–15, applies to the 50 mmol L^{-1}

TABLE 2 Sodium Adsorption Ratio (SAR)[a] and Exchangeable Sodium Percentage (ESP) Values Associated with 20% Reduction in Relative Saturated Hydraulic Conductivity (RSHC) for Pembroke and Uniontown Soils at Three pH Values

	Pembroke						Uniontown					
Cl	pH 4.3		pH 6.1		pH 7.5		pH 4.3		pH 6.3		pH 7.7	
(mmol L^{-1})	SAR	ESP	SAR	ESP	SAR	ESP	SAR	ESP	SAR	ESP	SAR	ESP
5	2.6	5.5	1.6	1.1	0.4	0.6	2.4	2.8	1.1	0.8	0.3	0.5
50	49.6	59.1	29.4	5.5	20.8	0.8	19.8	32.5	16.9	7.5	14.8	2.1
200	—[b]	—	—	—	90.4	0.5	—[b]	—	—	—	83.2	68.5

[a] SAR in $(\text{mmol } L^{-1})^{-1/2}$.
[b] Threshold values are not reported because the reduction on SHC is less than 20%.

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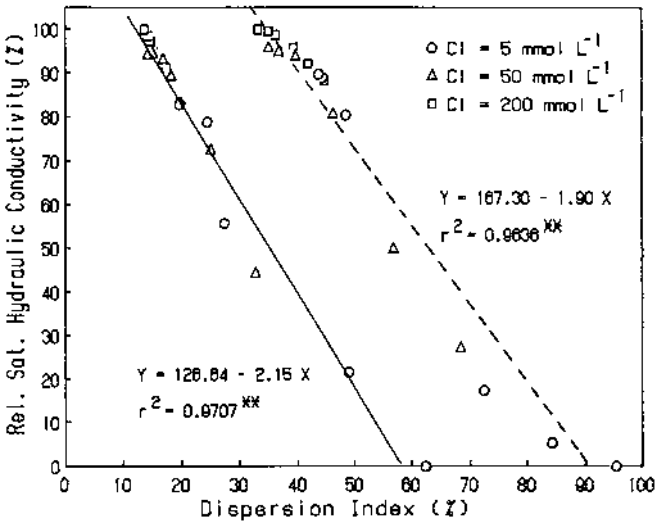

FIGURE 10 Relationship between relative saturated hydraulic conductivity (RSHC) and dispersion index (DI) of the Pembroke (solid line) and Uniontown (dashed line) soils at pH 4.3 with three chloride (Cl) concentrations. (From Ref. 92.)

Cl concentration of the Uniontown soil only. The Pembroke soil (Table 2) at the 50 mmol L^{-1} Cl concentration exhibits a much greater critical threshold.

The data presented in Table 2 show that the RSHC of the Uniontown soil is more sensitive to ionic strength and solution composition than that of the Pembroke soil. These sensitivity differences are probably a result of the differences in mineralogy between the two soils. The effect of clay mineralogy on the critical SAR was also reported by McNeal and Coleman [79].

Imhoff Cone–Saturated Hydraulic Conductivity

Values of relative saturated hydraulic conductivity correlated with Imhoff cone results, expressed as dispersion index (DI) to predict the RSHC for the soils, when salt affected, are shown in Figures 10 through 14.

The data in Figure 10 show that near pH 4, the RSHC-DI relationship was independent of Cl concentrations, but at pH of approximately 7.5, the soils (Fig. 11) exhibited two unique RSHC-DI relationships. The first RSHC-DI relationship belongs to the 50 and 200 mmol L^{-1} Cl system and the second belongs to the 5 mmol L^{-1} Cl system.

The data in Figure 12a revealed that the Pembroke soil, at 5 mmol L^{-1} Cl solution, showed two unique RHSC-DI relationships. One occurred at pH 4.3 and the other occurred at pH 6.1 and 7.5. The Uniontown soil (Fig. 12) showed a unique RSHC-DI relationship for each of the pH values tested. When the Cl concentration was raised to 200 mmol L^{-1}, the RSHC-DI relationship became independent of pH for both soils (Fig. 13).

In all of the data displayed in Figures 10 through 13, one piece of specific information stands out. Generally, the slope of the RSHC-DI relationship was greater for the Pembroke soil than the Uniontown soil. This is also shown in Figure 14. This suggested that the SHC of Uniontown soil was less affected by changes in DI than was the Pembroke soil. Moreover, these data also show that to attain similar relative suppression in SHC, a greater DI was needed for the Uniontown soil than the Pembroke soil. This is probably due to soil texture. The Pembroke soil contained 59% clay; the Uniontown contained only 28%. Hamid and Mustafa [81] reported that RSHC-DI relationships are highly affected by soil texture as well as pore size distribution.

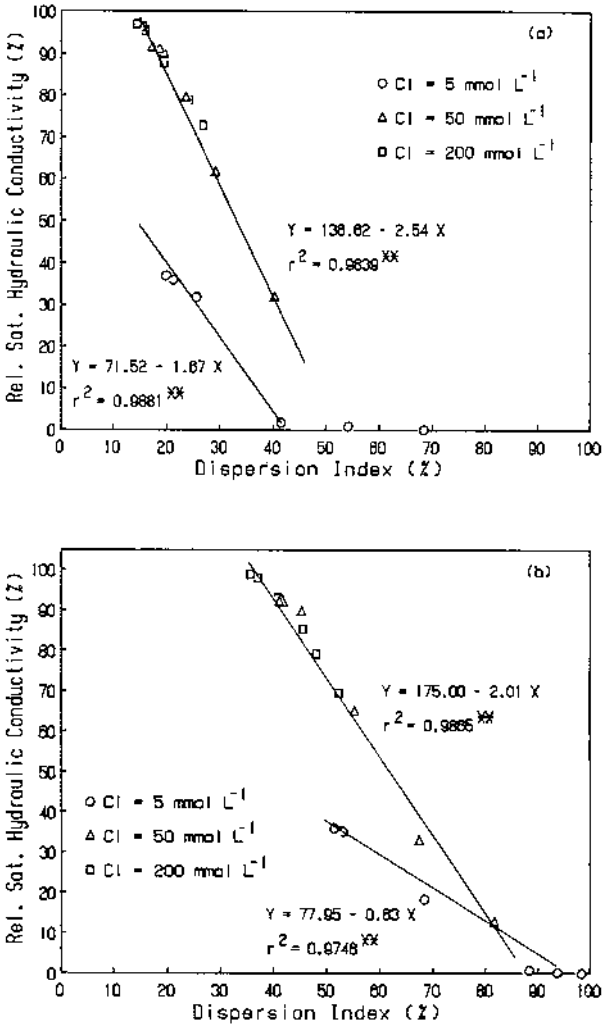

FIGURE 11 Relationship between relative saturated hydraulic conductivity (RSHC) and dispersion index (DI) of the Pembroke soil at pH 7.5 (a) and Uniontown soil at pH 7.7 (b) with three chloride (Cl) concentrations. (From Ref. 92.)

Figure 11 shows that for each of the two soils there was a unique RSHC-DI relationship at the 5 mmol L^{-1} chloride concentration. More importantly, at this Cl concentration a lower DI was needed than with the higher Cl concentrations to suppress to a large degree the SCH. This suggests that at the lower salt concentration, clay swelling is also implicated in reducing SHC [20,74, 79,80,93].

A swelling effect could therefore be implicated in the results shown in Figure 12. As pH increases, a small DI imposes a large suppression in the SHC. The increase in pH could be implicated in increasing swelling potential. This is likely because of the removal of Al-OH polymers from the interlayer (Fig. 15). The presence of Al-OH polymers at the lower pH values may limit interlayer swelling [94]. Clays that have the basic 2:1 mineral structure may exhibit limited expansion because of the presence of hydroxy-Al islands which block their interlayer spaces (see Fig. 3). It is well

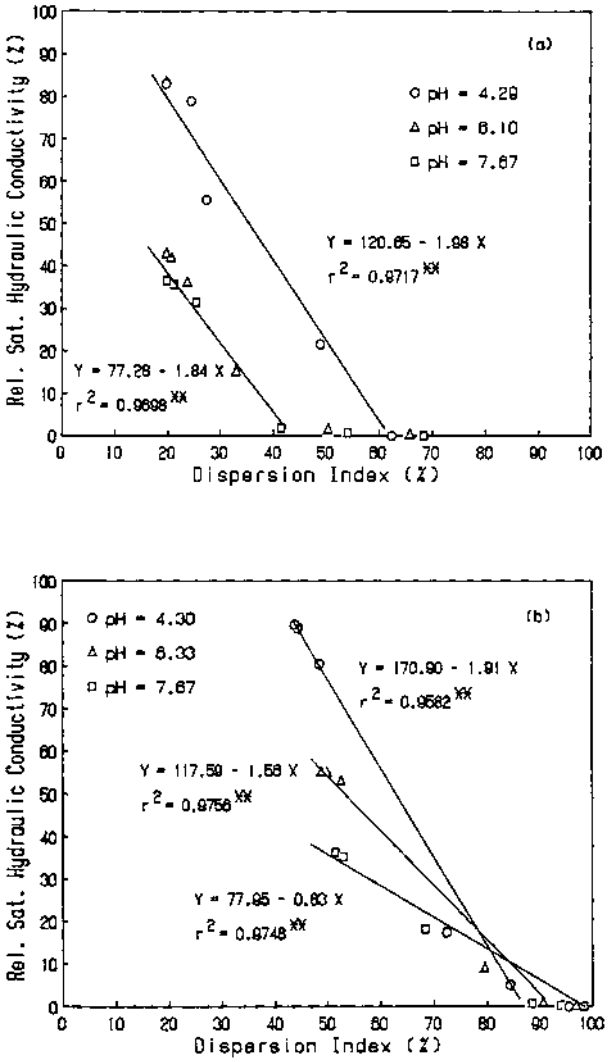

FIGURE 12 Relationship between relative saturated hydraulic conductivity (RSHC) and dispersion index (DI) of the Pembroke (a) and Uniontown (b) soils equilibrated with solutions of 5 mmol L^{-1} of chloride (Cl) at three pH values (DI = amount of dispersed clay divided by the amount of clay in 1 g soil). (From Ref. 92.)

known that these Al interlayer components are completely removed at pH values 9.0–10.0 through dissolution mechanisms [95]. This interlayer removal is expected to increase the dispersion potential of the mineral by allowing free expansion. Similar phenomena of hydroxy-Al interlayer removal have been demonstrated to be the cause for failed septic systems [96] under a far less dramatic chemical regimen than that often encountered in salt brine–contaminated systems. In addition to increased swelling, dispersion can also be enhanced in such systems as a result of the increased mineral surface charge following removal of Al-hydroxy from the interlayer. When removed from interlayer positions, these positively charged hydroxy-Al components would increase the effective

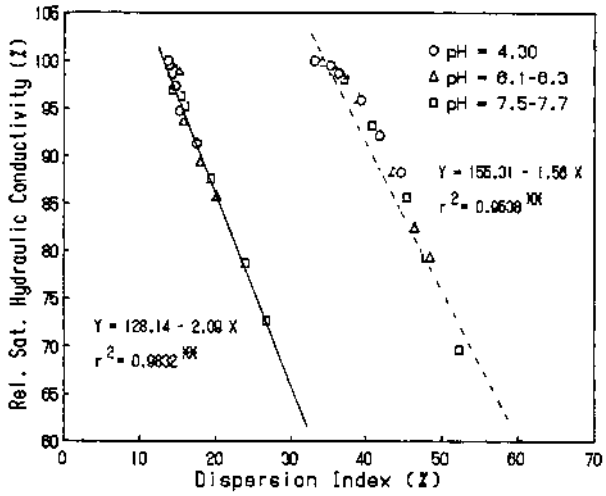

FIGURE 13 Relationship between relative saturated hydraulic conductivity (RSHC) and dispersion index (DI) of the Pembroke (solid line) and Uniontown (dashed line) soils equilibrated with solutions of 200 mmol L^{-1} of chloride (Cl) at three pH values (DI = amount of dispersed clay divided by the amount of clay in 1 g soil). (From Ref. 92.)

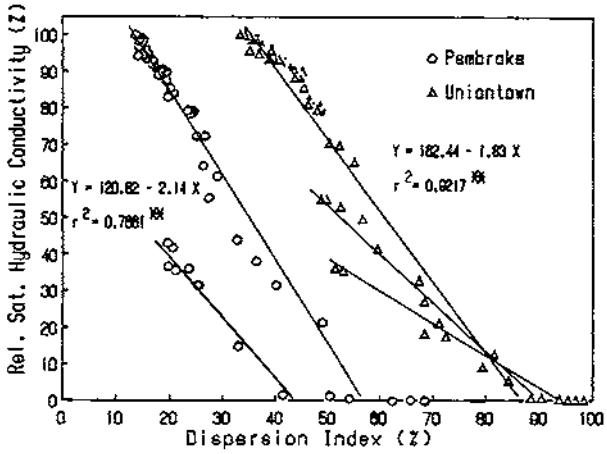

FIGURE 14 Relationship between relative saturated hydraulic conductivity (RSHC) and dispersion index (DI) of the Pembroke and Uniontown soils equilibrated with three chloride (Cl) concentrations at three pH values (DI = amount of dispersed clay divided by the amount of clay in 1 g soil). (From Ref. 92.)

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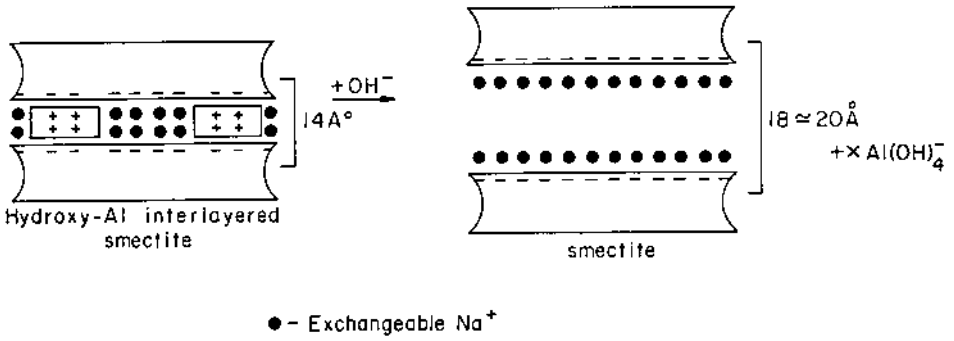

FIGURE 15 Al-OH polymer removal from the interlayer space of 2:1 clay minerals.

surface charge available for Na adsorption, thus increasing the probability of soil structural destabilization.

CONCLUSIONS

The dispersion index could predict RSHC. The relationship between RSHC and DI is not universal; however, it is unique to a particular soil under a given set of leaching conditions. The properties that appear to influence the RSHC-DI relationship of soils in humid regions are soil mineralogy, soil texture, soil pH, ionic strength, and solution composition. Information on humid region soils clearly demonstrates the following points: (a) the RSHC is related to the clay dispersion index, (b) the relationship between RSHC and DI is dependent upon ionic strength and pH, and (c) soils exhibit different RSHC-DI relationships. Furthermore, soils of the humid regions appear to behave differently with respect to Na^+-Ca^{2+} exchange and physical stability in relationship to soils of arid regions.

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Impact of Soil pH on Nutrient Uptake by Crop Plants

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INTRODUCTION

Soil pH is one of the most indicative measurements of the chemical properties of a soil, which exerts far-reaching and potentially favorable or adverse effects on the growth and nutrient uptake by crop plants.

SOIL pH–INDUCED STRESS

The pH of the growth medium has significant effects on the properties of soils and consequently on the nutrient uptake by crop plants. Soil pH is one of the most indicative measurements of the chemical properties of a soil. Whether a soil is acidic, neutral, or basic has much to do with the solubility of various compounds, the relative bonding of ions to exchange sites, and the activity of various microorganisms in the soil systems. Thomas [1] noted that three soil pH ranges are particularly informative: a pH less than 4 indicates the presence of free acids generally from oxidation of sulfides; a pH less than 5.5 suggests the likely occurrence of exchangeable Al; and a pH from 7.8 to 8.2 indicates the presence of calcium carbonate, an important agent of calcareous soil.

Soils with pH values ranging from 4 to 7 [2] are extensively distributed throughout the tropical and subtropical regions of the world. Soils with pH values less than 4 also exist and are commonly found as acid sulfate soils and in mine soils. Plant growth in acid soils may be limited by a variety of factors, including the direct effect of pH (excess H ion concentration) as well as pH-induced toxicities (e.g., Al, Mn) and/or insufficiencies (e.g., Ca, Mg, P, Mo) [3]. Increase in the hydrogen-ion concentration of the medium generally causes a decrease in the rate of absorption of cations, probably as a result of competition between the similarly charged ions for binding and carrier sites. Similarly, the role of high pH has often been considered to be detrimental in causing deficient nutrient availability and ionic imbalance.

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Depending on the predominant clay-type soil, pH can indicate the percentage base saturation. It also can indicate something about the degree of dissociation of H^+ from cation exchange sites or the extent of H^+ formation by hydrolysis of Al. Since the availability of most plant essential elements depends on soil pH, it is an indication of the relative availability of plant nutrients. Thus, soil pH is generally an indicator of both the soil condition and of the reactions that occur in the soil.

Soil pH is an important factor influencing the growth of most crops and pastures and the distribution of native plant species [4,5]. Often the effects of pH on the growth of plants are complex and it is difficult to separate direct effects of excess hydrogen (H^+) or hydroxyl (OH^-) ions from indirect effects associated with numerous chemical changes in the solubility and availability of various biologically important plant nutrients [6,7].

Among the various plant parts, the roots are directly affected by the pH of the growth medium. Low pH injury or H^+ injury is one of the factors responsible for growth retardation in acid soils. Hydrogen ions (H^+) increase the solubility of Al, Mn, and Fe in acid soils [8]. The presence of hydrogen ions in the growth medium generally inhibits root elongation, and this phenomenon is observed at extremely low pH [9,10]. It has generally been considered that H^+ injury is negligible in a medium at a pH above 4. However, even in this case, the contents of mineral nutrients in plants decrease with the decrease of the pH [11], and, in some cases, mineral ions flow out of the roots [12]. Excess H^+ in the growth medium affects plant growth by two processes: (a) Nonspecific inhibition of root elongation, lateral branching, and water absorption; and (b) specific effects on root ion fluxes via H^+ competition with base cations for uptake and H^+ damage to the ion-selective carrier in root membranes.

It is generally recognized that poor growth in acid soils is not caused by the Ca deficiency of the soils but by other factors such as Al or Mn excess, because plant growth does not improve by the addition of calcium sulfate to the acid soils. In acid soils, it may be difficult to observe the ameliorating effect of Ca, because Al injury is predominant [13]. In solution culture, however, a high Ca concentration in the growth medium alleviates Al injury or low-pH injury [10] and prevents K loss associated with H^+ injury [12].

Calcium plays an important role in raising the pH of the growth medium. It is required to sustain cell membrane integrity plus facilitate the active uptake of otherwise competitive cations. This ''Viets'' effect of Ca can be demonstrated with other polyvalent cations (including Al) [14], and it has been shown to alleviate the toxic effects of high H^+ activities. At pH levels of less than 4, H^+ may out compete Ca^{2+}, preventing their absorption, and even displacing Ca present in the root apoplast. Once Ca absorption is repressed, cell membranes lose integrity and the selective ion carrier mechanism dysfunctions resulting in reduced base cation absorption and efflux of cations. Loss of root membrane integrity can also produce the wilting symptoms of low turgor pressure observed with H toxicity.

The important role of Al in acid soil chemistry has been reviewed, as have Al effects on plant growth in predominantly horticultural and agronomic species. Three general processes by which Al affects plant growth in acid soils are: (a) reduced divalent cation (especially, Ca) uptake by plant roots due to the presence of excess Al in the rhizosphere or in the root apoplast; (b) dysfunction of cell division in the root meristematic tissue due to penetration of Al into the root protoplasm and the production of abnormal root morphology; and (c) decreased anion (SO_4^{2-}, PO_4^{3-}, Cl^-) adsorption by roots due to increased positive adsorption sites in the rhizosphere and root apoplast. Aluminium activity is critical in the above processes, because at low activities, a synergistic response with plant growth can occur. Aluminium is believed to facilitate monovalent cation uptake (especially K uptake via the Viets effect), and increased P sorption, as hydroxy-Al-P-complexes of low positive charge density have been proposed [15].

Several studies have shown that solution pH greatly affects the absorption of inorganic nutrients by plants [4,5,16]. Short-term studies have shown that, at low pH, ion transport may be impaired, especially at low Ca concentrations, and sufficient membrane damage may occur to allow the loss of previously absorbed solutes. Similarly, long-term studies on several plant species have shown

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that prolonged exposure of roots to low pH leads to suppression of lateral root development and, in extreme cases, to death of the root tips [17].

The solubility and plant availability of micronutrient cations in soils generally decreases with increasing pH owing to adsorption-precipitation reactions. The pH of the nutrient solution will affect the availability of certain elements, particularly the micronutrients, stimulating excessive uptake at a low pH, and resulting in removal from the nutrient solution by precipitation at high pH. The pH of the nutrient solution is thought to be best when kept between 6.0 and 6.5, although most nutrient solutions when constituted will have a pH between 5.0 and 6.0. In their experiments, Islam et al. [5] have found that tissue concentrations of all essential elements were adequate for healthy plant growth at pH 5.5.

In a solution culture experiment, the concentrations of N, P, K, Ca, Mg, and Mn generally increased in rice leaves with increasing pH values [6] and were higher with NO_3-N than with NH_4-N, whereas Fe content decreased in rice shoots but increased in roots with high pH. The result suggests that a pH of 5–6 is reasonably good for normal growth and nutrient uptake by rice plants with these N sources.

Nitrogen concentration in plant tops decreased with decreasing pH over the range of 5.5–3.3, and in tomato, the concentrations at pH 3.3–4.0 (1.2 and 1.3%, respectively) were clearly in the deficient range [5]. Nitrogen concentrations in cassava (*Manihot esculenta* Crantz.) tops at pH 3.3 and 4.0 (2.3 and 2.6%, respectively) were also well below the critical N concentration of 5.1% in the fourth and fifth fully expanded leaves of cv. Llanera [18] and the normal N concentrations ranging from 4.5 to 6.5% in young fully opened leaves. No satisfactory explanation can be given for the decline in plant N concentrations at low pH. Bassioni [19] observed that NO^-_3 uptake by excised barley roots was less at pH 4 than at pH 6. However, this result is somewhat suspicious, as the test solution apparently did not contain Ca. Rao and Rains [20] reported higher rates of NO_3^- absorption in short-term uptake experiments with barley roots at pH 4.0 than at pH 5.7 or 8.5. Similarly, in flowing solution culture experiment, Forno [21] found that mean rates of N uptake by cassava (as NO^-_3) per unit root weight were either higher at pH 4.4 than at pH 6.8 (Cassava cv. M. Aus. 3) or approximately the same (Cassava cvs. Nina and Ceiba). In the roots of sunflower (*Helianthus annuus* L.) and flax, the total N concentrations were strongly reduced at pH 4 [22]. In soils with pH 7 or below, high concentrations of NH_4^+ can be toxic to raddish (*Raphanus sativus* L.) [23], and NH_4^+ toxicity is particularly deleterious to young seedlings, limiting plant yields.

Ammonium absorption by plants can rapidly depress solution pH to injurious levels in noncalcareous soils (NH_4^+ and Al^{3+} toxicities), because NH_4NO_3 contributes to soil acidity. Ammonium toxicity occurs in many plant species, but it is not considered a problem when the plants are grown in calcareous soils (free $CaCO_3$). Barker and Mills [24] noted that even when all of the N is ammonical, near-normal growth can be obtained if the pH of the medium is buffered to near neutrality (e.g., calcareous soil). Less N plant tissue was found at pH 5.5 under all redox potential conditions in the soil, with the highest pH being 7.5 [25].

Nitrification of NH_4-N based N fertilizers is known to increase soil acidity. Legumes increase soil acidity, because they absorb more cations than anions from soil [26]. Nitrogen, in the NO_3^- form, seems almost universally to lead to an increase in pH. The observed effect of pH on NO_3^- uptake suggests that both H^+ and OH^- are involved in the absorption process. At low pH values, H^+ may cause injury to the root tissue, whereas at higher pH values, competition with OH^- reduces NO_3^- uptake.

Breemen et al. [27] have indicated that nitrification of NH_4^+ and accompanying soil acidification can occur even at a pH of less than 4. Lowering of pH has been found to be associated with the uptake of N as NH_4^+ [28]. Uptake of NH_4^+ by the roots results in a release of H^+. The rhizosphere (or nutrient solution) becomes acidified and root integrity is impaired. This type of NH_4^+ toxicity can be avoided by pH control of the rooting medium. In their experiment with Kentucky bluegrass (*Poa pratensis* L.) and using N sources, Davis and Dernoeden [29] observed that soil pH was affected by N sources. In the years 1987 and 1988, the $NaNO_3$-treated plots had the highest pH, whereas SCU- (sulfur-coated urea) and NH_4Cl-treated plots had the lowest pH. Acidification was greatest

(pH 5.3) in NH_4Cl-treated turf, whereas pH was highest (pH 6.0) in plots subjected to NaCl. They further stated that NH_4Cl-treated plots generally exhibited severe disease injury. Sodium nitrate-treated plots which had the highest soil pH were associated with more disease injury when compared with SCU-treated turf. The data provide no clear evidence for a relationship between disease and soil reaction as influenced by the N sources.

The concentration of H^+ in the growth medium has an especially important effect on phosphate absorption, because over the physiological range of pH values, the predominant ionic form shifts from univalent ($H_2PO_4^-$) to bivalent ($HPO_4^=$), and finally to trivalent ($PO_4^{\equiv}$) as the medium becomes more alkaline.

Decreases in rate of phosphate absorption with increasing pH are well documented [30]. Arnon and Johnson [10] considered that P deficiency contributed to the poor growth of their plants at higher pH values. However, Islam et al. [5] reported that tissue phosphate concentrations were adequate in their plants at pH 8.5. Khalid et al. [31] reported that the availability rates of P depended on the differential sorption under the influences of changing pH. The increase in pH of the test solution from 5.3 to 7.4 may increase P absorption. Arnon et al. [11] found that for tomato, maximum absorption of P occurred at pH 7 and decreased toward pH 3 and 9. Ponnamperuma [32] reported that the increase in pH of acid soils due to submergence is beneficial to plants, as it increases the P availability.

The shifting of pH from acidity to neutrality increases the P mineralization. Several essential elements become limiting to plant growth at alkaline soil pH. For example, the availability of phosphate, Fe, B, Zn, and Mn has been shown to decrease at high pH. Precipitation of phosphate by Ca and the cations by carbonate, hydroxide, or phosphate is responsible for decreased availability of these elements. Hagen and Hopkins [30] observed that excised barley roots absorbed both univalent and bivalent phosphate from the growth medium. This may be due to the fact that roots absorbing more anions than cations excrete OH^- rather than H^+, leading to an increase in solution pH [33].

Soil pH can indirectly reflect the P distribution pattern of soils to a certain degree but not perfectly. The pH of the soil solution determines the form of P absorbed by plants, however, P is absorbed mainly as the inorganic dihydrogen ion ($H_2PO_4^-$). It is known that Ca-P is found in large amounts in alkaline soils, and Al-P and Fe-P are found in acid soils. Therefore, the concentration of phosphorus in the soil solution depends mainly on soil pH, and a decrease in pH can reduce P concentration by causing precipitation of Al-phosphate or Fe-phosphate as amorphous polynuclear complexes with high surface area.

Addition of NH_4^+ rather than NO_3^- increases P uptake from the neutral soils [28]. Generally, absorption of the NH_4^+ tends to lower the pH in the rhizosphere, and in the soil studies, there was a corresponding increase in the concentration of phosphate in the solution. At 1 mM $NaNO_3$ and lower pH [4], the ion uptake (N, P, K, and S) and growth of wheat and rice were severely affected [34]. There was a great decrease in the P concentration with the increase in $CaCO_3$, which was mostly due to the transformation of available P to di- and tricalcium phosphates and also to apatites owing to formation of ferric phosphate/hydroxy phosphate.

Decreases in the K content of plants were observed under low pH conditions [12], and the movement of K in roots was symplastic. It is assumed that in the plants which exhibit a low K content in a medium with a low pH, the function of the plasma membrane is impeded by H^+. One of the main physiological roles of K is to maintain the osmotic pressure of cells (maintenance of turgor). As the roots elongate rapidly by the successive production and thickening of new cells, they must absorb a large amount of K in order to maintain the K concentration of these newly formed cells at a suitable level [7]. Therefore, it is assumed that H^+ decreases the function of the plasma membrane and promotes K loss or the inhibition of K uptake, and consequently brings about poor root growth.

Potassium loss associated with a low pH can be alleviated by the increase Ca concentration in the growth medium [12]. In gramineous crops, the index of the K content tended to increase with the increase of the Ca concentration in the medium. Many experimental results have been published on the stimulation of K absorption by Ca in plant roots [35].

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Large decreases in the rate of absorption of Ca with decreasing pH have been reported. The availability of Ca has been limited at low pH; this is because as the amount of H^+ increases, the amount of Ca decreases. A strong antagonism between H^+ and Ca^{2+} in legume nodulation has been documented [36], and calcium accumulation in maize has been associated with soil pH. In their experiments, Inoue et al. [7] observed that under both conditions of low pH and low Ca concentration, the Ca uptake was strongly inhibited by H^+, and consequently the corn shoots suffered Ca deficiency, leading secondarily to poor root growth of several gramineous crops. This inhibition of Ca uptake appears to be caused by the antagonism between H^+ and Ca^{2+} at the position of the substitution radical of the cell wall and/or of plasma membrane. Both the contents of K and Ca in barley, wheat, and rye were low in medium with a low pH. It is, thus, considered that since the functions of the plasma membrane and Ca uptake were inhibited by H^+, the root growth was considerably poor. The roots of gramineous crops generally display a low ability to absorb Ca and to transfer Ca to the top. Calcium plays an important role in the strengthening or maintenance of the cell wall and plasma membrane. Assuming that H^+ and Ca^{2+} antagonize each other at the position of the substitution radical of the cell wall or plasma membrane, the increase of the Ca concentration in the medium may alleviate the H^+ injury.

Calcium concentrations in maize at pH 3.3 and 4.0 (0.39 and 0.37%, respectively) were below the concentration normally considered adequate for healthy growth [5]. However, Loneragan and Snowball [37] obtained maximum yield of young maize plants in flowing solution culture when the Ca concentration in the tops was only 0.12%. In the same experiment, tomato and the wheat cultivars Gabo and Wongoondy achieved maximum yield, with Ca concentrations in the plant tops of 1.29, 0.15, and 0.32%, respectively. These Ca concentrations are well below those obtained in the tops of tomato and wheat cv. Gatcher in the experiment carried out by Islam et al. [5] at low pH with different plant species.

Arnon and Johnson [10] attributed much of their growth reduction below pH 5 to inadequate Ca absorption. In a subsidiary experiment with lettuce (*Lactuca sativa* L.) and tomato, these investigators showed that raising the initial Ca concentration in the nutrient solution from 2000 to 7000 μM increased yields at both pH 4 and 5, whereas lowering the initial Ca concentration to 500 μM lowered plant yields. Further evidence of the interation between effects of low pH and Ca concentration comes from studies in legume nutrition. Lucerne (*Medicago sativa* L.) plants supplied with combined N grew equally well at pH 4 and 5, with a Ca concentration of 5000 μM, but that growth was poorer at pH 4 when lower Ca concentrations were used.

In an unsuitable environment, limitation in Ca supply to the plant roots caused disturbance in the growth and metabolic processes in plants. Deficiency of Ca reduces the absorption and accumulation of monovalent cations and increases the uptake of divalent cations [38]. Accumulation of P, K, and Na decreases in all part of Ca-deficient potato plants. This view strengthens the argument that the absence of Ca in the growth medium will cause a decrease in the uptake of K and Na and increases in the accumulation of Mg in plants. Calcium deficiency causes accumulation of oxalic acid in such a quantity as to become injurious to the plants. Calcium helps in the precipitation of oxalic acid and soluble oxalates in the form of Ca-oxalate and protects plants from being affected by more H^+ concentrations. Large decreases in the rate of Mg absorption by different crop plants have been reported with decreasing pH [11]. Magnesium concentrations in the tops of plant species at pH 3.3 and 4.0 (0.03–0.16%) were sufficiently low to be either deficient or marginally limiting for plant growth [6].

The solubility of Fe salts in soils are reported to be governed by the pH of the system, which affects the availability of Fe to the plants. The increase in pH of acid soils is due mainly to reduction of ferric-Fe to ferrous-Fe. The decrease in pH of sodic and calcareous soils and the check on the pH rise of acid soils are the result of the accumulation of CO_2, soil reduction, and organic acid production. Increased Fe availability on calcareous soils can also be achieved by lowering the pH of the bulk soil with the application of S and sulfuric acid. In a short-term experiment, increasing the solution pH from 3.5 to 8.5 decreased the concentration of Fe by the tops of rice plants, whereas in roots, the Fe content increased [4]. At a high-solution pH, the new leaves become chlorotic. The

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appearance of Fe deficiency in rice plants at high pH may be explained by the low solubility of Fe in the rooting medium by the fast oxidation of ferrous-Fe and by immobilization in the roots. The action of rice roots in oxidizing Fe^{2+} to Fe^{3+} is believed to be responsible for the oxidation of H_2S by the roots, suggesting that chlorosis at high pH may be related to a more rapid oxidation of ferrous-Fe to higher forms [9].

When the pH of the growth medium is high, Fe phosphate is precipitated in the stem and both high phosphate and increased pH are known to enhance Fe chlorosis. High levels of P and Al in the growth medium often have been found to reduce Fe absorption and utilization, especially under neutral or alkaline condition [13]. Rice plants given excess P in the growth medium progressively accumulated Fe [39]. The activity of iron is affected by P in the plant tissue or nutrient media. Poor Fe nutrition depressed the growth of maize and wheat at pH 7.5 and 8.5 despite the use of Fe N,N-dihydroxyethylethylene-diamineacetic acid (HEDDA) (Sequestrene 138) as an Fe source. This compound is reported to be stable over the pH range of 4–9 [40]. Iron concentration in the tops of maize grown at pH 8.5 (85 μg/g) is in the range that has been considered deficient for this species. This observation is confirmed by the development of severe symptoms of Fe chlorosis [41].

The high hydroxyl and bicarbonate ion concentrations associated with the alkaline soil solution in a calcareous soil keep available Fe^{2+} concentrations too low to supply sufficient Fe for normal plant uptake. Similarly, bicarbonate induced Fe stress for plants grown in nutrient solution and in alkaline soils. Some studies have indicated a combination of bicarbonate and high P–induced Fe chlorosis. Iron chlorosis is also enhanced under conditions of increased soil moisture and high Fe to P ratio.

When large amounts of NO_3^- are taken up, more hydroxyl ions are released by roots resulting in decreased availability of soil Fe [42]. The availability of Fe, Mn, Zn, and Cu was low in calcareous soils, and added P antagonized micronutrient deficiencies more under high pH conditions. Therefore, under conditions where Fe is highly insoluble and immobile, the main mechanism of Fe uptake may be by direct contact between insoluble Fe compounds and plant roots. Inhibition of lateral root development would have detrimental effects on the ability of the roots to reduce Fe^{2+}, since the iron-reducing activity occurs at or near the surface of young lateral roots.

Soil pH is often the determining factor in whether a soil will respond to Mn fertilization. Liming coastal plain soils from pH 6.0 to 6.5 intensified Mn deficiency symptoms on soybeans [43,44]. Fitts et al. [45] observed yield responses to Mn only where the soil pH was neutral or alkaline. These investigators found that liming above pH 6 reduced leaching of Mn and decreased plant Mn. The decrease in soil pH from 6.8 to 6.0 during the course of a greenhouse experiment prevented Mn deficiencies from developing in soybeans. Jones and Nelson [46] reported that liming soils to a pH 5.5 or above reduced extractable soil Mn, decreased foliar Mn concentration, eliminated toxic effects, and increased soybean yields. Manganese availability is inversely related to soil pH and its oxidation-reduction potential. Plants take up the divalent form of Mn for their normal growth. The oxidation of divalent Mn to less soluble forms occur in the pH range of 7–8, primarily as a result of microbial activity. At soil pH less than 7, Mn was sufficiently available for normal turf appearance and growth of Bermuda grass (*Cynodon dactylon* L.), and Mn deficiencies observed were pH induced rather than attributable to insufficient total Mn in the soil [43]. A soil pH of 5.3 resulted in highest concentration of Mn in the soybean leaf, whereas pH 7.0 showed the lowest Mn concentration in the leaf [47]. Higher concentration of Mn in the leaf tissue at pH 5.3 was due to the greater solubility of Mn under the strongly acid solution of the soil and consequently absorption by the soybean.

With decreasing pH, the concentration of Mn decreased in crop plants. Similarly, decreasing the solution pH from 7.0 to 5.4 resulted in decreased Mn concentration in the tops of two *Medicago* species. Apparently, in the poorly buffered solution, high Ca levels ameliorated the adverse effects of an acidic pH on Mn uptake. Manganese concentrations in tops of maize (*Zea mays* L.) plants at pH 3.3 and 4.0 (12 and 14 μg/g, respectively) decreased [5] and were in the range that is considered to be inadequate for healthy growth.

Zinc deficiency is prevalent in acid, leached sandy soils having a low Zn content and in neutral

and alkaline soils having high levels of available P and organic matter. Availability of Zn in soils may become critical at soil pH values as low as 5.3. Zinc uptake by corn was significantly correlated with soil pH between 4.3 and 7.5. Lime reduced Zn uptake by red clover (*Trifolium pratense* L.), timothy, and bromegrass (*Bromus marginatus* L.). The N fertilizers affect the availability of Zn, and these effects were attributed to changes in soil pH, and $NaNO_3$ decreased the Zn uptake and $(NH_4)_2SO_4$ increased it [48]. Severe Zn deficiency in subterranean clover with increasing N supply is due to formation of a Zn-protein complex in the roots. The addition of $CaCO_3$ generally decreased the Zn content of sorghum at soil pH levels between 5.7 and 6.6 [49]. The reduction in Zn uptake induced by $CaCO_3$ was attributed to the increased soil pH and not the Ca added. Similarly, the addition of lime to sandy Alabama soils to a pH near 6.5 produced Zn deficiency in corn.

Reducing the pH of the test solution from 5.5 to 4.5 decreased Zn absorption rates by factors of 1000 and 10000 in the rice cultivars IR6 and Basmati-370 [50]. Similarly, reducing the solution pH from 5 to 3 reduced zinc absorption by a factor of about 100 in wheat seedlings [51]. Zinc absorption by plants usually decreases as the concentration of H^+ increases, presumbly because of the direct effect of H^+ toxicity and because of an indirect effect of competition between Zn^{2+} and H^+ ions from uptake sites on the root surface. At low pH in presence of citrate (pH 4.0 and 4.6), when the toxicity effect of H^+ ions was greatest, the roots did not respond to an increasing concentration of Zn [52]. Soil pH, organic matter content, and the presence of other cations affect the availability of Cu to plants in soils. At a pH value above 4.7, Cu is probably precipitated as $Cu(OH)_2$ in the presence of organic matter. Increasing the soil pH decreases the solubility and availability of Cu to plants [53]. However, the pH at which Cu availability is highest appears to vary with the orgnic matter content and the presence of other ions.

The chemistry of boron (B) in the soil is still poorly understood. It is probably present in the soil solution as boric acid, $B(OH)_3$. Liming of acid soils to a pH of 7 and above has often resulted in B deficiencies. The fixation of applied B in soils was much greater at pH 7 and above. The work of Sims and Bingham [54] indicates that hydroxy Al and Fe materials are responsible for B fixation when acid soils are limed. Retention of B by hydroxy Al and Fe compounds was pH dependent. According to Sims and Bingham [55], retention of B was maximum at pH 7 with hydroxy Al compounds and at pH 8.5 with hydroxy Fe compounds. These investigators postulated that the retention of B is due to anion exchange reactions in which borate ions replace hydroxyl ions.

Soil pH also had an effect on the availability of water-soluble B. As the pH was increased from 5.2 or 6.3 to 7.4, the concentration of B in the plant decreased [56]. Boron absorption by plants decreased much more when both pH and Ca concentrations were increased. The uptake of B has been shown to decrease as the Ca uptake has increased. Availability and plant uptake of native or added B was generally lower in calcareous soils than in noncalcareous soils. A high pH and high Ca concentrations of the nutrient solution decreased B uptake by cotton. Neither high pH nor high Ca alone had any effect on the absorption rate of B. It was suggested that the presence of a high concentration of Ca^{2+} and OH^- affected the B adsorption mechanism. Therefore, pH appeared to have a physiological effect on B absorption by plants when the supply of Ca was high.

The S requirements of crops are very similar to their P requirements. Sulfur deficiency is most widely found in leguminous crops. The atmosphere contains S compounds, partly as aerosols and partly as gaseous SO_2. In an experiment, Kamprath et al. [57] observed that there was a marked decrease in the amount of sulfate adsorbed when the pH of a soil was increased from 5 to 6. The effects of pH on sulfate adsorption were much more pronounced on soils that contained appreciable amounts of Al oxides and hydrous Fe. Chang and Thomas [58] have suggested that sulfate adsorption increases when the pH is lowered, because the replaced hydroxyl ions are more effectively neutralized by H^+ resulting from the hydrolysis of Al replaced by the cations added with the sulfate in the soil. The adsorption of sulfate was greater from a solution of $CaSO_4$ than from K_2SO_4. However, the soil pH had a greater effect on sulfate adsorption than did the nature of the cation.

It is well known that acid soils and those rich in Fe stone can strongly fix Mo. In a review on factors affecting availability of Mo, Davis [59] stated that many investigators have shown that Mo availability increases as the pH of the acid soil is increased. Stephens and Oertel [60] suggested

that this might be due to hydroxyl ion replacing adsorbed molybdate ions. The amount of Mo sorbed by soils and the amount of hydrous oxides increased as the pH was decreased. Molybdate ions replaced surface hydroxyls of hydrous oxides of Fe and Al in acid soils. Water-soluble Mo increased sixfold as the pH increased from 4.7 to 7.5. Generally, replacement of tightly adsorbed Mo by OH^- ions is responsible for the increase in the water-soluble Mo as the pH is increased.

CONCLUSIONS

As natural stress, soil pH has far-reaching effects on the growth and nutrient uptake by crop plants. It is difficult to minimize the abnormal effects of pH exerting on the growth of crop plants. However, efforts should be made to reduce the ill effects of pH in order to maintain the normal growth of plants in a growing medium.

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Plant Adaptation to Phosphorus–Limited Tropical Soils

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INTRODUCTION

The world population has increased during this century from about 1.5 to over 5.5 billion people. Every year, approximately 90 million more people are added to the global population, which is expected to reach 8.5 billion by the year 2025. Since the total area of arable land is stable or declining, the average area of cropable land per person must decrease as population grows. During the recent past, the successful implementation of several technologies that form the basis of modern agriculture has contributed to the production of enough food today, on a global scale, to meet the basic requirements of every person in the world. Mechanization of agricultural production, developments in irrigation systems, cost-effective crop protection chemicals and fertilizers, and genetic enhancement of crops are the major contributors. Yet one-fifth of the developing world's population remains chronically hungry owing to inequalities in availability and distribution. Equitable food distribution is restricted by lack of purchasing power among poor countries and within countries. The threat of famine is greatest in rural areas where approximately two-thirds of the population of developing nations live. Predictions of population growth in developing countries give cause to anticipate further disparities.

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Despite the advances of modern agriculture, current average yields of our major crops are only a small fraction of the record yields realized with best management practices (Table 1). The major causes for the shortfall can be separated and attributed, directly or indirectly, to biotic and abiotic factors. Biotic factors, including insects, diseases, and weeds, are responsible for losses representing less than 20% of the record yields of most crop species [1–3]. Abiotic factors, including edaphic and climatic constraints, account for the major portion of the yield losses. Among the edaphic factors, low availability of phosphorus (P) is a major constraint to crop production in the tropics.

Crops grow by acquiring resources from their environment: nutrients and water from soil, oxygen, carbon dioxide, and light from the atmosphere. Agriculture is concerned with making conditions favorable for the acquisition of these resources, so as to produce crops profitably and with minimal environmental damage. Agriculture is also concerned with improving genetic adaptation of crops to abiotic and biotic constraints. Increasing demand for food and fiber requires new approaches to further enhance crop yields and quality. There is also a need to reduce the energy inputs in modern intensive crop production by improving the acquisition and utilization of native and applied nutrients in soil.

Soil and agriculture are the foundation for sustaining human societies through production of food and renewable forms of energy [4–6]. Soils exert production, filtering, and biological functions. Therefore, soils not only produce food, feed, fiber, and fuel but also play a central role in determining the quality of our environment. Land productivity is viewed as decreasing when withdrawals of nutrients exceed their inputs. Maintaining long-term land productivity, therefore, requires that agricultural system management activities minimize exportation of soil resources and, when necessary, replenish depleted resources with inputs.

Global soil maps show that poor soils dominate the tropical latitudes, whereas the most fertile soils are found in certain areas of the Temperate Zone [5]. The inherent infertility of many tropical soils is a consequence of their formation on geological parent materials that were low in essential mineral elements coupled with the intense rates of weathering they have experienced under warm humid tropical conditions. Under these conditions, accelerated chemical and biological processes and high rainfall have resulted in the loss of most nutrients by leaching and the development of a highly acidic solum dominated by the endproducts of mineral weathering: kaolinite and the oxides and hydrous oxides of iron and aluminum [7]. Geological stability and the lack of glaciation have reduced the input of fresh, mineral-rich substrate for soil formation so that highly fertile soils in the tropics are generally limited to areas of active volcanism or alluvial sediments from young mountain ranges [8].

The latitudinal gradient of soil fertility has global economic significance, because most developing countries are located in the tropical latitudes. One tragic consequence of this is that rural poverty is likely to be much more severe in tropical than temperate countries. Economies of Third World countries in the tropics are based disproportionately on agriculture. Owing to their potential

Table 1 Record Yields, Average Yields, and Causes of the Yield Losses of Major Crops

	Mg ha^{-1} (% of record yield)			
Crop	Record yield	Average yield	Yield loss due to biotic factors	Yield loss due to abiotic factors
Corn	19.3	6.6 (34)	2.2 (11)	10.5 (54)
Wheat	14.5	1.9 (13)	0.7 (5)	11.9 (82)
Sorghum	20.1	3.6 (18)	1.0 (5)	16.3 (81)
Soybean	7.4	1.6 (22)	1.3 (17)	5.1 (69)
Cotton	4.0	0.7 (17)	0.9 (22)	2.4 (60)
Sugarbeet	121.0	42.6 (35)	17.1 (14)	61.3 (51)

Source: Adapted from Refs. 1–3.

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to increase yields, the use of nutrient inputs has benefited countless individual farmers as well as the economies of these countries, contributing to agricultural development in general. However, weak economies coupled with inherently low soil fertility often are unable to support the investment needed to improve agricultural productivity.

Phosphorus deficiency is one of the most widespread nutrient constraints to agricultural productivity in soils of the tropics [9]. In a sample of 500 soils collected from 42 countries in the tropics, the World Phosphate Institute classified 65% as acutely deficient in P, whereas only 8% were classified as not deficient [10]. Amelioration of P deficiency with fertilizers is not a viable option for many resource-poor farmers. Moreover, as a nonrenewable resource with relatively low concentrations in the biosphere, the use of fertilizer P inputs in any agricultural system must be carefully rationalized [11]. Crop and forage genotypes that can acquire and utilize scarce P resources more efficiently from low-P tropical soils could both improve and stabilize agricultural production.

One advantage of applied P over other nutrients is that once applied it is adsorbed and retained in the soil-plant system and is not subject to the large losses by leaching that occur with N and K fertilizers. Genotypes which can better exploit the residues of fertilizer P would substantially improve the returns on strategic P inputs as well as ''capital investments'' in large basal or corrective P fertilizer applications. The sustained agricultural productivity of low P tropical soils, therefore, requires that crops and forages make the most efficient use of available soil P in order to reduce the demand for P applications.

The genetic potentials of tropical crop and forage cultivars and the environments in which they are grown influence growth and productivity. Adaptation of crop and forage plants to low P-supplying soils could be due to plant mechanisms which contribute to a high P uptake ability at low P concentrations and/or more efficient internal use of P for increased crop/forage yield (Fig. 1). Genetic variability in the ability of plants to absorb, translocate, distribute, accumulate, and use P is important in adapting plants to low P-supplying tropical soils. However, only recently has this

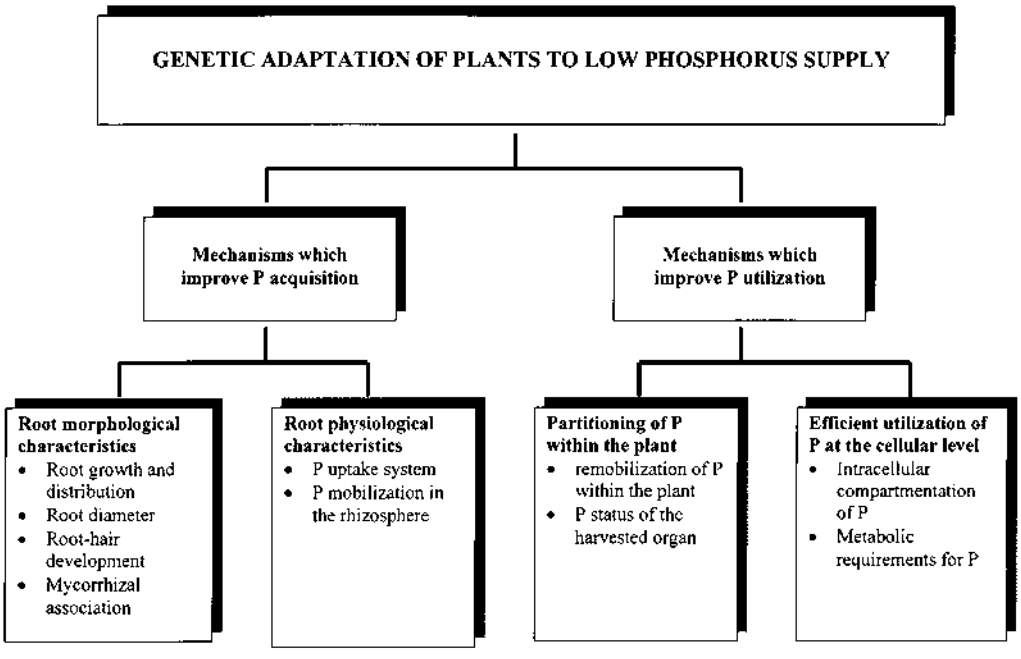

FIGURE 1 Mechanisms which improve genetic adaptation of plants to phosphorus-limited tropical soils.

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variability been conscientiously considered for the purpose of adapting plants to low-P soil conditions [12]. Although inter- and intraspecies differences in P uptake, accumulation and use are well known [13,14], the mechanisms responsible for the differential abilities of tropical crop and forage species to grow at low or high P supply are not completely understood and few have been described to any extent [12,15]. Understanding these mechanisms is a prerequisite to the identification, selection, and improvement of adapted germplasm for low-P soils.

PHOSPHORUS AVAILABILITY IN TROPICAL SOILS

Tropical soils have been broadly defined as those soils which have an ''iso-'' soil temperature regimen (i.e., mean annual variation of $<5°C$). Approximately 40% of the earth's land surface occurs within the intertropical zone (below 23½° latitude) and more than one-third of world soils are classified as tropical soils [16]. Because they have developed under immense environmental and ecological diversity on both very old and recent land surfaces, soils of the tropics are extremely diverse and variable. All 11 Orders of the U.S. Soil Taxonomy are represented in the tropics. Some 36% (1.7 billion ha) of tropical soils have low nutrient reserves (defined as containing $<10\%$ weatherable minerals in the sand + silt fraction), whereas 23% (1.1 billion ha) have a high P fixation capacity [17]. Phosphorus is probably the major limiting nutrient on the vast majority of these soils [18], particularly the acid savanna soils in South America which are primarily classified as Oxisols [19,20].

Phosphorus-limited soils in the tropics generally fall within the soil taxonomy orders of Oxisols and Ultisols (43% of tropical soils), whereas relatively minor but demographically important areas (in Latin America, cultivated by the poorest sector of the rural population) occur under the Andisol and Spodosol orders (2% of tropical soils). (Approximately 7 and 6%, respectively, of temperate region soils also belong to these orders.) Soils belonging to other soil orders, notably rhodic or oxic subgroups of Alfisols and Inceptisols, also suffer from limited P availability owing to the mineralogical composition; clayey phases may also be high P-fixers [21]. Other less weathered soils of the tropics may also have P limitations for crops because of overexploitation during centuries of cultivation with low inputs.

Characteristics of P-Limited Tropical Soils

Oxisols and Ultisols are characterized by their low content of weatherable minerals and high content of low-activity clays and oxides of iron (Fe) and aluminum (Al), with these minerals being the endproducts of chemical weathering in which primary P in the form of calcium phosphate minerals is hydrolyzed and moves through the soil solution to various adsorbed, precipitated, and organically immobilized forms [22,23]. Weathering results also in the loss of basic cations, the desilication of clay minerals and the generation of iron and aluminum hydrous oxides onto which phosphates adsorb or precipitate. With time the more labile adsorbed phosphate and amorphous precipitates are occluded with fresh coatings of oxides or become more crystalline, reducing P solubility and the concentration in soil solution [24,25]. Through geological time, P is also lost from the system and total P content declines stabilizing typically in the range of 200–400 $\mu g\text{-}P\ g^{-1}$ soil depending on soil texture [26]. Organic P (Po) forms between 20 and 80% of the total P content and constitutes an increasingly important fraction of the total P content of soils as they weather [26–29].

Oxisols and Ultisols also have a moderate to high P-''fixation'' capacity owing to the large surface area for phosphate adsorption presented by the significant contents of amorphous and microcrystalline iron and aluminum oxyhydroxides. An estimated 110–450 mg P/kg soil are required to obtain 0.1 mg P/L in equilibrium soil solution in these soils [30–32], a level considered adequate for crop growth.

Andisols, in contrast to Oxisols and Ultisols, are relatively young soils that most usually developed on volcanic parent materials [23]. Although they often contain high total amounts of P,

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it usually occurs in highly stabilized inorganic and organic forms in highly amorphous associations with the allophane, imogolite, ferrihydrite, and/or Al-humus complexes which dominate the clay fraction [33,34], and is virtually unavailable to crops. The allophanic materials also impart to these soils a P-fixation capacity which requires even greater amounts of applied P than is usually necessary in Oxisols and Ultisols to attain the required equilibrium solution concentration to support adequate crop growth [21].

Although young soils often show a relatively uniform distribution of total P in the profile, pedogenesis brings about a redistribution in which calcium (Ca)–phosphates are gradually depleted, beginning in the surface horizons, and replaced by organic P [22]. This is the result of biogeochemical cycling of P by deep rooted plant species and the deposition of this P on the soil surface through litterfall and decomposition. Hence, the profile gradient in P concentration increases with increasing age of the soil and is greatest in highly weathered soils. The gradient is also increased by fertilizer applications to the plow layer [35,36]. Phosphorus deposited at the soil surface moves exceedingly slowly in inorganic form into the soil profile, especially in medium to heavier textured soils, owing to the strong adsorption reactions which bind phosphate onto clay colloids [37]. Organic P forms are similarly bound in the surface horizons of allophanic soils [33]. As a result of this significant P gradient with soil depth and the fact that P applications remain close to where they are applied in soil, plant root architecture can markedly affect the accessibility of soil P. Although deeper rooting plants may exploit a greater volume of the soil profile, it is pertinent to ask whether they might better invest their photosynthate in developing roots in the more P-enriched soil layers.

Plants take up P as orthophosphate ions (Pi) from soil solution. Soil solution Pi is in dynamic equilibrium with labile Pi forms adsorbed onto mineral surfaces, and it is replenished as solution concentration is depleted by plant uptake. As labile Pi itself is depleted, less soluble (primary or secondary) Pi forms control the Pi concentration in soil solution [38–40]. Soil solution Pi is also replenished by mineralization of labile organic P forms in processes mediated largely by inter- and extracellular phosphatase enzymes [41,42]. The role of extracellular enzymes in Po mineralization led McGill and Cole [43] to hypothesize that the process is driven more by Pi availability than the need for energy. Thus, microbial activity is controlled both by availability of substrate (e.g., litter and crop residues) and solution Pi concentration (i.e., demand). The availability and contribution of labile Po forms to plant P nutrition, therefore, depends on microbial activity.

As soils weather, an increasingly greater proportion of their labile P content is derived from organic P forms Tiessen et al. [44] showed that, in Mollisols, 86% of the labile P is associated with inorganic P (Pi) forms, whereas in Ultisols, 80% of the labile P is associated with organic P forms. Similarly, Sharpley et al. [28] found an increasing proportion of total P as organic P in more highly weathered soils. This implies that Ultisols and Oxisols rely much more on organic P forms to resupply Pi removed from soil solution by plants. Logically, as shown by Sharpley [45], available P (by Bray P) is correlated with soil Pi content in fertilized soils but with Po content in depleted unfertilized soils.

Availability and Fate of P Inputs

There is an extensive literature (reviewed by Sample et al. [24]) on the reactions and fate of P fertilizers applied to soils. Reaction products differ among soluble and insoluble as well as between inorganic and organic P sources. The nature of the products also depends on the mineralogy of the soil with which they react.

Soluble P fertilizers hydrolyze in soil to produce an acidic, supersaturated solution which diffuses outward from the point of application, dissolving soil mineral constituents as it does so [24]. For Ca-phosphate fertilizers (''superphosphates''), an insoluble Ca-P product (dicalcium phosphate) is initially precipitated at the dissolution site, whereas other secondary phosphates precipitate from the diffusing solution. In calcareous soils, these will mainly be Ca-phosphates, whereas in acidic soils, the main products will be Fe- and Al-phosphates. As the solution radiates outward and insoluble P compounds precipitate, solution P concentration declines eventually to the point where

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dissolution/precipitation reactions are no longer possible and chemisorption and surface adsorption reactions dominate. These initial reactions occur within a very short time after P fertilizer application (approximately 1–3 weeks) and within a very short distance (<25 mm) from the point of P placement. Because of their amorphous nature, these reaction products are highly labile in soil. With time, however, the amorphous and more soluble products are gradually converted to less soluble more crystalline compounds which, in acid soils, are predominantly variscite and strengite-like compounds.

The availability of insoluble P fertilizers such as phosphate rocks (PR) depends largely on the solubility product of the mineral which is controlled by its mineralogical characteristics [46]. Since the products of PR dissolution are Ca and phosphate ions, and since dissolution involves an acid reaction, Ca and Pi concentration in soil solution and soil pH are important external factors governing PR availability [47]. Of these three, Ca sink size seems to be the strongest factor driving dissolution [48,49]. This suggests that a plant with strong Ca acquisition characteristics may encourage PR dissolution and efficient use of P from these sources. Because it forms a very dilute equilibrium solution, Pi derived from PR dissolution enters primarily into adsorption reactions with soil mineral constituents. Thereafter it may be expected to undergo slow transformations similar to those described above which render it less available with time to plants. Since the PR dissolution rate is slow, it has been argued that release would be more in synchrony with plant demand enabling roots to capture a greater proportion of the Pi before fixation. To the contrary, however, there is strong evidence that soluble P sources are less affected by increased fixation capacity than PR sources [50].

As with soil organic P forms, release of organically bound P from organic inputs (crop residues, green manures, animal manures) is mediated by soil microbial activity (these sources may also contain significant amounts of Pi) [51]. Although not fully understood, there is evidence that the rate of P release from residues is influenced by nitrogen (N) mineralization rates which in turn are affected by substrate composition (C/N ratio, lignin and polyphenol contents) as well as by the C/P ratio [52]. Pi immobilization during residue decomposition has been reported under tropical conditions [53]. On the other hand, the half-lives of P release from residues have generally been observed in the range of 3–18 weeks [52,54,55]. Pi released from organic sources, as with PR dissolution, is either adsorbed onto mineral surfaces or enters into the organic P cycle through plant or microbial absorption. Radioisotopic tracer studies with ^{32}P indicate that mineral surfaces compete very strongly with plants for P released from residues [56].

Forms of P in Low-P Soils and Implications for Improved Adaptation

Phosphorus-limited tropical soils range from highly weathered soils, containing low total (and hence low available) P, to young soils derived from or influenced by volcanic ash, containing large but highly stabilized total P contents. The chemical forms of P in these soils differ widely and organic P pools constitute a significant, indeed often a substantial, fraction of the total P content. Plant adaptation to low-P soils may depend on how the plant can influence the availability of P in the various soil P pools. As will be discussed in more detail below, this can occur in a number of ways, including rhizosphere influences on Pi sorption, dissolution of precipitated Ca-, Al-, and Fe-phosphate forms, and mineralization of organic P forms. This influence occurs primarily through root exudation of protons to maintain internal charge balance or organic acids [57].

Phosphate sorption on Fe and Al oxide surfaces depends on pH, although the literature reports contradictory effects [58–60]. According to Barrow [60], these can be explained by the confounding effects of solution electrolyte composition, the variable charge properties of the oxide surfaces, the form (monobasic or dibasic) of the phosphate ion, and the direction of Pi movement—onto the surface or from the surface. In the absence of cation interferences, increasing pH reduces Pi adsorption owing to increased negative charge on the variable charge oxide surfaces [60]. Paradoxically, and more importantly from the point of view of rhizosphere influence on P availability, decreasing

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pH results in increased desorption of labile Pi. Phosphate sorption may also be reduced by organic anions such as citrate, malate, oxalate, and phytate which compete for adsorption sites on oxide surfaces (recently reviewed by Iyamuremye and Dick [61]). The effectiveness of anion competition depends on the anion, the nature of the mineral surface, and pH. Similarly, organic acids may release Pi from adsorption sites on Fe and Al hydrous oxide surfaces by ligand exchange reactions [62].

Dissolution-precipitation equilibria of Ca-, Al-, and Fe-phosphate minerals involve H^+ and OH^- ions [63]. As illustrated in phase diagrams [63], the solubility of these minerals (and their amorphous precursors) depends on pH; Al- and Fe-phosphate solubility increases with increasing pH, whereas the solubility of Ca-phosphates, including phosphate rocks, decreases [64]. Changes in pH in the root rhizosphere can therefore influence the dissolution of secondary P forms in soils, although the rates of dissolution may be too slow to have significant impact on plant growth. On the other hand, decreasing pH in the root rhizosphere of legumes as a result of an alkaline uptake pattern has been shown to increase the availability of PR fertilizers [65]. Organic anions may also enhance dissolution of mineral phosphates by forming complexes with metallic ions such as Al and Fe [61,66–69]. Complexation lowers the activity of metal ions in solutions shifting the dissolution equilibrium to the right and bringing more phosphate into solution.

Mineralization of P from organic pools may be stimulated by exudation of organic acids which become substrates for microbial and enzymatic processes in the rhizosphere [41,70]. A large part of soil organic P is in the form of phosphate esters. Phosphate is cleaved from these esters by enzymes such as phosphatase which is produced by roots of higher plants [71]. Helal and Sauerbeck [41] showed that phosphatase activity was much greater in the rhizosphere of maize roots than in bulk soil.

IMPORTANCE OF PHOSPHORUS SUPPLY TO PLANT GROWTH

No soil can sustain high yields if it is deficient in P. As an essential plant nutrient, P is involved in a wide range of plant processes from permitting cell division to the development of a good root system to ensuring timely and uniform ripening of the crop. P is needed most by young, fast-growing tissues, and performs a number of functions related to growth, development, photosynthesis, and utilization of carbohydrates [72–76]. P is a constituent of adenosine diphosphate (ADP) and adenosine triphosphate (ATP), two of the most important substances in life processes. Because of the importance of P for plant growth and yield, many compound fertilizers (NPK) used to correct major deficiencies in soil contain P as a major element [11].

Optimal plant growth requires P in the range of 0.3–0.5% of dry matter during the vegetative growth stage. Dry-matter P contents in excess of 1% may be toxic for most crops. However, many tropical food legumes are more sensitive to excess P, and toxicity may occur at much lower shoot P contents, for example, 0.3–0.4% in pigeonpea and 0.6–0.7% in black gram [77]. The partial productive efficiency of P for grain or seed is higher at early growth stages than at later stages, because P is needed for tillering or branching. If sufficient P is absorbed at early growth stages, it will be redistributed to other growing organs.

The most striking effects of P deficiency are reduction in leaf expansion and leaf surface area as well as total number of leaves [78–81]. The reduction in leaf expansion in low-P leaves is strongly related to the extension of leaf epidermal cells, which may be attributed to their low P content [82]. The reduction in leaf expansion was found to be related to a decrease in root hydraulic conductivity [83]. Reduced leaf expansion, auxillary bud growth, and, therefore, shoot canopy reduce the plant's photosynthetic surface area and carbohydrate utilization [75,76]. Since cell and leaf expansion are more retarded than chloroplast and chlorophyll formation [84], a low P supply increases the soluble protein and chlorophyll content per unit leaf area, resulting in small and darker green leaves [79]. Nevertheless, an inadequate supply of Pi limits the rate of photosynthesis owing to both the short- and long-term effects of Pi on the development of photosynthetic machinery and metabolism [76].

In the short term, low Pi might restrict photophosphorylation which should lead to increased energization of the thylakoid membrane, decreased electron flow, and associated inhibition of photosynthesis. In contrast to the short-term effects, inadequate Pi supply over the long term decreases the rate of photosynthesis by limiting the capacity for regeneration of ribulose 1,5-bisphosphate in the photosynthetic carbon reduction cycle. However, the long-term effects of Pi deprivation on photosynthesis are reversible [85].

Often P deficiency is not easily recognized, because plants may not show symptoms or the symptoms may be confused with those of other nutrients. The effects of P deficiency, for example, may resemble those of N deficiency. Stunted growth, suppression of tillering (in monocots) or branching (in dicots), shorter and more erect leaves, and delayed flowering are common effects of P deficiency in many crops. As indicated above, older leaves may be darker green or, in more extreme deficiency, may turn purple.

Root growth in P-deficient plants is relatively less inhibited than shoot growth, leading to an increase in root to shoot dry weight ratio [85–88]. In the forage legume, *Stylosanthes hamata*, shoot growth declines rapidly, but roots continue to grow under low P supply, not only because most P is retained but also because there is additional net translocation of P from the shoot to the root [89]. The maintenance of root growth at the expense of shoot growth is correlated with an increase in partitioning of carbohydrates toward the roots of P-deficient maize [90] and beans [91], which is indicated particularly by a steep increase in the sucrose content of the roots. The greater assimilate importation and sugar accumulation in the roots appears to be an early plant response to P deficiency [92]. The increase in dry weight and carbohydrate status in P-deficient bean roots was also associated with an increase in alternative respiration (cyanide-resistant pathway) which can be reversed within a few hours after P resupply to low-P plants [93]. The absence of chlorosis and maintenance of a high level of root growth in P-deficient bean plants was attributed to stimulated sucrose transport to roots due to unimpaired phloem loading [94]. This stimulation is probably related to the repressed sink activity in shoots; that is, reduced leaf expansion and shoot growth rate [85,95,96]. There is also evidence for an enhanced elongation rate of individual root cells and roots of low-P plants [97]. In certain plant species, a low P supply results in the formation of ''proteoid roots'' which are clusters of determinate lateral roots [98].

In addition to the aforementioned effects on vegetative growth, a low P supply also limits the formation of reproductive organs. Premature leaf senescence, delayed flower initiation [99], decreased number of flowers [100], and restricted seed formation [101] all contribute to yield reductions under P-limited conditions.

PHOSPHORUS REQUIREMENTS OF TROPICAL CROPS

The P requirements of plants are defined both in terms of their ''internal'' requirements and their ''external'' requirements for plant growth and yield. Genetic variation in plant adaptation to low-P soils may be related to external and internal P requirements. The internal requirement is the minimum uptake by a plant associated with a specific yield, usually near maximum growth [38]. It may also be expressed in terms of concentration; hence the term *critical concentration* for optimal crop growth or yield. Plants take up P as phosphate ions from soil solution and, hence, the external P requirement of plants is the P concentration in soil solution associated with adequate nutrition or growth [38].

Concentration of P in soil solution is very dilute (in the order of 0.01–0.6μg P mL^{-1}) [40,102] and is rapidly depleted by growing roots in soil. Depletion of Pi in the soil solution at the root surface results in the establishment of a concentration gradient between the root surface and the bulk soil solution, a short distance away from the surface. As solution Pi falls below its equilibrium concentration, it is replenished by labile Pi desorbed from clay mineral surfaces adjacent to the roots [38]. Pi thus moves from the adsorbed forms on clay surfaces into solution and along a concentration gradient to the root where the concentration is lowest. The depletion of P observed at the root surface

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TABLE 2 Internal and External P Requirements of Seven Plant Species for 80% of Maximum Growth

Plant species	P content in dry matter (g kg^{-1})	Soil solution (μM P)	Fertilizer application (mg P kg^{-1} soil)
Onion	1.4	6.9	170
French bean	2.0	4.6	90
Winter wheat	2.8	1.2	40
Ryegrass	3.3	1.4	50
Rape	3.9	1.4	50
Tomato	4.5	5.7	110
Spinach	8.3	4.6	90

Source: Adapted from Ref. 107.

shows that plants are able to create almost the maximum possible P concentration gradient between bulk soil and the root surface [103]. This is important for the movement of P toward the root surface, because gradient is the driving force of diffusive flux. On the other hand, P depletion may imply severe restriction of P influx into plants, because P influx depends on the concentration at the root surface.

Although the external P requirement for a particular plant varies little between soils, the amount of labile Pi needed to provide a certain Pi concentration in soil solution depends on soil mineralogy and texture [38,104]. In P-limited tropical soils, the quantity of labile P may be insufficient to maintain Pi solution concentration against depletion by plant roots. Adsorption isotherms have been used to establish the relationship between adsorbed (labile) Pi and solution Pi concentration for a wide variety of soils [38]. Based on these, P fertilizer applications are used to adjust solution Pi to the desired concentration for adequate crop nutrition. With their capacity to adsorb large quantities of P from solution, P-limited tropical soils often require much higher applications than Temperate Zone soils.

The P concentration in soil solution (external P requirement) necessary to achieve maximum growth differs widely among crops. Using flowing solution cultures, Asher and Longeragan [105] showed a 25-fold difference in external P requirements among 8 plant species and Asher [106] reported a 200-fold difference for 18 species ranging from *Stylosanthes guianensis* to cassava. External P requirements of a range of crops and vegetables estimated in the field on Hawaiian Oxisols using adsorption isotherms were equally variable [38]. Kamprath and Watson [102] summarized external P requirements for several temperate and tropical crop species in the range of 0.06–0.68 μg P mL^{-1}. Requirements summarized by Föhse et al [107] also demonstrate wide variation in external P requirements as well as internal requirements (Table 2). There was no correlation between internal and external P requirements (e.g., wheat vs onion). These data indicate that the differences were mainly due to differences in the P-acquisition efficiency of the root systems.

MECHANISMS WHICH IMPROVE PHOSPHORUS ACQUISITION

Phosphorus acquisition by plants depends on the morphological and physiological characteristics of the root system, because the relative immobility of P in soil makes P acquisition by the plant very dependent on soil exploration in time and space [14,103,108,109]. Research in germplasm development for the past 20 years has resulted in the identification of superior tropical crop and forage germplasm adapted to low P soils [9,15,110–120]. Crops and forages that are genetically adapted to low P–supplying tropical soils are often characterized by a low P requirement and/or

increased efficiency in absorbing P from soils of low P status, and in utilizing P for plant growth. Identification of plant attributes and mechanisms that contribute to the P efficiency of these crop and forage genotypes, however, remains a major research challenge. Below we consider both root morphological and physiological characteristics implicated in improved P acquisition.

Root Morphological Characteristics

The roots of annual crops have a volume that is usually less than 1% of the soil volume they occupy [103]. Therefore, crop roots contact less than 1% of the total available P in the soil, an amount which is usually only a small fraction of the crop's requirement. Several root morphological characteristics including length, diameter, number, and duration and length of root hairs, as well as mycorrhizal associations, are very important in determining the efficiency of P acquisition from low-P soils.

Root Growth and Distribution

Efficiency in P acquisition depends markedly on rooting density and root distribution in the soil profile. Both parameters depend on plant genotype, soil chemical and physical properties, and cropping system (e.g., rotation). The rooting depth of most annual crops increases as the growing season progresses, although it is rare for it to increase much after anthesis in determinate crops such as the cereals. Indeterminate legume crops continue to allocate assimilate to the root system during early pod filling. Consequently, the total size of the root system continues to increase, although usually at a lower rate than before flowering. Compared with annual crops, perennial forage species, particularly grasses, develop more vigorous root systems as an adaptive feature to low P availability in tropical soils [55,121]. Differences in root growth and distribution to a large extent explain the differences among cultivars in P acquisition [122–124] or the competitive advantages of grasses over nongrasses at low P supply [121,125].

The importance of root size in P acquisition was convincingly demonstrated using maize isolines differing in the ''rootless'' gene [126,127]. A monofactorial inherited mutation caused a drastic reduction in the growth of crown roots of the ''rootless'' isoline [128]. Under limited P–supply conditions, total dry matter production of the ''normal'' line significantly exceeded that of the ''rootless'' line and P acquisition was strongly correlated with root dry weight for both isolines. However, the advantage of the ''normal'' line vanished with the increase in P supply. Otani and Ae [129] found the opposite effect in their examination of the relationships between P uptake and root length, as affected by soil volume and soil P status, in field and pot experiments for several crops, including buckwheat, castor, peanut, pigeonpea, sorghum, and soybean. P uptake by crops was strongly correlated with root length in soils where P availability was high, but not in soils with low P availability or where volume is limited. These results suggest that additional mechanisms besides root length are involved in P acquisition.

Root Diameter

The fineness of the root system (root diameter) is an important attribute that determines P acquisition from low-P soils [103,130]. This is because root diameter defines the maximum volume of soil which can be exploited with a given amount of photosynthate. If a fixed proportion of photoassimilates is used for root growth, a much greater root length can be achieved by reducing root diameter; that is, specific root length (length of root per unit root weight) increases [131]. Root diameter varies between species and cultivars and changes as plants age [132,133]. There are large variations between monocotyledonous and dicotyledonous species [134,135] (Table 3). The greater root diameter of dicots than monocots could be due to (a) a need for a greater surface for symplastic loading and (b) a greater need for basic cations. The variation in root diameter among closely related species was found to be greatest when plant growth is limited by P supply [131]. A comparative study of two wheat cultivars, a modern (''Cosir'') and a traditional (''Peragis''), indicated that the larger specific root length of the modern cultivar contributed to the greater P-acquisition efficiency [136].

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TABLE 3 Morphological Characteristics of Roots of Seven Plant Species

Plant species	Root radius (mm)	Root-hairs			
		Density[a]	Average length[b]	Total length[c]	Surface area[d]
Onion	0.029	1	0.05	0.3	0.1
French bean	0.145	49	0.20	11.8	0.4
Winter wheat	0.077	46	0.33	20.4	1.3
Ryegrass	0.066	45	0.34	17.1	1.3
Rape	0.073	44	0.31	18.6	0.7
Tomato	0.100	58	0.17	13.7	0.7
Spinach	0.107	71	0.62	41.4	1.9

[a] Number per millimeter of root length.
[b] Millimeter per root-hair.
[c] Millimeter of root-hairs per millimeter of root length.
[d] Square millimeter of root-hairs per square millimeter of root axis.
Source: Adapted from Ref. 137.

Root-hair Development

The formation of root-hairs is one of the most efficient ways to increase P acquisition. Jungk and Claassen [137] found the influx of P per unit root length greatly enhanced by root-hairs. This can be explained by the enlargement of the root surface area and because root-hairs penetrate the soil perpendicular to the root axis, giving access to a larger volume of soil per unit root length. Consequently, P-depletion profiles are found to differ in their radial extension depending on root-hair length [138]. Assuming a frequency of 100 mm^{-1}, a radius of 0.005 mm, and a dry matter content of 5%, Clarkson [139] calculated a threefold increase in surface area could be achieved at an expense of less than 2% of root dry matter.

There is marked variation among crops in the number and surface area of root-hairs per unit root cylinder (see Table 3). Differences in P acquisition among several crops may be explained by differences in root-hair length [140,141]. Root-hair length was 0.28 mm in cotton and 0.77 mm in rape, so that 1-cm length of cotton root would utilize a volume of 19 mm^3, whereas an equal length of rape root would utilize 41 mm^3 [141]. Thus, for cotton, the soil would be completely explored by a root length density of 52 $cm\text{-}cm^{-3}$ but rape would require a density of only 24 $cm\text{-}cm^{-3}$. The major benefit of root hairs in P acquisition seems to be that they enable the root system to operate effectively with low P concentrations in the soil solution. This demonstrates the importance of root diameter and the peculiar geometrical arrangement of root hairs in soil.

Plant breeding for low-P soils could involve selection for root-hair length. Selection for root-hair length improved P-acquisition efficiency of white clover [142]. In the case of wheat, the P-efficient variety had longer root-hairs (1.37 mm) than the less efficient variety (1.19 mm). However, a major limitation for using root-hair length as a selection criterion could be the high environmental variability of this trait [143,144].

Mycorrhizal Association

Mycorrhizae colonize the root systems of most plants and serve as an extended link between plant roots and soil [145]. These links are very important in increasing the efficiency with which root systems can acquire P, since the external hyphal system enables the roots to exploit a larger soil volume. When root exploration of the soil is restricted by low P supply, up to 80% of the plant P can be delivered to the host plant by the external arbuscular-mycorrhizal (AM) hyphae which explore soil to a distance of more than 10 cm from the root surface [146].

Associations of AM fungi are almost universal in the roots of plants in the tropics. Although

roots of most crops are colonized with AM fungi, the mycorrhizal efficiency of P acquisition probably varies markedly among crop species. Cassava has a higher AM dependence than *Stylosanthes guianensis*, cowpea, beans, *Andropogon gayanus*, maize, or rice [110]. Because of its thick roots and poorly branched root system, cassava will not grow without AM infection except in soils with extremely high available P [147–149]. Many other important tropical crops in addition to cassava are also highly dependent on mycorrhizal associations for adequate supplies of P when no P fertilizer is applied [110].

The impact of crop management (e.g., rotations and green manures versus monocultures) on P acquisition may be an indirect way of affecting mycorrhizal infection potential in the soil and root colonization with AM. In natural ecosystems, significant associations between roots, mycorrhizal fungi, and decomposing organic materials can be observed [150]. By this means, plants can achieve very close contact with sites where nutrients are being released by the processes of decomposition. Thus, systems which promote mycorrhizal associations may well be used to influence P acquisition by components and directly reduce P-fixation processes and increase P cycling.

Soil and crop management practices (crop sequence, tillage, fertilizers, pesticides) can influence the total quantity of AM development [151]. Thus, changes in cropping systems and fertilizer applications that affect the soil environment should affect both roots and AM colonization [152]. In continuous monocultures of corn or soybeans, detrimental species in the mycorrhizal fungal community increase relative to beneficial species [153]. In time, crop vigor declines under monoculture, because populations of beneficial AM fungal species decrease. Using different plant hosts, different populations of AM can be built up in the soil around the root system [154].

Mycorrhizal association could reduce the overall retention of carbon in the plant-fungus symbiosis by increasing carbon in roots and belowground respiration, and reducing its retention and release aboveground [155]. A shift in allocation of carbon in mycorrhizal plants to pools that are rapidly turned over (primarily to fine roots and fungal hyphae) could alter the size of belowground carbon pools as well as the quality and, therefore, the retention time of carbon belowground. Should this occur, mycorrhizal associations could significantly increase the rate of cycling of N, P, and S through organic pools in litter and soil organic matter. There is some evidence that mycorrhizal fungi, in certain cases, may directly recycle P from litter [156]. In addition, there is evidence that roots influence decay rates. It is therefore important to define both the magnitude of these effects and the circumstances under which they operate.

In view of the various possible effects of mycorrhizae on plant growth [145,157], a better understanding of the host-mycorrhizal interactions is necessary to be able to predict the capacity of external mycelium to acquire P for the host under various conditions. There is also a need to characterize the conditions at the hyphae-soil interface which may influence P availability in soil.

Root Physiological Characteristics

Phosphorus-Uptake System

The uptake of Pi across the plasma membrane of a plant cell proceeds via $2H^+/H_2PO_4$-cotransport driven by an electrochemical proton gradient [158]. This proton-cotransport mechanism has been found in the Pi-uptake system of *Lemna gibba* [158], corn [159], and *Catharanthus roseus* [160]. Recent studies on phosphate transporters of different species indicate that the mechanism of P uptake at the cellular level is remarkably similar among organisms [161–164].

Under conditions of P deficiency, an enhanced P-uptake system may be induced [165]. This enhanced P-uptake system causes a rapid accumulation of P in leaves once the availability of Pi to roots is improved [85,166]. Under conditions in which the rate-determining step in P uptake is located in the root, P uptake will increase if root length per unit plant weight and maximal net influx per centimeter of root length (I_{max}) increase and the Michaelis-Menten constant (K_m) and minimum concentration (C_{min}) decrease [167].

Large genotypic differences in the efficiency and kinetics of P uptake from soil have been

reported [168]. However, the assessment of the kinetic parameters (I_{max}, K_m, and C_{min}) characterizing the uptake system of a genotype is complicated by at least three factors [136]: (a) the plasticity of the system in response to the P status of the plant [137,169], (b) the differences in P uptake along roots [170,171], and (3) the dependence of P uptake on plant growth rate [172]. Thus there is general agreement that the efficiency of the uptake system is of minor importance for P acquisition from soils, because transport of P to the root surface rather than the uptake is the limiting step [103]. Therefore, it is less likely that selection for an efficient P-uptake system will contribute to more efficient P acquisition from low-P soils.

Phosphorus Mobilization in the Rhizosphere

In low-P soils, root-induced changes in the rhizosphere may be particularly important in P acquisition. There is increasing evidence that root release of organic acids (especially malic acid, citric acid, and perhaps oxalic acid) are key components in this respect. Organic acids differ markedly in their capacity to complex Fe and Al and thus solubilize the respective P compounds in soil bound by these ions. An example of this high specificity was seen in pigeonpea which releases a particular acid (piscidic acid) that complexes Fe but not Ca [111]. Accordingly, pigeonpea is highly P efficient on Alfisols where P is bound predominantly as Fe-phosphates but not on Vertisols where P occurs predominantly as Ca-phosphates. The release of organic acids is enhanced under conditions of P deficiency [173–175]. Aluminum tolerance in certain crop species is associated with the release of organic acids stimulated by monomeric Al species in soil solution [176]. It is expected that both mechanisms are important in mobilizing P from sparingly soluble sources in the root rhizosphere in low P-supplying tropical soils. In addition to these two mechanisms, Ae et al. [177] proposed that cell walls of plant roots are involved in P-solubilizing activity. Their results indicated that groundnut root cell walls had a higher P-solubilizing activity than those of soybean or sorghum. Thus, there is a need to further examine ''direct contact reactions'' between the root surface and P minerals.

Organic P forms in infertile tropical soils probably contribute significantly to plant P nutrition, particularly in natural ecosystems [178]. Root exudation of acid phosphatases (ectoenzymes) is common in plants and is usually enhanced under P deficiency [179]. The kinetic constants of secreted acid phosphatase enzymes from roots may be used as an indicator of the P stress tolerance of plants [180]. Secretory acid phosphatase can liberate bound P from soil [181] and have been shown to deplete organic P in the rhizosphere of lupin roots within about 2.5 mm of the root surface [182].

A comparative study using 16 plant species indicated a marked variation in the secretion of phytase from roots of P-deficient plants [183]. Secretion of phytase was highest in *Brachiaria decumbens* CIAT 606, *Stylosanthes guianensis* CIAT 184, and tomato. It is speculated that the secretory phytase could provide an efficient mechanism for wide adaptation of the tropical forage grass *B. decumbens* CIAT 606 (planted on over 40 million ha) to the low P–supplying tropical soils of Latin America. Studies of enzyme activity at the soil-root interface [180,184] may help to identify genotypes which are more efficient in mobilizing organic P sources in soil.

At least some species of AM fungi also show acid phosphatase activity at the external hyphae, effectively utilizing organic P (Po) (e.g., Na phytate) and supplying it to the host plant [185]. There is a need to evaluate the capacity of hyphae, roots, and mycorrhizal roots to utilize these various forms of Po using compartmented pots [185,186].

MECHANISMS WHICH IMPROVE PHOSPHORUS UTILIZATION

In addition to possible genotypic differences in P acquisition, plant adaptation to P-limited tropical soils can be partially attributed to inherent genotypic differences in P use efficiency (PUE). From the agronomic point of view, the amount of total biomass and/or economic yield produced per unit of acquired P indicates PUE [187–189]. As defined, such efficiency is controlled directly or indi-

rectly by plant traits and mechanisms related to basic metabolism [190], by patterns of partitioning and remobilization of P among different organs and tissues [12], and perhaps mostly by the capacity of the plant to accumulate dry matter owing to efficient utilization of P in plant metabolic processes. A comparison of the amount of P taken up by various crops to produce 1 ton of yield is shown in Table 4 [149,191–193]. Beans and soybeans stand out as the most P-demanding crops.

PUE is sometimes considered to be the inverse of P concentration [194–197]. This definition may be more useful in describing the current dynamics of P acquisition in relation to P utilization. PUE can also be defined as a response (measured in dry weight) for a given increase in P content during a given increment of time [14]. All plants exhibit an increase in PUE under conditions of P deficiency [86,198–200], because (a) a larger proportion of plant biomass is allocated to tissues with low P concentration (e.g., roots as contrasted with leaves or reproductive organs); and (b) P storage in vacuoles declines [201] and structural and nonstructural carbohydrates increase [76,86,202].

Efficient utilization of P acquired from low-P soils is dependent on a number of plant attributes [12,15], including (a) high–dry matter yield per unit of P acquired, (b) growth duration and plant type, (c) partitioning of P between different pools within the plant, (d) translocation and partitioning of P within the plant, (e) redistribution of previously assimilated P, (f) leaf death rate, and (g) partitioning of a greater proportion of biomass to harvestable yield. When the P supply limits plant growth, higher plants undergo changes in a number of shoot and root attributes [86,121,201,203]. Among them are a marked reduction in leaf area production [78,79], an increase in P-uptake capacity per unit root length [204–208], an increase in root to shoot ratio [79,209,210], changes in the morphology of the root system [107,118,211], and an increase in the proportion of total P partitioned to roots [209,212,213]. Several studies indicate that P-efficient species have a high ability to retranslocate P from inactive to active tissues [201,214–216]. Several of these plant attributes may be significantly affected by association with AM fungi [14,150].

Table 4 Comparison of P Uptake and P Taken Up per Unit of Economic Yield by Various Crops

Crop	Plant part	Yield (kg ha^{-1})	Total P uptake and removal (kg P ha^{-1})	Total P uptake per unit yield (kg P t^{-1} yield)
Maize	Grain	9,416	26	4.67
	Total	19,496	44	
Rice	Grain	5,380	10	2.97
	Total	10,990	16	
Wheat	Grain	2,690	12	5.58
	Total	6,050	15	
Beans	Grain	940	3.6	9.68
	Total		9.1	
Soybeans	Grain	3,000	22	8.33
	Total	6,700	25	
Cassava	Roots	13,530	13.2	1.75
	Total		23.7	
Sweet potato	Tubers	10,520	18	2.85
	Total	16,660	30	
Potato	Tubers	11,850	34	3.71
	Total	18,250	44	
Sugarbeet	Tubers	11,180	29	4.11
	Total	17,040	46	

Source: Adapted from Refs. 149 and 191–193.

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When the P supply is limiting in soil, plants have to make most efficient use of the P that they have acquired. Improvements in P use efficiency can be achieved by at least two major mechanisms: (a) changing the partitioning of P among plant parts and (b) increasing the metabolic efficiency of P at the cellular level. These are discussed in the following sections.

Partitioning of Phosphorus Within the Plant

Remobilization of Phosphorus Within the Plant

The ability of crop plants to remobilize P from vegetative to reproductive organs and forage plants from senescing to growing points may form an important mechanism that allows plants to improve the utilization of acquired P [15]. Any factor that affects plant growth (and therefore P demand) will alter PUE. For example, the degree of P remobilization from leaves increases when there are P sinks such as reproductive organs present; this results in reduced leaf P concentration (i.e., increased PUE). On average, about 50–75% of the P contents are retranslocated from a leaf before it is shed [217,218]. Comparison of two white clover cultivars has shown that the more P-responsive cultivar was better able to remobilize P from senescing tissue to growing points than the less P-responsive cultivar [219]. Populations of white clover collected from low-P soils had a lower proportion of dead leaf to total leaf than populations from high-P soils when grown in solution culture [220]. Several researchers found that species adapted to low-P soils generate a lower proportion of dead leaf to total leaf material when under P stress than species from high-P soils [221–224].

Based on nutrient harvest indices in soybean, it has been suggested that more seed P than N is derived from remobilization [225,226]. From these studies, it appeared that P nutrition and the remobilization of vegetative P to reproductive structures may be closely associated with leaf senescence and productivity. But subsequent studies using soil P treatments showed that P nutrition, in general, and specifically P remobilization from leaves, does not exert any regulatory control on the process of leaf senescence [227], and seed development in soybean may occur independently of net P remobilization [228].

Snapp and Lynch [229] measured P remobilization from roots and leaves and examined the influence of P nutrition on remobilization patterns and tissue longevity in the common bean cultivar, ''Calima.'' Using a split-root system and ^{32}P tracer, they demonstrated that low-P roots successfully competed with reproductive tissues for available P. Retention of P in low-P roots was in contrast to remobilization of P from leaf and stem to grain in both low-and high-P plants. They suggested that root P retention may allow roots to sustain nutrient and water uptake late in the ontogeny.

Common bean lines with low P concentration in shoot tissue retained more P in roots and older leaves under P-deficient conditions than lines with a high P concentration [230]. The greater remobilization of P in bean lines with a high P concentration could be attributed to greater P requirement to maintain normal metabolic activity in growing tissues. But in the case of a forage legume, white clover, there was no evidence that populations from low-P soils were more effective in remobilizing P from senescing leaves than populations adapted to high-P soils [220]. When both populations were grown in low-P soils, the P concentration in dead leaves did not differ, indicating that differences in remobilization of P from leaves prior to senescence or abscission were not a significant adaptive feature of white clover populations growing on low-P soils [231]. Thus, the contrasting patterns of P remobilization from older leaves between common bean and white clover may indicate the importance of maintaining greater P concentration in the grain of crop plants.

Phosphorus Status of the Harvested Organ

The concentration of P in the harvested organ (grain) is important, because (a) it indicates the amount of P used to produce a kilogram of harvested organ; (b) high-yielding genotypes with low grain P concentration would remove less P from the soil and therefore reduce the cost to produce each ton of grain; and (c) reduction of grain P concentration would also lower the concentration of phytic acid, an antinutritional factor [232,233]. However, high grain P concentration may have some bene-

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fits, including greater seedling vigor and higher grain yield if the seed is used to grow the following crop [234,235].

Glasshouse and field studies using a genetically diverse range of wheats indicated that the concentration of P in grain was negatively correlated to the harvest index (grain dry weight/grain + straw dry weight) [236,237]. However, the strength of this relationship varied between seasons and the level of fertilizer P application [237]. Based on these data, it appears that, if adequate selection pressure is applied, higher or lower grain P concentrations can be achieved. However, attempts to retain P in vegetative tissues may be counterproductive, because a reduction in the supply of P to developing grains could result in smaller grain size [238].

Efficient Utilization of Phosphorus at the Cellular Level

A number of tropical crop and forage species can grow normally with low tissue P concentrations owing to efficient utilization of P among the major biochemical fractions. Several studies have indicated that the differences in utilization of P fractions (soluble P, Lipid P, and residue P) may form the basis for the identification of plants tolerant to low-P environments [73,196,210,239]. Comparative studies between lotus and white clover suggested that changes in the pi concentration in tissues may affect the ability of species to survive under low-P environments [240]. Lotus, which maintained relatively low tissue Pi concentrations, was found to be more tolerant to low-P conditions than white clover, which exhibited high-Pi concentrations in the tissues. Leaf Pi concentrations are also known to regulate plant growth, photosynthesis, and carbon partitioning [76,85]. Leaf Pi values correlated most closely with relative grain yields ($R^2 = 0.89$) of pot-grown barley in mineral soils [241].

It is possible that species which have a relatively small ''pool size'' of Pi would be able to maintain high metabolic activities at the low external Pi supply and therefore be adaptable to low-P soils. On the other hand, Chisholm and Blair [196] concluded in their experiment with white clover and stylo that differences between species or cultivars in lipid P stability may form the basis for the selection of plants tolerant to low-P conditions. Adu-Gyamfi et al. [242] compared the degree of P tolerance in soybean and pigeonpea based on the utilization of P fractions, especially at low-P conditions. They found that pigeonpea is more tolerant to low-P conditions compared with soybean, because it maintains relatively low tissue concentration of Pi owing to the efficient incorporation of the external Pi into residue P. The importance of intracellular compartmentation of P and metabolic utilization of P for plant adaptation is discussed below.

Intracellular Compartmentation of P

The pioneering work of Bieleski and his associates [73], using radioisotopes, showed that the cytoplasm and vacuole work as distinct compartments for Pi at the cellular level in plants. Intracellular Pi compartmentation studies using ^{31}P-NMR (nuclear magnetic resonance) indicate that, under Pi deficiency, the vacuole acts as a Pi reservoir to maintain a constant cytoplasmic Pi concentration [76,243,244]. Measurements of in vivo changes in intracellular distribution of Pi in soybean leaves as affected by P nutrition, using ^{31}P-NMR, indicated that the cytoplasmic P pool, and the leaf carbon metabolism dependent on it, are buffered by the vacuolar P pool until the late stages of reproductive growth [245]. Using ^{32}P-labeled Pi, and an autoradiographic measuring system highly sensitive to beta irradiation, Mimura et al. [246] visualized and measured the retranslocation of Pi in the same plantlet. Under Pi deficiency, the cytoplasmic Pi concentration of the first leaf remained constant until 16 days after sowing whereas vacuolar Pi was completely exhausted after 8–10 days. The exhaustion of vacuolar Pi in the first leaf coincided with the appearance of the second leaf. They suggested that various membrane-transport systems, that is, the plasma membrane and the tonoplast, play important roles in Pi homeostasis and translocation. Further research is needed to measure the changes in the Pi-transport activities of these membranes during plant growth with a limited Pi supply.

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Metabolic Requirements of P

Differences in the utilization of leaf P fractions (soluble P, lipid P, and residue P) may form the basis for genetic differences in plant adaptation to a low P supply in soil [15]. Species with a low leaf Pi ''pool size'' may be able to maintain high metabolic activity at a low external Pi supply and therefore be adapted to low-P soils. Jeschke et al. [247] compared, for the first time, complete inventories of uptake, transport, and utilization of C, N, and H_2O between and within organs of intact castor bean plants deprived of P at a given growth state. Despite much lower intakes under P deficiency, the general patterns of flows and partitioning of C, N, and H_2O, in comparison with the P-sufficient plants, appear to be well coordinated and well adapted toward allowing the plants to withstand the disadvantageous P deficient conditions. They suggested that the formation of a proportionally larger root system in P-starved plants would clearly offer a means of adaptation to a deficiency of P supply.

GENETIC ADAPTATION TO LOW PHOSPHORUS SUPPLY IN SOIL

Several physiological attributes of plants can exhibit considerable genotypic variability in their expression, a high degree of stability in genotype ranking across environments, and in some cases a high narrow sense heritability so that improvement through recombination and selection may be both effective and relatively straightforward [248]. Genetic variability for the trait or traits in question and the ability to manipulate this genetic variability for improvement of desirable traits are two essential components for enhanced genetic adaptation of crop and forage species and cultivars to a low P supply in tropical soils. However, plant breeders are aware of the fact that selection for a single desirable trait can often have deleterious effects such as (a) poor field adaptation as a result of ignoring the general agronomy of the crop and (b) concomitant changes in other desirable traits which are of adaptive significance. Genotypic variation in plant traits related to P acquisition and utilization has been observed in a number of crop and forage species [112,115,123,168,188,233,249–265] (Table 5). Since a number of shoot and root traits contribute to P acquisition and utilization, determining the genetic control of these traits becomes a major research objective [13,266].

Shoot Traits

Reciprocal grafting experiments indicate that shoot factors rather than root factors regulate P uptake per unit root size [267]. This is because removing a part of the shoot, by cutting, reduced P uptake per unit root weight. But removal of half of the root system from the P supply, either by splitting root systems or root pruning alone for approximately 3 days, had no effect on P uptake per unit root weight [268]. It may be that the rate of P absorption is quite strictly regulated by biochemical factors which vary with the rate at which P is utilized for plant growth [269]. There is evidence that P uptake is regulated primarily by Pi concentration of the root cell [270] which largely reflects the P status of the shoot [271]. Increase in the root Pi concentration improved P-acquisition efficiency of the tropical forage legume *Arachis pintoi* [265].

Screening of several hundred accessions of wheat for tolerance to a low P supply in soil showed that lines with a higher harvest index had greater and more consistent grain yields compared with lines with a low harvest index [272]. High P efficiency in other wheat genotypes was also associated with high harvest indices [233].

Field and pot studies were conducted to evaluate genetic variation in diverse bean germplasm for P efficiency on soil types with contrasting P chemistry and to assess possible relationships between dry matter distribution, P partitioning, and yield [254,255]. They found no evidence for specific adaptation to low P availability in volcanic or mineral soils. They also showed that vegetative and reproductive responses to low P availability are not correlated.

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TABLE 5 Summary of Studies Identifying Genotypic Differences in P Acquisition and Utilization in a Range of Crop and Forage Species

Species	No. of genotypes	Shoot and root traits measured	Reference
Crops			
wheat	23	Grain yield, P uptake, root dry weight, P harvest index	188
	20	Harvest index, grain P concentration	233
	9	P uptake, root length	123
corn	9 inbreds	Dry matter yield, P uptake, root to shoot ratio	112
rice (upland)	20	Dry matter yield, root length, P uptake, P use efficiency	249
barley	7	Dry matter yield, P uptake, net P influx rate	168
sorghum	8 parents and 16 hybrids	Dry matter yield, P uptake	250
	2	Dry matter distribution, P distribution, P uptake	251
pearl millet	12	Grain yield, dry matter, P uptake, P use efficiency	252
cassava	4	Root yield, root length density, P uptake, P use efficiency	115
bean	26	Shoot growth, P distribution	253
	16	Shoot growth, root growth, P accumulation	254
	12	Grain yield, yield components, P distribution	255
	6	Shoot biomass, root biomass, P accumulation	256
cowpea	20	Grain yield, root dry weight, P use efficiency	257
	5	Shoot dry matter, P uptake	258
pigeonpea	2	Dry matter yield, root length, root surface area, P translocation, P distribution	259
soybean	2	Root carbohydrates, P uptake	260
mungbean	3	Shoot dry matter, nodulation, N_2 fixation	261
Forages			
white clover	98	Shoot dry wt., root dry weight, shoot P, root P	262
	6	Shoot dry wt., root dry weight, root hairs, P uptake	263
lucerne	2	Shoot dry wt., root dry weight, P uptake, P use efficiency	264
stylosanthes	4	Shoot dry wt., root dry weight, P uptake, P use efficiency	265
centrosema	4	Shoot dry wt., root dry weight, P uptake, P use efficiency	265
brachiaria	4	Shoot dry wt., root dry weight, P uptake, P use efficiency	265

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Root Traits

Evaluation of the root system as a selection criterion can help to increase productivity and yield stability [273]. A special synthetic variety of lucerne (*Medicago sativa*) with a large root system has been developed which is notable for performance, stability, and persistence [274]. A recent review [266] summarized the extent of genotypic variation in a number of root traits. Traits that reflect root system size include root weight, root length, root number, and root volume. Traits that reflect root morphology include root diameter, primary root length, number of adventitious roots, root branching, root length density, and root-hair length. In addition to these traits, those that increase the solubility of sparingly soluble soil P such as, root exudates, root exocellular phosphatases and phytases, and root-induced pH changes are also important. At low levels of available P, total root length, root weight, and extensiveness (mass and surface area) of all roots and root-hairs were found to be important traits in genetic adaptation of tomato [275] and white clover [142] to a low P supply. However, these traits were not important when plants were grown with adequate levels of available P or with mycorrhizal association at low P.

Genetic Manipulation of Traits

The existence of, or potential to create adequate genetic variability in important shoot and root traits, is a prerequisite to favorable genetic manipulation. Classic plant breeding techniques and/or modern molecular and cellular biological techniques for gene transfer can be employed to improve plant adaptation to P-limited environments [13,118,119,276,277], as discussed below.

Use of Classic Plant Breeding Techniques

Coltman et al. [275] used yield at a low P supply as a selection criterion to identify strains of tomato that are adapted to P deficiency. They found that broad-sense heritability for yield at a low P supply varied from 0.61 to 0.67 depending on generation. In the expression of low-P tolerance, dominance effects were more important than additive genetic variance. Additive gene effects indicate that a genetic trait is altered by each additional allele whereas dominant gene effects indicate gene action deviating from an additive condition, such that the heterozygote is more like one parent than like another.

A study of P-efficiency traits in wheat indicated that P uptake in shoots per unit root dry weight, which describes the ability of the plant to obtain P from the soil, is a far more beneficial measure for use in breeding programs than either grain yield per unit P uptake or grain P content as a percentage of total P uptake [188]. Selection for shoot P concentration has been successful with realized heritabilities for increased P concentration of up to 0.36 for alfalfa [278] and narrow-sense heritabilities of 0.42 for wheat [279]. Three major genes [280], probably located on chromosome 9 [281], seem to control P concentration of the ear leaf of maize. In sorghum, dominant effects were found to be more important [250], whereas in wheat, additive genes were responsible for P utilization (the inverse of P concentration) differences [279]. In common bean, epistatic effects (i.e., gene interactions where one gene interferes with the phenotypic expression of another nonallelic gene[s]) were important [282]. Genetic component analysis in rice suggested that both additive and dominance gene effects are involved in the inheritance of P-deficiency tolerance [283].

Selections for extremes of root system size have been successful in alfalfa [284], ryegrass [285], white clover [286], maize [287], spring wheat [288], peas [289], and rice [290,291]. Heritabilities for root weight have been estimated frequently for a number of crop and forage species by several researchers [266], with a median narrow-sense heritability of 0.53 (mean 0.52 $\pm$ 0.05). Heritabilities increased with depth of rooting for root number and root surface area of creeping bentgrass [292]. For root to shoot ratio and root growth rate, the median narrow sense heritabilities were 0.52 and less than 0.4, respectively [292].

There has been some inconsistency among studies in determining the importance of additive and dominance effects on root system size [266]. Both additive and dominance components of

genetic variation were important for root number in maize [287], root length and weight in spring wheat [288], root weight and root volume in peas [289], and root length, root number, root to shoot ratio, and root volume in rice [290,291]. However, only additive gene effects were important for root length and diameter in rice [293].

Genotypic variation in responsiveness to P application (i.e., growth rate per unit of P applied) was observed in rice [249,294,295], maize [294], sorghum [12,250], beans [296], and white clover [297]. In white clover, superior genotypes were identified that combined both tolerance to low P (i.e., high yield at low P) and an ability to respond to added P [298]. Genetic studies indicated that high P response (higher dry weight increase per unit of P applied) was dominant over low P response, and that narrow-sense heritabilities for P response were moderate (0.33–0.66) [299]. The ratio of dominant to recessive genes in all white clover parents was approximately 2 for P response. Moreover, it was estimated that at least four individual or groups of genes are involved in the P response [299].

In a breeding program to improve dry bean performance under P deficiency, Schettini et al. [300] successfully employed one accession of an exotic snap bean germplasm and demonstrated that quantitative traits such as P efficiency can be transferred into an agriculturally useful genetic background using the inbred backcross line method. They found, in general, that the lines which performed well in nutrient solution culture also performed well in a field test in soil moderately deficient in P.

Use of Molecular and Cellular Biological Techniques

During the past decade, much progress has been made in the use of molecular and cellular biological techniques to improve plant adaptation to biotic and abiotic constraints. Many of the agronomically and economically important crops have been transformed so that genes for pest and disease resistance, improved grain or fruit quality, herbicide resistance and more recently aluminum tolerance from a variety of sources can be and are being inserted [301,302,303,304]. Molecular markers and associated technologies can assist in map construction and the analysis of the molecular and genetic basis of quantitative and qualitative traits [305]. Rapid progress in this field has enabled high-density genetic maps to be made and the location of the genes regulating the expression of physiological and agronomic characteristics that are inherited in a quantitative manner to be determined using a combination of statistical analysis, multiple regression, and maximum likelihood techniques [306,307]. By analyzing these quantitative trait loci (QTL) for coincidence among traits, it is now possible to test whether the characteristics are causally related.

The molecular maps produced by restriction fragment length polymorphisms (RFLP) and random amplified polymorphic DNA (RAPD) have excellent potential for use as tools for gene mapping of root and shoot traits associated with plant adaptation to a low P supply in soil. QTL analysis has been used to identify the number of loci in a maize population segregating for tolerance to low-P stress, their approximate location, and the magnitude of their effect [308]. Six RFLP marker loci were found to be significantly associated with performance under low-P stress. One marker locus accounted for 25% of the total phenotypic variation. Additive gene action was predominant for all of the QTL identified.

Further progress in the use of molecular and cellular biological techniques is dependent on finding common ground among molecular/cell biologists, breeders, physiologists, and agronomists to test genes for low-P adaptation and to identify genes that will be useful for yield improvement under low P availability in soil.

SUMMARY AND CONCLUSIONS

The supply of P to plant roots depends on soil properties such as P content, chemical form of P compounds, and the mobility of this P in soil. These parameters constitute the P availability of a soil. The amount of P that a plant can acquire from this available P depends on its root length and

on several other morphological and physiological properties of the root, including association with AM fungi. Furthermore, availability and acquisition of P are markedly affected by root-induced changes in the rhizosphere such as P mobilization by root exudates. Different crops and forages differ in their ability to extract P from soil, presumably owing to differences in rooting characteristics and root-AM symbiosis and to differences in their ability to influence and modify the rhizosphere soil.

When the P supply limits plant growth, higher plants undergo changes in a number of shoot and root attributes. Among them are a marked reduction in leaf area production, an increase in P uptake capacity per unit root length, an increase in root to shoot ratio, changes in the morphology of the root system, and an increase in the proportion of total P partitioned to roots. Several studies indicate that P-efficient species have a high ability to retranslocate P from inactive to active tissues. Several of these plant attributes may be significantly affected by association with AM fungi.

Poor adaptation of plants to a low P supply in soil is mostly the result of the inability of roots to absorb P from soil solution that is low in P supply and then to function metabolically and physiologically because of the low available P concentration in the plant. Plant adaptation to low-P soils can be maximized by manipulating the genetic characteristics related to P acquisition and utilization, by enhancing the symbiosis with mycorrhizal fungi that may increase the soil volume from which P can be acquired, and by increasing the P supply of the soil through application of P-containing fertilizers or adjustment in soil pH.

It must be recognized that strategic P inputs are essential components to increased and sustained agricultural production in any agricultural system and in infertile tropical soils in particular. Resource-poor farmers in the tropics, however, often cannot afford fertilizer inputs. Their best short-term option for increased production is therefore to use germplasm adapted to poor soils. This situation conflicts with the demand for a higher food supply to support increasing populations and the need to protect the resource base against nutrient mining and further degradation. However, more efficient genotypes may also extract the greatest benefit from applied P and thereby provide farmers with a greater incentive to apply P fertilizer. Since P acquisition by even the most efficient genotypes is unlikely to exceed much more than 20% of the total fertilizer P applied to low-P soils, small strategic P applications based on soil P availability and reduced crop P requirements will gradually build up the level of available P in the soil. Consequently, the frequency and amounts of P applications required to sustain production will decrease with time. The challenge, however, will be to provide farmers with the incentive to begin the process of strategic P application. This is more likely to succeed if the fertilizer requirements needed to produce an economic return can be reduced through the use of more efficient crop and forage germplasm.

FUTURE RESEARCH PRIORITIES

Approaches to improve P nutrition of plants involve either manipulation of the plant to improve its ability to acquire and utilize P or manipulation of the plant's environment to improve the physical and chemical availability of P in soil [309]. The former approach involves both the identification of plant attributes and traits which confer greater efficiency in P acquisition and the selection of species and genotypes that have a greater capacity to utilize P for maximizing crop/forage yield within the plant (that is, greater internal P use efficiency). Greater efficiency in P acquisition may also be achieved by manipulating the symbiosis between plants and AM fungi in soil to maximize P uptake. Manipulation of the plant's environment to enable greater acquisition of P may involve removal of physical and chemical limitations to root growth or activity (such as improving soil tilth and penetrability and reducing toxicities such as Al). It may also involve increasing the availability of soil and applied P by reducing the rate of fixation and increasing P cycling in crop-livestock production systems [55]. There exists a great potential for genetic manipulation of plant efficiency in P acquisition and utilization.

In view of the various possible effects of mycorrhizae on plant growth [145,157], a better

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understanding of the host-mycorrhizal interactions is necessary to be able to predict the capacity of external mycelium to acquire P for the host under various conditions. There is also a need to characterize the conditions at the hyphae-soil interface which may influence P availability.

It is essential to identify differences in P acquisition among crop and forage components from different P sources and soil P pools in P-limited tropical soils in order to design crop/pasture production systems that optimize the use of strategic P inputs. Introduction of forage/cover legume components with crop components could stimulate soil P transformations and P cycling that improve the profitability of P applications to P-limited tropical soils. Economically viable and ecologically sound P management in tropical soils can contribute not only to sustained crop/animal production but also to reduced soil degradation.

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5

Mechanisms Involved in Salt Tolerance of Plants

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INTRODUCTION

A large part (about 70%) of the surface of the earth is covered by oceans that comprise a salt solution with an osmotic potential of about −2.0 MP, derived primarily from sodium and chloride—about 0.5 and 0.6 M, respectively [1]. It is further estimated that a third of the world's irrigated land has been salinized to various degrees. This salinization results from an accumulation of salts dissolved in the irrigation water. Many wild as well as cultivated plants have thus to deal with saline environments.

A saline environment imposes two principal kinds of stress on plants: an osmotic stress and a toxicity stress.

OSMOTIC STRESS

The water potential of plant cells generally equilibrates with that of their environment. The water relations of plant cells and their environment are given by Equation (1) [2]:

$$\Psi_w^o = \Psi_w^i = \Psi_\pi^i + \Psi_p \tag{1}$$

where Ψ_w = water potential, Ψ_π = osmotic (or solute) potential, Ψ_p = turgor, o = outside and i = inside. The water potential of the saline environment, Ψ_w^o, is primarily determined by its salt concentration (Ψ_π). Exposure of wall-encased plant cells to the low Ψ_w^o of a saline environment results in equilibration of Ψ_w, by cell-water loss and an accompanying decreases of Ψ_π^i and turgor (Ψ_p), according to Equation 1. In wall-less cells, such as those of some microalgae, turgor is almost nonexistent and $\Psi_w^i = \Psi_\pi^i$. In such cells, the lowering of Ψ_w^o, the consequent water loss, and the decrease of Ψ_w^i, are accompanied by a decrease of Ψ_w^i and of cell volume.

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Turgor is a prerequisite for plant cell expansion and growth. A simplified description of the growth in relation to turgor is given in Equation (2) [3]:

$$G = m\ (\Psi_p - y) \tag{2}$$

where G = growth rate, m = plasticity of cell walls, and y = threshold turgor for cell enlargement. In a saline environment, growth should, hence, cease if turgor is not regulated. Salt-resistant plants are able to regulate their turgor within the range of their salt resistance, or they are able to adjust cell-wall plasticity and threshold values.

Turgor Regulation

Bisson and Gutknecht [4] described the sequence of events occurring in plant cells on external salinization and decrease of Ψ_w^o (Fig. 1): Water exits from the cell, turgor decreases, and water potentials equilibrate. The turgor decrease is sensed by a ''turgor sensor,'' apparently in the plasma membrane. The sensor emits an ''error signal'' that is transduced to the activation of some biochemical processes, such as increased solute accumulation or synthesis. Changes in the physical tension of the cytoskeleton during water stress might be involved in triggering the responses [5]. Enhanced accumulation and synthesis results in an increase of the amount of solutes in the cell, a transient decrease of Ψ_π^i and Ψ_w^i water influx, and eventually recovery of the original (regular) turgor pressure. During the recovery phase, Ψ_w^i and Ψ_π^i do not change, but the amount of solutes in the cell and turgor increase concurrently. In wall-less cells, a similar sequence of events regulates volume instead of turgor.

Some initial error signals resulting from turgor decrease have been investigated. In the salt-resistant Characean *Lamprothamnium* [6], a hypertonic salt shock induced a hyperpolarization of the plasma membrane potential. Concordantly, in red beet tissue slices and some plant roots, a nonplasmolysing hypertonic DASW, (dilute artificial sea water −0.5 MPa) shock induced an enhancement of plasma membrane (PM) adenosine triphosphatase (ATPase) activity; in response to

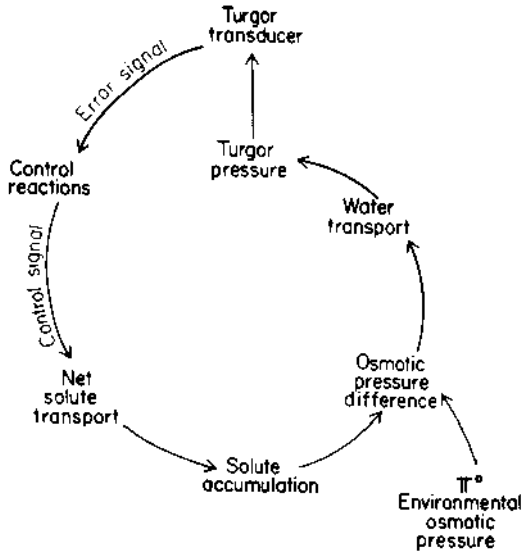

FIGURE 1 Basic elements of turgor regulation system based on solute and water transport. Input of system is random fluctuations in environmental water potential and output is turgor pressure. (From, Ref. 4.)

a similar (−0.5 Mpa) mannitol shock, enhanced K^+ uptake could be measured as well. The DASW shock also induced an increase of the inositol-1,4,5-trisphosphate (1,4,5-IP_3) content in the cells [7] (Table 1), a decrease in PM phosphatidylinositol-4,5-bisphosphate ($PtdInsP_2$) (Table 1), and phosphorylation of some PM membrane proteins [7] (Table 1). The effects of a DASW shock on ATPase activity, 1,4,5-IP_3 and $PtdInsP_2$ were observed 1 min after shock application and before enhancement of protein phosphorylation was evident [8] (Table 1). This sequence of events implied that protein phosphorylation was not a prerequisite for DASW-induced enhancement of ATPase activity. All the cited effects of DASW were inhibited by neomycin, an inhibitor phospholipid interconversion and hydrolysis in animals [9] and plants [10]. These cited DASW effects could be induced by secondary butanol in the absence of a DASW shock (Table 1); the latter compound artificially activates G-proteins [11]. These results indicated that the initial, turgor loss–induced, *error* signal involves G-proteins and the phosphoinositide cascade [12]. Changes in PM phosphoinositide composition may activate the PM ATPase [9,12,13]. Protein phosphorylation may be involved in subsequent activation of processes responsible for long-term turgor regulation, such as synthesis of osmoprotective compounds. For example, osmotic stress increased the phosphorylation of spinach leaf sucrosephosphate synthase, catylized by a Ca^{2+}-dependent protein kinase [14]. In yeast the protein phosphatase calcineurin was essential for salt tolerance. The latter data indicated that NaCl adaptation in yeast depended on signal transduction involving Ca^{2+} and protein phosphorylation/dephosphorylation.

Calcium ions also seem to be involved as a second messenger in transduction of the error signal in the unicellular, wall-less alga *Poterioochromonas*. In response to an osmotic shock, this alga regulates volume first by enhanced K^+ uptake and later by isofloridozide synthesis. The synthesis depends on Ca^{2+}-mediated activation of the enzyme isofloridozidephosphate synthase [15]. Volume regulation was not hinged on the presence of external Ca^{2+}. The Ca^{2+} needed for activation of isofloridozide synthesis should, hence, have originated from an internal compartment, apparently the vacuole. Calcium release from the vacuoles of plant cells is induced by elevation of cytosolic 1,4,5 -IP_3 [16]. Increased cytosolic Ca^{2+} concentration seems to induce the release of a membrane-bound protease in *Poterioochromonas* cells. The protease, in turn, activates isofloridosidephosphate synthase [15].

Joset et al. [17] distinguish between immediate responses to salt stress, such as those cited above and long-term adaptations that are protein synthesis dependent. The latter kind of adaptations reported for higher plants include synthesis of neutral organic compounds; induction of salt stress–associated proteins, such as osmotin [18] and gluthatione peroxidase [19]; and upregulation of PM [20] and tonoplast [21] H^+-ATPases. Some of the stress-inducible genes that encode proteins, such as Δ^1-pyrroline 5-carboxylate synthetase, a key enzyme for proline biosynthesis, were overexpressed in transgenic plants to produce a salt-tolerant phenotype of the plants [22]; the latter results indicated that the gene products really function in stress tolerance.

Genes induced during water- and salt-stress conditions are thought to function not only in protecting cells by the production of important metabolic proteins but also in the regulation of genes for factors involved in the signal transduction cascades of the stress response [23]. The latter include such factors as protein kinases and phosphlipase C [5,24].

Solutes Employed for Turgor Regulation in Plants

Various organic solutes, as well as mineral ions, in particular Na^+, K^+, and Cl^-, are accumulated in plants during turgor or volume regulation. Some halophytes, the native flora of saline environments [25], adjust their solute content mainly with inorganic ions. *Suaeda maritima* plants grown in 370 mM NaCl (−1.76 MPa) maintained the Ψ_π of their leaves at −2.5 MPa and NaCl accounted for 93% of the accumulated salt [26]. In other plants, such as the marine alga (*Porphyra purpurea* L.) [27], sodium is excluded or excreted, and KCl is the major solute accumulated for turgor regulation. Potassium chloride also comprises most of the solute accumulated in the extremely halophylic bacteria *Halobacterium halobium* grown in 3 M NaCl, whereas Na^+ is excreted and maintained at a low

Table 1 Initial Responses in Plasma Membranes of Aged Red Beet Slices to a Dilute Artificial Sea Water (−0.52 MPa) Shock

DASW shock	ATPase: μmol (h mg protein)$^{-1}$	1,4,5-IP_3 pmol (g FW)$^{-1}$	$PtdInsP_2$: % of ^{32}P-labeled phosphoinositides	20-kDa poly-peptide phosphorylation
None	98 ± 6.7[a]	9.1 ± 0.9[a]	1.36 ± 0.16[a]	100 ± 4.2[a]
1 min	149 ± 3.0[c]	18.9 ± 1.6[c]	0.62 ± 0.07[c]	75 ± 4.2[a]
2 min	155 ± 3.3[c]	19.2 ± 1.8[c]	0.74 ± 0.06[c]	325 ± 29[c]
None, neomycin	110 ± 7.1[a]	5.2 ± 0.4[a]	1.32 ± 0.1[a]	
2 min + neomycin	102 ± 6.8[a]	7.8 ± 0.6[a]	0.99 ± 0.04[a]	112 ± 5.2[a]
None, 0.8% secondary-butanol	180 ± 7.2[c]	17 ± 1.0[c]	0.58 ± 13[c]	

PM ATPase activity; 1,4,5-IP_3 (inositol-1,4,5-trisphosphate) content; PM $PtdInsP_2$ (phosphatidylinositol Bisphosphate) content; and phosphorylation of 20-kDa PM polypeptide. Relative Density of SDS-PAGE autoradiographs. Mean ± SE. Different superscripts in each column indicate significant differences at $P < .01$.

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internal concentration [28]. In other plants, a larger part of the solutes comprise organic compounds. Thus, in mature leaves of *Thinopyrum bessarabicum*, a salt-tolerant perennial grass [29], K^+ and Na^+ salts accounted for only 50–60% of the sap Ψ_π in both control and salt-treated plants. In control plants, the K^+/Na^+ ratio was 60, and it changed to 1.0 in plants treated with 0.37 mM NaCl in the medium. A survey of salt marsh plants [30] showed low K^+/Na^+ ratios in dicotyledonous halophytes and high ratios for monocotyledons. The range of K^+/Na^+ ratios for dicotyledons was 0.06–1.19 with a mean of 0.38 ± 0.3, and for monocotyledons, it was 0.27–14.2 with a mean of 2.4 ± 0.6.

Neutral organic solutes make major contributions to turgor regulation in unicellular, slightly vacuolated algae [31]. A large part of the biomass of plants would have to be diverted to turgor regulation if organic solutes were the main compound employed for this in highly vacuolated plant cells. Greenway [32] calculated that for adaptation to 100 mM external NaCl with hexoses, 20–30% of the total biomass would be needed. Raven [33] analyzed the cost benefit of turgor regulation with different solutes. These calculations show that 2–4 mol photons of light energy are needed for the accumulation of 1 osmol KCl or NaCl, whereas 68–78 mol photons are needed for the synthesis of 1 osmol sorbitol or mannitol, 70–93 mol photons for 1 osmol proline, and 78–101 mol photons for 1 osmol glycinebetain. The exact amount of mol photons needed in each case depends on whether the solutes are accumulated in the roots or shoots, and for proline and glycinebetain, also on the N source—NH_4^+ or NO_3^-.

Energy inexpensive turgor regulation with mineral ions, seems to be limited by the inhibitory effects of high salt concentrations on various metabolic processes in the cytoplasm. Hence, adjustment to low Ψ_w^o with mineral salts is limited in the cytoplasm and largely confined to the vacuoles. Slightly vacuolated organisms, such as *Chlorella, Ochromonas*, and *Dunaliella*, have to use compatible organic compounds for a large part of the adjustment. The same seems to be true for the cytoplasmic compartment of vacuolated cells.

Cytoplasmic Compartmentation of Organic Solutes

Various lines of evidence indicate that, in response to salt stress, organic solute accumulation in vacuolated plant cells is primarily restricted to the cytoplasmic compartment (cytosol and cytoplasmic organells). As the cytoplasm constitutes only 5–10% of the osmotic volume [34] of vacuolated cells [35], relatively small amounts of solute can account for the adjustment therein to high external salt concentrations.

Cytoplasmic confinement of digeneaside (2-D-glyceric acid α-D-mannopyranoside) accumulated under saline conditions is indicated for the marine red alga *Griffithia monilis* L. [36]. Digeneaside concentration decreased in the cells of this alga with cell size and concomittant vacuolization. The digeneaside/chlorophyll *a* ratio of the cells however did not change (Table 2). These relations indicated that digeneaside accumulation was restricted to the cytoplasm that also contains the chlorophyll. Confinement of organic solutes to the cytoplasm was also shown for *Mesembryanthemum crystallinum*. Exposure of this plant to 0.4 M NaCl was accompanied by pinitol (1-D-3-O-methyl-

TABLE 2 Variation of Digeneaside Concentration with Size of *Griffithia monilis* Cells[a]

Cell size	Digeneaside (μmol g^{-1} FW)	Chlorophyll a (mg g^{-1} FW)	Digeneaside (μmol g^{-1}/Chlorophylla *a*)
Large	1.97	0.097	20.3
Small	5.87	0.280	20.9

[a] Large cells were those with about 50% >2 mm; small cells were those with few >2 mm.
Source: From Ref. 36.

chiro-inositol) accumulation in the leaves to 10–14 mmol (kg frwt)$^{-1}$ [37]. Leaf-cell protoplasts, chloroplasts, and vacuoles were separated and analyzed. Calculations indicated a pinitol concentration of 230 mM in the chloroplasts and of 100 mM in the cytosol; none was detected in the vacuoles.

Transmission electron microscopy and x-ray microanalysis were employed by Hall et al. [38] to localize glycinebetaine in shoot cells of *Suaeda maritima*. Glycinebetaine was shown to be accumulated under saline conditions and to be restricted to the cytoplasm (Fig. 2).

Adjustment of Cell Wall Characteristics

Equation (2) [$G = m(\Psi_p - y)$] shows that the growth rate (G) of plant cells depends on cell wall plasticity (m) and on the turgor above a threshold value (y). Hence, in order to maintain growth under saline conditions, plants may either increase the amounts of solutes in the cells and regulate turgor or adjust plasticity and/or threshold turgor. Adjustment of threshold turgor can indeed be considered as regulation of the effective turgor ($\Psi_p - y$). Plasticity and threshold turgor are both cell wall characteristics.

Munns et al. [39] found only partial turgor regulation in the unicellular microalga *Chlorella emersonii* L. when exposed to low external Ψ_w. However, growth decreased much less than turgor (Table 3). They found a large decrease in the volumetric elastic modulus ε of the cells. This modulus is the relation between turgor change ($\Delta\Psi_p$) and relative volume change (ΔV) during variations in water content of plant cells ($\varepsilon = \Delta\Psi_p \times V_{\text{initial}}/\Delta V$). The decrease of ε was not related to a decrease in wall thickness; the latter, indeed, increased with water stress. The investigators concluded that the decrease of ε indicates a change of cell wall properties that also effects plasticity and threshold turgor. The latter changes would explain the relatively small effect of turgor decrease on the growth rate of *Chlorella emersoni*.

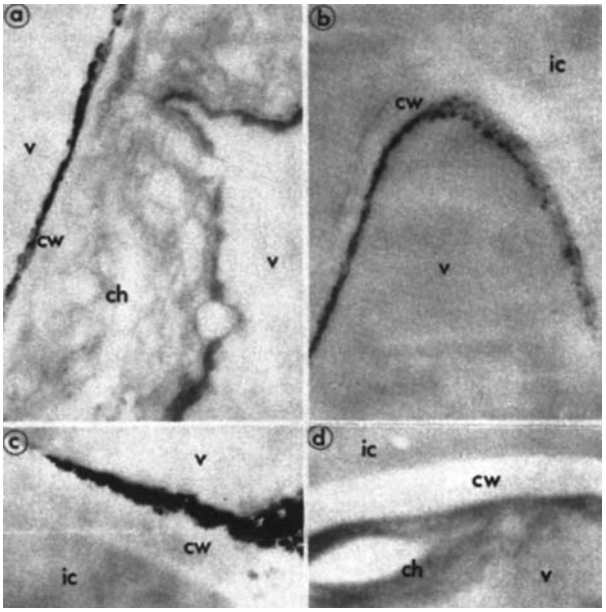

FIGURE 2 Electron micrograph of *Suaeda maritima* cells freeze-substituted in the presence of iodoplatimate stain **a, b**. Grown in the presence of 1 % NaCl showing dense betaine deposits in the cytoplasm and no staining in the vacuole **a,** ×25,500, **b,** ×38,000. **c**. Grown in the presence of 3% NaCl showing dense cytoplasmic deposits ×30,000. **d**. Grown on tap water showing no staining ×25,500. (From, Ref. 38.)

TABLE 3 Relative Growth Rate (RGR), Turgor, and Volumetric Elastic Modulus (VEM) of *Chlorella emersonii* Grown for 6–10 Days at Various NaCl Concentrations

Growth medium		RGR (% of rate	Turgor	VEM
NaCl (mM)	Ψ_π (MPa)	at 0.08 MPa	(MPa ± SE[a])	MPa ± SE
1	0.08	100	0.54 ± 0.18	8.5 ± 1.7
200	1.02	90	0.16 ± 0.009	1.4 ± 0.7
300	1.64	55–70	0.012 ± 0.023	0.9 ± 0.6

[a] Standard error of the mean.
Source: From Ref. 23.

SALT TOXICITY

Sodium chloride is the most important constituent of saline environments. The accumulation of NaCl by plant cells for turgor regulation is limited by the toxicity of a high salt concentration. Such cytoplasmic Na^+ toxicity is ubiquitous in all eucaryotes and bacteria. Even the ancient halophilic *Halobacteria* [40] accumulate K^+ and Cl^- to concentrations of several mols L^{-1}, but not Na^+. The accumulated K^+ and Cl^- ions are located in the cytoplasm of these bacteria and the enzymes are adapted to the high KCl concentration. Enzymes extracted from salt-adapted halophytes are NaCl sensitive. These enzymes are severely inhibited in vitro at salt concentrations similar to those that are optimal in the medium for growth of these plants [41,42]. The in vitro salt sensitivity of amino acid incorporation into proteins by microsomes from salt-adapted halophytes (Fig. 3) did indeed not differ from that of microsomes obtained from glycophytes [43].

The specific harmful effect of NaCl, in addition to its osmotic effect, was elegantly demonstrated by Cramer et al. [44]. They monitored the growth of maize roots in the presence of mannitol

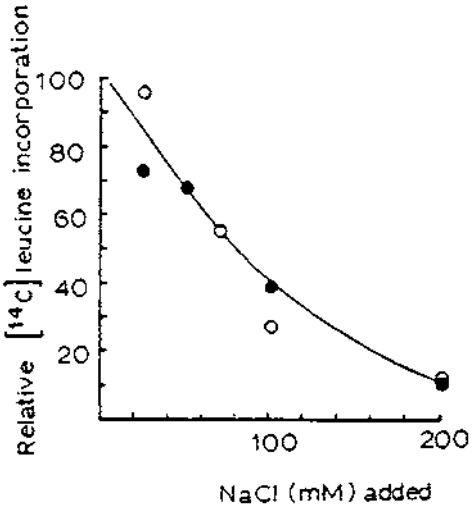

FIGURE 3 The effect of NaCl on the incorporation of leucine into protein by microsomal fractions prepared from *Suaeda* grown in the presence (open circles) and absence (closed circles) of salt. (From, Ref. 43.)

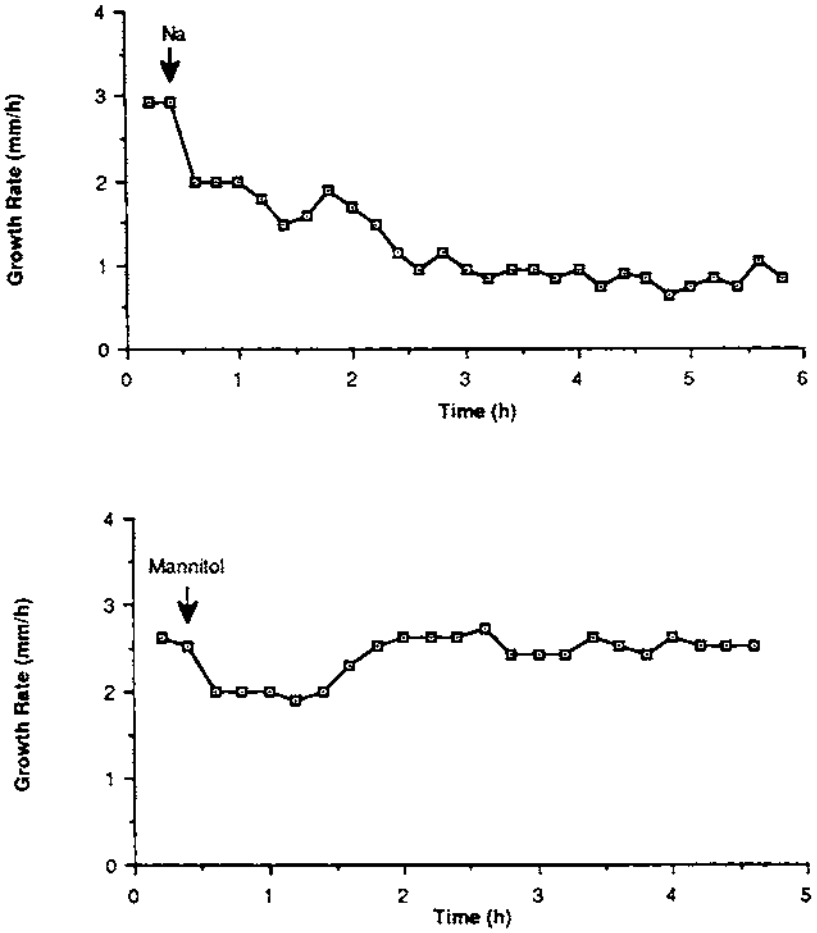

FIGURE 4 The effects of NaCl (above) and mannitol (below) on root elongation over time. At the time indicated by the arrow, 75 mM NaCl or 138 mM (isotonic) mannitol were added. (From, Ref. 44.)

and isotonic NaCl (Fig. 4). In mannitol, an initial decrease of growth rate occurred followed by gradual recovery. In NaCl, the growth rate declined to 20% of that before salt addition and did not recover.

Plant Strategies for Sodium Avoidance

Plants have apparently evolved two principal strategies for avoiding high sodium concentrations in the cytoplasm: compartmentation and exclusion.

Sodium Compartmentation and Compatible Solutes

Many halophytes regulate turgor by NaCl accumulation to a concentration higher than that in the saline medium. Numerous essential enzymes are severely inhibited in vitro at such Na^+ concentrations. Flowers et al. [45] compiled a list of enzymes that are 50% inhibited when exposed in vitro

to the salt concentration found in their source tissue. Wyn Jones et al. [46] suggested compartmentation of salts in plant cells. Thus, in plants, such as the halophilic grass *Distichis spicata* L. [47] that accumulate large amounts of sodium salts in their cells, these salts seem to be occluded in the vacuole, where they serve for turgor regulation. Organic solutes that are compatible with enzyme function apparently have a large share in turgor regulation in the cytoplasmic compartment of the plant cells.

Compatible osmolytes found in higher plants comprise a relatively small number of low molecular weight organic compounds, mainly proline [47–55], glycinebetaine [29,38,51,55–57], some sugars [29,58–60], polyols [37,60], and malate [60]. A larger variety of such compounds is found in lower plants [31,47]. Compatible solutes are supposed to provide an environment that is compatible with macromolecular structure and function [61]. It was proposed that these solutes are preferentially excluded from the surface of proteins and their immediate hydration sphere. Thus, the addition of these solutes to a protein suspension creates a thermodynamically unfavorable situation, since the chemical potentials of both the protein and the additive are increased. This situation stabilizes the native conformation of the proteins, because denaturation would lead to a greater contact surface between the protein and the solvent, thus augmenting the unfavorable effect [62]. Steward and Lee [50] demonstrated the compatibility of proline with glutamate dehydrogenase extracted from the halophyte *Triglochin maritima*. The enzyme was not inhibited in vitro by proline up to a concentration of 0.6 M. Similar results were obtained for barley leaf malate dehydrogenase and barley-embryo pyruvate kinase [63]. These enzymes were not inhibited in vitro by up to 0.5 M glycinebetaine. In addition, glycinebetaine, and to a lesser extent dimethylglycine, partially restored malate dehydrogenase activity in the presence of NaCl. The enzyme was 70% inhibited in the presence of 0.3 M NaCl alone. The inhibition decreased linearly with addition of glycinebetaine to 50% at 0.5 M glycinebetaine (Fig. 5).

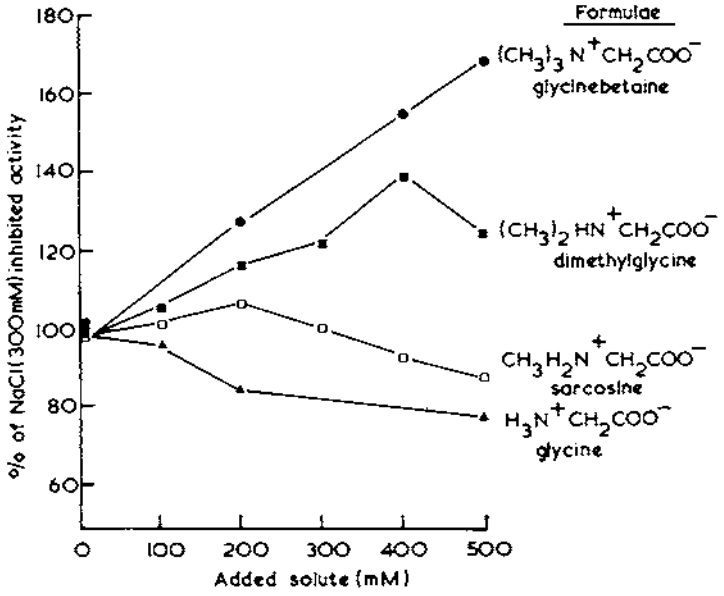

FIGURE 5 Comparative effects of successively methylated derivatives of glycine on inhibition of barley leaf malate dehydrogenase (decarboxylating) by 0.3 M NaCl. Activity was 70% inhibited by 0.3 M NaCl alone. (From, Ref. 63.)

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Mechanisms of Sodium Compartmentation

Various lines of evidence show that Na^+ is occluded in the cell vacuoles of many plants, particularly in halophytes, and is excluded from the cytoplasm of all plants. Indirect evidence for such compartmentation comes from measurements of longitudinal profiles of Na^+ and K^+ concentrations in roots. In such experiments with *Hordeum distichum* grown in the presence of 1 M NaCl [64], Na^+ concentration in meristemic, nonvacuolated, cells at the root tip was 10 mM. Sodium concentration increased rapidly with distance from the root tip and with cell vacuolization to 65 mM at 2 mm from the tip. Potassium concentration changed in the opposite direction; that is, it decreased with distance from the root tip. Comparable results were obtained for *Atriplex hortensis* and *Plantago maritima* roots [65].

More direct evidence for compartmentation was obtained with electron probe x-ray microanalysis. Harvey et al. [66] examined compartmentation of the major mineral ions in leaf cells of *Suaeda maritima* grown in the presence of 350 mM NaCl (Table 4). They found a large accumulation of Na^+ and Cl^- in the vacuoles and relatively low concentrations in the cytoplasm; the K^+ concentration was similar in both compartments. The data for glycinebetaine presented in Table 4 were taken by the authors from their earlier work, where the concentration [67] and cytoplasmic localization of this solute [38] were established. Glycinebetaine accounted for more than 75% of the osmolality of the cytoplasm. Hijibagheri and Flowers [68] found similar Na^+ compartmentation in the roots of *S. maritima* 118 mM in the cytoplasm and 432 mM in the vacuoles.

Mechanisms of Na^+ Transport

Sodium transport from the environment into the cytoplasm of plant cells is a passive process. It depends on the electrochemical-potential gradient of Na^+ and the presence of Na-permeable channels in the plasma membrane. In principle, Na^+ could accumulate in the cytoplasm to a few hundred times of its concentration in the environment. For steady-state conditions and 30°C, the relation is $E_M/60 = \log [Na^+]^\circ/[Na^+]^i$, where E_M = membrane potential [69]. Thus, at an E_M of −120 mV (cytoplasm negative), Na^+ could accumulate in the cytoplasm to 100 times the external concentration. Such accumulation is prevented in salt-tolerant plants by control of influx (channel gating) and/or by active export from the cytoplasm to the vacuoles and also back to the environment.

Active sodium transport in plant cells is performed by Na^+/H^+ antiport [70] that is ordinarily driven by an ATPase-activity derived protonmotive force [71]. Such antiport has been documented at plasma membranes and tonoplasts of some plants [72]. In yeast, gene amplification at a locus encoding a putative Na^+/H^+ antiporter conferred Na^+ tolerance [73].

A survey of 16 crop plants [72], however, showed that the presence of a Na^+/H^+ antiporter is not ubiquitous in plants. It could not be demonstrated in 10 of the 16 surveyed plants, including *Zea mays*, *Phaseolus vulgaris*, and *Gossipium hirsutum*. In *Chara longifolia*, a salt-tolerant charophyte, Na^+/H^+ antiport at the PM was induced by 24 h preculture in artificial sea water [74].

The presence of a Na^+/H^+ antiporter would be expected in the tonoplasts of plant cells that

Table 4 Compartmentation of Na^+, K^+, Cl^-, and Glycinebetaine in *Suaeda maritima* Leaf Cells

	Concentration (mM)	
Solute	cytoplasm	vacuole
Na^+	109	565
K^+	16	24
Cl^-	830	388
Glycinebetain	830	—

Source: From Refs. 38,66,67.

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tolerate Na^+ by its excretion to and occlusion in the vacuoles. Plants that have not conserved this antiporter during their phylogenesis should have to regulate cytoplasmic Na^+ concentration by Na^+ exclusion.

Ion Channels and Sodium Exclusion

The sodium permeability of biological membranes is 10^2–10^6 times higher than that of artificial phospholipid bilayers [75]. This permeability is facilitated by intrinsic proteins that constitute ion channels in the phospholipid bilayer [76]. Sodium-specific channels have hitherto not been demonstrated in the plasma membranes of plant cells. Sodium apparently moves through a general cation channel with different permeabilities for the various ions [77]. Calculations for cells of the Characean alga (*Nitella obtusa* L.) [78] indicated that the measured permeability and density of such channels could quantitatively account for Na^+ influx in salt-stressed cells. Regulation of gating and selectivity of such channels seem to be responsible for sodium exclusion in many salt tolerant crop plants. The presence of K^+ and in particular Ca^{2+} ions has been shown to decrease Na^+ influx to plant cells (Fig. 6) [79–85], and consequently to decrease Na^+ damage [80] and yield reduction [83,84].

The existence of two kinds of channels that allow Na^+ permeation has been reported for the plasma membrane of plant cells. One is an inward rectified channel (closes on membrane depolarization) with P_K/P_{Na} (K^+/Na^+ permeability ratio) of 5–10 [86] and an outward rectified one (opens on depolarization) with P_K/P_{Na} of 20–60 [74]. The latter channel may serve as a possible route for Na^+ entry and K^+ loss under high salt conditions [87]. Schachtman et al. [77] suggested that depolarization opens the outward rectified channel allowing Na^+ influx and K^+ efflux under saline conditions and increasing conductivity. Indeed, Katsuhara and Tazawa [82] showed that 0.1 M NaCl depolarized the plasma membrane of *N. obtusa*, increased its electrical conductivity (EC), increased Na^+ content of the cells, and decreased their K^+ content.

Regulation of the inward rectified cation chanel seems to be involved in salt adaptation [87]. Adaptation of tobacco cells to 50 or 100 mM NaCl resulted in an about twofold reduction of the PM outward rectifying cation-channel permeability. Such reduction in the permeability to K^+ and Na^+ of the PM cation channels, caused by adaptation to salt stress, would decrease the entry of

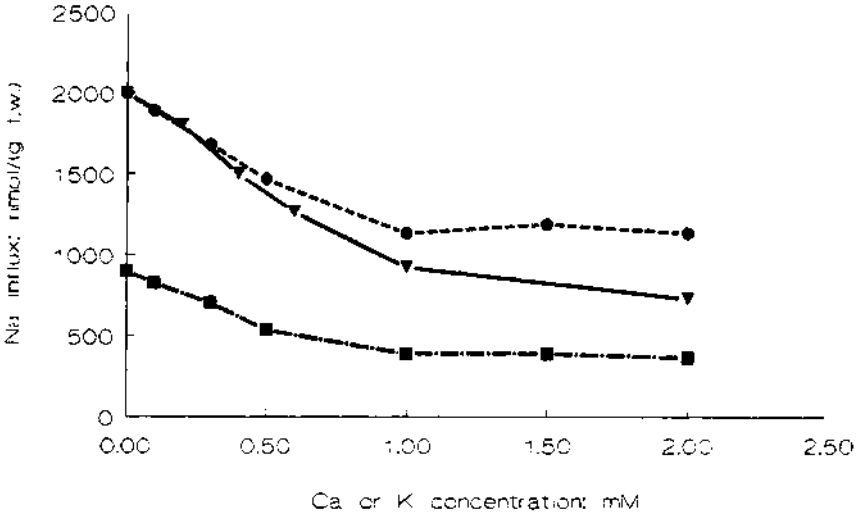

FIGURE 6 Effects of Ca^{2+} (triangles), K^+ (circles), and K^+ in the presence of 10 mM $CaSO_4$ (squares) on Na^+ influx from 10 mM NaCl for 30 min into corn root segments. (Compiled from Ref. 81.)

Na^+ ions into cells and the leakage of K^+ ions out of cells under high salt conditions. The latter study revealed no significant difference between NaCl–adapted and NaCl–unadapted cells in the K^+/Na^+ permeability ratio (P_K/P_{Na}). Similar results were reported for the P_K/P_{Na} of the outward rectfying PM channels of root cells from a NaCl-tolerant and a NaCl-sensitve species of wheat [80]. The investigators concluded that salt-induced reduction of conductivity should be ascribed to a reduction in the frequency of channel opening and/or in the number of channels. A different situation was reported for yeast (see Ref. 88 and references therein). Yeast cells absorb Na^+ by the K^+ uptake system, and the ratio between K^+ and Na^+ K_M values (affinities; low K_M = high affinity) varies depending on the growth conditions. When this system was in the low-affinity state, the ratio between K_M values for Na^+ and K^+ was approximately 15; in the high-affinity state, this ratio increased to 300. Under Na^+ stress, the uptake system converted to the high-affinity system, thus increasing the discrimination between K^+ and Na^+. *TRK1* is *a gene* required for the expression of the high-K– affinity mode of transport. The salt tolerance of a yeast strain carrying a disruption in *TRK1* was 125 mM NaCl, whereas that of the wild type was 400 mM.

Membrane potential–dependent Na^+ influx to corn root was abolished in the presence of K^+ [81] and Ca^{2+} [82]. These cations thus seem to prevent Na^+ movement across the inward rectified channel.

Katsuhara and Tazawa [82] investigated the effect of Ca^{2+} on the salt tolerance of *N. obtusa*. They showed that Ca^{2+} inhibits the Na^+-induced depolarization of the plasma membrane, its increase in electrical conductivity, the increase of Na^+ content of the cells, and the decrease of their K^+ content. Investigations by Hoffmann et al. [89] with *Chara* showed that addition of Ca^{2+} drastically decreased P_{Na} and, hence, Na^+ fluxes at all concentrations.

The sites of Na^+ action and its prevention by Ca^{2+} as well as the sequence of these events are still not clear. Cramer et al. [90] speculated that displacement of Ca^{2+} by Na^+ from the surface of the plasma membrane may be the primary event, and that this is prevented by increased external Ca^{2+} concentration. The investigations further suggested that the opening of K^+ channels and K^+ leakage may either be a direct result of Ca^{2+} displacement from membrane surfaces or from membrane depolarization and a rise of intracellular Ca^{2+}. Either way, potassium leakage should probably be preceded by a change in the direction of the electrochemical K^+ gradient. Such a change would be induced by membrane depolarization, and it should also open the outward rectified K^+ channel.

Evidence for a possible intracellular action of Ca^{2+} is provided by Lynch et al. [91] for maize root protoplasts showing an increase of cytosolic Ca^{2+} concentration in the presence of external 120—150 mM NaCl. The investigations proposed that this Ca^{2+} originated from an internal compartment. However, the possibility that Ca^{2+} may have permeated from the outside, where the Ca^{2+} concentration was 0.1 mM, can not be excluded. Membrane depolarization has been shown to increase Ca^{2+} influx [92], apparently due to Ca^{2+} channel opening [93].

Sodium-induced membrane depolarization may indeed, be activated by Ca^{2+} displacement from membrane surfaces [90,94], or alternatively by Na^+ influx and increased cytoplasmic Na^+ concentration. In *N. obtusa* cells, the protective effect of externally supplied Ca^{2+} depended on the concurrent intracellular presence of ATP or ADP [95]. The presence of the adenine nucleotides decreased the opening frequency of a Na^+-permeable channel [78]. The data for *N. obtusa* [95] further indicate that Ca^{2+} does partially prevent Na^+-induced membrane depolarization (Fig. 7). In the absence of Ca^{2+}, externally supplied Na^+induced a complete depolarization of the plasma membrane. In the presence of Ca^{2+}, only partial and transient depolarization was induced by Na^+; E_M then recovered and receded to -116 mV instead of -131 mV in the absence of Na^+. A transient depolarization induced by Na^+ influx could cause Ca^{2+} channel opening and Ca^{2+} influx. Elevated cytosolic Ca^{2+} concentration may then regulate Na^+ permeability in concert with adenine nucleotides and prevent further Na^+-dependent malfunction of the cells.

Effect of Salinity on Potassium Content

The deleterious effects of salt, reported for *N. obtusa*, included excess Na^+ accumulation as well as K^+ leakage [82]. Both are prevented by Ca^{2+}. Thus, the presence of Ca^{2+} seems to increase K^+/

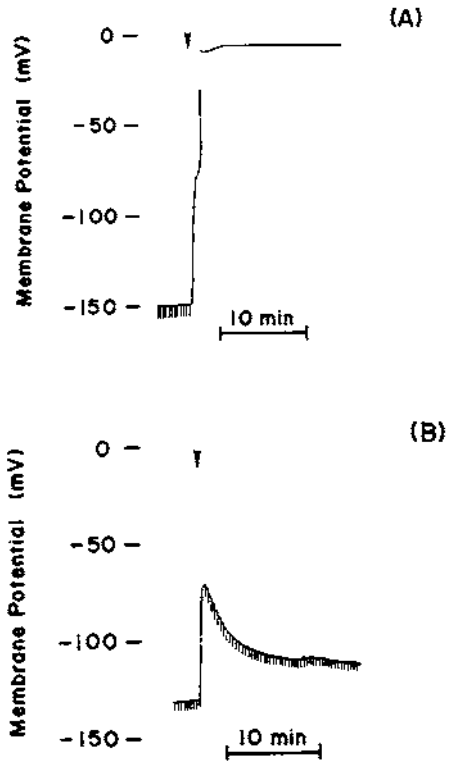

FIGURE 7 Changes in membrane potential of tonoplast free *Nitellopsis* cells perfused with a medium containing 1 mM ATP. Cells were first incubated in APW (artificial pond water) and then treated with APW + 0.1 M NaCl (A) or APW + 0.1 M NaCL + 10 mM $CaCl_2$ (B) at the time indicated. (From, Ref. 95.)

Na^+ selectivity [79] and to be necessary for the maintenance of an appropriate K^+ concentration in plant cells. The importance of Ca^{2+} for adequate K^+ absorption and growth under saline conditions was demonstrated in *Citrus* cell cultures grown on a range of NaCl concentrations in the presence of various $CaCl_2$ concentrations [84]. The growth rate of these cell cultures was related to their K^+ content. The capability of plants to maintain an adequate K^+ content under saline conditions is also enhanced by ample K^+ supply. Thus, salt-adapted *Sorghum* plants [96] were able to grow on 0.3 M NaCl in the presence of a full-strength Hoagland solution or half-strength Hoagland solution supplemented with K^+ to its concentration in full-strength Hoagland solution. The plants did not grow in 0.3 M NaCl with unsupplemented half-strength Hoagland solution.

The response of K^+ content in different plants to external Na^+ increments is not uniform, as shown in Table 5. Many plants, in particular relatively salt-tolerant glycophytes such as *Atylosia sericea* and *Glycine max* cv. Lee, maintain K^+ content constant or even increase it in the presence of salt. More sensitive glycophytes fail to maintain K^+ content in the presence of a high salt concentration. Such decrease of K^+ content may indicate damage [97]. This is demonstrated by two *Atylosia* species [98] and two *G. max* cultivars [99] differing in salt tolerance. The tolerant plants, *A. sericea* and *G. max* cv. Lee, are capable of increasing leaf K^+ content in the presence of salt as well as excluding Na^+ more efficiently than the sensitive ones, *A. acutifolia* and *G. max* cv. Jackson (Table 5). On the other hand, halophytes such as *Suaeda maritima* and *Simondsia chinensis*, as well as tolerant glycophytes that accumulate Na^+ such as *Lycopersicum peruvium, Solanum pennellii* [100], and *Sorghum bicolor* (Table 5), decrease their K^+ content with increasing external salt concentration

TABLE 5 Effect of Na^+ Concentration in the Medium on Na^+ and K^+ Concentration in Some Plant Species and Cultivars

Species	Medium Na^+ (mM)	Concentration in plants		Units[a]	Plant organ	Reference
		Na^+	K^+			
Atylosia sericea (tolerant)	0	80	350	1	Leaf	98
	50	133	500			
A. acutifolia (sensitive)	0	40	350	1	Leaf	98
	50	850	115			
Chlorella emersonii	0	9	282	2	Cell	39
	335	21	342			
Glycine max cv. Jackson (sensitive)	0	50	600	1	Leaf	99
	100	650	700			
Glycine max cv. Lee (tolerant)	0	58	588	1	Leaf	99
	100	176	882			
Lycopersium peruvium	0	200	2500	1	Callus	100
	350	1800	2200			
Simondsia sinensis	0	150	550	1	Leaf	55
	600	1300	250			
Solanum pennellii	0	250	2200	1	Callus	100
	35	2700	700			
Sorghum biocolor	0	0.9	349	3	Leaf	50
	184	51	140			
Suaeda maritima	0	50	1600	1	Shoot	26
	340	5000	330			

[a] (1) mmol/kg DW; (2) mM; (3) nmol/kg FW.

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without concomitant damage. This decrease seems to be related to the replacement of vacuolar K^+ with Na^+ [101]. The maintenance of adequate K^+ content under saline conditions seems to depend on selective K^+ uptake as well as selective K^+ and Na^+ compartmentation in the cells and distribution in the shoots.

Sodium Distribution in the Plant

Most plants, when grown in the presence of salt, accumulate some Na^+ in their roots even when it is excluded from the shoots. Collander [102] distinguished between Na^+ accumulator plants and nonaccumulators. The former plants, transport large amounts of Na^+ to their shoots, whereas the latter exclude Na^+ from their shoots and retain it in their roots. Dicotyledonous halophytes are the most prominent Na^+ accumulators, but some salt-resistant glycophytes, such as barley, also belong to that group. Generally, salt-sensitive plants, such as beans and corn, are the most prominent Na^+ excluders. Table 6 compares Na^+ distribution in corn and barley.

Sodium retention in the roots of bean (*Phaseolus vulgaris L.*) plants was shown to result from metabolic energy–dependent depletion of Na^+ in the ascending xylem sap and in roots as well as stems [103,104]. Derooted bean plants retained Na^+ at the base of the stem. Absorption from the xylem was Na^+ specific as compared with K^+ and Cl^-. Sodium depletion of the xylem sap is accomplished by stelar cells lining the xylem [105,106]; transfer cells also have been implicated in this process [107]. Sodium that is removed from the xylem is transferred to the phloem and retransported to the roots [108,109]. Preferential removal of Na^+ from the xylem sap and recirculation to the roots occurs also in petioles [110] and veins of mature leaves [111,112]. In the absence of an inward directed electrochemical Na^+ gradient in the roots, Na^+ leaks to the medium [109,113]; otherwise, it is recirculated.

Sodium recirculation is a mechanism for Na^+ exclusion from the shoots employed by relatively salt-sensitive plants. It breaks down at high salt concentrations [103,104]. Cell membranes of sodium nonaccumulators, such as beans and apparently many other crop plants, seem not to comprise a Na^+/H^+ antiporter at the tonoplast [72] and, hence, cannot excrete Na^+ from the cytoplasm to the vacuoles. Sodium influx to the root and xylem is passive uniport via channels and also possibly by apoplastic bypass flow [114]. The latter flow bypasses the Casparian strips of endodermal cell walls. It is suggested to occur at sites of secondary root emergence [115,116] or through the apical region of the roots [117]. Bypass flow seems to increase under conditions of stress damage. Under saline conditions, bypass flow contributed substantially to the total quantity of Na^+ reaching the xylem of rice plants [114].

The mechanism of selective Na^+ absorption from the xylem is still being explored. It is inhibited by anoxia and depends on energy metabolism [104,118]. It cannot be envisioned as simple

TABLE 6 Distribution of ^{22}Na in Corn and Barley Grown for 25 h in 0.2 m $MCaSO_4$ and 10 mM $^{22}NaCl$

	^{22}Na distribution (% of absorbed)	
Plant part	corn	barley
Roots[a]	98.1	65
Stem base, 0–30 cm	0.8	10
Stem base, 30–70 cm	0.6	5
Rest of stem and leaves	0.5	20
Total export from roots	1.9	35

[a] Washed in 10 mM $CaSO_4$.

Na^+/H^+ antiport, because stelar cell plasma membrane ATPases secrete protons into the xylem [119], and the proton gradient is in the wrong direction—as evidenced by the relative acidity of the xylem sap. Lacan and Duran [120] suggested that the absorption of Na^+ from the ascending sap is primarily accomplished by indirect K^+-Na^+ exchange; namely, reverse H^+/Na^+ antiport (against the proton gradient) linked to K^+/H^+ antiport (with the proton gradient) and anion-proton symport to the symplast of cells bordering the xylem. They hypothesize that the process is primarily driven by a proton gradient resulting from proton pumping into the xylem by adjacent cells. This proton gradient is then utilized for K^+ transport to the xylem by H^+/K^+ antiport and for H^+-anion symport. The latter proton movements decrease the cytosolic pH of stellar cells lining the xylem and facilitate H^+/Na^+ antiport and Na^+ depletion of the xylem. The assumption of indirect Na^+-K^+ exchange was supported by the absence of a fixed stoichiometry between K^+ and Na^+ transport [121] and by the pH sensitivity of the Na^+/K^+ exchange. Also, increased xylem K^+ concentration resulted in decreased K^+ extrusion but not in decreased Na^+ uptake. The investigators do not provide direct evidence for reversed H^+/Na^+ antiport. Indeed, the proposed indirect Na^+/K^+ exchange could be sustained by K^+/H^+ antiport, as suggested, and electrophoretic Na^+ transport via cation channels (uniport). Such Na^+ transport would depend on the negative E_M of the living cells sorrounding the xylem, and hence on proton pumping as suggested in Lacan and Durand's [120] model. A previous proposal for reversed H^+/Na^+ antiport [122], cited by Lacan and Duran [120], concerns cells acidified by propionic acid.

Sodium recirculation has been found to contribute to salt resistance in many plants such as reed [123], the relatively salt tolerant soybean variety Lee [124], castor bean [125], trifoliate orange [126], *Trifolium alexandrinum* [127], *Atylosia albicans*, and *A. platicarpa* [98].

Chloride Toxicity

Chloride is the prevalent anion accompanying Na^+ and K^+, hence its concentration in vacuoles, as well as cytoplasm, is usually in the same range as the sum of Na^+ and K^+. This concurrence of Na^+ and Cl^- complicates the evaluation of Cl^--specific toxicity. Only a small number of experiments have been published that attempt to determine the direct toxicity of Cl^-, and their interpretation is not straightforward. Leopold and Willing [128] exposed soybean cotyledonary leaf slices to different salts and determined their effect on membrane integrity by measuring the subsequent leakage of organic solutes into water. They found a 28% increase of leakage when 133 mM Na_2SO_4 was replaced with near-isotonic (200 mM) NaCl. These results may be explained as a specific Cl^- toxicity, but Cl^- concentration was higher than that of SO_4^{2-}, and absorption as well as subsequent internal Cl^- concentration may have been much larger than that of SO_4^{2-}. In other experiments by Meiri et al. [129], 96 mM NaCl was less detrimental to the growth of bean plants than 72 mM (isotonic) Na_2SO_4.

Greenway and Munns [130] compared Na^+ and Cl^- contents in the leaves of seven salt-tolerant and salt-sensitive varieties or subspecies. In four of these plants, tolerance was related to lower contents of Na^+ as well as Cl^-. In two cases, there was little difference in concentration of either ion, or there was some increase in the concentration of both ions in the tolerant plants. In one case (avocado), a large decrease of Na^+ concentration was found in the tolerant variety but no difference in Cl^- concentration. In summary, these data do not indicate, that high Cl^- concentration in the leaves may have been related to sensitivity in any of the cases. A similar conclusion may be drawn from the comparison of Na^+ and Cl^- contents in salt-tolerant and salt-sensitive corn varieties [131] and *Atylosia* species [98]. In both cases, Na^+ and Cl^- were excluded from the leaves of the tolerant varieties and species, but exclusion was much more efficient for Na^+ than it was for Cl^-. Furthermore, in some salt-sensitive species, such as *Phaseolus coccineus* [107] and *P. vulgaris* [104,105], Na^+ was found to be excluded from the shoots but not Cl^-.

The growth rate of castor bean at different salinities [125] was not related to Cl^- content of the leaves but rather to Na^+ content. The growth rate was not affected by external NaCl up to 70 mM and decreased by about 80% at concentrations between 80 and 160 mM. Chloride

content of the leaves increased linearly with external NaCl concentration, whereas Na^+ was excluded from the leaves (up to 70 mM NaCl outside), and its leaf content was correlated with growth inhibition.

Although the cited experiments indicate that many salt-tolerant species can deal with higher Cl^- than Na^+ contents in the shoots, a greater Na^+ than Cl^- toxicity in the cytoplasm cannot be deduced. The apparently greater Cl^- than Na^+ tolerance may result from different capabilities for compartmentation of these ions in the vacuoles. All plants seem to be able to accumulate Cl^- in the vacuoles of their cells, whereas many are deficient in the Na^+/H^+ antiporter needed for Na^+ occlusion in the vacuoles [72].

SALT SECRETION

The transpiration stream continuously carries salts to plant shoots. Large amounts of salt should hence be delivered to the leaves of plants growing in a saline environment if the salts are not excluded from the shoots. Even in halophytes that accumulate Na^+ and Cl^- in their leaf cells, the amount of salt carried to the shoot is much in excess of that needed for turgor regulation. Secretion by special salt glands is one important mechanism for the removal of excess mineral ions from the leaves [132].

Structure of Salt Glands

The structural details of various kinds of plant salt glands (Fig. 8) were recently reviewed [133,134]. Based on their structure, three principal types of salt glands may be distinguished: two-celled glands of the grasses, multicellular glands of various dicotyledonous plants, and bladder hairs of the *Chenopodiacea*. The glands eliminate salts to the leaf surface, whereas bladder hairs eliminate them to the central vacuole of the bladder hair.

Some unifying principles in the structure of the different kinds of salt glands may be summarized. They all contain one or more subtending cells that are in apoplastic as well as symplastic continuum with both the adjacent mesophyll and the distal, secreting gland cells. These subtending cells are the basal cells in the two-celled glands, the inermost secretory cells in multicellular glands, and the stalk cells in bladders (see Fig. 8). The exterior walls of secretory cells in all salt glands are covered by a cuticle. The cuticle extends inward along the lateral walls of the external gland cells but not into the walls between the secretory and basal cells. In glands that excrete to the leaf surface, the cuticle is continuous with that of the epidermal cells and partially detached from the exterior walls of the secretory cells. The space formed between the detached cuticle and the walls forms a collecting compartment for the excreted solution. Small pores occur in the detached portion of the cuticle in all glands examined except those of *Aegiceras corniculatum* [135].

Pathway of Salts

As pointed out, structural investigations reveal the existence of an apoplastic continuum from the mesophyll to the subtending gland cells in all three types of glands. The availability of this route to solute transport was shown with the aid of La^{3+} [136,137]. This ion is able to move in the apoplast with the transpiration stream but is unable to penetrate into the symplast. The ion is visible as a precipitate in the electron microscope [138].

The existence of a symplastic continuum between the mesophyll and the gland cells suggests that symplastic flow can also occur. Cytochemical studies utilizing silver precipitates of Cl^- show the presence of Cl^- in plasmodesmata connecting the mesophyll and proximal gland cells of *Limonium* [139] and *Tamarix* [140]. Campbell and Thomson [140] concluded that salt moves to the salt glands apoplastically as well as symplastically, but the predominant route was probably the apoplast.

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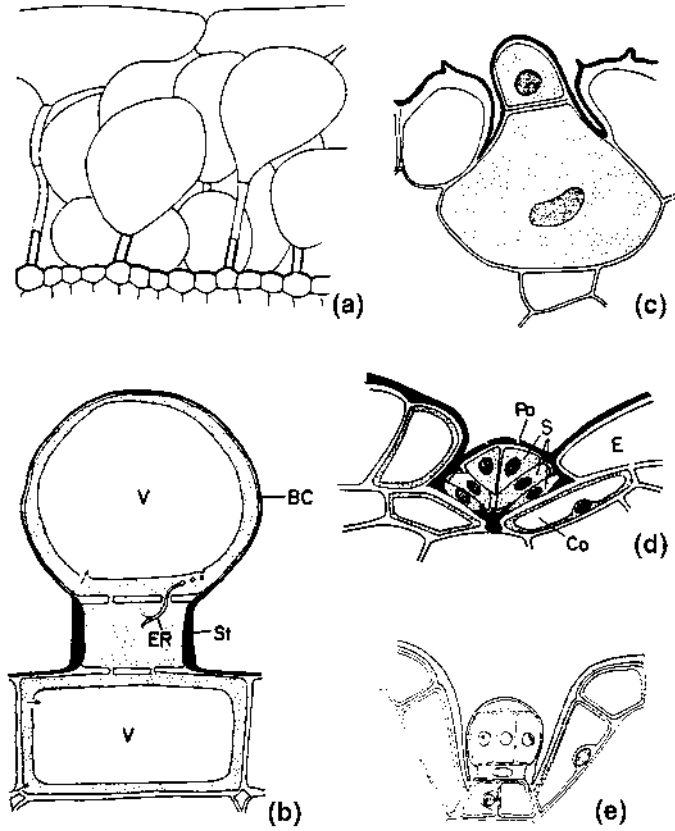

FIGURE 8 Salt glands (a,b) *Atriplex halimus*. L. (a) Epidermis and bladder hairs; the lateral walls of the lowest stalk cell are completely cutinized. (b) Diagram of a bladder hair showing possible routes of chloride transfer to the bladder cell and its vacuole. Arrows indicate active transport through membranes. One vesicle is seen fusing with the bladder tonoplast. BC, bladder cell; ER, endoplasmic reticulum; St, stalk cell; V, vacuole. (c) *Spartina townsendii*. H. and J. Groves. (d) *Tamarix aphylla*. Co, collecting cell; E, epidermal cell; Po, pore in the cuticle; S, secretory cell. (e) *Avicennia marina*. (From Ref. 134.)

Function of Salt Glands

The qualitative composition of salts secreted by glands was usually found to be similar to that of the native environment [141] or the culture solution [142]. However, the proportions and concentrations of the various ions are different. Selectivity, therefore, occurs at some site in the path from the roots to the glands. Different orders of mineral-ion selectivity have been reported for different plants [143–145].

Ionic concentration and Ψ_π of solution secreted by salt glands were found to be higher than those of the root medium or the challenging solution in experiments with excised leaves or leaf tissues [146–149]. Similarly, Mozafar and Goodin [150] found higher NaCl concentrations in the bladder hairs of *Atriplex* than in the medium. The salt concentration of the secreted solution was also found to be higher than that in the xylem sap [141,143]. These concentration gradients indicate the involvement of a metabolic energy–dependent process in secretion. This was explicitly demonstrated by Arisz et al. [146], who measured the effect of light and inhibitors of energy metabolism on

salt secretion by *Limonium* leaf disks. The requirement for a metabolic energy source [147,151,152] and the involvement of the PM H^+-ATPase [153] were confirmed by other investigators.

Thomson et al. [133] proposed two possible mechanisms for secretion by salt glands. One proposal assumes symplastic transport to the secreting cells and metabolic energy–dependent secretion of the respective ions to the collecting chamber or vacuole in bladder hairs. Water movement should follow this salt secretion and expand the collecting chamber. This expansion is supposed to open the cuticular pores and enable outflow of the solution. The second proposal assumes apoplastic flow of solution to the subtending gland cells and metabolic energy–dependent accumulation of the respective ions by the latter cells. The ions are then supposed to move down their electrochemical potential gradient to the secreting cells.

The passive permeation of an accumulated salt solution from secreting cells to the collecting chamber could be regarded as a special case of turgor downregulation as described for the charophyte *Lamprothamnium* [93,154–156]. In this series of publications, turgor downregulation, in response to a hypotonic shock, was shown to be accompanied by depolarization and increased EC (electrical conductivity) of the plasma membrane in the involved cells. The presence of Ca^{2+} in the medium was needed for EC increase but not for depolarization. The proposed sequence of events is water influx from the hypotonic medium and turgor elevation; membrane depolarization; Ca^{2+} influx, apparently consequent to opening of Ca^{2+} channels; increased PM conductivity; and ion efflux accompanied by water. In the special case of salt glands, the initial water influx and turgor elevation would be induced by salt accumulation in the subtending gland cells.

SALT ADAPTATION

Suspension cultures and calli of plant cells have been adapted to NaCl by stepwise transfer to increasing salt concentrations. With this procedure, cell lines evincing enhanced resistance to salt have been isolated from various plants [45,52,157–163]. Dry weight production of some of the adapted cell lines, in the presence of salt, was similar to that of the wild lines in the absence of salt (Fig. 9) [158,161,164,165]. Such adapted cell lines may retain their resistance for many generations even after growth in the absence of salt [157,161,162,164].

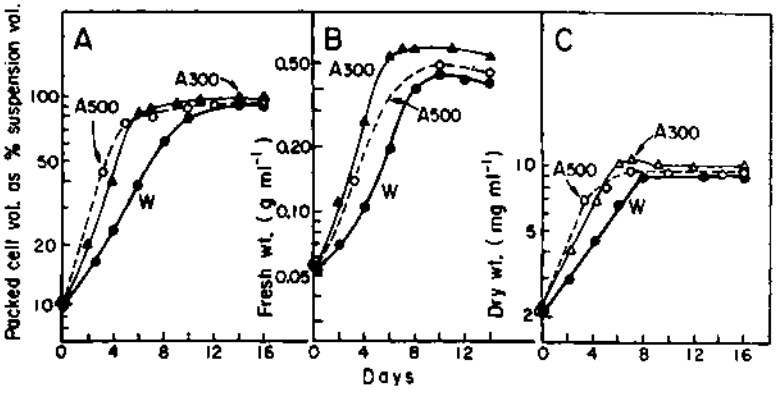

FIGURE 9 Growth curve (dry weight) for various NaCl adapted lines of *Nicotiana* cells growing in media to which they were adapted; also for wild-type cells growing in standard medium and in 0.3 M NaCl (closed circles), wild-type cells in standard medium and (open circles) in 0.3 M NaCl; (closed triangles), cells adapted to 0.3 M NaCl in 0.3 M NaCl; (open triangles) cells adapted to 0.4 M NaCl in 0.4 M NaCl; (open squares) cells adapted to 0.5 M NaCl in 0.5 M NaCL. Growth measured as packed cell volume (A); fresh weight (B); and dry weight (C) (From Ref. 164.)

Increased salt tolerance of salt-adapted cultured cells has rarely led to increased salt tolerance in normal regenerated plants [160,165,166]. Selected cultures, however, are systems where nearly isogenic cells differ, at least in theory, only in the desired tolerance trait [167]. Cell cultures and stress-adapted cell lines from such cultures provide a convenient tool for elucidating salt-resistance mechanisms at the cellular level.

Both of the strategies employed by intact salt-resistant plants can be found in salt-adapted cell lines. Thus, in the presence of salt, tolerant cell lines of *Citrus* [162] and potato [163] more efficiently excluded Na^+ and prevented the decrease of K^+ content than unadapted lines. In cultured *Citrus sinensis* cell lines, the most pronounced characteristic of adapted cells was indeed their capability for larger accumulation of K^+ [168]. A similar trait was reported for NaCl-selected alfalfa cell lines [160]. On the other hand, in tobacco cell lines, salt tolerance was associated with a decrease in K^+ content in concert with increasing salinity [159,169], and an increase of Na^+ [159,169] as well as Cl^- [169], as principle solutes for turgor regulation. Organic compounds also accumulated with salinity, in particular, proline [159,169] and sucrose [169]. Sodium and Cl^- were occluded in the vacuoles of adapted tobacco cells. In cells adapted to 428 mM NaCl, the vacuolar contents of Na^+ and Cl^- were 780 and 624 mM, respectively, whereas cytoplasmic concentrations were maintained at 96 mM [170].

Abscisic acid (ABA) accelerated the adaptation of cultured tobacco cells to high salt concentrations [171]. Abscisic acid, as well as exposure to salt, enhanced the synthesis of a number of proteins [172]. The most striking effect of both treatments on previously unadapted cells was induction of the synthesis of a cross-reactive 26-kDa protein. This protein appeared to be associated with adaptation. When induced by ABA, it was transient unless the cells were simultaneously exposed to salt. Salt-induced changes in the amounts of several proteins were also reported for salt adapted *Citrus* and tomato cell lines [167].

Salt adaptation was also accomplished with whole plants. Eight-day-old *Sorghum* seedlings could be adapted to high salinity by growth in 150 mM NaCl for 20 days [173]. At that time, NaCl could be increased to 300 mM without an effect on the relative growth rate and dry weight produced. The adaptative treatment (150 mM NaCl), however, decreased shoot dry weight production by about 70% as compared with unsalinized control plants. The salt adaptation of *Sorghum* plants was accompanied by an increased capability to exclude Na^+ [173] and an increase in phosphoenolpyruvate carboxylase activity [174]. Treatments with 40 mM ABA increased the growth of salt-treated *Sorghum* seedlings and inhibited the growth of the controls. Abscisic acid also accelerated the adaptation of *Sorghum* plants [174] similar to its effect on the salt adaptation of cultured tobacco cells [171]. The time needed for adaptation of *Sorghum* plants in the presence of 150 mM NaCl was decreased by ABA from 20 to 10 days [174].

CONCLUSIONS

Salt-resistant plants have to maintain growth in the presence of an osmotic stress and, concomitantly, avoid high salt concentration in their cytoplasm. Growth is primarily maintained by an increase of the amount of solutes in the cells and by subsequent turgor regulation. This mechanism may be supplemented by increased cell wall plasticity and decreased threshold turgor. The turgor decrease is sensed by a ''turgor sensor'' apparently in the plasma membrane. The sensor emits an ''error signal'' that is transduced to the activation of adaptive processes.

Salt toxicity is avoided by employing compatible solutes for osmotic cytoplasm adjustment and by confining salt, in particular Na^+, to the vacuoles. Some plants excrete Na^+ from the cytoplasm by active Na^+/H^+ antiport into the vacuole and also to the apoplast. The leaves of such plants may also contain salt glands. These glands accumulate excess salts and subsequently excrete it. This excretion may be explained as a special case of turgor downregulation. Other plants that apparently lack the Na^+/H^+ antiporter accumulate organic solutes and K^+ salts; they prevent Na^+ influx to the roots and its translocation to the more sensitive shoots. The latter is accomplished by selective Na^+ absorption from the ascending xylem sap and its recirculation to the roots via the phloem.

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Sodium ions permeate into plant cells through outward rectified cation channels that apparently open in response to Na^+-induced depolarization. The presence of Ca^{2+} and K^+ enhances Na^+ exclusion by controlling channel selectivity. High potassium concentration in the medium also ensures its adequate supply to the plant in the presence of excess Na^+.

Some plant tissue cultures and intact plants can be adapted to salinity. The same strategies for maintaining growth employed by salt tolerant plants can be induced in response to adaptation.

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6

Plants in Saline Environments

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INTRODUCTION

Saline habitats are those whose soils contain a high percentage of soluble salts, and one or more of these salt components is usually present in excess. There are mainly two types of saline habitats, wet and dry. Wet saline habitats are usually maritime salt marshes. These are areas bordering the sea and subject to periodic inundations as a result of which the level of salinity fluctuates. Dry saline habitats are usually located inland and bordering deserts. Other types of saline habitats are seashore dunes, where salt spray is an important factor, and dry salt lakes. The common denominator for these types of saline habitats is the salinity of the soil and/or of the water resources, as well as the type of vegetation. The most abundant kinds of salinity are NaCl and Na_2SO_4, sometimes together with Mg^{2+} salts [1]. The vegetation of saline habitats is designated ''halophytic,'' distinguished from the vegetation of nonsaline habitats, sometimes referred to as ''glycophytic.'' Phenologically the halophytic plants may be succulent or xeromorphic, with small or grasslike leaves and often also having salt-secreting glands.

Halophytes in their saline environment are exposed not only to salt stress: the roots may also be exposed to osmotic water and low oxygen pressure stress. The latter occurs often in saline-alkaline soils in which aeration is very poor or only periodic during floods at high tide.

Glycophytes, like halophytes, vary in the degree of salt tolerance, so that it is difficult to draw a dividing line between the two groups [2]. Stocker [3] suggested a division by a critical salt concentration, stating that ''a halophyte is a plant that, at any stage of its life, will tolerate this critical salt concentration which will not be tolerated by a 'normal' nonhalophyte.'' Stocker [3] suggested the concentration of 0.5% (≈88 mM) NaCl as this critical value. Flowers et al. [4] mentioned 300 mM NaCl as the critical value. This definition implies that a halophyte will grow in a nonsaline medium as well as a glycophyte, but this is not always the case. Some halophytes do not grow in the absence of NaCl; for example, *Salicornia* spp. germinate but do not elongate in the absence of NaCl. However, with seawater irrigation, it has been possible to obtain a reasonable yield of *Salicornia* for use as fodder crop.

Individuals of the genus *Salicornia* show phenotypic variability in response to different exter-

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nal conditions, but practically no genetic variability could be shown (assayed as electrophoretic mobility of enzymes). Among all plants, collected at different sites and examined, only two types could be distinguished: one typical of the upper marsh and the other typical of the lower marsh. In their distribution within an ecological gradient, each of the *Salicornia* types inhabits its specific ecological niche where salinity is presumably the dominant factor [5,6]. Usually, the external salt concentration for maximal relative growth rate is much lower than the maximal salt concentration endured by the halophytic plant [4,7]. For many halophytes, the optimal NaCl concentration for growth depends on other external conditions.

The interest in halophytes has two aspects: a purely scientific and theoretical interest, to understand their behavior and their adaptation to this specific, harsh habitat. A more applied interest [4] concerns the hope of understanding the response and metabolism of halophytes and how they differ from glycophytes. This will help in the selection or development of crops that are more salt tolerant than existing crops. These interests may be achieved by conventional methods or more recently by genetic engineering [8,9]. Although their growth was inhibited by salinity, Galapagos wild tomatoes (*Lycopersicon cheesmani L.*) could grow in full-strength seawater, but the cultivated, salt-tolerant cultivar of *L. esculentum* could hardly survive in 50% seawater. The two types of tomatoes differed markedly in nitrogen metabolism [10]. In studies of the differences between halophytes and nonhalophytes in their response to salinity, the following properties have been emphasized:

1. Ability to accumulate or to exclude ions selectively [11]
2. Control of ion uptake by the root and control of transport to the shoot and leaf [4]
3. Selectivity in xylem release [12]
4. Role of accumulated ions in osmotic adaptation [4,13]
5. Compartmentation of ions at the cellular and at the whole-plant level [4]
6. Accumulation of so-called compatible solutes and their role in salt tolerance [14]

Nevertheless, none of these characteristics could be used as markers for breeding salt-tolerant crops. In fact, none of these factors alone can be a basis even for a definition of a halophyte. It is difficult to define a halophyte by physiological traits, such as ion accumulation or synthesis and accumulation of compatible solutes. The difficulty arises from the fact that expression of such traits changes with the age of the plant, its physiological stage of development, and under changing environmental conditions. Such traits are multigenic in their origin; they are regulated by many genes located in a large number of loci and on different groups of chromosomes [5,6,15,16]. In addition to these genetic difficulties, the data resulting from the preceding research directions are very controversial and the conclusions drawn are in dispute [17].

Because of the high correlation between salt tolerance and vigor in wheat and barley addition lines, it has been suggested that breeding for agricultural traits may be more productive than breeding for physiological traits. However, the vigor genes and the tolerance genes were located on different groups of chromosomes having potentially opposing effects. These experiments were not successful, but the investigators believe that the idea can be developed further and that this approach has potential for success [15].

The large variability in the response of the different halophytes to the type and the level of salinity brought about attempts to classify them into groups based on either of the following: (a) their ability to accumulate or exclude Na^+ and/or K^+ [18,19] or (b) their ability to synthesize and accumulate sucrose and polyols as opposed to preferentially synthesizing methylated onium compounds [20]. It was suggested by Gorham et al. [18] that the monocotyledonous halophytes are mostly those that exclude Na^+ and accumulate organic solutes. Most of the data presented by Briens and Larher [20] conform to this concept, but some of the 16 plant species investigated by them did not conform, suggesting that the response to salinity is more complex. Greenway and Munns [16] considered high ion uptake the principal adaptation of the halophytes. These investigations [16] classified the halophytes further according to their ability to grow rapidly or slowly in salinities of 200–800 mM NaCl. These reporters [16] considered avoidance of high internal salinity as a

Table 1 Distribution (%) of Dry Matter Produced Between Plant Parts of Two Halophytes at Different Substrate Salinities[a]

	Atriplex triangularis 1985 data				Kosteletzkya virginica 1984 data			
	root	stem	leaf	fruit	root	stem	leaf	fruit
Nonsaline substrate (control)	15.7	42.6	16.2	25.5	39.0	31.5	25.4	4.2
NaCl, 15 g kg^{-1} H_2O (≈263 mM)	10.2	42.6	11.1	36.0	47.1	26.5	22.7	3.7
NaCl, 30 g kg^{-1} H_2O (≈526 mM)	14.3	30.3	14.9	40.5	68.9	14.4	16.8	0.0

[a] Plants grown in lysimeters.
Source: Compiled from data in Ref. 24.

more typical characteristic of nonhalophytes. One of the ways for avoidance is elimination of the ions from the xylem sap on the way from root to shoot. It has been suggested that the ions accumulate in the root or in the basal part of the shoot from where they are returned to the root system and excreted back into the medium [21]. Otherwise, salt may accumulate in the lower, older leaves, as in bean plants, leaving the upper younger leaves with low salt content [22]. A similar ion distribution along the plant shoot was also reported for such halophytes as *Kosteletzkya virginica* [23].

Philipp [24], in controlled lysimeter experiments, demonstrated the different life habits of different halophytes. This reporter [24] compared two halophytes, *Atriplex triangularis* (an annual) and *Kosteletzkya virginica* (a perennial) by percentage dry matter production and allocation to different plant parts (Table 1). In parallel, Philipp [24] measured the mineral distribution in the same plant parts. In both plants, grown in nonsaline substrate, most of the minerals were accumulated in root tissue. In *Atriplex*, under saline conditions, the mineral content of the roots markedly decreased (as a percentage of total) approximately by 50%, but in the leaves, it increased significantly. At a substrate salinity of 30 g kg^{-1} water (approximately 526 mM), the leaf mineral content increased by about 300%. In *Kosteletzkya* plants in nonsaline substrate, minerals also accumulated in the roots, but under saline conditions, the situation did not change much and most of the minerals remained in the roots. In general, these two plants show different strategies in response to salinity that are in accord with their mode of life. In *Atriplex*, the new dry matter production was allocated to the fruit; in *Kosteletzkya*, it was allocated to the root (Table 1), which remained in the soil and sprouted again during the next season.

The variability in the responses of plants to salinity, as well as their variability in maximal salinity level a plant can endure, makes it difficult to characterize the specific trait responsible for salt tolerance.

The most evident effect of salinity is disturbances in growth, and growth is affected by phytohormones. Indeed, evidence shows that many environmental factors (i.e., changes in the concentration of nutrients), including stresses, affect the level of endogenous plant hormones (Table 2), and thus the hormonal balance of the plant is disturbed. It therefore seems logical to assume a relationship between the effect of the stress on hormonal balance and the effect of the disturbed hormonal balance on the growth and development of the plant. Recently, a considerable amount of evidence has suggested that phytohormones are the signals sent between root and shoot, triggering responses to external stress [34] (see section on The Root as a Sensing Organ below). The information collected in our laboratory suggests that the endogenous hormonal balance has an important regulating role in the response of plants to salinity, and it may be possible to ameliorate the endogenous balance by application of exogenous hormones. In the following, we discuss plant-environment interactions with an emphasis on the role of phytohormones.

TABLE 2 Effects of Changes in Concentration of the Mineral Nutrients in the Medium on the Level of Endogenous Phytohormones[a]

Mineral nutrient	Concentration change (mol m^{-3})	Concentration change in phytohormone	Plant species	Reference
NO_3^- ↓	14–0	GA ↓, IAA ↓	Tomato	25
P_i ↓	1–0	CK ↓	Tomato	26
NH_4^+ ↑	0–3	GA ↑, IAA ↓	Pine	27
NO_3^- ↑	0–18	CK ↑	Apple	28
NH_4^+ ↑	0–16	CK ↑		
NO_3^- ↓	10–1	CK ↓	Sunflower	29
P_i ↓	1–0.1	CK ↓		
K^+ ↓	6–0.5	CK ↓		
N ↓	8–0.8	CK ↓	Birch,	30
P_i ↓	1–0.1	CK ↓	Sycamore	
K^+ ↑	0–1	GA ↑	Pine	31
Mineral nutrients ↑	30–260	ABA ↑ temp CK ↓ perm	Tomato	32
Mineral nutrients ↓	30–0.6	CK ↓	Plantago	33

GA, gibberlin; IAA, indole acetic acid; CK, cytokinin; ABA, abscisic acid.
[a] Decreased or increased concentration indicated by direction of arrow; temp = temporarily, perm = permanently. Indicated concentration changes are approximate values.

ROOTS IN THE SOIL ENVIRONMENT

A plant is an organism exposed simultaneously to two environments, the soil and the atmosphere. The aerial part of the plant, the shoot, depends on the root for its supply of water, minerals, nitrogenous components, and possibly other substances that are absorbed from the soil or synthesized or transformed by the root and transported to the shoot. On the other hand the root depends on the shoot for photosynthates and probably other substances synthesized in the shoot and transported to the root. Since root-shoot growth, development, and ratio are coordinated, there must be a regulatory system. About 60 years ago, the existence of rhizocaline and caulocaline was suggested [35]; now it is well known that plant growth substances play an important role in this system.

The soil is the environment of the root system, and the root is exposed directly to the changing conditions of the soil. It is through the root that the whole plant is affected by changing soil conditions. The root may be considered the plant's sensor in the soil. The most drastic and frequent changes occurring in the soil are in the availability of water (see section on The Root as a Sensing Organ below).

The Soil as the Root's Environment

The soil itself is a heterogeneous multiphasic system composed of minerals and organic particles that differ in chemical nature, size, and arrangement. The mode of packing of these particles determines the size and properties of the interparticular spaces. These spaces contain water and gases. Besides the mineral component, the solids of the soil contain colloidal components that imbibe water and bind ions. The soil water is therefore composed of free water and imbibed water. The free soil water is actually a solution of ions, gases, and other solutes; the ions are distributed between the imbibed water and the free water (soil solution).

The ratio between the volume of the interparticular spaces occupied by air and that occupied by soil solution varies in different soils and changes with time as a result of irrigation, rains, floods, and evaporation. In water-logged soils, practically all the interparticular volume is saturated with liquid solution; the air is driven out and the conditions in the soil become practically anaerobic. With cessation of flood or irrigation, a considerable part of the water is drained down gravimetrically, and aeration is reestablished. The soil particles are drawn together, and shrinkage of the soil may occur. The drainage becomes slower with time until it is so slow that it becomes insignificant (usually 2–3 days after flooding). At this stage, the water is retained in the soil mainly by surface tension effects.

Root Growth and Salinity

Root growth is a result of two processes, cell division and cell expansion. These two processes are independent, but sequential, and must be coordinated for growth to occur. Cell division in roots is localized in the apical meristem of the root tip and, to a certain extent, in the inner mature layers of the root tissue, the pericycle and the endodermis, for development of laterals. An accepted definition of growth is an irreversible increase in size through cell extension. Green et al. [36], for *Nitella*, and Green and Cummins [37], for coleoptiles, have proposed a mathematical expression describing rate of growth, also taking into account cell wall properties. The ''driving force'' for cell expansion is turgor, but for growth to occur, turgor pressure must be higher than a critical value defined as the yield pressure Y. The cell wall properties were defined as extensibility m, and P is the turgor pressure. The growth rate r is therefore

$$r = m(P - Y)$$

Growth modifiers, such as phytohormones, affect growth by changing m values, which eventually change Y. Salinity can affect P, m, and Y.

Turgor is a function of the water relation of the plant with its environment and is usually considered a purely osmotic phenomenon. However, by measurements of stress relaxation of turgor in vivo, it was shown that, at least in growth of young stems, water uptake was a consequence mainly of wall relaxation and turgor affected growth only slightly [38–40]. While discussing the relationship between osmotic adjustment and the role of turgor in growth, Munns [41] concluded that there are probably other factors that control growth. Later in this chapter (see section on The Shoot in the Aerial Environment—Sink Source, Photosynthesis, and Hormones), we present data collected from the literature suggesting that these factors could be phytohormones. The phytohormonal balance of the plant probably controls both photosynthesis (source activity) and growth (sink strength).

Cell division is apparently affected by other factors that regulate entry into mitosis, or cessation of the cell cycle, when the cell reaches a certain distance from the apex, where onset of cell differentiation occurs. These processes may be affected by yet another substance that accumulates in plant tissue in response to stress-the polyamines. Stresses (temperature, osmotic, drought, and others) stimulate the accumulation of polyamines. Polyamines affect the cell cycle at the transition from state G_1 to S [42]. Meristematic cells in the root apex, for example, age with distance from the tip; with aging, the level of polyamines also decreases and may cause cessation of mitosis by locking the cells in the G_1 state. This hypothesis may be supported by the finding of Bagni and Pistocchi [43], who showed that cell division requires polyamines. If the growing tissue is low in polyamines ($<10\mu M$), exogenous polyamines must be added to sustain cell division.

Extension growth and cell division must be coordinated and regulated. Plant growth regulators (plant hormones) must therefore play an important role in growth and development of the root and its response to external stimuli [44,45]. The plant hormone that was studied most extensively for its effect on root growth is indole acetic acid (IAA). Pilet [46] showed that the growth of maize roots was regulated by endogenous IAA and abscisic acid (ABA). Both hormones have been shown

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to be present in elongating roots. The highest concentration of IAA was found in the root cap, in the first 0.5 mm of the root, 357 μg kg^{-1} fresh weight (FW). The highest concentration of ABA was found in the root apex located immediately above the root cap, 67 μg kg^{-1} FW. The IAA content in the region of the apex was half that in the cap itself. The ABA was lower both above and below the apical region. An interesting point is that 10^{-8} M ABA stimulated root growth, but higher concentrations inhibited it. Exogenous ABA induced a reduction in the endogenous IAA. In roots placed horizontally, the upper side grew more during the first 3h, but the growth of the lower side was inhibited. However, the distribution of phytohormones in the tissue showed that both IAA and ABA moved to the lower part of the horizontal root, making interpretation difficult. The IAA apparently did not originate in the root but moved acropetally (from the shoot) and accumulated in the root cap. ABA was synthesized, or released, in the cells of the root tips and moved basipetally (from root tip to shoot). Exposure to salinity has been shown to increase the ABA level and thus further complicates the interpretation.

The growth of the root system and increase in absorbing area occur to a large extent by development of laterals. Exogenous IAA inhibits the growth of laterals but not their initiation in the pericycle [47]. Wightman and Thimann [48] suggested that endogenous IAA stimulates development of laterals; endogenous ABA and cytokinins (CK) inhibit it. They are also of the opinion that IAA moves acropetally, but both ABA and CK are transported basipetally. They consider the gradients of these hormones, resulting from their movement in opposite directions, as the decisive factor affecting the development of laterals. However, there is no evidence yet that the plant is capable of sensing such gradients.

The growth induced by IAA is mainly due to cell extension, including extension of the cell wall. It has been suggested that cell wall extension is regulated by auxin-induced acidification caused by proton extrusion (acid growth theory). However, the suggestion of Key [49] that auxin induces the synthesis of specific mRNAs and their respective proteins seems more likely. Theologis [50], on the other hand, suggested that both mechanisms may be involved in cell elongation. This reporter [50] presented evidence showing that proton extrusion is not the initial driving force for growth. Although it eventually affects cell wall elongation, it is itself a result of a long process initiated by the induction by IAA of specific mRNAs. Theologis [50] did not mention the involvement of CK, gibberellins (GA), or ABA in the regulation of growth. The antagonistic effect of these hormones on growth was mentioned earlier.

Salinity induces growth inhibition, and in many cases the shoot is affected more than the root. Moreover, in many of the plants studied, glycophytes and halophytes, the effect of salinity on root growth is a function of salt concentration. Some concentrations can stimulate root growth while inhibiting shoot growth. This was the case, for example, in pea seedlings (a glycophyte); in *Kosteletzkya virginica* (a halophyte), the situation was more complicated. In pea plants 4–5 days after imbibition, the daily increment in root length, in the presence of 120 mM NaCl, was higher than in roots of the control plants (Fig. 1A), but decreased after that period. Higher NaCl concentration was inhibitory throughout. In shoots, no stimulation of growth by salinity was observed (Fig. 1B). The ABA content was measured in the cotyledons, as they were the first organs to be exposed to salinity stress at the beginning of germination. The ABA reached its peak on days 5–6 of germination (Fig. 1C); at that time, the relative growth of the root started to decline [51]. In *K. virginica* [52], the mean relative growth rate ($\overline{RGR}$) of the root was significantly stimulated by low salinity (85 mM) during the first 14 days of exposure, but after 30 days, it did not differ significantly from control values. In high salinities (175 and 255 mM NaCl), stimulation of the growth rate occurred only during the second week of exposure to salinity (Fig. 2A). During the first week, the plants were probably recovering from the osmotic shock or undergoing other steps of accommodation to salinity. The shoot responded to salinity in a similar way but more mildly (Fig. 2B).

Salinity may be considered to cause earlier aging of tissues, as can be seen by earlier differentiation of the xylem (lower down in the root) and more extensive lignification of the xylem elements [53].

Salinity inhibits growth, and this inhibition is usually measured in the laboratory as inhibition

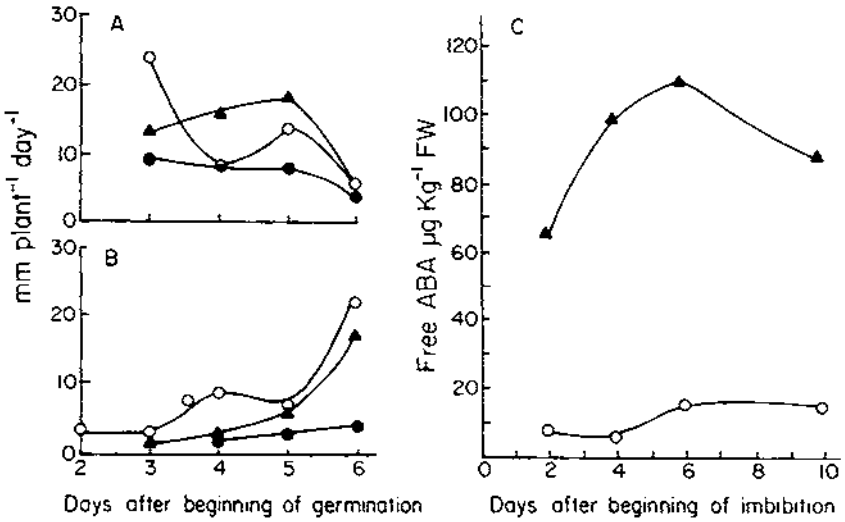

FIGURE 1 Effect of salinity of substrate on growth rate of pea seedlings. Growth as mm day^{-1} per plant: (A) roots, (B) shoots. Concentration of NaCl: open circles, 0 mM; closed triangles, 120 mM; closed circles, 195 mM. (C) Free ABA content of cotyledons of the seeds (µg kg^{-1} FW), grown in 192 mM NaCl. (Calculated from data in Ref. 53.)

of elongation, dry matter accumulation, or $\overline{R}\overline{G}\overline{R}$. However, as shown earlier, not all plant organs respond to salinity in the same way. Therefore, from an agricultural perspective, the ''damage'' of salinity differs in different crop plants depending on the part of the plant—seeds, fruits, roots, or leaves—being harvested.

Root-Soil Water Relations

The soil-root osmotic gradient is the main force responsible for water absorption by the plant. In normal soils (nonsaline), the osmotic contribution to water retention is rather small, but in saline soils, it may be considerably higher, causing reduced water availability for glycophytes. Halophytes apparently have the ability to maintain the necessary gradient even in saline soils. In agricultural practice, a soil water potential of −15 bars (−1.5 MPa) was considered the limit for water availability to the plants and the permanent wilting coefficient. This value is actually the average of the range −10–−20 bars collected from many experiments of permanent wilting [54]. The water present in the soil at field capacity is slowly depleted by the plants and by evaporation until the water potential of the soil reaches approximately −15 bars (−1.5 MPa). Tardieu et al. [55] considered the soil water between −11.0 and −1.5 MPa as transpirable soil water. This concept of a soil-plant water relation was later replaced by a new approach that regarded the soil-water-plant-atmosphere as one continuum.

Wilting occurs from loss of turgor in the leaves and thus depends on the osmotic equilibrium and a dynamic balance between tissue water potential and soil water potential. However, the roots of a transpiring plant can extract water from the soil at a lower soil water potential than those of a nontranspiring plant. Army and Kozlowski [56] showed that a transpiring tomato plant could absorb water from a sucrose solution with an osmotic potential of −17.8 bars (−1.78 MPa), but the detopped root system ceased to absorb water from a solution of only −2 bars (−0.2 MPa). The plant's water relations do not depend absolutely on the simultaneous activity of the whole root system, as shown by ''split-root'' experiments in sour orange seedlings [57]. In these experiments,

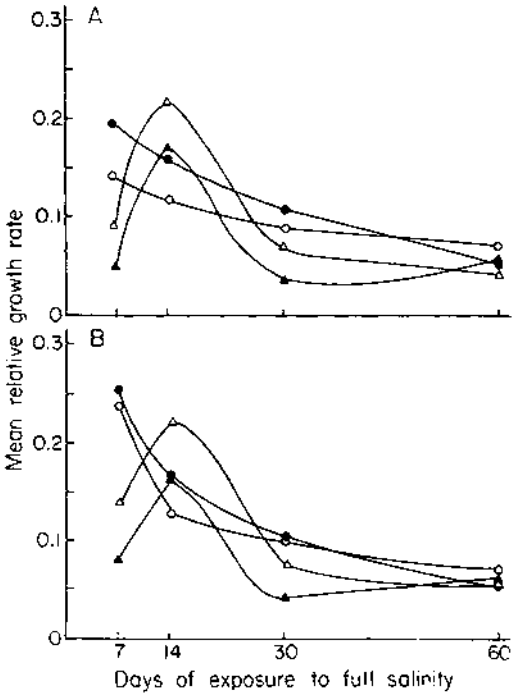

FIGURE 2 Relative growth rate of roots (A) and shoots (B) of seedlings of *Kostelezkya virginica* exposed to three levels of salinity. Concentration of NaCl: open circles, 0 mM; closed circles, 85 mM; open triangles, 175 mM; closed triangles, 225 mM. (Drawn from data in Refs. 23 and 52.)

the root system of a seedling was divided between two containers. One contained saline medium (−0.1 MPa NaCl) and the other contained normal medium. After 4 months of exposure, growth of the plants (with roots divided in NaCl and control containers) was only 9% lower than that of control plants with both halves of the root system in normal medium. If both halves of the root system were exposed to salinity, growth inhibition was 45%. Kirkham [58] reported a split-root experiment in which half the roots were in soil and the other half in a nutrient solution containing cadmium. In this setup, water and cadmium were transported from the liquid medium to the soil. Since cadmium was detected in the leaves, it was assumed that cadmium and water were taken up by the roots from the liquid medium, transported to the shoot, returned to the half-root system in the soil, and excreted to the soil. These data suggest that under drought conditions, when the soil is relatively dry, or in plants growing on saline patches, reasonable amounts of water can be supplied by only a small part of the root system, which is located in a place where water is available. Moreover, Caldwell [59] suggested that in arid soils, in deep-rooted plants with access to water, efflux of water may occur into the dry upper soil layer. This water may be available for the neighboring shallow-rooted vegetation. Such a rare situation is defined by the investigator as ''water parasitism.''

As already mentioned in the beginning of this section, in normal soil, salinity is not as high as to affect the osmotic gradient for water uptake. It is only in saline soils that it can be a problem. Within a reasonable range of salinity, however, there is an effective osmotic adjustment of the root and the shoot [13]. Slatyer [54] has shown that during such adjustment, turgor is restored to very

close to the initial value. It is now accepted that osmotic adjustment occurs at first as a result of ion absorption. Synthesis of organic solutes is another mechanism of osmotic accommodation.

THE ROOT AS A SENSING ORGAN

It is often observed that response to salinity in the shoot is observed earlier than in the root. This occurs despite that the root is the organ exposed to the soil salinity. The most sensitive processes are stomatal conductance and leaf growth. These effects do not show a high correlation with leaf salt content, but correlation is relatively high with the salinity of the root substrate. Therefore, the root seems to be a mediator capable of monitoring the changing conditions in the soil and transmitting the information to the shoot. As mentioned before, the existence of such an exchange of information between root and shoot must exist, but there was not enough evidence presented to support it. In this section, evidence from the literature is presented to demonstrate and support such an exchange of information in reference to various types of stresses exerted on the root with the response being observed in the shoot.

Water Stress

Davies et al. [34] suggested that the root is capable of monitoring the availability of soil water and of transferring this information to the shoot as a positive chemical signal. Recently, Gowing et al. [60] demonstrated this by a split-root experiment. The root of a whole plant was divided in two; each half was made to grow in a separate pot. The two pots were at first well watered, and after the roots were well established in their respective pots, irrigation was discontinued in one pot for 25–30 days. In the other pot, irrigation was continued. Measurements of the rate of leaf growth showed that in plants in which irrigation of one of the root halves was stopped, the growth rate was lower than in the control plants (both halves watered). This decrease in growth was not accompanied by any change in the leaf water status (water potential, solute potential, and turgor), which remained identical in the plants that only had half of their roots watered and those that had both halves watered. The investigators interpreted these results as a demonstration that the signal responsible for the decrease in growth was not a hydraulic effect. In the group in which half the root system was not watered for a few weeks, when the plants were watered again, or when this half of the root system was cut off completely, leaf growth rate increased with time and slowly became comparable to the growth of the well-watered control plants. This response indicates not only that half a root system is sufficient to sustain leaf growth [57] but also that the decrease in leaf growth of plants with half of their roots unwatered resulted from a positive root signal. An example of such a positive signal is an increase in ABA transported from the root to the shoot via the xylem, as suggested by several authors (see Ref. 61 and references therein). Negative signals probably also exist; for example, a decrease in cytokinins transported from root to shoot via the xylem [62].

These data and hypotheses suggest ways in which the root can function as a sensory organ. It senses the soil environment by the effect of environmental factors on the level of phytohormones reaching the xylem and thus affects shoot growth accordingly. Information in the literature shows that, besides soil water status, the root also senses several other soil parameters.

Salinity and Mineral Stress

The literature indicates that changes in mineral nutrient concentration of the medium result in a modification of endogenous phytohormone concentration (see Table 2). The data suggest that a deviation, either an increase or a decrease, from the optimum mineral concentration result in a decrease in the concentration of cytokinins and gibberellins.

Itai et al. [63] and Vaadia [64] reported that exposure of sunflower plants to NaCl resulted in a decrease in CK in the xylem exudate. Downton and Loveys [65] showed, in grapevine, a temporary increase in leaf ABA following salinization.

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Low Oxygen Pressure Stress

Bradford and Yang [66], Jackson and Campbell [67], and Jackson [68] reported that decreased root aeration in tomato caused by flooding resulted in increased root ACC (1-aminocyclopropane-1-carboxylic acid) concentration and its increased export from the root to the shoot via the xylem. The ACC is thought to be converted to ethylene in petiole and leaves.

Exposure of pea root to anaerobiosis caused an increase in leaf ABA concentration, resulting in stomatal closure in the absence of dehydration of the leaf [68,69].

Temperature Stress

Atkin et al. [70] studied the effect of root temperature between 8 and 33°C on the growth of corn while shoot temperature was kept constant. Leaf growth increased with increasing root temperature. After 17 days of treatment, xylem exudate showed a maximum ABA concentration at 18°C and maximum CK and GA concentrations at a root temperature of 28°C. Sattin et al. [71] measured the rapid response of leaf growth in bean as a function of change in root temperature using a rotary variable displacement transducer (RVTD). The decrease in root temperature from 23 to 10°C caused a fall in leaf growth rate within 20 min to less than 10%. In parallel, leaf ABA concentration increased rapidly [72], but the hydraulic conductivity of water in the root decreased. When root temperature was returned from 10 to 23°C, growth rate increased within a few minutes, showing an overshoot of leaf growth for several minutes.

Soil Compactness Stress

With increasing soil compactness, a decrease in shoot and root growth was observed. Growth of the shoot was inhibited more than that of the root. Furthermore, increasing soil compactness resulted in increased leaf resistance to gas diffusion and decreased transpiration [73]. The signal from root to shoot has not been identified.

Table 3 summarizes the data in the literature on changes in phytohormone concentrations occurring in response to modifications of parameters of the soil environment. The information available is certainly not complete, but it is clear that there is a correlation between the soil environment, plant growth, and changes in the concentration of phytohormones. These data are in agreement with the idea that changes in the phytohormones transported via the xylem from root to shoot may serve as either positive or negative signals carrying the message of changing soil conditions.

TABLE 3 Response of Plants to Environmental Parameters in the Soil[a]

Parameter	Whole-plant response	Changes in phytohormone concentration
Water ↓	Growth ↓	ABA ↑
Oxygen ↓	Growth ↓	ABA ↑, ethylene (ACC) ↑
Mineral nutrients ↓	Growth ↓	CK ↓, GA ↓
Mineral nutrients ↑	Growth ↓	CK ↓, ABA ↑ temp
Salinity (NaCl) ↑	Growth ↓	CK ↓, ABA ↑ temp
Temperature ↓	Growth ↓	CK ↓, GA ↓, ABA ↑
Soil compactness ↑	Growth ↓	No data

ABA, abscisic acid; ACC, 1-aminocyclopropane-1-carbohylic acid; CK, cytokinins; GA, gibberellin.

[a] Decreased or increased concentration indicated by direction of arrows.

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THE SHOOT IN THE AERIAL ENVIRONMENT

The plant's shoot in its aerial environment is exposed to the effect of external conditions. These include light, temperature, relative humidity, pollution, the mechanical effect of wind, and, in specific habitats, salt spray. The aerial environment is much less stable than the soil environment. Climatic factors are continuously changing in annual, seasonal, and diurnal cycles, and various unpredicted climatic changes are liable to occur. In addition, the shoot is affected by ''messages'' from the root, which fall in two categories: qualitative, such as the supply of water, minerals, and various substances, originating in the root; and quantitative, the change in the rate of supply of these substances, or some of them, as a result of changing conditions in the soil. The factors in the aerial environment are not directly affected by substrate salinity, but soil salinity may affect the signals sent from the root to the shoot. On the other hand, messages are also being sent from the shoot to the root; for instance, products of photosynthesis, various nitrogenous substances, plant hormones (such as IAA), and others.

The water balance of the plant is actually the equilibrium between supply from the soil through, and by, the root and loss by transpiration from the leaves through the stomata. Stomatal resistance and the evaporative demand of the atmosphere (which is an interaction between relative humidity [RH] and temperature) affect the rate of transpiration, which in turn also affects the CO_2 supply for photosynthesis. When transpiration exceeds supply, transitional water stress may develop in the plant even when the soil is wet, and wilting and/or heat shock may occur. Closure of stomata thus decreases transpiration and enables the plant to balance the water economy and restore turgor.

Shoot Growth in Salinity

Salinity affects the growth of different plants in different ways according to the mode of life of the plants (see Table 1). Although inhibition of growth by salinity is the most evident effect, it is not a simple phenomenon that affects all types of plants, or all organs of the plant, in the same way. As can be seen from Figures 1 and 2, shoot growth may be inhibited by salinity levels that stimulate root growth. In pea seedlings, inhibition of stem growth is mainly due to shortening the internodes, not to a decrease in their number. In *Kosteletzkya*, the relative growth rate of root and shoot are affected similarly. In rice, Yeo et al. [74] distinguished two stages of leaf growth inhibition by salinity: Exposure of rice plants to 50 mM NaCl caused immediate cessation of growth, but within 30 min the growth rate recovered. The investigators believed that it was difficult to assume that anything other than water supply could be perceived, transmitted, and translated in such a short time. On long exposure, growth was inhibited, longevity of leaves was reduced, and leaf mortality was high. Although the investigators ascribed these phenomena to excessive ion accumulation, they did not discard the possibility that hormonal signals from the roots may have been involved.

Now it is generally accepted that, in most plants, stress induces an accumulation of ABA and ABA plays an important role in the growth and development of the plants and also serves as a signal conveying information (see section on The Root as a Sensing Organ above).

Recently, Saab et al. [75] studied, in great detail, millimeter by millimeter, the extension growth of maize mesocotyl and the primary root under conditions of low water potential of the substrate. Earlier, Saab et al. [76] found that, under low water potential, in contrast to conditions of high water potential, the endogenous ABA enhanced root growth but inhibited shoot and mesocotyl growth. Treatment of the plant with fluridone actually reversed the effect of the endogenous ABA. After measuring endogenous ABA, water content, and elongation of millimeter segments, these investigators [76] concluded that a gradient of responsiveness to ABA developed in the cells of the elongation zone. The ability of ABA to protect cell expansion of the elongation zone, for instance in the root at low water potential, decreased with distance from the tip. In the mesocotyl at low water potential, ABA became more inhibiting to cell expansion with increasing distance from the meristem [75].

The different growth responses to salinity or stress can in general be interpreted as resulting

from changes in the allocation of resources (i.e., products of photosynthesis). Plants usually maintain a characteristic root-shoot ratio. When this ratio is disturbed, either through loss of part of the shoot by cutting or grazing or similarly by loss of a part of the root, the immediate response is a reduction in dry matter accumulation in both the root and the shoot. However, eventually the plant compensates by increased growth of the damaged part until the ratio is restored [77]. Klepper [77] presented ample evidence showing that removal of a part of the root system was followed by acceleration of root growth (relative to the shoot). If, on the other hand, the plant was partly defoliated, the remaining shoot parts showed enhanced photosynthesis and growth. However, this is by no means a universal behavior. The return to the initial root-shoot ratio is designated ''homeostasis'' by Klepper [77]. This can perhaps be interpreted as a change induced in the allocation of resources and partitioning of dry matter.

Carmi and Koller [78] showed that in bean plants detopped above the primary leaves compared with intact plants, neither the net assimilation rate (NAR) nor the net photosynthesis (NP) of the primary leaves was affected. The assimilates normally allocated to the top leaves in the intact plant were distributed in what remained from the top in the detopped plant. If the petioles or the stem below the primary leaves was girdled or steamed, thus not allowing the photosynthates to flow out of the leaf, the NAR and NP were still not affected, and the assimilates formed, remained, and accumulated in the primary leaf as starch grains (see the next section). From the following, it appears that, in nongirdled detopped plants, the distribution of resources may be directed by phytohormones. Treatment of the detopped stump with IAA diverted the allocation of dry matter to the root and increased root growth occurred. Treatment of the stump with GA directed the assimilate flow to the shoot. Even with this distribution of assimilates from the primary leaves, however, neither the NP nor the NAR was affected (see the next section). If instead of detopping, or in addition to it, $\approx$80% of the root system was excised, a considerable reduction in the photosynthesis rate of the primary leaves was observed [79]. This reduction was shown not to be due to inadequate supply of water or minerals but apparently to an inadequate supply of some essential substances activating the photosynthetic apparatus. The exogenous addition of benzyladenine could substitute for the missing part of the root system, indicating that cytokinin-like substances originating in the root participate in regulation of the rate of photosynthesis in the leaves, in this case, the primary leaves.

This information implies that loss of part of the plant's canopy, or loss of a part of the root system, changes the hormonal balance of the plant as a whole. Substrate salinity, as shown, to some extent mimics this ''mechanical'' effect, at least in the root system, and induces a change in the hormonal balance of the plant and a change in the allocation of resources.

Tshaplinsky and Blake [80], in their experiment with young poplar trees, showed a behavior different from that of the bean plant in the experiments of Carmi and Koller [79]. Decapitation was followed by reinvigoration of growth in the remaining stump leaf. Diurnal photosynthetic patterns of the retained stump leaves showed that 15 days after decapitation, the photosynthetic potential was increased by increasing NP in the early afternoon, thus eliminating the afternoon reduction in photosynthesis typical of the control leaves. The increase in NP was accompanied by increased transpiration rate and increased stomatal conductance. Thus, photosynthesis was increased without requiring activation of, or increase in, the photosynthetic apparatus, but water loss was not controlled.

In the experiments described by Carmi and Koller [79], the primary leaves of the detopped or partially defoliated plant are the source of the photosynthates. The roots and/or the leaves, and the shoots above these primary leaves, are the sink for these photosynthates. It was shown that phytohormones are capable of changing the direction of flow of the assimilates; that is, they may change ''the strength of the sink.''

Sink-Source, Photosynthesis, and Hormones

A considerable amount of information in the literature suggests that the accumulation of sucrose and/or sugar-phosphate intermediates in source leaves affects photosynthetic rates by feedback inhibition. This was proposed by several investigators in the past, for example Herold [81], and was

reaffirmed lately by Stitt [82]. Some investigators, however, for example Geiger [83], were not able to demonstrate this relationship in their experiments. Table 4 presents data of plants tested. In most of them (sunflower, tobacco, *Amaranthus*, peanut, soybean, cotton, and wheat), a decrease in the export of photoassimilates from source leaf rapidly resulted in an increase in soluble sugar concentration, as well as in a decrease in photosynthesis. This correlation can also exist in cucumber, although it requires 5, 6, or even up to 16 days to be observed (see Table 4). In bean, the phenomenon could not be demonstrated [83,96], possibly because the experiments were too short—4 and 2 days, respectively. The earlier study on bean [102] showed that decreased export of photosynthates resulted in a considerable increase in the dry weight of the source leaf, which the investigators suggested might be due to starch accumulation. The data in Table 4 show a negative correlation between photosynthesis and accumulation of soluble photosynthates in source leaves. Sometimes the two are tightly coupled and the response is rapid; in other cases, they are more loosely coupled, probably when relatively larger amounts of starch accumulate in the chloroplast, thereby decreasing sucrose and sugar-phosphate accumulation in the cytoplasm of source leaves. This phenomenon, although common, is not always observable, as exemplified by the following: (a) in certain cases, the phenomenon is only observed after a very prolonged lag and (b) the data of Carmi and Koller [78] show that starch accumulates when there is no outlet for the photosynthates from the primary leaves; no effect was observed on NP or NAR, and the decrease in photosynthesis occurred only when the supply of CK substance was decreased.

The data from the literature, summarized in Table 5, show that changes in auxin, ABA, GA, or CK concentration in many cases affect the sink-source relationship. Reports are sometimes contradictory, because phytohormonal effects depend on the developmental stage of the plant being studied; hormones can have an effect only if the plant tissue can perceive and respond to the stimulus. In general, the data in Table 5 suggest that auxin enhances export of photoassimilates from source to sink, apparently by enhancing phloem loading. The effect of ABA is the most unpredictable, because the sensitivity of the plant depends on its developmental stage. It often enhances phloem unloading. The GA seems to enhance phloem unloading, possibly by induction of invertase activity. The CK seems to enhance phloem unloading but through a mechanism other than that of GA. These generalizations are tentative, since the mechanisms of phytohormone action are not known. However, they strongly suggest that phytohormones may play a central role in the partitioning of photoassimilates.

Sucrose is the main product of photosynthesis exported from source leaves to sinks. The data in the literature indicate that its concentration is affected by salinity. The sucrose concentration in source leaves either increases or decreases depending on the particular plant being studied (Table 6). The data presented earlier permit the formulation of a working hypothesis that describes the response of plants to NaCl salinity. Salinization of the root environment causes a change in the hormonal balance of the plant, such as decreases in CK and GA and a temporary increase in ABA concentration. This change in the phytohormonal balance in the plant results in a decrease in photosynthetic activity as well as in the activity of the sinks. When the decrease in photosynthetic activity induced by the phytohormonal change is greater than the decrease in the activity of the sinks, the sucrose concentration in source leaves decreases. However, when the decrease in photosynthetic activity is relatively small compared with the decrease in sink activities, the sucrose concentration in source leaves increases. This increase in sucrose together with an accumulation of phosphorylated photosynthesis intermediates in source leaves eventually results in decreased photosynthesis by a feedback inhibition mechanism, and the sucrose concentration remains higher than in the absence of salinity.

To explain the effect of salinity on nonhalophytes, Munns and Termaat [127] suggested that phytohormones of root origin regulated metabolic processes in the leaf. However, since these investigators [127] were not able to counteract the effects of salinity by exogenous phytohormonal treatment, they did not pursue the idea further. Amzallag et al. [128], however, were able to show such an effect of phytohormones on salt-affected sorghum plants. Sorghum plants adapted to 150 mM NaCl salinity [129] did not grow when the salinity was increased to 300 mM NaCl, if the mineral

Table 4 Source-Sink Relationship and Photosynthesis[a]

Mechanism decreasing export from source leaf	Plant, species, organism	Effect on sugars in source leaf	Effect on photosynthesis in source leaf	Reference
Fruit removal	Cucumber	No effect	↓ After 16 days	84
Cooling	Sunflower	Sucrose ↑	↓ After 12 h	85
Transgenic plant with invertase in apoplast	Tobacco	Sucrose ↑, glucose ↑, fructose ↑, starch ↑	↓ After 6 h	86, 87
Petiole cooling	*Amaranthus*	Sucrose ↑, glucose ↑	↓ After 1 h	88
Continuous light, cooling	Soybean	Sucrose and P intermediates ↑	↓ After 6 days	89–91
Cooling	Peanut	Not determined	↓ After 1 day	92
Starchless mutant	*Arabidopsis*	Sucrose ↑, starch ↓	↓	93, 94
Continuous light	Soybean, spinach, barley	Not determined	All species first ↑ for 6 h then ↓	95
Removal of sinks (young leaves, fruits, flowers, or buds)	Cucumber[b]		↓ After 6 days	96
	Cotton		↓ After 1 day	
	Radish		↓ After 9 days	
	Pepper		No effect after 6 days	
	Eggplant		No effect after 6 days	
	P. vulgaris		No effect after 4 days	
	Castor bean		No effect after 4 days	

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Girdling or removal of aerial sinks	Cucumber	Starch ↑	↓ After 5 days	97
High CO_2	Cotton	Starch ↑	↓ After 1 day	98
Low O_2, high CO_2, cooling, petiole cooling	Wheat	Carbohydrates ↑ (glucose, fructose, sucrose, starch)	↓ After 4 h	99
Leaf excision	Soybean	Sucrose ↑, starch ↑	↓ After 1 h	100
Removal of shoot apices, petiole cooling	*P. vulgaris*	Not determined	No effect after 2 days	83[c]
Removal of sink	Wheat	Not determined	↓ After 1 day	101
Cooling, removal of sink	*P. vulgaris*	Dry matter accumulation (probably starch)	Mild ↓	102, 103

[a] Effect of modification of photosynthate export on source leaf sugar and starch concentration. Decreased or increased concentration indicated by direction of arrows.
[b] Sucrose ↑ and starch ↓ measured only for cotton. For cucumber, see Ref. 97.
[c] The author presents a summary of previous studies. In most cases, photosynthesis in the source leaf was affected by changes in the export of photosynthates; however, in some studies, there was no effect on rate of photosynthesis.

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TABLE 5 Effect of Phytohormones on Translocation of Photosynthates or Sink Source Activity.[a]

Phytohormone	Plant species	Affected organ	Effect	Reference
Auxin				
Exo	Pea	Epicotyl	Sugar, ABA transport ↑	104
Exo	Celery	Phloem	Uptake ↑	105
		Source leaf	Export ↑	
Exo	*P. vulgaris*	Sink leaf	Sucrose uptake K_m ↑ V_{max} ↑	106
Exo	Castor bean	Petiole (phloem)	Sucrose loading ↑	107
Exo, endo	Strawberry	Berry	Growth ↑	108
Abscisic acid (ABA)				
Exo	*P. vulgaris*	Seed coat	Photosynthate unloading ↑	109
Exo, endo	Soybean	Seed	Seed filling ↑ with increased (ABA)	110
Exo, endo	Barley	Flag leaf ([^{14}C] sucrose), ear (ABA)	Sucrose uptake by seed ↑ or ↓ as function of stage of plant development	111
Exo	Castor bean	Petiole (phloem)	Sucrose loading ↓	107
Gibberellins (GA)				
Exo	Pea	Epicotyl	Sugar uptake ↑, apoplast invertase ↑	112, 113
Exo	Pea	Ovary	^{11}Cpa uptake ↑	114, 115
		Apex	^{11}Cpa uptake ↓	
		Root	^{11}Cpa uptake ↑	
Exo	Celery	Phloem	Uptake ↑	105
		Source leaf	Export ↑	
Exo GA_3	*V. faba*	Source leaf	Photosynthate export ↑	116
Exo	*P. vulgaris*	GA on root	Sucrose trans to elongating stem ↑, to hypocotyl and root ↓; invertase activity in elongating stem ↑, in hypocotyl and root ↓	117
Cytokinins (CK)				
Exo CK	*P. vulgaris*	Seed coat	Photosynthate unloading ↑	109
Endo CK	Barley	Grain	Filling ↑	118

Exo, exogenous; endo, endogenous; trans, transport; ^{11}Cpa, ^{11}C = labeled photassimilates.

[a] Decreased or increased concentration indicated by direction of arrows.

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Table 6 Effect of Medium Salinity on Leaf Carbohydrate Concentrations

Species	Medium	Leaf carbohydrate concentration				Reference
Eucalyptus microtheca	[NaCl]	Soluble sugars mg g^{-1} FW				119
	Control	17 100%				
	100 mM	24 141%				
	300 mM	65 382%				
	500 mM	81 476%				
Pistachio plant	[Cl^-] mM	Sucrose mM	Glucose mM	Fructose mM	Starch mg g^{-1} DW	120
	0.5	52 100%	49 100%	55 100%	16 100%	
	100	49 94%	60 122%	59 107%	11 69%	
	175	43 83%	61 124%	56 102%	7 44%	
Sebania bispinosa	[NaCl] mM	Sucrose mM	Fructose mM	Glucose mM		121
	Control	3 100%	6 100%	7 100%		
	75	2 67%	7 117%	14 200%		
	150	2 67%	7 117%	15 214%		
Leucaena leucocephala	Control	17 100%	6 100%			121
	100	44 159%	19 317%			
Barley	[NaCl] mM	Soluble sugars mM				122
	Control	51 100%				
	120	50 98%				
	180	41 80%				
Aster tripolium	[NaCl] mM	Sucrose mM	Glucose mM	Fructose mM		123
	Control	0.70 100%	0.16 100%	0.21 100%		
	300	2.19 313%	0.11 69%	0.81 386%		
Daucus carota	Control	8.7 100%	21.4 100%	14.1 100%		123
	75	15.5 178%	11.8 55%	10.0 70%		
	150	26.0 299%	9.7 45%	5.8 41%		

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Table 6 Continued

Species	Medium				Reference
Honkenya peploides	Control	1.3 100%	8.4 100%	6.7 100%	123
	75	2.4 185%	4.6 55%	3.7 55%	
	150	4.7 361%	3.6 43%	3.0 45%	
	250	13.0 1000%	4.3 51%	2.4 28%	
Eleocharis uniglumis	Control	13.5 100%	1.3 100%	1.3 100%	123
	200	60.1 445%	2.8 215%	2.8 215%	
Carex extensa	Control	95 100%	77 100%	34 100%	123
	200	83 87%	38 49%	20 59%	
Schoenoplectus tabernaemontani	Control	18 100%	13 100%	11 100%	123
	300	27 150%	5 38%	4 36%	
Juncus maritimus	Control	28 100%	8 100%	5 100%	123
	300	35 125%	30 375%	18 360%	
Plantago coronopus		Soluble sugars, glucose equivalents mM			123
	Control	45 100%			
	200	60 133%			
Sueda maritima	[NaCl]	Sucrose μmol g^{-1} FW	Glucose μmol g^{-1} FW	Fructose μmol g^{-1} FW	124
	Control	4.1 100	1.6 100%	1.0 100%	
	340 mM	3.2 78%	2.0 125%	2.0 200%	
Grapevine	[Cl^-]	Sucrose μmol g^{-1} FW	Reducing sugars μmol g^{-1} FW	Starch μmol glucose Eq g^{-1} FW	
	Control	30 100%	115 100%	170 100%	
	100 mM	10 33%	175 152%	110 65%	
Barley		Sucrose mg per 10 g FW	Reducing sugars mg per 10 g FW	Starch mg per 10 g FW	126
	Control	48100%	17 100%	1.0 100%	
	100 mM	81 169%	17 100%	1.2 120%	

FW, fresh weight; DW, dry weight.

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nutrient medium was maintained at the concentration of half-strength Hoagland solution. When the concentration of the nutrient solution was increased to full-strength Hoagland, RGR was restored to the level of the controls. However, addition of an appropriate concentration of CK (10^{-7} M), GA (10^{-8} M), or, even better, a mixture of CK plus GA (both at 10^{-9} M) substituted for the increased mineral concentration, and RGR was restored to that of control plants. These results, as well as the data in Table 2, show an interrelationship between mineral nutrients and phytohormones in the regulation of growth.

Modification of the Effect of NaCl on Sink Activity by Increased Ambient CO_2 Partial Pressure

Zeroni and Gale [130] reported that rose plants (*Rosa hybrida*, Sonia, grafted on *Rosa indica*) showed a change in sensitivity toward salinity during prolonged exposure to a high CO_2 concentration. Exposure to 29 mM NaCl inhibited growth (dry weight) to 74% of control when CO_2 concentration in the air was 320 μmol mol^{-1}. At a CO_2 concentration of 600 μmol mol^{-1}, exposure to a similar salinity enhanced growth to 146% of control (plants exposed to 600 μmol mol^{-1} of CO_2, but no NaCl). If our hypothesis presented in the previous section is correct, these data suggest that at 320 μmol mol^{-1} of CO_2, addition of NaCl causes a decrease in sink activity; at 600 μmol mol^{-1} of CO_2, the salinity causes increased sink activity. At present, not enough data are available to explain this interesting effect. Similar data on the effect of CO_2 concentration in response to salinity were obtained by Bowman and Strain [131] in a study on *Andropogon glomeratus*. Increased CO_2 concentration in the air is known to effect numerous phenomena. It has been reported that increased CO_2 concentration affects the synthesis of ethylene [132], and at higher concentration, CO_2 also acts as a competitive inhibitor of ethylene activity [133]. The effect of increased CO_2 concentration on the response of the rose plants to salinity could result from this effect of CO_2 or perhaps from the effect of a change in sucrose concentration on genome expression [134].

ADAPTATION TO SALINITY

As outlined at the beginning of this chapter, most plants are capable of tolerating a certain range of salinity. This range varies in different species, varieties, and ecotypes. In some plants, this range is rather narrow; in others (i.e., in halophytes), it is wide. A large part of the research on salinity is carried out with the intention of accommodating crop plants to grow in salinities outside the natural range of tolerance and nevertheless obtain appropriate agricultural yields. Such expressions as accommodation, adaptation, and acclimation are used as synonyms in the literature. Since two types of plant responses to salinity have been distinguished, we prefer to use different terms: *preexisting resistance mechanisms* that enable the plant to cope with salinity within its natural range of tolerance, and *adaptation* [129]. Adaptation is achieved during a specific treatment and involves changes in the plant's behavior and expression of properties that were not evident before the treatment. A plant is considered ''adapted'' to salinity when at least one of the following cases occurs after the treatment that induces adaptation: (a) an increase in the mean relative growth rate of the salt-treated plant occurs, so that the growth is restored to a value more or less similar to that of the control plant; or (b) when the plant has acquired the capacity to complete its life cycle in a saline environment in which the nonadapted plant is not able to do so [129]. In the following, we present a few examples of adaptation.

Adaptation at the Whole-Plant Level

Phaseolus

The response of *Phaseolus vulgaris* to salinity was reported by Wignarajah et al. [135–137]. They exposed the bean plants to 48 mM NaCl 8 days following germination. At first, growth was inhibited

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and leaf Na^+ and Cl^- concentrations increased rapidly. However, 25 days after the beginning of salinization, $\overline{RGR}$ was restored to a value similar to that of the control plants. The leaf Na^+ and Cl^- concentrations decreased to low and controlled values. The decreased ion concentration results both from dilution by growth as well as retranslocation from leaf to root followed by excretion to the medium. Both these properties, normal growth and controlled ion concentrations, suggest that the plants had adapted to salinity.

Sorghum

A period of 20 days of exposure of 8-day-old *Sorghum bicolor* plants to sublethal NaCl concentrations (above a threshold of about 30 mM) induced the ability to survive the presence of the otherwise lethal NaCl concentration of 300 mM NaCl [129]. Moreover, the plants grew, flowered, and set seeds if the Hoagland solution was brought to full-strength concentration, while the control plants died. Pretreatment of the plants with the low NaCl concentration resulted in adaptation to salinity. Adapted *Sorghum* plants exposed to 300 mM NaCl have $\overline{RGR}$ values similar to those of control plants. Furthermore, shoot Na^+ and Cl^- concentrations were stable and controlled. The process of adaptation to salinity is sensitive to exogenous plant growth regulators. The period required for adaptation was shortened by ABA treatment, but the process of adaptation was inhibited by exogenous CK and/or GA [128,138]. Adaptation to salinity occurred only if the pretreatment was initiated not later than 10 days following germination. After this period, adaptation was no longer possible. The short and defined period of time during which adaptation was possible was considered a ''developmental window.'' The growth of adapted *Sorghum* plants exposed to 300 mM NaCl showed a very high degree of variability, indicating that individual plants reached different levels of adaptation.

C_3 to CAM Shift

In facultative CAM (crassulacean acid metabolism) in plants, water stress or salinity induces a shift from C_3 to CAM photosynthesis. This shift has been extensively studied in *Mesembryanthemum crystallinum*. The plant has the capacity to shift from one physiological mode to the other. The shift, which takes about 10 days to occur, is composed of a series of events. The first event observed is the appearance of the early stress proteins and accumulation of proline. This is followed by an increase in PEPCase mRNA and its protein. Finally, the plant shifts into CAM [139]. Ostrem et al. [140] reported that this response is only inducible in plants that are at least 6 weeks old. Chu et al. [141] have shown that 10 μM exogenous ABA applied to the leaf induced the C_3 to CAM shift, and preliminary results reported by Piepenbrock and Schmitt [142] showed that 100 μM CK added to the medium inhibited NaCl induction of PEPCase in *M. crystallinum*.

There are many similarities between the increase in tolerance to salinity induced by NaCl in *Phaseolus, Sorghum*, and *Mesembryanthemum*. For *Phaseolus* and *Sorghum*, growth rates that are first inhibited by exposure to salinity are restored to values similar to those in the absence of salinity. Furthermore, the shoot Na^+ and Cl^- concentrations seem to be controlled as a result of adaptation. In *Sorghum* and *Mesembryanthemum*, phytohormones are seen to play a role in the adaptation process. The ABA accelerates the process, and CK and GA prevent its development.

Although three examples of adaptation have been presented here, the response of plants to salinity is often not by adaptation but rather through preexisting tolerance mechanisms. For example, the prolonged growth kinetic study by Greenway [143] on two varieties of *Hordeum vulgare* under saline conditions did not show any increase in salt tolerance of the plants.

Adaptation at the Cell Level

It is possible to adapt cells in suspension culture by a stepwise increase in the NaCl concentration. Using this technique, tobacco cells were made to grow in a medium containing 500 mM NaCl [144,145]. Adapted cells can be returned, progressively, to a medium containing no NaCl and grow for many generations. When these salt-adapted cells are transferred directly back to medium con-

taining 500 mM NaCl, they begin to grow within a few days. At first they grow slowly, but after several generations, the growth rate becomes similar to that of cells grown continuously at high salinity. These cells retain their adapted character even if grown for many generations in the absence of salinity. La Rosa et al. [146,147] showed that the process of adaptation is accelerated by the addition of 10 μM ABA to the medium.

The adaptation of cultured cells has often been performed in view of the possibility of regenerating plants with enhanced salinity tolerance. McCoy [148] compared the salt tolerance of the whole plant with that of its cells in culture for several species of *Medicago*. This investigator [148] did not find any correlation between the tolerance at the cultured cell level and that at the whole-plant level. In other experiments, McCoy [149] adapted cells of two *Medicago* species to salinity and compared the regenerated plants obtained from the adapted cells to plants regenerated from control cells. The plants regenerated from control cells all looked normal. In one species, all the plants showed normal chromosomes, in the other species, one type of chromosomal aberration occurred in 74% of the plants. In contrast, all the plants regenerated from the salt-adapted cells looked very abnormal; all of them showed multiple types of chromosomal aberrations. Exposure of cultured cells to salinity greatly enhances the frequency of occurrence of chromosomal aberrations, which suggests that NaCl causes gross changes in the organization of the genome in cultured cells.

CONCLUSIONS

In the past, the effects of salt on plants have been considered by some investigators as resulting from the physicochemical properties of the saline solution, and it was supposed that the tolerance of the plant resides in the tolerance of its cells. This has led to the development of several concepts concerning the growth inhibition observed following salinization of salt-sensitive species:

1. The decreased water potential of the medium results in a decrease in turgor, which is necessary for cell elongation. This was defined as physiological drought. To grow under saline conditions, the plants must accumulate solutes, either inorganic (such as NaCl itself) or organic.
2. The NaCl toxicity is a major reason for growth inhibition. Compartmentation of Na^+ and Cl^- in the vacuole and accumulation of compatible solutes in the cytoplasm prevent toxicity damage.
3. Plants regenerated from salt-adapted cells may yield more resistant species.

These concepts, however, are not adequate to interpret the data available at present on the response of plants to salinity. Salt tolerance may well be a property of the whole plant, not the sum of the tolerance of its cells [63]. This is supported by the unsuccessful attempts to produce salt-tolerant plants through regeneration from salt-adapted cell lines. In plants, different cell types cooperate to form an integrated organism. Moreover, interactions between different cell types result in characteristics that are proper to the whole organism.

Phytohormones play an important role in the integration of developmental processes in plants, including the responses of the whole plant to changing environmental factors. As described, deviations in the characteristics of the soil environment from optimal conditions result in changes in the phytohormonal balance of the plants that are correlated with inhibition of growth. The decrease in growth may be a necessary intermediary stage during which various changes occur, which may result in accommodation of the plant to the new environmental conditions. The plant may respond to salinity by using its preexisting resistance mechanisms. Under specific conditions, it may ''adapt'' to salinity; this adaptation is expressed, for example, by an expanded range of salt tolerance or a changed photosynthetic mechanism. In general, such a change may be described as a change in the plant's physiological ''mode.'' Whole plants, in the course of their development, may have only a short time period, a developmental window, during which they are susceptible to the treatment resulting in adaptation.

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Many mechanisms play a role in the response of plants to salinity, but it seems that hormonal balance is a major factor affected by salinity. The disturbed hormonal balance seems to be one of the main factors responsible for growth inhibition.

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7

Germination of Seeds and Propagules Under Salt Stress

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INTRODUCTION

Germination is a complex phenomenon involving many physiological and biochemical changes and leading to the activation of embryo [1,2]. However, during initial phases of germination, propagules may behave differently as compared with seed, but fundamentally embryonic tissues in both of them show more or less the same pattern of growth. Any unfavorable change may jeopardize the process of germination to a large extent.

Salinity, as an abiotic hazard, induces numerous disorders in seeds and propagules during germination. It either completely inhibits germination at higher levels or induces a state of dormancy at lower levels [3,4]. It first reduces imbibition of water because of the lowered osmotic potential of the medium [5,6]. Second, it causes toxicity; that is, it changes enzymatic activity [7,8], hampers protein metabolism [9,10], upsets plant growth regulators balance [11], and reduces the utilization of seed reserves [12,13,14]. It may elicit changes at ultrastructural [5], cellular and tissue [15,16], or even at organ levels [17].

Salinity interacts with certain plant and environmental factors during germination. Among the plant factors, seed coat [18], dormancy [4], seed age [19], seed polymorphism [20,21], and seedling vigor [22,23] are prominent. Environmental factors include temperature [24], light [3], availability of water [25], and oxygen [26].

Efforts have been made to ameliorate the adverse effects of salinity on germination by employing certain chemical and biochemical agents. Gibberellic acid initiates germination by breaking salt-induced dormancy [27,28], whereas kinetin stimulates it [29,30,31]. Similarly, polyamines [32], thiourea [33], amino acids [34], betaines [6], and sugars [35] have been successfully used to accomplish a higher germination rate and seedling growth. The role of calcium has been well documented in the mitigation of ionic toxicity and regulation of membrane processes during germination [36,37]. Moreover ammonium, nitrate [38], potassium, and magnesium [23] have also proved their worth in germination and seedling development. This chapter encompasses the details of salt-induced

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changes on the process of germination, interaction of some factors with germination of seeds (including caryopses) and propagules (e.g., buds, tubers, stem cuttings), and impact of some ameliorants to enhance germination under salinity.

PROCESS OF GERMINATION UNDER SALINITY

Germination of a viable seed or propagule starts with the imbibition of water and terminates with emergence of embryonic tissues. This involves the hydration of proteins, subcellular structural changes, respiration, synthesis of macromolecules, and cell elongation [1]. Whereas, Chong and Bible [39] have regarded growth of embryonic tissues as an important step in the completion of germination, some workers have given due emphasis to the establishment of seedlings under stressful conditions [40,41]. The latter seems crucial under salinity, as without a successful crop stand simple emergence of embryonic tissues will prove futile. The process of germination is greatly influenced by the nature and extent of salinity and, above all, on the behavior of seeds or propagules. For a better understanding of the adverse effects of salinity, we have categorized the process of germination into four events: (a) imbibition, (b) active metabolism, (c) emergence and elongation of embryonic tissues, and (d) establishment of seedlings (Table 1).

Imbibition of Water

Hydration of stored materials is the initial step for the onset of germination [60]. The osmotic component of salinity poses a strong inhibitory effect on the hydration of the embryo, cotyledon, and endosperm [16,61,62]. It is independent of types of salinity and growth media whatsoever [44,62], as use of any salt induces an osmotic effect.

Active Metabolism

The ions are inevitably taken up by seeds, during exposure to salinity, which cause toxicity to various physiological and biochemical processes. The activities of enzymes are hampered [9,63], leading to the altered and reduced synthesis of micro- and macromolecules [49] and their reduced mobilization to the developing tissues [16,49,59]. The synthesis of new proteins in response to saline stress was observed in wheat embryo which ceased on return to water [10]. This pattern of synthesis was attributed to the specific effect of ions on the activities of enzymes [9]. Salinity also causes the accumulation of soluble sugars, free proline, and soluble proteins [6,52]. These metabolites may prove to be beneficial to the germination; first by reducing osmotic inhibition and second by providing substrates for the growth of embryonic tissues [16,64].

Emergence and Elongation of Embryonic Tissues

Seed reserves are utilized in the growth of the embryo and the elongation of young tissues and involve the turnover and de novo synthesis of macromolecules. Germinating seeds in saline media exhibit a lowered and delayed production of radicle and plumule [54–56]. Sodium chloride affects the emergence of young tissues more adversely than other salinities [30,40,58].

Establishment of Seedlings

A successful crop stand depends on the establishment of young seedlings. Prolonged exposure to substrate salinity results in an extremely poor stand [14] caused by seedling mortality [42]. This may be more pronounced in the case of glycophytes owing to their high sensitivity to salinity [65]. A

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TABLE 1 Effect of Different Types of Salinity on the Process of Germination of Seeds and Propagules

Germination event	Salinity applied	Response elicited	Reference
Imbibition	NaCl	Reduced hydration of embryo and cotyledon.	6,16,40,42,43
	$NaCl + Na_2SO_4 + CaCl_2\ MgCl_2$	Reduced water uptake.	44
Active metabolism	NaCl	Disaggregation of intermembrane particles: leakage of solutes; reduced mobilization of reserves; inhibited activities of carbohydrates and fatty acid metabolism enzymes; altered pattern of protein synthesis; production of osmotically active solutes.	9,10,16,19,45–50
Emergence and elongation of embryonic tissues	NaCl	Delayed and reduced emergence of radicle and plumule; reduced elongation of embryonic tissues.	5,16,22,36,40,48,51–55
	$CaCl_2$	Reduced emergence of seedlings.	41
	$Na_2SO_4 + CaCl_2 + MgCl_2 + NaCl$	Inhibited radicle emergence.	56
	$NaCl + Na_2SO_4 + MgCl_2 + CaSO_4$ (ψ from -2.5 to -15.0 bars)	Delayed emergence and final suppression of embryonic tissues growth.	57
	$NaCl + CaSO_4$	Hampered emergence of cotyledon.	58
	Sea water	Delayed rate and emergence of seedlings.	3
	$NaCl + Na_2SO_4 + MgSO_4$	Inhibited growth of seedlings.	59
Establishment of seedlings	NaCl	Enhanced seedling mortality; reduced seedling growth.	40,42,48
	$NaCl + Na_2SO_4 + MgCl_2 + CaSO_4$ (ψ from -2.5 to -15.0 bars)	Reduced growth, vigor and establishment of seedlings.	57

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good stand of crop was achieved in *Sorghum halepense* under mild salinity owing to the rapid rate of germination [57]. Plants having higher seedling vigor also show better stand under salinity [66].

GERMINATION OF SEEDS UNDER SALINITY

Germination and Salinity—Osmotic or Toxicity Effect?

Germination and salinity interaction is often studied on the premise that it has dual action; that is, osmotic and toxic actions [67]. Attempts to separate both the components of salinity by using isotonic solutions of salts and nonpermeating osmotica yielded conflicting data [43,68]. Some regard the osmotic effect as the crippling factor [22,27], whereas the majority consider ion toxicity as being a noxious component [7,9,42,64], or that both the components are equally detrimental to germination [57,69]. Wahid et al. [16] reported that incubation of seeds in salt solution followed by reduced germination in water gave credence to the major role of ion toxicity.

Metabolism of Stored Materials

Seeds, whether from monocots or dicots, comprise of storage tissue (endosperm and cotyledons respectively) and an embryo. The nature and extent of stored materials may be different in different species. Major stored materials include proteins, sugars, and oils, whereas nucleic acids, plant growth regulators, nitrogenous compounds other than proteins, and some nutrients may form a small component [1]. Salt stress hampers the metabolism of stored materials and the growth of the embryo. At the onset of germination, synthesis of enzymes and changes in the metabolic pattern are initiated [24], but salt stress either alters it or does not permit the synthesis of specific metabolites required for germination [7–9]. Application of salinity hampers the utilization and mobilization of materials required for the production of seedlings by affecting the enzymatic activities of seed essential for these reactions (Table 2).

Proteins

Salinity creates an impact on the activities of the enzymes for protein metabolism [9,47]. Protease, which catalyzes the turnover and solubilization of proteins to soluble nitrogen in seed is largely inhibited by salinity [38,62]. It interferes with the incorporation of [^{3}H]leucine and [^{35}S]methionine during protein synthesis in the wheat embryo [10,43] and modulates the production of a selected group of proteins not synthesized otherwise [10]. Ramagopal [50] found the synthesis of qualitatively and quantitatively different eight new proteins in germinating barley embryo under salt stress and seven during recovery.

Carbohydrates

Carbohydrates (as starch) constitute a major bulk of storage material in some seeds (e.g., caryopses). Amylases mainly regulate the metabolism of carbohydrates, and their activity is greatly attenuated by salinity. The activity of α-amylase is reduced under salinity in a concentration-dependent manner, depressing the growth of seedlings [34]. Greater salt tolerance of sorghum during germination was attributed to the enhanced activity of α-amylase [62]. Salt-treated lentil seeds indicated no variation in different solute contents; however, the activity of α-galactosidase increased and caused the accumulation of sucrose, galactose, and mannose in the embryonic tissues [68]. An accumulation of osmotically active sugars and proline was noted in different plants [16,52], which played an important role during and after relief from salinity.

Nucleic Acids

The most important factor in nucleic acid metabolism is the synthesis and activation of ribonuclease (RNase). Salinity delays the de novo synthesis and/or activation of RNase in *Vigna* cotyledons due

TABLE 2 Physiological and Biochemical Changes in Germinating Seeds Under Salinity

Metabolic activity	Effect of salinity	Reference
Proteins	Toxic to the protein phosphatase and protein kinase specific activities; strong inhibition of methionine and leucine uptake and incorporation into proteins chain; de novo synthesis of eight new heat-shock proteins during stress and seven during recovery; a major change in protein phosphorylation-dephosphorylation and a depression in seedling growth.	9,10,13,43,47,50
Carbohydrates	Reduction in endospermic α-amylase activity in a concentration-dependent manner; promotion of cotyledonary α-glycosidase activity; decrease in reducing and nonreducing sugars; accumulation of sugars as osmotica.	13,35,52,68
Nucleic acids	Slow rise in activity or inhibited de novo synthesis of RNase; inhibition of incorporation of precursor into nucleic acids reflecting their suppressed biosynthesis; decrease in DNA level throughout germination period; condensed chromatin material.	7,43,46
Lipid	Reduction in the activity of glyoxysomal enzymes; reduction in utilization of total lipids and triacylglycerol.	49
Polyamine	Induction of putrescine synthesis with the activation of arginine decarboxylase; no effect on spermine; significant increase in spermidine; accumulation of total polyamines.	32,70,71
Other compounds (nitrogenous and nonnitrogenous)	Increase in betaine aldehyde dehydrogenase; rise in betaine content; progressive accumulation of free proline and sugars and soluble proteins; decrease in the level of soluble amino nitrogen.	6,16,40,52,63

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to its toxic effect [7]. A slight increase in the cotyledonary RNA level during the first day of germination was noted, but it decreased subsequently up to 7 days; however, DNA decreased continuously during this period [46]. Petruzzelli et al. [43] suggested that the suppression of nucleic acid biosynthesis in wheat embryo was due to salt-induced inhibition of the incorporation of precursors into nucleic acids.

Lipids

Glyoxysomal enzymes are responsible for the metabolism of stored lipids. Salinity exerts an inhibitory effect on glyoxysomal catalase, malate synthase, and iso-citrate lyase, decreasing the levels of triacylglycerol, diacylglycerol, and monoacylglycerol and increasing the levels of free fatty acids and polar lipids [49].

Polyamines

Polyamines have recently gained importance in the escape of seedlings from the adverse effect of salinity. They promote seedling growth by the production of ethylene-forming enzymes [32]. Lin and Kao [70] reported an increase in the level of spermidine under salinity but a low level of putrescine in the shoot and roots of rice seedlings. Accumulation of putrescine and spermidine, with the activity of arginine decarboxylase in rice seedlings, plays a specific role in salt tolerance owing to production of ethylene [71,72].

Other Organic Compounds

Various endogenous compounds are differentially metabolized during germination and seedling growth. The glycine betaine, a compatible solute, either disappears [6], exhibits no change [40], or accumulates as a result of salt-stimulated betaine aldehyde dehydrogenase activity and rescues the seed from the adverse effect of salt [63]. Similarly, the rise in the level of free proline and soluble sugars of seeds or seedlings also plays a beneficial role [6,16,52].

Seed Nutrients

The higher content of seed nutrients is of vital importance for germination, but salinity suppresses their role in the metabolism of seed and the production of seedlings [73]. During germination of sorghum caryopses, a higher content of potassium, calcium, phosphorus, and nitrogen was partitioned into the plumule and radicle as a strategy of tolerance to salinity [16]. Guerrier [74] attributed the reduced salt tolerance of tomato to its inability to accumulate and transport lower amounts of calcium and potassium.

GERMINATION OF PROPAGULES UNDER SALINITY

The initial events of propagule germination may be different from seeds. However, bud activation, elongation, and establishment of seedlings seem almost similar. Germination of sugar cane sets (stem cuttings) exhibited significant reduction in the rate and percentage of germination due to NaCl damage [55]. These plants had an enhanced content of Na^+ and Cl^-, a concomitantly reduced content of potassium, calcium, nitrogen, and phosphorus and reduced elongation and dry matter of seedlings. Citrus rootstocks used to raise plantlets had a negative correlation of Cl^- with certain nutrients [75]. Resting buds of salt-stressed poplar plant, grown in vitro, did not accumulate glycinebetaine and proline and thus had reduced growth of seedlings [76]. Similarly, tubers of hydrilla indicated the signs of salt damage and reduced germination [77]. There is a dearth of information particularly about the salt tolerance of propagules during germination.

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REGULATION OF IONS IN SEEDS AND SEEDLINGS

Exposure of seeds or seedlings to salinity results in the influx of ions with the imbibition of water, which exerts an adverse effect on the growth of embryo [6,78]. This may lead to a marked decrease in the internal potassium concentration [43], a vital nutrient for protein synthesis and plant growth [79]. Seedlings exposed to salinity are highly prone to excessive ions, sometimes leading to their death shortly after emergence [42,80]. The ability of plants to cope with ion toxicity is principally related to the greater transport of ions to shoot. Grasses show a strategy of salt tolerance by storing toxic ions in the mesocotyl up to a certain limit [81,82]. This has significance in that the epicotyl and hypocotyl avoid ion toxicity, thus ensuring their better growth [16].

STRUCTURAL CHANGES IN SEEDS AND SEEDLINGS UNDER SALINITY

Salinity triggers structural changes at various levels of organization in seeds and seedlings (Table 3). At the subcellular level, major changes were found in (a) nuclear chromatin, which was condensed indicating suppressed nucleic acid biosynthesis; (b) formation of small provacuoles instead of single large vacuole; and (c) damaged mitochondrial apparatus and reduced oxygen uptake [5,43]. Salinity caused the contraction of plasmalemma away from the cell walls [43], which may be due to disaggregation of intramembranous particles [45]. The cell wall of salt-treated cotton roots and the sorghum mesocotyl became considerably thickened [16,17].

At cell and tissue levels, the salinity reduced the cortical cell area and as a result the mesocotyl of sorghum was considerably constricted and appeared to act as a repository of ions [16]. Furthermore, there was the induction of exodermis with a casparian band having suberin lamellae close to the root base and in the transition zone of the hypocotyl of cotton [17]. This protected the root from water loss and/or leakage of solutes important for osmotic adjustment. Salinity also stimulated the development and lignification of secondary tissues and enhanced the number of water-storage cells in the epidermis and cortical layer of the hypocotyl [15].

TABLE 3 Salt-Induced Changes in Anatomical Characteristics During Germination in Various Tissues

Level of organization	Salt-induced change	Reference
Subcellular	Formation of small provacuoles in coleorhiza cells.	5
	Diffusion and condensation of chromatin material in embryo.	5,43
	Reduced size of plasmalemma and mitochondria.	43
	Lignification and thickening of cell wall.	16,17
Cellular	Reduced size of cortical cells in mesocotyl of sorghum.	16
	Induction of endodermis with Casparian band and suberin lamellae close to root base.	17
Tissue	Earlier development and differentiation of secondary xylem in hypocotyl.	17
	Constriction of cortical tissue of mesocotyl.	16
	Increased lignification of secondary tissues.	15

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FACTORS INTERACTING WITH SALINITY DURING GERMINATION

Plant Factors

Seed Coat

The seed coat is the first barrier to the entry of water and ions into the seed. The hard and thick seed coat offers resistance to the entry of water into the seed and minimizes the contact of ions with the embryo. It also acts as a buffering agent to ionic toxicity [6,18] and enhances germination.

Dormancy

One of the primary impacts of salinity is the induction of dormancy in the seed due either to inhibition of the synthesis of nucleic acids [83] or plant growth regulator imbalance [11]. Although dormancy carries no consistent relationship with salinity [84], it is important for the halophytes, since it permits the seed to remain viable for the period until the environment becomes conducive to germination [24,85].

Seed Age

Aging or prolonged storage of a seed affects its germinability [86]. This has been used to test and predict the salt-tolerance potential during germination. Smith and Dobrenz [19] found a strong negative relationship between salt tolerance and seed age in a sensitive but a significant decline in the solute leachate during imbibition of water in a tolerant alfalfa genotype.

Seed Polymorphism

The seed size of a species also shows a differential response to salinity [87]. The greater the seed size, the greater is the salt tolerance [20,88]. Smaller seeds containing a higher amount of toxic ions and a low amount of reserves per unit weight show higher dormancy-delayed germination and reduced weight of seedlings [21].

Seedling Growth and Vigor

Seedling growth and vigor is an important factor for the establishment of plants. Root growth, being the most important factor, determines the establishment of a stand under salinity [34,53]. This problem may be partially solved either by using a higher seed rate to obtain high seedling density or by selecting the crop for high seedling vigor, especially in arable farming [22].

Environmental Factors

Temperature

A slight variation in temperature may change the germination greatly. The adverse effect of high salinity is further aggravated by higher temperature regimens, which may prolong the time taken for emergence of seedling [24,89]. However, a synergistic effect of low temperature and high salinity has been noted in halophilic barley seeds [90].

Light

Light has a profound effect on the germination of many species [91]. The light may be effective in breaking the dormancy and promoting germination in some halophyte species. This may result in better establishment of a stand in marginally saline areas [3,24].

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Water

Salt-induced lowering in the water potential of the germination medium enhances toxicity, whereas scarcity of water further aggravates it. Major interaction of water stress under salinity conditions includes a differential pattern of protein synthesis [43], delayed emergence of embryonic tissues [57], and a decrease in final rate and percentage of germination [92]. The supply of water to seeds or seedlings reverses these processes to a great extent [90,93], as it minimizes ionic toxicity.

Oxygen

Salt-induced dormancy reduces the availability of oxygen to the embryo for metabolic activities. High salinity coupled with hypoxia significantly reduces both emergence and elongation of the radicle and plumule [26]. Anoxia completely restricted the process of germination; however, no specific difference was discernible with respect to salinity and anoxia tolerance. *Spartina alterniflora* could better tolerate salinity and anoxia than *Phragmites australis*, as the former showed a rapid rate of coleoptile and mesocotyl growth [54].

ALLEVIATION OF SALT STRESS

Various chemical agents have been employed to ameliorate the adverse effects of salts. The plant-growth regulators are the most widely used. Some nitrogenous compounds, sugars, and certain nutrients have also been employed (Table 4).

Implication of Plant Growth Regulators

Both naturally derived and synthetic plant-growth regulators have been employed separately or in combination [2,31,97]. Kahn [27] suggested that primary action of osmotic inhibition is the reduction in water uptake, and plant-growth regulators may offset this inhibition and promote the process of germination. Presoaking in gibberellin after salt stress releases the seed from physiological dormancy [11,29], enhances water uptake, mobilizes starch [35], and improves the rate and percentage of germination. Auxins like indole butyric acid and indole acetic acid promote germination and seedling growth better than kinetin by eliminating osmotic effect of salinity [94].

Bozcuk [29] reported that kinetin releases the seed from salt-induced dormancy and enhances seed protein synthesis. Kinetin applied to salt-stressed seeds promotes germination by enhanced production of pregermination ethylene with the synthesis of 1-aminocyclopropane-1-carboxylic acid [31,95]. The use of kinetin in combination with gibberellin not only promotes germination but also stimulates the growth of seedlings under salinity [30]. It is likely that salinity suppresses the endogenous level of plant-growth regulators, and their exogenous supply fulfills this requirement for the initiation of germination.

Polyamines

The polyamines compete with the ethylene pathway, as S-adenosylmethionine is a common precursor [98]. The exogenous application of putrescine to seed not only counteracts the adverse effect of salinity, but also induces tolerance up to the seedling stage owing to their de novo synthesis in response to salinity [71]. Lin and Kao [70] found an increase in the level of putrescine by exogenously applying the precursors of putrescine biosynthesis (L-arginine and L-ornithine), but they did not induce a significant mitigation of salt toxicity.

Other Organic Compounds

Some organic chemicals have also been employed to lessen the adverse effect of salinity. Application of sucrose and glucose partially reverses the salt-inhibition of germination [35]. Noor and Khan

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TABLE 4 Role of Organic and Inorganic Chemical Agents in the Alleviation of Adverse Effects of Salinity

Chemical agents	Class	Role in alleviation of salt stress	Reference
Growth hormones	Gibberellin	Counteracting inhibition of α-amylase activity in seed; breaking dormancy; stimulating rate and percentage of germination; promotion of seedling growth and development.	11,19,28,35,94
	Auxins	Breaking dormancy; eliminating osmotic inhibition; enhancing water uptake.	25,83,94
	Cytokinin	Breaking dormancy; enhancing protein synthesis; promoting radicle emergence; offsetting salt damage; increased water uptake.	11,30,25,29,31,83,94
	Ethylene	Promoting germination by its endogenous production.	31,95
Other organic agents	Fusicoccin	Counterinhibiting decreased water potential; enhancing protein synthesis; promoting seedling growth.	5,96
	Polyamines	Stimulating germination by their endogenous biosynthesis; enhancing salt tolerance; promoting seedling growth.	32,70,71
	Glutamine and glutamic acid	Increasing glutamine synthase and glutamine synthetase activity and counteracted growth inhibition due to salt.	34
	Thiourea	Reversal of salt-inhibited germination.	33
	Sugars	Promoting seedling growth.	6
	Proline	Enhancing hypocotyl growth; promoting seedling growth.	6,35
Inorganic agents	Calcium	Promotion of membrane activity; stimulating plumule emergence.	5,23,36,37,92
	Magnesium	Counterinhibition of root growth; ameliorating Na^+ toxicity.	23
	Ammonium	Promoting protease activity and enhanced solubilization of endospermic proteins.	38
	Nitrate	Higher counteractive effect of salinity than calcium; enhancing germination and seedling emergence.	37,38
	Potassium	Counterinhibition of root growth.	23

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[33] reported the efficacy of a low amount of thiourea in breaking salt-induced dormancy. The application of glycinebetaine and proline increased the root growth but did not affect the hypocotyl growth [6]. Fusicoccin also counteracts the inhibitory effect of NaCl on the process of germination by cell wall loosening, promoting transport activity, incorporating amino acids in the protein chain [96], and enhancing potassium uptake owing to stimulation of proton efflux [25]. Addition of methionine sulfoximine was found to reduce the seedling growth of salt-stressed plants, which was reversed by stimulation of glutamine synthetase and glutamate synthase activity, and with the addition of glutamine and glutamic acid to the growth medium [34].

Inorganic Agents

Calcium has been extensively used to alleviate salt toxicity because of its crucial role in the maintenance of membrane processes, modulation of enzyme activities, and buffering of Na^+-toxicity [18,36,38]. Although presoaking of seed in $CaCl_2$ does not initiate germination, its application to a saline medium significantly improves the rate and percentage of germination [37]. In addition, supplying calcium stimulates plumule emergence [5] and root growth as well [23].

Application of ammonium promotes the protease activity in the endosperm, whereas nitrate enhances the seed germinability [37,38] owing to the solubilization and availability of catabolites for the synthesis of embryonic structures [38]. Furthermore, the use of potassium and magnesium also counteract the NaCl inhibition of the root growth of rice seedlings [23].

CONCLUSIONS

All the events of germination starting from imbibition of water to the establishment of seedling are adversely affected by increased levels of salinity. It cripples the rate and percentage of germination, partially through the osmotic effect on the imbibition of water and is mainly due to its toxicity to the metabolism of seed reserves. Salinity also induces structural changes at subcellular, cellular, tissue, and organ levels and affects the rate of respiration, transport of materials, and induction of new tissues in the seeds or seedlings.

Certain internal and external factors substantially interact with the germination and seedling growth under salinity. The seed coat minimizes the access of ions to the embryo. Dormancy allows the halophytes to escape the adverse effect of salinity. Aging has been used to test seed viability and to predict salt-tolerance ability. Seeds of large size exhibit greater germination and seedling vigor because of a higher content of seed reserves and absorb low content of toxic ions per unit weight. Water and temperature stresses further aggravate the impact of salinity on germination, whereas light may break dormancy in certain halophytes. Salinity-induced dormancy creates an anoxic condition and inhibits seed germination. The seedlings with vigorous growth may escape salinity successfully.

The osmotic and toxic effects of salinity can be successfully alleviated with the help of plant-growth regulators, polyamines, sugars, and certain nutrients. Among them the plant-growth regulators and some nutrients, including calcium, ammonium, nitrate, potassium, and magnesium, have been successfully used to promote the process of germination.

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Crop Response and Management of Salt-Affected Soils

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INTRODUCTION

Salinity is a major factor reducing plant growth and productivity throughout the world [1]. Approximately 10% of the world's 7×10^9 ha arable land surface consists of saline or sodic soils. The percentage of cultivated lands affected by salts is even greater. Of the 1.5×10^9 ha cultivated lands, 23% are considered saline and another 37% are sodic. Although the data are tenuous, it has been estimated that one-half of all irrigated lands (about 2.5×10^8 ha) are seriously affected by salinity or waterlogging [2]. Historically, soil salinity contributed to the decline of several ancient civilizations [3]. Despite the advanced management technologies available today, salinization of millions of hectares of land continues to reduce crop production severely in the United States and worldwide [4]. The National Academy of Sciences [5] includes salinization of soils and waters as one of the leading processes contributing to a worldwide biological catastrophe.

Sustained and profitable production of crops on salt-affected soils is possible if appropriate on-farm management decisions are made. To be successful, growers require an understanding of how plants respond to salinity, the relative tolerances of different crops and their sensitivity at different stages of growth, and how different soil and environmental conditions affect salt-stressed plants. This chapter discusses the effects of soil and water salinity on agronomic and horticultural crop plants, presents data on the tolerance of crops to salinity, and considers consequences of various cultural and management practices on crop yield responses.

PLANT RESPONSE TO THE SOIL ENVIRONMENT

Saline Soils

All soils contain a mixture of soluble salts, some of which are essential for plant growth. When the total concentration of salts becomes excessive, plant growth is suppressed. The suppression increases

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as the salt concentration increases until the plant dies. Although all plants are subject to stunting, their tolerance threshold and the rate of growth reduction at concentrations above the threshold vary widely among different crop species. Growth suppression seems to be a nonspecific salt effect that is directly related to the total concentration of soluble salts or osmotic potential of the soil water. Within limits, isosmotic concentrations of different combinations of salts cause nearly equal reductions in growth. On the other hand, single salts or extreme ion ratios are likely to cause specific ion effects; namely, ion toxicities or nutritional imbalances. Since saline soils in the field generally consist of a mixture of different salts, specific ion effects are minimal and osmotic effects predominate. Some exceptions to this generalization exist. Woody fruit and nut crops tend to accumulate toxic levels of Cl^- or Na^+ that cause leaf burn, necrosis, and defoliation. Some herbaceous crops, such as soybean, are also susceptible to ion toxicities, but most do not exhibit leaf-injury symptoms even though some accumulate levels of Cl^- or Na^+ that cause injury in woody species.

The relative contribution of osmotic effects and specific ion toxicities on yield are difficult to quantify, however. With most crops, including tree species, yield losses from osmotic stress can be significant before foliar injury is apparent. Reports that citrus yield reductions occur without excessive accumulations of Cl^- or Na^+ and without apparent toxicity symptoms indicate that the dominant effect is osmotic [6–11]. However, salts tend to accumulate in woody tissues over several years before toxic symptoms appear; consequently, the effects of leaf injury and loss can occur dramatically when the salts reach the leaves. When specific ion toxicities occur, the effects on yield are generally additive with the growth-suppressive effects of osmotic stress. Besides causing specific toxic effects, salinity can induce nutritional disorders in plants [12,13]. Some specific nutrient deficiencies or imbalances, which vary among species and even among varieties of a given crop, are described later in this chapter and by Grattan and Grieve [14].

Sodic Soils

Sodic soils, previously called alkali soils, contain excess exchangeable Na^+, with 15% or more of the cation-exchange sites in the soil being occupied by Na^+ [15]. These soils may be either saline or nonsaline depending on the concentration of salts present in the soil solution. In nonsaline-sodic soils, the total salt concentrations are low, and this, coupled with high ratios of exchangeable Na^+ to Ca^{2+} and Mg^{2+}, can lead to Ca^{2+} and/or Mg^{2+} deficiencies. These deficiencies, rather than Na^+ toxicity, are frequently the cause of poor growth among nonwoody species. In contrast, saline-sodic soils contain higher concentrations of Ca^{2+} and Mg^{2+} and may therefore remain nutritionally adequate. With saline-sodic soils, salinity effects predominate and the nutritional effects of sodicity are usually absent.

In addition to the nutritional imbalances encountered in sodic soils, the hydraulic conductivity and permeability of both water and air are significantly affected by the deterioration of the soil physical condition caused by the high exchangeable Na^+ content. To alleviate the poor permeability of these soils, the electrolyte concentration in the soil water must be increased. This is accomplished by the addition of gypsum ($CaSO_4$), sulfuric acid, or acid-forming compounds to the soil or irrigation water [16]. The acid and acid-forming compounds react with the soil lime ($CaCO_3$) to release Ca^{2+} into the soil solution. The use of gypsum and the importance of Ca^{2+} in relation to sodic soils and their reclamation have been extensively reviewed by Oster [17] and Rengasamy [18].

Soil Fertility

Plants grown on infertile soils may appear to be more salt tolerant than those grown with adequate fertility. This is because inadequate nutrition depresses yields more under nonsaline than under saline conditions [19,20]. When fertility is low, proper fertilizer applications increase yields regardless of the soil salinity, but proportionally more if the soil is nonsaline [21]. When both salinity and fertility limit yields, decreasing salinity or increasing fertility is beneficial.

Despite some claims to the contrary, fertilizer applications exceeding those required on nonsa-

line soils do not increase the salt tolerance of plants. Unless salinity causes certain nutritional deficiencies or imbalances, excess applications of N, P, or K rarely alleviate the inhibition of growth by salinity [14]. In fact, additional fertilizer adds to the salinity already present in the soil profile and may aggravate salt injury.

Irrigation Water Quality and Management

The principal criteria to determine irrigation water quality are salinity, sodicity, and specific ion concentrations. However, the effects on crops of a given water are not determined solely by its solute composition. These water quality factors should be considered in relation to the specific conditions under which the water is to be used [22,23]; that is, soil properties, irrigation methods, cultural practices, climatic conditions, and the crop to be grown.

Salinity control is frequently a major concern of irrigation management even though the primary objective of irrigation is to maintain soil water in a range suitable for optimum crop yield. To avoid plant water stress, saline soils should be irrigated when the soil water content is appreciably above the permanent-wilting percentage of the soil, as determined under nonsaline conditions. Plant water stress is a function of total soil water potential, which includes both matric and osmotic potential components. As the soil dries, the matric potential decreases, and because the salts are concentrated, the osmotic potential also decreases, further decreasing the total soil water potential.

The extent of permissible water depletion for a given crop is determined by the maximum acceptable salt concentration for that crop [24]. When additional water depletion occurs and no irrigation water is applied to recharge the root zone and dilute this concentrated soil water, yield is reduced. Therefore, increased irrigation frequency is generally required under saline conditions [2]. With shorter irrigation intervals, the concentrating effect for evapotranspiration on soil salinity is minimized [25,26].

Evidence indicates that plants respond primarily to the soil salinity in that part of the root zone with the highest total water potential [25,27]. With more frequent irrigations, this zone corresponds primarily to the upper part of the root zone, where soil salinity is influenced primarily by the salinity of the irrigation water. With infrequent irrigations, the zone of maximum water uptake becomes larger as the plant extracts water from increasingly saline solutions at greater depths.

In soils that are not well drained, the frequency and amount of irrigation water must be closely monitored. Application of excess water over that required for the crop and for leaching should be avoided. Not only are valuable nutrients lost with overirrigation, but flooded or poorly drained soils suffer from poor aeration, which may affect the crop's response to salt stress. Studies have shown that low levels of oxygen interact with salinity to affect shoot growth of tomato [28]. If drainage is inadequate, a shallow water table may develop, which can directly affect the crop response. Plants can extract water directly from this source and, depending on the quality of the water, respond much differently than expected from the level of salinity in the soil.

Most irrigation waters contain more salts than are removed by the crop, so that continued irrigation without leaching progressively salinizes the land. Water in excess of consumptive use (evapotranspiration) must therefore be applied to carry the residual salts out of the root zone. In addition, soils must be sufficiently permeable to allow the extra water needed for leaching to infiltrate in a reasonable time. In practice, it is usually necessary to grow crops for which evapotranspiration is sufficiently less than attainable infiltration to achieve the necessary drainage and salinity control.

Previous studies have shown that salt can be stored in the lower portion of the root zone with only moderate yield reduction, provided the upper portion of the root zone is maintained relatively free of salinity [27,29]. With most irrigation waters and crops, regularity of leaching is not critical. Even when salinities in the lower root zone approximate the tolerable limit for a crop, leaching intermittently can be as effective as leaching every irrigation [25].

Sensitive crops require the drainage of larger percentages of applied water from the root zone to maintain soil water concentrations within tolerable limits. Generally stated, the leaching requirement is inversely proportional to the salt tolerance of the crop [24].

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The goal of irrigation management should be increased irrigation efficiency to reduce the amount of infiltrated water that is not used by the plant but passes beyond the root zone as deep percolation. The irrigation reuse of this water and the disadvantages of blending this water with low-salinity water for reuse has been thoroughly reviewed by Rhoades and colleagues [30–32].

PLANT RESPONSE TO CULTURAL PRACTICES

Planting Patterns and Population Density

Failure to obtain a satisfactory stand of furrow-irrigated row crops planted on raised beds is a serious problem in many places. The practice of planting a single row in the center of the bed has frequently resulted in poor seed germination even when the soil is only slightly saline at the time of planting. This is because the wetting fronts from both furrows transport salt in the soil to the center of the bed, where it accumulates. Therefore, whether a single row or double row bed is used, the seed row should be planted near the bed shoulder, where the salt accumulation is the lowest. Another method used to minimize salt accumulation when using single-row beds is to irrigate alternate furrows, so the wetting front carries the salt beyond the seed row to the nonirrigated side of the bed.

With either single- or double-row plants, increasing the depth of water in the furrow can also improve germination in salt-affected soils. Salinity can be controlled even better by using sloping beds, with the seed row planted on the slope just above the irrigation water line. Irrigations move the salt past the seed row to the peak of the bed with this method. Planting in furrows is satisfactory from the standpoint of salinity control but may cause emergence problems from soil crusting or poor aeration.

Increasing plant populations in cotton has been shown effectively to lessen the yield reduction associated with salinity [33,34]. Since nearly all crops are stunted to some degree by salinity, a large portion of the field is without canopy cover. When canopy closure is incomplete and solar radiation is lost to the soil, potential yield is lost. Increasing the number of plants per unit area by decreasing row width compensates for the smaller plant size [33,34]. In contrast, reducing intrarow spacing of cotton showed no effect in maintaining yield [34].

Irrigation Methods

The response of crops to soil and water salinity depends on the method of irrigation and the frequency of water application [35–38]. Numerous irrigation systems are used to apply water to crops, but except for minor variations, they all fall within one of the following categories: gravity, sprinkler, or drip. The differences in water distribution by these systems directly affect the distribution of soil salinity in the root zone. In flooded or fully sprinkled soils, water and salt movement is essentially downward, or one dimensional. In furrow-irrigated soils, water flow is two dimensional; that is, both downward and lateral. When water is applied in small flooded basins or by minisprinklers or drip emitters, flow is three dimensional. This method is used primarily with tree or vine crops. Because water and salt move radially away from the source, salts tend to accumulate at the periphery of the wetted zone. This concentration of salts at the outer edges of the root zone can be a problem for plants when winter rains wash the salts back into the root zone.

Crops irrigated with sprinkler irrigation are subject to injury not only from salts in the soil but also from salts absorbed directly through wetted leaf surfaces [39]. In tree crops, the extent that leaves are wetted can be minimized by sprinkling under the canopy. However, even with undercanopy sprinklers, severe damage of the lower leaves can occur [40]. The extent of foliar injury depends on the concentration of salt in the leaves, but weather conditions and water stress can influence the onset of injury. For instance, salt concentrations that cause severe leaf injury and necrosis after a day or two of hot, dry weather may not cause any symptoms while the weather remains cool and humid. Numerous factors affect the amount of salt accumulated by leaves, including the leaf age, shape, angle, and position on the plant, the type and concentration of salt, the ambient temperature

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TABLE 1 Relative Susceptibility of Crops to Foliar Injury from Saline Sprinkling Waters: Na or Cl Concentration ($mmol_c\ L^{-1}$) Causing Foliar Injury[a]

<5	5–10	10–20	>20
Almond	Grape	Alfalfa	Cauliflower
Apricot	Pepper	Barley	Cotton
Citrus	Potato	Corn	Sugar beet
Plum	Tomato	Cucumber	Sunflower
		Safflower	
		Sesame	
		Sorghum	

[a] Susceptibility based on direct accumulation of salts through the leaves. Foliar injury is influenced by cultural and environmental conditions. These data are presented only as general guidelines for daytime sprinkling.
Source: Data compiled from Refs. 38 and 41–44.

and humidity, and the length of time the leaf remains wet. In addition, the leaf surface properties, such as a waxy cuticular layer or pubescence, may restrict ion absorption.

Susceptibility to foliar injury varies considerably among crop species (Table 1). A comparative study by Maas et al. [44] with 11 herbaceous species revealed wide differences in the rates of Na^+ and Cl^- absorption when the plants were sprinkled with saline water. Leaves of deciduous fruit trees (almond, apricot, and plum) appear to absorb Na^+ and Cl^- even more readily than herbaceous crops [41]. Citrus leaves absorbed these ions more slowly but in amounts adequate to cause severe leaf burn [40].

Francois and Clark [42] reported a linear increase in Na^+ and Cl^- concentration in grape leaves when sprinkled with saline water. When Cl^- is readily absorbed directly by the leaves, chloride-resistant grape rootstocks that reduce Cl^- uptake by the roots would be of little benefit with sprinkler irrigation.

If sprinkler irrigation must be used, then good water management is essential. Since foliar injury is related more to frequency of sprinkling then duration [42,43], infrequent, heavy irrigations should be applied rather than frequent, light irrigations. Slowly rotating sprinklers that allow drying between cycles should be avoided, since this increases the wetting-drying frequency. Sprinkling should be done at night or in the early morning when evaporation is less. Hot, dry, windy days should be avoided. In general, poorer quality water can be used for surface-applied irrigation than can be used for sprinkler irrigation.

PLANT RESPONSE TO THE AERIAL ENVIRONMENT

The influence of environmental factors significantly affects the response of plants to salinity. Most crops can tolerate greater salt stress when the weather is cool and humid than when it is hot and dry. Magistad et al. [45], working with identical soil salinities, showed that crops grown in a coastal climate (cool and humid) consistently produced higher yields than those grown in a desert climate (hot and dry). Hoffman and Rawlins [46] reported that the salt tolerance of kidney beans grown with cool temperatures and high relative humidity was more than double that obtained with high-temperature, low-humidity conditions.

These factors also affect the expression of specific salt-injury symptoms. Fruit crops and woody plants, susceptible to leaf injury by excess Cl^- or Na^+ accumulation, often develop leaf necrosis with the onset of hot, dry weather in late spring or early summer [47]. Ehlig [48] reported

similar results with grapes, which showed no leaf-injury symptoms during cool, cloudy spring weather even though the leaves contained levels of Cl^- considered toxic.

Although high humidity has been shown consistently to improve growth under salt stress [49], temperature is believed to be the dominant factor in plant response to saline conditions [50]. Other studies have confirmed that temperature influences plant salt tolerance to a greater degree than relative humidity [46,51].

Light intensity has also been implicated in growth reduction caused by salinity. Studies have shown that growth depression from salinity is generally greater under higher than under low-light intensities [52–54]. With citrus, leaf toxicity symptoms are frequently observed on the south side of trees in response to higher light intensities, whereas leaves on the north side may remain symptom free [55].

It is likely that at least part of the reduction in plant growth on saline media is a result of increased transpiration, since high temperature, low relative humidity, and exposure to light are conditions that favor a high rate of transpiration. This may explain why some crops grown outside, where these environmental conditions exist, are more salt sensitive than when the same crop is grown in the greenhouse.

Ozone, a major air pollutant, decreases the yield of some oxidant-sensitive crops more under nonsaline than saline conditions [56–59]. This aberration has the tendency to make many crops grown in air-polluted regions appear to be more salt tolerant than they really are. This salinity-ozone interaction may be agronomically important in air-polluted areas. However, the increased ozone tolerance induced by salinity may be more than offset by the detrimental effects of salinity on the harvestable product [57,58,60].

In contrast to ozone, higher CO_2 concentrations in the atmosphere have been shown to increase the salt tolerance of bean, corn, and tomato [61,62]. This increased tolerance is believed to be the result of an increased rate of photosynthesis [63].

PLANT RESPONSE IN RELATION TO BIOLOGICAL FACTORS

Stage of Growth

Information about the salt tolerance of crops at different stages of growth is extremely limited. Most salt-tolerance data have been obtained from studies in which salinity was relatively constant from seeding to harvest or from the late seedling stage to harvest. These studies provided no information about the salt sensitivity or tolerance at individual stages of growth.

What data are available generally agree that the early seedling stage of growth is the most salt sensitive for most crops [64–68]. It is during this stage of growth with cereal crops that leaf and spikelet primordia are initiated and tiller buds are formed [69]. Consequently, high soil salinity during this stage can severely affect final seed yield.

Although salt stress delays germination and emergence, most crops are capable of germinating at higher salinity levels than they would normally tolerate at the vegetative or reproductive stages of growth [69]. However, this high tolerance is of little benefit when the plants are so much less tolerant during the following seedling stage.

It is generally agreed that after the seedling stage, most plants become increasingly tolerant as growth proceeds through the vegetative, reproductive, and grain-filling stages. Rice may be an exception. Pearson and Bernstein [70] reported that rice yields are significantly reduced if salt stress is imposed at either the seedling stage or during pollination and fertilization. However, a subsequent study by Kaddah [65] did not confirm the salt sensitivity at this latter stage of growth. Increased tolerance with age has also been observed in asparagus, a perennial crop that is much more tolerant after the first year's growth [71].

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Influence of Rootstocks

The tolerance of many fruit trees and vine crops can be significantly improved by selecting rootstocks that restrict Cl^- and/or Na^+ accumulation. The Cl^- tolerance levels presented in Table 2 indicate the maximum Cl^- concentrations permissible in soil water that do not cause leaf injury. However, yield of some crops may be decreased without obvious injury symptoms when the osmotic thresholds of the rootstocks are less than these limits.

Although citrus is not considered very salt tolerant, there are differences in salt tolerance among the various rootstocks [55,73,74]. These differences are attributed to salt exclusion, particularly to chloride exclusion [75,76]. Citrus apparently excludes Cl^- from shoots, not by sequestering it in the root but by restricting its entry into and/or movement within the roots. The Cl^- concentration differences found in leaves and to a lesser extent in stems emphasize pronounced rootstock differences in transport of chloride from the root to the shoot [76]. The scion appears to have no major influence on Cl^- transport from the roots to the shoot [77].

Differences among rootstocks is much greater for Cl^- accumulation than for Na^+, and there appears to be no correlation between Cl^- tolerance and Na^+ tolerance [78]. These differences are due to the existence of apparent separate mechanisms that operate to limit or regulate the transport of Na^+ or Cl^- to the leaves [72].

The Cl^- tolerance range for avocado rootstocks is much narrower than for citrus. In addition,

TABLE 2 Chloride Tolerance Limits of Some Fruit Crop Rootstocks

Crop	Rootstock	Maximum permissible Cl^- in soil water without leaf injury[a] (mol m^{-3})
Citrus		
(*Citrus* spp.)	Mandarin (Sunki, Cleopatra), grapefruit, Rangpur lime	50
	Rough lemon,[a] tangelo (Sampson, Minneola), sour orange, Ponkan mandarin	30
	Citrumelo 4475, Calamondin, sweet orange, trifoliate orange, Cuban shaddock, Citrange (Savage, Rusk, Troyer)	20
Grape		
(*Vitis* spp.)	Salt Creek, 1613-3	80
	Dog Ridge	60
	Thompson seedless, Perlette	40
	Cardinal, black rose	20
Stone fruit		
(*Prunus* sp.)	Marianna	50
	Lovell, Shalil	20
	Yunnan	15
Avocado		
(*Persea americana*)	West Indian	15
	Guatemalan	12
	Mexican	10

[a] For some crops, these concentrations may exceed the osmotic threshold and cause some yield reduction. Data from Australia indicate that rough lemon is more sensitive to Cl^- than sweet orange [72].
Source: Adapted from Ref. 21.

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because of the wide variation among varieties of the same rootstock, the rootstock tolerances tend to overlap [79]. However, it is generally agreed that the average Cl^- tolerance is West Indian > Guatemalan > Mexican [78–80]. The general pattern for Na^+ accumulation with avocado rootstocks tends to follow that for Cl^- accumulation and, like Cl^-, shows differences among varieties on the same rootstock [80,81].

Cold hardiness has been implicated in the salt tolerance of citrus and avocado rootstocks. Wutscher [82] reported that citrus rootstocks, which have good Cl^--excluding characteristics, tend to be relatively cold hardy. For some citrus species, a short-term, moderate salt stress has been shown to enhance cold hardiness in seedlings by modifying growth, water relations, and mineral nutrition [83].

In contrast to citrus, the more salt tolerant avocado rootstocks, such as West Indian and West Indian–Guatemalan hybrids, are the least cold tolerant. Likewise, the salt-sensitive Mexican rootstock is the most cold-tolerant [84].

Chloride toxicity has been the principal limiting factor for grapevines grown on their own roots. However, a significant reduction in Cl^- accumulation has been shown to occur in Cl^--sensitive scions grown on Dog Ridge or 1613-3 rootstocks [85]. The salt tolerance of these two rootstocks is probably limited by soil osmotic effects long before Cl^- reaches toxic levels.

Differences Among Cultivars

Most commercially grown cultivars are developed under nonsaline conditions and are not bred to endure salt stress. Therefore, their relative tolerances to salinity are often similar and difficult to measure. In addition, many cultivars developed in the past were derived from a narrow genetic base and thus possessed similar traits. Currently developed cultivars are from a much more diverse genetic base and may therefore possess a wider range of salt tolerance.

Among the crop species that already show some diversity in salt tolerance are Bermuda grass, brome grass, creeping bent grass, rice, wheat, barley, soybean, berseem clover, squash, muskmelon, and strawberry. Cotton and sugar cane also show significant cultivar differences, but these differences occur only at high salinity where yields are below commercially acceptable levels [86,87].

Salt Effects on Nitrogen Fixation and Nodulation

Most *Rhizobium* species are relatively unaffected at soil salinity levels that are less than the tolerance threshold values reported for most leguminous crops (Table 3). At soil salinities greater than their threshold, their ability to survive and fix N may be severely reduced [142–144]. This is particularly important, since legumes that are already weakened by salinity stress will be deprived of essential N fertilization as well.

There appears to be a wide range of tolerance to salinity among the various species of rhizobia. Some strains of *R. meliloti* can survive soil water salinities greater than that of seawater (≈46 dS m^{-1}), but most strains of *R. japonicum* grow poorly at salinities of 12 dS m^{-1} [145]. Studies comparing various *Rhizobium* species report the salt tolerance of *R. meliloti* > *R. trifolii* > *R. leguminosarum* > *R. japonicum* [145,146].

The salt effect on rhizobia appeared to be ion specific, with Cl^- salts of Na^+, K^+, and Mg^{2+} being more toxic than corresponding SO_4^{2-} salts [147,148]. In addition, Mg^{2+} ions inhibited growth at a much lower concentration than Na^+ or K^+ [149,150].

Since rhizobia can withstand large increases in salinity, they must be able to regulate and adjust their internal solute concentration. Osmoregulation in *Rhizobium* species grown at high external salt concentrations involves the accumulation of organic and/or inorganic solutes. Although some strains respond to salt stress by increasing their intracellular K^+ level [151], others accumulate organic compounds, such as amino acids, betaine, and carbohydrates, in the cytoplasm [152,153].

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TABLE 3 Salt Tolerance of Herbaceous Crops[a]

Crop		Tolerance based on	Salt-tolerance parameters		Rating[d]	Reference
Common name	Botanical name[b]		Threshold[c] EC_e (dS m^{-1})	Slope (% per dS m^{-1})		
Fiber, grain, and special crops						
Artichoke, Jerusalem	*Helianthus tuberosus* L.	Tuber yield	0.4	9.6	MS	88
Barley[e]	*Hordeum vulgare* L.	Grain yield	8.0	5.0	T	89
Bean	*Phaseolus vulgaris* L.	Seed or pods	1.0	19	S	89
Canola	*Brassica campestris* L. [syn. *B. rapa* L.]	Seed yield	—	—	T	U[f]
Canola	*B. napus* L.	Seed yield	—	—	T	U
Chickpea	*Cicer arietinum* L.	Seed yield	—	—	MS	90, 91
Corn[g]	*Zea mays* L.	Ear FW	1.7	12	MS	89
Cotton	*Gossypium hirsutum* L.	Seed cotton yield	7.7	5.2	T	89
Flax	*Linum usitatissimum* L.	Seed yield	1.7	12	MS	89
Guar	*Cyamopsis tetragonoloba* (L.) Taub.	Seed yield	8.8	17	T	92
Kenaf	*Hibiscus cannabinus* L.	Stem DW	8.1	11.6	T	93
Millet, channel	*Echinochloa tumerana* (Domin) J. M. Black	Grain yield	—	—	T	94
Oats	*Avena sativa* L.	Grain yield	—	—	T	95, U
Peanut	*Arachis hypogaea* L.	Seed yield	3.2	29	MS	89
Rice, paddy	*Oryza sativa* L.	Grain yield	3.0[h]	12[h]	S	89
Roselle	*Hibiscus sabdariffa* L.	Stem DW	—	—	MT	96
Rye	*Secale cereale* L.	Grain yield	11.4	10.8	T	97
Safflower	*Carthamus tinctorius* L.	Seed yield	—	—	MT	89
Sesame[i]	*Sesamum indicum* L.	Pod DW	—	—	S	98
Sorghum	*Sorghum bicolor* (L.) Moench	Grain yield	6.8	16	MT	99
Soybean	*Glycine max* (L.) Merrill	Seed yield	5.0	20	MT	89
Sugar beet[j]	*Beta vulgaris* L.	Storage root	7.0	5.9	T	89
Sugar cane	*Saccharum officinarum* L.	Shoot DW	1.7	5.9	MS	89
Sunflower	*Helianthus annuus* L.	Seed yield	—	—	MT	100, U
Triticale	× *Triticosecale* Wittmack	Grain yield	6.1	2.5	T	101

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TABLE 3 Continued

Crop		Tolerance based on	Salt-tolerance parameters		Rating[d]	Reference
Common name	Botanical name[b]		Threshold[c] EC_e (dS m^{-1})	Slope (% per dS m^{-1})		
Wheat	*Triticum aestivum* L.	Grain yield	6.0	7.1	MT	89
Wheat (semidwarf)[k]	*T. aestivum* L.	Grain yield	8.6	3.0	T	102
Wheat, durum	*T. turgidum* L. var. *durum* Desf.	Grain yield	5.9	3.8	T	102
Grasses and forage crops						
Alfalfa	*Medicago sativa* L.	Shoot DW	2.0	7.3	MS	89
Alkali grass, Nuttall	*Puccinellia airoides* (Nutt.) Wats. & Coult.	Shoot DW	—	—	T*	15
Alkali sacaton	*Sporobolus airoides* Torr.	Shoot DW	—	—	T*	15
Barley (forage)[e]	*Hordeum vulgare* L.	Shoot DW	6.0	7.1	MT	89
Bent grass, creeping	*Agrostis stolonifera* L.	Shoot DW	—	—	MS	89
Bermuda grass[l]	*Cynodon dactylon* (L.) Pers.	Shoot DW	6.9	6.4	T	89
Bluestem, Angleton	*Dichanthium aristatum* (Poir.) C. E. Hubb. [syn. *Andropogon nodosus* (Willem.) Nash]	Shoot DW	—	—	MS*	103
Broad bean	*Vicia faba* L.	Shoot DW	1.6	9.6	MS	89
Brome grass, mountain	*Bromus marginatus* Nees ex Steud.	Shoot DW	—	—	MT*	15
Brome grass, smooth	*B. inermis* Leyss	Shoot DW	—	—	MT	89
Buffelgrass	*Pennisetum ciliare* (L.) Link. [syn. *Cenchurs ciliaris*]	Shoot DW	—	—	MS*	103
Burnet	*Poterium sanguisorba* L.	Shoot DW	—	—	MS*	15
Canary grass, reed	*Phalaris arundinacea* L.	Shoot DW	—	—	MT	89
Clover, alsike	*Trifolium hybridum* L.	Shoot DW	1.5	12	MS	89
Clover, berseem	*T. alexandrinum* L.	Shoot DW	1.5	5.7	MS	89
Clover, Hubam	*Melilotus alba* Dest. var. *annua* H. S. Coe	Shoot DW	—	—	MT*	15
Clover, ladino	*Trifolium repens* L.	Shoot DW	1.5	12	MS	89
Clover, Persian	*T. resupinatum* L.	Shoot DW	—	—	MS*	104

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Clover, red	*T. pratense* L.	Shoot DW	1.5	12	MS	89
Clover, strawberry	*T. fragiferum* L.	Shoot DW	1.5	12	MS	89
Clover, sweet	*Melilotus* sp. Mill.	Shoot DW	—	—	MT*	15
Clover, white Dutch	*Trifolium repens* L.	Shoot DW	—	—	MS*	15
Corn (forage)[g]	*Zea mays* L.	Shoot DW	1.8	7.4	MS	89
Cowpea (forage)	*Vigna unguiculata* (L.) Walp.	Shoot DW	2.5	11	MS	105
Dallis grass	*Paspalum dilatatum* Poir.	Shoot DW	—	—	MS*	106
Dhaincha	*Sesbania bispinosa* (Linn.) W.F. Wight [syn. *Sesbania aculeata* (Willd.) Poir]	Shoot DW	—	—	MT	107, 108
Fescue, meadow	*Festuca pratensis* Huds.	Shoot DW	—	—	MT*	15
Fescue, tall	*Festuca elatior* L.	Shoot DW	3.9	5.3	MT	89
Foxtail, meadow	*Alopecurus pratensis* L.	Shoot DW	1.5	9.6	MS	89
Glycine	*Neonotonia wightii* [formerly *Glycine wightii* or *javanica*]	Shoot DW	—	—	MS	106, 109
Gram; black	*Vigna mungo* (L.) Hepper [syn. *Phaseolus mungo* L.]	Shoot DW	—	—	S	110
Grama, blue	*Bouteloua gracilis* (HBK) Lag. ex Steud.	Shoot DW	—	—	MS*	15
Guinea grass	*Panicum maximum* Jacq.	Shoot DW	—	—	MT	106
Harding grass	*Phalaris tuberosa* L. var. *stenoptera* (Hack) A. S. Hitchc.	Shoot DW	4.6	7.6	MT	89
Kallar grass	*Leptochloa fusca* (L.) Kunth., formerly *Diplachne fusca* Beauv.	Shoot DW	—	—	T	111
Lablab bean	*Lablab purpureus* (L.) Sweet (syn. Dolichos lablab L.)	Shoot DW	—	—	MS	106
Love grass[m]	*Eragrostis* sp. N. M. Wolf	Shoot DW	2.0	8.4	MS	89
Milk vetch, Cicer	*Astragalus cicer* L.	Shoot DW	—	—	MS*	15
Millet, foxtail	*Setaria italica* (L.) Beauvois	Dry matter	—	—	MS	89
Oat grass, tall	*Arrhenatherum elatius* (L.) Beauvois. ex J. Presl & K. Presl	Shoot DW	—	—	MS*	15
Oats (forage)	*Avena sativa* L.	Straw DW	—	—	T	95, U
Orchard grass	*Dactylis glomerata* L.	Shoot DW	1.5	6.2	MS	89

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TABLE 3 Continued

Crop		Tolerance based on	Salt-tolerance parameters			
Common name	Botanical name[b]		Threshold[c] EC_e (dS m^{-1})	Slope (% per dS m^{-1})	Rating[d]	Reference
Panic grass, blue	*Panicum antidotale* Retz.	Shoot DW	—	—	MS*	103, 112
Pigeon pea	*Cajanus cajan* (L.) Huth [syn. *C. indicus* (K.) Spreng.]	Shoot DW	—	—	S	110, 113
Rape (forage)	*Brassica napus* L.		—	—	MT*	15
Rescue grass	*Bromus unioloides* HBK	Shoot DW	—	—	MT*	15
Rhodes grass	*Chloris gayana* Kunth.	Shoot DW	—	—	MT	103, 112
Rye (forage)	*Secale cereale* L.	Shoot DW	7.6	4.9	T	97
Rye grass, Italian	*Lolium multiflorum* Lam.	Shoot DW	—	—	MT*	114
Rye grass, perennial	*Lolium perenne* L.	Shoot DW	5.6	7.6	MT	89
Rye grass, Wimmera	*L. rigidum* Gaud.		—	—	MT*	115
Salt grass, desert	*Distichlis spicta* L. var. *stricta* (Torr.) Bettle	Shoot DW	—	—	T*	15
Sesbania	*Sesbania exaltata* (Raf.) V. L. Cory	Shoot DW	2.3	7.0	MS	89
Siratro	*Macroptilium atropurpureum* (DC) Urb.	Shoot DW	—	—	MS	106
Sphaerophysa	*Sphaerophysa salsula* (Pall.) DC	Shoot DW	2.2	7.0	MS	116
Sudan grass	*Sorghum sudanense* (Piper) Stapf	Shoot DW	2.8	4.3	MT	89
Timothy	*Phleum pratense* L.	Shoot DW	—	—	MS*	89
Trefoil, big	*Lotus pedunculatus* Cav.	Shoot DW	2.3	19	MS	89
Trefoil, broadleaf birdsfoot	*L. corniculatus* L. var. *arvenis* (Schkuhr) Ser. ex DC	Shoot DW	—	—	MS	117
Trefoil, narrowleaf birdsfoot	*L. corniculatus* var. *tenuifolium* L.	Shoot DW	5.0	10	MT	89
Vetch, common	*Vicia angustifolia* L.	Shoot DW	3.0	11	MS	89
Wheat (forage)[j]	*Triticum aestivum* L.	Shoot DW	4.5	2.6	MT	102
Wheat durum (forage)	*T. turgidum* L. var. *durum* Desf.	Shoot DW	2.1	2.5	MT	102

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Wheat grass, fairway crested	*Agropyron cristatum* (L.) Gaertn.	Shoot DW	7.5	6.9	T	89
Wheat grass, intermediate	*A. intermedium* (Host) Beauvois	Shoot DW	—	—	MT*	118
Wheat grass, slender	*A trachycaulum* (Link) Malte	Shoot DW	—	—	MT	89
Wheat grass, standard crested	*A. sibiricum* (Willd.) Beauvois	Shoot DW	3.5	4.0	MT	89
Wheat grass, tall	*A. elongatum* (Hort) Beauvois	Shoot DW	7.5	4.2	T	89
Wheat grass, western	*A. smithii* Rydb.	Shoot DW	—	—	MT*	15
Wild rye, Altai	*Elymus angustus* Trin.	Shoot DW	—	—	T	89
Wild rye, beardless	*E. Triticoides* Buckl.	Shoot DW	2.7	6.0	MT	89
Wild rye, Canadian	*E. canadensis* L.	Shoot DW	—	—	MT*	15
Wild rye, Russian	*E. junceus* Fisch.	Shoot DW	—	—	T	89
Vegetables and fruit crops						
Artichoke	*Cynara scolymus* L.	Head yield	—	—	MT*	104
Asparagus	*Asparagus officinalis* L.	Spear yield	4.1	2.0	T	71
Bean, common	*Phaseolus vulgaris* L.	Seed yield	1.0	19	S	89
Bean, lima	*P. lunatus* L.	Seed yield	—	—	MT*	119
Bean, mung	*Vigna radiata* (L.) R. Wilcz.	Seed yield	1.8	20.7	S	120
Beet, red[i]	*Beta vulgaris* L.	Storage root	4.0	9.0	MT	89
Broccoli	*Brassica oleracea* L. (botrytis group)	Shoot FW	2.8	9.2	MS	89
Brussels sprouts	*B. oleracea* L. (gemmifera group)		—	—	MS*	
Cabbage	*B. oleracea* L. (capitata group)	Head FW	1.8	9.7	MS	89
Carrot	*Daucus carota* L.	Storge root	1.0	14	S	89
Cassava	*Manihot esculenta* Crantz	Tuber yield	—	—	MS	121, 122
Cauliflower	*Brassica oleracea* L. (botrytis group)		—	—	MS*	
Celery	*Apium graveolens* L. var. *dulce* (Mill.) Pers	Petiole FW	1.8	6.2	MS	123
Corn, sweet	*Zea mays* L.	Ear FW	1.7	12	MS	89
Cowpea	*Vigna unguiculata* (L.) Walp.	Seed yield	4.9	12	MT	105
Cucumber	*Cucumis sativus* L.	Fruit yield	2.5	13	MS	89

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TABLE 3 Continued

Crop		Tolerance based on	Salt-tolerance parameters			
Common name	Botanical name[b]		Threshold[c] EC_e (dS m^{-1})	Slope (% per dS m^{-1})	Rating[d]	Reference
Eggplant	*Solanum melongena* L. var. *esculentum* Nees.	Fruit yield	1.1	6.9	MS	124
Garlic	*Allium sativum* L.	Bulb yield	1.7	10	MS	125
Gram, black or urd bean	*Vigna mungo* (L.) Hepper [syn. *Phaseolus mungo* L.]	Shoot DW	—	—	S	110
Kale	*Brassica oleracea* L. (acephala group)		—	—	MS*	115
Kohlrabi	*Brassica oleracea* L. (gongylodes group)		—	—	MS*	
Lettuce	*Lactuca sativa* L.	Top FW	1.3	13	MS	89
Muskmelon	*Cucumis melo* L. (reticulatus group)	Fruit yield	1.0	8.4	MS	126, 127
Okra	*Abelmoschus esculentus* (L.) Moench	Pod yield	—	—	MS	128, 129
Onion (bulb)	*Allium cepa* L.	Bulb yield	1.2	16	S	89
Onion (seed)	*Allium cepa* L.	Seed yield	1.0	8.0	MS	130
Parsnip	*Pastinaca sativa* L.		—	—	S*	115
Pea	*Pisum sativum* L.	Seed FW	3.4	10.6	MS	131
Pepper	*Capsicum annuum* L.	Fruit yield	1.5	14	MS	89
Pigeon Pea	*Cajanus cajan* (L.) Huth [syn. *C. indicus* (K.) Spreng.]	Shoot DW	—	—	S	110, 113
Potato	*Solanum tuberosum* L.	Tuber yield	1.7	12	MS	89
Pumpkin	*Cucurbita pepo* L. var. *Pepo*		—	—	MS*	
Purslane	*Portulaca oleracea* L.	Shoot FW	6.3	9.6	MT	132
Radish	*Raphanus sativus* L.	Storage root	1.2	13	MS	89
Spinach	*Spinacia oleracea* L.	Top FW	2.0	7.6	MS	89
Squash, scallop	*Cucurbita pepo* L. var. *melopepo* (L.) Alef.	Fruit yield	3.2	16	MS	133

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Squash, zucchini	*C. pepo* L. var. *melopepo* (L.) Alef.	Fruit yield	4.7	9.4	MT	133
Strawberry	*Fragaria x Ananassa* Duch.	Fruit yield	1.0	33	S	89
Sweet potato	*Ipomoea batatas* (L.) Lam.	Fleshy root	1.5	11	MS	89
Tepary bean	*Phaseolus acutifolius* Gray		—	—	MS*	134–136
Tomato	*Lycopersicon lycopersicum* (L.) Karst. ex Farw.	Fruit yield	2.5	9.9	MS	89
Tomato, cherry	*L. lycopersicum* L. var. *cerasiforme* (Dunal) Alef.	Fruit yield	1.7	9.1	MS	137
Turnip	*Brassica rapa* L. (rapifera group)	Storage root	0.9	9.0	MS	138
Turnip (greens)	*Brassica rapa* L. (rapifera group)	Top FW	3.3	4.3	MT	138
Watermelon	*Citrullus lanatus* (Thunb.) Matsum. & Nalai	Fruit yield	—	—	MS*	104
Winged bean	*Psophocarpus tetragonolobus* L. DC	Shoot DW	—	—	MT	139

EC_e, electrical conductivity of the saturated-soil extract; FW, fresh weight; DW, dry weight; S, sensitive; MS, moderately sensitive; MT, moderately Tolerant; T, tolerant.

[a] These data serve only as a guideline to relative tolerances among crops. Absolute tolerances vary depending on climate, soil conditions, and cultural practices.

[b] Botanical and common names follow the convention of *Hortus Third* [140] when possible.

[c] In gypsiferous soils, plants tolerate EC_e about 2 dS m^{-1} higher than indicated.

[d] Ratings are defined by the boundaries in Figure 1. Ratings marked by an asterisk are estimates

[e] Less tolerant during seedling stage, EC_e at this stage should not exceed 4 or 5 dS m^{-1}.

[f] Unpublished U.S. Salinity Laboratory data.

[g] Grain and forage yields of DeKalb XL-75 grown on an organic muck soil decreased about 26% per dS m^{-1} above a threshold of 1.9 dS m^{-1} [141].

[h] Because paddy rice is grown under flooded conditions, values refer to the electrical conductivity of the soil water while the plants are submerged. Less tolerant during seedling stage.

[i] Sesame cultivars Sesaco 7 and 8 may be more tolerant than indicated by the sensitivity rating.

[j] Sensitive during germination and emergence, EC_e should not exceed 3 dS m^{-1}.

[k] Data from one cultivar, Probred.

[l] Average of several varieties. Suwannee and Coastal are about 20% more tolerant and common and Greenfield are about 20% less tolerant than the average.

[m] Average for Boer, Wilman, Sand, and Weeping cultivars. Lehmann seems about 50% more tolerant.

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SALT–TOLERANCE DATA

Yield-Response Functions

Yield-response curves indicate that most crops tolerate salinity up to a threshold level above which yields decrease approximately linearly as salinity increases. Maas and Hoffman [89] proposed a two-piece linear response model to characterize the curves. The two parameters obtained from this model are the threshold, the maximum allowable salinity without yield reduction, and the slope, the percentage yield decrease per unit increase in salinity beyond the threshold. Table 3 presents these yield-response parameters for many field, forage, vegetable, and fruit crops. The data are presented in terms of the electrical conductivity of the saturated-soil extract, EC_e [15] at 25°C with units of decisiemens per meter (1 dS m^{-1} = 1 mmho cm^{-1}). These data serve only as a guideline to relative tolerances among crops. Absolute tolerances vary, depending on climate, soil conditions, and cultural practices.

The threshold and slope obtained from the model can be used to calculate relative yield Y_r for any given soil salinity exceeding the threshold by using the equation

$$Y_r = 100 - B(EC_e - A)$$

where A = the salinity threshold expressed in dS m^{-1}; B = the slope expressed in % per dS m^{-1}; and EC_e is the mean electrical conductivity of the saturated-soil extract of the root zone [89].

The data in Table 3 apply to soils in which Cl^- is the predominant anion. The EC_e of saturated soil paste from gypsiferous soils (nonsodic, low Mg^{2+}) generally ranges from 1 to 3 dS m^{-1} higher than that from nongypsiferous soils with the same conductivity in the soil water at field capacity [154]. The higher salinities are the result of gypsum dissolution during preparation of the soil paste. The extent of this dissolution depends on the exchangeable ion composition, cation-exchange capacity, and solution composition. Therefore, plants grown on gypsiferous soils tolerate salinity levels approximately 2 dS m^{-1} higher than those indicated in Table 3.

The salt-tolerance classifications in Figure 1 are presented for quick comparisons among crops. Division boundaries for the classes were chosen to correspond with previously published salt-tolerance terminology ranging from sensitive to tolerant. Generally, the threshold and linear slope for

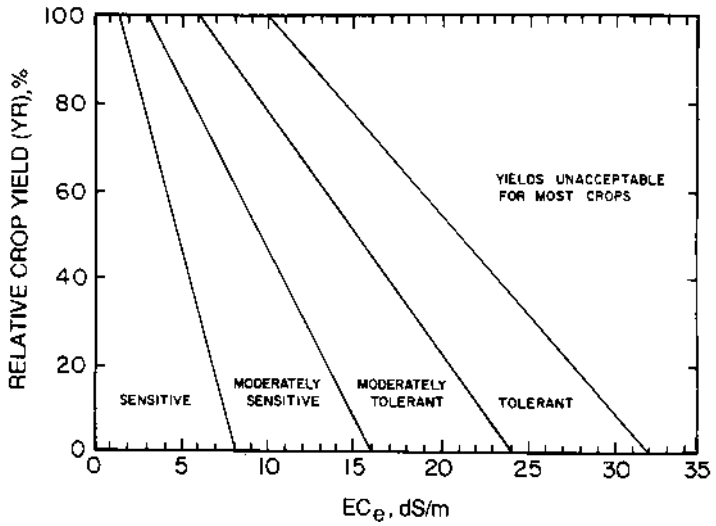

FIGURE 1 Divisions for classifying crop tolerance to salinity.

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a crop remain within one class. Where the linear curve for a crop crossed division boundaries, the crop was classified based on the tolerance at lower salinity levels at which yields are commercially acceptable. Classification for some crops in Table 3 are listed with only a qualitative rating, because the data are insufficient to calculate the threshold and slope.

Salt Tolerance of Vegetable Crops

Vegetable crops tend to fall into the more sensitive salt-tolerant categories. The only notable exceptions are asparagus, red beet, and zucchini squash. Since most vegetables are salt sensitive, the choice of land and/or irrigation water where they can be successfully grown is severely restricted. Under marginal conditions of salinity, the growth of many vegetables is stunted without showing other visible injury symptoms [155]. At high salinity levels, some vegetables exhibit pronounced injury symptoms in the later stages of growth. Bean leaves develop a marginal chlorosis-necrosis with an upward cupping of the leaves [156]. Onions have also been shown to develop a leaf necrosis [157]. In addition to growth suppression, some vegetable crops exhibit symptoms of nutritional imbalance or deficiency. Some lettuce cultivars develop calcium-deficiency symptoms when sulfate levels in the soil are too high. Excessive calcium may restrict the uptake of potassium, which may be a factor in reduced yields of bean and carrot [158]. With most vegetable crops, however, the osmotic effect predominates and nutritional effects are either absent or of decidedly secondary importance.

High levels of exchangeable Na^+ frequently restrict vegetative growth because of the unfavorable physical conditions associated with sodic soils. Most vegetable crops appear to be at least moderately tolerant to exchangeable Na^+. Bean plants, however, are sensitive to nutritional factors in sodic soils and may be severely affected even before the physical condition of the soil is impaired.

Most vegetable crops produced on saline soils are not of prime market quality. This is seen in such diverse ways as smaller fruit size of tomatoes and peppers [158], reduced petiole length of celery [123], and misshapen potatoes [159]. It has been generally observed, however, that tomato yields are reduced more by decreases in fruit number than in fruit size or weight [160,161]. Not all salinity effects on quality are detrimental. The flavor of carrots [162] and asparagus [71] is enhanced by a measurable increase in sugar content when grown under saline conditions. Likewise, a number of studies [160,163–165] have shown that total soluble solids in tomatoes is significantly increased as salt stress is increased. Unfortunately, this gain in quality is more than offset by lower yields.

Salt Tolerance of Cereal Crops

Most of the major cereal crops exhibit high tolerance to soil salinity. In this group are sorghum, wheat, triticale, rye, oats, and barley. The only exceptions are corn and rice [21].

Regardless of the overall salt tolerance, all cereals tend to follow the same sensitivity or tolerance pattern in relation to their stage of growth. The seedling or early vegetative stage appears to be the most sensitive, with subsequent stages showing increased tolerance. This phenomenon has been reported for sorghum [67], wheat [66], barley [64], corn [166], and rice [70]. The other cereal crops, although not tested but with similar growth patterns, are also expected to show sensitivity at the early vegetative stage of growth.

Since the life cycle of cereals is an orderly sequence of developmental events, salinity stress can have a significant effect on the developmental process occurring at a particular time. The sequence of events has been separated into three distinct but continuous developmental phases [69]. In the first phase, which encompasses the early vegetative growth stage, leaf and spikelet primordia are initiated, leaf growth occurs, and tiller buds are produced in the axils of the leaves. High soil salinity at this time reduces the number of leaves per culm, the number of spikelets per spike, and

the number of tillers per plant [69,167]. Differentiation of the terminal spikelet signals completion of this phase.

During phase II, the tillers grow, mainstem and tiller culms elongate, and the final number of florets is set [168]. Salinity stress during this phase may affect tiller survival and reduce the number of functional florets per spikelet. This phase ends with anthesis. Carpel fertilization and grain filling occur during the final phase [168]. At this time, salinity affects seed number and seed size.

The effect of salinity on spikelet and tiller number established during phase I has a greater influence on final seed yield than the effects exerted on yield components in the latter two phases [66,67,166].

Salt Tolerance of Forage Crops

Forage crops fall into two broad salt-tolerance categories. Most grasses belong to the tolerant group, with the majority of legumes being in the sensitive group. Exceptions to this generalization are meadow foxtail (*Alopecurus pratensis*), love grass (*Eragrostis* spp.), and orchard grass (*Dactylis glomerata*), which are moderately sensitive to salt stress, and birdsfoot trefoil (*Lotus corniculatus* var. *tenuifolium*) and the sweet clovers (*Melilotus* spp.), which are moderately tolerant [89].

Many of the forage grasses possess the same growth habit as the cereal grasses and, like the cereals, are more sensitive to salinity during the early seedling stage of growth [169]. Unlike the cereals, however, many of the grasses are maintained in a perpetual vegetative stage of growth from continued grazing or mowing. Therefore, it appears that these grasses, once they are beyond the early seedling stage and well established, are less sensitive to soil salinity.

Because of their fibrous roots, grasses alone or in combination with forage legumes are frequently used in the reclamation of saline and sodic soils to restore good soil structure [170]. Under nonirrigated conditions, grasses that accumulate significantly high concentrations of Na^+ and Cl^- in the shoots may be used to restore soil structure and also to remove these ions from the soil profile [171]. Grasses used for this purpose may be unfit for animal feed because of the high salt content [170].

Clovers are the predominant legume of pastures and are frequently grown in combination with various grass species. However, salt-sensitive clovers tend to die out on saline soils as the more tolerant grass becomes the predominant vegetation. Loss of the clover from the pasture mixture significantly reduces the nutritional value of the pasture [172].

The salt tolerance of clovers [173] and alfalfa [174] is highly dependent on the stage of growth at which salinity is first imposed. The salt tolerance of alfalfa has been reported to be closely associated with Cl^- accumulation in the leaves [174,175]. Salt-affected plants are characterized initially by a dark green leaf coloration and reduced leaf size [175] followed by a general reduction in plant size [12].

Although the salt tolerance of alfalfa appears to depend on a salt-exclusion mechanism [175], no consistent correlation seems to exist between salt tolerance and salt exclusion for legumes in general [176]. There appears to be sufficient evidence that the genetic variability that exists among the grass and legume species and cultivars offers the possibility of developing strains with higher salt tolerance [169,173,176–178].

Salt Tolerance of Fruit Tree and Vine Crops

With the exception of date palm and a few others believed to be moderately tolerant, most fruit trees are relatively sensitive to salinity (Table 4). Stone fruits, citrus, and avocado have all shown specific sensitivity to foliar accumulations of Cl^- and Na^+. The accumulation of these ions to harmful levels, as well as the general osmotic growth inhibition, contribute to the reduction in tree growth and fruit yield.

Different cultivars and rootstocks absorb Cl^- and Na^+ at different rates, so tolerance can vary

TABLE 4 Salt Tolerance of Woody Crops[a]

Crop			Salt tolerance parameters			
Common name	Botanical Name[b]	Tolerance based on	Threshold[c] EC_e (dS m^{-1})	Slope (% per dS m^{-1})	Rating[d]	Reference
Almond	*Prunus duclis* (Mill.) D. A. Webb	Shoot growth	1.5	19	S	89
Apple	*Malus sylvestris* Mill.		—	—	S	89
Apricot	*Prunus armeniaca* L.	Shoot growth	1.6	24	S	89
Avocado	*Persea americana* Mill.	Shoot growth	—	—	S	89
Banana	*Musa acuminata* Colla	Fruit yield	—	—	S	179
Blackberry	*Rubus macropetalus* Doug. ex Hook	Fruit yield	1.5	22	S	89
Boysenberry	*Rubus ursinus* Cham. & Schlechtend	Fruit yield	1.5	22	S	89
Castor bean	*Ricinus communis* L.		—	—	MS*	15
Cherimoya	*Annona cherimola* Mill.	Foliar injury	—	—	S	180
Cherry, sand	*Prunus besseyi* L. H. Bailey	Foliar injury, stem growth	—	—	S*	182
Cherry, sweet	*Prunus avium* L.		—	—	S*	181
Coconut	*Cocos nucifera* L.		—	—	MT*	183
Currant	*Ribes* sp. L.	Foliar injury, stem growth	—	—	S*	181,182
Date palm	*Phoenix dactylifera* L.	Fruit yield	4.0	3.6	T	89
Fig	*Ficus carica* L.	Plant DW	—	—	MT*	15,184
Gooseberry	*Ribes* sp. L.		—	—	S*	181
Grape	*Vitis vinifera* L.	Shoot growth	1.5	9.6	MS	89
Grapefruit	*Citrus* × *paradisi* Macfady.	Fruit yield	1.2	13.5	S	7
Guava	*Psidium guajava* L.	Shoot and root growth	4.7	9.8	MT	185

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TABLE 4 Continued

Crop			Salt tolerance parameters			
Common name	Botanical Name[b]	Tolerance based on	Threshold[c] EC_e (dS m^{-1})	Slope (% per dS m^{-1})	Rating[d]	Reference
Guayule	*Parthenium argentatum* A. Gray	Shoot DW	8.7	11.6	T	186
Guayule	*Parthenium argentatum* A. Gray	Rubber yield	7.8	10.8	T	186
Jambolan plum	*Syzygium cumini* L.	Shoot growth	—	—	MT	187
Jojoba	*Simmondsia chinensis* (Link) C. K. Schneid	Shoot growth	—	—	T	188,189
Jujube, Indian	*Ziziphus mauritiana* Lam.	Fruit yield	—	—	MT	190
Lemon	*Citrus limon* (L.) Burm. f.	Fruit yield	1.5	12.8	S	9
Lime	*Citrus aurantiifolia* (Christm.) Swingle		—	—	S*	
Loquat	*Eriobotrya japonica* (Thunb.) Lindl.	Foliar injury	—	—	S*	115,191
Macadamia	*Macadamia integrifolia* Maiden & Betche	Seedling growth	—	—	MS*	192
Mandarin orange; tangerine	*Citrus reticulata* Blanco	Shoot growth	—	—	S*	193
Mango	*Mangifera indica* L.	Foliar injury	—	—	S	180
Natal plum	*Carissa grandiflora* (E. H. Mey.) A. DC	Shoot growth	—	—	T	47
Olive	*Olea europaea* L.	Seedling growth, Fruit yield	—	—	MT	89
Orange	*Citrus sinensis* (L.) Osbeck	Fruit yield	1.3	13.1	S	6,8,10,194

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Papaya	*Carica papaya* L.	Seedling growth, foliar injury	—	—	MS	195,196
Passion fruit	*Passiflora edulis* Sims.		—	—	S*	115
Peach	*Prunus persica* (L.) Batsch	Shoot growth, Fruit yield	1.7	21	S	89
Pear	*Pyrus communis* L.		—	—	S*	15
Pecan	*Carya illinoinensis* (Wangenh.) C. Koch	Nut yield, trunk growth	—	—	MS	197
Persimmon	*Diospyros virginiana* L.		—	—	S*	115
Pineapple	*Ananas comosus* (L.) Merrill	Shoot DW	—	—	MT	198
Pistachio	*Pistacia vera* L.	Shoot growth	—	—	MS	199,200
Plum, prune	*Prunus domestica* L.	Fruit yield	2.6	31	MS	201
Pomegranate	*Punica granatum* L.	Shoot growth	—	—	MS	202
Popinac, white	*Leucaena leucocephalia* (Lam.) de Wit [syn. *Leucaena glauca* Benth.]	Shoot DW	—	—	MS	203,204
Pummelo	*Citrus maxima* (Burm.)	Foliar injury	—	—	S*	205
Raspberry	*Rubus idaeus* L.	Fruit yield	—	—	S	89
Rose apple	*Syzygium jambos* (L.) Alston	Foliar injury	—	—	S*	206
Sapote, white	*Casimiroa edulis* Llave	Foliar injury	—	—	S*	180
Scarlet wisteria	*Sesbania grandiflora*	Shoot DW	—	—	MT	207
Tamarugo	*Prosopis tamarugo* Phil.	Observation	—	—	T	208
Walnut	*Juglans* spp.		—	—	S*	181

Abbreviations as in Table 3.

[a] These data serve only as a guideline to relative tolerances among crops. Absolute tolerances vary depending on climate, soil conditions, and cultural practices. The data are applicable when rootstocks are used that do not accumulate Na^+ or Cl^- rapidly or when these ions do not predominate in the soil.

[b] Botanical and common names follow the convention of *Hortus Third* [140] when possible.

[c] In gypsiferous soils, plants tolerate EC_e about 2 dS m^{-1} higher than indicated.

[d] Ratings are defined by the boundaries in Figure 1. Ratings marked by an asterisk are estimates.

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considerably within a species. In the absence of specific ion effects, however, the tolerance of these crops can be expressed as a function of the concentration of total soluble salts or the osmotic potential of the soil solution.

Some of the more sensitive fruit crops may accumulate toxic levels of Na^+ and/or Cl^- over a period of years from soils that would be classified as nonsaline and nonsodic [209,210]. Chloride toxicity in woody species is generally more severe and is observed on a wider range of species than Na^+ toxicity. The differences among species, cultivars, or rootstocks in susceptibility to Cl^- usually reflect the capability of the plant to prevent or retard Cl^- accumulation in the plant tops.

The initial symptoms of excess Cl^- accumulation in fruit crops is leaf tip necrosis developing into marginal necrosis. With citrus, a chlorosis and bronzing of the leaves occur without a well-defined necrosis. As Cl^- continues to accumulate, the effects become more severe with premature leaf drop, complete defoliation, twig dieback, and in extreme cases death of the tree or vine [210,211].

Injury by Na^+ can occur at concentrations as low as 5 mol m^{-3} in the soil solution [21]. However, injury symptoms, which are characterized as tip, marginal, and/or interveinal necrosis, may not appear for a considerable time after exposure to salinity. Initially, the Na^+ is thought to be retained in the sapwood of the tree. With the conversion of sapwood to heartwood, the Na^+ is released and then translocated to the leaves, causing leaf burn [212]. This may partly explain why stone fruits and grapes appear to be more sensitive to salinity as the plants grow older. With succeeding years, the Cl^- and Na^+ accumulate more rapidly in the leaves, causing leaf burn to develop earlier and with increasing severity [201].

Recent studies have shown that Na^+ accumulation in plum leaves did not significantly increase until the leaves were already severely damaged by Cl^- accumulation [201].

These studies indicate that when Cl^- and Na^+ are present in the soil solution, Cl^- is the primary damaging ion on stone fruits. Sodium accumulation only occurs after the leaf membranes have already been damaged.

Growth and yield reduction may occur with woody fruit species in the absence of specific ion toxicity. Francois and Clark [213], working with Valencia orange, reported a 50% reduction in fruit yield from salinity with no visible leaf-injury symptoms. Once salts have accumulated to toxic levels, however, growth and yield are suppressed by the additive effects of osmotic stress and specific ion toxicities [210].

Salt Tolerance of Ornamentals, Trees, and Flowers

In contrast to crop species that produce a marketable product, the salt tolerance of ornamental shrubs, trees, and flowers is determined by the esthetic value of the plant species. Injury or loss of leaves or flowers caused by salt stress is unacceptable even though growth may be unaffected. A significant growth reduction might be acceptable and possibly desirable for some species, as long as they appear to be healthy and attractive. The salt tolerance limits presented in Table 5 for some ornamental shrubs, trees, and ground covers indicate the maximum permissible EC_e for an acceptable appearance.

The type of injury seen on woody ornamentals and trees is similar to damage recorded for fruit trees and vines. A number of reports have shown that although some species accumulate Na^+, salt tolerance is closely associated with their ability to limit Cl^- uptake and accumulation [214,216,217].

In northern climates, where NaCl and/or $CaCl_2$ are used as deicing salts, typical salt-injury symptoms occur on roadside trees. These trees are subjected to both soil salinity from runoff and saline spray from passing automobiles. Although salt spray is thought to be the more detrimental of the two modes of deposition [218,219], soil-salinity effects may be accumulative and over a period of years may result in a slow but progressive decline of the trees.

A limited number of floricultural plants have been tested for salt tolerance. Chrysanthemum, carnation, and stock are considered moderately tolerant to salt stress; aster, poinsettia, gladiolus,

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TABLE 5 Salt Tolerance of Ornamental Shrubs, Trees, and Ground Cover[a]

Common name	Botanical name	Maximum permissible soil salinity[b] EC_e (dS m^{-1})
Very sensitive		
Star jasmine	*Trachelospermum jasminoides* (Lindl.) Lem.	1–2
Pyrenees cotoneaster	*Cotoneaster congestus* Bak.	1–2
Oregon grape	*Mahonia aquifolium* (Pursh) Nutt.	1–2
Photinia	*Photinia* × *Fraseri* Dress.	1–2
Sensitive		
Pineapple guava	*Feijoa sellowiana* O. Berg	2–3
Chinese holly, cv. Burford	*Ilex cornuta* Lindl & Paxt.	2–3
Rose, cv. Grenoble	*Rosa* sp. L.	2–3
Glossy abelia	*Abelia* × *grandiflora* (Andre) Rehd.	2–3
Southern yew	*Podocarpus macrophyllus* (Thunb.) D. Don	2–3
Tulip tree	*Liriodendron tulipifera* L.	2–3
Algerian ivy	*Hedera canariensis* Willd.	3–4
Japanese pittosporum	*Pittosporum tobira* (Thunb.) Ait.	3–4
Heavenly bamboo	*Nandina domestica* Thunb.	3–4
Chinese hibiscus	*Hibiscus rosa-sinensis* L.	3–4
Laurustinus, cv. Robustum	*Viburnum tinus* L.	3–4
Strawberry tree, cv. Compact	*Arbutus unedo* L.	3–4
Crape myrtle	*Lagerstroemia indica* L.	3–4
Moderately sensitive		
Glossy privet	*Ligustrum lucidum* Ait.	4–6
Yellow sage	*Lantana camara* L.	4–6
Orchid tree	*Bauhinia purpurea* L.	4–6
Southern magnolia	*Magnolia grandiflora* L.	4–6
Japanese boxwood	*Buxus microphylla* Siebold & Zucc. var. *japonica* (Mull. Arg) Rehd. & E. H. Wils.	4–6
Xylosma	*Xylosma congestum* (Lour.) Merrill	4–6
Japanese black pine	*Pinus thunbergiana* Franco	4–6
Indian hawthorn	*Raphiolepis indica* (L.) Lindl.	4–6
Dodonaea, cv. atropurpurea	*Dodonaea viscosa* (L.) Jacq.	4–6
Oriental arborvitae	*Platycladus orientalis* (L.) Franco	4–6
Thorny elaeagnus	*Elaeagnus pungens* Thunb.	4–6
Spreading juniper	*Juniperus chinensis* L.	4–6
Pyracantha, cv. Graberi	*Pyracantha fortuneana* (Maxim.) H. L. Li.	4–6
Cherry plum	*Prunus cerasifera* J. F. Ehrh.	4–6
Moderately tolerant		
Weeping bottlebrush	*Callistemon viminalis* (Soland. ex Gaertn.) Cheel.	6–8
Oleander	*Nerium oleander* L.	6–8
European fan palm	*Chamaerops humilis* L.	6–8
Blue dracaena	*Cordyline indivisa* (G. Forst.) Steud	6–8

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TABLE 5 Continued

Common name	Botanical name	Maximum permissible soil salinity[b] EC_e (dS m^{-1})
Spindle tree, cv. Grandiflora	*Euonymus japonica* Thunb.	6–8
Rosemary	*Rosmarinus officinalis* L.	6–8
Aleppo pine	*Pinus halepensis* Mill.	6–8
Sweet gum	*Liquidambar styraciflua* L.	6–8
Tolerant		
Brush cherry	*Syzygium paniculatum* Gaertn.	>8[c]
Ceniza	*Leucophyllum frutescens* (Berland.) I. M. Johnst.	>8[c]
Natal plum	*Carissa grandiflora* (E. H. Mey.) A. DC.	>8[c]
Evergreen pear	*Pyrus kawakamii* Hayata	>8[c]
Bougainvillea	*Bougainvillea spectabilis* Willd.	>8[c]
Italian stone pine	*Pinus pinea* L.	>8[c]
Very tolerant		
White iceplant	*Delosperma alba* N. E. Br.	>10[c]
Rosea iceplant	*Drosanthemum hispidum* (L.) Schwant	>10[c]
Purple iceplant	*Lampranthus productus* N. E. Br.	>10[c]
Croceum iceplant	*Mesembryanthemum croceus* Jacq.	>10[c]

EC_e, electrical conductivity of the saturated-soil extract.
[a] Species are listed in order of increasing tolerance based on appearance as well as growth reduction.
[b] Salinities exceeding the maximum permissible EC_e may cause leaf burn, loss of leaves, and/or excessive stunting.
[c] Maximum permissible EC_e is unknown. No injury symptoms or growth reduction was apparent at 7 dS m^{-1}. The growth of all iceplant species was increased by soil salinity of 7 dS m^{-1}.
Source: Data compiled from Refs. 47, 214, and 215.

azalea, gardenia, gerbera, amaryllis, and African violet are considered somewhat sensitive [220,221]. Like other ornamental species, the esthetic value of floral plants is the determining factor for salt tolerance.

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9

Mineral Nutrient Acquisition and Response by Plants Grown in Saline Environments

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INTRODUCTION

Plants acquire mineral nutrients from their native soil environments. Most crop plants are glycophytes and have evolved under conditions of low soil salinity. Consequently, they have developed mechanisms for absorbing, transporting, and utilizing mineral nutrients in nonsaline soils. Under saline conditions, which are characterized by low nutrient-ion activities and extreme ratios of Na^+/Ca^{2+}, Na^+/K^+, Mg^{2+}/Ca^{2+}, and Cl^-/NO_3^-, nutritional disorders can develop and crop growth and quality may be reduced. This is not surprising, since under saline conditions, Na^+ and/or Cl^- often exceed macronutrient concentrations by one or two orders of magnitude and even more in the case of micronutrients. Halophytes, native to saline environments, may also develop nutrient disorders despite their remarkable ability to absorb nutrients selectively from soil solutions dominated by Na^+ and Cl^-.

Nutrient imbalance can develop in salt-stressed plants in different ways. It may result from the effect of salinity on nutrient availability, uptake, transport, or partitioning within the plant or may be caused by physiological inactivation of a given nutrient resulting in an increase in the plant's internal requirement for that essential element. It is likely that salinity may affect one or more of these processes at the same time, but whether or not this imbalance results in loss of crop yield or quality depends on its severity, which is influenced by a number of environmental factors.

Nutrient availability and uptake by plants grown in saline environments is related to (a) the activity of the nutrient ion in the soil solution which depends on pH, pE[1] (the negative log of

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the activity of the electron), concentration, and composition; (b) the concentration and ratios of accompanying elements that influence the uptake and transport of this nutrient by roots; and (c) numerous environmental factors. Unless the salinizing ions are nutrients (e.g., Ca^{2+}, Mg^{2+}, SO_4^{2-}), increasing salinity generally decreases nutrient availability.

Plants vary not only in the rate by which they absorb an available nutrient element but also in the manner by which they spatially distribute the element within the plant. Moreover salinity can affect this internal distribution. For example, sodium can have a profound influence on calcium mobility and distribution within organs, particularly when it is the sole salinizing cation.

Even in the absence of salinity, nutrient availability, uptake, transport, and distribution in plants are affected by a number of biotic and abiotic factors resulting in complex interactions [1]. The presence of salinity, however, adds a new level of complexity to the mineral nutrition of plants.

NUTRIENT CONCENTRATION AND PLANT RESPONSE

In the absence of salinity, plant growth in relation to the concentration of an essential nutrient element in the root media is often described by the function illustrated in Figure 1. This relationship is a modification of the ''generalized dose response curve'' illustrated by Berry and Wallace [2]. Plant growth, usually expressed as absolute or relative biomass, is suboptimal when the concentration or activity of the essential nutrient element is less than A and optimal when the concentration is between A and B. Nutrient concentrations that exceed B may inhibit growth owing to either a toxicity or to a salt-induced nutrient deficiency.

A substantial body of information in the literature indicates that the plant may not exhibit the same response function under saline conditions as it does under nonsaline conditions. In some cases, the optimal range may be widened, narrowed, or it may shift in one direction or the other depending on the plant species (or cultivar), the particular nutrient, the salinity level, or environmental conditions. In most studies, salinity (either concentration or composition) is a major variable, and the experiment may only have a few treatments that vary in nutrient concentration. Therefore, most reported studies present insufficient data under saline and nonsaline conditions to develop response functions similar to Figure 1. Nevertheless, many studies demonstrated that an optimal concentration or activity of a particular nutrient element in nonsaline conditions may be deficient or, in a few cases, excessive under saline conditions.

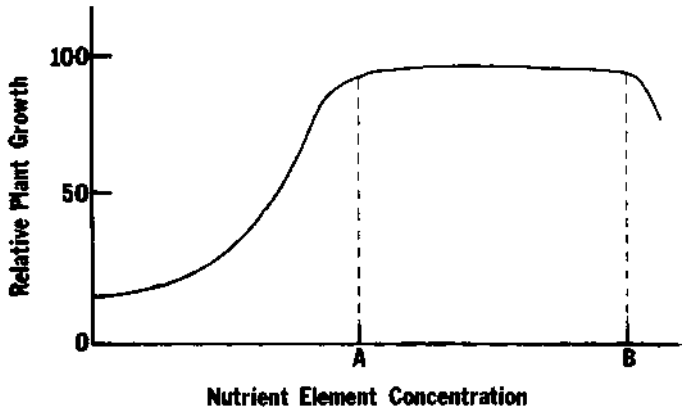

FIGURE 1 Relative growth of plants in relation to a wide range of concentrations of an essential nutrient element.

INTERPRETATION OF SALINITY–NUTRIENT INTERACTIONS

Salinity and mineral nutrient interaction studies are conducted in the laboratory, greenhouse, and the field and correspondingly test a broad range of agronomic, horticultural, or physiological hypotheses.

In many salinity-fertility studies conducted in the field, a major objective is to test if fertilizing salt-stressed plants either alleviates the growth-limiting effect of salinity or actually increases crop salt tolerance. In most of these field studies, two major factors operate simultaneously to limit growth and development: the presence of salinity and the imbalance of a particular nutrient element. The ''salt-tolerance'' of a crop, as defined by Maas and Hoffman [3], may vary depending on whether salinity or nutrition is the factor more limiting to growth. Bernstein et al. [4] defined three different types of idealized salinity and nutrition interactions that could occur: (a) no effect on salt tolerance, (b) increased salt tolerance, and (c) decreased salt tolerance. In contrast to the definition of Bernstein et al. [4], we prefer to define the interactions based on plant performance at optimal fertility relative to the performance at suboptimal fertility, and this interpretation is shown in Figure 2(a–c). Generally, plant growth will be promoted more if the limiting factor is relieved rather than the next limiting factor. For example, if nutrient deficiency limits growth more than salinity, a crop may appear to be more salt tolerant than it would if the plant was adequately supplied with that nutrient. That is, improving soil fertility to an adequate level would improve plant performance proportionally more in nonsaline conditions than under saline conditions.

Bernstein et al. [4] concluded that the effects of salinity and nutrition on grains and several vegetables are independent and additive when stresses imposed on them by nutrient deficiency and salinity are moderate. When either of these factors severely limit growth, the other has little influence on yield. Ten years later, the work of Okusanya and Ungar [5] with two halophytes and a glycophyte gave results that support Bernstein's salinity and fertility interaction model [4]. In the study by Okusanya and Ungar [5], nutrient applications increased the growth of the halophytes in saline conditions, presumably because salinity was moderately growth limiting. On the other hand, nutrient applications did not improve plant growth of the glycophyte under saline conditions, presumably because salinity was severely growth limiting.

It should be made clear, however, that the salinity and fertility interactions described by Bernstein et al. [4] are idealized and, therefore, can be misleading if interpreted improperly. These investigators emphasized that growth (or yield) is controlled by the factor (salinity or nutrient deficiency) that is most growth limiting. Yet the interactions are based on the plant response to salinity as it increases from nonlimiting to severely limiting levels. In many experiments, the nutrient concentration is the most limiting factor in low-salinity conditions, yet when the identical concentration is present in a highly saline environment, salinity will be the limiting factor. This point was emphasized by Champagnol [6] in his literature review on the relationship between salinity and phosphorus nutrition of plants.

Much of the data in the literature that describes salinity $\times$ N or salinity $\times$ P response functions can be reanalyzed by examining the interactions under low-, moderate-, and high-salinity levels. In many cases, a response function similar to that illustrated in Figure 3 will be obtained. Under low-salinity stress, nutrient deficiency limits plant growth more than salinity and a positive (+) interaction or an increased salt-tolerance response occurs. Under moderate salinity, nutrient deficiency and salinity stress may be equally limiting plant growth and no interaction (0) occurs. Under high-salinity conditions, salinity limits growth more than nutrient deficiency. In fact, plant performance would always exhibit a negative (−) interaction or a ''decreased salt tolerance'' (see Fig. 2c) response if a nutrient element was limiting growth under nonsaline conditions and the upper salinity treatment was lethal or severely growth limiting. In this case, only plants grown in nonsaline environments would respond to a nutrient addition.

In light of the above discussion and the multitude of interactions that could occur, results reported by various scientists on this subject may not be as contradictory as reviewers (e.g., see Refs. 7–10) have suggested.

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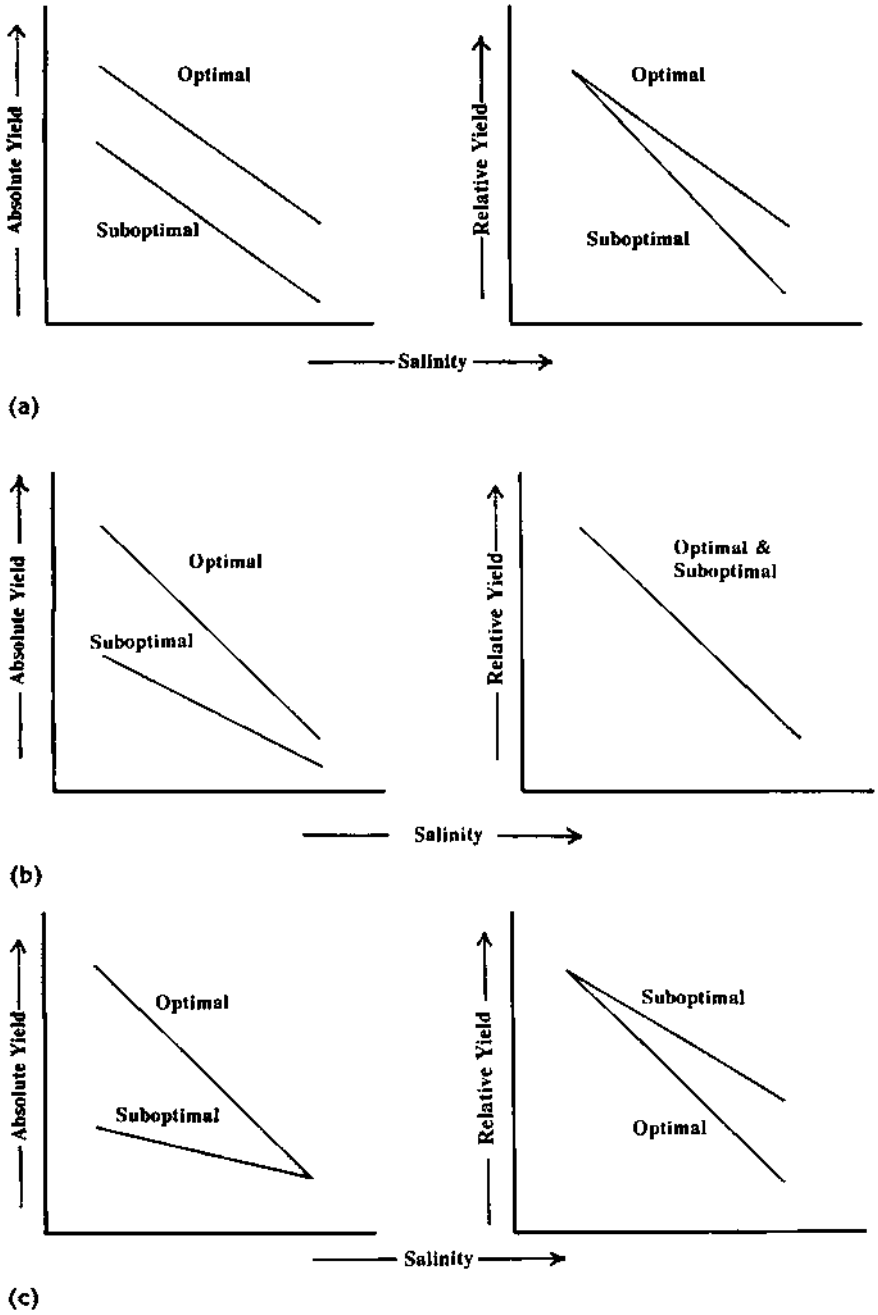

FIGURE 2 (a–c) Types of growth responses a plant can exhibit under variable salinity as the nutrient status within the substrate increases from suboptimal to optimal levels.

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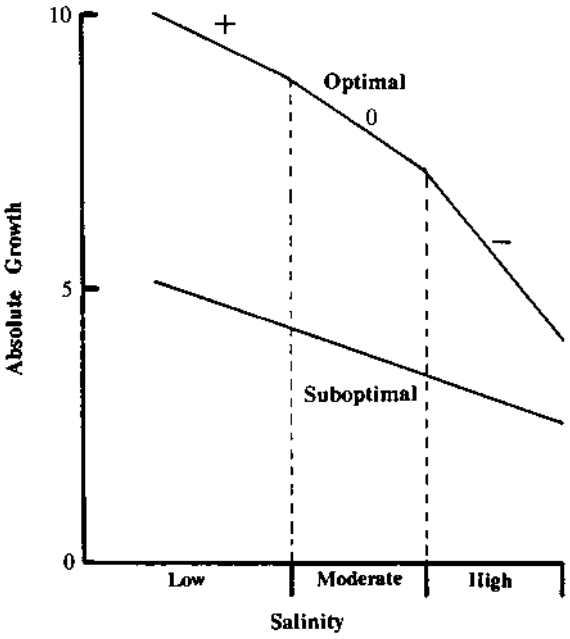

FIGURE 3 Influence of low, moderate, and high levels of salinity at sub-optimal and optimal levels of nutrient supply on plant growth. Symbols indicate increase (+), no effect (0), and decrease (−) in plant tolerance to salinity, respectively.

SOIL AND SOLUTION CULTURE STUDIES

Many of the studies in the area of plant nutrition and salinity interactions have been conducted in sand or solution cultures. A major difficulty in understanding plant nutrition as it is affected by soil salinity is reconciling results obtained in experiments conducted in the field and in solution cultures [7]. In the field, the concentrations of some nutrients in the soil solution, particularly, P, K^+, and the micronutrients, are controlled by the solid phase and concentrations are much lower than those in nutrient solutions. To complicate matters further, field studies must contend with extreme variability in salinity, nutrient concentration, soil moisture, and soil texture, all of which vary with depth, location, and time. In solution cultures, nutrient ratios are much different than those found in soil solutions and concentrations of nutrients and salts are controlled over the course of the experiment. Furthermore, it is well known that root development is entirely different in both systems, so it is likely that plant responses and interactions observed in artificial media may not necessarily occur, at least with the same magnitude, as they would under natural conditions. Nevertheless, solution culture studies are extremely beneficial, since they have advanced our understanding of plant salt tolerance and of the physiological mechanisms responsible for nutrient uptake and discrimination.

STUDIES WITH SINGLE VERSUS MIXED SALTS

Salinity and salt type vary among soils and water supplies across the globe. In most cases, the major cations in the water supply or soil solution are Na^+, Ca^{2+}, and Mg^{2+}, whereas the major anions are Cl^- SO_4^{2-}, and HCO_3^-. Despite the variation in composition, there is a general relationship between salinity and the ratio of $Na^+/(Na^+ + Ca^{2+})$ in waters around the world [11]. Those waters very high in salinity such as those in oceans and seas have a ratio near 1, indicating that Na^+ is the major

salinizing cation. However, the bulk of the inland waters with low (60 mg/L total dissolved salts) to moderate (1300 mg/L) salinity have ratios between 0.1 and 0.7, indicating that Ca^{2+}, although not the major cation, is a substantial contributor to the salinizing media.

Based on this information, one would expect that using single salts, for example, NaCl, would be more appropriate when conducting salinity-nutrient studies with halophytes, whereas a mixed salt solution of some type would be more suitable for nonhalophytes (i.e., most crop plants). Surprisingly, a large percentage of salinity studies on agronomic and horticultural crops use NaCl as the sole salinizing agent, which limits the extent by which one can interpret the results or relate them to field conditions.

The same argument for the cations can also be taken for the anions. The majority of salinity studies use Cl^- as the sole salinizing anion, yet most agricultural fields affected by salinity have a substantial amount of SO_4^{2-} and HCO_3^-. We argue that much more can be learned if a larger fraction of future salinity-nutrient studies, regardless of experimental scale or objectives, are conducted with more realistic ion ratios.

The remaining portion of this chapter is directed toward plant performance and acquisition of the major nutrient elements (N, P, K^+, Ca^{2+}, Mg^{2+}, and S) and micronutrient elements in saline environments. This chapter includes references to both soil and solution culture studies as well as those that use single-salt (i.e., NaCl) and mixed-salt compositions. It is beyond the scope of this chapter to address salinity-nutrient interactions at the biochemical or molecular level, nor are salinity-microbe interactions addressed despite their importance in mineral nutrition. The emphasis is placed on glycophytes at the organ and whole-plant level. Discussion of halophytes is included where appropriate and where information is available.

NITROGEN

In most soils, saline or nonsaline, N is usually the most growth-limiting plant nutrient. Consequently, addition of N usually improves plant growth and yield. In many field studies, researchers set out to test the hypothesis that N fertilizer additions alleviate, at least to some extent, the deleterious effect of salinity on plants.

Most salinity and N interaction studies were conducted on soils deficient in N. Therefore, additions of N improved growth and/or yield of apple [12], barley [13], bean [14–16], carrots, cowpea, tomato, corn, clover, beans, millet and vetch [17], coastal bermudagrass [18], corn and cotton [19], corn and millet [20], tomato [21], spinach [22], wheat [23,24], and rice [23] when the degree of salinity was not severe. In most of these studies, the fact that applied N did not improve the growth under extreme saline conditions suggests that applied N decreased plant salt tolerance (see response in Fig. 2c). On the other hand, only one study showed an increase in crop yield under saline conditions where N was applied above a level considered optimal under nonsaline conditions [25,26]. In this case, additional N did in fact increase the salt tolerance of millet and clover. Selassie and Wagenet [27] also reported that the salt tolerance of well-watered corn may have been increased with urea additions up to 375 kg/ha to a soil initially supplied with sufficient N. This practice, however, is not necessarily practical and would most likely be undesirable from both economical and environmental perspectives.

Despite the majority of evidence indicating that N applied to saline soils above a level considered optimal under nonsaline conditions does not improve plant growth or yield, a number of laboratory and greenhouse studies have shown that salinity reduces N accumulation in plants [28–33]. This is not surprising, since with few exceptions [8,34], an increase in Cl^- uptake and accumulation is accompanied by a decrease in shoot nitrogen concentration. Examples of this effect are also found in barley [35–38], cotton [39], cucumber [40], eggplant [41], tomato [42], tomato and melon [43], and wheat [44,45]. Many attribute this reduction to Cl^- antagonism on NO_3^- uptake [42,43,46], whereas others attributed the response to salinity's effect on reduced water uptake [47].

Gorham et al. [48] observed that despite drastic reductions in leaf NO_3^- concentrations in

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response to salinity, other nitrogen-containing fractions either increased (e.g., proline, glycinebetaine, total soluble protein) or were not greatly reduced (e.g., total amino acid content). These results argue against N deficiency per se as a mechanism of salt injury. This conclusion is also supported by Munns and Termaat [49]. In their review, these investigators suggested that although NaCl-treated plants may contain less N than nonstressed plants, there is no strong evidence to support that this effect is growth limiting.

In contrast to the effect of Cl^- on NO_3^- uptake, reported data indicate that increased NO_3^- in the substrate decreased Cl^- uptake and accumulation [4,42,43,50]. This type of interaction may be particularly important to tree and vine crops that are susceptible to Cl^- toxicity. In one study with citrus and avocado, additions of NO_3^- above concentrations considered sufficient for optimal growth decreased the Cl^- concentrations in leaves to the extent that foliar injury was reduced, thereby lessening growth inhibition by salinity [46]. The authors did, however, caution the reader that such practices could promote NO_3^- contamination of the ground water.

The form in which N is supplied to salt-stressed plants may influence salinity-N relations as well as affect salinity's relationship with other nutrients [50–52], although the form of N did not influence the yield of moderately salt-stressed wheat [53]. NH_4^+-fed maize and wheat plants were more sensitive to salinity than NO_3^--fed plants when grown in solution cultures [51,52]. Similar responses were found in melon [54]. Addition of Ca^{2+} to the media improved the growth rate of the plants in the NO_3^- treatment but not those treated with NH_4^+ [51]. In addition, Martinez and Cerdá [50] found that Cl^- uptake was enhanced in cucumber when half the NO_3^- in the solution was replaced by NH_4^+. These investigators further noted that when NO_3^- was the only N source, accumulation of K^+ in the plant was increased in saline conditions. When the media contained both NO_3^- and NH_4^+, K^+ was reduced. Similar effects were found in salt-stressed melon [54]. As the NH_4^+/NO_3^- ratio was increased, plants accumulated more Na^+ and Cl^- and less Ca^{2+} and K^+ in their leaves. Based on the results of their nutrient solution experiments, Leidi et al. [55] suggested that NO_3^- is a better N source than NH_4^+ for wheat. This conclusion was supported by Silberbush and Lips [56,57], who reported that mean grain weight of wheat grown in sand cultures was negatively correlated with the NH_4^+/NO_3^- ratio. In a more recent study [58], however, it was recommended that the best source of N for wheat was a mixture of NH_4^+ and NO_3^-.

The results of salinity and N-source studies conducted in hydroponic or sand cultures, cited above, contrast to those where plants were grown in soil. Shaviv et al. [59] found that wheat grown in soil salinized with NaCl was more tolerant in terms of grain yield under a combination of NH_4^+ and NO_3^- than NO_3^- alone. This was also found for peanut in a salinity study testing the plant response to different forms of N in soil and solution cultures [60]. This is a classic example of how plant-nutritional experiments conducted in solution cultures alone may lead to inappropriate fertilizer recommendations for the field.

Halophytes grown in highly saline, N-deficient environments and glycophytes grown in mildly saline, N-deficient environments respond similarly to added N [5,61–64]. Skeffington and Jeffrey [63] found that N additions increased the growth of *Plantago maritima* L. even when grown in seawater. Furthermore, N additions increased plant survival. Okusanya and Ungar [5] found that the poor growth of two *Spergularia* species grown in 50% seawater was improved by $Ca(NO_3)_2$ additions. Naidoo [62] studied the interactive effects of N and NaCl salinity on young mangroves (*Avicennia marina* [Forsk.] Vierh.). The N was supplied as NH_4^+ rather than NO_3^- to simulate the saturated, and thus anaerobic, environments that are typical of the natural habitat of mangroves. Therefore, nitrate reduction is prominent and most plant-available N is in the NH_4^+ form. Naidoo [62] found that increased salinity decreased N and K^+ in tissues. The decrease in tissue N is probably caused by NH_4^+/Na^+ competition, since Bradley and Morris [65] found that sea-salt salinity reduced the kinetics of NH_4^+ uptake in *Spartina alterniflora* Lois. Furthermore, as NH_4^+-N increased from 1.4 to 14 mg/L, shoot growth increased in the 100- and 300-mM NaCl treatment, but not in the 500-mM NaCl treatment. Therefore, in agreement with most of the work with glycophytes, it would be interpreted that added N decreased salt tolerance of these halophytic species if the response was characterized over the entire range of salinity.

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In some halophytes, the minimum internal shoot N concentration required for biomass accumulation may be affected by salinity. Bradley and Morris [66] found that the minimum tissue concentration of N required to sustain biomass accumulation in *Spartina alterniflora* increased with increasing salinity.

Some halophytes have salt glands, a unique anatomical feature that allows the plant selectively to excrete salt (particularly NaCl) from its shoot. Not only does this feature allow the plant to reduce its internal salt load, at least to some extent, it improves the nutrient relations within the plant. Waisel et al. [67] suggested that salt glands, by selective removal of Na^+ and Cl^- from the leaves of *Avicennia marina* (Forsk.) Vierh., may help this mangrove species metabolize normally by decreasing the ratios of Cl^-/NO_3^-, $Cl^-/H_2PO_4^-$, and Na^+/K^+ within its leaves.

PHOSPHORUS

The interaction between salinity and phosphorus (P) nutrition of plants is perhaps as complex as that between salinity and N. The interaction is highly dependent on the plant species (or cultivar), plant developmental age [68], the composition and level of salinity, and the concentration of P in the substrate. Therefore, depending on plants selected and conditions of the experiment, different results can occur.

Nearly two decades have elapsed since Champagnol [6] reviewed 17 publications and found that P, added to saline soils, increased crop growth and yield in 34 of the 37 crops studied. However, added P did not necessarily increase crop salt tolerance as defined by the nutrient × salinity response model originally developed by Bernstein et al. [4]. After analyzing studies with barley, carrot, clover, maize, millet, sorghum, sugar beet, tomato, vetch, and wheat, Champagnol [6] concluded that added P increased, had no effect, or decreased salt tolerance as salinity increased from low, to moderate, to high levels, respectively. This demonstrates the complexity of interpreting salinity-fertility studies regarding whether or not the addition of P to deficient soils or media increases crop salt tolerance. The most useful conclusion from studies reviewed by Champagnol [6] is that P additions to P-deficient soils are beneficial providing that the crop is not experiencing severe salt stress.

The influence of salinity on P accumulation in crop plants is variable and depends on the plant and experimental conditions [6]. In most cases, salinity decreased the concentration of P in plant tissue [69], whereas in others, it increased P or had no effect. It is not surprising that these differences among studies occur, since plant type and environmental conditions play a large role in P accumulation and P concentrations vary widely in different experiments. Champagnol [6] concluded that it is unlikely that Cl^- and $H_2PO_4^-$ ions are competitive in terms of plant uptake. However, Papadopoulos and Rendig [21] concluded that Cl^- may have suppressed P uptake and accumulation in tomato shoots. Martinez and Läuchli [70,71] found that not only did NaCl salinity reduce phosphate uptake by cotton roots but also showed a reduction in P transport within the roots and from the roots to the shoots. Zhukovskaya [68] found that Cl^- as well as SO_4^{2-} salts reduce P uptake in barley and sunflower. In other cases, a reduction in plant P concentration by salinity may result from the reduced activity of P in the soil solution owing to the high ionic strength of the media [72].

Many of the studies that show salinity-reduced P concentrations in plant tissues were conducted in soils. Phosphate availability is reduced in saline soils not only because of ionic strength effects that reduce the activity of P but also because P concentrations in soil solution are tightly controlled by sorption processes and by the low solubility of Ca-P minerals. Therefore, it is understandable that P concentrations in field-grown agronomic crops decreased as salinity ($NaCl + CaCl_2$) increased [69]. In many cases, tissue P concentration was reduced between 20 and 50%, yet there was no evidence of P deficiency in the crops. In cases where plants are P deficient, they may be more sensitive to salinity. Gibson [73] found that P-deficient wheat plants were more sensitive to salinity than those with adequate P and that deficient plants had a lower cellular tolerance for the accumulated ion.

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Since the solubility of P in the solutions of saline soils containing high levels of Ca^{2+} is controlled by sorption processes on Al hydroxides and by the solid phase of Ca-P minerals, it is reasonable to question why some plants respond positively to added P. Evidently the kinetics of sorption and/or precipitation are relatively slow and initial forms of calcium phosphate are thermodynamically unstable (D.L. Suarez, U.S. Salinity Laboratory, personal communication, 1990). Later, more stable phases are formed, plant availability decreases, and repeated P applications to saline/calcareous soils are required.

Some research indicates that salinity may increase the P requirement of certain plants. Awad et al. [72] found that when NaCl increased in the substrate from 10 to 50 and 100 mM, the P concentrations in the youngest mature tomato leaf necessary to obtain 50% yield increased from 58 to 77 and 97 mM/kg dry weight (DW), respectively. Their conclusion was also supported by foliar symptoms of P deficiency that were evident on plants grown at high NaCl but were not evident on others at lower salinity with equal leaf P concentrations.

Unlike studies conducted in the field, most studies which demonstrated that salinity increased tissue P were conducted in sand or solution cultures. Phosphate concentration in solution cultures is often orders of magnitude higher than that in soil solutions (e.g., 2 mM vs 2 μM). Several studies conducted in solution cultures have shown that P concentrations that are optimal in nonsaline solutions may adversely affect growth or be toxic to corn [4,74], lupin [75], sesame [76], and certain soybean cultivars [77] when grown in saline solutions. This is evidence that the optimal P range (A to B in Fig. 1), in these instances, narrows under saline conditions. In all these studies, salinity increased P accumulation in plants at the highest substrate P level. The increased P accumulation in the shoot is independent of the composition of salts in the growth media and is presumably controlled at the root level [78]. However, the actual mechanism of this salinity-enhanced uptake rate of P by roots is still unknown [79]. It should be emphasized, however, these adverse interactions in the studies described above would rarely occur under field conditions, because P concentrations in soil solutions are usually orders of magnitude less than those used in these studies. Nevertheless, these interactions are important from an academic viewpoint and pose interesting questions regarding the mechanisms of P uptake and transport within the plant.

Although the majority of studies that report salinity-induced P toxicities to crops have been conducted in solution cultures, this phenomena has also been observed in field conditions [80]. Additions of P greater than 18 kg/ha to paddy rice under salt stress were found to be toxic and resulted is a substantial reduction in yield.

Phosphate additions to halophytes grown in highly saline environments have also resulted in increased plant growth. Okusanya and Fawole [81] showed that phosphate stimulated the growth of *Lavatera arborea* L. much more at 40 and 50% strength seawater than under nonsaline conditions. The magnitude of this effect may be partly due to the increase in the shoot/root ratio by salinity. When no phosphate was added, salinity reduced plant growth. However, when 0.05 and 0.25 mM phosphate was added to the nutrient sand culture, salinity, at the concentration of 40% seawater, actually increased plant growth. Therefore, addition of phosphate increased the salt tolerance of *L. arborea*.

POTASSIUM

Potassium is the most prominent inorganic plant solute, and as such it makes a major contribution to reducing the osmotic potential in root cells to facilitate turgor pressure–driven solute transport processes and to sustain the overall water balance of the plant [1]. Therefore, maintenance of adequate levels of K^+ is essential for plant survival in saline habitats.

Potassium, like P, is present in relatively low concentrations in the soil solution. Potassium is readily adsorbed onto the surface of soil particles and is fixed, and thus unavailable, within layers of expandable 2:1 clay minerals. In some vermiculitic soils, applications of K as high as 700 kg/ha were ineffective at correcting visual symptoms in K-deficient cotton [82]. Because of the plant's

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requirement for an adequate amount of K^+, it is fortunate that the plasma membranes of root cortical cells have a high affinity for K^+ over Na^+ even though the degree of selectivity can vary quite drastically among species [83]. This is particularly important in saline/sodic and sodic environments, where concentrations of Na^+ in the soil solution are orders of magnitude higher than that of K^+. The high K^+/Na^+ selectivity within plants is maintained provided that the calcium status in the root is adequate [84–87] and that the roots have a sufficient supply of O_2 [88].

Although plants selectively absorb and translocate K^+ in preference to Na^+, the degree of selectivity varies among species as well as cultivars within a species. Kafkafi [10] reported the data of Bower and Wadleigh [89] as the fraction of monovalent cations (Na[Na+K] or K[Na+K]) in the exchange complex versus that within the roots of bean and beet. Kafkafi [10] concluded that the roots of the salt-tolerant species (beet) had a higher affinity for K^+, in exchange for Na^+, than the salt-sensitive species (bean). Rathert [90] found that salinity ($Na^+/K^+ = 9$) reduced the concentration of K^+ in the leaves of the salt-sensitive cotton cultivar (Dandara) more than that in the salt-tolerant cultivar (Giza 45).

There is evidence that Na^+ can partially substitute for K^+ in many glycophytic species without affecting growth. Marschner [1] classified many crop species into four groups depending on the extent by which Na^+ can replace K^+. Crop species in group A can replace a high proportion of K^+ by Na^+ (e.g., beets, turnip, and swiss chard), whereas in crop species in group D (e.g., maize, bean, and lettuce) no substitution of K^+ is possible.

Rice has been classified as a group C crop where only a minor substitution of K^+ by Na^+ is possible and Na^+ has no specific effect on growth, which is unlike those crops in groups A and B [1]. However, the addition of 17 mM NaCl to solution cultures low in available K^+ improved vegetative growth and increased panicle yield [91]. Sodium chloride decreased the K^+ content only when the K^+ supply was low. Thus, a relatively high Na^+ content may benefit K nutrition in rice under saline conditions when the supply of K^+ is low [91]. Supplemental K^+ improved all yield components in salt-stressed rice and decreased Na^+, Ca^{2+}, and Mg^{2+} in the straw [92]. Despite the plant's high affinity for K^+ over Na^+, the K^+ status in plants is related to the ratio of Na^+/K^+ in the saturated-soil extract [93]. If it is assumed that the composition of the soil solution is at least close to equilibrium with that on the exchange phase, then it would follow that K^+ accumulation by the root would be reduced if the exchangeable sodium percentage (ESP) on the exchange phase were increased. This effect was observed in bean and beet [89].

Numerous studies have shown that the K^+ concentration in plant tissue is reduced as Na^+ salinity or the Na^+/Ca^{2+} ratio in the root media is increased (e.g., see Refs. 5,86,87,94–96). Reduction in K^+ uptake in plants by Na^+ is a competitive process and occurs regardless of whether the solution is dominated by Na^+ salts of Cl^- or SO_4^{2-}. Janzen and Chang [95] found that barley plants exposed to Na_2SO_4 salinity contained only one-third the concentration of K^+ in their shoots than those grown in nonsalinized solutions. Sodium-induced K^+ deficiency has been implicated in growth and yield reductions of various crops, including tomato [97,98], spinach [99], fennel [100], and maize [101].

Halophytes, like glycophytes, have also shown a high degree of K^+ selectivity and increasing Na^+ concentrations in the substrate have caused reduced K^+ concentrations in their shoots. Excised leaf tissue of the mangrove, *Avicennia marina* (Forsk.) Vierh., was highly selective for K^+ over Na^+ [102] and *Hordeum jubatum* L. was found selectively to transport K^+ to the shoot against a strong external concentration gradient of Na^+ [103]. Nevertheless, increased NaCl salinity decreased shoot K^+ in the same mangrove species even though there was no effect on root K^+ [62]. Ball et al. [104] concluded that NaCl salinity produced a salinity-induced K^+ deficiency in *A. marina*. In contrast, Clough [105] found no differences in leaf or stem K^+ in *A. marina* when plants were grown in different dilutions of seawater. The author did note, however, that the K^+ concentration in the media increased ninefold as the percentage of seawater increased from 0 to 100.

Although plants show high selectivity of K^+ over Na^+, excessive amounts of K^+ may be detrimental to some plants. Rush and Epstein [106] found that the wild tomato species (*Lycopersicon cheesmanii* ssp. minor [Hook.] C.H. Mull.) could tolerate 200 mM Na^+ but 200 mM K^+ was toxic.

On the other hand, the domestic and more salt-sensitive tomato species (*Lycopersicon esculentum* Mill.) showed the opposite behavior; it could tolerate K^+, but not Na^+, at the same concentration. The adverse effects of high K^+/Na^+ at high total salt concentration have been observed in both halophytes (e.g., *Atriplex amnicola, A. inflata, A. nummularia* Lindl., *Suaeda maritima* [L.] Dum., and *Vigna radiata*) [107,108] and glycophytes [90,93,108].

Despite the overwhelming amount of data that show reduced uptake and translocation of K^+ by plants grown in high Na^+ substrates, there are little data that show that the addition of K^+ to sodium-dominated soils improved plant growth or yield. Bernstein et al. [4] found that increasing solution K^+ from 0.4 to 2.0 mM did not affect leaf K^+ or yield of corn. Bar-Tal et al. [109] did find an increase in the yield of corn grown in sandy soil, but the response was proportional at all salinity levels. These investigators concluded that despite its beneficial effects on increasing K^+/Na^+ within the plant, K^+ fertilization did not reduce the deleterious effects of salinity. Using solution cultures, Muhammed et al. [110] found that shoot and root growth of rice plants grown in 100-mM NaCl solutions were increased when substrate K^+ increased from 1 to 7 mM. In other nutrient culture studies, Chow et al. [99] showed that differences in the shoot growth of spinach, between plants grown at low (50 mM NaCl) and high (250 mM NaCl) salinity at a given level of K^+, can be reduced when K^+ is added to the highest salinity treatment. However, plant growth at the low-salinity level only doubled when K^+ in the solution was increased from 0.01 to 10.0 mM. In field conditions, soil solution K^+ remains relatively low even after fertilizer additions of K^+. Therefore, it is difficult to imagine many situations where reasonable amounts of K^+ added to the soil would completely correct Na^+-induced K^+ deficiencies in plants suffering from this disorder.

CALCIUM

Calcium (Ca) plays a vital nutritional and physiological role in plant metabolism. It is essential in processes that preserve the structural and functional integrity of plant membranes [111], stabilize cell wall structures, regulate ion transport, and control ion-exchange behavior as well as cell wall enzyme activities [112]. Because Ca^{2+} is readily displaced from its extracellular binding sites by other cations, these functions may become seriously impaired by reduced Ca^{2+} availability. Root growth and function may be restricted by high Na^+/Ca^{2+} [85,113–116]. From the results of a quantitative study of plasma membrane–bound Ca^{2+}, Yermiyahu et al. [117] concluded that salt-resistant genotypes may have a lower requirement for the fraction of surface charges bound to Ca^{2+}. Solomon et al. [118] observed abnormal root morphology and anatomy of pea (*Pisum sativum* L.) grown in nutrient cultures containing 120 mM NaCl as the sole salinizing salt. These ''salinity-induced'' changes, characterized by curvature of the root tip as well as constriction and thickening above the apex, were completely reversed by the addition of 10 mM Ca^{2+} [119]. Sodium-induced Ca^{2+} deficiencies have notorious growth-distorting effects on developing leaves as illustrated on several grass species grown in solution cultures [120–122].

The presence of Ca^{2+} as the dominant cation in agricultural soils generally ensures that the absolute Ca^{2+} level is not a primary growth-limiting factor. As salinity increases, the requirements of plants for Ca^{2+} increases [123]. In saline soils, as contrasted with sodic soils, Ca^{2+} concentrations usually increase as the total salt concentration increases. At the same time, however, the uptake of Ca^{2+} from the soil solution may decrease because of ion interactions, precipitation, and increases in ionic strength that reduce the activity of Ca^{2+}. These combined effects are at least partially responsible for reduced yields under saline or sodic conditions [95,124–126]. Therefore, in reference to Figure 1, the optimum range is shifted to the right for most crops grown under saline conditions, particularly, if the solution is dominated by Na^+ salts.

The critical Ca^{2+} requirement for plants has been estimated as the ratio of soluble Ca^{2+} to the total cations (Ca^{2+}/TC) rather than to the absolute concentration of Ca^{2+} in the soil solution. Physiological disorders that are related to Ca^{2+} deficiency occur when the Ca^{2+}/TC falls below a critical level [127,128]. In the Solonetzic soils of the Canadian prairie, ion imbalances result from

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high Na^+ and low Ca^{2+} together with predominately sulfate salinity. Severe Ca^{2+} deficiency in barley occurs in these regions when the Ca^{2+}/Mg^{2+} molar ratio or the Ca^{2+}/TC ratio is less than 0.15 [129]. The critical Ca^{2+} requirement for the optimum rate of extension of cotton root has been related to the molar Ca^{2+}/TC ratio [130]. Subsequently, the Ca^{2+}/TC ratio, expressed in terms of ion activity, was considered to be a more accurate measure of Ca^{2+} availability [131–133]. However, it would seem preferable to distinguish specific ion competition; for example, Ca^{2+}/Na^+ and Ca^{2+}/Mg^{2+} rather than Ca^{2+}/TC.

The Ca^{2+}/TC in the soil solution has been related to the Ca^{2+}/TC in saturated-paste extracts [95]. Carter and Webster [134] used this relationship to predict plant-available Ca^{2+} as well as Ca^{2+} accumulation in plant tissues. Critical levels of Ca^{2+} in barley and wheat (63 mM/kg DW) and alfalfa (250 mM/kg DW.) corresponded to a Ca^{2+}/TC ratio of 0.10 in the soil extract.

Although NaCl salinity reduced shoot Ca concentration in barley, this decrease was not due to reduced influx of Ca^{2+} into the roots by the salinizing salts [135]. Lynch and Läuchli [135] proposed that sodium may inhibit the radial movement of Ca^{2+} from the external solution to the root xylem by screening of cation exchange sites in the apoplast. Cramer et al. [94,136] suggested that the primary response to NaCl stress in cotton roots is the displacement of membrane-associated Ca^{2+} by Na^+ leading to increased membrane permeability and to loss of K^+/Na^+ selectivity. The addition of 10 mM Ca^{2+} to the saline cultures preserved membrane integrity and prevented leakage of K^+. Exchange constants, calculated from the relationship between the activities of Ca^{2+} and Na^+ in nutrient cultures and the equivalent fraction of Ca^{2+} and Na^+ in corn shoots indicated that the cation uptake process is strongly selective for Ca^{2+} against Na^+. As the activity of Na^+ in the substrate increases, however, the system becomes less discriminating and the selectivity for Ca^{2+} is impaired [137]. Likewise, Davenport et al. [138] concluded that the maintenance of a critical Ca^{2+} activity in the substrate rather than a specific Ca^{2+}/Na^+ ratio was essential for normal growth of salt-stressed wheat.

Nutritional imbalances in salt-stressed cereals have been studied in isosmotic nutrient solutions salinized with various molar ratios of Na^+ and Ca^{2+}. This investigation included corn [122], rice [139], and sorghum [121] as well as wheat, barley, rye, and oats (E.V. Maas and C.M. Grieve, unpublished data, 1984). The cereals show striking intergeneric differences in their response to different Na^+/Ca^{2+} molar ratios in cultures of equal osmotic potential (OP). A salt stress of -0.6 MPa with $Na^+/Ca^{2+} = 52$ reduced the relative dry matter yield of wheat less than that of rye or oats. At -0.4 MPa, rice was more sensitive at $Na^+/Ca^{2+} = 5$ than was corn.

In a comparative study of a cultivated barley and a wild barley variety that exhibits higher salt tolerance, the wild species was able to maintain higher tissue concentrations of calcium and was more effective at compartmentalizing Na^+ in the root rather than the shoot [140]. This difference between barley species may partly explain why increasing the $Ca^{2+}/(Ca^{2+} + Mg^{2+} + Na^+)$ ratio from 0.02 to 0.09 in the solution culture only benefited the cultivated species. Wild barley (*Hordeum jubatum* L.) populations also differ in their response to salinity. Wang et al. [141] identified three ecotypes, two of which were more tolerant of $MgSO_4$-salinity and high Na^+ than was the third. The investigators attributed the enhanced growth of the tolerant ecotypes to their superior Ca^{2+}-use efficiency and their ability to restrict Na^+ and Mg^{2+} translocation to the leaves.

Genotypes may also vary in their susceptibility to Ca^{2+} disorders at high substrate Na^+/Ca^{2+}. Grieve and Maas [121] compared the response of three sorghum cultivars and suggested that the Na^+ tolerance of Hegari cultivar was related to the efficiency of Ca^{2+} transport to the developing leaves. At $Na^+/Ca^{2+} = 34.6$ and OP $= -0.40$ MPa, many of the expanding blades of the sensitive cultivars NK 265 and NB 9040 were deeply serrated and tightly rolled with withered, often necrotic, tips. These symptoms have been associated with severe Ca^{2+} deficiency [142], and this diagnosis was confirmed by mineral analysis. Yeo and Flowers [143] reported that the elite breeding line (IR 2153) of rice was very unresponsive to external Ca^{2+}. Shoot growth of this line was not affected over a wide range (5–500) of Na^+/Ca^{2+} ratios and Ca^{2+} concentration had a limited effect on NaCl uptake. In contrast, high Na^+/Ca^{2+} inhibited shoot growth in two rice cultivars (M9 and M201) developed for specific regions of California [139]. Ca^{2+}-deficiency symptoms were observed at an

OP of −0.4 MPa and Na^+/Ca^{2+} molar ratios of 198 and 78. Shoot growth improved and the Ca^{2+} disorder was eliminated when the Na^+/Ca^{2+} ratio was reduced to 17.8 [139]. The shoot and root growth of the rice cultivar KS282 was significantly influenced by external Na^+/Ca^{2+} [110]. Rolling and bleaching of the young leaves occurred when the Na^+/Ca^{2+} ratio exceeded 100. Muhammed et al. [110] also attributed differences in root growth to an interaction between Na^+/Ca^{2+} and Na^+/K^+ ratios in the root media. Norlyn and Epstein [144] observed that triticale lines differed in tolerance to high Na^+/Ca^{2+} (i.e., 500) during emergence and germination. Emergence of only one line improved when the Na^+/Ca^{2+} was reduced to 37, whereas other lines showed no effect of added Ca^{2+}. Kingsbury and Epstein [145] contrasted the response of two wheat genotypes to isosmotic solutions that varied in ionic composition. One line was highly resistant to Na^+ toxicity and, in response to high external Mg^{2+}/Ca^{2+}, showed superior Ca^{2+} -use efficiency. Ashraf and O'Leary [146] reported that in response to varying external Na^+/Ca^{2+}, transpiration and stomatal conductance in a salt-tolerant sunflower were unchanged, whereas these parameters in a salt-sensitive line decreased significantly. In closely related *Brassica* species, Ashraf and Naqvi [147] found that although supplemental Ca^{2+} improved dry matter production of *B. napus* and *B. juncea*, growth of *B. campestris* and *B. carinata* was unaffected by increases in Ca^{2+} concentration in growth media salinized with 150 mM NaCl (13 dS/m). In contrast, growth of these four species, along with *B. oleraceae* and *B. nigra*, was not influenced by the addition of Ca^{2+} to saline solution cultures (Instant Ocean*, 8 dS/m) containing a mixture of salts [148].

Several studies [110,121] have shown that as the injured cereal leaves mature and become less dependent on root pressure for their supply of water and nutrients, their Ca^{2+} demands are then met via increased transpiration rates. Eventually, the Ca^{2+} concentration in the older blades of salinized plants was as high as in those in the nonsaline controls.

The limited capacity of plants to regulate internal Ca^{2+} distribution in relation to the demands of low-transpiring organs has been implicated in numerous Ca^{2+} -related physiological disorders [149–151]. Even under nonsaline conditions, failure to meet the Ca^{2+} requirements of developing leaves may result in necrosis of these tissues as in blackheart of celery [152], tipburn of lettuce and Chinese cabbage [153], and internal browning of cauliflower and Brussels sprouts [151]. Root crops may be similarly affected; for example, cracking and cavity spot of carrots and parsnips [150]. Calcium disorders in reproductive tissues are associated with heavy economic losses in the fruit industry due to such diseases as blossom-end rot (BER) of tomato, pepper, and melon, bitter pit of apples and pears; and end spot of avocado. Under saline or sodic conditions, the calcium status of these organs may become even more impaired, which will affect their marketability.

The use of low to moderately saline irrigation waters or nutrient cultures for tomato production has been advocated to improve fruit quality by increasing firmness as well as the levels of total soluble solids; for example, reducing sugars and organic acids [154–156]. Depending on the cultivar, this practice may, however, reduce Ca^{2+} content of the fruit and increase the incidence of BER [157,158].

Ca^{2+}-related disorders are profoundly influenced by the interaction of salinity with environmental factors [159]. Although conditions that increase transpiration rates may stimulate the uptake of Ca^{2+}, it may be disproportionately delivered to older leaves and cause localized deficiency in young tissues. Reduction of Ca^{2+} accumulation in fruit was associated with increased incidence of BER of salt-stressed tomatoes grown under low daytime humidity [160], high root temperature, and high irradiance [161]. Differences in environmental conditions most probably account for the disparate results obtained by two separate teams of researchers investigating the response of artichoke to salinity. In the first instance, plants were grown under controlled greenhouse conditions in pot cultures irrigated with saline (NaCl) waters with an electrical conductivity (EC) ranging from 0.74

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to 15 dS/m. Plant injury due to salinity was restricted to moderate necrosis on the older leaves [162,163]. The second study was a field trial conducted during two consecutive years in a desert area whose high daytime temperatures and desiccating winds resulted in very high transpiration rates. Although irrigation waters were salinized with NaCl and $CaCl_2$, the Ca^{2+} requirements of the developing buds were not met and internal browning occurred. More than half the plants irrigated with saline water at 10 dS/m produced buds that were unmarketable. These investigators [164,165] attributed this disorder to poor Ca^{2+} partitioning rather than restricted uptake inasmuch as the transpiration-mediated Ca^{2+} transport to the leaf blades increased, whereas root pressure–driven Ca^{2+} transport to the shoot apex decreased.

Salinity's effect on root pressure has also been associated with Ca^{2+}-related injury in young tissues that depend on diffusion to meet their demands for plant resources, for example, Chinese cabbage [166], lettuce [167], and celery [168]. In wheat and barley, the reduction of spikelet primordium numbers may also occur through a similar process or through Ca^{2+-}-mediated signaling of salinity stress [169].

Increased root permeability, caused by reduction in the availability of external Ca^{2+}, may lead to increased Cl^- uptake. Elevated internal Cl^- concentrations have been associated with decreased shoot growth in several species such as cowpea [170], tobacco [171], pigeon pea [87], and *Leucaena leucocephala* [115,116].

Maintaining an adequate supply of Ca^{2+} in the soil solution is an important factor in controlling the severity of specific ion toxicities [172]. This is particularly important for tree and vine crops, which are more prone to Na^+ and Cl^- injury than most annual crops. In citrus, calcium was found to be effective at reducing the transport of both Na^+ and Cl^- from the roots to leaves, thereby reducing foliar injury [173–176].

The importance of maintaining a balanced nutrient solution to optimize plant performance of glycophytes under saline conditions has been known for over 80 years [177], yet an alarming percentage of salinity studies conducted to date use NaCl as the only salinizing salt. We must, therefore, emphasize that the use of extreme ratios of Na^+ and Ca^{2+} may introduce unique nutritional problems and result in misleading and erroneous interpretations about the plant response to salinity.

MAGNESIUM

Many studies have analyzed the plant tissue for Mg^{2+}, yet most salinity-nutrient studies have directed little attention to magnesium nutrition as affected by salinity.

Calcium is strongly competitive with Mg^{2+}, and the binding sites on the root plasma membrane appear to have less affinity for the highly hydrated Mg^{2+} than for Ca^{2+} [1]. Thus, high concentrations of substrate Ca^{2+} usually result in increased leaf Ca along with a marked reduction in leaf Mg [178]. Increased concentration of $CaSO_4$ in the nutrient solution decreased Mg^{2+} in roots, stems, and leaves of *Leucaena leucocephala* [116]. Calcium-induced Mg^{2+} deficiency has been observed in sesame [179] as well as corn [180]. Both the photosynthetic rate and water-use efficiency declined in salt-stressed corn (OP = −0.4 MPa) as the external Ca^{2+}/Mg^{2+} ratio increased [180]. Carter et al. [129] found that barley growth was reduced as the Mg^{2+}/Ca^{2+} ratio increased above 1. Similarly, grain yield of wheat decreased significantly in soil cultures irrigated with saline waters (EC = 6 dS/m) containing an Mg^{2+}/Ca^{2+} ratio of 4. With increasing external Mg^{2+}/Ca^{2+}, Na^+ and P in the straw increased, whereas K^+ decreased [181].

Increases in salinity, however, are not always associated with reductions in leaf Mg^{2+} concentration. Bernstein et al. [4] found that increases in salinity (NaCl + $CaCl_2$) reduced leaf Mg^{2+} concentration in beet but had little or no effect in leaves from the five other vegetable crops that they tested.

In the case of plants grown in seawater or dilutions of seawater, it is possible that nutrient disorders could develop because of the high Mg^{2+}/Ca^{2+} ratio. In most seawater compositions, Mg^{2+}: Ca^{2+} is 5:1 on a molar basis. It has been known for over 30 years that solutions with an Mg^{2+}/

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Ca^{2+} ratio greater than 1 reduces the growth of corn and soybean [182]. In a more recent study, Mg-salts reduced root growth of eucalyptus more than Na-salts [183]. Reduced root growth was associated with low Ca^{2+} concentrations in the root.

SULFUR

Most salinity studies that include sulfur as an external variable have not examined the influence of salinity on sulfur nutrition in the plant. Rather these studies were directed more toward how the plant responds to sulfate salinity as compared to chloride salinity. Differences in crop response to chloride and sulfate salinity have been measured in terms of identical electrical conductivities [184–186], molar or equivalent basis [187–193], or isosmotic potentials [194–199]. Whether or not differences in plant response are found between chloride and sulfate salinity may depend on the salinity indices chosen [10].

Very little attention is given to salinity's influence on sulfur uptake and accumulation in crops. In one study that compared the effects of both Cl^- and SO_4^{2-}-salinity on pea, Mor and Manchanda [186] found that chloride salinity reduced the sulfur content in the straw. Sulfur accumulation in the roots, however, was enhanced by Cl-salinity.

Many crops are very sensitive to high internal chloride levels, and species are generally more tolerant of sulfate salinity; possibly owing to enhanced P or N nutrition [199,200]. Consequently, Bernstein [201] suggested that for most vegetable crops, the salt tolerance would be 2 dS/m greater in a sulfate system as opposed to chloride system. At low salt levels, the response of salinized sorghum followed this general rule [195,202]; however, as salinity increased (-0.6 MPa), growth was inhibited more by sulfate than chloride salinity, probably through disruption of the Na^+-exclusion mechanism which resulted in an increase in shoot Na^+ and concurrent nutrient ion imbalances [195]. Likewise, sulfate salinity was more damaging to the halophyte *Chenopodium rubrum* than NaCl salinity, particularly at high concentrations [203].

MICRONUTRIENTS

The concentrations of micronutrients in soil solutions, with the exception of Cl^-, are low (μM range) and depend on the physical and chemical characteristics of the soil. The availability of most micronutrients depends on the pH and pE of the soil solution as well as the nature of binding sites on organic and inorganic particle surfaces. Consequently, the relationship between salinity and trace element nutrition is complex [204]. In saline and/or sodic soils, the solubility of micronutrients (e.g., Cu, Fe, Mn, and Zn) is particularly low, and plants grown in these soils often experience deficiencies in these elements [205,206]. Nevertheless, the micronutrient concentration in plant shoots may increase, decrease, or have no effect depending on the type of plant, tissue, salinity, micronutrient concentration, and environmental conditions.

Zinc (Zn) concentration has been found to increase in shoots of salt-stressed barley [204,207], bean [208], squash, tomato [209], pepper [210], and rice grain [211] but decrease in corn [212], bean [213], and mesquite [214]. Salinity increased the manganese (Mn) concentration in the shoots of barley [204,207], rice [211], sugar beet [215], and tomato [209] but decreased its concentration in the shoots of barley (cultivar CM72) [216], squash [209], pea [217], corn [212], peanut, and cucumber [218]. In the study with sugar beets [215], salt ($NaCl + CaCl_2$) additions increased Mn in the saturated soil extract. Others did not find an effect of salinity on shoot Mn, but increasing sodicity in soil-grown maize had a significant reduction in shoot concentration [219].

Although differences were found in the literature regarding salinity's effect on shoot Mn concentration in barley, the differences may be explained, in part, by the composition of the salinizing salts [204]. Saline solutions rich in divalent cations increase shoot-Mn concentration, whereas a saline environment dominated by monovalent cations reduces shoot Mn concentration. Likewise,

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the accompanying anion in the salinizing media was important in Zn nutrition of soybean. Shoot Zn was higher in chloride-dominated salinity than in the sulfate system [190].

Reports on the influence of salinity on the iron (Fe) concentration in plants are as inconsistent as those that concern Zn and Mn concentration. Salinity increased the Fe concentration in the shoots of pea [217], tomato, soybean, squash [209], bean [213], and rice [211] and decreased its concentration in the shoots of barley, corn [207,212], peanut, and cucumber [218]. In other investigations with barley, salinity had no effect on shoot Fe concentration, but at low Ca^{2+}, salinity increased root Fe in certain *Hordeum vulgare* L. species [204]. This was not observed with foxtail barley (*H. jubatum* L.).

Although the influence of salinity stress on the micronutrient concentration in plants is highly variable, there is evidence that NaCl salinity may induce an Fe deficiency. In the presence of 100–400 mM NaCl, root epidermal cells of *Atriplex hastata* L. and *A. hortensis* L. developed features that are characteristic of transfer cells; for example, bladder-shaped root hairs and thickened convolutions on the outer peripheral cell wall. Further evaluation of these results showed that alterations were not a specific response to salinity but were a symptom of Na^+-induced iron deficiency [220].

BORON

For most crops, the optimal concentration range of plant-available B is very narrow, and various criteria have been proposed to define those levels that are required for adequate B nutrition but at the same time are not so high as to induce B toxicity [221–223]. Although B deficiency is more widespread than B toxicity, particularly in humid climates, B toxicity is more of a concern in arid environments where salinity problems also exist [224].

Toxicity occurs in horticultural crops when boron concentrations increase in either stem and leaf tissues to lethal levels, but soil and plant-tissue analyses can only be used as general guidelines for assessing the risk of B toxicity [223]. Although experimental evidence indicates that plants absorb B passively as H_3BO_3, contradictions between experimental results and observations in the field suggest that other factors, yet unknown, may affect B uptake [225]. Once B has accumulated in a particular organ within the shoot, it has restricted mobility in most plant species but not all [226]. In some plant species, particularly those that produce substantial amounts of polyols, B is readily translocated as B-polyol complexes.

Despite the common occurrence of high boron and high salinity in many parts of the world, very little research has been done to study the interaction of the two. From sand culture experiments conducted in a greenhouse, researchers found that wheat responded to boron in the soil solution independently of salinity ($NaCl + CaCl_2$) [227]. The salinity-B interaction was insignificant with respect to leaf B concentrations. Other investigators found that mixed salt solutions (i.e., Na^+, Ca^{2+}, Cl^-, and SO_4^{2-}) reduced leaf B concentrations in chickpea [228] and wheat [229,230] grown in pot cultures. Grain yield of salt-stressed wheat declined markedly with increasing concentrations of external B, whereas the yield under nonsaline conditions was unaffected by added B [230]. From these results, Manchanda and Sharma [230] concluded that increasing soil salinity decreased the B tolerance of wheat even though B levels in the plant decreased significantly. In other studies using a mixture of chloride and sulfate salts, El-Motaium et al. [231] found that salinity reduced B uptake and accumulation in the stem of several *Prunus* rootstocks, thereby decreasing B-toxicity symptoms. They also found a negative relationship between B and SO_4^{2-} concentrations in tissue suggesting that SO_4^{2-} could be responsible for the salinity-induced reduction in tissue B. Others have also found that a mixture of chloride and sulfate salinity reduces leaf B accumulation in *Eucalyptus camaldulensis* Dehnh. [232]. In neither study were the investigators able to suggest the actual mechanism that supports this phenomenon such as direct ion interactions, reduced transpiration in salt-stressed conditions, or both.

In addition to the potential sulfate-boron interaction, the interaction between B and Ca^{2+} in the nutrition of both mono- and dicotyledonous plants has long been recognized [233,234]. High

concentrations of substrate Ca^{2+}, particularly under calcareous conditions, decreased B absorption and can induce a B deficiency [223]. Therefore, in reference to experiments with mixtures of salts where salinity reduced B uptake and transport to the shoot [228,231,232], it is difficult to distinguish influences of either sulfate or calcium on B uptake since in each case, these ions increased in the substrate with increasing salinity.

CONCLUSIONS

The relations between salinity and the mineral nutrition of plants are extremely complex, and a full understanding of these interactions would require a multidisciplinary team of scientists. It is no easy task to reconcile results from salinity-nutrition experiments conducted in the field versus the greenhouse; in soils versus solution cultures; using single salts versus mixed salts; under one set of environmental conditions versus another set; or studies conducted over the short term versus the long term. Nevertheless, by accounting for these differences in experimental parameters, one can begin to see more consistencies in salinity-nutrient interactions and obtain a better understanding of the salinity-nutrient relations in plants overall.

Plant performance, usually expressed as a crop yield or plant biomass, may be adversely affected by salinity-induced nutritional disorders. In the field, additions of nutrients have increased the growth of both glycophytes and halophytes provided that the plants were not experiencing severe salt stress. Relief of the growth-limiting stress, salinity or nutrient deficiency, promotes growth more than relief of the next limiting factor. Therefore, addition of a limiting nutrient may increase, decrease, or have no effect on plant salt tolerance depending on the severity of salinity stress. Consequently, interpretation of plant salt tolerance expressed on a relative basis under variable soil fertility can be misleading.

Salinity-induced nutritional disorders may develop on plants from the effect of salinity on nutrient availability, competitive uptake, transport, or partitioning within the plant. For example, salinity reduces phosphate uptake and accumulation in crops grown in soils primarily by reducing phosphate availability, whereas in solution cultures, reductions may be due to a competitive process. Salinity dominated by Na^+ salts not only reduces Ca^{2+} availability but also reduces its transport and mobility to growing regions of the plant, thereby affecting the quality of both vegetative and reproductive organs. These disorders are aggravated when transpirational demands are high. Salinity can directly affect nutrient uptake, as has been observed in the reduction in K^+ uptake by Na^+ or NO_3^- uptake by Cl^-. The occurrence of these disorders and their ultimate effect on crop yield or quality depends on the plant species and the experimental conditions where the study was conducted.

Salinity can cause a combination of complex interactions affecting plant metabolism or susceptibility to injury. In several studies, it has been shown that salinity increases the internal requirement for a particular nutrient. Examples were given for N in the halophyte *Spartina alterniflora*, P in tomato, and K^+ in spinach. In other studies, it was shown that salinity can cause plants that are deficient in an element to have a lower cellular tolerance for a specific ion. Moreover, there are undoubtedly a multitude of other interactions yet to be found.

Despite a large number of studies that demonstrate that salinity reduces nutrient uptake and accumulation or affects nutrient partitioning within the plant, little evidence exists that adding nutrients at levels above what is considered optimal in nonsaline environments improves plant growth or crop yield in saline environments. Nutrient additions, on the other hand, have been more successful in improving crop quality. For example, Ca^{2+} additions to soils or as foliar sprays can sometimes correct disorders caused by Na-induced Ca^{2+} deficiencies.

Nutrient additions may also reduce the incidence of injury. An adequate supply of Ca^{2+} maintains membrane integrity and selectivity, thereby reducing Na^+ and Cl^- toxicity in tree and vine crops. Benefits from added Ca^{2+} are usually observed in solution culture studies when NaCl is the sole salinizing agent. There are also studies that have shown that increased concentrations of NO_3^-

can reduce Cl^- toxicity in certain tree crops. Although these studies may have practical implications, there is a danger that this practice may increase NO_3^- concentrations in the groundwater.

It is reasonable to believe that numerous salinity-nutrient interactions are occurring at the same time, but whether they ultimately affect crop yield or quality depends on the salinity level and composition of salts, the crop species, the nutrient in question, and a number of environmental factors.

In the area of salinity-mineral nutrition relations, halophytes have received less attention than have glycophytes. Nevertheless, some halophytes, despite their remarkable ability to absorb nutrients selectively from solutions dominated by Na^+ and Cl^-, may also exhibit symptoms of mineral imbalances and disorders.

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10

Plant Response to Water-Deficit Conditions

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INTRODUCTION

Organisms which live in aerobic environments must constantly cope with the threat of oxidation, and almost any cell process that involves oxygen can create activated oxygen [1]. Reactions involving free radicals are a common feature of plant stress and appear to contribute to a process of oxidative deterioration which leads to cell death when the water potential (Ψ_w) is very low.

Water stress conditions may trigger an increased production of reactive oxygen forms, which can explain the remarkable damage to the enzymes with active sulfydrylic groups, the chloroplast pigments, the membrane lipids and proteins, and the alteration of their structural integrity [2,3]. This formation is a consequence of the Mehler reaction, which provides a pathway for the removal of excess electrochemical energy caused by drought stress [4].

During water depletion, $O_2^{\bullet -}$ can also react nonenzymatically with H_2O_2 giving rise to products, such as hydroxyl radicals and singlet oxygen, which are even more reactive than $O_2^{\bullet -}$ itself [3,5]. Photosynthetic cells can tolerate elevated oxygen levels because of the endogenous mechanisms that effectively scavenge and remove the toxic products before cellular damage occurs [6]. In photosynthetically active cells, it is clear, however, that in the light, reductants generated as a consequence of electron transport can participate in the process of free radical scavenging, whereas in the dark or in heterotrophic tissue, various respiratory pathways must serve as a comparable source of the reductants themselves [7].

ACTIVE OXYGEN SPECIES AND WATER STRESS

Mechanisms of Active Oxygen Production in Plant Cells

The production of active oxygen species (AOS) is an unavoidable consequence of life with oxygen. Atmospheric oxygen ($^3O_2$), being a diradical with a parallel spin state, is particularly unreactive, so

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that its divalent reduction is kinetically limited by the relatively slow spin inversion process. The spin conservation rule states that spin must be conserved during the time necessary for a chemical reaction to occur, so that O_2 in the ground state cannot accept a pair of electrons of opposite spin. For this reason, ground-state O_2 tends to accept additional electrons one at a time. When $^3O_2$ is involved in metabolic oxidation, in order to have productive collision, it must be transformed into more reduced or electronically excited status via a univalent pathway (Equations. [1–4]).

$$O_2 + 1\ e^- \rightarrow O_2^{\bullet -} \quad (1)$$

$$\downarrow +\ e^- + 2\ H^+$$

$$H_2O_2 \quad (2)$$

$$\downarrow +\ e^- + H^+$$

$$^{\bullet}OH \quad (3)$$

$$\downarrow +\ e^- + H^+$$

$$H_2O \quad (4)$$

The first reduced product (Equation [1]) of this univalent pathway is superoxide ($O_2^{\bullet -}$). Superoxide in a nonpolar environment is a powerful nucleophile and base (proton acceptor), which can act as both an oxidant ($E_0 = O_2^{\bullet -}/H_2O_2 = 0.87$ V) with compounds that can donate H^+ such as ascorbate, tocopherol, and catechol as well as a mild reductant ($E_0\ O_2/O_2^{\bullet -} = -0.33$ V).

The successive univalent reductions produce (Equation [2]) hydrogen peroxide (H_2O_2) and (Equation [3]) hydroxyl radical ($^{\bullet}OH$). The redox potential of $^{\bullet}OH$ at approximately 2 V clearly marks this radical as a highly oxidizing agent. This species in biological systems has a very short lifetime (in the range of a few microseconds), because it randomly reacts with almost all organic molecules. Likely candidates are small molecules, enzymes, nucleic acid, proteins, and lipids of membranes.

Hydrogen peroxide is the first stable product of the intermediate reduction of oxygen without radical properties. The formation of H_2O_2 at physiological pH is rather slow, with its rate constant being $1 \times 10^5\ M^{-1}\ s^{-1}$, but superoxide dismutase (SOD, EC 1.15.1.1), an enzyme present in all cell compartments, increases the reaction rate constant to $2 \times 10^9\ M^{-1}\ s^{-1}$ [8]. Hydrogen peroxide, like $O_2^{\bullet -}$, can be a mild reductant as well as an oxidant but, with a standard reduction potential of 1.77 V, it is also a nucleophilic oxidizing agent. Hydrogen peroxide in the chloroplasts is a highly toxic molecule, because, even at low concentrations, it inhibits several Calvin cycle enzymes, so that it is a powerful inhibitor of the photosynthetic CO_2 assimilation. The most sensitive enzymes are fructose-1,6-bisphosphatase and sedoeptulose bisphosphatase, although $NADP^+$-dependent glyceraldehyde-3-phosphate dehydrogenase and phosphoribulokinase can also be attacked [9]. Superoxide radicals and H_2O_2 can either directly attack lipid membranes and inactivate SH-containing enzymes or, in the course of the chain reaction, interact to generate the more toxic $^{\bullet}OH$ (Equation [5]), which can also damage membrane lipids and proteins [10,11].

$$O_2^{\bullet -} + H_2O_2 \rightarrow OH^- + {}^{\bullet}OH + O_2 \quad (5)$$

The Haber-Weiss reaction (Equation [5]) is very slow, but the presence of transition metals such as iron and copper [12,13] catalyzes the reaction (Equations [6 and 7]) [14].

$$O_2^{\bullet -} + M^{n+1} \rightarrow O_2 + M^n \quad (6)$$

$$M^n + H_2O_2 \rightarrow {}^{\bullet}OH + OH^- + M^{n+1} \quad (7)$$

The Fenton reaction (Equation [6]) may also occur when the antioxidant ascorbate is present in the cells at high concentrations [14].

Singlet oxygen ($^1O_2$) is an excited species of oxygen not produced by redox reactions but by absorption of electromagnetic energy. It possesses two electrons with antiparallel spins formed from

the ground state of atmospheric oxygen ($^3O_2$) by transfer of energy from photoexcited compounds (photosensitizers) naturally present in plants, such as chlorophyll, protoporphyrin IX, and many other secondary compounds, including quinones [15]. The change in the spin state from parallel ($^3O_2$) to antiparallel ($^1O_2$) increases its reactivity and eliminates the spin restriction. Singlet oxygen is highly destructive, reacting with most biological molecules almost at diffusion rates [15,16].

Oxygen activation may potentially occur in all compartments of plant cells [17]. The sources of superoxide in plant cells are electron transport activities in which electrons are diverted from their normal course and leak to oxygen. Normally, this leakage is very limited and proportional to the local oxygen concentration, but under certain conditions, such as in water-deficit stress, the possibility of electron leakage to O_2 is enhanced. In mitochondria, the flavoprotein region of the NADPH dehydrogenase segment of the respiratory chain has been identified as a site of production of $O_2^{\bullet -}$ insensitive to cyanide and antimycin A. A second site has been identified close to complexes I and III at the ubiquinone level [18]. When the electron transport is blocked beyond ubiquinone by cyanide or antimycin A, the rate of $O_2^{\bullet -}$ formation increases [19]. In peroxisomal and glyoxisomal membranes, a NADPH-dependent superoxide formation by cytochrome b_5 reductase has been shown to occur [20,21]. A microsomal redox system can form superoxide at the expense of NADPH via cytochrome P-450 or cytochrome P-450 reductase [19]. Moreover, microsomal $O_2^{\bullet -}$ generation in soybean seedlings has been monitored in the presence of either NADPH or NADH as cofactors [22]. It has been suggested that the superoxide found in microsomes of senescent carnation flowers is generated enzymatically, presumably by a membrane-associated oxidase [23].

The concurrence in chloroplasts of a high energy level, high oxygen tension, and abundant catalyst such as chlorophyll, iron-sulfur proteins, and quinones increases the probability of energy transfer to ground-state oxygen [5,17,24,25]. In the chloroplasts, from a thermodynamic viewpoint, O_2 can accept electrons from both photosystems forming superoxide [26]. In addition, chloroplasts, besides being the major site of superoxide production (see section Generation of AOS in Chloroplasts in Water-Deficit Conditions below), are likely sites of singlet oxygen production and action.

Little is known about the enzymes responsible for the reduction of O_2 to form $O_2^{\bullet -}$. An apoplastic NADH, NAD radical, and NAD^+ pathway has been suggested for the production of superoxide by means of cell wall peroxidases [17,27]. A plasma membrane peroxidase is responsible for the formation of $O_2^{\bullet -}$ at the plasmalemma surface [28,29]. More recently, it has been demonstrated that the enzyme responsible for the synthesis of $O_2^{\bullet -}$ by elicitor-treated plant cells is similar to mammalian neutrophil NADPH oxidase [30], and that one function of the flavins and *b*-type cytochromes may be to produce $O_2^{\bullet -}$ in plant plasma membranes [31].

Effects of Oxidative Stress in Cells

It is clear that the toxicity of O_2 comes from its reduction products or from its excitation to the single state and perturbation in cell metabolism would markedly affect AOS levels. The destructive potential of AOS on pigments, lipids, proteins and DNA is well documented [15,17,24,32,33]. AOS can destroy membranes since their target includes phospolipids and glycolipids, whose deesterification produces free fatty acids which, remaining in membranes, lead to more rigid and disassembled structures [34]. Furthermore, oxidation of polyunsaturated fatty acids leads to many different products, such as short-chain alcohols, aldehydes, and alkanes. Under most oxidative conditions, malondialdehyde (MDA) is a product too often considered as a marker of peroxidative damage. It is important to interpret such measurements with caution, since there are a lot of drawbacks linked to the thiobarbituric acid (TBA) test for MDA determination [35–39]. In particular, in regard to its use to monitor peroxidative damage due to water-deficit conditions, we must consider that TBA may react with several oxidized products of amino acids, among which proline, and carbohydrates, which are known to accumulate for osmotic adjustment [40].

The formation of toxic oxy radicals is a facet of normal cell metabolism. Indeed the rate of O_2 photoreduction has been estimated to range from 15 to 25 μmol (mg Chl)$^{-1}$ h^{-1} even under normal

conditions [41]. Superoxide, produced by the transport of electrons to oxygen, is not compatible with metabolism; hence all organisms that have evolved in aerobic environments must possess an efficient mechanism capable of preventing or removing oxidation of cellular components. Thus the equilibrium between the oxidative and antioxidative capacities determines the fate of the plant.

Essentially, antioxidant defenses fall into three general classes comprising: (a) the water-soluble reductants, for example, compounds that contain thiol groups (e.g., cysteine, glutathione), ascorbate, urate, cathecols (epinephrine); (b) liposoluble vitamins, for example, α-tocopherol and carotene; and (c) enzymatic antioxidants, for example, SOD, catalase, and peroxidases.

A low steady-state concentration of superoxide may be maintained by the several isozymes of SOD localized in the subcellular compartments where superoxide is produced [42], since superoxide, being a charged molecule, cannot cross biological membranes. Detoxification of H_2O_2, arising from dismutation of superoxides by SOD (Equation [9]) is mediated by catalase (EC 1.11.1.6). However, catalase has a very low affinity for H_2O_2 and it is mostly localized in peroxisomes and glyoxisomes [43], being that its activity is either extremely low or not detectable in the cytosol, mitochondria, and chloroplasts [44]. In plant cells, the ascorbate-glutathione cycle (Asada-Halliwell cycle) represents an alternative and more effective detoxification mechanism against H_2O_2. It may remove H_2O_2 in a series of reactions involving glutathione and ascorbate [45,46] and related enzymes such as ascorbate peroxidase (APX, EC 1.11.1.11), monodehydroascorbate reductase (MDHAR, EC 1.6.5.4), dehydroascorbate reductase (DHAR, EC 1.8.5.1), and glutathione reductase (GR, EC 1.6.4.2). Rapid operation of the cycle and regeneration of the intermediates through photosynthetically generated reductant are necessary, since at least one of its constituent enzymes, APX, is inhibited by H_2O_2 itself [47]. The high affinity of APX for H_2O_2 indicates that this enzyme, rather than catalase, is responsible for most H_2O_2 removal outside the peroxisomes. This pathway may exist in the cytosol, in the chloroplast, and in other compartments as well as in nonphotosynthetic tissues such as roots, although the level of enzyme activity at these locations appears to be lower than in chloroplasts [48–52].

It is clear that an efficient removal of the first two intermediates of oxygen reduction (Equations [1 and 2]) will prevent the formation of the third (Equation [3]). This is fortunate, since the hydroxyl radical is a highly powerful reagent and its specific enzymatic scavenging would be impossible [53].

Furthermore, glutathione peroxidase (GP, EC 1.11.1.9) and glutathione transferase (GT_s) reduce organic peroxides, thus protecting cell proteins and cell membranes against oxidation [50,54,55]. In addition, reduced glutathione (GSH) can break the chain propagation of lipid peroxidation by regenerating the liposoluble α-tocopherol [50].

Active Oxygen Species in Water-Deficit Conditions

During water deficit, when stomata close in order to limit water loss, there is either a restricted CO_2 supply or a CO_2-limited carbon fixation and a decreased availability of oxidized nicotinamide adenine dinucleotide ($NADP^+$) as an electron acceptor for photosystem I. Photosynthetic electron transport is, however, maintained at a relatively higher rate in the stressed leaves as compared with the large decrease in the rate of CO_2 fixation [56,57]. This unbalance between electron transport and CO_2 fixation rates may result in the overreduction of the electron transport chain components and facilitate the transfer of electrons to O_2. This is probably a mechanism that plants adopt to protect the photosynthetic electron transport chain components from photodamage during water stress. Under these conditions, O_2 in the thylakoids can compete with $NADP^+$ as a Hill reductant and can produce superoxide [2]. In addition, when plants are exposed to excess energy or when there is a limitation in CO_2 availability, the possibility of an increase in singlet oxygen production may exist. A consequence of the drought-induced limitation of photosynthesis is indeed the exposure of plants to an excess of energy. If this is not dissipated in a harmless way, it may damage the photosynthetic apparatus because of the overreduction of the photosynthetic reaction centers [58] and the increased production of AOS species in leaf tissue [40].

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The O_2-dependent flow, as well as photorespiration, may be regarded as being regulatory mechanisms for the protection of the photosynthetic apparatus against damage by $^1O_2$, especially when its production is not sufficiently prevented by carotenoids. Carotenoids, and particularly β-carotene, may react with excited triplet chlorophyll and $^1O_2$ [58]. On the other hand, it is now a widely held view that the excess excitation energy is normally dissipated via nonradiative energy dissipation in the pigment bed [58]. Development of excessive energy is also accompanied by deepoxidation of violaxanthin to zeaxanthin (Fig. 1), and there is increasing evidence that zeaxanthin is involved in mediating nonradiative energy dissipation, although the mechanism is not well known [59,60].

Furthermore, the increase in the production of $O_2^{\bullet -}$ gives rise to an increased formation of H_2O_2 and $^{\bullet}OH$, highly reactive oxygen forms, which together with $^1O_2$ can damage cell structure and function. An extensive literature [19,33,61,62] suggests that the origin of the peroxidative damage occurring under water-stress conditions is likely due to the formation of transient but highly reactive, partially reduced or activated forms of oxygen. Evidence for increased generation of activated oxygen producing superoxide as the first product of radical-mediated reactions is scanty. Such evidence comes mainly from the fact that the activities of enzymes, such as SOD, peroxidases, and Asada-Halliwell pathway recycling enzymes, as well as the concentrations of antioxidant molecules, are generally increased in plants exposed to stressful conditions [63–66]. This increase correlates with increased stress tolerance [67].

A stable, C-centered, free radical, determined by electron paramagnetic resonance (EPR), which is formed in vivo has been also used as marker of previous oxidative stress [68–70]. The increase in the amplitude of the EPR signal of water-stressed sunflower seedlings [69] indicates that water-deficit conditions have determined the further production of the stable radicals as found during the loss of desiccation tolerance in seed tissues [68,71–73] and during the desiccation of two species of mosses [74].

Measurements of $O_2^{\bullet -}$ in biological systems are technically difficult. Generally, the methods used lack specificity and/or sensitivity, making it difficult to determine the rate of $O_2^{\bullet -}$ production accurately, as the radicals are very unstable and terminate quickly by disproportionation or other mechanisms [75]. However, 1,2-dihydroxybenzene-3,5-disulfonic acid (Tiron), a disulfonate catechol that is readily oxidized to the corresponding semiquinone (a more stable radical) by $O_2^{\bullet -}$, can be used to detect the superoxide as soon as its production has been induced by illumination of

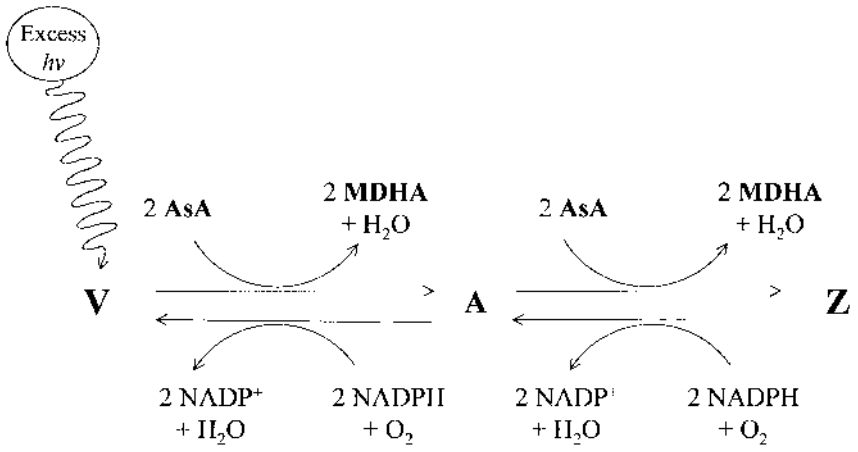

FIGURE 1 Nonradiative energy dissipation via the xanthophyll cycle. The energy dissipation process involves deepoxidation of violaxanthin (V) to antheraxanthin (A) and to zeaxanthin (Z). Epoxidation of zeaxanthin (Z) regenerates violaxanthin (V).

isolated thylakoids or other biological systems [3,49,75–77]. The generation of the signal of the Tiron radical depends on oxygen and is obscured by scavenger of $O_2^{\bullet-}$, such as ascorbate, L-adrenaline or reduced glutathione, showing that the Tiron radical is derived from $O_2^{\bullet-}$ [49]. Exogenous SOD is less effective in obscuring the signal, because the larger size of the enzyme, in comparison with Tiron, makes it less able to permeate the site of $O_2^{\bullet-}$ production. This reflects a steric problem in which the SOD is unable to access the $O_2^{\bullet-}$ which is available for the much smaller Tiron molecule [75,76,78].

Several factors seem to be involved in $O_2^{\bullet-}$ production under drought conditions: electron transport rate, composition of membranes, higher exposure of chlorophyll molecules to oxygen, plant species, intensity of stress, repeated stress periods, and plant age [3,5,49,63,77,79].

Attempts have also been made to compare directly the formation of reactive oxygen species from stressed and unstressed plants. These investigations provide some evidence for an increased rate of formation of $O_2^{\bullet-}$ in stressed plants. Isolated thylakoids from drought-stressed wheat show increased superoxide formation [78]. Superoxide formation in thylakoids from wheat and sunflower seedlings subjected to increasing stress by water deficit is higher than in the control [49,79]. An increase in superoxide in wheat mitochondria after desiccation has been also found [18].

Generation of AOS in Chloroplasts in Water-Deficit Conditions

Chloroplasts are likely to be particularly subjected to oxidative injury, because they are the most aerobic compartment in plant cells. This is due to the fact that they both consume and produce oxygen and their functionality depends on the redox capacity of their photosynthetic apparatus. Furthermore, they contain a large amount of polar lipids and polyunsaturated fatty acid residues. The photosensitizing pigments absorb light and as the electron transport chain operates in a high O_2 environment, its tendency to leak electrons to oxygen is correspondently greater. Molecular oxygen possesses the physicochemical properties that permit this molecule to serve as an alternative Hill oxidant within the chloroplasts in vivo [41]. The solubility of O_2 in the aprotic interior of membranes is higher than in water [80] and $O_2^{\bullet-}$ production is not limited by O_2 availability [81]. In the interior of the chloroplasts, the O_2 concentration has been estimated to range from 275 to 300 μM, and even at moderate light intensities (350 $\mu E\ m^{-2}s^{-1}$), the rate of superoxide formation may be as high as 15 $\mu mol\ (mg\ Chl)^{-1}\ h^{-1}$ [41].

The production of $O_2^{\bullet-}$ in chloroplasts has been extensively reviewed [80]. Briefly, thermodynamically feasible sites for superoxide production in the chloroplasts can be either photosystem I (PSI) and/or photosystem II (PSII). Two mechanisms are associated with $O_2^{\bullet-}$ generation on the reducing site of PSI. Most superoxide production proceeds via the univalent reduction of oxygen by reduced ferredoxin. Ferredoxin undergoes an oxidation in a one-electron step when the availability of $NADP^+$ is low (i.e., under water-deficit conditions). An alternative source is from the nonhaem Fe-S center [80]. At the PSII acceptor side, superoxide may be formed as the result of passing electrons from pheophytin to plastoquinone (Q_A) to O_2 [40,41,82–84]. In this way, when the CO_2 supply is limited, O_2 can act as an alternative acceptor, thereby maintaining Q_A in a partially oxidized state and maintaining electron transport. The possibility that superoxide may also arise by autooxidation of Q_B cannot be excluded. A direct oxidation of plastoquinone to produce $O_2^{\bullet-}$ has not yet been demonstrated under physiological conditions, although several nonendogenous quinones have been shown to produce H_2O_2 on illumination of chloroplasts. Cytochrome *f* and plastocyanin can instead accept electrons from $O_2^{\bullet-}$, and in physiological conditions, a superoxide-mediated cyclic transport around PSI cannot be excluded [80].

When plants are exposed to light levels exceeding those they have experienced during growth, photoinhibition may occur, because there is an exposure to more light that can be used for CO_2 fixation. Water deficit could also lead to exposure of the leaves to excess energy when plants are grown in natural sunlight. In these conditions, the light becomes excessive, because the utilization of energy through photosynthetic carbon assimilation decreases. This occurs whether the limita-

tion of photosynthesis is caused by stomatal limitation to the carbon dioxide supply or by direct inhibition of carbon fixation without significantly decreasing the rate of electron transport.

Moreover, type I photodynamic reactions (Equation [8]), undergoing charge separation within the excited pigment, have been postulated in the production of superoxide radicals [5], and the direct involvement of chlorophyll in the production of the Tiron radical signal, via superoxide, has been demonstrated [75].

$$P + h\nu \rightarrow P^* \rightarrow {}_+P^- \text{ (charge separation)}; \; {}_+P^- + {}^3O_2 \rightarrow {}_+P + O_2^{\bullet -} \tag{8}$$

When normal pathways and acceptors of photosynthetic electron transport are restricted under water-stress conditions, photodynamic reaction type I (Equation [8]) may be intensified.

The reaction initiated by light is mediated by the photosynthetic electron chain [7,77,85,86]. The impairment by drought of photosynthesis, without significantly decreasing the flow of electrons through the photosystems, and the transient disruption of the photosystems during water stress, making them ''leaky,'' both result in the transfer of electrons to molecular oxygen, thus increasing the production of superoxide (Fig. 2).

Chloroplasts lack glycolate oxidase which in leaf peroxisomes, through a two-electron pathway, produces hydrogen peroxide. Therefore, in the absence of such oxidase, hydrogen peroxide in chloroplasts derives from the dismutation of superoxide catalyzed by SOD (Equation [9]).

$$O_2^{\bullet -} + O_2^{\bullet -} + H^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2 \tag{9}$$

In addition, other reductants (ascorbate, Mn^{2+}, GSH, reduced ferredoxin) may reduce superoxide, but the low stromal concentrations of these reductants and their low reaction rate constants with superoxide make their contribution to the formation of hydrogen peroxide very unlikely [80].

Trace amounts of transition metals, such as Fe^{2+} and Cu^+ [87–89], or reduced ferredoxin

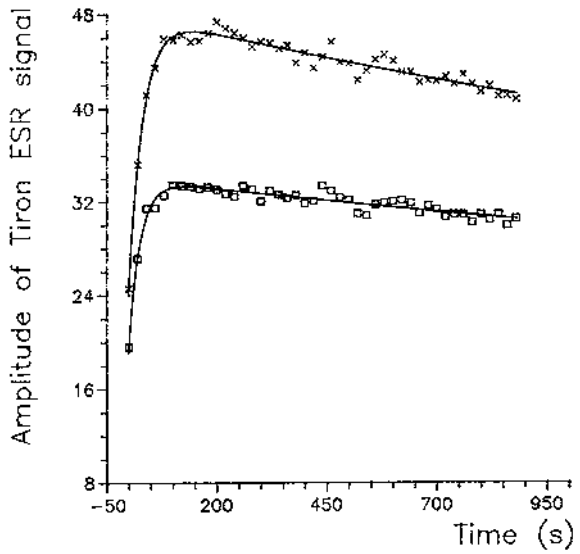

FIGURE 2 Kinetic measurements of $O_2^{\bullet -}$ production by illuminated thylakoids in plants of *Triticum durum* L cv. Adamello subjected to water deficit. □, Control; ×, Stressed; continous lines, least-squares-fit curves. Amplitude is given in arbitrary units. (From Ref. 50.)

[90] may catalyze the formation of hydroxyl radicals from hydrogen peroxide (Equations [5–7]). Superoxide, ascorbate, and/or GSH reduce back the oxidized metals.

ANTIOXIDANT PROTECTION IN CHLOROPLASTS

Ascorbate-Glutathione System in Chloroplasts and Its Role in Water-Deficit Conditions

The steady state of AOS in the chloroplasts depends on the critical balance between those factors that would tend to generate them and the mechanisms that protect cells from their production [26,91,92]. Under normal conditions, this balance is tightly controlled, but environmental constraints can lead to an increase in the steady-state concentration of AOS. Thus AOS production overcomes the protection afforded by antioxidant defense mechanisms; thereby leading to oxidative damage to tissues. An increase in low molecular mass antioxidants, as well as in the activities of antioxidant enzymes [62,93–95], plays an irreplaceable role in stressful conditions and those augmentations are correlated with enhanced tolerance [96].

An important strategy in the ability of antioxidants to protect cells is an early intervention in order to break the sequence of chain reactions determined by the production of AOS. Thus the mobility, as well as a radical-trapping ability, of antioxidants are important in determining their effectiveness in biological systems. The liposoluble antioxidants and membrane-bound antioxidative enzymes of higher plants serve as the first line of defense against AOS produced within membranes, whereas the water-soluble antioxidants serve to eliminate AOS in the aqueous phase. Active oxygen species, except for hydrogen peroxide, cannot diffuse very far from their locus of formation, since in biological systems, they have a very small average radius of diffusion and a half-life of only a few microseconds [97]. For this reason, in order to prevent damage, protective measures have to be available in the immediate surroundings of those cellular compartments where the production of AOS takes place. It is reasonable to assume that chloroplasts are a primary site for AOS injury (see Section Generation of AOS in Chloroplasts in Water-Deficit Conditions below) and their efficient removal is critical, since H_2O_2 can inhibit photosynthesis even at concentrations as low as 10 μM [98]. For this reason, specific mechanisms of detoxification have evolved that act at both stromal and thylakoid level. The lipophilic antioxidants tocopherol and carotenoids fulfill essential antioxidant action in thylakoid membranes. Furthermore, in the chloroplasts, two separate oxygen radical–scavenging systems are present, a soluble system comprising GSH and reduced ascorbate (AsA) and a membrane-bound system that comprises SOD and APX [80]. The proposed pathway is as in Figure 3.

As soon as $O_2^{\bullet -}$ has been generated from the activity of PSI and PSII (see section Generation of AOS Chloroplasts in Water-Deficit Conditions below), it is rapidly dismutated by SOD (Equation [9]) attached to thylakoids [99,100]. The H_2O_2 produced from this dismutation is reduced to H_2O by the thylakoid-bound APX; the monodehydroascorbate radical (MDHA) so generated is reduced back to AsA by photoreduced ferredoxin or by reducing equivalents of NAD(P)H through the FAD enzyme MDHAR. As compared with the half-time of linear electron transport of the thylakoids (10 ms), the half-times of $O_2^{\bullet -}$, H_2O_2 and MDHA (0.1–10.0 μs) are shorter by several orders of magnitude [101]. Superoxide and H_2O_2, which escape this first line of defense, spread into the stroma where they are scavenged by a stromal SOD and via a reaction cycle initiated by stromal APX. Any MDHA radical formed by APX, which is not reduced to ascorbate by MDHAR, alternatively can disproportionate into ascorbic acid and dehydroascorbic acid, which is unstable at physiological pH [102]. Dehydroascorbate is converted into ascorbic acid by DHAR, which uses GSH as an electron donor. The regeneration of GSH requires GR and NADPH. Ascorbate and glutathione have little influence on the spread of oxygen radicals along or within the membranes, so it seems reasonable to consider that they may intercept only the oxygen radicals spreading outward. The more likely function of GSH is its involvement in the ascorbate-glutathione cycle, but a function in the

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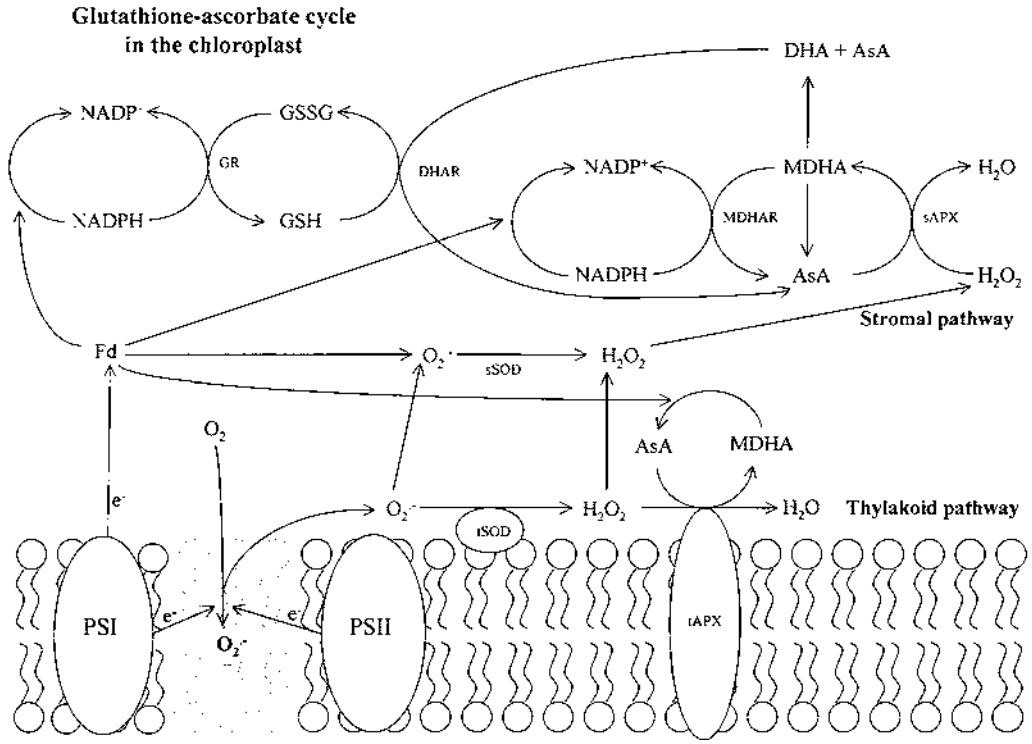

FIGURE 3 Thylakoid and stromal scavenging systems in chloroplasts. Superoxide radicals ($O_2^{\bullet -}$) generated by PSI and PSII are dismutated by thylakoid-bound superoxide dismutase (t-SOD). Hydrogen peroxide formed is reduced to water by the thylakoid-bound ascorbate peroxidase (t-APX). Monodehydroascorbate radical (MDHA) is reduced back to ascorbate (AsA) by the photoreduced ferredoxin (Fd). The escaped MDHA would be reduced back to AsA with FAD enzyme monodehydroascorbate reductase (MDHAR). The $O_2^{\bullet -}$ and H_2O_2 escaped to thylakoid system are scavenged by a stromal superoxide dismutase (sSOD) and by a glutathione-ascorbate cycle initiated by stromal APX (sAPX). Dehydroascorbate (DHAR) formed by MDHA is converted into AsA by dehydroascorbate reductase (DHAR). (Adapted from Ref. 80).

direct removal of oxygen radicals cannot be excluded [103], so that the reaction between GSH and $O_2^{\bullet -}$ may potentially be of importance in oxidative stress and/or in conditions of lowered SOD activity [104]. However, both ascorbate and glutathione may serve as mediators between hydrophylic and lipophilic phases to maintain the antioxidant properties of membrane-localized protective systems (see Section Lipid Oxidation Chain Reaction Breaking in Chloroplasts below). The above depicted cycle in chloroplasts is a mean of photoprotection both when the carbon dioxide supply is reduced and/or when there is an excess of excitation energy.

Effect of Water-Deficit on the Antioxidant Systems

Physiological and genetic evidence suggest that the increase in the AOS-scavenging systems in plants exposed to oxidative stress is an important component of stress-protective mechanisms [62,94,96]. During stress by water deficit, the water status of the plants plays a key role in the activation of defense mechanisms. Contrasting results under the same experimental conditions can

be related to the fact that, besides the difference in species, growth conditions, and stage of the plants, different levels of water deficit experienced by the plants should modulate the enzyme activities. The SOD activity responds to water deficit differently in different experiments and species: increasing, decreasing, or remaining unchanged [40,69,70,105–112]. Wheat and barley experiencing a gradual imposition of water deficit, obtained by withholding water from the soil, increased the activity of SOD [40,70], whereas sunflower showed a decrease [40,69]. Catalase activity seemed to be little affected in the early phase of water-deficit imposition, but with an increased degree of water depletion, a decrease in activity generally occurred [4,70,106–108,111,112]. Inhibition of protein synthesis induced by water depletion [113,114] could explain, at least in part, the decrease in catalase activity when a severe water deficit is imposed. Catalase turns over very rapidly in the light if protein synthesis is inhibited [40]. Catalase is not a robust enzyme. It is susceptible to photoinactivation and degradation, and it is also limited in its effectiveness by the relatively poor affinity for H_2O_2 as well as for the subcellular localization in the peroxisomes [1,94,95]. The GR activity catalyzes the rate-limiting step of the ascorbate-glutathione cycle. Comparison of two maize strains with different sensitivity to water stress showed in the more tolerant cultivar a higher GR activity, which further increased when the two cultivars were submitted to oxidative stress by paraquat or H_2O_2 [115]. Drought tolerance in maize strains was correlated with a high level of both SOD and GR [116], but the criteria used in the classification of tolerant and sensitive strains were not clearly stated. In the leaves of nonirrigated field-grown winter wheat and cotton, the observed increase in GR arises primarily from a smaller inhibition in activity as the leaves mature in comparison with control leaves rather than from an increase from the control level [4,117]. When a leaf water deficit was induced by withholding water, an increase was also monitored in the GR of wheat, sunflower, and sorghum [107,112] and in the GR and MDHAR of *Eragrostis tef* [118]. In sunflower seedlings submitted to a water deficit of increasing intensity, the specific activities of GR, APX, and DHAR have been seen to be dependent on water stress intensity [113], as it has also been observed for SOD and peroxidase activities [69,70,106]. Thus, after 5 days of water depletion, decreases of 0.2 MPa in water potential and osmotic potential were sufficient to induce oxidative stress, to increase H_2O_2, DHA, and GSSG, and to reduce the specific activities of APX, DHAR, and GR to 30–50% of the control seedlings [113]. At a moderate level of stress, when an osmotic adjustment occurred in the seedlings, H_2O_2 did not increase further, and the specific activity of the enzymes of the ascorbate-glutathione cycle were activated [113]. Transcriptional and translational events have been seen to be implicated in metabolic responses to oxidative stress [91], although the possibility of the mobilization of ordinarily inactive enzyme pools and adaptative changes in their catalytic properties must be remembered. At severe water stress, the decrease in enzyme activities was accompanied by the highest H_2O_2 level. The conservation or induction of enzyme activities might be linked to the rate of decline in water potential, which is in agreement with the fact that plants are more tolerant when water deficit stress is slowly imposed [119]. Consistent with the previous findings, in wheat subjected to two water-deficit periods, obtained by withholding water and rewatering at the end of the first period of stress, the decreased defense activities of GR, DHAR, and APX following the second period of stress might be a consequence of a reduced rate of activated oxygen production (Table 1) [49]. In maize, when drought imposition was gradual and acclimation may have occurred, GR activity and H_2O_2 levels were unaffected by dehydration [120]. It has been suggested that when plants are allowed to acclimate to drought, the $O_2^{\bullet -}$ may not accumulate [77]. The previous findings are in agreement with other reports on the decreased activity of GR, DHAR, and APX in plants in which H_2O_2 did not accumulate [109]. It remains to be clarified whether under drought the changes in activity shown in the previous reports are related to particular isoforms. Studies on the individual responses of the different isozymes to drought might in fact provide more useful information. In tomato, for example, it was found that the cytoplasmatic, but not the chloroplastic, CuZn-SOD was induced under drought [62]. GR isoforms have been associated with different cell compartments [121], MDHAR in tomato leaves has at least two isoforms [40], and soluble APX has six isoforms of which at least three are chloroplastic [99].

In pea nodules subjected to a water stress of −2.03 MPa, a general decrease in all the above

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Table 1 Formation Rate of $O_2^{\cdot-}$ in Illuminated Thylakoids and Specific Activities of the Enzymes of the H_2O_2 Detoxification cycle of *Triticum durum* L. cv. Ofanto Subjected to Two Periods of Water Depletion

	First period		Second period	
parameter	control	stressed	control	stressed
Ψ_w	−0.50 a	−1.10 b	−0.60 a	−1.30 b
k	18.50 a	21.30 b	23.10 b	16.30 a
GR	0.19 a	0.18 a	0.15 b	0.11 a
DHAR	0.85 a	1.02 a	1.37 b	0.65 a
APX	0.48 a	0.45 a	0.60 b	0.36 a

Results are the means of three replicates of four separate experiments. For comparisons among means the analysis of variance was used. For each period, means in rows followed by different letters are significantly different at $P \leq .01$. APX, ascorbate peroxidase (U mg^{-1} protein); DHAR, dehydroascorbate reductase (U mg^{-1} protein); GR, glutathione reductase (U mg^{-1} protein); k, $O_2^{\cdot-}$ formation rate constant (10^3 s^{-1}); Ψ_w, leaf water potential (MPa).
Source: From Ref. 49.

reported enzymes but MDHAR and in the content of ascorbate and reduced and oxidized glutathione has been reported as being due to a restricted supply of NAD(P)H in vivo for the ascorbate-glutathione pathway [122]. The MDHAR appears to be more important than DHAR in AsA regeneration [26]. The high concentrations of soluble antioxidants ascorbate and glutathione found in the cells suggest that their roles are not restricted to being substrates for APX and DHAR (see section Ascorbate-Glutathione System in Chloroplasts and Its Role in Water-Deficit Conditions above). Both metabolites may contribute to maintain the highly reducing conditions required by the cells and to protect them by direct scavenging of activated oxygen, especially organic radicals [37]. The GSH during drying may protect, via thiol-disulfide exchange, the thiol status of proteins, therefore maintaining their metabolically active form and the activity of the enzymes which possess exposed thiol groups. In addition to the previous reported functions, GSH is involved as a substrate for the glutathione peroxidase (GP, EC 1.11.1.9), which reduces H_2O_2 and organic peroxides, thus protecting cell proteins and cell membranes against oxidation, as found in wheat and in *Boea hygroscopica* [123,124]. The importance of glutathione in the establishment of water-deficit stress tolerance has been pointed out [51,52]. The water content required to cause GSH oxidation has been reported to be very near the limit of survival for desiccation-sensitive species [40]. In wheat plants subjected to a moderate level of water stress by withholding water, the maintenance of a low GSSG/GSH ratio, despite a decrease in total glutathione content as a consequence of its decreased synthesis and/or its increased degradation, has been established [49,123]. Therefore, the greatest portion of the glutathione in the cell is maintained in the reduced form, which plays an important role in the stabilization of many enzymes and a more general role as an oxidant scavenger. In pea plants, drought caused a 25% decrease in GSH [109], which also has been observed in sunflower seedlings where, as seen in resurrection plants (see section Production of AOS and Protection Mechanisms in Resurrection Plants below), GSH oxidation at the beginning of water depletion may have been the trigger for increased glutathione synthesis at a moderate level of water-deficit stress. In droughted seedlings, GSSG/GSH ratios, always higher than in the control, increased further when under severe water-deficit stress owing to the degradation in GSH [112,113]. This indicates that the induction of defense mechanisms was established late in response to higher oxidative stress. An increase in the GSSG/GSH ratio with increasing drought conditions has also been reported in pea nodules, with most glutathione pools remaining in the GSSG form [122]. At a late stage of drought, however, the GSH level increased in sorghum and the GSSG level decreased at the middle and late stages

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[112]. Other investigators do not distinguish between reduced and oxidized glutathione and ascorbate. In addition, the imposition of water deficit by removing roots from the nutrient medium for some hours each day, may have rapidly induced very large changes in water status. Contrasting patterns in the GSH pool emerge from these studies. Although total GSH increased in some grasses [110], no change occurred in *Armeria maritima* and a decrease was observed in *Cochlearia atlantica* [108]. On the contrary, the total ascorbate pool has been seen to decrease largely in all species investigated by the same workers. A decrease in reduced ascorbate was observed in sunflower seedlings subjected to increasing water-deficit stress. During drying, a maintained total ascorbate pool was observed in the seedlings up until the point of severe stress [113]. Similarly, a reduction in AsA was observed in other species [112,125]. When plants are slowly dehydrated, a different picture emerges in that total ascorbate is maintained at high levels, owing to a good functionality of ascorbate-glutathione cycle, and resulting in being less susceptible to changes than GSH [49,109]. During drying in wheat, the higher AsA/DHA ratio in comparison with the control, despite the decrease in DHAR, may be an indication of the key role of MDHAR in the regenerating AsA [40,49] and of the decrease due to drought in APX, so that ascorbate may accumulate without being consumed. A high level of AsA is necessary for a plant's defense in water-deficit conditions, since in addition to its role in the H_2O_2 detoxification cycle, in the regeneration of tocopherol, and in the zeaxanthine synthesis, AsA can also directly act as a scavenger of hydroxyl radicals.

WATER DEFICIT INDUCES MEMBRANE LIPID CHANGES

Oxidative Stress and Damage to Membranes

Oxidative damage can be described as a consequence of insufficient antioxidant potential or an excessive oxidative stress. Several models, among which is AOS-induced peroxidative damage to membrane lipids, have been regarded as critical initiating events leading to injury of cell membranes during dehydration [126–129]. There is an increasing body of evidence that under water-deficit conditions, plants undergo increased exposure to AOS and accumulation of free radicals, which are associated with damage to membranes and degradation of lipid components. Reduced damage has been linked to increased synthesis of antioxidants and induction of enzymatic defenses against AOS [3,4,11,50,68,69,74,77–79,108,110,114,130–134]. Among AOS, the superoxide radical can promote degradative reactions by a nucleophilic attack on the carbonyl groups of the ester bonds linking fatty acids to the glycerol backbone [135,136], thus releasing free fatty acids (FFA) and thereby changing membrane polar lipid composition. Furthermore, the hydroperoxyl radical ($HOO^{\bullet}$), derived from the reaction of superoxide with protons (Equation [5]), and the hydroxyl radical ($^{\bullet}OH$), derived from the reaction of superoxide with hydrogen peroxide (Equations [6 and 7]), are able to initiate the peroxidation of lipids [137]. Peroxidation of membranes interferes with the normal membrane functioning, and lipid peroxides can decompose to form a wide range of highly cytotoxic products. The AOS are able to produce chemical modifications and/or damage to proteins, lipids, carbohydrates, and nucleotides [62,138]. The AOS may injure cells (a) by covalent binding to membrane components, enzymes, and/or receptors; (b) by impairing transport processes through sulfydryl group oxidation, change in lipid/protein ratio, or covalent binding; (c) by deesterification of polar lipids; and (d) by initiation of lipid peroxidation.

Evidence supporting the above statements derives from the fact that (a) stress conditions increase the production of free radicals [69,78,114,139]; (b) chemical and physical changes observed in membranes isolated from stressed plant tissues can be simulated in vitro by exposure to oxygen free radicals [140,141]; (c) membranes from tolerant plants tissues are more tolerant of free radical treatment than those from susceptible tissues [140,141]; (d) membranes from dehydrated plants producing more superoxide suffer higher decreases in fluidity and changes in composition than those producing less superoxide [11]; and (e) the production of $O_2^{\bullet -}$ increases by about two orders of magnitude when the integrity of thylakoids is damaged by detergents [142]. All the above-mentioned

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observations indicate that the integrity of membranes depends on the acquisition of dehydration tolerance, which makes the membranes more resistant to AOS.

Oxidative Stress and Membrane Lipids

The generally accepted mechanism of free radical attack involves acyl chain oxidation which should lead to a decrease in the unsaturation level of membrane lipids. Such a phenomenon has not been observed in water-stressed tissues [11,69,129,140,143,144], in seeds exposed to the action of free radicals [141], and in resurrection plants during desiccation [3,139,145]. This suggests that changes in the unsaturation level of plants, which are often considered to be the only result of oxidative stress, are in fact a comparatively minor response to the action of free radicals [34,69,127]. There are alternative mechanisms of free radical attack on plant membrane lipids [34,97,135], so that the target for oxidative damage need not necessarily be unsaturated lipids. As reported in a previous section on the effects of oxidative stress in cells, the fatty acid ester linkage may be broken as a result of the nucleophilic addition of a superoxide radical to the ester bond; thus the fatty acids so liberated remain in membranes and destabilize them by changing the fluidity.

The free radical reactions in model membrane systems and plant membranes are quite distinctly different. Liposomes exposed to oxygen free radicals resulted in peroxidative reactions leading to degradation of the unsaturated fatty acids. In contrast, in winter wheat treated with free radicals, in spite of the lack of change in fatty acid unsaturation, there was a substantial loss of esterified fatty acids without a subsequent increase in peroxidation, suggesting the presence of terminating antioxidants that prevent the chain reaction from continuing [127]. Under conditions of oxidative stress, changes in membrane polar lipids and accumulation of free fatty acids with no changes in unsaturation level were also observed in sunflower and barley seedlings [69,135].

In the absence of changes in fatty acid unsaturation, the inherent danger of AOS lies in their ability to mediate the degradation of polar lipids, with accumulation of free fatty acids (FFA) or other uncharged lipids such as triacylglycerols (TG) [11,69,132,135]. The accumulation of these neutral lipids destabilizes the bilayer, leading to the formation of gel phase domains [127]. Both nucleophilic attack and peroxidation may result in membrane disorganization owing to changes in membrane composition that determine the formation of nonbilayer configuration and possible displacement of membrane proteins, with the consequence being a loss of functional integrity of membranes.

In thylakoids, lipid damage is an ever-present problem because of the high quantity of polar lipids and polyunsaturated fatty acid residues, which are particularly susceptible to free radical attack [146], Furthermore, alterations in bulk membrane lipids and in lipids of the boundary layer surrounding proteins [147], such as those caused by dehydration, hinder cell function by inducing changes in the structure and function of several intrinsic protein complexes [148,149]. Therefore, removal of activated oxygen species and hydroperoxyl fatty acid radicals is a priority if the functional integrity of thylakoid membranes is to be preserved.

Lipid Oxidative Chain Reaction Breaking in Chloroplasts

The process of lipid peroxidation has been shown to be associated with loss of membrane polyunsaturated fatty acids and the formation of hydroperoxides, free radical intermediates, and other secondary products. Lipid peroxidation products generated, such as hydroperoxides, aldehydes, and epoxides, may react with and inactivate essential proteins, enzymes, and nucleic acids. Thus, the process of lipid peroxidation, if not interrupted, may lead to the alteration of cell membranes and to the release of destructive enzymes.

The ascorbate-glutathione system in chloroplasts play an irreplaceable role in protection against oxidative damage (see section Ascorbate-Glutathione System in Chloroplasts and its Role in Water-Deficit Conditions above), but other antioxidative protective mechanisms must be present in chloroplasts which serve to break the peroxidative chain reaction. Ascorbate and glutathione may

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serve as mediators between the hydrophilic and lipophilic phase to maintain the antioxidant properties of membrane-protective systems. In combination with tocopherol, they can result in synergistic inhibition of oxidative damage to cell membranes [150] by trapping the lipid radicals and suppressing lipid peroxidation rather than by scavenging $O_2^{\bullet -}$ or singlet oxygen [151,152]. The chain-breaking activity of tocopherol is predominantly maintained by ascorbate, whereas GSH predominantly acts as a preventive antioxidant [153]. Ascorbate has been seen to protect against lipid peroxidation as long as tocopherol is present, but in tocopherol-deficient microsomes, ascorbate initiates lipid peroxidation immediately, demonstrating its pro-oxidant effect. On the contrary, GSH is effective at low tocopherol concentrations, thereby also providing a protective role in tocopherol deficiency. GSH may prevent lipid peroxidation from entering the propagation stage by scavenging lipid alkyl or lipoxyl radicals formed during the initiation stage of peroxidation [154].

Tocopherol is able to quench and scavenge singlet oxygen even if carotenoids are probably more important in this role, since they react with singlet oxygen at a diffusion-controlled rate and they can also quench those excited triplet states of chlorophyll that lead to singlet oxygen formation [61]. Chloroplasts are the site of synthesis of tocopherol and the thylakoid membrane is very rich in tocopherol. Tocopherol is an amphipatic molecule with its hydrophobic phytil tail located in the membrane, associated with the acyl chains of fatty acids, whereas its polar ''head'' group (chromanol) is oriented toward the membrane surface, with the phenolic hydroxyl group being located near the polar group of the lipid matrix [155]. Tocopherol is an unspecific trap for the activated oxygen species, but it reacts rapidly ($2.4 \times 10^6\ M^{-1}\ s^{-1}$) with alkyl peroxyl radicals formed in the lipid phase [156] and becomes irreversibly oxidized [157]. The tocopheroxyl radical must be reduced back to act again as a chain-breaking antioxidant, but no enzyme systems for such a function have been described until now. Fast regeneration of the tocopheroxyl radical by ascorbate and GSH has been detected by EPR [97,158]. Direct interaction at the membrane surface with either ascorbate [154] or reduced glutathione [153] allows tocopherol many chain-breaking events before its degradation, producing the monodehydroascorbate and glutathiyl radicals. However, ascorbate and GSH will act as radical scavengers only when there is an efficient removal of their radical forms. The radicals formed to regenerate tocopherol are in turn recycled by available reducing equivalents, such as NAD(P)H [26], in association with their specific reductase enzymes. This mechanism creates a link between the free radical reactions initiated in the lipid phase and the scavenging activity in the aqueous phase (see section Ascorbate-Glutathione System in Chloroplasts and its Role in Water-Deficit Conditions above).

Changes in Lipid Composition

Water-deficit conditions are frequent in crops even in a normal season during some steps of their cultural cycle, with the result being a limitation in their growth and production. Limited growth and production are the result of structural modification of membrane components, particularly lipids [159–161]. Many vital activities of the cell originate in the membrane, the structure and function of which are profoundly altered following water stress that leads to destructive events, such as transition from a liquid-crystalline to a gel phase, fusion, and increased permeability [128,162–164]. Therefore lipids, being one of the major components of membrane, are obviously affected by water stress. The composition and physical state of the lipid bilayer influence the lipid-protein and the protein-protein associations, enzyme activities, and transport capacity of membranes [148,165,166].

Studies concerning the effects of water deficit on lipids have demonstrated that lipids are affected by drought [143,167], but the data have often been contradictory owing to the lack of information on the water status of the plants. Very often only the number of days without watering is given, and this is unable to provide a clear description of stress effects on plants. Further uncertainties arise from the numerous factors that are implicated in the plant response. The nature and the extent of changes in lipids are a function of intensity and duration of stress and of the tissue examined and growth stage, as well as of the genetically determined ability of the plant to cope with the

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environment [144,168–170]. The main lipids in leaves are polar acyl lipids [171,172], which are associated with membraneous structures in plant cells. More than 60% of chloroplast membranes are composed of glycolipids (GL), whereas the phospholipids (PL) are generally considered to be the most important mitochondrial and plasma membrane lipids [173]. Evidence from cotton, barley, and wheat indicates that GL amounts decrease in response to water deficit [162,174], but the authors cited failed to specify the stress intensity imposed or else exposed the plants to excessive levels of stress. When the water status of the plants is monitored, it has been shown that the levels of GL depended on stress severity, so that GL may decrease, remain constant, or even increase [144,170,175,176]. This disparity in behavior may be a further indication of the great impact that stress severity has on plant metabolic responses. The adaptation of plants to water depletion may maintain or even stimulate the production of GL, which are an important structural feature of photosynthesis and play an essential role in the maintenance of the electron transport system and in thylakoid stabilization [177]. With regard to the main glycolipid, it is known that monogalactosyldiacylglycerol (MGDG), on isolation, forms a cylindrical inverted hexagonal configuration instead of the bilayer configuration adopted by the other thylakoid lipids [178]. Stabilization of thylakoids can be achieved by reducing the tendency of MGDG to form nonbilayer arrangements. Furthermore, increasing the saturation reduces the tendency of MGDG to form a nonbilayer structure [179]. The more MGDG is present in the lipid mixture, the larger is the tendency to form a nonbilayer configuration [180]. Dry land conditions caused different rearrangements in two cultivars of wheat with different sensitivities to water-deficit conditions (Table 2) [11]. Whereas oligalactosyldiacylglycerol (DGDG) was unaffected by water deficit in both cultivars, an increase in the MGDG/DGDG molar ratio occurred in the more sensitive cultivar and a decreased ratio in the less sensitive, which also showed less unsaturation in MGDG. The lower MGDG and the lower unsaturation found in thylakoid membranes of the tolerant cultivar in comparison with the sensitive one may have maintained membrane fluidity, although the potential of acyl lipids to form lamellar or nonlamellar configurations is dependent on several other factors, such as temperature, water content, pH, and cation type and concentration [181]. Water-deficit conditions increased the proportion of FFA in the sensitive wheat, whereas the level was unchanged in the tolerant plant. The FFA accumulated in the stressed sensitive wheat may have induced an increase in membrane microviscosity and may have changed

TABLE 2 Chemical and Physical Changes and $O_2^{\cdot-}$ Formation Rate in Thylakoids of Two Wheat Cultivars Differently Sensitive to Water Deficit Conditions

	cv Adamello (sensitive)		Cv Ofanto (tolerant)	
parameter	control	stressed	control	stressed
Ψ_w	−0.60 a	−1.70 b	−0.50 a	−1.80 b
FFA	0.80 a	1.40 b	2.00 a	1.80 a
MGDG/DGDG	1.90 a	2.10 b	2.00 b	1.40 a
MGDG uns	92.00 a	97.00 b	85.00 b	80.00 a
τ	0.13 a	0.22 b	0.16 a	0.19 b
Lipid/protein	0.63 a	0.67 a	0.46 a	0.57 b
K	100.00 a	125.00 b	100.00 b	69.00 a

For each cultivar, means in rows followed by different letters are significantly different from control by an analysis of variance test ($P \leq .01$). Ψ_w, leaf water potential (MPa); FFA, free fatty acids (mol%); MGDG/DGDG, monogalactosyldiacylglycerol to digalactosyldiacylglycerol (molar ratio); uns, unsaturation (%); τ, spin label rotational correlation time (ns); k, $O_2^{\cdot-}$ formation rate (% of control).
Source: From Refs. 11 and 50.

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lipid-protein interactions and protein conformation. In the stressed drought-sensitive cultivar, a higher superoxide radical production has also been found in comparison with the tolerant cultivar, in which no increase was observed following water depletion [182].

Investigations on various crop species [69,144,170,176,183–187] report a general decrease in the PL of leaf tissue exposed to long periods of water deficit even though only a few of the previous works specify the intensity of the stress applied. In some cases, a degradation of PL with a decrease in water potential has been found, but a PL decrease is not always accompanied by an accumulation of FFA. Other lipid components, such as TG, resulting from the esterification of stress-induced increase in FFA and diacylglycerols (DAG), may play a role in destabilizing the membrane, as demonstrated in senescent tissue. In this tissue, discrete gel and liquid crystalline domains are formed because of the presence of several components, with the exception of FFA, in the neutral lipid fraction [188]. These components, identified as TG, 1,2-diglicerides, free sterols, and sterol esters, could interact with PL and induce a lateral phase separation resulting in a mixture of gel phase lipid in the liquid-crystalline domains [140,141]. As the polar lipid level of the cell has been correlated with the content of its membraneous system [189], the decrease following water depletion may indicate a disorganization of the cellular membranes with accompanying loss of permeability, electron transport capacity, and enzyme-bound activity [190,191].

It is important to point out that in the previously reported investigations the changes in PL and in Gl in stressed plants take place without significant changes in the fatty acid unsaturation. The loss of PL following stress seems to be related to enhanced hydrolysis rather than to an inhibition of PL synthesis, since it is accounted for by an increase in the neutral TG [144,170,176,192]. The breakdown of PL, in fact, may support TG accumulation through the formation of the precursors DAG, which in some cases have been seen to increase following water depletion [144,160,175]. The accumulation of TG may be considered to be a way of storing fixed carbon and a greater energy reserve than starch. It provides an adaptative advantage, because TG accumulated during water deficit can be readily utilized when drought stress is relieved, and it makes no demands on the available water for accumulation. In further support of this hypothesis, starch, sucrose, and the export of photosynthates are severely decreased by water deficit [193]. Such conditions would be conducive to the storage of fixed carbon such as TG during drought and may indicate an alteration of normal translocation processes. However, oleosome lipids, consisting mainly of TG, are not involved in the storage of products of photosynthesis [194], and the increased levels of TG and DAG have been attributed to de novo synthesis rather than to breakdown or utilization of existing glycerolipids and fatty acids [175]. In addition, it must be borne in mind that every membrane has a characteristic lipid composition and lipid/protein ratio, which represent two important factors in the biochemical functioning of membranes [172]. Therefore, it may be postulated that the presence in stressed seedlings of increased amounts of TG, which are uncharged lipids, disturbs the normally orderly arrangement of the membrane by aggregating to form lipid bodies between its amphiphilic phospholipid-protein layers. The increase in TG levels would induce a certain instability in the bilayer and may play a role in the transport of protein across membranes, changing the overall physical status of the latter [144].

It has been suggested that the molecular basis of water-deficit stress sensitivity in plants is related to the nature of the fatty acyl residue associated with membrane lipids. Compared with sensitive strains, drought-resistant strains of *Vigna unguiculata*, which contain a high proportion of linolenic acid, increased the unsaturation level when droughted, whereas in the drought-sensitive plants, it decreased [195]. On the contrary, in two genotypes of *Lupinus albus* [196], one resistant and the other sensitive to water-deficit conditions, a high percentage of linolenic acid was found in both watered and unwatered plants. The total unsaturation in both sets of lines was remarkably stable, as also seen in other plants [70,132,144,170,187]. From studies with [1-^{14}C]-acetate, it emerges that under water stress, a marked decrease of precursor incorporation into cotton leaf lipids occurs, particularly in phosphatidylcholine (PC) and in galactolipids. On the contrary, the relative incorporation into neutral lipids increases [186]. An inhibition of the fatty acid desaturation, further evidenced in experiments with [1-^{14}C]-oleate and [1-^{14}C] linoleate, has also been demonstrated [197].

The overall physical state of membranes is also regulated by free sterols, which are membrane stabilizers. When their relative proportion increases, they reduce the mobility of the acyl chains and thus the fluidity of the bilayer by inserting themselves into the PL cavities [198,199]. Therefore, in order to have a complete picture, one must take into account whether or not free sterol changes occur in water-deficit conditions. Mild water stress did not lead to alterations or reduced slightly the free sterol level [70,169,192,200], whereas severe water stress or repeated stress periods increased their amounts significantly [144,201]. It is likely that mild water-deficit stress involves some metabolic adjustments in membrane lipids which stabilize cell membranes when severe stress occurs. Furthermore, several inducive cycles or a critical intensity of water-deficit stress may be required in order to obtain modifications in sterol metabolism. Besides the amount, the composition of FS also alters membrane status because of the specific effect of the individual sterols. In *Zea mays* hybrids exposed to mild osmotic stress, an increased molar ratio of stigmasterol to sitosterol in stressed tissues has been observed [200]. The same thing occurred in sunflower and maize grown in the field under water-deficit conditions [144,170]. Changes in this ratio may be an example of an early event that may control membrane function. The increase in the stigmasterol/sitosterol ratio could be an index of metabolic disorder, because the increase in stigmasterol is indicative of cell disorganization and of a reduced adaptative capacity to stress conditions [202]. The ''more planar'' sterols (cholesterol + campesterol) are far more effective in stabilizing membranes and reducing membrane permeability than the ''less planar'' sterols (stigmasterol + sitosterol), and their ratio may be used as an index of membrane permeability [202,203]. This ratio has been seen to be maintained on stress in maize and sunflower which experienced a water deficit of −1.05 MPa [144,170]. The stability of this ratio and the constant level of lipid unsaturation, together with the increase in the amount of free sterols, may reflect the relative abilities of plants to regulate membrane stability and permeability under conditions of water deficit in an attempt to maintain normal cell function.

The reported changes, however, are relative to lipids from all intracellular membranes and show only more general changes. In water-stressed plants, the first cellular perturbation was found to be an increase in solute leakage, which is positively correlated to the decrease in water potential. This provides evidence that plasma membranes (PM), which limit solute efflux, had been damaged. There is little information available on changes in lipids from purified PM in response to water stress. The main lipid components of PM are PL and FS [168,204], and there is some evidence that water stress can induce changes in these lipids. In plasma membrane isolated from sunflower seedlings exposed to a water deficit of − 1.3 MPa, a loss of PL and glucocerebrosides together with an increase in TG, a non–bilayer-forming lipid, have been observed [132]. The decrease in the membrane PL and the increase in FS, resulting in an increase in the FS/PL molar ratio [132,185], could initiate changes in the fluidity of the membrane [168]. Greater disorganization of the PM may be also due to a change in the more planar/less planar sterol ratio and to the increase in the stigmasterol/sitosterol ratio already observed in the whole plants [144,194,170,200,202]. The unsaturation of PM lipids has been seen to remain constant under water-deficit stress [132,185] as often observed at the whole plant level [170,176] and higher than 60%, which is an adequate value to ensure correct membrane functioning without the need for any specific qualitative or quantitative fatty acid composition [205].

In the plasma membrane of sunflower, in accordance with other findings on oat roots and wheat shoots, the PC/PE ratio increased during desiccation [132,184,206]. It is known that PC tends to form a bilayer configuration under physiological conditions, whereas PE, a non–bilayer-forming lipid, orients into a hexagonal H_{II} configuration. The balance between non–bilayer-forming and bilayer-forming lipids is an important factor in determining membrane function. Therefore, accumulation of PC during drought may result in increased membrane fluidity and prevents the formation of a nonbilayer lipid configuration under stress conditions. Methylated species, such as PC, hydrate to nearly twice the extent of other lipids and may also influence the structure of the water layer adjacent to the membrane, since the interfacial water layer is more ordered in the crystalline than in the gel phase owing to increased interactions between the polar head groups and water [207]. Therefore, higher levels of PC can limit the increase in the transition temperature and the formation

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of the increased proportion of gel phase in the liquid crystalline domain in contrast to the effect of PE [164]. On the other hand, a tighter lipid packing, such as that obtained with PE, reduces the accessibility of the polar head groups to water.

Plants are more tolerant of drought when water stress is slowly imposed, since such a condition favors osmotic adjustment, which, in sorghum for instance, becomes negligible only when the dehydration rate is increased to 1.2 MPa/day [208]. The dehydration rate in sunflower seedlings was about 0.1 MPa/day, which is a decrease that is quite consistent with the above considerations. On the other hand, acclimation to dehydration reveals a different picture, since in the PM of oat roots, a decrease in the PC/PE ratio occurred, which was regarded as a system capable of increasing the curvature of the membrane and facilitating its repeated invaginations [185]. A different water status experienced by the plants may explain these contrasting results even if the different response may also be due to the system used by Norberg and Liljinberg [185] to impose water-deficit conditions. Indeed, the oat plants were periodically removed from the nutrient medium and placed on a coarse stainless steel net to allow the roots to air dry.

AN EXTREME CASE OF DESICCATION TOLERANCE: RESURRECTION PLANTS

Production of AOS and Protective Mechanisms in Resurrection Plants

Under conditions of low relative humidity, the viability of higher plant tissues may become seriously affected because of the evaporation of cellular water. Adaptation to cellular dehydration is one of the most important characteristics that determines the distribution and the yield of crops. Most desiccation-tolerant species show definite limits of tolerance beyond which tissue damage or death occurs. In only a few cases is an organism able to withstand severe dehydration. In higher plants, the ability to survive water loss is normally limited to embryos in developed seeds and to a small group of plants called ''resurrection'' plants. These are a group of plants whose fully differentiated tissues have the ability to withstand dehydration down to air dryness and to resume full biological activity on rehydration [209–212]. In these poikyloidric plants, the water content closely follows the changes in the dryness of the environment. The remarkable tolerance to prolonged anhydrobiosis in these plants suggests that they are able to maintain essential structure and physiological integrity in the dry state or are able to repair dehydration rehydration-induced damage. Several structural, chemical, and molecular aspects have been suggested to explain the ability of resurrection plants to survive a severe reduction in water content [3,51,124,139,213–218]. The distinction between desiccation tolerance and intolerance has been suggested over the years to be a function of the plant's ability to (a) process AOS by maintaining their defensive mechanisms or amplify them during the desiccation and rehydration phase, (b) retain integrity in the dry state, and (c) develop repair mechanisms on rehydration. The free radical damage hypothesis of desiccation injury states that the molecular defenses are unable to detoxify AOS during dehydration and rehydration.

The use of EPR and electron nuclear double magnetic resonance (ENDOR) showed the formation of a stable carbonium-centered free radical both in desiccation-tolerant and desiccation-intolerant mosses, but damage only occurs in sensitive species [74] that have a smaller amount of the antioxidants tocopherol and glutathione and antioxidant-recycling enzymes [133]. *B. hygroscopica* is a resurrection plant that experiences either viable (slowly dried leaves) or nonviable (rapidly dried leaves) dehydration. Illuminated thylakoids of dried leaves showed a lower superoxide production in comparison with the control and rehydrated leaves. In addition, viable dehydrated leaves had a lower production of superoxide in comparison with nonviable dehydrated leaves [3]. Both viable and nonviable dehydrated leaves decreased their electron transport rate notably (F. Navari-Izzo, personal communication), and this may account for the decrease in superoxide production in comparison with control and rehydrated samples. Furthermore, the destabilization of membranes due to lipid changes during nonviable drying (see section Membrane Lipid Composition During Dehydration and

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Rehydration below) might take into account the increase in superoxide production in comparison with slowly dried leaves.

Only a few studies deal with the antioxidative defenses during the dehydration and rehydration cycle in resurrection plants. In droughted plants, conservation or resumption of enzyme activities depend on species, rate of water loss, and enzyme considered. Generally, a rapid water loss is more harmful than a slow water loss for the redevelopment of enzyme activities during rehydration [219]. In *Selaginella lepidophylla*, the mean of photosynthetic enzyme conservation is significantly lower than the mean of glycolitic enzymes [220], thus explaining the rapid resumption of respiratory activity and the delayed development of photosynthetic activity seen in this, as in other resurrection species, on rehydration. In detached leaves of *Sporobolus stapfianus*, the enzymes related to the glutathione-ascorbate cycle respond individually to a rapid water loss; the specific activities of GR and DHAR were more than twice as high as in control leaves, whereas the APX activity decreased to 40% of the well-hydrated sample [52]. Two major mechanisms seem to be involved in the survival of resurrection plants: the synthesis of glutathione during dehydration and the activation of the ascorbate-glutathione cycle during rehydration. Glutathione seems to play a very important role in the survival of detached leaves of *B. hygroscopica*, which during dehydration increase the amount of constitutive GSH by up to 50 times and utilize it when rehydrated [51]. The enhanced formation of reduced glutathione plays an irreplaceable role in limiting lipid peroxidation [3,51,221] and in protecting enzymes which possess exposed thiol groups [123]. In the moss *Tortula ruralis* and in the resurrection plant *S. lephidophylla*, the $NADP^+$–glyceraldehyde-3-phosphate dehydrogenase activity was severely reduced following GSH oxidation and was restored only after the addition of GSH [222–225]. Notably, during dehydration of *B. hygroscopica* plants, the activity of the enzyme remained constant [124]. Dehydration of *B. hygroscopica* plants to 80% relative water content (RWC) determined a dramatic decrease in GSH, which on further dehydration began to accumulate by up to 20 times at 23% RWC [124]. The release of feedback inhibition of the synthesis of GSH through an initial decrease in GSH might have been involved [226]. In flowering plants, as an oxidative stress is imposed, there is also a rapid decline in the GSH level, which is immediately restored over time [113,227]. It is quite interesting that an induction in ABA has been observed in *B. hygroscopica* dehydrated to 80% RWC (A. Bochicchio, personal communication), with ABA presumably being involved in the induction of gene expression during water loss [228]. A more reduced status of the cells during desiccation may also be maintained through the induction in the synthesis of ascorbate, and the decrease of DHAR and GR activities on drying is probably due to the accumulation of AsA and GSH [51]. The activation of MDAR, DHAR, and APX has been observed during dehydration of *S. stapfianus* plants [84]. Antioxidants such as AsA and GSH, which accumulate during drying, might constitute a reserve which allows the plant to tolerate oxidative damage during desiccation and rehydration, when the injury caused by desiccation must be repaired, and when plants recover their catabolic and/or anabolic activities. Indeed detached leaves of *S. stapfianus* which during drying do not accumulate reduced ascorbate and glutathione [52] are not able to revive on rehydration [215]. Both GSH [51,124] and AsA [84] may have helped to break the chain of peroxidative reactions by maintaining tocopherol in a reduced form, thus preventing lipid peroxidation and/or oxidation of protein sulfydryl groups in desiccated resurrection plants [84]. During rehydration oxidative processes intensify and resurrection plants might be more exposed to AOS, since the levels of $O_2^{\bullet -}$ [3] and H_2O_2 as well as the oxidation of GSH and AsA have been seen to increase [51]. During this phase the oxidation of these two antioxidants stimulates the activity of the enzymes of the ascorbate-glutathione cycle [51], which may thus play an important role in the resurrection of these plants and in the regeneration of AsA and GSH at the end of rehydration.

Membrane Lipid Composition During Dehydration and Rehydration

Drastic changes in the volume of the cells and in cellular components due to dehydration and rehydration subject endomembranes to multiple stress leading to structural changes and possible breakdown and perturbation of membrane stability [11,77,132]. Mechanisms of desiccation tolerance are

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thought to be based on membrane behavior [40], and an efficient recovery and full reconstitution of membrane structure and composition during rehydration may be a requisite to cell survival [229]. Removal of water is likely to induce demixing and/or liquid crystalline to gel phase transitions, but when water enters the cells, an efficient repair mechanism is necessary to restore membrane integrity much more than during dehydration. Rehydration of membranes causes a part of them to pass again to the crystalline phase and leads to solute leakage [230]. During dehydration-rehydration, cycle changes in lipid composition and in activities of enzymes involved in lipid metabolism have been found in desiccation-tolerant mosses [131,231] and in the resurrection flowering species *Ramonda, Haberlea* [229,232], *B. hygroscopica* [139], and *S. stapfianus* [233]. Thylakoids of *B. hygroscopica* have a lipid composition characteristic of bryophites and of plants grown in arid or semiarid climates [234,235], showing a low MGDG content, a low MGDG/DGDG molar ratio, a low degree of unsaturation, and a low proportion of linolenic acid [18:3]. Dehydration causes a large decrease in MGDG levels and in MGDG/DGDG molar ratios. Similar results have been reported in the resurrection plants *Haberlea rhodopensis* [232], *Ramonda serbica* [229], and *S. stapfianus* [233]. A decrease in this ratio has been also monitored on water-deficit stress in a drought-tolerant wheat [11,182] and in a tolerant variety of *V. unguiculata* [195], whereas sensitive varieties showed an increase in this ratio [11,182,195].

A higher proportion of DGDG, a bilayer-forming lipid, may play a role in the control of ionic permeability in the chloroplasts [232]. Even though biological membranes are a complex mixture of several lipids, variations induced by water deficit in the relative proportions of MGDG and DGDG influence the physical state of membranes and their functionality [11,178] because of their different arrangements within thylakoid membranes (see section Changes in Lipid Composition above). During dehydration, the metabolic activities of resurrection plants, including photosynthesis, decline but they undergo functional recovery at rehydration. A decrease in MGDG could be a mechanism for maintaining a low efficiency of photosystems, in agreement with the role of $MGDG_s$ in electron transfer between the antennae and the cores of the photosystems [236]. The only lipid organization found to be compatible with functional membranes is the bilayer or lamellar phase [237]; for this reason, the decrease in MGDG/DGDG molar ratio following dehydration may help the survival of the plant by retaining the bilayer arrangement, since DGDG adopts only the lamellar phase [178].

In photosynthetic membranes, the most abundant fatty acid is the polyunsaturated acid 18:3, and its high level suggests that it is important in maintaining photosynthetic activity. On the other hand, linoleic acid 18:2 is generally only a minor component of chloroplast membranes (1–3% of chloroplast lipids), whereas it is the main constituent of PL in nonphotosynthetic tissues.

It is also present at a fairly high level (30% of polar lipids) in algae and bryophites [238]. In *Ramonda* species and *H. rhodopensis*, 18:3 was found to be the main fatty acid component in MGDG, but it was present in lower percentage compared with the levels present in the typical flowering plants [239]. Dehydration decreased its content, whereas it sharply increased the 18:2 content [229,232]. Thylakoid membranes of *B. hygroscopica* [139] as well as the whole leaves of other resurrection plants [145,229] have a peculiarly low 18:3 content, and in addition, in *B. hygroscopica*, a high level of 18:2 has also been detected. An 18:3 deficiency in chloroplast membranes has been seen to decrease the photosynthetic capacity [240]. Therefore, it seems likely that the low 18:3 and the high 18:2 levels found in *B. hygroscopica* may play a role in maintaining a low photosynthetic activity in these plants. Thylakoids of *B. hygroscopica* [139] as well as leaves of other resurrection plants [229,232,233] are rich in neutral lipids in comparison with other higher plants [132,135,144]. Among neutral lipids, TGs accumulate on dehydration [139,229,233] and decrease during rehydration [139,233]. The storage of neutral lipids in dehydrated leaves and their reduction during rehydration indicate that the reserves are used for restoration of respiration, which is more rapidly reactivated than the photosynthetic membrane reaction [241–243], as soon as the water is available again. On rewatering, the synthesis of lipids is strikingly fast [229,231,233] owing to the stability of mRNA under desiccation, which leads to a rapid synthesis of polyribosomes on rehydration [244].

During dehydration and rehydration of *B. hygroscopica* and *S. stapfianus*, peroxidative dam-

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age to polyunsaturated fatty acids does not occur. The absence of peroxidative processes in the dehydrated and rewatered plants can be due, at least in part, to the increase in carotenoids on dehydration [233] and to the maintained levels of the lipophilic antioxidant tocopherol (F. Navari-Izzo, personal communication). The storage of higher amounts of FFA and TG, although usually leading to concomitant changes in the packing, fluidity, and/or physical arrangement of the membranes [3,11,139,233], may be considered to be a readily available source of precursors for the reconstitution of the lipids during rehydration and for energy supply as soon as water is available again [139], since the properties of membranes have been maintained. In addition, an increased or maintained unsaturation level was observed on desiccation in these as well as in other resurrection plants. This may indicate that FFA accumulation is caused not only by polar lipid degradation and unsaturated MGDG in particular, but that it is likely to be due to active synthesis during the early dehydration period to produce precursors to be used when rewatering is resumed. Following rehydration, the unsaturation level returns again to that of undesiccated leaves [3,145]. The changes in unsaturation on desiccation may be the result of the decrease in both enzymic oxidation [245] and oxidation due to AOS and of the increase of superoxide radicals on rehydration [3]. Furthermore, in dried *Craterogstigma plantagineum* and in *S. stapfianus* colneleic and colnelenic acids, inhibitors of lipoxigenase, have been found [245,246]. According to these results, the target for oxidative damage needs not necessarily to be unsaturated lipids and the free radical–induced polar lipid deesterification hypothesis has to be considered [34,135]. On rewatering, a general trend toward recovery has been observed in the individual lipids [3,139,229,233], showing that an efficient repair occurs in these plants.

The rate of water loss during the desiccation phase of the dehydration-rehydration cycle is particularly significant. Dehydrated leaves of most resurrection plants do not survive desiccation unless they are slowly dried as was observed in *B. hygroscopica* [3,51,139] and in *Borya nitida* [215]. Apparently, only slow desiccation stimulates some processes which allow the plant to survive dehydration. This may suggest that only slow drying could increase the chances for survival by giving the plants additional time to carry out the structural and compositional changes necessary to achieve a viable dehydrated status. The revival of the plants might also be due to a higher water content of the slowly dried leaves to 1.8% RWC in comparison with the rapidly dried leaves to 0.3% RWC, as shown in *B. hygroscopica* [3,51], which has been seen to lose the ability to survive when slowly dehydrated plants were dehydrated to the same water content of rapidly dried leaves [247]. Rapid dehydration results in a higher accumulation of FFA, which is known to destabilize the membrane bilayer and to alter the conformation of proteins [248]. Other differences that may be correlated to the different capacity of slowly and rapidly dried leaves to withstand dehydration are a higher total polar lipid/free sterol molar ratio, a lower proportion of free sterols, and a lower MGDG/DGDG molar ratio. These are all changes that all together may lead to the destabilization of membranes [3,139].

Cell Structure Preservation

Cell preservation in a desiccated state is not related only to functional conditions but also depends on a structural basis. The safeguard of both a physiological integrity and a certain structural organization can be seen as intrinsically linked [249]. For this reason, the ultrastructural changes which occur during dehydration and rehydration of desiccation-tolerant cells assume a major significance, being able to provide useful information on cell structure stability and on their contribution in surviving an extreme water loss.

The preservation of structural integrity of cellular membranes is a determinant factor in surviving desiccation and subsequent rewatering. Electron microscopy on freeze-fractured cells of different resurrection plants shows that in the dry state, membranes exhibit the bilayer configuration with normally dispersed intramembranal particles [250]. This is noticed in the fern *S. lipidophylla* and also in the moss *T. ruralis*, a species with a desiccation tolerance essentially based on a repair system [251]. Moreover, the ability to preserve cell membrane integrity results from the far lower differences

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in the rate of electrolyte leakage among fully hydrated, dry, and rehydrated cells of desiccation-tolerant plants compared with that of desiccation-sensitive ones [252]. The structural and functional features of the membranes in completely dried cells of desiccation-tolerant species mean that, besides those already described, additional protective mechanisms have to operate during dehydration in order to defend the membrane against the rigor of extreme water loss and to preserve the bilayer organization far below 20% hydration, which is regarded as the critical value to maintain this configuration [253]. However, most of these mechanisms, which are essentially based on the accumulation of soluble sugars and proteins, are not exclusive to the vegetative cells of resurrection plants but are shared with seeds, embryos, and organisms which can acquire tolerance to different environmental conditions causing a water deficit [214,216,254–256].

The production of soluble sugars, particularly disaccharides, as protectants against the dry damage of cell membranes is a method common to all living beings able to cope with complete dehydration [257] and seems to be a very ancient mechanism, since even archaebacteria can activate it to tolerate extreme drought stress [258]. In animal anhydrobiotic organisms, the disaccharide employed is trehalose, whereas in plants, it is usually substituted by saccharose [145,245,259–261]. However, in desiccation-tolerant embryos and in leaves of some resurrection plants other sugars, with the same trehalose among them, have been found [215,246,261,262–264]. The synthesis of soluble sugars is an early and massive event in desiccation-tolerance induction. This is shown in drying cells of *C. plantagineum* by the immediate increase in transcripts of saccharose phosphate synthase and saccharose synthase [216], which leads to a saccharose content up to 40% of dry weight in fully dehydrated leaves [265]. At the ultrastructural level, this can account for the observed disappearance of starch from chloroplasts of resurrection plants during cell desiccation [252,266–270]. In desiccation-tolerant leaves of *S. stapfianus*, the loss of starch occurs during dehydration (Figs. 4 and 5), whereas in detached drought-sensitive leaves of the same species, starch persists in chloroplasts of dried cells (Fig. 6) [233]. The soluble sugars seem to play different roles in protecting the cell against desiccation damage. First of all, they can preserve membranes by stabilizing the dry double layer through direct interaction between their —OH groups and the phosphate ones of membrane phospholipids [216,257]. In this way, the packing of phospholipid head groups, due to water removal, is prevented, the phase transition temperature is depressed, and dry phospho-

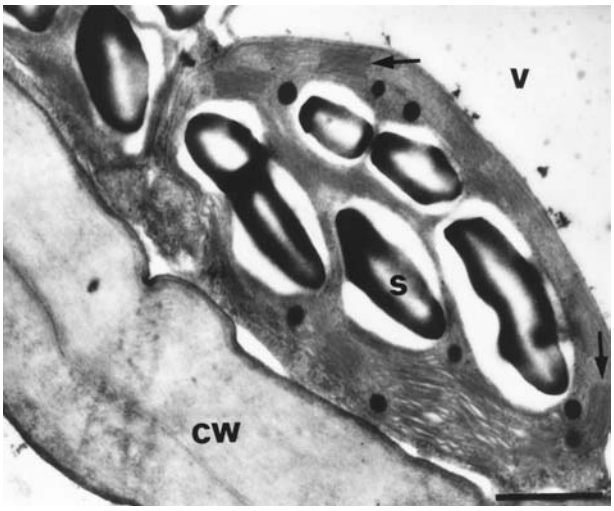

FIGURE 4 Bundle sheath chloroplast in a fully hydrated cell of a leaf of *Sporobolus stapfianus.* The organelle contains a granal (arrow) thylakoid system and large stach grains (s). (cw = cell wall; v = vacuole) (bar = 1 μm). (From Ref. 233.)

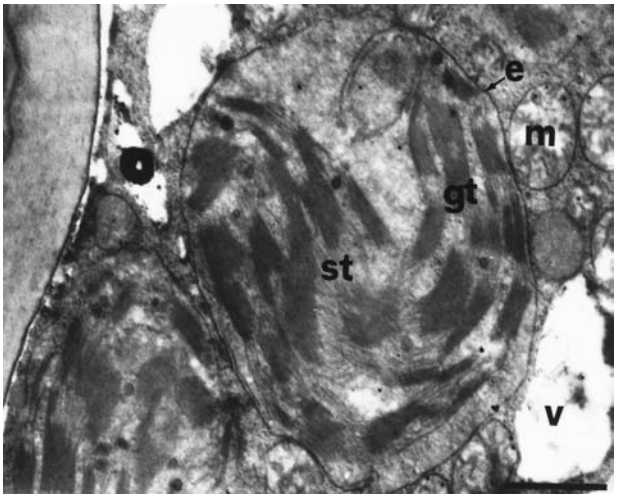

FIGURE 5 In the dry bundle sheath cell of a desiccation-tolerant leaf of *Sporobolus stapfianus*, a chloroplast with well-defined envelope (e), several grana (gt), and stroma (st) thylakoids but devoid of starch can be seen. Note also the well-preserved mitochondrion (m) and small vacuoles (v) (bar = 1 μm). (From Ref. 233.)

lipids remain in the liquid crystalline phase in the absence of water. As a consequence, the leakage which should occur during rehydration with the phospholipid bilayer passage from gel to liquid crystalline phase can be avoided. Apart from the stabilizing action on membranes, disaccharides are able to protect the protoplast against severe desiccation by forming glass. Thus, during dehydration, a supersaturated liquid, with the mechanical properties of a solid, is produced, which hinders cellular collapse and maintains a stable quiescent state [271]. Moreover, disaccharides can preserve the

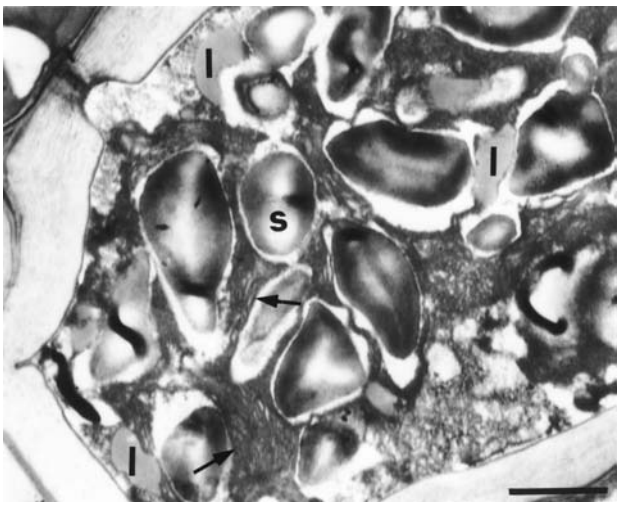

FIGURE 6 Rather indistinct chloroplasts containing swollen thylakoids (arrows), lipid-like inclusions (l), and several starch grains (s) are present in the dry bundle sheath cell of a desiccation-sensitive leaf of *Sporobolus stapfianus* (bar = 1 μm). (From Ref. 233.)

activity of enzymatic molecules in drying cells, probably through formation of hydrogen bonds between their —OH groups and the protein polar residues [257]. It has been demonstrated, for instance, that the tetrameric enzyme phosphofructokinase, which irreversibly dissociates into inactive dimers when dehydrated, is stabilized in vitro in the active form by disaccharides [272].

The production of specific proteins involved in preservation of the structural integrity and functionality of drying cells is another defense strategy adopted by resurrection plants. In the cytosol of dehydrating cells of *C. plantagineum*, three desiccation-related proteins are synthesized [273], which exhibit homology with a family of late embryogenesis–abundant (LEA) proteins accumulated during the final stage of seed development [274]. LEA-like proteins, also termed ''dehydrins'' [275], have been found not only in angiosperms but also in desiccation-tolerant species taxonomically different, such as ferns [276], liverwort [277], mosses [278], and even cyanobacteria [279], so that their presence as components of dehydration-tolerance pattern seems to be ubiquitous among photosynthetic organisms [275]. Dehydrins are cytosolic and nuclear soluble proteins characterized by an extreme hydrophilic nature, being rich in charged and polar amino acids. This feature makes dehydrins able to operate in desiccation tolerance by replacing the water of the hydration shell of macromolecules with their superficial hydroxilated groups and with a protective action superior to that of saccharose because of the fact that they are less likely to crystallize [216]. Synergically with compatible solutes, such as proline and glycine-betaine, dehydrins seem also to act as ''chaperones'' maintaining the drying proteins in their correct folded state. Moreover, the dehydrin bonds with both anions and cations can counteract the damaging effects caused by the increasing ionic strength in dehydrating cells [216]. Thus, this kind of proteins seems to occupy a key place in cell desiccation tolerance.

The desiccation-tolerant *T. ruralis* lacks any water-conservation mechanism, so the rate of drying is directly controlled by the water status of the environment and can be too fast [280] to permit the activation of protecting systems such as the production of soluble sugars and dehydrins. This may account for the fact that in this species two major dehydrins are constitutively expressed and are already present in fully hydrated tissues [278] together with saccharose quantities (10% dry weight) sufficient to offer membrane protection during cell drying [281]. Thus, *T. ruralis*, and probably other truly ''desiccation-tolerant'' or ''truly poikilohydric'' species [218] appear to adopt a tolerance strategy which, during the dehydration phase, is based on constitutive protection systems. On the contrary, in pteridophytes and angiosperms, which have more complex ways of water use and regulation, the mechanisms of drying tolerance are inductive and require a certain amount of time to be put in place. These modified ''desiccation-tolerant'' plants [218] do not survive rapid dehydration [218,282] and employ morphological and physiological systems to control and retard the rate of water loss [210,218,283]. In resurrection plants belonging to pteridophytes and angiosperms, a major role in desiccation-tolerance induction seems to be played by abscisic acid (ABA) [218,284–287]. However, this hormone may be also involved in acquisition of desiccation tolerance in lower plants that do not possess constitutive systems of cell protection, such as the moss *Funaria hygrometrica* [288] and the liverwort *Exormotheca holstii* [277].

Through all the protecting mechanisms so far described, their interactions [257], and the possible complementary action of further more species-specific factors [289], resurrection plants succeed in preserving the basic cell structures and in maintaining a certain degree of cellular order. This fact is confirmed by the numerous ultrastructural observations of dry cells, which show defined plasmalemma and tonoplast outlines, cytoplasm rich in ribosomes, an intact nucleus surrounded by the envelope, and well-preserved mitochondria, although with few cristae [230,249,250,252,266–269,290].

Differently from the other cell components, chloroplasts can undergo great changes during dehydration of leaf cells. Resurrection plants can adopt different strategies in order to defend the photosynthetic apparatus, which is very sensible and liable to injury, against desiccation stress. The so-called ''poikilochlorophyllous'' species lose the photosynthetic pigments and the entire thylakoid system during dehydration, with formation in dried tissues of organelles devoid of any inner membranal structure termed ''desiccoplasts'' [291]. On the contrary, the ''homoiochlorophyllous'' ones

maintain most of the pigments and thylakoids in the dry state [233]. In the homoiochlorophyllous resurrection plants, the preservation of a membrane system with quite a good level of ultrastructural organization (see Fig. 5) [233,250,252] also requires the activation in chloroplasts of protecting mechanisms like those operating in the cytoplasm. In *C. plantagineum*, which is a homoiochlorophyllous resurrection plant, three ABA-inducible desiccation-stress proteins (dsp), termed dps21, dps22 and dps34, are synthesized in the drying chloroplasts [273,292,293]. One of them (dps21) is a stromal protein which shows homology with dehydrins, and the other two are inserted in the thylakoid membranes. The dps34 does not exhibit homology with any other so far known stress-related protein, but studies are now being carried out to characterize a protein with the same molecular weight found in thylakoids of drought-stressed *Solanum tuberosum* [294]. On the contrary, dps22 shows homology with early light-inducible proteins (ELIPs), a class of stress-related proteins expressed in greening etiolated leaves and in green leaves exposed to excess light [295]. It is interesting to note that, as in the case of ELIPs [295], the expression of dsp22 transcripts in drought-stressed leaves, besides being stimulated by light, also seems to depend on a circadian oscillator [295]. The functions of the two thylakoidal dsps have been suggested to be structural and protective [273,292]. They could play major roles in maintaining membrane order and in cooperating with other photoprotective systems [296] to cope with the light-damaging effects due to the photoinhibitory condition which always occurs in thylakoid membranes during severe water stress [295,297]. The stroma dehydrin, besides stabilizing the membranes, might operate in preserving the structural and functional integrity of soluble proteins. In the homoiochlorophyllous plants, the stromal enzymes are not degraded. Active ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) can be found in the dry state [298], and CO_2 fixation can occur at an extremely low osmotic potential [299]. Thus, in this kind of resurrection plant, the photosynthetic apparatus is maintained in a recoverable form during dehydration. In the poikilochlorophyllous species, on the contrary, the drying chloroplasts lose chlorophylls, most carotenoids, and the entire thylakoid system, so the photosynthetic apparatus has to be completely reconstructed during rewatering. However, pigment loss and demolition of the other thylakoid components are highly organized responses to desiccation [267] realized according to regulated metabolic pathways. In dehydrating poikilochlorophyllous plants, mitochondria remain functional for a longer time with respect to the homoiochlorophyllous ones, thus maintaining the respiration (desiccation respiration) necessary for the energy supply which allows the controlled transformation of chloroplasts into desiccoplasts.

On rewatering, the desiccation-tolerant species are able to recover the complete organization and functionality of their cells in a time which can depend on the kind of plant, the dehydration rate, the pattern of events occurring on drying and the ability to put in place repair mechanisms. In *T. ruralis*, the mitochondrion and chloroplast reactivation occurs in a few minutes. Photosynthesis can be fully restored in less than 1 h and, despite the high rate of dark respiration, a positive carbon balance can be achieved within 20 min [300]. In this species, cell recovery is as fast as the previous water loss was slow. This is because a quickly dried moss suffers a greater amount of damage on rehydration than a slowly dried one. However, *T. ruralis* can rely on very efficient repair mechanisms which enable it to cope with the added damage associated with a too rapid dehydration [250]. During the rewatering phase, new proteins termed ''rehydrins'' are synthesized, owing to a gene expression regulated in large measure at the translational level by recruitment of specific mRNA into polysomes [218,301]. The precise functions of rehydrins are so far unknown, although it appears likely that they might be enzymes somehow involved in the rapid cellular repair of eventual desiccation-induced damage. Interestingly, some of these proteins show homology with proteins related to seed imbibition and dormancy [218]. In modified desiccation-tolerant plants, cell recovery on rewatering is far slower and can require several hours or even days. Normally it takes a shorter time in homoiochlorophyllous than in poikilochlorophyllous species. This is because in these latter plants, the entire photosynthetic apparatus had to be reconstructed during rehydration. In poichilochlorophyllous resurrection plants, an intensive respiration (rehydration respiration) starts early on rewatering and characteristically lasts up to 30 h [242]. During this period, the whole recovery of mitochondria occurs, whereas 3–8 days are required to rebuild well-structured and fully functional chloro-

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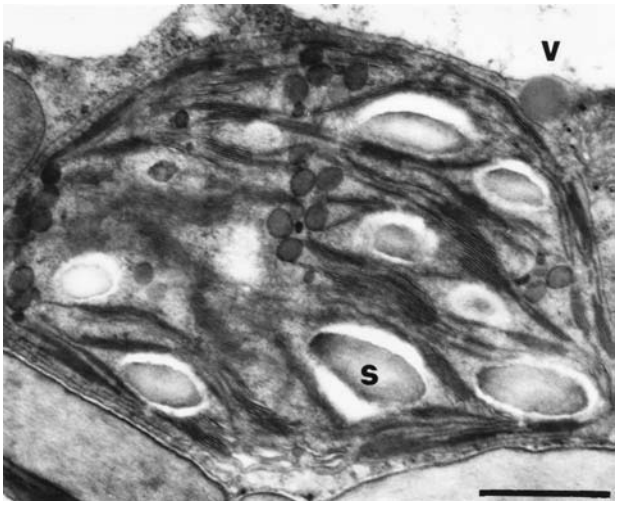

Figure 7 Well-organized bundle sheath chloroplast in a fully rehydrated cell of a desiccation-tolerant leaf of *Sporobolus stapfianus* (s = starch, v = vacuole) (bar = 1 μm). (From Ref. 233.)

plasts [242,252,270,291,302]. In homoiochlorophyllous species, the dark respiration is restored within few hours of rewatering [241], and chloroplasts reach the height of their functionality in 1–2 days [252,290].

At the beginning of rehydration, all the desiccation-related proteins synthesized during cell drying disappear in *C. plantagineum*, whereas the transcription of new rehydration-specific genes is activated [302]. Since these rehydration genes essentially codify for components of the photosyn-

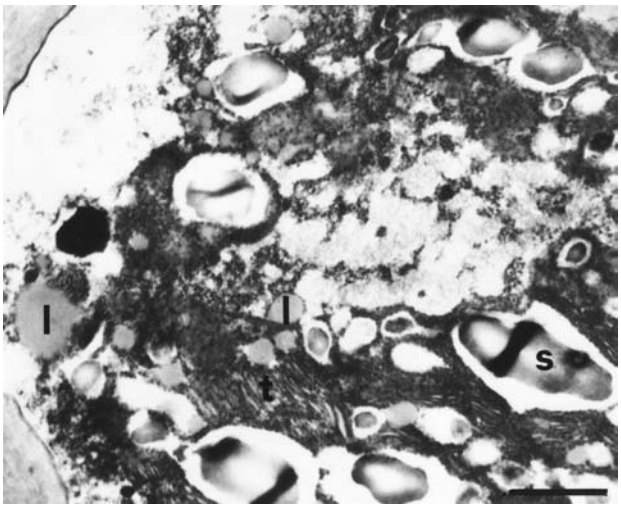

Figure 8 The rehydrated bundle sheath cell of desiccation-sensitive leaf of *Sporobolus stapfianus* shows widespread chloroplast remnants in a dismantled cytoplasm (l = lipid-like inclusions, s = starch, t = thylakoids) (bar 0 1 μm). (From Ref. 233.)

thetic apparatus, such as the Rubisco small subunits (SSU) and a chl *a/b* binding protein, and for enzymes such as a transketolase, the molecular events which occur on rehydration are not related to repair mechanisms but only contribute to a rapid restoration of the metabolism. Thus, in this species as well as in other higher resurrection plants, the desiccation tolerance seems essentially to depend on the events occurring during the dehydration phase. In *S. stapfianus* [233] cells dried in conditions which induce desiccation tolerance totally recover their original organization on rewatering (Fig. 7), whereas cells which cannot activate tolerance mechanisms during dehydration undergo dramatic damage when remoistened (Fig. 8).

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Constraints by Water Stress on Plant Growth

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INTRODUCTION

In regions where temperature allows plant growth, water is among the most limiting factors for plant productivity and growth rates are proportional to water availability. Because of its essential role in plant metabolism, at both the cellular and whole-plant levels, any decrease in water availability has an immediate effect on plant growth, and processes ranging from photosynthesis to solute transport and accumulation are seriously affected (Fig. 1) [1]. Plants are generally subjected to shortages in water availability varying in length from hours to days. Water lost by transpiration causes transient water deficits even in plants growing in wet places, so that most plants suffer at least regular and daily water shortages [2]. When drying soil causes water absorption to lag behind loss by transpiration, permanent water deficits develop that may result in permanent wilting and death by dehydration. Therefore, most plants must deal with some water stress. Plants have evolved physiological responses as well as ecological strategies to cope with water shortages by either stress avoidance or stress tolerance. These responses allow them to survive and even to maintain some growth under very harsh circumstances [3].

Water stress has been defined as the induction of turgor pressure below the maximal potential pressure [4,5]. The magnitude of such stress is determined by the extent and duration of the deprivation. Therefore, plant responses depend on the nature of the water shortage and may be classified as (a) physiological responses to short-term changes, (b) acclimation to a certain level of water availability, and (c) adaptations to drought. Short-term responses to water stress, acting within seconds after the onset of stress, are primarily linked to stomatal regulation, thereby reducing water loss by transpiration and maximizing CO_2 intake. An optimum efficiency in this process would lead

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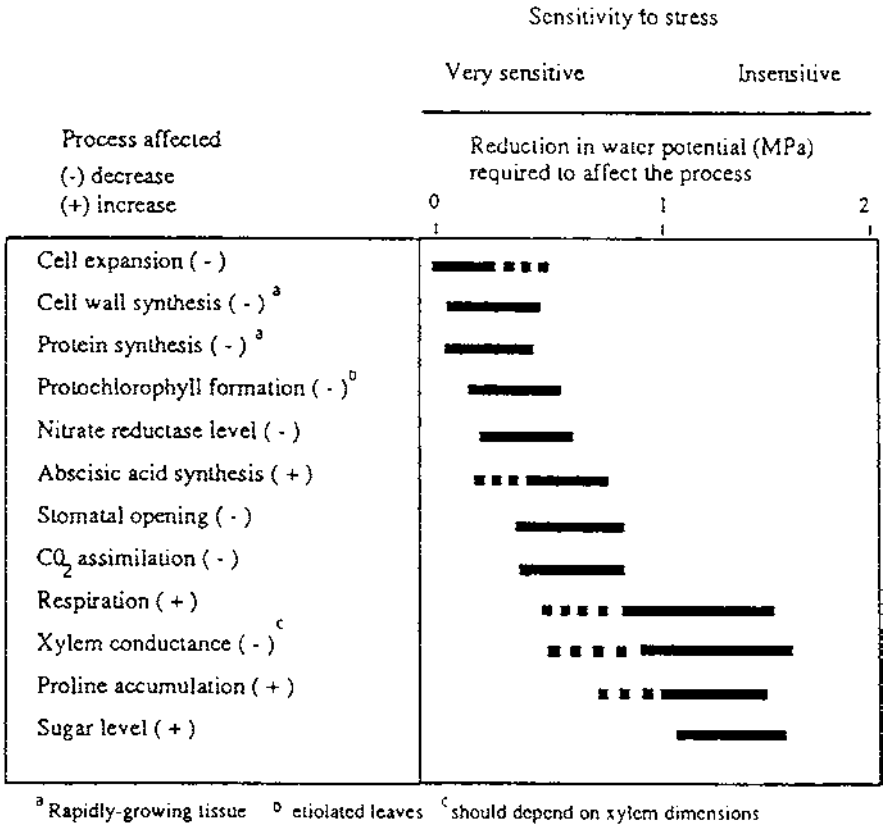

FIGURE 1 Relative sensitivity to water stress of various plant processes. The solid horizontal bars indicate the range of stress levels within which a process is first affected; the broken bars refer to the portion of the water potential range in which the response is not well established. (From Ref. 1.)

to a constant ratio of transpiration to photosynthesis [6]. Midterm responses (acclimation) include the adjustment of the osmotic potential by solute accumulation, changes in cell wall elasticity, and morphological changes. Long-term adaptation to drought includes genetically fixed patterns of biomass allocation, specific anatomical modifications, and sophisticated physiological mechanisms, with an overall growth reduction to balance resource acquisition [7,8].

EFFECT OF WATER STRESS ON NUTRIENT UPTAKE

Nutrients are less mobile in a drying soil, because the pores between soil particles are replaced by air and the pathway from the soil to the root surface is less direct [9]. Since the rate of ion diffusion to the root is very often the step limiting nutrient uptake, a decrease in soil water availability can affect plant growth. Whenever water stress limits growth more strongly than it limits nutrient uptake, tissue nutrient concentrations are higher than if water stress limits nutrient uptake more than growth [10]. Normally, the concentrations of growth-limiting nutrients decline during water stress, showing

that the indirect effects of soil water content on nutrient uptake may be as important as the direct effects of water stress on plant growth [8].

CONTROL OF STOMATAL CLOSURE

Gas-Exchange Dynamics

A certain degree of water stress is generally experienced by plants irrespective of life cycle and habitat [2]. Particularly in trees, the decrease in water potential may be greater, since hydraulic resistance increases through embolism in the xylem. The plant water content recovers at night, equalizing the soil water potential and allowing the plant to reach its highest water potential just before dawn.

In light, stomata open and begin to lose water; the leaf reaches its lowest water content when transpiration is maximum near midday. Stomata have a high capacity of response to changes in the plant water status, and they close as the leaf water potencial decreases. They are even more sensitive to changes in atmospheric humidity [11,12], however, and they close as the vapor pressure deficit between the leaf and the air increases (Fig. 2). Stomatal response to ambient humidity is a species-specific trait of the guard cells [4]. Since stomata are the way by which CO_2 enters the leaf, the changes that water stress induces on stomatal apertures affect CO_2 intake and assimilation and therefore plant growth. Apparently the evolution of leaf structures favorable for high rates of photosynthesis had more survival value than that of structures favorable to low rates of transpiration except in

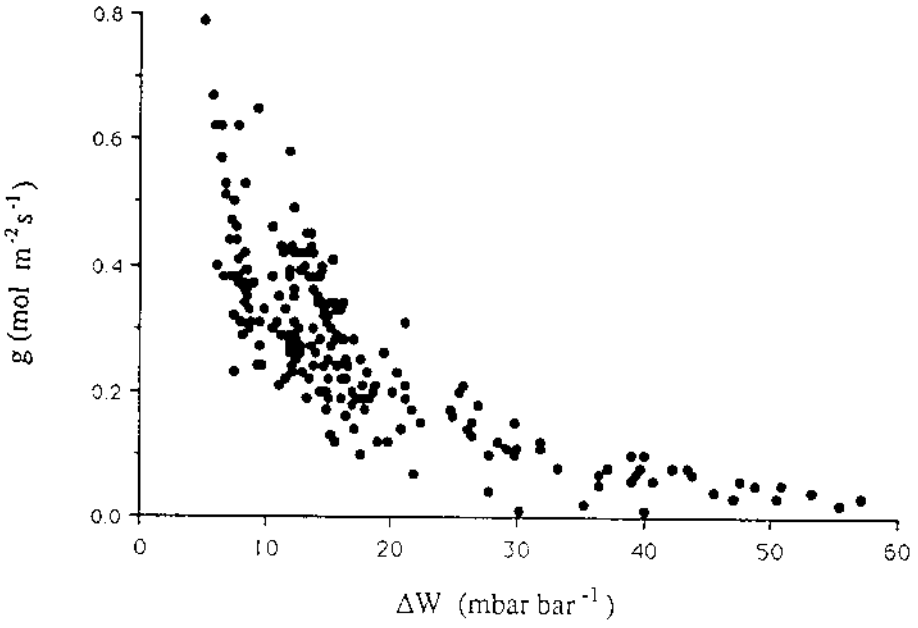

FIGURE 2 Decrease in stomatal conductance (g) as leaf-to-air water vapor mole fraction difference (ΔW) increased in field-grown Eucalyptus globulus trees. Conductance and ΔW were measured at midday between February and December 1991. Temperature ranged from 21 to 40°C and dawn water potential from −0.21 to −2.6 MPa during this period. (From L. Serrano and J. Pardos, unpublished observations.)

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very dry habitats [13]. Stomatal opening is affected by the CO_2 concentration, and responses of isolated pairs of guard cells suggest a sensing mechanism that responds to low levels of internal CO_2 [14]. Under water stress, internal CO_2 drops in the stomatic chamber, thereby decreasing CO_2 assimilation. Since water stress directly affects photosynthetic capacity at the chloroplast level, the stomatal limitation to growth is presumed to be modest [15]. However, the stomatal or nonstomatal inhibition of photosynthesis is still a controversial topic [6].

Many trees often reach xylem pressures close to that provoking cavitation [16]. This small safety margin between minimum pressures experienced by trees and that at which cavitation is initiated induces a reduction in the transpiration rate and consequently in the stomatal conductance [17].

The process of cavitation involves a restriction on xylem pressure and a decrease on hydraulic conductance that affects the stomatal response to water stress in order to regulate leaf water potential [18]. Changes in stomatal conductance related to cavitation tend to match the progressive reduction in leaf-specific hydraulic conductance. Under these conditions, plants avoid an uncontrolled reduction in leaf water potential that otherwise would cause cavitation to continue until all xylem is embolized [18,19].

When water content in the soil diminishes, cavitation could be interpreted to be an adaptive mechanism having important implications in the control of water use [18].

Role of Growth Regulators

There is substantial evidence for the physiological role played by abscisic acid (ABA) in the regulation of the stomatal aperture [20]. Endogenous ABA increases after a period of wilting, and when applied to plants, ABA strongly inhibits transpiration. It has been hypothesized that ABAs accumulation in leaves during water stress is responsible for stomatal closure, but its overall role at the whole-plant level is still not clear. Some aspects concerning the form (free or conjugated) and location of ABA within the mesophyll cells (mainly determined by pH) must be taken into consideration.

The stomatal aperture is regulated by ABA, which is synthesized in the cytosol and accumulates in chloroplasts of the mesophyll cells [21]. Water stress results in the release of the accumulated ABA to the apoplast, from which it is carried by the transpiration stream through the leaf to the guard cells [15]. Epidermal water relations have been suggested [22] as modulators of the responses of stomata to ABA. Environmental factors and plant development also influence the process, so N-deficient media increase the release of ABA, with older leaves being more responsive than younger ones [23]. Plants with dried root systems may exhibit increased stomatal resistance despite unchanged leaf water potential, indicating that this reaction is the result of a hormonal sign sent by the roots to the shoots [24–27].

Other growth regulators influence stomatal opening. Cytokinins open stomata, but usually in environmentally stressed plants (e.g., with some nutrient deficiency) [28], and both cytokinins and auxin antagonize the action of ABA [27].

Overall, water stress triggers a change in hormonal balance, including an increase in leaf ABA and/or a decline in cytokinins. The increase in leaf ABA reduces cell wall extensibility and therefore causes a decline in leaf elongation. In other plants, the altered hormonal balance reduces root hydraulic conductance and tissue turgor, thereby reducing leaf growth. Regardless of the mechanism by which it is achieved, the decline in growth reduces the plant demand for carbon, so carbohydrates accumulate and photosynthesis declines to match the reduced requirement for carbohydrates. These rapid changes in response to environmental stress serve as an early warning system that reduces plant growth and alters allocation before there is a severe imbalance in C- and N-containing metabolites [8,29].

TURGOR AND GROWTH

Because plant growth is the result of cell division and enlargement, water stress directly reduces growth by decreasing CO_2 assimilation and reducing cell division and elongation. The effect of

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water stress is more evident on cell wall expansion [13], because cell enlargement involves the extensibility of the cell wall under turgor pressure. Therefore, any loss in turgor pressure as a consequence of the imbalance in the plant water content could result in reduced growth and even in the total absence of growth under dry environmental conditions. Nevertheless, the relationship between turgor loss and cell enlargement is unclear [30].

Cell growth rate, Gr, can be expressed as a function of turgor pressure, P, and the extensibility coefficient, Φ, by the equation

$$\mathrm{Gr} = \Phi\,(P - Y)$$

where Y is the yield threshold pressure [31]. The equation shows that growth rate decreases as P decreases, but it could also be maintained if either Φ increases or Y decreases. Therefore, reduced growth rate may not rely only on reduced turgor caused by desiccation [32]. There is some evidence of reduced growth without loss of turgor in plants subjected to desiccation stress [33], but this reduction may be part of the osmotic adjustment process [34]. Some mechanism may control cell wall extensibility through the perception of soil dryness [32], giving rise to smaller plants and, hence, lower water requirements and higher survival.

RESPONSES TO DROUGHT STRESS

Conversion of light energy into carbon-based energy implies loss of water. Indeed, water loss is considerably greater than C gain on a molar basis, because the diffusion gradient from water vapor in the leaf to the atmosphere is steeper than the gradient in CO_2 from the atmosphere to the leaf. Therefore, plant adaptations dealing with water conservation have a special meaning in dry environments when water stress is either permanent or temporary and severely limits plant growth. Since a large proportion of the Earth's surface is arid or semiarid, and since even in temperate regions, those environments with a Mediterranean-type climate suffer seasonal water stress, the distribution of natural vegetation and yield of cultivated plants are largely restricted by water availability. Plants living in such environments have adapted by increased drought tolerance and water use efficiency.

There are a number of modifications in plant structures and processes as a consequence of drought stress. These include sensitivity of stomatal response, osmotic adjustment, smaller cell volume, reduced leaf area, increased leaf thickness, hairy leaves, and increased root-shoot ratio, as well as several changes in enzyme and hormone production and activity.

Depending on their response to drought, plants may be classified as drought avoiders or drought tolerators [3,13,35]. Drought-avoidance strategies include short seasonal cycles, as in desert annuals, or earlier maturity, as in C_3 grasses in Mediterranean climates [36]. The drought-tolerance strategy includes either dehydration postponement or dehydration tolerance.

Drought Avoidance

Plants avoiding drought show adaptations leading to the acquisition of the maximum amount of available water or restrict their activities to the periods of water availability. A greater allocation to roots is a main feature of drought-avoidance plants in dry environments where roots consist of 60–90% of plant biomass. In contrast, in coniferous forests, the root biomass is 21–25%, and this figure reaches 30–40% in drier, tropical savanna woodlands [4]. With decreasing water availability, root growth is enhanced at the cost of aboveground biomass production [7,37].

Under well-watered conditions, plants extract water very intensively from the upper soil layers; deep rooting and subsoil water extraction become increasingly important under limited water supply. Perennial shrubs in dry habitats usually have unbranched root systems tapping water to 30 m below the surface [38,39]. In tropical savanna grasslands and North American prairies, the pattern of rooting is a profuse branching in the top layer of soil and deep roots, so that water and nutrients are efficiently absorbed from the top soil layers during wet periods and deep stored moisture is

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tapped during the dry season [4]. Root growth and distribution follow the water reserves of the soil, but severe drought may promote initiation and elongation of lateral roots [40]. As a consequence of greater root allocation, aboveground biomass is smaller and the growth rate is decreased to reduce overall resource requirements [8,41]. Most plants adapted to dry environments also have mycorrhizal symbiosis, which improves water and nutrient supply but is also a sink for carbohydrates and may consume 5–10% of total photosynthate [42,43].

Among the plant adaptations to water stress, leaf modifications are especially important [44]. Since the diffusive resistance offered by a leaf to CO_2 uptake is greater than that offered to water loss, any change in the resistance of the common part of the pathway has a greater influence on the transpirational loss of water than on CO_2 intake. Therefore, many species have features that favor photosynthesis over transpiration by increasing the diffusive resistance of stomata using depressions in the epidermis, pores, or cutin or waxes. [4]. By reducing their evaporative surface, plants may reduce water loss, and for this reason, leaves tend to be smaller (Fig. 3) and thicker in dry habitats [45] but maintain a high photosynthetic rate [46]. Also, by reducing leaf size, the convective heat flux to the atmosphere is increased, and by adjustment of leaf angle, the interception of solar radiation can be reduced [47]. Leaf pubescence is a feature of dry habitats that increases light reflectance, decreases leaf temperature, and allows the leaf to gain a higher rate of carbon under arid conditions than the leaf could acquire without hairs. Pubescence also allows the plant to avoid potentially lethal high leaf temperatures and to lower daily water loss, allowing the plant to extend its growth for a longer period into the drought [48]. All these mechanisms help to maintain the leaf energy balance and tend to optimize plant growth and functioning.

High water use efficiency (expressed as a ratio between A and E) could be considered as an adaptive feature of plants submitted to extended periods of drought or growing under competition [49]. Variations observed in A and E after environmental changes occurring as a consequence of stomatal regulation can be optimized when a constant ratio is maintained through time [50]. This optimal stomatal behavior might be seen as one of many possible functional adaptations against drought [17]. However, this only has been confirmed in some species [12,51].

Sclerophylly is regarded as being a typical feature of Mediterranean-type plants and is interpreted as an adaptation to drought [52,53]. However, similar sclerophyllous plants differ broadly

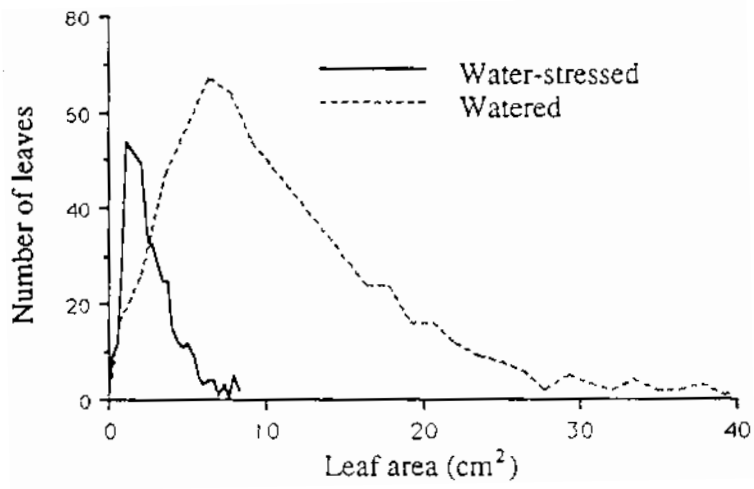

FIGURE 3 Frequency of leaves by size in clonal plants of Eucalyptus globulus after being watered and after several cycles of drought reaching the wilting point. (From L. Serrano and J. Pardos, unpublished observations.)

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in water relations [54], leading to the hypothesis [55] that sclerophylly cannot be considered significantly related to a drought-avoiding strategy but rather to nutrient limitation [56]. Wax of sclerophyllous leaves keeps cuticular transpiration at a minimum once stomatal transpiration and CO_2 exchange have ceased, thereby conserving water. Since the production and maintenance costs of schlerophyllous leaves are higher than the costs of more mesic leaves [57], the slow growth of Mediterranean evergreen shrubs may be partially due to a greater investment in leaves along with resource limitation.

Drought deciduous shrubs (the most characteristic desert group) rely on morphological changes in the quality and the quantity of their foliar biomass to remain metabolically active through most of the year. Typically, these species develop a relatively large canopy of mesomorphic leaves when water is available and therefore maintain a relatively high rate of productivity. These leaves are replaced by smaller and more xeromorphic summer leaves as seasonal water stress increases [58]. These changes in total canopy leaf area reduce sharply the productivity of xeromorphic plants. However, the increase in water use efficiency (WUE), combined with adaptations in tissue water relations, allows photosynthetic activity through all but the most extreme water stress. Early shedding of leaves during drought often prevents death by desiccation in tropical and temperate zone woody plants [6]. A gradual leaf fall seems to be an adaptation in water stress–prone environments to maximize photosynthetic gain and nutrient cycling [59]. The capacity for leaf shedding during drought varies appreciably among species, but because water deficits frequently limit the growth and survival of trees, selective pressure for adaptation to drought is often high [6]. Because nutrient cycling and nutrient use efficiency are related to leaf fall [60,61], water stress at the time of leaf shedding may severely affect the plant's nutrient budget by decreasing nutrient resorption from leaves [62]. Leaflessness is another feature of dry habitats that allows for a reduced water loss, relying on photosynthetic stem tissues. Cortical stem tissue is structurally very similar to leaf tissue but maintains a net positive rate of photosynthesis even in drought-stressed shrubs and allows a quick recovery from herbivory [63].

Dehydration Postponement

Increased stomatal sensitivity is a functional mechanism that allows plants to maintain high water status during drought periods. This response occurs as a consequence of various events: soil water depletion, increase in the vapor pressure deficit (VPD) in the atmosphere, or both together [17].

The effect of water stress acclimation has been shown in different species to be a further reduction in stomatal conductance [64]. Other species exhibit a variation in stomatal conductance concomitant with changes in VPD and no great variations in leaf water potential [12].

Stomatal closure, although an effective means of postponing dehydration, can reduce photosynthesis to below the compensation point and, especially in dry environments, may cause heat imbalance because of the reduced transpiration rate and photoinhibition [65,66]. Furthermore, no general statement can be made concerning the adaptive value of sensitivity of guard cells to ambient humidity [67], since the response to humidity in a large number of species surveyed was not related to their natural habitat [68]. Thus, changes in the stomatal sensitivity are quite variable; nevertheless, they can be considered as an adaptive response to drought which is species-specific [4].

Metabolic adaptations to water stress cause plants with different photosynthetic pathways to differ in their sensitivity to atmospheric humidity and the resultant gradient in water vapor pressure from leaf to air. Clearly associated with dehydration postponement are CAM and C_4 photosynthetic pathways.

In CAM plants, the daytime closure of stomata combined with dark fixation of CO_2 reduces water loss without limiting photosynthesis. CAM plants, mostly desert succulents, show the highest water use efficiency but the lowest growth rate. Nevertheless, the productivity of some CAM plants may be high, for example, *Opuntia ficusindica* in Mexico and Chile (47 ton ha^{-1} $year^{-1}$) or some *Agave* species (38–42 ton ha^{-1} $year^{-1}$), which surpass the average 30–40 ton ha^{-1} $year^{-1}$ of such crops as wheat, sugar beet, and alfalfa or many tree species over a range of productive soils [69].

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The C_4 species evolved as a response to a reduction in atmospheric CO_2 levels that began during the Cretaceous era and continued until the Miocene [70]. Stomata of C_4 species are less sensitive to a desiccating atmosphere than those of C_3 plants, which would provide a greater C gain in low-humidity atmospheres [71]. The ecological advantage of a C_4 photosynthetic pathway is still unclear even though it allows a greater WUE than in the C_3 species [72]. Plant traits other than those related to the photosynthetic pathway should be responsible for the adaptation of some C_4 species to dry habitats [67,73]. When water and N are available, C_4 plants show a high growth rate, and photosynthetic N use efficiency is highest [46]. When limited in either of them, however, C_4 productivity is lower than in ecologically similar C_3 species [72].

Water storage is generally of little importance in drought avoidance because of the high leaf water turnover. Only in a few plants, such as baobab (*Adansonia digitata* L.) and saguaro (*Carnegia gigantea* L.), is water stored in a significant amount [13]. In general, the cost of water storage is high, and most plants have little or nothing in terms of water-storing structures [74].

Dehydration Tolerance

During dry periods, plants may delay dehydration, but as drought continues, dehydration may become severe, causing injury and death. Dehydration tolerance is a species-specific trait, ranging from -1.2 MPa in aquatic plants to -10 MPa or higher in some xerophytes, but differences in species tolerance are not well understood [13]. Many species of algae, lichens, and mosses, as well as some 70 higher plant species, can be air dried and later recover [75].

Dehydration usually causes severe damage and disorganization of membranes and organelles, mechanical rupture of protoplasm, degradation of cell membranes, protein denaturation, and gene mutations. Chlorophyll content remains relatively unaffected by water stress, but the content of proteins, glycolipids, and phospholipids in chloroplasts generally decreases [76]. Damage caused by desiccation particularly affects photosystem II [77]. Reduction in C assimilation by water stress is also caused by the decreased activity of many enzymes of the Calvin cycle. This effect is completely reversible as long as the water stress is not too severe [78]. The activity of nitrate reductase is also depressed [79] and dark respiration enzymes are enhanced, so that dissimilation processes are more than doubled.

Different experiments have indicated that desiccation tolerance involves changes in the viscosity of the cytoplasm during drought hardening, the protection of membrane properties by the release of organic solutes, and a reduction in the number and reactivity of thiol groups carried by macromolecules [4].

A means of increasing drought tolerance is by decreasing osmotic potential by accumulation of solutes, so that turgor and turgor-dependent processes may be maintained at a significantly lower water availability. This osmotic adjustment allows cell enlargement and plant growth at high water stress and keeps open stomata and CO_2 assimilation at otherwise inhibitory levels [13]. However, evidence indicates that osmotic adjustment may maintain growth only for short periods of time and may not contribute greatly to continued leaf growth in water-stressed plants [80] or play a major role in the distribution of the species [81]. Nevertheless, osmotic adjustment can accomplish two functions: (a) extend the lifetime of active tissues between ephemeral showers and (b) extend the period of tissue preparation for drought (drought hardening) [71]. Furthermore, although stomatal control or reduction in leaf area gives an almost certain reduction in productivity, osmotic adjustment provides the potential for maintaining photosynthesis and growth of at least some parts of the plant as the water deficit increases. Thus, in terms of growth, the cost of osmotic adjustment must be lower even though the solute accumulated cannot be used elsewhere [35]. Osmotic adjustment is reversed when water stress is removed and may reach up to -0.7 MPa in daily changes, although values of -0.1 MPa are more usual [35,80]. It seems that there is a metabolic ceiling for each species [80].

Many solutes may be used in osmotic adjustment. Inorganic ions, such as Na^+, K^+, and Cl^-, accounted for most of the osmotic potential in several species [82,83], but sugars and amino acids,

especially proline [84,85], are major osmoregulators in vascular plants [86]. The reason is probably the convenience of osmolyte storage in large, osmotically inactive molecules, such as starch or protein, which may serve several functions and from which they can be retrieved under conditions of stress. It appears that neither the synthesis of new compounds nor biochemical pathways are involved during osmotic adjustment [35]. Rather, it appears that the disturbance of normal metabolic pathways by water stress is responsible for producing the solutes involved in osmotic adjustment.

Some studies have indicated that the degree of drought tolerance is associated with the ability to undergo changes in the cell elastic properties. A drought-induced increase in the bulk modulus of elasticity, ϵ, would permit the maintenance of a large water potential gradient through the soil-plant-atmosphere continuum, with little change in the relative water content [87], therefore increasing the ability to extract soil moisture from progressively drier soil. Although increases in ϵ have been observed in response to drought stress [88], seasonal patterns differed among wild plants under the same environmental stress [54] and were inconclusive in cultivated plants [81].

CONCLUSIONS

Generally, the daily or seasonal water stress to which a plant is subjected induces a range of plant responses that depends on the extent of the water shortage. Water stress causes primarily stomatal closure, decreasing assimilation and therefore growth. Water stress also reduces plant growth by reducing cell division and enlargement and causes a decline in ion transport to the root surface, which leads to a further decrease in plant growth.

Multiple responses allow the plant to tolerate water stress. These range from stomatal sensibility to soil and atmosphere dehydration to changes in cell wall elasticity and osmotic adjustment. In plants adapted to dry environments, anatomical and morphological changes at the leaf and whole-plant levels (such as reduced leaf size, hairy leaves, sclerophyll, or higher allocation to roots) prevent metabolic imbalance and help to improve water relations. These adaptations impose a cost on plant growth, with the overall effect of reducing growth to match all levels of resource acquisition. The C_4 photosynthetic pathway may have some remarkable advantages in water-limited environments, but CAM plants represent a higher degree of plant adaptation to dry environments.

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12

Nutrient Uptake by Plants Under Stress Conditions

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INTRODUCTION

The term *stress* is defined as an environmental change that tends to inhibit the normal cycle systems from functioning. Plant species or varieties differ in terms of their optimal environments and their susceptibility to particular stress. Some workers prefer to consider as stressful only those environments that actually damage the plants and cause a qualitative change, such as membrane damage or cell death, whereas others consider that in stressed systems, energy expenditure is increased or potential energy of the system is decreased. Commonly, plants are considered to be under stress when they experience a relatively severe shortage of an essential constituent or an excess of a potentially toxic or damaging substance.

The external constraints or form of stresses may be biotic (e.g., pests or diseases) or they may be physical and related to shortage or excesses in the supply of solar energy, water, mineral nutrients, and/or atmospheric pollutants. Sometimes the stresses are chronic and sometimes they are imposed for short periods.

Stress factors do not usually operate alone, so that interactions between the covariation of stresses are the norm in the natural environment. Stress may also have a greater damaging effect during certain phases of the plants life cycle than others. Seedlings establishment and floral development are often particularly sensitive. To predict the impact of stress on plants, we need to know something about (a) the temporal variation in stresses, (b) the plant's potential to acclimatize to stress, and (c) interactions between different stresses and the plant responses. In both natural and agricultural communities, the environment is seldom optimal for plant growth. Environmental stress limits the overall productivity of agricultural crops. It is a well-documented fact that maximum growth potential of horticultural and agronomic crops seldom is attained under natural conditions because of limitations imposed by large seasonal fluctuations in light, moisture, high soil temperature, high soil strength, flooding, cold, heat, low soil oxygen, acid soil complex, salt toxicities, imbalance of nutrients, combination of Al-Mn-H toxicities and/or deficiencies of Mg-Ca-K-P, soil pH, climatic changes, and other environmental stresses [1]. Moreover, most natural environments

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are continuously suboptimal with respect to one or more environmental parameters, such as water or nutrient availability.

At present, most research on the physiological responses of plants to environmental stress has focused on the responses of plants to specific stress. For example, plants adjust osmotically in response to salt and water stresses, increase their potential to absorb nutrients in response to nutrient stress, and alter the quantity and balance of photosynthetic enzymes in response to shade or light stress.

Soil is a basic anchor to support plant growth, and it is one of our most valuable natural resources. When properly fertilized, a handful of soil gives a meaningful crop yield. Many soils are fragile, especially in tropical areas, and overuse generally leads to a continuing problem of millions of hectares of land every year becoming unproductive and affecting the growth of plants. The amount of a nutrient that a plant may need for growth and reproduction varies among plant species and/or varieties. A common perception is that plant response to insufficient nutrient supply involves physiological changes that are unique to nutrient stress. Nutrient uptake by crop plants grown in soil is greatly influenced by root morphology, soil properties, climate, cultural and management practices, and plant species [2,3]. Similarly, soil water potential at the soil-root interface appears to be the main soil characteristic controlling the availability of soil water for plant growth, and nutrient concentrations at the root surface directly control nutrient uptake. It has also been reported that the uptake of water and ions by a plant root creates a concentration gradient in response to which water and ions flow from the surrounding soil to the root [4].

The quantity of a nutrient taken up by a plant generally depends on the configuration and growth rate of the root extension, mean root radius, mean root-hair density, and length of the root. Among the various physiological factors contributing to plant growth, nutrient element availability plays a vital role. However, these factors may simultaneously interact, antagonistically or synergistically, in the nutrient solution, in the soil, in the plant, and or at the root absorption sites of the plants [5,6].

Numerous essential plant nutrient elements are known to regulate the plant metabolism even under stress conditions by acting as cofactors or activators of enzymes. Examples of these are Mg as a component of chlorophyll, Fe as a component of ferridoxin and cytochromes; Zn as a component of glutamic, alcohol and lactic dehydrogenase, and carbonic anhydrase; Cu as a component of laccase, cytochrome oxidase, ascorbic acid oxidase, and polyphenol oxidase; Mn as a component of arginase and phosphotransferase; and Mo as a component of nitrogenase, nitrate reductase, and aldehyde oxidase [7]. The mobilization and utilization of certain mineral elements by plants may be influenced by the adverse or favorable environmental growth conditions.

Mineral stresses include both deficiencies of essential nutrient elements and/or excesses of toxic elements. Mineral nutrients generally play fundamental cellular roles. Likewise, the toxic ions affect injury via the disruption of fundamental cellular mechanism; for example, competition with essential elements for uptake, inactivation of enzymes, and displacement of essential elements from the functional sites [8].

In agricultural productivity, soil fertility is considered to be the status of a soil with respect to its capacity to provide plants with a sufficient amount of nutrients, rate, and balance, needed for optimum growth. Fertility depends on (a) the presence of water, oxygen, and adequate nutrients in the forms the plants can absorb; (b) soil capacity to deliver oxygen and nutrients by mass flow and diffusion to the root surface; (c) the presence of a favorable ionic composition; and (d) the absence of substances that interfere with the movement of nutrients in balanced amounts into roots. The poor soil aeration, which normally indicates an insufficient supply of oxygen to plant roots, generally influences the uptake and utilization of important plant nutrients, such as, for example, N, P, K, Ca, Mg, Fe, Mn, Zn, Cu, B, Mo, Na, and Cl, and all of which are essential to plant growth.

Many nutrient elements are actively taken up by plants. Potential energy is required for active uptake of nutrients, and aerobic respiration in the soil system is the chief supplier of this energy. During the period of respiration, oxygen is taken up and carbon dioxide is given off. For adequate

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aeration, plant roots generally need air in the soil to survive. Warm aerobic soil conditions provide a better environment for the uptake of elements than cool and anaerobic conditions.

The major stresses of the world which are considered to be of massive nature and create gigantic problems on a global basis in the normal growth and nutrient uptake by crop plants are mainly flooding, water stress (drought), and salinity, which are discussed below.

STRESS AND ITS EFFECT ON NUTRIENT UPTAKE BY PLANTS

Flooding—Induced Stress

Flooding stress results in extensive destruction or impairment of goods, services, health, and crops. The growth, distribution, and cultivation of crop plants are controlled chiefly by too little or too much available water. Temporary or continuous flooding with either fresh or brakish water is very common for cultivation of crops throughout the world, with about 72% of the Earth's surface being covered by submerged soils [9]. It is a well-established fact that for most agricultural crops and native species not adapted to wetland conditions, flooding the soil reduces the shoot and root growth, dry matter accumulation, and final crops yield [10–16]. When a soil is temporarily flooded, a number of physical and chemical changes can take place, any of which may profoundly influence the soil properties, which ultimately reduces the growth of plants. In addition, the physiological damage arises within the plant roots, because incompletely oxidized metabolic products may generally accumulate to toxic levels. Plant growth may be severely affected, because root systems deprived of oxygen can no longer perform, energy-requiring processes, such as the active uptake and retention of essential plant nutrients. Flooding damage to plants follows the depletion of dissolved oxygen from the soil by the respiration of root and soil microorganisms [17]. Shortly after they are flooded, plants exhibit sequential changes in metabolism and physiological processes. Reduced absorption and closure of stomata leading to a lowered rate of photosynthesis are among the earliest plant responses to flooding [18]. Subsequent changes include decreased permeability of roots [19], reduced or increased mineral uptake [20–24], alterations in growth hormone balances [25–29], lowered the permeability of roots to water [30–35], leaf epinasty, chlorosis, and abscission [36].

A further complication is that, in a flooded soil, the soil population of microorganisms, reacts vigorously to a deficiency of lifesaving oxygen. But on the other hand, the potentially inhibitory concentrations of carbon dioxide, hydrogen sulfide, methane, ethylene, manganese, iron, sulfate, and many organic substances may accumulate abnormally in large concentrations and lead to plant damage as a result of a reduction in the oxygen level [37,38].

Flooding stress causes an increase in abscisic acid (ABA) that could be responsible for changes in growth [39]. After reduction in the growth rate, there are decreases in the potential to absorb nutrients, concentrations of photosynthetic enzymes, rate of photosynthesis, root hydraulic conductance, tissue water potential, and turgor [39].

The ways in which flooding influences plant mineral nutrition are very complex, being determined by several concomitant flooding effects on the soil, initial soil conditions, and nutrient absorption mechanisms, as well as other physiological processes and responses of the particular plant species under study. It has been reported that soil flooding stress may severely reduce water and ion uptake directly by increasing the permeability of roots to water and indirectly by reducing the size and volume of the plant roots, as well as by decreasing the ion uptake per unit weight of roots. Such uptake implies a disruption in root metabolism [40] and reducing the effective root surface area available for ion uptake. Much evidence indicates that dysfunction in nutrient absorption by roots under flooding conditions is largely caused by a lack of O_2 and attendant deleterious metabolic effects. The accumulation of dioxygen in the Earth's atmosphere allowed for evolution of aerobic organisms that use O_2 as the terminal electron acceptor, thus providing a higher yield of energy compared with fermentation and anaerobic respiration. For example, in aerobic metabolism, the

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complete breakdown of one molecule of glucose yields a total of 38 molecules of adenosine triphosphate (ATP), whereas the anaerobic breakdown of this same glucose molecule to ethanol and CO_2 yields only 8 molecules of ATP [41]. Oxygen stress may inhibit guttation either by increasing the root resistance to water movement or by inhibition of ion transport to the xylem and arrest of vegetation and reproductive growth.

In addition, ammonia volatilization, denitrification, and leaching can lead to a major loss of nitrate from the soil solution [11,42–46], so that plants may suffer from nitrogen-deficiency symptoms. It is considered to be true that the N decline in plant parts reflects a lack of carbohydrate, which is a situation that delays conversion of NH_4^+ to the amines. Nitrogen leaching losses reported from pot studies range from 11 to 60% for ammonium sulfate, and about 4–30% in field studies under wet soil condition [14]. The larger losses might be expected with urea, but measured only 17% loss from a basal urea application in the flooded field.

The reduced uptake of nitrate as a result of the effects of low O_2 tension on root metabolism also appears to play an important role in reducing nitrogen levels in flooded plants. In waterlogged soils, slowing down of shoot and root growth was more closely related to the declining O_2 concentration in the soil solution than to the concentration of dissolved inorganic nitrogen [47]. In waterlogged soils, there is a rapid depletion of NO_3^- N, as free O_2 is quickly consumed by soil biota, anaerobic conditions develop, and loss of active soil N is freely promoted through denitrification. The normal aerobic N cycle is arrested in the mineralization stage in anaerobic soils, since oxygen is not available for oxidation of NH_4^+ to NO_3^- except in the oxidized layer [48]. This situation encourages in the presence of an excess of NH_4^+ in the anoxic layer. Flooding enhanced ammonification of soil N but retarded the nitrification process. On the other hand, under aerobic conditions, the NH_4^+ form of soil mineral N is oxidized to NO_3^-, which may accumulate in the soil or be utilized by crops from there [49].

Flooding of soybean plants grown on Crowley silt loam at R_2 growth stage adversely affected N nutrition in terms of concentration and total amount accumulated. The soybean plants recovered from this effect 2 weeks after the floodwater was removed [50]. The decrease in the accumulation of N by shoots of legumes in waterlogging soil is partly the consequence of the reduced nodulation of the roots by nitrogen-fixing bacteriods (*Rhizobium* spp.) [51]. For maize plants, diminished N uptake, proved to be a major limiting factor, its concentration generally decreased to the maximum extent, and deficiency symptoms appeared on the tops within 2–3 days of initiation of flooding stress. Brown et al. [52] found that soil moisture contents from 20 to 50% by weight resulted in a significant decrease in N uptake by soybean. Constable and Hearn [53] noted the coincidence of visual symptoms of flooding and reduced rates of N uptake. Cotton plant susceptibility to flooding may be due to a lowered soil oxygen supply or to reduced availability and uptake of N [54]. Flooding and the occurrence of prolonged soil moisture content above the field capacity suppressed the accumulation of N by cowpea (*Vigna unguiculata* L.) [55] as a result of decreases in O_2. Singh and Ghildyal [56] attributed part of the reduction in N concentration in corn shoots to reduced NO_3^- availability in the soil. In an experiment, Sallam and Scott [50] found N concentration of the whole soybean plant to be decreased immediately after flooding and remained lower in the flooded than in the nonflooded soybean plants for the first 3 weeks following the flood. Because nitrates may be quickly lost during flooding and mineralization is slow down at high soil water pressures following flooding, N deficiency was probably at least partially responsible for the slow recovery of the growth.

In a series of experiments, Drew and Lynch [57] studied growth and nitrogen-uptake patterns of wheat subjected to several anaerobic regimens. Waterlogged wheat plants exhibited reduced nitrogen concentration, chlorosis, and generally accelerated senescence of older leaves, with the latter two occurring with the onset of remobilization of N from old to young leaves [58]. Generally a lack of O_2 in the root environment causes an immediate decrease in nutrient uptake and redistribution of N from older to younger leaves [8].

Under conditions of low N availability, there was a decline in leaf allocation and water uptake as a result of decreased demand by the plants [40]. The concentration of N in the leaves, stems,

and branches were lower in the flooded soybean than in the nonflooded soybean plants [50]. On the other hand, the pods of the flooded soybean plants had higher N concentrations (4.3–5.1%) than those of the nonflooded soybean plants (3.5–4.60%). Both the N uptake and grain yield of wheat were found to increase linearly with an increase in water use [14]. The flooding of rice generally increased the tissue concentrations of N [20,22]. Primary tillage depth exerted a significant influence on mineral N availability in submerged soil [59]. The addition of NO_3^- to soil apparently prevented flooding stress in barley [23], corn [60], and other crops [61].

The crop plants, tolerant of flooding stress, often grow well and take up more available nutrients in response to flooding compared with the well-watered control plants. Such beneficial responses have been reported for rice (*Oryza sativa* L.) plants and for several species of flood-tolerant woody angiosperms and conifers [62]. Several morphological adaptations of flood-tolerant species allow continued nutrient absorption under waterlogging conditions. Many flood-tolerant species initiate vigorous adventitious roots that proliferate most abundantly in the upper, well-aerated portion of submerged soil [62].

It is an accepted fact that, under flooding stress, the N concentrations in plant parts of wheat (*Triticum aestivum* L.) [63], barley (*Hordeum vulgare* L.) [23,34,64], field corn (*Zea mays* L.) [34,56,65–67], pea (*Pisum sativum* L.) [68], cotton (*Gossypium hirsutum* L.) [33], sunflower (*Helianthus annuus* L.) [33], soybean (*Glycine max*. L. Merrill) [13,50], subterranean clover (*Trifolium subterraneum* L.) [67], bent grass (*Agrostis stolonifera* L.) [31], orchard grass (*Dactylis glomerata*), perennial ryegrass (*Lolium perenne* L.), timothy (*Phleum pratense* L.), fescue (*Festuca arundinacea* L.) [69], avocado (*Persea americana* L.) [70], sweet gum (*Liquidambar styraciflua* L.) [71], hackberry (*Celtis laevigata*) [71], and orange (*Citrus sinensis* L.) [72] are reported to be significantly decreased. One contributor to the reduction of N in tissues of flooded plants is that, in waterlogged soils, NO_3^- N is rapidly depleted as oxygen is quickly consumed by soil biota and anaerobic conditions develop. As a result, volatilization and loss of N are promoted through denitrification in which nitrates serve as a terminal electron acceptor for anaerobic microbes [9].

Phosphorus is one of the most important nutrient element in the growth and development of plants. It plays a key role in cellular energy transfer, respiration, and photosynthesis. Phosphorus is present in nucleic acids, phospholipids, and sugar phosphate. A phosphorus deficiency causes immediate and severe disruptions of metabolism and development [8]. Phosphorus is generally present at a very low concentration in the soil solution in comparison with the other essential plant nutrients, and it diffuses only slowly in the soil media. Soil solution P is an immediate P source for the plant, and standard solution P concentration (0.2 mg P/L) provides P adequately for many crops if it is continuously maintained in the growing medium [73]. Soils vary greatly in the amount of fertilizer P required to provide an adequate supply of available P to plants, and plants also vary in their P requirements for optimum growth.

The P composition of flooded plants, like the N composition, is greatly influenced by both soil conditions and plant uptake responses to soil inundation. Where the amounts of soluble P available in the soil are adequate, flooding stress of intolerant plants generally lowers both the tissue concentration and total content of P [74]. Flood-intolerant plants that have shown the lower uptake of P are barley [23], cowpea [55], *Citrus sinensis* [72], corn [65,66], wheat [17], *Helianthus annuus* [33], *Liquidambar styraciflua* [71], maize [66], *Persea americana* [70], ryegrass [69], and jojoba (*Simmondsia chinensis*) [35]. These declines in P concentration have been attributed to the inhibited uptake under anaerobiosis [57]. However, the situation is more complex for soils that are moderately or severely deficient in P. In such well-aerated soils, much P may be held in unavailable forms. When soil is flooded, soil pH moves toward neutrality and soil reduction levels increase; as a result, P can be released from insoluble adsorbed and bound forms [9], thereby becoming more available for uptake by roots. The increase in the concentration of water-soluble phosphate and desorption of sulfate caused by flooding the soils may be the result of a decrease in anion exchange capacity and an increase in the bicarbonate concentration. Hence, if P uptake is not severely limited by the imposed level of anaerobiosis and the levels of soil P before flooding are not inordinately high, flooding can result in temporarily increased plant P content [67].

In addition, some flood-intolerant plants, such as subterranean clover [67], hackberry (*Celtis laevigata*) [71], and pea [68], have been shown to increase the uptake of P from flooded soils. Similarly, plants tolerant to flooding often grow better and take up more P in response to flooding compared with well-watered controls. Such responses have been reported for cereal species and several species of flood-tolerant woody angiosperms (*Fraxinus pennsylvanica, Frs. profunds, Nyssa aquatica, Salix nigra, Acer negundo, A. rubrum, A. saccharinum, Populus deltoides*, and *Platanus occidentialis; conifers* (*Taxodium distichum*); and rice (*Oryza* spp.) [71,75,76]. Reducing conditions that develop when soils are flooded for rice production appear to increase the availability of soil P. Some reports also stated that lowland rice generally responds to P fertilization under flooded condition [77].

The mechanism of P release in flooded soil generally includes the reduction of insoluble ferric phosphate to more soluble ferrous phosphate, release of occluded P by reduction of hydrated ferric oxide coatings, displacement of P from ferric and aluminium phosphates by organic anions, hydrolysis of ferric and aluminium phosphates as a result of the increase in alkalinity, and anionic phosphate exchange between clay and organic anions. It was reported [43] that more soil P was released by reduced soils than by oxidized soils. Plants subjected to prolonged flooding generally have reduced tissue P concentration and total content, because increased P availability cannot compensate for the severe degeneration of the plant root system.

In general, the inhibitory effects of flooding stress on potassium uptake are similar to those for N. Severe inhibition of K uptake characteristically follows soil submergence, and this response may limit plant growth in certain flooded crops. It has generally been reported that on giving flooding stress to soils, the K content in crop plants, such as barley [23,64] wheat [58], corn [65], subterranean clover [67], avocado (*Persea americana*) [70], *Liquidambar stayraciflua* [71], *Celtis laevigata* [71], *Pinus elliotti* [79], *Citrus sinensis* [72], *Gossypium hirsutum* [33], *Simmondsia chinensis* [35], *Dactylis glomerata* [69], *Phleum pratense* [69], *Lolium perenne* [34], sweet gum and hackberry [71], and orchard grass and bent grass [31] generally decreased.

Reduction in K absorption is most likely attributable to the effects of anaerobiosis on uptake mechanisms of roots [30,56,58,80]. If organic matter is available and cation exchange capacity of the soil is low, submergence may increase soluble K somewhat in the soil solution through displacement of exchangeable K from the exchange complex by competing ions [81]. However, the flood-associated increases in K available to the plants are generally too small to overcome the large inhibitory effects of anaerobic conditions on K uptake by crop plants. Some other investigators [82–84] have found greater efficiency of K fertilizer with increasing soil moisture of the growth medium. The K content also decreased even in flooded tolerant rice plants [85]. Lack of K was found drastically to reduce the oxidizing power of rice roots [86].

In a 4-month-old loblolly pine (*Pinus taeda* L.), seedling flooding stress reduced total concentration of N, P, K, Ca, Mg, Zn, and Mn as compared with those grown in well-drained conditions [87]. Flooding stress appears to have much less pronounced inhibitory effects on the accumulation of Ca and Mg than on N, P, or K. Hence, Ca and Mg concentrations are not altered as much by flooding as are those of N, P, and K. However, concentrations may decrease slightly and their total contents decline appreciably because of severely reduced growth. It has been suggested that the absorption of Ca and Mg may be metabolically mediated, and therefore may be dependent on an adequate supply of O_2, but some data suggested that Ca and Mg ions are actively extruded from the plasmalemma [88]. Moreover, based on comparisons of mineral element, analyses of xylem exudate, and culture solutions, Trought and Drew [47] suggested that Ca and Mg were excluded relative to water movement in anaerobically cultured wheat root systems and that the Ca and Mg contents of the exudate could be accounted for by simple mass flow. Accordingly, the lack of close coupling between active uptake mechanisms and Ca and Mg concentrations by crop plants may explain the reduced effect of flooding on tissue concentrations of these two secondary elements. Flooding of rice increased the tissue concentrations of Ca, N, P, and Fe, whereas those of Mg were not significantly altered. On the other hand, the concentrations of Ca and Mg were generally decreased in crop plants, such as wheat [58] corn [66], subterranean clover [67], *Persea ameri-*

cana [70], *Celtis laevigata* [71], *Citrus sinensis* [72], *Helianthus annuus* [33], and *Agrostis stolonifera* [31].

Sulfur occurs in most soil largely in organic forms and is subject to numerous biological transformations. All forms of organic S in soil contribute to S mineralization and greater availability to crops [89]. Elemental S, which is biologically oxidized to H_2SO_4 under aerobic conditions, is often applied to reduce soil pH and dissolve insoluble nutrients [90]. In calcareous soils with low organic matter, addition of organic matter with S stimulates S oxidation [91].

Sulfur deficiency has been recognized as an important growth-limiting factor for both dryland crops and wetland rice [92]. Sulfur deficiency of wetland rice has been also reported in many Asian countries [92]. Sulfate concentrations in most of the soil solutions decreased to 1.8 mg/L within 8 weeks of submergence [93].

Sulfate-containing fertilizers applied to flooded or submerged rice soils may undergo reduction to sulfide in the paddy fields, with the subsequent problem of plant availability and/or H_2S production. Reduced or flooded conditions in the paddy soil may also inhibit the oxidation of elemental S and render this form of fertilizer useless to rice. Incubation studies of S transformations in flooded soils are of limited value in predicting fertilizer reactions in the presence of rice plants because of the modifying effect of oxidized zone adjacent to the rice root. The change in pH range that occurs when a soil is flooded means a drastic change in sulfate adsorption. It is pH dependent, with adsorption being negligible above the pH value of 6.5. The flooding of an acid soil raises the pH to an equilibrium value ranging from 6.7 to 7.2 [94], and at this pH, sulfate adsorption is negligible. Since crop plants can take S only in the sulfate form (SO_4^{2-}), the oxidation state of the S present in the paddy soil is important. Because of the reduction of sulfate to S, the availability of S to rice has been found to be lower under flooded than upland conditions.

It has been found that sulfur uptake from $K_2{}^{35}SO_4$ and $Na_2{}^{35}S$ sources was found to be equal in experiments conducted at the International Rice Research Institute (IRRI) Manila, the Philippines [95]. Blair et al. [92] reported that all the sources of fertilizer S were equally effective for rice growth when applied at transplanting. This contradicts the suggestion of Wang [96] that, ''chemicals containing S, other than sulfate are not particularly useful for rice,'' and shows that the assumption that a rice paddy is anaerobic below the surface-oxidized layer may be incorrect. Gypsum ($CaSO_4$, $2H_2O$) is often considered as an insoluble or slightly soluble sulfate source because of its solubility in chemical terms (241 mg/100 mL cold water). However, a rice paddy covered with 5 cm of water has the capacity to dissolve 1205 kg gypsum ha^{-1}, which is well above the rate used for agricultural and horticultural purposes. Gypsum is a good source of Ca, and an increase in its application increased the leaching of Mg, K, Na, and Mn [97]. Gypsum provided a better soil environment, and it is known to increase the depth of root penetration [98].

Under submerged soil conditions, the iron and manganese solubilities generally increase as first the ferric and manganic forms are converted to the more reduced and soluble ferrous and manganous forms [9]. The change in availabilities of these two elements in waterlogged soils is reflected in increased tissue concentrations by several plants, including wheat [17], cotton [33], and some other flood-intolerant plants [71]. Some workers have not found any change [64] or a decrease [70] in the plant uptake of Fe and Mn. However, the total content of Fe and Mn most often declines because of severely inhibited growth.

Flooding of tolerant plants such as rice also increased the content of Fe and Mn [20,22,99,100]. Uptake of both Fe and Mn increased in French bean (*Phaseolus vulgaris* L.) and maize as a result of flooding, although the reoxidation of the soil affected Fe more than Mn [101]. It appears that a high moisture content, by reducing aeration, causes an increased in the ferrous Fe in the soil solution, which reduces from ferric iron and thus promotes Fe uptake and an increase in the total Fe content of the plant leaf tissue. The reduction of Fe is a consequence of anaerobic metabolism, and it appears to involve chemical reduction by bacterial metabolites. It has been observed that 5–50% of the active ferric iron present in a soil may be reduced within a few weeks of submergence depending on temperature, organic matter content, nitrate concentration, and crystallinity of the oxides. The reduced Fe acts as a sink for oxygen diffusing into the soil and is a source of ferrous ions. The

concentrations of several nutrients (Fe, Mn, Zn, Cu, and P) were higher in mycorrhizal wetland rice than in nonmycorrhizal rice under flooded conditions [102]. Reduced aeration also restricts carbon dioxide escape from the soil causing, in alkaline soils, an increased bicarbonate concentration in the soil solution, which is known to reduce the availability of Fe in the plants. The bicarbonate ion is reported to inhibit the activity of cytochrome oxidase in the roots of soybean (*Glycine max* L.) and spinach (*Spinacea oleracea* L.) [103].

A high soil moisture content caused poor utilization of Fe by peanut (*Arachis hypogaea* L.) from alkaline soil. However, Alam and Azmi [104] have reported an increase in available Fe, Mn, and P in alkaline calcareous soil due to flooding. Factors including poor soil aeration, a high concentration of phosphate, the presence of heavy metals (Ni, Zn, Co, and Cr), extreme light, plant root damage, and viral incidence have also been reported to cause plants to fail either to absorb Fe from the soil or to utilize it efficiently. Couto et al. [105] conducted a glasshouse experiment to determine how soil-reducing conditions would affect plant nutrient availability and uptake by two tropical forage species in a red-yellow latosol. They found that the grass andropogon (*Andropogon gayanus* kunth) and the legume stylo (*Stylosanthes capitata* Vog.) responded differently to reducing conditions. Andropogon showed a low Fe and Mn, P, Ca, and Mg content in the shoots, but an intense coating of oxidized Fe was observed on the surface of roots. The stylo plant, on the other hand, showed no Fe deposition on the root surface but a high Fe content in the shoots. No decreased in P, Ca, or Mg content was observed in this case. They concluded that, in water-saturated soil, reduction took place and plant performance was affected not only by restricted root development but by preventing P, Ca, and Mg uptake in andropogon and increasing Fe uptake in stylo plants.

The amounts of Fe and Mn of the root coating extracted from trees under reduced soil conditions were much higher for the green ash (*Fraxinus pennsylvanica* Marsh) and water oak (*Quercus nigra* L.) root [106]. It is argued that this reflects differences in the ability of these two species to maintain rhizosphere oxidation under a continuous prolonged periods of flooding and to prevent the accumulation of reduced potentially phytotoxic growth-inhibiting compounds. Flooding causes the microbial reduction of Fe and Mn in the soil systems. Varying the water conditions is expected to modify the flooding effects on Fe and Mn and, consequently, the uptake of these elements by crop plants.

Under flooding condition, soil organic matter contributes to Fe and Mn availability through the formation of metallo-organic complexes with organic substances. This phenomenon may be attributed to the production of chelating agents from compost that generally keep the micronutrient elements soluble and, consequently, more available to crop plants. Increased Fe and Mn solubility in flooded soils benefits rice, which has a higher requirement for these elements than the other plants. There was an increase in pH, CO_3^{2-}, and DTPA extractable Fe and Mn on the submergence of a lowland rice plants [107].

The abundance of ferrous and manganous ions in flooded soils may result in acute phytotoxic effects in certain plant species. Differential responses of plant species to the build-up of soluble ferrous and manganous ions have been suggested as the potential factors in species ecology and habitat distribution. The cut shoots of a dune-slack species (*Erica cinerea*) characteristically found on waterlogged soils were much less injured by Fe sulfate introduced into the water supply than were those of a drier-site dune species (*E. tetralix*). These results suggest that high soluble-iron concentrations in wet habitats might exclude or reduce the abundance of the latter species [81].

It has been observed that generally high levels of Fe are found in the soil solution of submerged soil. In dry soil that has low pH and abundant sulfate, extremely high amounts of soluble ferrous-Fe are found soon after submergence, causing bronzing of rice leaves [108]. As the pH rises toward neutrality with prolonged flooding, Fe availability decreases, as does Fe toxicity. Excessive Fe can also interfere with the uptake of other nutrients. High concentrations of Fe in flooded soil can induce P-deficiency symptoms in rice plants. Manganese concentrations of flooded rice plants grown on certain soils may reach 3000 mg/L, but visual toxicity symptoms on plants are unusual.

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Under flooding conditions, the solubility of Zn, Cu, B, and Mo generally change with time and growth environmental conditions. It has been found that, under flooded conditions, the production of organic complexing compounds and reductions of Fe and Mn tend to enhance the solubility of Zn and Cu in the growth media. Increase in soil pH in acidic to near neutral soils on submergence plays the most dominant role in depressing Zn and Cu availability in flooded rice soils, whereas the role of increased concentrations of CO_2 and S, although considerable, is less than that of pH. On calcareous soils, the rice plants frequently exhibit Zn deficiency possibly as a result of Zn fixation. Deficiency occurs primarily during early growth of the crop and during this period may be exacerbated by immobilization of Zn in roots by bicarbonate ions that are produced in alkaline soils soon after submergence [108]. Alkaline soils subjected to prolonged flooding exhibit increased Zn availability as pH declines with increasing soil reduction [9].

Submergence increases the accumulation of CO_2 in soil solution resulting in an increase in the formation of H_2CO_3, HCO_3^-, and CO_3^-, which has been shown to depress the Zn availability in flooded soils. In an experiment, the percentage decrease in Zn and Cu to 57 and 59%, respectively, in soil on submergence are partially due to their insoluble precipitation as sulfides, hydroxide, carbonate, phosphate, oxide, and chelate and their adsorption-precipitation by iron compounds [107].

Long-term flooding of noncalcareous soils generally tends to increase the availability of Cu and Mo and depress that of Zn [108]. It has been observed that tissue concentrations and the total content of Zn generally decline in flood-intolerant plants, such as wheat, corn, bent grass, and subterranean clover [65,70]. In an experiment, it was found that urea and ammonium sulfate had more effects than ammonium nitrate on the availability of Zn under flooded conditions. The total contents of Cu and B decreases in plants and the growth is markedly inhibited. It has been reported that, under flooding conditions, the tissue concentration of Mo increased in the ear leaf of corn. The behavior of Cu and Zn under flooded conditions seems to be complex; both decreases and increases in readily available forms have been reported. When a soil has undergone reduction by flooding, the breakdown of Fe and Mn oxides can provide an increased surface area with a high adsorptive capacity onto which Cu and Zn may be firmly adsorbed [101].

It has been reported by a number of workers that, under flooding stress or anaerobiosis conditions, the uptake and transport of Na ions generally increased in a number of crop plants, such as *Persea americana* [70], *Citrus sinensis* [72], cotton [33], sunflower [33], and jojoba [35]. Contrary to this, some workers have reported substantial decreased in the Na content by crop plants like subterranean clover [67] and *Persea americana* [70] under flooding stress.

Waterlogging of the rootzone of tomato resulted in significantly higher concentration of Na ions in plant parts when tested at temperatures of 20 and 28°C [109]. This response is consistent with the current understanding of nutrient element metabolism in that plant roots are thought to extrude Na^+ ions at the plasmalemma [88]. It is also possible that under flooding stress, with the O_2 depletion, exclusion of Na from the growth media becomes less efficient and the tissue Na concentrations rise away to a considerable extent. Some evidence, however, shows that Na uptake by root systems occurs under both aerobic and anaerobic conditions; hence that anaerobic condition itself cannot account for observed changes of Na in flooded plants. In any case, the tendency for Na to accumulate in flooded plants suggests the possibility of Na toxicity, particularly in Na-sensitive species.

There is some evidence that flooding stress effects on nutrient contents vary among the plant organs. Although not apparent in all studies, high concentration of elements in the roots of flooded plants may be coupled with decreased shoot concentration of some minerals like N, P, and K [35]. To account for these responses, it has been suggested that reduced O_2 availability to roots inhibited translocation of ions from roots to shoots, which decreased ion uptake [66]. In control, the Na concentration in flooded plants was sometimes increased in shoots and decreased in roots [35]. As root system of unflooded plants in these studies had a higher Na concentration compared with shoots, metabolically related reduction in the efficiency with which Na is excluded from the shoot may occur in flooded plants.

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Salinity-Induced Stress

Salinity stress is a major environmental factor that drastically affects the crop productivity throughout the world [110–123]. It is a menace to both agriculture and the soil body. Historically, soil salinity contributed greatly to the decline of several ancient civilizations. Despite the advanced management technologies available today, salinization of millions of hectares of land continues severely to reduce crop production on a worldwide basis. Soil salinity is thus threatening human civilization by persistently reducing the areas of agricultural crop production all over the world.

All soils contain a mixture of soluble salts, some of which are essential for plant growth. When the total concentration of salts become excessive, plant growth is suppressed. The suppression increases as the salt concentration increases until the plant dies. The most common cations associated with soil salinity are Ca^{2+}, Mg^{2+}, and Na^{+}, and the anions are Cl^{-}, SO_4^{2-}, and HCO_3^{-}. In some instances, K^{+} and NO_3^{-} may contribute to salinity, and when the pH of the medium is greater than 9, CO_3^{2-} becomes an important anion. Excessive Na^{+} causes deterioration of the physical structure of the soil and can be toxic to plants. Chloride and B are also toxic. Boron has received considerable attention, because it has been identified in a number of saline waters.

Plant growth is severely affected by excessive concentrations of soluble salts in the growth media. Soluble salts decrease the solubility and availability of water to the plants by decreasing the free energy of water. Generally plant biomass is inhibited by an excess of solute taken up by plants from saline growth media. Salts may exert detrimental effects on plant growth through the toxicity of one or more specific ions present in higher relative concentrations.

The salinity stress problem arises when semiarid or arid lands are subjected to cultivation either because the soils are already saline and/or irrigated with saline water, which adds to the salinity of the soil. In addition, the excessive use of chemical fertilizers and irrigation have turned hundreds of hectares of cultivated fertile lands into saline lands. Plant responses to salinity depend on the kinds of salts (sulfates and chlorides) contributing to salinity as well as the total electrolyte concentrations [124].

Generally plants react to salinity by a reduction in growth, but there are quantitative differences in the degree of response. The growth response of a plant to salinity, expressed as yield, decreased under saline conditions compared with nonsaline is called plant salt tolerance [125]. Studies have shown considerable variations in salt tolerance between species of the same genus, between cultivars, or within varieties [126]. These differences are very often correlated with differences in translocation of Na^{+} and Cl^{-} in aboveground parts of plants.

The mechanism of salt tolerance of cultivated crop species that differ considerably in tolerance to salinity generally range from restricted ion uptake and translocation into the shoot to structural metabolic changes that decrease salt injury. The reduced water potential at high salt concentrations may further aggravate the effects. Salt tolerance under such conditions is generally related to the ability to regulate Na^{+} and Cl^{-} uptake by plant roots and subsequent translocation to the shoots [127–129]. The toxicity caused by salinity is considered to be due to water stress, which is a result of osmotic imbalance between plant and soil or ionic imbalance due to excessive salt uptake.

Salinity stress is known to retard growth [112,130] through its influence on several vital facets of plant metabolism, like osmotic adjustment [131], ion uptake [110,112,130,132–138], protein and nucleic acid synthesis, photosynthesis, organic solute accumulation, enzyme activities, hormonal balance, injury to tissue, alteration in respiration rates, interaction of salt with microbial activity, and reduced water availability to crop plants [139].

Salinity stress under certain experimental conditions may curtail or promote nutrient uptake by plant species by affecting the mobility of a nutrient within the plant or by increasing the nutrient requirement by plants in the cells. The simultaneous presence of salts and nutrient elements in the rootzones can influence nutrient uptake by plants and thereby affect their chemical composition. Synergistic and antagonistic effects may increase or decrease the intensity of these processes. At high salt stress, leaf scorching was predominant in older leaves and also was observed in the younger ones. The relationship between this toxic symptom and internal Cl^{-} level has been earlier demon-

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strated. Specific injury through Na^+ and Cl^- accumulation rather than osmotic stress was suggested to be the main reason for NaCl susceptibility. Symptom of Na^+ toxicity can be easily seen when the leaves of sensitive plants contain approximately 0.25% Na on a dry weight basis. The Na^+ toxicity is characterized by leaf burn, necrotic spots, and limited leaf expansion, which in turn directly reduces plant photosynthesis and yield [140]. Salinity stress is generally recognized as being injurious to the growth of many crops owing to a disturbance in the electrolyte balance resulting in the deficiency of some essential nutrient elements and in an excess of certain unwanted salts in the plant tissue.

Preferential accumulation of either Na [141,142], Cl [143,144], and/or both Na and Cl [127,136,145,146] is also reported to account for salt tolerance in crop plants. The harmful effects of a high Na concentration in the medium on plant growth can be divided into three groups: (a) inhibition of water uptake due to osmotic potential of the culture solution [147], (b) disturbance of normal metabolism caused by high Na concentration in plant tissues [148], and (c) inhibition of the absorption of other essential cations by plants [149].

Increased Na content generally disturbs the nutrient balance and osmotic regulation and causes specific ion toxicity. It is the ionic balance of a growth medium rather than absolute Na content which determines the salt tolerance of a plant. The Na accumulations were significantly higher in salinity treatments compared with control for barley cultivars, and they were of the order about 10-fold at the 20 dSm^{-1} salinity level [150].

In a saline environment, plants take up excessive amounts of Na at the cost of K and Ca. High Na/Ca and Na/K ratios in a saline nutrient solution may cause an increase in membrane permeability [151], and this may have result in the passive accumulation of Na^+ and Cl^- in the root and shoot of salt-stressed plants. The higher K/Na ratio in shoots of barley cultivars compared with that in root medium solution indicated selective uptake of K, which seems to be among the processes involved in tolerance of cultivars to salinity stress [150]. Addition of K suppressed the uptake of other cations by rice and tomato plants in the order of Na>Mg>Ca. The depression of Na uptake by K could be due to the antagonism between the two cations [152]. It is widely recognized that a high Na concentration inhibits K uptake by plants [153]. On the other hand, Na appeared to stimulate the K uptake by plants [154]. The greater accumulation of Na in plant roots may be due to a regulatory mechanism located within the roots which prevents the translocation of excessive cations such as Na from roots to aerial parts resulting in Na retention [142].

Salt tolerance in many crop plants depends on the efficiency of root system, which can regulate the excess of Na^+ and Cl^- and/or SO_4^{2-} ions to reach the shoot. Roots have a definite capacity to act as storage for Na and other ions taken up from the external medium [143]. The acquisition of mineral nutrients and water and tolerance of the plant to the presence of potentially toxic levels of elements such as Na in the soil solution may, therefore, depend on the continued growth of the adventitious roots. The adventitious roots in salt-stressed plants represent a potential reservoir for the storage of Na^+ and Cl^- [143]. The presence of Na and Cl in the rooting environment have been shown to affect plant metabolism by affecting ion uptake.

The generally higher root Na^+ and Cl^- concentrations of various plants and lower leaf Na^+ and Cl^- concentrations suggested that some plants have a relatively high tolerance of Na^+ and Cl^- in roots coupled with the mechanisms for reduced translocation of Na^+ and Cl^- to the shoots. Such finding was observed for love grass (*Eragrostis tenella* L.) [155]. In a field experiment, Bhatti and Wienke [156] studied the uptake and distribution of Na^+ and Cl^- in ''salt-tolerant'' Kallar grass (*Diplachne fusca* L.) and found that increasing NaCl concentrations significantly raised Na^+ and Cl^- concentrations in the roots, as well as in the shoots, without showing any visual toxic symptoms in the leaves. Furthermore, these investigators reported that the old leaves were able to extrude a larger percentage (30–40%) of their total Na and Cl. Flowers and Yeo [126] obtained an inverse relationship between the Na uptake to the leaves and plant survival. Jones [157] reported that, when Na was present in high concentration in the growth medium, the transpiration rate of peas was reduced to proportion to salinity. High Na in soil solution also has an antagonistic effect on Ca^{2+} and Mg^{2+} uptake. Sodium salinity caused Ca-deficiency symptoms in tomato, pepper, and celery

(*Apium graveoleus*) plants. This is most likely caused by Na^+ displacing Ca^{2+} from membranes of root cells rendering the membranes nonfunctional.

Chloride is a more sensitive indicator of salt damage than Na, since it is stored by the plant, whereas Na is absorbed in smaller quantities despite high Na concentrations in the soil. The chloride content of pigeon pea (*Cajanus cajan* L.) was greater than Na, which is in agreement with the increased Cl contents in plants irrigated with salt water [158]. Leaf burning caused by the effects of salinity on peach trees was attributed to the accumulation of Cl^- in the leaves. These conclusions may have been based on the study of plants that take more Cl^- than Na^+ into the leaves. In fact, many of the more salt-susceptible species, such as maize, cress, sunflower, pepper, and bean, have higher Cl^- than Na^+ concentrations in their leaves [159]. This is also true for crops like *Acer Saccharum, Glycine max, Panicum repens, Spinacea oleracea*, and *Fagus sylvatica*. On the other hand, some of the most salt-tolerant species have higher concentrations of Na^+ than of Cl^- in the leaves; for example, *Plantago maritima, Suaeda monoica, Atriplex spongiosa, Suaeda maritima*, and *Puccinellia maritima* [160].

Salinity stress has significant inhibitory effects on the concentrations of K, Ca, and Mg [127,131,132,134,135,145,146,148,161–163] as well as stimulatory effects of these nutrient elements [131,132,145,148] on different crop plants.

Potassium, which is an essential cytoplasmic element [164], because of its involvement in osmotic regulation and its competitive effect against Na, is frequently considered to be important under saline conditions. Potassium participates not only in osmotic adjustment under saline conditions but also plays an important role in turgor-mediated responses such as stomatal and leaf movement. Numerous studies have shown that the K^+ concentration in plant tissue is reduced as the Na^+ salinity or the Na^+/Ca^{2+} ratio in the root media is increased [130,144]. Reduction in K^+ uptake by Na^+ is a competitive process and occurs regardless of whether the solution is dominated by Na^+ salts of Cl^- and SO_4^{2-}. In sheaths of sorghum, K was found to be the predominant cation, suggesting that the K ion may be operating as counter ion to Na, thereby contributing to osmotic adjustment in salt-stressed sorghum (*Sorghum bicolor* L.) [143]. With the increasing concentration of NaCl salts, K concentration decreased in the leaves, stems, and roots and was accompanied by a substantial increase of Na^+ in the organs. In Na_2SO_4–treated sorghum plants, an increase in Na^+ and SO_4^{2-} and a decrease in K^+ uptake was observed with increasing concentrations of the salt [130]. The dry weight of rice decreased markedly with the increase of NaCl concentration (50–200 mM NaCl) in the nutrient medium compared with that of barley. The salt tolerance of barley was higher than that of rice. The high salt tolerance of barley was ascribed to the fact that Na translocation from root to shoot was prevented at high NaCl concentration in the growth medium [165]. Excessive Na decreased the dry weight contents of essential cations, especially K in rice and tomato plants, and that the addition of K was able to improve their growth [152]. Using nutrient culture solution, Muhammad et al. [166] found that the shoot and root growth of rice plants grown in 100 mM NaCl solutions were increased when substrate K increased from 1 to 7 mM. In another nutrient solution experiment, Chow et al. [167] showed that differences in the shoot growth of spinach between plants grown at low (50 mM NaCl) and high (250 mM NaCl) salinity at a given level of K^+ can be reduced when K^+ is added to the highest salinity treatment. Application of 150 mg K kg^{-1} soil to the high Na–treated plants reduced seed Na concentration by greater than 50% [168].

Calcium plays a vital nutritional and physiological role in plant metabolism. Calcium, which like K also is an essentail mineral nutrient, helps in maintaining membrane integrity, is important in senescence processes, and is known to counteract the harmful effects of Na on crops [169]. Plant growth is dependent on Ca^{2+}, and both cell division and cell elongation processes are affected by the Ca^{2+} ion concentration. Calcium also plays a crucial role in controlling cell membranes' permeability and selectivity. It is thus important in regulating the salt economy of plants, and it may protect them against some of the deleterious effects of salinity. Several studies have highlighted the role of Ca in mediating salt responses in plants [170].

Calcium, which is a component of the cell membrane, contributes to the maintenance of the structure and function of the membrane. Calcium is needed preferentially in actively growing tissues.

A high Na concentration inhibits Ca absorption by plants. Alternatively, the addition of Ca improved plant growth under saline conditions by depressing Na absorption and accelerating K absorption. Also, Ca reduced the efflux of K^+, NO_3^-, and $H_2PO_4^-$ caused by the high Na concentration [149]. The addition of 10 mmol Ca/L improved rice growth by decreasing the Na uptake and increasing the K and Ca uptake [152].

The presence of Ca^{2+} as the dominant cation in agricultural soils generally ensures that the absolute Ca^{2+} level is not a primary growth-limiting factor. As salinity increases, the requirement of plants for Ca^{2+} increases. The uptake of Ca^{2+} from the soil solution may decrease because of ion interactions, precipitation, and increases in ionic strength that reduce the activity of Ca^{2+}. These combined effects are at least partially responsible for reduced yield under saline conditions. Calcium protects cell membranes from the adverse effect of Na and minimizes the leakage of cytosolic K. The vital roles of Ca^{2+} are the regulation of ionic relations in plants and improving the soil physical conditions.

It is wellknown that Ca^{2+} is readily displaced from its extracellular binding sites by other cations, and these functions may become seriously impaired by reduced Ca^{2+} availability. Root growth and function may be restricted by high Na^+/Ca^{2+}. Soloman et al. [170] observed abnormal root growth and anatomy of pea plants grown in nutrient culture containing 120 mM NaCl as the sole salinizing salt. These salinity-induced changes, characterized by curvature of the root tip as well as constriction and thickening above the apex, were completely reversed by the addition of 10 mM Ca^{2+}. Sodium-induced Ca^{2+} deficiencies have notorious growth-distorting effects on developing leaves, as illustrated in several grass species grown in solution cultures. A case of Na-induced Ca deficiency in corn salinized with NaCl has been documented. The symptoms of Ca deficiency disappeared when part of the NaCl was replaced with $CaCl_2$. From a study comparing the salt tolerance of a commercial barley cultivar with that of wild barley (*Hordium jubatum* L.), Suhayda et al. [171] concluded that the greater salt tolerance of the wild species was partly attributable to its ability to maintain high tissue levels of Ca under salt stress. Further, the commercial barley cultivar was more tolerant to salinity when the Ca concentration in the salt medium was increased.

Cramer et al. [172] concluded that the primary response to NaCl stress in cotton roots is the displacement of membrane-associated Ca^{2+} by Na^+ leading to increased membrane permeability and loss of K^+/Na^+ selectivity. The addition of 10 mM Ca^{2+} to the saline culture preserved membrane integrity and prevented leakage of K^+. Under saline conditions, a high Ca^{2+} supply alleviated the inhibition of NO_3^- uptake and increased Na^+/K^+ selectivity [173]. Elevated Ca^{2+} levels may protect the plant from NaCl toxicity by reducing displacement of membrane-associated Ca^{2+} and by reducing Na^+ uptake and transport to the shoots. In citrus, Ca was found to be effective at reducing the transport of both Na^+ and Cl^- from the roots to leaves, thereby reducing the foliar injury. Maintaining an adequate supply of Ca^{2+} in the soil solution is an important factor in controlling the severity of specific ion toxicities. This is particularly important for tree and vine crops, which are more prone to Na^+ and Cl^- injury than most annual crops [174].

The magnesium content of the leaves of saline-treated bean plants increased, whereas it decreased in the root. Hodson et al. [175] found potentially toxic concentrations of Mg^+ in salt-marsh soil solution samples and demonstrated that a salt-marsh clone, *Agrostis stolonifera*, was considerably more tolerant to Mg^{2+} than was an inland clone. Magnesium concentration of avocado leaves was decreased with an increase in the exchangeable Na^+ in the soil. In rice, Mg transport to the tops was suppressed by Na compared with Mg uptake [152]. The Mg content in the roots revealed the competition between Mg and Na uptake and transport to the tops [163].

Under salt-stress conditions, the uptake of N by crop plants is generally affected. Reports show inhibitory [134,145,176] and stimulatory [177,178] effects on the plant N uptake under salinity stress. A substantial number of laboratory and greenhouse studies have shown that salinity reduces N accumulation in plants. It has been reported that an increase in Cl^- uptake and accumulation is accompanied by a decrease in shoot nitrate concentration. Examples of these effects are also found in barley, cotton, watermelon (*Citrullus lanatus*), and wheat. In his experiment, Aslam et al. [173] have reported that Cl^- inhibited NO_3^- uptake more than SO_4^{2-} when these anions were present on

an equal osmolarity basis. In contrast to the effect of Cl^- on NO_3^- uptake, reported data indicated that increased NO_3^- in the substrate decreased Cl^- uptake and accumulation. The possible decrease in N uptake by increasing salinity has been partly attributed to a probable substitution of Cl^- for NO_3^- [179]. For example, the N-deficiency symptom increased the Cl level in corn, barley, and some other crops. Sodium chloride salinity significantly decreased the amount of total N in all parts of the wheat plants possibly as a result of the antagonism of nitrate by chloride in the growth medium [180]. Both the chloride salts of Na and K inhibited the nitrate uptake similarly, suggesting that the process was more sensitive to anionic salinity than to cationic salinity [173].

Although, Cl^- salts were primarily responsible for reduced NO_3^- uptake by plants, NO_3^- reduction in plants was not affected by salinity in studies with barley [173]. Salinity also stimulated nitrate reductase activity in peanut plants as well as decreased the nitrate reductase activity in tomato and cucumber (*Cucumis sativus* L.) plants, and reduction in NRA may be due to inhibition of NO_3^- uptake by Cl^- in plant species [180].

The source in which N is applied to salt-treated plants also is important. In an experiment, the NH_4-fed maize and wheat plants were more sensitive to salinity than NO_3-fed plants grown in nutrient solution culture. Supplementation of Ca^{2+} to the growth media improved the growth rate of the plants in the NO_3 treatment but not those treated with NH_4^+. Based on the results of their nutrient solution experiments, Leidi et al. [181] suggested that NO_3^- is a better N source than NH_4^+ for wheat grown in salt-affected soils.

Phosphorus, which has a crucial role in the energy metabolism of cells, is involved in a number of anabolic and catabolic pathways. A recent study indicates that salinity may increase the P requirement of certain plants. Awad et al. [182] found that when NaCl increased in the substrate from 10 to 50 or 100 mM, the P content in the tomato leaf increased from 58 to 70 and 97 mmol kg^{-1} dry weight.

The influence of salinity on P accumulation in crop plants is variable and depends on the plant and experimental conditions. In many cases, salinity decreased the P concentration in plant tissue [183]. It is unlikely that Cl^- and $H_2PO_4^-$ ions are competitive in terms of plant uptake. However, it has also been observed that Cl^- may have a suppressing effect on P uptake in tomato shoots [179].

The presence of Cl^- as well as SO_4^{2-} reduced P uptake in barley and sunflower plants. In other cases, a reduction in plant P concentration by salinity may result from the reduced activity of P in the soil solution due to the high ionic strength of the growth media [182]. Phosphate solubility-availability is reduced in saline soils not only because of ionic-strength effects that reduce the activity of phosphate but also because the P concentration in soil solution is tightly controlled by sorption processes and by the low solubility of Ca-P minerals. It is, therefore, understandable that P concentrations in field-grown agronomic crops decreased as salinity increased in the media. When plants are P deficient, they may be more sensitive to salinity [183].

The concentrations of micronutrients in the soil solutions, with the exception of Cl^-, seem to be low and depend on the physical and chemical characteristics of the soil bodies. The availability of most micronutrients depends on the pH of the soil solution as well as the nature of binding sites on organic and inorganic particle surfaces. In saline soils, the solubility of micronutrients such as Fe, Mn, Zn, and Cl is particularly low and plants grown in these soils often experience deficiencies in these elements [184]. Nevertheless, the micronutrient concentration in plant shoots may increase, decrease, or have no effect depending on the type of plant tissue, salt tolerance of plant species, salinity, micronutrient concentration, environmental conditions, and/or abrupt changes in the permeability of the crop cell membranes.

Both Fe and Mn contents were reported to increase in all parts of the salt-treated peanut plants [158]. The increase in Fe contents was more prominent than that of Mn. Salinity increased the Fe concentration in the shoots of pea and rice and decreased its concentration in the shoots of barley and corn [185]. In other investigations with barley, salinity had no effect on shoot Fe concentrations, but at low Ca, salinity increased root Fe in certain barley (*Hordeum vulgare* L.) species [171].

Salinity increased the manganese concentration in the shoots of barley, rice sugar beet (*Beta*

vulgaris L.), soybean, and tomato plants, but decreased in concentrations in the shoots of barley, squash, pea, and corn [171,185,186] plants. In the study with the sugar beet, the addition of NaCl–$CaCl_2$ increased Mn in the saturated soil extract. Other investigators did not find the effect of salinity on shoot Mn, but did find that increasing the sodicity in soil grown maize had a significant reduction in shoot concentration. Saline solutions rich in divalent cations increase shoot Mn concentration, whereas a saline environment dominated by monovalent cations reduces shoot Mn concentration. Zinc concentration has been found to increase in salt-stressed bean, barley, soybean, squash, and tomato plants but to decrease in corn and mesquite (*Prosopis juliflora* L.) plants [185].

Drought-Induced Stress

Water availability plays a major role in the regulation of plant growth and seed development [187]. Water is generally considered as one of the limiting factors which affect the numerous metabolic physiological and biochemical process affecting crop productivity [188–192]. On the global basis, water stress limits plant growth and yield more than any other single environmental factor [1].

Water stress develops when the water efflux from the plant is greater than the water influx into the plant. Although plant growth rates are generally reduced when the soil water supply is limited, the shoot growth is often more inhibited than the root growth.

Water, which is a combination of oxygen and hydrogen gases, is the Earth's most distinctive constituent and is an essential ingredient of all creatures living on the Earth's surface. Its availability is one of the most limiting environmental factors affecting crop productivity and numerous human applications. It is a well known fact that crop growth is frequently subjected to water stress during the course of its lifetime. However, certain growth stages, such as germination, seedling, and flowering, are the most critical for water-stress damage. Stress imposed during these periods drastically affects crop growth, ultimately leading to a massive loss in yield and quality [193–197]. Water stressing or droughting can be intensified by gradually lengthening the drought period each day or by applying drought over a period of days or weeks.

As a natural hazard, drought is a unique syndrome. It differs from other natural hazards in that it is a creeping phenomenon that is pervasive in nature. The effects of drought accumulate gradually and may persist over a long period of time, making it difficult to determine when a drought has begun or when it has ended. Water deficits are very common in the production of most crops, and numerous studies have indicated that they can have substantial negative impacts on plant growth and development [198–203].

Water-stress conditions of soils have wide-ranging effects on several morphological and biochemical alterations in plants. It causes a decrease in the cytokinin transport from roots to shoots and/or an increase in the amount of leaf abscisic acid. These changes in hormone balance cause changes in the cell wall extensibility, a decline in the concentrations of photosynthetic enzymes, and growth of the biomass. Water stress leads to a reduction in the efficiency of key plant processes, including protein synthesis, photosynthesis, respiration, and nucleic acid synthesis, causes activation or inhibition of the activities of many enzymes, and leads to changes in the ultrastructures of plant tissues.

The potential capacity of plant roots to absorb water and nutrients generally declines in water-stressed plants, presumably because of a decline in the nutrient element demand. There is some evidence that roots are the primary sensors of water deficit in the soil, causing the observed physiological and biochemical perturbations in the stems and the decline in growth to be generally interconnected with changes in plant nutrition, carbon dioxide balance, and water relations.

The internal transport of plant nutrients largely depends on the synthesis, utilization, and translocation of photosynthesis, because the switching over of these processes is regulated by nutrient metabolism. Any perturbation in the system may severely affect both the supply and demand of nutrients for crop plants. Plant nutrient elements and available water are absorbed by plant roots in independent processes, but they are closely related to one another. In soil systems, water relationships affect all of the physiological processes, which are closely associated with the nutrient element

solubility or availability. These processes involve element concentrations in the soil solutions, because of nutrient diffusion and mass flow to the root surface, and then absorption of the elements by the roots, translocation from roots to shoots, and utilization of minerals by the other plant parts.

Nutrient uptake by crop plants is generally decreased under water-stress conditions owing to a substantial decrease in transpiration rates and imparied active transport and membrane permeability [190], and resulting in a reduced root-absorbing power of crop plants. Nutrient uptake from the soil solution is also closely linked to the plant root and soil water status. A decline in the soil moisture content is associated with a decrease in the diffusion rate of nutrients from the soil matrix to the absorbing root surface. The plant water status and an internal water deficit are related to root system development, and during water stress, root activity and mainly root permeability may change substantially to lower levels. The reductions in uptake and transpiration are usually associated with a reduction in the water content of the shoots and stomatal aperture, suggesting that water stress has developed in the leaves. Maximum water uptake occurs in young roots, but continued aging of the roots after cessation of growth would result in a reduction in the root permeability to water and nutrients. Inadequate water availability and nutrient element uptake owing to reduced root permeability causes a disturbance in the root metabolism [204]. The changes in the soil moisture regimen can alter the root morphology and anatomy, the pore size distribution, and the angle of roots penetration, which affect root proliferation.

It is a bit difficult clearly to deliniate the potential effects of water stress on the mineral uptake and accumulation in crop plants. Many workers have reported varied effects of plant species and genotypes on nutrient concentrations, and most studies have reported that mineral uptakes are decreased when the intensity of water stress is decreased.

Under water-stress conditions, the uptake of N decreased in soybean plants [205]. This decline in shoot N uptake can be attributed to the decreased transpiration rate to transport N from roots to shoots. The high N level under water stress has largely been attributed to the proline accumulation in grasses, as evidenced by others [206]. Nitrogen deficiency sensitized cotton plants to water stress, causing the effects of stress to occur at a higher water potential. The common opinion extended by Barnett and Naylor [207] holds that the high N level of crop plants subjected to water stress is primarily due to the fast accumulation of free amino acids that are not converted into proteins. It is also plausible that the slower growth rate of plant crops under moisture stress prevents the dilution effect of nutrient elements. As such, the availability or solubility of N can be reduced significantly by leaching under normal irrigation. Although water and nutrient uptake by plants are the independent phenomena, the transpirational equilibrium under irrigation favors the leaching down of the soluble forms of N from the soil strata. Therefore, it has been clearly emphasized that water acts as a vector for nutrient transport within the system. The main effects of moisture stress on anion transport to the live green component of *Dichanthium annulatum*, a dominant perennial, and a weed, *Polypogon monspeliensis*, are due to retardation of the mass flow of actively absorbed ions arresting their regular delivery to the active sites of uptake.

Tanguilig et al. [205] reported that total shoot N uptake of water-stressed plants decreased in rice plants despite its high root dry matter weight. It has been reported that under water-stress conditions, absorption of both NH_4^+ and NO_3^- decreased, and the decrease was greater for NO_3^- than for NH_4^+. In water-stressed plants, dry matter production and total N/plant decreased, but N concentrations increased and proline synthesis was inhibited. Plant concentrations of N decreased rapidly when osmotic water stresses were initially imposed. This decrease in N was apparent for wheat within 24 h at -1 bar potential and was more pronounced in plants stressed for longer times. The effects of water stress on N concentration was as severe or more severe at -1 bar as at higher water stress. Viets [208] generalized the opinion that moisture stress induces a definite increase in the N level in plants, whereas it induces a decrease in the P level and variable effects on the K level. However, besides the increase in N and the decrease in the P level, a definite reduction in the K level was observed in all grasses.

During the wilting of tomato (*Lycopersicon esculentum* L.) leaves, the uptake of N and P was reduced and both N and P were translocated from the laminae to the petioles and stems. When

plants were rewatered, the active uptake of N and P resumed and both passed preferentially to the laminae rather than remaining in the stems. Nitrogen accumulation in stems under water stress was also noted [209]. Nitrogen was less liable than P, and wilting depressed the uptake of P more than that of N. The uptake of N and P was decreased relatively early in the drying cycle and preceded water-stress effects on dry-matter yield. Dry-matter yield usually decreased faster than total nutrient uptake [204]. In mature maize plants grown with inadequate water, component accumulations of P, N, Mg, K, and Ca were 40, 50, 65, 71, and 91%, respectively, of those found in mature plants grown with adequate water [210].

In contrast to the reduction of mineral element uptake by plants with water stress, nutrient elements in many range and forage plant species increased with water stress [204]. Water stress generally favored the increases of N, K, Ca, Mg, Na, and Cl and the decreases of P and Fe [211]. The relative deficit of N accumulation as a function of increased water stress was less than the deficits for dry-matter yields and the decrease in the accumulation of other nutrients. For several grassland plants, total nutrients generally decreased with increasing water stress [204].

Phosphorus is essential for plant growth, as it is involved in most metabolic processes. Phosphorus is a constituent of nucleic acids, phospholipids, phosphoproteins, dinucleotides, and adenosine triphosphate. Hence, P is required for the storage and transfer of energy, photosynthesis, the regulation of some enzymes, and the transport of carbohydrates. Phosphorus is phloem mobile, and the purple symptoms of P deficiency appear first in old leaves as P is redirected to young leaves.

Water-stress conditions of the growth media has far-reaching effects on the uptake and concentration of phosphorus by crop plants. Gerakis et al. [204] pointed out that of the many studies conducted before about the mid 1950s, 12 of the 21 papers reported that water stress decreased P concentrations in plants, and 9 papers reported that water stress did not affect the P status of plants. Since then studies have reported both decreases [212–214] and increases [204,211] in P and other mineral element concentrations with increased water stress. It is generally accepted that the uptake of phosphorus by crop plants is reduced in dry soil conditions [215–217], although it has been reported [213] that only severe water stress reduced plant phosphorus absorption.

In an experiment [218], phosphate and water were added differently to surface and subsurface layers of reconstituted soil profiles. The layers were subjected to brief periods of drying and to partial or complete remoistening at frequencies ranging from daily to every 2 week. Their findings have revealed that P uptake from surface applications and shoot yield were proportional to the frequency of remoistening of the soil surface to field capacity, and the response to additional subsurface phosphate presence diminshed as the amount of surface water increased. In the treatments where moistening did not achieve field capacity, yield was linearly proportional to the amount of water applied to the surface. These investigators have further described that watering the surface to field capacity twice a week led to a 50% reduction in phosphorus uptake by subterranean clover (*Trifolium subterraneum* L.) compared with daily watering. It also appeared that rapid P uptake occurred only at high moisture contents, and that uptake was proportional to the volume of soil brought close to the field capaicty and the length of time that it remained moist. It seems that plants were able to obtain their entire P requirements from the surface layer only when it was kept continuously mosit. Furthermore, the amount of P absorbed by the plants was directly proportional to the amount of water applied to the soil surface. This research finding is in direct contrast to that of Fawcett and Quirk [213], who finally concluded that the rate of P absorption and utilization by crop plants was not greatly affected by increasing water stress until the plants were subjected to wilting, at which point, absorption decreased markedly. However, the true meaning of this result [213] is uncertain, since groups of wheat plants were grown in small containers of soil (300–600 g) through which phosphate had been intimately mixed. Moisture depletion must have occurred very rapidly in such small containers, and so frequent remoistening would be necessary to avoid wilting. This experimental set-up and design was thus completely artificial, and it did not simulate the usual field situation in which phosphate is banded or layered in position separate from the location of the main soil moisture reserves.

Mouatt and Nes [219] have suggested that an adequate supply of phosphate can be maintained

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only when there is a high level of available water in the growth medium. Of the several plants put under water stresses beyond the available moisture range, three had markedly lower concentrations of $H_2PO_4^-$ than other plants allowed to grow in the available moisture range. The reduction in the relative uptake of $H_2PO_4^-$ by maize seedlings was nearly linear with the soil moisture stress and decreased progressively from 100, 94, 80, and 54 to 35% of the control for water stresses of -0.3, -0.5, -1, -3, and -9 bar, respectively [220]. Regardless of water stress, P uptake by plants increased with increased P levels in the soil. Olsen et al. [221] observed that monovalent phosphate uptake depended on the soil P levels as much as on the magnitude of water stress. There was an increase in the concentration of P in alfalfa (*Medicago sativa* L.) and that of Ca, Mg, and Zn both in alfalfa and soinfoin (*Onobrychis viciifolia* Scop.) with decreasing soil moisture supply [222]. Compared with alfalfa, soinfoin had higher concentrations of P and Mg and lower concentrations of Ca, K, and Cu and a similar Ca/P ratio.

Nuttall [223] reported that increased soil moisture resulted in increased P but decreased S in alfalfa. Gomez-Beltrano [224] did not find any influence of moisture stress on P, N, and K concentrations. The P concentration was found to decrease at low leaf water potentials in pepper (*Capsicum annuum* L.) plants [225], and P deficiency appears to be one of the earliest effects of mild to moderate levels of water stress in soil-grown plants. The effects of water stress on P uptake depended on the magnitude and intensity of the water stress and on the concentration of P ion. Increasing the water stress from -0.4 bar to -5.5 bar had no effect on the accumulation of P or B in root cells or in xylem sap [226]. However, the absorption of $H_2PO_4^-$ was severely inhibited when water stresses were increased to -12 and -15 bar [227]. Nevertheless, translocation of P to the shoots was severely restricted even at a relatively low water stress condition [226]. Water stresses for the inhibition of P translocation in maize was about -5 bar, but for the inhibition of $H_2PO_4^-$ uptake, water stress had to be greater than -15 bar.

In experiments with elements at different concentrations in the uptake medium, the effects of water stress intensity on $H_2PO_4^-$ uptake in maize roots were greater at relatively high P levels (10^{-3} M or higher) than at lower levels [226]. Reports have indicated that at -10 bar, P absorption and translocation to shoots were inhibited with mannitol-induced water stress, but only P translocation was inhibited with PEG-induced water stress [226].

Under water stress, the uptake of K and Ca by maize plant increased [205]. Increased K uptake in maize suggests that, under stress conditions, K is absorbed preferably to N and P. Sinha [228] observed that drought-tolerant wheat varieties can accumulate more K than do the more susceptible varieties, and plants well supplied with K had a higher stomatal resistance, which resulted in a low transpiration rate. Furthermore, Maurya and Gupta [229] observed an increased water potential of wheat plants with increased K fertilization. Kuchenbuch et al. [230] have shown that low levels of soil moisture reduced both the root growth and the rate of K inflow per unit of root length of onion (*Allium cepa* L.) plants. It is, therefore, assumed that the water content of the soil influences the rate of K uptake by its effect on the transport of K from the soil to the root surface. Soil moisture content affects the availability of K in soil [231]. Van der Paauw [232] observed a positive relation between the number of days without rain in the growing season and the K response of grass, potato (*Solanum tuberosum* L.), and wheat. This indicates that soil moisture influences both the diffusion of K in the soil and plant root growth. This in turn is apparently the reason for the increasing rate of K uptake per unit of root surface and the total uptake of K with increasing soil moisture. In addition, variations in K uptake with moisture stress in sugar beet were such that under equally increased with water stresses, K increased in one variety and decreased in the other. The relative amounts of K, Ca, and Mg increased considerably more in barley than in rye when water stresses were imposed. It is suggested that nutrient uptake under water-stress conditions is influenced by the capacity of the roots to absorb nutrients.

An experiment [233] showed that drying of the upper layer of a siliceous soil profile strongly reduced the absorption of Mn by rye grass but that the uptake of Cu and Zn were relatively unaffected. It has been noted that an increased water-stress condition of a growth medium not only depressed the uptake and solubility of nutrient elements but also increased Ca/K and Ca/P ratios

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in herbage. Calcium was depressed only slightly compared with P and K. The functional capacity of the plant root system may be reduced as a result of a shortage of soil moisture content. Furthermore, in a water-stress situation, the older roots surrounded by dry soil apparently lost their ability to function and the nutrients were supplied exclusively by the more active root tips. This led to a low uptake of anions and a greater uptake of bivalent cations compared with monovalent cations. The undesirable Ca/K ratios could develop in plants and cause other complications in the growth of the plants. Ratios of bivalent to monovalent cations were higher for dicotyledonous than for monocotyledonous plants [204]. In an experiment, Khan and Soltanpur [234] noted another anomaly in plants and suggested that high moisture levels could enhance P availability in the plant. This enahnced P availability could have caused the Zn deficiency that they observed in bean (*Phaselous vulgaris* L.) plants.

In general, the uptake of Cl^- was not affected immediately after the removal of roots from a water stress medium, but after 36 hs, the Cl^- uptake was only 2 μM/h compared with 7 μM/h for unstressed plants. In this same study, the plants subjected to water stress by decreasing the amount of the root system in solution transported less water after removal of stress than the unstressed plants. In addition, plants repeatedly subjected to water stress and then allowed to recover had enhanced rates of ion losses by leakage [227]. Greenway et al. [235] favorably suggested that the depressed uptake and accumulation of ions in water-stressed plants was caused by excessive leakage of ions from the cell membranes.

Numerous studies on water stresses have revealed that water stress, in most cases, restricted the uptake of nutrient elements by crop plants. Uptake of the ions from the growth media was closely related to plant water content, transpiration, and/or water flow. The length of time the plant roots could be exposed without permanent damage depended on the method used to impose water stress, the level of stress, and the plant species. Apparently the active transport systems of the crop plants were imparied or destroyed at high water stresses, and thereafter the various ions present in the growth medium responded differently.

CONCLUSIONS

The major types of stresses which potentially affect plant growth and nutrient uptake on a global basis are flooding, salinity, and water stress (drought). In addition, the other stresses which also potentially and perhaps simultaneously affect the normal growth of plants and ultimately the nutrient uptake scenario are, for example, seedbed preparation, plant population, planting time, types of soil properties, soil N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, B, Mo, Co, Si, Cd, Cr, Se, and Al, nutrient imbalances and their interactions, soil organisms, diseases, weeds, toxic metals, air pollution, growth regulators, wind, hailstorms, water-table, and allelopathy.

Flooding is the saturation of the soil rootzone with water. The soil pore space, normally filled with air, is filled with water. Flooding (waterlogging) occurs when inundation persists as a result of inadequate surface and/or subsurface drainage and the aeration status of the soil system decreases below critical limits. Aerobic systems are oxygen sufficient, whereas anaerobic or anoxic systems are oxygen depleted. When soil is saturated, the concentration of O_2 available to the roots decreases rapidly, because it is consumed by roots and soil microorganisms. Anaerobiosis affects the plant metabolism and plant nutrient availability as a result of low oxygen concentrations in the rooting medium. Salinity is a condition of excess salts in the soil, which affects crop productivity by increasing the osmotic pressure of the soil solution interfering with the normal nutrient uptake and inducing ionic toxicity and associated nutrient imbalances. Salinity poses a severe threat for cultivation of crops. Water stress (drought) is also an important limitation to crop production. Reduction in photosynthetic activity and increases in leaf senescence are symptomatic of water stress and adversely affect crop growth. Other effects of water stress include a reduction in nutrient uptake, reduced cell growth and enlargement, leaf expansion, assimilate translocation and transpiration. Water stress also reduces the net CO_2 assimilation.

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Photosynthesis Under Stress: Stress Signals and Adaptive Response of Chloroplasts

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INTRODUCTION

The term *plant stress* is difficult to define because of several factors. The major factor, however, is the complex interaction between plants and the environment. If a stress is defined in terms of a plant's response in field conditions, then the stress cannot precisely be described, because the response of any individual stress factor is not easy to identify. In nature, it is almost impossible to find a condition where a single stress operates without the interference of other stress factors. In certain conditions, many environmental factors individually may not exhibit any stress effect but in different combinations can create a stressful environment for the plants. However, most of the studies in this area are conducted in defined conditions; the stress, therefore, is defined in a very general way as a biotic or abiotic factor that prevents plants from normal functioning and thus results in a reduction in their growth and reproduction [1]. Many environmental factors at their extremes have been recognized as stress factors. The threshhold level of different environmental factors exhibiting the stress response depends on many factors, including prehistoric growth and the structure of plants.

Since plants cannot flee from the stressful environment, they have developed various strategies during evolution to adapt to the changing environmental conditions and thus counter the stress effects. Plant adaptation involves three major events; namely, stress perception, transduction of stress signals, and the final response. In most stressful environments, plants can sense, correctly recognize stress signals, and use the signals as cues to bring specific changes at various levels, like alterations in morphological structures, physiological behavior, modification in biochemical pathways, and expression of stress-specific genes as a response for the adaptations.

In this chapter, the photosynthetic response of green plants to the environmental stress factors, including extremes of light, water, and temperature, are described. Some other factors, like heavy metals and nutrition stress, also are discussed in regard to their effects on photosynthesis. Emphasis

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has been given to various pathways of transduction of stress signals and adaptive responses of chloroplasts experiencing these stresses.

STRESS SIGNALS, PERCEPTION, AND SIGNAL-RESPONSE COUPLING IN PLANTS

The mechanisms of recognition and perception of a stress signal followed by the conversion of the signal to a biochemical response in plants largely remain unclear. The perception of the stress signals and their initial interaction with the cells could be recognized by various physical perturbations that may include changes in cell volume, structure of biomembranes, ionic balance, total content, and composition of cellular solutes or alteration in protein-ligand interactions.

The bilayer lipid membrane, a boundary between the cell and its environment, is considered to be one of the major sites for perception of the stress signals [2,3]. In addition to the plasma membrane, the membranes of the nucleus, mitochondria, and chloroplast are well-organized structures that constitute not only lipids and proteins but also ions and various kinds of receptors that recognize both intrinsic and environmental signals. The stress-induced changes in the lipid structures and/or perturbations in lipoprotein complexes may subsequently be transmitted to various types of cellular responses through appropriate biochemical changes for developing adaptive mechanisms to counter the stress effect. The perception of stress signal that leads to changes in membrane fluidity and triggers a series of events, and the expression of genes for stress adaptation have recently been critically discussed by Murata and Loss [3].

The coupling of stress signals to the plant response appears to be very complex [3–5]. Different stress signals may have a common response. For example, the synthesis of many scavenging enzymes against the oxy free radicals in plants has been shown as a common response to most of environmental stresses. These stresses, however, may have different receptors and may produce the radicals in different biochemical routes. With some other environmental stress factors, the perception and initial transduction of stress signals may be similar, but the transduction of the signals at a later stage may diverge at the biochemical level, leading to individual stress-specific responses. This proposition is supported by the observation of expression of stress-specific genes as controlled by different regions of stress-specific promoters [6]. On the other hand, a single stress may induce different signal transduction pathways for stress adaptation. Abscisic acid (ABA) production and its role for expression of water stress–related genes are recognized as being a major signal transduction pathway, but there may be other signal transduction routes induced by the same stress independent of the hormone route [4].

Thus it appears that the stress signal transduction is complex in nature and is, therefore difficult to study. Second, most of the studies in this area are made at cellular levels. Such studies may be considered as models for predicting how the stress signals are recognized and transmitted through various cellular events and finally to the response in a whole-plant system. The signal transduction route, therefore, becomes much more complicated when discussed in the background of an integrated system with various tissue and cell types.

CHLOROPLAST AS THE MAJOR CELLULAR TARGET FOR PERCEPTION OF STRESS SIGNALS

Chloroplast, the Photosynthetic Organelle

Excellent reviews are available on the structure and function of chloroplasts [7–9]. The photosynthetic organelle consists of two major components (a) a lamellar network, collectively referred to as thylakoids, and (b) a stroma matrix with soluble enzymes of the Calvin cycle. The thylakoid membranes vary in their organization from simple structure in bacteria to a very complex organization in the chloroplasts of higher plants. The so-called light reactions comprizing the primary photo-

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chemical reactions result in the formation of NADPH and ATP and liberation of O_2 from water. Thylakoid membranes, as classified to grana and stroma lamellae, consist of photosynthetic pigments like Chla, Chlb, carotenes, and xanthophylls in higher plants, where as different types of phycobilins constitute major light-harvesting pigments in cyanobacteria. The pigments associated with specific membrane-bound proteins form pigment assemblies for optimization of light energy absorption. The light reactions in the thylakoids are driven primarily by two photosystems which are coupled by an intersystem electron transport chain. The products of light reactions are subsequently utilized in the so-called dark reactions associated with the Calvin cycle for formation of sugar. The question that has been addressed in this section is how the chloroplast with these structural and functional features acts as the major sensor of environmental stress in addition to its major task of making organic food materials through the process of photosynthesis.

Why is the Chloroplast a Sensor of Stress?

Most of the environmental stresses when experienced by green plants bring about oxidative damage of cell structures and consequently a loss in the cellular activities. The transfer of electrons to O_2 and subsequent redox reactions in plant cells may generate various toxic O_2 species. In addition to the production of these highly toxic species, the redox reactions may lead to the formation of other kinds of oxidants with a high positive redox potential that can oxidize most of the essential cellular molecules. Under normal nonstressed conditions, the cellular environment tends to maintain a redox homeostasis which, however, is disrupted by various intrinsic and extrinsic factors. A shift of the redox steady state induced by various stresses creates an oxidative environment.

Chloroplasts in plant cells not only possess pigments which can absorb light and drive redox reactions of thylakoids, but the organelles also are the sites in the cell where O_2 is liberated from water. The availability of O_2 in the vicinity of the electron transport chain may lead to the formation of oxy free radicals in the cellular environment. Chloroplasts are also capable of producing strong oxidants associated with photosystemII (PSII) of thylakoids. Although these oxidants with high oxidizing potential are responsible for the splitting of H_2O molecules, they can oxidize pigments, proteins, and lipids of the thylakoid membranes as well. These special characteristics in fact make the photosynthetic organelle a major stress sensor in green plants.

The other important factor that makes the chloroplast sensitive to stress is the differential response of primary events associated with charge separation and enzyme-mediated electron transport systems of thylakoids. The primary photochemistry involved in the separation of charges within photosystem reaction centers is independent of temperature. The events like the transfer of energy and the subsequent oxidation of reaction centers occur in the time scale of the femtosecond to microsecond range. On the other hand, temperature-dependent redox reactions of the electron transport chain or fixation of CO_2 in the Calvin cycle mediated by enzymes are relatively slow processes that occur in the time range of a milisecond to minutes. A change in environmental factors, including a change in temperature, is likely to affect the electron transport efficiency and CO_2 fixation, the sink for energy utilization, without disturbing the light absorption and the initial events associated with charge separation at the reaction centers. This in turn may create a kind of excitation pressure on the photosystems of thylakoids. The excess quanta become harmful to the organelle in many ways, and they are discussed later in this chapter.

There appear to be two major events that regulate the induction of stress-signaling systems in chloroplasts. One event is the building up of excitation pressure on thylakoids because of high light stress, and the other event is the nonutilization of products of the light reaction in the Calvin cycle during CO_2 fixation. This situation is induced by stress factors other than high light stress and leads ultimately to generation of a redox back pressure on PSII of thylakoids. Both these events, however, are coupled under high light intensity and during operation of other environmental stress factors in the presence of light even at moderate intensity. The high light stress syndrome, therefore, could also be exhibited by the plants experiencing other abiotic stress conditions in the field where damage to any part of the electron transport chain of the thylakoid membrane or loss in the efficiency

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of the Calvin cycle induced by these stresses may result in an imbalance between the amount of light absorbed and its utilization, leading to building up of the excitation pressure for the induction of photoinhibitory damage. The threshhold of light intensity for induction of such damage depends on the interaction of other stress factors operating in different combinations in the field.

Stress-Induced Alterations in the Structure and Function of the Photosynthetic Apparatus

The modifications of the chloroplast brought about by various environmental stresses are manifested at the levels of pigment composition, structural organizations of the lamellar network, primary photochemistry, and the CO_2 fixation efficiency of the organelle [10–12]. The major stress factors that are monitored either in the laboratory or in the field to examine their effects on the metabolism of chloroplasts include light stress [13–15], drought [15–19], temperature extremes [15,20–25], nutritional stress [26–29], heavy metals [30–32], ultraviolet light [33–39], ozone [40–43], and elevated CO_2 [44–46]. A quantitative loss of photosynthetic pigments, differential response of Chla and Chlb, and remarkable changes in carotenoid composition, particularly an alteration in the composition of xanthophyll cycle components induced by various environmental stress factors, have been widely reported [10–12,15]. These stresses are also known to result in structural modifications in general and electron transport complexes in particular [11,12,15]. The changes in pigments and membrane structures of thylakoids are accompanied by alterations in the rates of primary photochemical reactions associated with PSI and PSII and in the activity of the enzymes of the Calvin cycle [15]. A vast literature, however, is available on the susceptibility of PSII of the thylakoid membrane that has been reported to be a major target of stress [15,47]. This photosystem is known to be associated with various adaptational mechanisms and therefore has been well examined as a key component during the transduction of stress signals for the acclimation of the photosynthetic organelle.

PHOTOSYNTHETIC ADAPTATIONS

Short-Term Stress Adaptation

Although it is difficult clearly to distinguish between short- and long-term stress adaptations, a quick adaptational response of plants to stress is normally referred to as a short-term adaptation. This kind of adaptation may not necessarily need any specific stress-sensing receptors or de novo synthesis of new proteins. The signal transduction may involve a change in the concentration of certain metabolites, phosphorylation-dephosphorylation of proteins changes in protein conformation and dislocation, alteration in membrane-bound complexes, membrane fluidity, and membrane topology in general. Most of these changes are physicochemical in the sense that the changes may ultimately lead to reallocation of matter and energy which favor the chloroplasts to adapt efficiently to the stress. During short-term adaptation, plants without major changes in the synthesis of new proteins or other metabolites make a coordinated interaction between light-harvesting antenna and energy conversion as well as electron transport activities of thylakoid complexes and operation of the Calvin cycle so as to optimize the photosynthetic rate under the changing environment. These kind of short-term adjustments are mostly reversible. Movement and changes in size of the chloroplasts, quenching of excess energy by interconversion of the xanthophyll cycle components, and redistribution of excitons between the PSI and PSII are a few examples of short-term adaptation.

Long-Term Stress Adaptation

During long-term adaptation of chloroplasts, the stress signals lead to the synthesis of proteins, lipids, and pigments, which is sometimes accompanied by the degradation of already existing proteins and other metabolites. Excess or limited production of light-harvesting Chla/b protein (LHCII), DI pro-

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teins of the PSII core leading to stoichiometric changes of photosystems of the thylakoids, and alterations in the quantity of Rubisco transcripts and its proteins are the stress-induced responses, considered to be in the category of long-term adaptation. The synthesis of stress-tolerant proteins like high light–induced, early light–inducible proteins (ELIPs), heat stress–induced heat-shock proteins (HSPs), and drought-induced dehydrin also is a typical example of the long-term adaptation of chloroplasts to environmental stress.

STRESS SIGNALS AND PSII PROTECTION

PSII Structure and Function

PhotosystemII of thylakoid has been recognized as the major target of environmental stress. A background description of the structure and function of PSII is necessary before various protective mechanisms adapted by the photosystem against different stresses are discussed.

The thylakoid of the photosynthetic organelle consists of several complexes, including PSI, PSII, the Cytbf complex, and ATPase. The primary photochemical events, including electron transport in the membrane, are mediated by these complexes. Since PSII initiates the first step of the photoelectron transport chain and is associated with the water-splitting complex, the photosystem has been extensively examined in different laboratories [13,47–50]. It is a multisubunit complex consisting of more than 25 different proteins. On the basis of their location on the membrane, some of the proteins are considered to be intrinsic and some others as extrinsic and are encoded by both plastid and nuclear genomes. The intrinsic proteins like D1 and D2 in addition to nonprotein components like the Chla dimer (P680), two additional Chla monomers, two pheophytins (pheo), quinone A (Q_A), and quinone B (Q_B), with a nonheme iron, constitute the core complex of PSII. The core complex is coupled to a cluster of 4 Mn and light-harvesting antennae like CP47 and CP43 [51]. Several extrinsic proteins, including a 33-kD Mn-stabilizing protein, are associated with the photosystem at the lumen side. The P680 acts as the primary electron donor and pheo as the primary electron acceptor. The photochemistry of PSII is initiated by charge separation that results in the formation of a $P680^+$ $Pheo^-$ primary radical pair. The Subsequent flow of electron from $Pheo^-$ to Q_A on D2 follows one electron gate pathway. Normally reduced Q_A is not protonated. On the other hand, Q_B on D1 has two electron gates, and after receiving the electrons gets protonated. The protonated Q_B is delocalized, moves to the lipid matrix, and transfers electrons to the Cytbf complex through a shuttling mechanism.

The donor-side electron transport component of PSII primarily consists of the oxygen-evolving complex (OEC) with the Mn cluster. The role of Mn in accumulating positive charges for evolution of O_2 is known [52,53]. The cluster is believed to be closely associated with D1 and D2 proteins. A number of amino acids in these proteins are suggested to be possible ligands for the metal binding. The ion like Ca^{2+} is reported to modulate the structure of OEC responsible for the splitting of H_2O molecules [54].

Chla/b binding proteins, namely LHCII, are known to be associated with PSII. Functionly active LHCII is formed when monomeric units are assembled at PSII in trimeric forms. Information is available on electron crystallographic structures [55] and the distribution of pigments in individual protein subunits of these complexes [56]. The arrangement of the structural components of PSII are shown in Figure 1.

Downregulation of PSII Photochemistry: An Adaptive Mechanism Against Stress

Downregulation of PSII is known as a major defense strategy of chloroplasts experiencing environmental stress. Two major signal transduction pathways induced by high light stress resulting in downregulation of photosynthesis are described below.

The Calvin cycle becomes a relatively poor sink when there is rapid electron transport activi-

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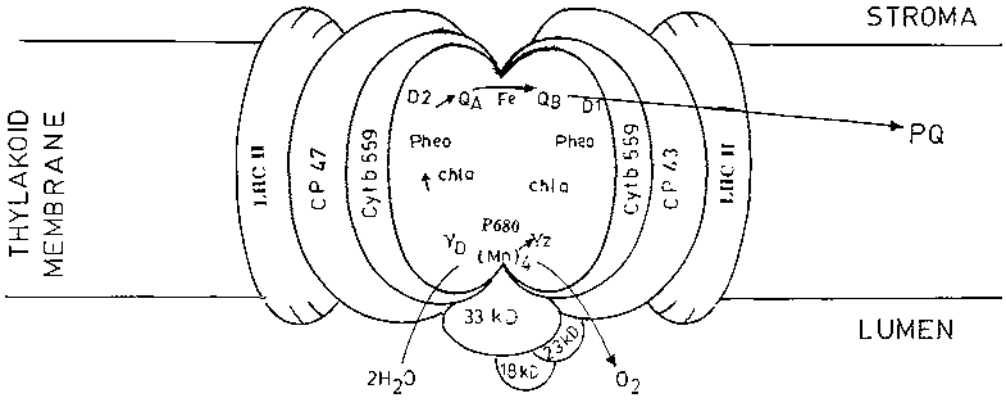

FIGURE 1 A scheme showing major polypeptides, donor and acceptor side components of PSII of thylakoids. The details for abbreviations and descriptions are given in the text.

ties induced by a high light condition. The mismatching between light and dark reactions generates a redox back pressure resulting in PSII inhibition. The inhibition is mostly mediated by overreduction of plastoquinones, particularly the double reduction of Q_A and the development of an acid pH of lumen. Both Q_A production and low lumen pH lead to downregulation of PSII through different mechanisms.

The overreduction of Q_A and its subsequent effects on D1 degradation are critically discussed below. The lowering of the lumen pH downregulates photosynthesis, primarily in two different signaling pathways, one by inactivating the OEC through the release of Ca^{2+} and the other through dissipation of light energy by the operation of xanthophyll cycle in light-harvesting antenna systems.

Inactivation of OEC and Reoxidation of Reduced Q_A

The Lowering of the lumen pH may lead to the release of Ca^{2+} from the OEC associated with PSII in the exchange of H^+ [57]. The release of the cations may cause an alteration in the specific geometry of the Mn cluster resulting in the inactivation of the water-splitting system, and consequently an accumulation and increased lifetime of $P680^+$ and YZ^+ [58]. Second, because of its potential for electrostatic interaction, released Ca^{2+} may bring certain changes in the core complex of PSII that possibly favor a charge recombination between $P680^+$ and Q_A^- and thus a decrease in back pressures. This recombination may lead to the formation of a triplet reaction center, which may degrade D1 protein, and thus have a control over turnover of the protein for photosynthetic adaptation. If the recombination follows a triplet-free route, the reoxidation of Q_A^- itself will decrease the back-pressure inhibition of PSII [58]. Both the signal transduction pathways, however, may be considered as the pathways for chloroplast adaptation against environmental stress.

Operation of the Xanthophyll Cycle

Induction of an acid lumen pH is the basic requirement for the operation of the xanthophyll cycle. Through dissipation of energy, the xanthophyll cycle protects chloroplasts against high-light stress. Since operation of the cycle dissipates excess light energy, it is therefore reported to minimize the requirement of D1 turnover, another adaptive response of PSII of chloroplasts against high-light stress [59]. The cycle basically involves the interconversion of violaxanthin to zeaxanthin through the formation of an intermediate (i.e., antheraxanthin). The enzymes involved for the interconversion are deepoxidase and epoxidase. The deepoxidase enzyme facing the lumen side is responsible for converting violaxanthin to zeaxanthin at low lumen pH induced by high light. On the other hand, the activity of epoxidase facing the stroma enhances the level of violaxanthin from zeaxanthin [15].

Although details of the characteristics of the enzymes are still not known, the violaxanthin deepoxidase has recently been purified as a 43-kDa protein. The gene for the enzyme has been cloned and sequenced [60]. The other enzyme, zeaxanthin epoxidase, has not been properly characterized yet. The pigments of the xanthophyll cycle are distributed throughout the thylakoid membrane mostly on small peripheral light-harvesting chlorophyll protein complexes [60].

Although considered primarily as being a response to high-light stress, the operation of the xanthophyll cycle can also be induced in other environmental stress conditions in the presence of varying light intensities. For example, a decrease in the chlorophyll content and the photosynthetic efficiency with a simultaneous increase in the dissipation of energy through the operation of the cycle have recently been reported by Verhoeven et al. [29] in higher plants under a limiting nitrogen supply. On the other hand, Biswal et al. [26,27] have observed a nitrogen deficiency–induced enhancement in the formation of zeaxanthin in a cyanobacterial system. The other stress factors like temperature extremes and drought may also result in a situation where absorbed light remains in excess and acts as a stress factor [14]. An enhancement in the level of zeaxanthin-antheraxanthin of the xanthophyll cycle may eventually be the response to these stress conditions. An increase in the level of zeaxanthin under a dehydration condition in *Chlorella* sp. to dissipate excess unutilized light energy as observed by Chen and Lai [61] supports this proposition. Similarly, a remarkable increase in the total pool of the xanthophyll cycle components and a relative enhancement in the level of zeaxanthin-antheraxanthin are found to be induced by low temperature even at moderate light conditions [62,63]. The operation of the xanthophyll cycle in a wide range of temperatures has been critically reviewed recently by Gilmore [64].

The signaling system for the induction of the operation of the xanthophyll cycle during high light intensity, low temperature, and drought conditions at moderate light appears to be the same with slight a difference. High light–induced rapid electron transport is known to induce an acid lumen pH, which is a prerequisite for the activation of deepoxidase for conversion of violaxanthin to zeaxanthin. On the other hand, a reduction in the primary photochemical reactions of thylakoids under drought and low temperature fails to develop low lumen pH by the same mechanism. These stress factors are likely to inactivate the function of the Calvin cycle and consequently a limited utilization of ATP molecules. The accumulation of unutilized ATP may prevent proper functioning of ATPase for H^+ transport. Hence, light-driven H^+ movement to the lumen becomes relatively more than its movement supported by ATPase from the lumen to the stroma and causing a low pH in the former. Thus, basically the mechanism of the signal transduction for dissipation of unutilized harmful quanta under high light and other abiotic stress conditions remains the same.

The exact nature of the signal transduction pathway with zeaxanthin as a major player during light stress is not clear. Different possible mechanisms are proposed to explain its role in the dissipation of light energy and the xanthophyll is extensively reported to be the effective quencher of excited singlet chlorophyll. The other major view on the mechanism is that the structural changes of light-harvesting systems induced by zeaxanthin result in the dissipation process. The light-induced lowering of the lumen pH may bring about specific structural changes of light harvesting Chl protein complexes through interconversion of violaxanthin to zeaxanthin. It is likely that a change in the structure may favor the aggregation of LHC and consequent dissipation of excess energy. It is also possible that the change in the structure of LHC could lead to the formation of Chl-carotenoid aggregates or specific, the aggregation of xanthophylls themselves. These pigment aggregates may directly serve as energy quenchers. However, the precise nature of the formation of these pigment aggregates is not known. It is possible that these aggregates are formed from the interaction between LHC trimers. The possibility of the protonation of LHC protein, and consequently aggregation also can not be ruled out [58]. Other possible explanation of energy quenching is the acid pH–induced protonation of amino acid residues of the LHC protein favoring not only the association of zeaxanthin to Chl but also the transfer of energy from singlet Chl to zeaxanthin [58]. The role of zeaxanthin in aggregating LHC with subsequent quenching of excess energy has been worked out in detail recently by Ruban et al. [65]. These investigators have clearly shown zeaxanthin-induced oligomerization of the major chla/b light-harvesting protein of PSII with a concomitant reduction in the

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yield of Chla fluorescence. Violaxanthin inhibits the aggregation, and consequently there is a rise in the fluorescence yield. These data are indicative of the fact that the excess unutilized light that behaves as a stress signal is transduced through a kind of physical aggregation of light-harvesting units.

On the other hand, the possible role of zeaxanthin in quenching excess energy in different routes has been recently discussed by Eskling et al. [60]. They have proposed that the xanthophyll cycle protects membrane lipids against oxidation stress. The cycle also may modulate the membrane fluidity and blue light response. ABA-induced modification of the stress response through the regulation of the synthesis of the phytohormone by the xanthophyll cycle also has been proposed.

It appears that the signal transduction for the dissipation of excess light energy through the zeaxanthin route still remains unclear and thus needs further investigations.

D1 Turnover

The rapid turnover of D1 protein under high-light stress is an excellent adaptive mechanism that permits thylakoids to avoid complete disassembly of PSII and other subsequent damages. The degradation of D1 is therefore a prerequisite for protecting other components of PSII against photodamage. In addition to high light, light-dependent D1 turnover has been reported when plants experience water stress [66] and nutritional stress [67]. The details of D1 turnover, particularly the possible mechanisms of its degradation, have been critically described in a recent review by Andersson and Barber [13]. Both synthesis and degradation of the protein are dependent on light during the repair cycle of PSII, with the damaged D1 being replaced by its newly synthesized copy followed by reassembly of fully functional PSII on the thylakoid surface.

Although reports are available on the stress-induced degradation of both the D1 and D2 proteins of the PSII core complex [13], the degradation of D2 protein appears to be relatively slow compared with rapid degradation of the D1 protein under high-light stress. It is, however, believed that the D2 degradation is a secondary event followed by the degradation and removal of D1 from the core. The degradation of D2 may not contribute to the PSII repair cycle. The precise nature of the signal transduction associated with the D1 degradation induced by various environmental stresses still remains unclear. To illucidate the mechanism, the degradation of the protein has been investigated in different experimental conditions [13]. It is likely that the protein undergoes a kind of conformational alteration during transmission of the stress signal, and its altered conformation possibly makes it prone to proteolytic degradation. The nature of protease and its action on the degradation of the reaction center core proteins, however, are not yet known even though some information is available on the cleaved fragments of D1 proteins of its N- and C-terminal origins [13,68]. The stress signal transduction leading ultimately to D1 degradation is normally considered as an adaptive response only when the damaged D1 is immediately replaced by a new copy of the protein, otherwise the PSII disassembly becomes irreversible. It appears there is an effective and perfect signal coordinating system that integrates the degradation and synthesis of this crucial protein. A tight coupling between its degradation and synthesis in vivo has been proposed as one of the possible mechanisms operating in higher plants [13]. In fact, the process of D1 degradation becomes slow unless a newly synthesized DNA is available for immediate replacement of the damaged one [13].

The slow degradation of the D1 protein is explained in terms of its phosphorylation [69]. Under certain conditions, this could again be the strategy of the photosynthetic orgenelle to have a compatibility between the rate of its degradation and its synthesis [70]. Recently, Ebbert and Godde [71] have done extensive experimentation on D1 phosphorylation and PSII stability. They have suggested that there are two pools of PSII centers under a high-light condition. The pool of PSII with relative stability is located in the grana lamellae, and these PSII centers are dimers existing in phosphorylated forms. On the other hand, the other pool consisting of monomers of PSII centers is located in the stroma lamellae and are involved in the rapid turnover of the D1 protein. This is

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the region where D1 is degraded and a new copy is inserted in place of the damaged one. The stability of dimeric and monomeric forms of PSII with reference to D1 degradation during the repair cycle of the photosystem has recently been described by Barber et al. [49].

STRESS-INDUCED SIGNAL TRANSDUCTION THROUGH THE CHANGES IN THE ELECTRON TRANSPORT SYSTEMS ASSOCIATED WITH PSII OF THYLAKOIDS

As discussed, one of the adaptive mechanisms of plants to environmental stress, particularly to high-light stress, is the rapid turnover of the D1 protein. This section briefly deals with how D1 degradation becomes a stress target by two different signal transduction pathways; namely, through the acceptor- and donor-side electron transport systems associated with the PSII reaction center which is believed to be the major victim of the stress. The degradation of the reaction center proteins is preceded by the acceptor- and/or donor-side inhibitions. The details of the components of the donor and acceptor sides are shown in Figure 1.

Signal Transduction Through the Donor-Side Route

The donor side of PSII with the OEC constitutes mainly a Mn cluster and two redox active tyrosine residues of D1 (Yz) and D2(YD) proteins. The Yz couples the Mn cluster to P680, the reaction center of PSII. When light is absorbed, coordinated action of these components at the donor side results in oxidation of water.

Light absorbed by P680 leads to separation of the charge with stable charge pairs like $Pheo^-$ and $P680^+$. The latter possibly extracts an electron from Yz resulting in the formation of Yz^+. Both $P680^+$ and Yz^+ are oxidants with a high oxidizing potential. Since OEC has a very delicate structure and is highly susceptible even to mild stress, which could initially trigger its damage and may result in an inability of the complex effectively to donate electrons to Yz^+ and $P680^+$. These oxidants consequently become long lived, and with a strong oxidizing capacity, they can oxidize pigments, lipids, and amino acids of proteins in the vicinity of PSII. Stress-induced destabilization of the OEC, inhibition at the donor side, and the possible locations of the inhibition have been critically discussed elsewhere [13,47]. The inactivation of the Mn cluster and the Yz may be the major stress-sensing signaling system operating on this side [13,47]. The events associated with the donor-side route may bring alteration in the D1 structure and its subsequent degradation. Jegerschold and Styring [72] have recently shown the damage of D1 protein by strongly oxidizing $P680^+$. They have proposed that with an inactivated Mn cluster, but with effective functioning of the acceptor side, $P680^+$ is the real oxidant that causes damage to the protein. The possible transduction of stress signals leading ultimately to the degradation of the D1 protein through the donor-side route of PSII is depicted in Figure 2.

Signal Transduction Through the Acceptor-Side Route

The transduction of a stress signal through changes in the electron flow of the acceptor side of PSII has been well investigated recently [13,47,73]. The signal transduction that results in the D1 structural change with subsequent proteolytic degradation could be initiated either by a low- or high-light condition in the presence or the absence of other environmental stress factors. The most important component of the pathway is the charge recombination process between Q_A^-/Q_B^- and the oxidized center of PSII. The charge recombination brings about the formation of $^3P680$ and the subsequent production of highly toxic $^1O_2$. Since the half-life of $^1O_2$ is short, its primary target therefore is PSII, particularly its D1 protein, which is known to degrade under this condition [74]. Different mechanisms are proposed to explain charge recombination processes in limiting excess-light stress

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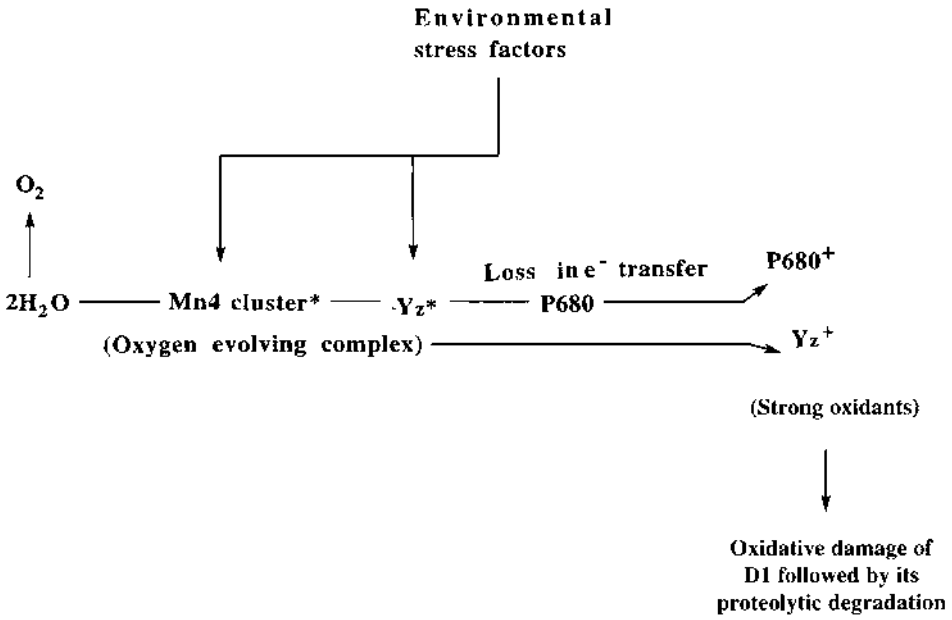

FIGURE 2 The possible mechanism of transmission of stress signals through the donor side of PSII. The stress factors are shown to affect the oxygen-evolving complex and thus reduce the flow of electrons from H_2O to P680, and there is a consequent accumulation of relatively long-lived Y_Z^+ and $P680^+$. These oxidants with high oxidizing potential can oxidize the D1 protein followed by its proteolytic degradation. * Indicates the possible targets of stress.

conditions resulting in the formation of $^1O_2$ via $^3P680$. In extreme low-light intensity, the rates of excitation may lead to a situation where Q_A^- and Q_B^- become relatively long lived. This makes the charge recombination between Q_A^-/Q_B^- of the acceptor side of PSII and the oxidized reaction centers at the donor side that favors formation of $^3P680$ and the subsequent formation of $^1O_2$ (Fig. 3). In extremely low irradiation, the charge recombination between Q_B^- and the oxidized center of PSII is possible before the double reduction of Q_B and its subsequent protonation to plastoquinol. Since this route does not operate in the anaerobic condition, it is $^1O_2$, not the oxidized species at the donor side, that is responsible for the D1 degradation [74]. In low irradiation conditions the possibility of the activation of any protective mechanism, including operation of the xanthophyll cycle or phosphorylation-dephosphorylation of PSII proteins normally induced by high-light conditions is less, the efficiency of low irradiation may therefore be higher than high-light stress for photodamage of PSII [74]. In high-light conditions, the basic mechanism of the signaling system for PSII damage, however, appears to remain the same; that is, through the charge recombination between Q_A^- and the oxidized centers of PSII. The signal transduction through the acceptor-side route resulting in D1 degradation is shown in Figure 3.

Whether it is the acceptor- or the donor-side route, the stress-induced inhibition results in the conformational alteration that signals the proteolytic degradation of the D1 protein [13], although the degradation pattern of the protein by the donor-side and acceptor-side inhibition may be different [68]. The acceptor-side or the donor-side signal transduction path may be induced by other stress factors in the presence of light. For example, drought, extremes of temperature, and other abiotic factors are known to affect CO_2 fixation in the Calvin cycle and thereby modulate electron transport efficiency at the acceptor side leading to excitation pressure at PSII [47] where photoinhibition takes over the rest of the damage, including D1 degradation (see Fig. 3).

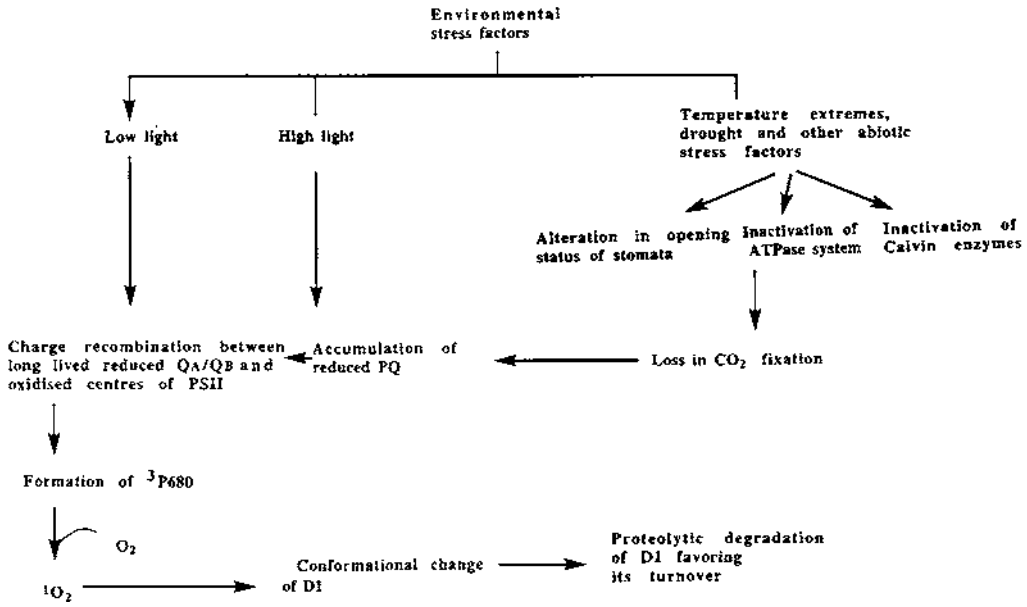

FIGURE 3 The possible stress-signaling system operating through the acceptor side of PSII. Various stress factors may cause closing of stomata, inactivation of enzymes for ATP synthesis, and CO_2 fixation in the Calvin cycle which as a consequence bring about saturation of the reduced PQ pool. The reduced pool leads to recombination of charges between Q_A^-/Q_B^- and oxidized centers of PSII and forms $^1O_2$ which finally degrades the D1, possibly through a protease. The figure also shows the production of $^1O_2$ through charge recombination processes that occur both in low- and high-light conditions.

MOLECULAR BIOLOGY OF STRESS AND CHLOROPLAST ADAPTATION: FROM STRESS PERCEPTION TO GENE EXPRESSION

In most of the stress adaptations, biochemical and physiological responses find their origin from the expression-repression of stress-related genes. The characteristics of such modifications are, however, determined by the interaction of genes with the stress within the genome limit of the plants. However, the question regarding the mechanism of the cellular perception of stress signals, and the subsequent induction of the signal transduction pathway leading to gene regulation has not been clearly answered [3,4,15].

There are three basic categories of stress-induced gene regulations. One of them is related to either overexpression or underexpression of certain genes, and the gene products may not necessarily be stress-specific proteins. The stress-induced changes may require quantitative readjustment of the existing chloroplast components, and depending on the nature of adjustment, underproduction or overproduction of structural proteins-enzymes becomes necessary as an adaptive response. The regulations for such readjustments are therefore considered to be nonspecific to stress. The genes expressed in response to stress in the second category are stress specific, and the gene products may not necessarily be related to the normal cellular activity. The third category of gene regulation involves the expression of genes for the synthesis of scavenging enzymes against oxy free radicals induced by almost all environmental stresses.

Stress-Sensing Signals and Regulation of Nonspecific Genes: Quantitative Readjustment of Chloroplast Components

An extensive literature is available on stress-induced overexpression or underexpression of genes for quantitative alterations of various components of the thylakoids and stroma of chloroplasts [15,75]. The differential regulation of cab gene expression for the synthesis of LHCII at varying light intensities by a redox signaling system is a well-studied example of this category [76]. The cab genes are nuclear genes, and the experiments on the run-on transcription and stability of mRNA clearly suggest that the light intensity, low or high, may act at the level of transcription through a signaling system generated by the redox status of the plastoquinone pool of PSII [77]. It is important to note that this redox-sensing signaling system is not only induced by high light alone but also by other stress factors which could reduce the capacity of the chloroplasts effectively to utilize light energy absorbed by the photosynthetic pigments. In addition to light, the extremes of temperature and water stress may result in a situation where the amount of light absorbed becomes more than its utilization in CO_2 fixation in the Calvin cycle [47]. This may lead to a redox pressure on PSII, a high-light syndrome, and thus generates the same kind of signaling system with the reduced plastoquinone pool as the sensor of these stresses (Fig. 4).

The proposition that the redox status of the plastoquinone pool as the stress-sensing system regulates cab gene expression is supported by the observation of the changes in the transcription of the gene by modulating the redox status of the pool with different types of inhibitors [75]. Escoubas et al. [75] propose that the signal generated by the redox status of plastoquinone from the chloroplast to the nucleus is mediated by a kinase-phosphatase cascade. High-light intensity that is capable of overreducing the plastoquinone pool may result in the phosphorylation of a chloroplast protein by redox-sensing kinase that may activate a protein kinase in the cytoplasm. The cytoplasmic

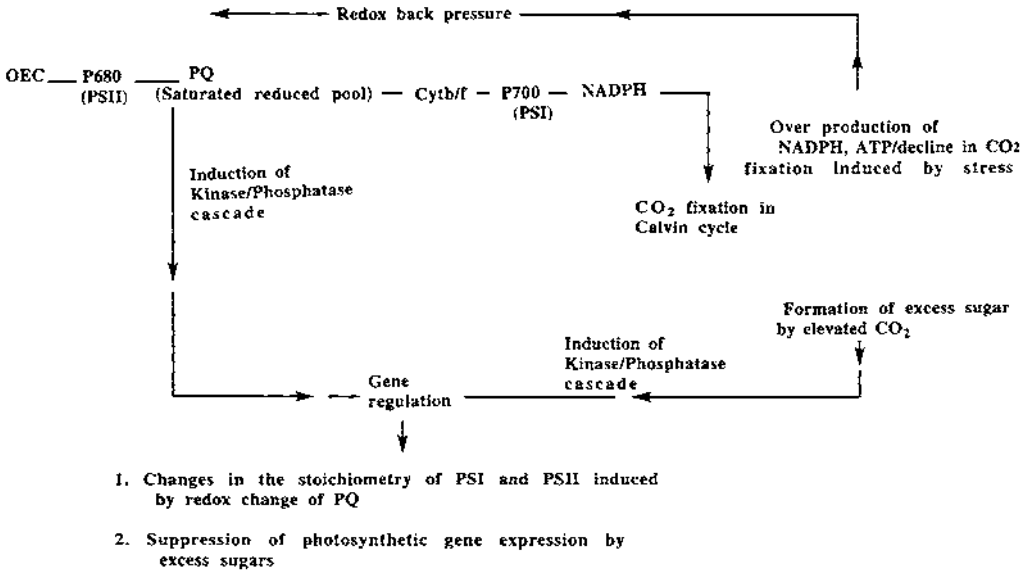

FIGURE 4 A scheme showing overexpression/underexpression of photosynthetic genes during varying environmental conditions. The figure exhibits the possible signal transduction paths of gene regulation for the synthesis of light harvesting and core proteins of photosystems of thylakoids induced by stress through a reduced pool of PQ and suppression of photosynthetic genes by excess sugar, possibly through hexokinase. The details of the stress-signaling systems operating through these paths are described in the text.

protein kinase possibly phosphorylates the cab gene repressor factor, which moves to the nucleus in its phosphorylated form, binds to cab gene promoter, and represses it [75]. In a low-light condition, the plastoquinone pool associated with PSII largely remains in the oxidized form that results in poor activity of the protein kinases and hence switches off this signal transduction pathway. These experiments clearly link a stress-induced signaling system generated through the changes in the rate of primary photochemical reactions and alteration in nuclear gene expression, an adaptive response of the photosynthetic organelle [76]. The signal of the changes in the light intensity not only affects the level of LHC of PSII, but the variation in the intensity could also lead to overexpression or limited expression of genes for core proteins of the photosystem. A high-light intensity is reported remarkably to enhance the level of the D1 protein [78] and the level, on the other hand, goes down when plants experience relatively low-light intensity [79]. It appears that the perception of the light stress, its recognition, and subsequent transduction are so perfect that plants can sense the right mode of the signal transduction pathway when the intensity of a single stress changes.

Another example of the regulation of photosynthetic genes induced by an elevated level of CO_2 is considered here. The production of excess sugar induced by elevated CO_2 as a stress factor leads to a kind of feedback inhibition of photosynthesis, and the inhibition is believed to be controlled at the level of genes [46]. This situation arises when the rate of photosynthesis is relatively high and the sugar-utilizing sinks are saturated. An elevated CO_2 level causes a remarkable loss in the amount of both large and small subunits of Rubisco in addition to the loss of thylakoid proteins like D1 and D2 of PSII core complex and the A1 protein of the PSI core [44,45]. Similar findings on the decline in the level of transcripts for the small subunit of Rubisco induced by excess sugars have been observed recently by Lee and Daie [80]. Although the nature of the signaling system that senses the excess sugar is not properly understood the phosphorylation of sugars mediated by hexokinase is considered as a possible component of the signal transduction pathway [46,81]. The proposition of this kind of signal transduction through hexokinase is supported by the observation of photosynthetic acclimation to a high CO_2 level by overexpression of the enzyme [46]. Different types of sugar-sensing signaling systems and possible mechanisms of the transduction of the signals have recently been discussed by Smeekens and Rook [5] and Ehness et al. [82].

These elegant experiments relating to the photosynthetic response to the changes in the level of light or CO_2 clearly suggest the operation of signaling systems that could rightly sense and recognize the variation in the intensity of a single stress and thus regulate overexpression-underexpression of genes as per the desired readjustment of the photosynthetic components during chloroplast acclimation (see Fig. 4).

Regulation of Stress-Specific Genes

Recently, many stress-responsive genes, particularly some of the genes relating to drought, chilling temperature, heat, and light stress have been isolated, cloned, and characterized. Because the accumulated data during the last 5 years is so vast, this chapter has limited the scope in describing them in detail. Attempts have been made to generalize and to discuss only a few of the major findings.

Induction of ELIP Synthesis: A Response to High-Light Stress

The ELIPs are nuclear encoded proteins that have structural similarities with the light-harvesting complex. These proteins are so named because they were initially thought to be synthesized during the early development of the photosynthetic organelle. However, it now has been established that these proteins accumulate as an adaptive response to high-light stress. Although the precise function of the protein has not been clearly worked out, these proteins may provide a surface for binding and the subsequent stability of free pigments which are released because of the high-light–induced degradation of Chl binding proteins of PSII. Since ELIPs are believed to be Chl binding proteins with four molecules of Chla, two luteins, and few other carotenoid species [83], they may function

as nonphotochemical quenchers of fluorescence and thus protect the photosynthetic organelle against high-light stress by dissipation of energy [84]. Under varying light conditions, a kind of inverse relationship is reported to exist between the accumulation of ELIPs and LHC. The induction of the accumulation of ELIPs with a concomitant decrease in the LHC antenna system may suggest an adaptive response of plants to high-light stress [85]. The details of structure, photoprotective functions, the regulation of synthesis, and the subsequent assembly of these proteins in thylakoids have been recently reviewed by Adamaska [86]. The high-light stress is reported to enhance the level of its transcripts by five- to eightf [85]. Both the transcripts and the proteins of ELIPs remain stable in relatively high- than in low-light conditions [15]. The induction of ELIPs and their regulation both at the transcription and posttranscription levels have been examined by Montane et al. [85] in varying light and temperature conditions.

The mechanism of a high-light–sensing signaling system responsible for the accumulation of ELIPs has not been worked out. In most of the cases, the turnover of the D1 protein induced by high-light stress is linked to the induction of ELIP transcription [86–88]. The stress-induced changes in the redox status of some component(s) of the electron transport chain associated with thylakoids may generate a signal for upregulation of ELIP transcription and translation [85]. Further studies in this direction may provide clues on that signal transduction pathway for the induction of this group of stress-responsive proteins.

Gene Expression and Synthesis of Specific Proteins Under Water and Salt Stress

Our knowledge on the perception of water stress signal, its transduction leading to specific gene expression, and finally processing of the gene products is still limited. It appears there are multiple pathways of signal transduction systems operating at the cellular level for gene regulation. The loss of water from the cells, one of the initial events of water deficit, may affect turgor and changes in size and membrane properties. Although the precise nature of signal-sensing mechanisms is not clearly understood, a few osmosensers associated with the membrane may transmit the signal through a cascade of phosphorylation-dephosphorylation leading to expression of specific genes [4].

Although the components of the signal transduction pathway are difficult to identify, ABA is well known as one of such components acting in one of the signal transduction pathways [4,89]. Experiments conducted so far clearly indicate ABA-dependent and ABA-independent pathways for the induction of stress-related genes. Participation of different elements of DNA regulating stress induction of gene expression supports this proposition [4].

In addition to the expression of genes for the production of late embryogenesis–abundant (LEA) proteins, including dehydrin, a set of stress-specific proteins synthesized as a response to water deficit [4], the stress has recently been reported to induce a chloroplastic lipoxygenase. The cDNA of the gene has been isolated, sequenced, and characterized [90]. The stress is also reported to induce two important chloroplastic proteins with a molecular weight of 32 and 34 kDa, which have been well characterized recently [91]. The 32-kDa protein is located in the stroma, and its synthesis is likely to be induced by a high osmolarity–related signal. On the other hand, stress-induced synthesis of a 34-kDa protein located in the thylakoids is mediated by an ABA-related signaling system. This protein may be involved in the reorganization of the thylakoid structure in order to tolerate the stress, and the 32-kDa protein may regulate the osmolarity of the stroma. Although synthesis of both the proteins is induced by water deficit, this signaling system for induction of their synthesis appears to have two different routes. It is interesting to note that the synthesis of the 34-kDa protein is induced not only by drought stress but also by low temperature and high salinity [91], which may suggest a single channel for transduction of signals generated by all the three kinds of stresses. But the drought stress resulting from low temperature and salinity and then operating in the channel cannot be ruled out.

It is well known that salt stress in addition to an ionic effect also results in dehydration.

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Therefore, salt stress–induced signal transduction is likely to have common routes with the signaling systems that are operative during drought and other environmental stresses causing dehydration. One of the pathways is mediated by the accumulation of osmolytes or compatible solutes, including sugars, amino acids, and proteins. These solutes maintain a kind of osmotic balance and then protect the cells-organelles against dehydration [4]. *Glycinebetaine*, a quaternary ammonium compound, is known to stabilize OEC [92], Rubisco [93] and against salt stress [94]. The codA gene responsible for the synthesis of choline oxidase and converts choline to glycinebetaine has already been cloned and characterized. The transgenic plants with overexpression of the gene have already been found to protect PSII activity in chloroplasts. Recently, the details of the gene regulation of the accumulation of proline, one of the major osmolytes induced by osmotic stress, have been critically reviewed by Yoshiba et al. [95]. A desired level of proline accumulates as a response to the stress by the regulation of genes activating the synthesis and inactivating the degradation of proline. A tilting of balance toward its synthesis may lead to overproduction of the osmolyte, and thus plants develop salt- or water-stress tolerance. However, the specific role of proline for photosynthetic acclimation still remains unclear.

Temperature Stress and Gene Expression

Reports are available on low temperature–induced gene expression in plants experiencing temperature stress [2,96–99]. Some of the genes relating to the stress also have been cloned and characterized [100]. However, the nature of the signaling system for gene expression and the mechanism of the photosynthetic adaptation are still not clearly understood.

The expression of two low temperature–induced genes (cor15a and cor15b) encoding for two proteins of a 15-kDa molecular weight each has been reported by Lin and Thomashow [101] and Wilhelm and Thomashow [102]. These proteins are targeted to the chloroplast. Since the induction is mediated by exogenous ABA [102], it is possible that the signal may be transmitted through the ABA route. The accumulation of compatible solutes like betaine in the cold-induced signal transduction pathway has recently been worked out both in cyanobacteria [103,104] and higher plants [94]. These investigators have shown expression of genes induced by low temperature followed by the accumulation of betaine that protects the photosynthetic organelle against the stress.

Extensive reports are available on the induction of gene expression by heat-shock treatments. The plastid-specific heat-shock proteins are linked to the protection of the photosynthetic organelle during light and heat stress [15].

The nature of the signal transduction system associated with the gene expression induced by heat shock and posttranscriptional as well as posttranslational modifications are not known, and the function of heat-shock proteins to protect chloroplasts is not clear. However, Debel et al. [105] have recently observed the accumulation of a 23-kDa nuclear encoded heat-shock protein in the mitochondria under light stress. High light, because of the production of heat, may result in the induction of heat-shock proteins. Debel et al. have proposed that under high-light conditions, the stress signal transduction for chloroplast proteins is very complex. A possible coupling of the chloroplast and mitochondrial functions through photorespiration under high-light conditions has been proposed. Since photorespiration is known to protect the chloroplast against photoinhibition, it is likely the response induced by heat-shock proteins in the mitochondria may have a link for photoprotection of chloroplasts through photorespiration, thus resulting in a long route signal transduction pathway involving two important organelles of the cell.

The studies on the expression of specific gene products relating to the stress factors other than light stress, drought, and temperature extremes are scanty and therefore are not discussed in this chapter.

Oxidative Stress and Regulation of Defense Genes

As discussed earlier, the excess light not utilized in the photochemical reactions of chloroplasts causes photodamage of the organelle. However, plants develop different kinds of adaptive mecha-

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nisms to counter this stress effect in different ways which have also been discussed. In spite of these defense strategies, there is a possibility of stress-induced disorganization of the thylakoid complexes and leakage of electrons to O_2 which results in the formation of various toxic O_2 species. The damage to the photosynthetic organelle induced by the formation of oxy free radicals is well known [106]. The plants therefore develop a second line of defense mechanism against these toxic O_2 species, including $O_2^{\cdot -}$, H_2O_2, $^{\cdot}OH$, and $^1O_2$. The stress-induced formation of these toxic species is reported to induce the expression of the genes responsible for the synthesis of superoxide dismutase, ascorbate peroxidase, and glutathione reductase that effectively scavenge these harmful species and thus protect plants in general and chloroplasts in particular against oxidative stress. The details of the regulation of the gene expression for the synthesis of these scavenging enzymes in plants experiencing drought, chilling, and salt stresses in different plant systems have been recently reviewed by Eshdat et al. [107] and Alscher et al. [108]. The induction of an oxidative environment is normally considered as a secondary event created by many abiotic and biotic stress factors that plants experience. The molecular mechanism of signal transduction induced by the events associated with oxidative stress resulting in the genetic response still remains unclear. However, two major components in the signal transduction pathway, namely, thiol-disulfide exchange reactions involving the glutathione pool and the production of H_2O_2, are proposed to be crucial for gene regulation [109]. Both thiol-disulfide exchange reactions and H_2O_2 are reported to couple the stress to the expression of genes for defense against O_2 free radicals [109]. Among the reactive O_2 species, H_2O_2 is considered to be the most stable one, and its production through the electron transport chain of thylakoids is well known. H_2O_2 as a possible stress-signaling molecule, acting at the level of genes, is supported by the observations of the induction of proteins reported to cause low-temperature tolerance on its exogenous application to higher plants [109]. Recently, Karpinski et al. [110] have shown a novel type of long route signal transduction path induced by O_2 free radicals under high-light conditions. High light–induced alteration in the redox status of the plastoquinone pool has been suggested to act as a light-sensing mechanism and is linked to the production of H_2O_2. The stress-induced event results in a remarkable increase in the transcripts of two ascorbate peroxidase genes. These enzymes are cytoplasmic and are involved in regulating the redox level of the glutathione pool. The unique feature of this signal transduction for the expression of genes to counter the oxidative stress is that the gene products do not participate directly in chloroplasts, although the initial signal is generated by redox reactions associated with the thylakoids of the organelle. The signal-induced expression of genes prevents photooxidative damage through scavenging of H_2O_2 produced by thylakoids but is located in the cytoplasm [110].

The literature on the induction of scavenging enzymes as a response to stress is rich, but the picture of the stress-induced signal transduction path leading to gene expression still remains hazy. There is no unambiguous experimental proof to accept H_2O_2 and the thiol pool as the stress-sensing components for gene regulation. This needs further clarification.

CONCLUSIONS AND PERSPECTIVE

Our knowledge of the plant response to environmental stress has remarkably progressed in the last two decades because of the availability of highly sophisticated techniques and the rapid expansion of our knowledge in plant science, particularly in the field of molecular biology. The literature on the molecular biology of the chloroplast is very rich. The organelle genome is well characterized, and information is available on the regulation of most of the photosynthetic genes. The response of some of the genes to stress also have been reported. But the central question of the recognition of the stress signal by the cell, its transduction for gene regulation, and photosynthetic modifications by gene products is not fully answered.

This chapter has focused primarily on the response of the chloroplast with particular reference to the photosynthetic acclimation in the background of the operation of possible stress-signaling systems. The field is so complex and our knowledge is so limited, we have been compelled to address some of the fundamental unanswered questions in this section.

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1. As discussed earlier, although the presence of green pigments and the potential of producing strong oxidants, make plants capable of splitting H_2O molecules for the liberation of O_2, this make them prone to environmental stress. Because of the association of the production of strong oxidants with PSII, the photosystem plays a key role in the stress-signaling system during photosynthetic acclimation. The stress response of PSII has, therefore recently become the major area of research. This chapter also reflects this view. However, the interpretation of most of the data is made on the background of PSII structure and function, which are extrapolated mostly from our knowledge of reaction centers of bacterial systems. Although O_2 evolution by green plants was discovered more than two centuries ago, the knowledge we have gained so far does not add to a clear understanding of the mechanism of H_2O splitting during photosynthesis. We do not know the precise topology of Mn in PSII, but the Mn cluster in the vicinity of the photosystem has been recognized as a major stress-sensing system. Unless we get a clear picture of the geometry of the Mn protein complex of PSII, it will be difficult to explain the mechanism of the transmission of the stress signal associated with it.

2. Most of the signal transduction pathways during photosynthetic acclimations are discussed with the kinase-phosphatase cascade as the stress-signaling system as reviewed in this chapter. But the details of the mechanism of protein phosphorylation leading to its conformational modifications have not been worked out. Again, the induction of nuclear gene regulation by protein phosphorylation in the chloroplast has to be explained in terms of transmission of the signal between these organelles. A clear understanding of the integration and coordination of the stress-signaling systems in the cellular environment during photosynthetic acclimation needs extensive experimentation.

3. This chapter has focused on the D1 turnover in PSII as a crucial event for chloroplast adaptation during light stress. But the nature of the degradation of the protein by the stress largely remains unclear. The stress-inducing signaling system involving charge recombination between Q_A^-/Q_B^- and the oxidized centers of PSII is reported to generate $^1O_2$ which is believed to bring oxidative damage to the D1. But the precise nature of the damage of the protein that makes it prone to proteolytic degradation is still obscure. The nature of so-called protease also remains ambiguous.

Similarly, stress signal transduction through the operation of the xanthophyll cycle has been considered as a photosynthetic adaptation. The mechanism of quenching of excess harmful quanta by zeaxanthin, however, remains rather controversial and needs further clarification.

4. The expression of the genes responsible for the synthesis of stress-specific proteins, including ELIPs, HSP, and LEA, in response to environmental stresses, has extensively been investigated. Most of these stress-responsive genes have been isolated, cloned, sequenced, and well characterized, but their precise functions in stress adaptation, particularly during photosynthetic acclimation, are not clearly understood.

We have several such unanswered questions in this area of research, but we have, at the same time, sufficient optimism to find answers to them in the near future because of our access to new technology and also because of our commitment and compulsion to study photosynthesis relating to stress, because the study is directly linked to plant productivity.

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Effect of High-Temperature Stress on the Photosynthetic Apparatus

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INTRODUCTION

The action of physiological stresses on plants has been a subject of numerous studies. Plants are able to survive and grow even under unfavorable environmental conditions. Living organisms can hardly survive below −3°C, because most of the biochemical reactions are hampered owing to the high content of water in the living tissues. Some plant species can grow under desert conditions and relatively high temperatures, but most species are sensitive to a variation of a few degrees below or above their normal growth temperature. In the higher temperature zone (37–47°C), tolerance mechanisms may allow some extent of adaptation. Hence, photosynthetic organisms can tolerate temperatures above their normal growth temperature. The latter may be very high for desert plants but relatively lower for other species. Each species has its own optimal temperature and its lower and higher temperature limits. When exposed to changing temperatures, various structural modifications are known to happen at the molecular level. Such modifications include changes in the rate of metabolic reactions as well as modifications of subcellular structures. A heat shock is induced when a plant is brought near to its higher temperature limit for growth. Of course, even a relatively weak temperature variation can alter the normal cellular biochemical processes which could be considered as a heat stress. However, an increase of 10–15°C above normal growth temperature will cause a deeper modification of growth without being necessarily lethal. Those changes involve protein denaturation, enzyme inactivation, and more specifically a reduction in the chloroplast's photosynthetic activity.

In this chapter, the action of high-temperature stress at the level of the photosynthetic apparatus will be examined. Heat stress induces significant modifications in the composition of the chloroplast membrane lipids and proteins together with structural changes of the thylakoid membranes. Those changes greatly affect the activity of the photosystems. However, some adaptation mechanisms can prevent excessive damage if plants are preexposed to high temperature. Acclimation

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mechanisms may involve some changes in the chloroplast membrane fluidity and lipid composition as well as the synthesis of heat-shock proteins.

DAMAGING EFFECTS OF HIGH TEMPERATURE ON THE PHOTOSYNTHETIC MEMBRANE

Heat stress results in a progressive decline of photosynthetic activity in terms of electron transport and CO_2 fixation [1–3]. In general, the photosynthetic activity remains stable up to 30°C but sharply decreases above this temperature to reach a complete inhibition at about 40°C [2]. Thylakoid membranes inside the intact chloroplast are somewhat less sensitive to heat in comparison with the isolated counterpart [4]. This is explained by the protective effect of stromal solutes [4]. Further, thermal damage to the thylakoid membrane in the intact chloroplast arises at a lower temperature in comparison with the chloroplast envelope membrane that is affected only between 53 and 57°C [5].

Alteration of Lipid and Protein Composition

The action of high temperature is not followed by major chemical modification of chloroplast membrane lipids such as the oxidation of unsaturated fatty acids. A 3-min exposure (in vivo or in vitro) at 48°C did not significantly influence the composition in fatty acids of the lipids in the thylakoid membranes in comparison with controls treated at 20°C [6]. However, the level of free fatty acids in the thylakoid membrane may increase by up to two- to threefold after incubation of the isolated membranes at high temperature. The most abundant fatty acids generated are the unsaturated ones such as linoleic acid and hexadecatrienoic acid [6]. Thylakoid membranes mainly contain monogalactosyl diacylglycerol (MGDG), digalactosyl diacyglycerol (DGDG), sulfoquinovosyl diacylglycerol, and phosphatidylglycerol. MGDG and DGDG are composed of a large proportion of highly unsaturated fatty acids. Thermal stress was also shown to induce a decrease in the MGDG/DGDG ratio and an increase in the incorporation of DGDG with saturated fatty acids into the thylakoid membrane [7].

It must be emphasized that even weak quantities of unsaturated fatty acids such as linoleic acid in the thylakoid membranes contribute to an increased sensitivity to thermal stress at 30°C [6]. It is suggested that endogenous lipases activated by thermal exposure may increase the content of free fatty acid with a resultant increase in polar interactions at the membrane surface which reduces the thermostability of the membrane structure. Thus, the formation of free fatty acids during heat stress could be an important mechanism leading to thermally induced damage. High-temperature treatment produces a raise in membrane fluidity and lateral diffusion of membrane lipids [8]. It was also suggested that membrane permeability increases during treatments at high temperature which resulted in a decrease in proton gradient formation across the thylakoid membrane [3]. The decreasing proton gradient formation as temperature is raised above 30°C is also suggested from the inhibition of the energy-dependent nonphotochemical fluorescence quenching qN at those temperatures [9,10].

It was shown by autoradiography that the synthesis of several thylakoid membrane proteins was strongly reduced during exposure at elevated temperatures [7]. This reduced synthesis includes the apoprotein of the reaction center of photosystem II (P680), subunits α and β of ATPase synthetase, cytochrome *f*, cytochrome *b*559, and the apoprotein of the core antenna complex CP47 [4]. All these polypeptides are coded by the chloroplastic genome. On the other hand, the light-activated protein kinase of the thylakoid membrane remained active as demonstrated by the phosphorylation of six-membrane polypeptides (9.0, 25.5, 26.0, 32.0, 33.0, and 43.0 kDa) during heat treatment at 50°C [7]. It was also noted that the small subunit of rubilose bisphosphate carboxylase and some polypeptides of the light-harvesting complex of photosystem II that are imported into the chloroplast in an ATP-dependent process are still accumulated even after inactivation of the ATPase synthetase.

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It is suggested that the conformational changes of the chloroplast membrane that occur during heat stress improved the membrane permeability for cytosolic ATP.

Structural Changes

Important structural changes modify the thylakoid membrane integrity following exposure at elevated temperatures [11,12]. The first changes appear in the 35–45°C interval as a decreased membrane stacking and a general reorganization of the thylakoid membranes. Above 45°C, a transformation of membranes into vesicles is observed. Freeze-fracture experiments have indicated the dissociation of the major light-harvesting complex of photosystem II from the core complex [13,14]. This dissociation is supposed to coincide with the decreased membrane stacking with the consequent formation of antenna-depleted photosystem II in the nonappressed region of the thylakoid membranes. A careful analysis of the protein composition of barley thylakoid membranes during heat stress at 46°C has also shown that the photosystem II core complex dissociates from a dimeric to a monomeric form, whereas the major light-harvesting complex is fractionated from a trimeric to a dimeric aggregate [15]. The above dissociations can be attributed to the increased fluidity of the thylakoid membrane mentioned in the previous section and to the modification in the hydrophylic and hydrophobic interactions.

Photosystem II cores with their full complement in light-harvesting complex were denoted as PSII-α, whereas the depleted photosystem II cores formed during heat stress were found to be similar to PSII-β located in the stromal lamellae [16,17]. An increase in PSII-β with increasing temperatures was recently confirmed from measurements of fast-induction chlorophyll fluorescence [18]. Heat treatment at 45°C thus results in an increased photosystem II/photosystem I ratio in the nonappressed region [16]. This progressive process is reversible up to 45°C; at this temperature, irreversible modifications are produced.

Changes in the antenna size of photosystem II units during heat stress also result in a modification of the distribution of absorbed energy between photosystem I and photosystem II. The reversible effects of moderately elevated temperature on excitation energy distribution are similar to the formation of state II, a state where the absorbed energy is preferentially redistributed toward photosystem I in contrast to the opposite state I [16,19,20]. This change in energy distribution could protect photosystem II against excessive damage caused by strong illumination (photoinhibition) that usually prevails simultaneously with high temperature in the environmental conditions [16,19]. It was shown that heat stress up to 42°C did not affect the possibility of state transition in the thylakoid membranes, which is however abolished at 47°C [9]. Thermal unstacking of the thylakoid membrane at this latter temperature should rather imply randomization of the protein complexes independently from state transition. It is proposed that the phosphorylated light-harvesting complexes of photosystem II move away from the photosystem and that photosystem II core complexes (PSII-β–like) migrate to the unstacked regions of the thylakoid membranes leaving the light-harvesting complexes in the grana region [21]. This reorganization does not necessarily imply an increased antenna size for photosystem I.

Inhibition of Photosynthetic Activity

Photosystem II

It has been known for some time that the process of oxygen evolution constitutes the most sensitive reaction to heat stress in photosynthesis and that photosystem II is much less resistant to heat in comparison with photosystem I [3,22]. Thus, artificial electron donors to photosystem II such as 1,5-diphenylcarbazide can reconstitute the electron transport activity after heat deactivation [22]. Experiments in the presence of ethylenediaminetetraacetic acid (EDTA) have shown that thermal inactivation is due to the extraction of divalent cations (Ca^{2+} and Mn^{2+}) from the oxygen-evolving complex of photosystem II [23]. Other studies have shown that inactivation of photosystem II was

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accelerated in the absence of Cl^- [24]. It was demonstrated that the addition of 1 mM Mn $(CH_3CO_2)_2$ decreases the temperature of half-inactivation by 2°C in isolated membranes enriched in photosystem II and fully complemented with Cl^-, whereas in the absence of Cl^-, this parameter decreased by 7–8°C [24]. This acceleration of the thermal inactivation was specific for Mn. Heat treatment also produced the release of three extrinsic polypeptides (18, 24, and 33 kDa) and half of the Mn associated with the oxygen-evolving complex from the membranes [24]. It was alternatively suggested that thermal inactivation of oxygen evolution was not specifically due to the release of Mn from the oxygen-evolving complex but rather to the dissociation of the 33-kDa extrinsic polypeptide that is involved in the stabilization of the Mn cluster [25].

The antenna complex of photosystem II is also sensitive to heat stress. Thus, chlorophyll fluorescence from chloroplasts decreases progressively with elevated temperature from 20 to 48°C [1]. Thermal inhibition of photosystem II is accompanied by a decrease of the variable portion of chlorophyll fluorescence associated with the photochemical activity of the photosystem and the so-called photochemical fluorescence quenching, qP [9,10,26,27]. This decline arises from a quenching of the maximal level of fluorescence, Fm, and an important increase of the minimal level, Fo, especially above 40°C [28]. The increase of Fo is either explained by the accumulation of reduced Q_A [29,30], or by the disconnection of the major light-harvesting complex from the photosystem II core [13,14]. However, a recent study using time-resolved fluorescence measurements in heat-treated leaves has shown that the major portion of the increase of Fo above 40°C is related to the disconnection from the photosystem II core of a protein complex containing only a small portion of the total antennae chlorophyll molecules [31].

The inhibition of photosystem II activity in intact leaves was shown to be largely attenuated if heat stress at 40°C was performed during illumination under low-light intensity [32,33]. However, this effect was attenuated by far-red light [32], and strong illumination accelerated the damaging process [34–36]. Photosystem II complexes devoid of the extrinsic proteins and of Mn involved in the process of oxygen evolution can be reassembled into an active complex by weak illumination in the appropriate medium. It is postulated that low light prevents thermal inactivation through a process of photoactivation that counterbalances the light-induced dissociation of the oxygen-evolving complex [37].

Photosystem I

Photosystem I is more resistant to heat stress and on inactivation of photosystem II in potato leaves, photosystem I catalyzes electron flow from endogenous stromal reductants [38] and may also retain its cyclic electron transport activity in leaves [39]. Optimal activity of electron transport from 2,6-dichlorophenolindophenol ($DCIPH_2$) to methylviologen was reported after incubation of thylakoid membranes at a temperature of 50°C [40]. Photosystem II activity is fully inhibited under such conditions [41–43]. A hypothesis to explain the above heat stress–stimulated electron transport rates is that new reduction sites or greater affinity for the artificial electron donors used in activity measurement was created at the level of the cytochrome b_6/f complex following membrane reorganization during heat stress [40,44,45]. This interpretation was based on the loss of the increased activity at 50°C in the presence of KCN or $HgCl_2$, two inhibitors affecting electron transport between the cytochrome b_6/f complex and the photosystem I reaction center complex. Further, the inhibitor 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone (DBMIB) that binds to a site prior to the cytochrome b_6/f complex presented no effect on the enhanced activity. The new reducing sites would be created owing to changes in lipid-protein interactions induced by the temperature treatment.

Another hypothesis to explain the high photosystem I electron transport activity after exposure to a temperature of 50°C concerns the dissociation of the antenna complex of photosystem II during heat stress. It was suggested that the dissociated light-harvesting complex could become associated with photosystem I [12,13] and reversibly increase its activity at elevated temperature in favor of the development of state II [46]. However, further experiments have shown that the enhanced activity at 50°C is also observed in isolated photosystem I-enriched submembrane fractions which demonstrate that dissociation of the light-harvesting complex from photosystem II is not required [47]. It was also proposed that this effect could be due to thermal uncoupling of ATP synthesis owing to

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a decreased proton gradient following an increased membrane fluidity after heat stress [12]. However, it was later shown that no enhancement was produced if the electron donor N,N,N′,N′-tetramethylphenylenediamine (TMPD) was used in place of $DCIPH_2$ [40,44,47].

The heat-induced stimulation was also lost when $NADP^+$ was used as the final electron acceptor instead of methylviologen [48]. It was deduced that the activity of the membrane-bound superoxide dismutase (SOD) decreases at elevated temperatures which would be at the origin of the activation of photosystem I activity. However, a more complete study using various electron donors in a photosystem I-enriched submembrane fraction [49] has shown that the stimulation was independent from the redox potential of the electron donor and from conformational changes at the level of the cytochrome b_6/f complex. Instead, thermal stress was suggested to release the membrane-bound SOD from the thylakoids, thus allowing artificial electron donors with appropriate properties such as hydrophobicity and capability to donate protons, directly to reduce superoxide radicals to hydrogen peroxide at the expense of the usual disproportionation of superoxide into oxygen and hydrogen peroxide with a resultant enhancement of oxygen-uptake activity [49].

Independently from the reactions involving oxygen uptake, higher rates of light-induced P700 oxidation were obtained after incubation of thylakoid membranes above 40°C with an optimal effect at 50°C [46,50]. This increased activity in whole thylakoid membranes was associated with a greater antenna cross-section in photosystem I after heat stress [50]. An increase in the fluorescence ratio F735/F685 measured at 77 K in the stromal portion of heat-stressed thylakoid membranes (50°C) was connected with changes of interaction between photosystem I and PSII-β that would favor energy distribution toward photosystem I [51].

ACCLIMATION TO HIGH TEMPERATURE

Changes Related to Membrane Fluidity and Structure

Plants can become adapted to elevated temperatures if they are exposed to moderately elevated temperature during their growth, with this property being dependent on their physiological state [52]. For example, senescent plants can hardly become adapted, because they are more sensitive to heat stress. Such thermal adaptation is associated with thylakoid membrane reorganization leading to better stability against further exposure to heat stress [8].

Isolated thylakoid membranes can be stabilized and better protected against elevated temperatures by immobilization in various media such as an albumin-glutaraldehyde cross-linked matrix or in polyvinylalcohol films [53,54]. Alternatively, the membrane stability under heat stress can be improved on addition of various solutes in the media. For example, glycinebetaine, glycerol, and sucrose were shown greatly to stabilize the oxygen-evolving function of photosystem II in thylakoid membranes or photosystem II-enriched membranes exposed to heat stress [55–57]. The temperature for inactivation of primary electron transport reactions in photosystem II-enriched membranes was also greatly reduced in the presence of glycinebetaine or sucrose [58]. Osmolytes are also present in plant cells, and osmotic pressure has been suggested to increase tolerance of the photosynthetic apparatus against heat stress [52]. Hence, desert plants increase their resistance by six- to ninefold at the end of their summer season owing to an increase in the cellular concentration of small molecules [52].

Transfer of photosynthetic organisms from 20 to 45°C results in a rapid modification of membrane fluidity [59]. There is no phase transition affecting the molecular order of membrane lipids during thermal adaptation; rather the changes are due to some modifications in the hydrophylic and hydrophobic forces. After adaptation, there is a significant accumulation of DGDG, and, more specifically, 16% of the molecular mass of DGDG is composed of saturated (18:1/16:0) fatty acyls. Also, the MGDG/DGDG ratio decreases from 1.3 to 0.9 [7,60]. The protective action of DGDG may be associated with its capability to form bilayers in contrast to MGDG [7,60]. Using homogeneous catalytic hydrogenation of thylakoid membranes, it was shown that saturation of *cis* double bonds of lipid alkyl chains resulted in a significant increase in the thermal stability of photosystem II and of general membrane structure [61]. Along those lines, mutants of *Arabidopsis thaliana* with

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reduced levels of polyunsaturated lipids presented a significant decrease in membrane stacking resulting from a decreased amount of thylakoid membranes per chloroplast and from an increased stability of photosynthesis toward heat stress [62,63]. However, recent studies using *Synechocystis* PCC6803 and *Anacystis nidulans* R2-SPc with modified desaturases demonstrated that elimination of dienoic lipids molecules, but not trienoic lipids molecules, produced some extent of decrease in heat tolerance [64,65]. It was concluded that an increase in the unsaturation of membrane lipids reduced sensitivity to chilling stress but not to heat stress [65]. Adversely, using a mutant of *Chlamydomonas reinhardtii* impaired in chloroplast fatty acid desaturation and which indeed presented a reduced level of unsaturation of thylakoid membrane lipids, it was shown that a lowered unsaturation level contributes to the high-temperature tolerance of photosystem II [66].

A role of the carotenoid zeaxanthin in the acquisition of thermostability has been suggested from experiments in which potato leaves or whole plants were exposed to a temperature of 35°C [67,68]. Elevated temperatures would promote the dissociation of violaxanthin molecules from the surface of the light-harvesting complexes which make this xanthophyll accessible for deepoxidation into zeaxanthin molecules by the membrane-bound xanthophyll deepoxidase [68]. Accumulation of zeaxanthin in the thylakoid membrane has been shown to reduce membrane fluidity [69]. It is thus proposed that the formation of rigid carotenoids such as zeaxanthin preserves membrane permeability and photosynthetic activity during heat stress [68,70].

Protein phosphorylation of the thylakoid membranes is less pronounced in thermotolerant plants. The lower phosphorylation state of the major light-harvesting complex may prevent its abusive dissociation from the core complex of photosystem II, with the phosphorylation being required for the migration of the light-harvesting complex [7]. Because the protein kinase remains active in plants exposed to a thermal stress, severe inhibition of the phosphoprotein phosphatase or possibly a conformational change of phosphoproteins of photosystem II that would decrease their accessibility to phosphorylation would be responsible for the low phosphorylation state of acclimated plants [7]. The light-harvesting complex is suggested to present a structural and stabilizing role in photosystem II under heat stress maintaining the oxygen-evolving complex in a functional state [37,71].

Finally, in the cyanobacterium *Synechoccus*, it was proposed that the presence of the low potential cytochrome *c*550 is responsible for the heat stability of oxygen evolution [72]. This cytochrome is supposed to be membrane bound to the lumenal side of the thylakoid membrane near the oxygen-evolving complex, but its mode of action remains unknown [72].

Synthesis of Heat-Shock Proteins

Synthesis of heat-shock proteins (HSPs) in plant species adapted to a temperate environment occurs mostly at temperatures above 32°C but is maximal at around 39–40°C for most species [73,74]. A large number of HSPs were initially found in the chloroplast. In fact, 19 proteins were reported to accumulate during heat stress and were detected in the soluble fraction of the chloroplast [7]. However, experiments using purification of the chloroplast fraction in Percoll gradients have shown three high molecular weight HSPs (96, 74, and 67 kDa) and 6 low molecular weight HSPs (26, 24, 22, 21, 19, and 17 kDa), and isolation with a sucrose density gradient resulted in the detection of only 7 HSPs [75].

Heat-shock proteins are synthesized in the cytosol and transported in the chloroplast. Indeed, although the synthesis of most proteins was inhibited by cycloheximide, an inhibitor of cytosolic ribosomes, the profile of synthesized HSPs remained unchanged in the presence of chloramphenicol, which affects the synthesis of proteins on organelle ribosomes [76]. It was shown that the most abundant HSPs imported have molecular masses of 22 and 25–27 kDa [76,77].

Fractionation of the soluble fraction from the membrane fraction of the chloroplast indicated that some of the HSPs may be associated with the thylakoid membranes [75,77,78]. Such association with the thylakoid membranes was found to be optimal between 36 and 40°C [77,79]. It was suggested that interactions of the proteins with the thylakoid membranes are facilitated at those temperatures owing to modifications of protein and lipid structure in the membrane. It has been shown that

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HSPs are stored in aggregated oligomeric forms of 200–800 kDa with mRNA. The formation of these grains is thought to constitute a mechanism for the protection of the mRNA against thermal denaturation during heat stress [80,81]. Heat-shock grains (HSGs) were also detected in the chloroplast [82,83]. The grains dissociate when the temperature returns to normal conditions [81]. Thus, it was also argued that the presence of HSPs with the membrane fraction is not due to direct association with the membrane but to the formation of HSGs that sediment with the membrane fraction [76,84].

The synthesis of HSPs is associated with resistance to heat stress, although it has not been demonstrated that they are directly involved. Several studies have shown a close correlation between the kinetics for synthesis of HSPs and thermotolerance [85–87]. Also, inhibition of the de novo synthesis of cytosolic proteins results in a strong decrease of photosystem II function after heat shock [88]. Localization of chloroplasts HSPs of 22 kDa has been suggested in the grana fraction [89], and reports of the light-controled synthesis and accumulation of this protein argue in favor of its implication in the protection of the photosynthetic apparatus [90–92]. HSPs of the family of 60, 70, and 90 kDa are known as ''chaperons.'' This type of polypeptide is involved in the transport and folding of some specific proteins. The polypeptides of the HSP70 family are involved in helping the formation of tertiary or multimeric structures or to translocate proteins across mitochondrial or chloroplastic membranes [93–95]. Polypeptides of the HSP70 family were also found in the chloroplast stroma and envelope [96,97]. To date, two chloroplastic functions of HSP70 homologues were suggested; namely, association with Cpn60 into the maturation of newly imported ferredoxin-$NADP^+$ reductase and integration of the apoprotein of the light-harvesting complex of photosystem II into the thylakoid membrane [98,99]. Interestingly, there has been reports of the ability of small HSPs (HSP20 family) and α-crystallins as well to inhibit aggregation and facilitate refolding of thermally denatured proteins [100–102]. However, the mode of action of HSPs in thermotolerance is still awaiting elucidation. A plausible hypothesis is that they may be involved in maintaining the tertiary structure of polypeptides to counterbalance the deleterious effects of heat.

CONCLUSIONS

Exposure of plants to temperatures above their usual environmental conditions results in reversible and irreversible modifications of the thylakoid membrane structure and composition that strongly alter photosynthetic efficiency, with photosystem II being very sensitive to high temperatures. Plants thus try to cope with temperature stress by controlling membrane fluidity and by the synthesis of heat-shock proteins. That these changes are directly involved in protection against elevated temperatures has not been demonstrated, although a correlation with an increased tolerance to heat stress has been fully documented. A better knowledge of plant adaptation mechanisms to heat stress may be useful in applying molecular engineering to the task of improving crop productivity.

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15

Altered Nitrogen Metabolism Under Environmental Stress Conditions

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INTRODUCTION

Specific nitrogen-containing compounds (NCCs) accumulate in plants subjected to specific environmental stress conditions. Books and review articles detailing the effects and consequences of environmental stress conditions are numerous [1–6]. Larcher [7] approached stress in plants from a somewhat philosophical viewpoint, defining it as the internal pressure reaction resulting from (usually) external forces. He also detailed the dynamics of the stress reaction into three phases: the ''alarm'' phase, characterized by a reduction in ''vitality''; a ''resistance'' phase (in response to prolonged stress), in which the plant can adapt and return to nearly normal functioning; and an ''exhaustion'' or ''depletion'' phase, in which the adaptive capacity is overstretched, leading to metabolic disturbances and sometimes death of the plant. Various proposals have been advanced that attempt to assign a biochemical role to the accumulation of NCCs during stress. Rabe [8] reviewed the relevant literature and attempted to provide a plausible, unified hypothesis to explain the accumulation of NCCs during stress conditions. This chapter revisits the evidence supporting the hypothesis advanced by Rabe [8] and elaborates on the practical significance of NCC accumulation, whether negative or positive. Not all the literature is recited, nor is the specific detail of NCC accumulation in a specific species in response to stress detailed again. The reader is therefore referred to Rabe [8] for additional information.

The specific environmental stress conditions to be discussed include mineral deficiencies—macronutrients (K, P, Mg, S, Cl, and Ca), and micronutrients (Fe, Mn, Zn, and Cu), some heavy metal toxicities, drought and salinity stress, temperature extremes, and disease infection (nematodes, root rot fungi, viruses, and bacteria). Documented evidence with respect to the physiological consequences of acid stress, anoxia, and externally induced NH_3-NH_4^+ toxicity is somewhat sparse but is briefly addressed.

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ACCUMULATION OF SPECIFIC NITROGEN-CONTAINING COMPOUNDS

The NCCs that normally accumulate during stress conditions include (a) protein amino acids (e.g., arginine, proline, lysine, histidine, glycine, and serine), (b) nonprotein amino acids (e.g., citrulline and ornithine), (c) the amides (glutamine and asparagine), and (d) diamines (e.g., agmatine, *N*-carbamoylputrescine, and putrescine) and polyamines (e.g., spermine and spermidine).

Characteristics general to plants subjected to stress are the increased levels of total free amino acids [9–16]. Reduced rates of protein synthesis or decreased protein levels also occur during stress [9–12].

In general, it seems as though the nature of the stress and the plant type govern which NCC accumulates (Table 1), because (a) during conditions of mineral deficiency, it is usually the amides, glutamine and asparagine, the basic amino acid arginine, and the diamine, putrescine, that accumulate; (b) stress related to altered water relations usually causes proline and putrescine to accumulate; and (c) disorders of a pathological nature increase arginine and proline levels. Details about which NCCs accumulate during various stress conditions are provided in Table 1. The reader is also referred to Rabe [8] for additional information on how specific crops are affected.

The NCCs accumulating during stress conditions all contain at least one amino group. The most frequently accumulating NCCs, that is, the basic amino acids and amides (arginine, citrulline, ornithine, glutamine, and asparagine) and the diamine, putrescine, contain two or more amino groups. The so-called N efficiency of these compounds is important in explaining why they preferentially accumulate under stress conditions. The close biochemical interrelationship between the NCCs accumulating during stress, either products or precursors of one another, is outlined in Figure 1.

CELLULAR FUNCTIONS OF NCCs

The functions attributed to some of the accumulating NCCs are quite diverse. For instance, during K deficiency, when excess organic acids are synthesized, putrescine has been reported to maintain the ionic balance of cells [24,68]. The role of proline accumulation is widely described as that of acting as cytoplasmic osmoticum (as is the case with betaines and related compounds, not discussed in this chapter; refer to Wyn Jones and Storey [83]), lowering cell water potential during periods of drought stress or under saline conditions. This enables the plant to take up moisture against external gradients [84]. Aspinall and Paleg [84] also list some other functional aspects of proline accumulation. These may include the hydration of biopolymers, serving as a readily utilizable energy source (carbon skeleton) and as a storage form of N during periods of nonoptimal growth conditions. Several papers also suggest that arginine and the amides can serve as N storage compounds during stress periods and resultant nonoptimal growth [40,45,46]. The synthesis of asparagine, glutamine, and arginine is expensive in terms of energy input [46]. The same applies to proline synthesis, if it solely acts as a carbon and nitrogen-storage form. This is energy that can be anabolically utilized in the process of normal protein synthesis rather than the accumulation of specific amino acids. Mertz et al. [46] did not explain why, under stress conditions, energy would be diverted to NCC synthesis at the expense of protein synthesis and continued growth, or why N would be stored when the growth rate is in any case impeded. Research results from Rabe and Lovatt [37] on the mechanisms underlying the accumulation of arginine during P deficiency may provide a possible explanation of this question. The mechanism proposed may be of general application and is discussed in more detail in the next section.

METABOLIC RESPONSE TO STRESS CONDITIONS

Response to Mineral Deficiencies

Arginine accumulation during phosphorus deficiency [37] is due to increased de novo synthesis rather than reduced catabolism or increased protein degradation. The increased activity of the argi-

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TABLE 1 Nitrogen-Containing Compounds Accumulating During Various Nutrient and Environmental Stress Conditions[a]

Stress conditions	NCC accumulation (references)
Low potassium	Arg [12,17–19], Asn [17,18,20–22], Gln [17,18], Pro [18], Ser [18], Orn [12], Citr [12], Pip [17], Urea [17], Putr [12,19,20,23–30], Agm [12,19,23,26,27,30], Spm [20], Spd [20,29,31], NCP [12], NCPase [32], ADCase [27,33], Total N [18]
Low phosphorus	Arg [9–11,17,34–40], Asn [20,22,36,41], Gln [10,36], Pro [15], Lys [15], His [15], Citr [15,17], Orn [15], Agm [26], NH_4^+[15,40], Total N [9,34–36]
Low magnesium	Arg [18,19], Asn [18], Gln [17,18], Pro [18,42,43], Try [43], Ser [43], FAA [43], Putr [23], Agm [19,26], Pip [17], Total N [18]
Low sulfur	Arg [22,42,44–46], Asn [22,44–47], Gln [42,44], Citr [44], Ser [44], Gly [44], Agm [26]
Low chloride	Arg [48], Asn [48], Gln [48], Pro [48], Pip [48]
Low calcium	Arg [17,18], Gly [17], Putr [19], Agm [26]
Low iron	Arg [13,14,16,43,49], Asn [43], Lys [13,14], His [13,14], Ser [43], Pip [16], NH_4^+ [13,14]
Low manganese	Arg [13,14,16], Lys [13,14], His [13,14], FAA [43], Agm [26], NH_4^+ [13,14]
Low zinc	Arg [16,43], Asn [16], Gln [16], Pro [43], FAA [43]
Low copper	Pro [43], Ser [43], FAA [43], GABA [43]
High cadmium	Putr [50,51], Spm [50], Spd [50]
High salinity	Asn [52], Pro [52–54], Put [19], FAA [52]
Water, osmotic stress	Arg [55–58], Asn [59], Orn [55,56], Pro [55,56,59–63], FAA [64], Putr [57,65–67]
Acid stress	Arg [68], Putr [68,69], Agm [68], ADCase [68,69], NCPase [68]
Exogenous ammonia	Gln [42,70,71], Asn [70,71], Arg [42,70,71], Ser [71], Ala [71], Lys [71], Orn [72], Putr [4,19,31,72–75], Spd [31,72,73], Spm [73]
High temperature	Pro [76]
Low temperature	Pro [76,77]
Anoxia	Arg [78], Pro [78], FAA [78]
Pathological stress	
Phytophthora	
Root rot	Pro [78], FAA [78]
Blight (in citrus)	Arg [67], FAA [67]
Virus infection	FAA [79], GABA [79], NH_4^+ [79]
Agrobacterium tumefaciens	Pro [80]
Nematodes	Arg [81], Asn [81], Gln [81], Pro [80–82], Lys [81], His [81], FAA [82]

[a] Abbreviations: Agm, agmatine; Ala, alanine; Arg, arginine; ADCase, arginine decarboxylase; Asn, asparagine; Citr, citrulline; FAA, free amino acids; GABA, gammaaminobutyric acid; Gln, glutamine; Gly, glycine; His, histidine; Lys, lysine; NCP, *N*-carbamoylputrescine; NCPase, NCP-amidohydrolase; Orn, ornithine; Pip, pipecolic acid; Pro, proline; Putr, putrescine; Ser, serine; Spd, spermidine; Spm, spermine; and Try, tryptophan.

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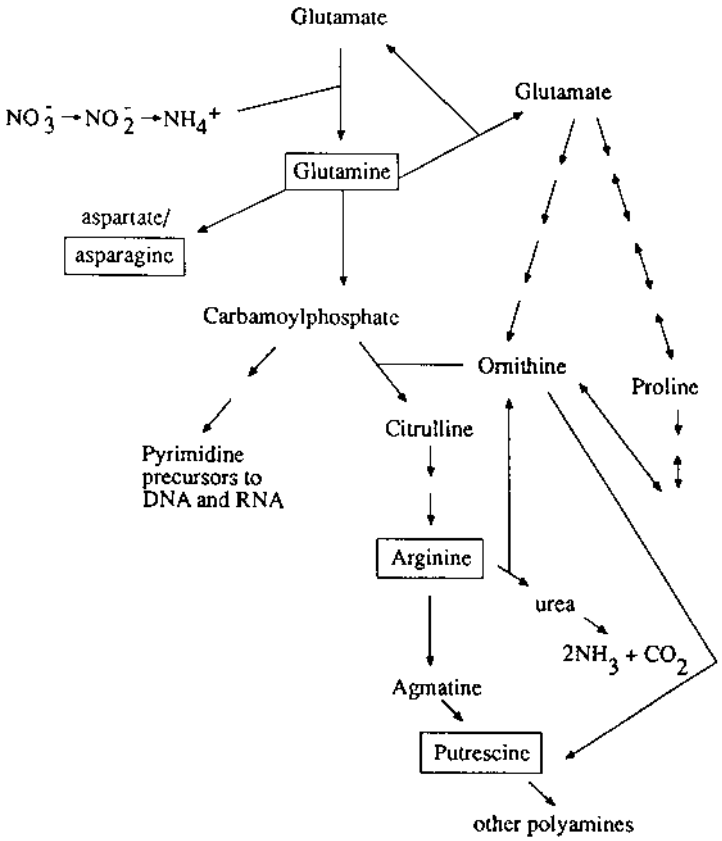

FIGURE 1 Biochemical interrelationship between NCCs that accumulate during stress conditions.

nine de novo biosynthetic pathway is an early response to P deficiency, within 10 days in squash (*Cucurbita pepo* L.) and 6 weeks in rough lemon seedlings (*Citrus limon*) in plants of two diverse families, that is, the Cucurbitaceae, an herbaceous annual, and a woody perennial, Rutaceae (Table 2) [38]. These workers also demonstrated that ammonia accumulated during P deficiency and that the amount of accumulated ammonia increased with the increasing severity of P deprivation. The source of ammonia appears to be twofold: conversion of accumulating nitrate and degradation of amino acids normally incorporated into protein. Phosphorus deficiency thus resulted in ammonium toxicity.

Rabe and Lovatt [38] hypothesized that any stress causing glucose depletion and/or reduced growth results in ammonia accumulation early in the stress period.

Arginine accumulation has also been recorded during Mg, K, S, Cl, Ca, Fe, Mn, and Zn deficiencies (refer to Table 1 for references). Many other NCCs accumulated during mineral-deficient situations (see Table 1).

Response to Other Environmental Stress Conditions

Elevated arginine levels have been recorded during osmotic stress [55–58], acid stress [68], stress arising from excess exogenously supplied ammonia [42,71,72], and certain pathological disorders

TABLE 2 Effect of P Deprivation on De Novo Arginine Biosynthesis in Young, Fully Expanded Leaves from Squash (*Cucurbita pepo* L.) and Rough Lemon (*Citrus limon* L.)

Plant	Treatment duration	De novo arginine biosynthesis[a]	
		P sufficient	P deficient
Squash	10 days	35 ± 3	78 ± 7 (7)[b]
Rough lemon	6 weeks	15 ± 3	149 ± 13 (6)
	12 weeks	15 ± 3	161 ± 17 (3)

[a] $NaH^{14}CO_3$ (nmol) incorporated into arginine plus urea per gram fresh weight tissue during 3 h incubation.
[b] Data are the mean ± SEM (standard error of the mean) with the number of experiments given in parentheses.
Source: Adapted from Ref. 38.

[67,81]. Proline accumulation has been reported to occur during water-deficit stress [84], following exposure of plants to high or low temperature [76], during salinity stress [58,84], during nutrient deficiency [5,85], and under pathological conditions [80]. Apart from arginine and proline, many other NCCs accumulate during the specific environmentally induced stress conditions described here (see Table 1). Elevated levels of ammonia were demonstrated during water stress [86] and low temperatures [87] in *Citrus* species.

MORPHOLOGICAL AND ANATOMICAL ASPECTS ASSOCIATED WITH ALTERED N METABOLISM

Symptoms of P deficiency seem to be identical to those induced by ammonia toxicity. In both cases, lesions appear on the blade of the leaf as darkened, water-soaked areas that later become necrotic [38]. The leaf margins of P-deficient *Citrus* plants appear burned [38], as was described for tomato [88]. The symptoms and metabolic changes associated with P deficiency could be duplicated via ammonia feeding [38]. Ammonia toxicity has been shown structurally to alter tomato chloroplasts [89], in which the chlorophyll and grana disappear.

Puritch and Barker [89] speculated that the disruption of the membrane structure may be due to chlorophyll loss or abnormal protein synthesis (e.g., short half-life of chloroplast proteins and low rate of synthesis). Chloroplast abnormalities similar to those shown for ammonia toxicity have also been shown for manganese-deficient spinach [90]; others [14] have reported that manganese deficiency, in addition to causing NCC accumulation, results in high endogenous ammonia levels (see Table 1).

NCC ACCUMULATION: A DETOXIFICATION MECHANISM?

The evidence of increased arginine levels and accelerated biosynthesis, discussed earlier with respect to the P-deficiency effects, is consistent with the hypothesis that the increased activity of the arginine biosynthetic pathway during P deficiency provides a mechanism for detoxifying leaf tissue of excess ammonia. This interpretation is supported by results obtained from ammonia-feeding experiments that resembled the P-deficiency symptoms or, stated differently, provided symptoms similar to those in plants deficient in P [38]. The incorporation of labeled bicarbonate into arginine plus urea also increased as the concentration of exogenously supplied ammonia or the length of exposure increased

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Table 3 De Novo Arginine Biosynthesis in Ammonia-Treated Young Fully Expanded Leaves from P-Sufficient Squash and Rough Lemon

Ammonia concentration (mM)	Duration (h)	$NaH_4^{+(14)}CO_3$ (nmol) incorporated into arginine plus urea per g fresh weight tissue during 3-h incubation	
		Squash	Rough lemon
None	—	4.0 ± 0.7 (5)	9.2 ± 1.4 (5)
30	3	8.7 ± 1.8 (3)	25.3 ± 1.2 (3)
10	15	14.7 ± 1.4 (3)	39.5
50	3	33.6	47.6
50	15	47.9	55.8

Figures in parentheses represent number of experiments conducted.
Source: Extracted from Ref. 38.

(Table 3). The elevated ammonia levels during water stress [86] and low temperatures [87] in *Citrus* species are additional evidence of altered N metabolism during stress.

The de novo arginine biosynthetic pathway is expensive in terms of ATP and carbon, causing additional stress to a plant that is ''functionally'' carbohydrate depleted. Thus, during P deprivation, the pathway should logically be prone to inhibition. This is not the case, however, supporting the argument that the de novo arginine pathway is important in detoxifying P-deficient leaf tissue of accumulating ammonia.

If the NCCs accumulate during P deficiency (arginine [9–11,17,34–40], asparagine [20,22,36,41], glutamine [10,36], proline [15], lysine [15], histidine [15], the amine, agmatine [26], and others [15,17]) as a response to high ammonia levels, is it true for NCC accumulation under other mineral or environmental stress conditions? First, I discuss the accumulation of proline. If it is assumed that proline accumulation is due, in each of the cases discussed earlier, to the same fundamental mechanism, then one should enquire about the features common to these seemingly disparate stress conditions. Proline accumulation in response to temperature extremes and salinity stress could be due to a disturbance in tissue water status comparable to that observed during drought stress [84]. Does this, however, fully explain proline accumulation during other stress conditions (e.g., nutrient deficiencies)? A more common denominator among all these stress conditions may be that they result in a general reduction in growth rate, as is the case with P deficiency, when arginine accumulates. There is no feedback inhibition for N uptake and nitrate reduction [91], but the lack of anabolic processes (protein synthesis and growth) leads to ammonia accumulation and subsequent detoxification by sequestering ammonia into NCCs (e.g., proline in this case). It is thus submitted that, based on evidence from the literature that NCCs accumulating during normally encountered environmental stress conditions can serve in detoxifying the cell of ammonia, as has been demonstrated for P deficiency in *Citrus* and cucurbits, this led to arginine accumulation. However, this does not exclude the possibility that in given situations these NCCs may indeed sometimes be a storage form for N and/or carbon or serve some other biochemical role.

We have discussed the endogenous generation of ammonia and the NCC accumulation response. How do plants react to high exogenous ammonia or nitrogen levels? Growing plants need to assimilate N ultimately in the fully reduced form. High exogenous concentrations of NH_3-NH_4^+ are toxic to plants, although the biochemical aspects of the toxicity are not always clear [89,92,93]. Bennett and Adams [94], in one of a few studies in which the concentration of exogenously supplied ammonia leading to incipient ammonia toxicity was quantified, concluded it to be between 0.15 and 0.20 mM ammonia in Sudan grass and cotton. Givan [95], in a review on the subject of metabolic

detoxification of ammonia in tissues of higher plants, where he mainly addressed externally induced toxicity, offered two ways in which plants may be able to assimilate and dispose of high concentrations of ammonia: detoxification of excess ammonia by simply accelerating the rate of N assimilation via the usual pathway or supplementing the normal pathway by additional ammonia-utilizing reactions, initiated only at times when the plant is subjected to excessive levels of ammonia. Both these mechanisms seem to be operative during certain nutrient deficiencies and environmental stress conditions with the ''additional ammonia-utilizing reactions'' being particularly important during NCC accumulation.

The effect of the N form in the nutrient solution on the amino acid composition can be quite significant (Table 4). Levels of asparagine, glutamine, serine, arginine, and histidine were elevated between two- and sixfold when NH_4^+ N was applied instead of NO_3^- N to sand-cultured tomato plants [71]. The same was found when the different N forms were supplied to plants grown in vermiculite, peat, or solution culture.

The de novo arginine biosynthetic pathway was accelerated significantly when subjecting excised leaves of both squash (*Cucurbita pepo* L.) and rough lemon (*Citrus limon* L.) to ammonia-containing solutions. The level of activity increased with increasing ammonia concentration or increased length of exposure [38].

Both free and bound putrescine and spermidine increased significantly when half of the NO_3^- N was substituted by NH_4^+ in sulfur-fumigated pea plants [72]. Amine levels were also increased in unfumigated, NH_4^+-supplied plants relative to exclusively NO_3^--supplied plants. Since both sulfur pollution and NH_4^+ nutrition increase the hydrogen ion concentration of the cells and cause a shift in the cation/anion ratio, it was concluded that both result in amines being synthesized to bind these hydrogen ions and to compensate for the relative cation deficit [72]. However, it should be investigated whether acid stress in itself does not also lead to elevated endogenous ammonia levels.

Increasing the supply of ammonia to tomato plants in sand culture resulted in a massive accumulation of glutamine in both the roots and shoots (within 8 h in the root [70]). It was suggested that this time course is probably too short for any ionic imbalance to initiate such a drastic response, especially since the glutamine levels started to decrease after 48 h and that one would have expected the ammonia nutrition to increase the solution acidity even further. Le Rudulier and Goas [74] and Klein et al. [31] also recorded increased putrescine levels in response to ammonium nutrition in soybeans and peas, respectively. Again, a reduction in cellular and exogenous pH was implicated as the cause of the putrescine accumulation [31]. The increase in endogenous ammonia concentration because of exogenous application, however, was not submitted as a possible reason for the accumulation of the NCCs.

TABLE 4 Levels of Certain Free Amino Acids in Shoots of Tomato Plants as Influenced by N Form and Growing Media (μmol g^{-1} dry weight)

	Solution		Sand		
Amino acid	NO_3^-	NH_4^+	NO_3^-	NH_4^+	5% LSD
Asparagine	32.5	42.0	22.5	61.5	16.1
Glutamine	8.8	26.0	8.6	49.1	3.9
Serine	9.1	27.1	9.0	22.6	1.8
Alanine	16.0	22.6	9.0	22.6	2.4
Arginine	4.4	20.9	4.8	16.5	0.7
Lysine	6.3	12.6	7.3	10.6	1.4
Total	227.2	301.1	219.0	325.1	

Source: Extracted from Ref. 71.

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The combined direct and indirect evidence from the literature of ammonia accumulation, accelerated arginine biosynthesis, NCC accumulation, and reduced anabolic processes like protein synthesis and growth during various environmental stress conditions all support the hypothesis that it is a mechanism of sequestering potentially toxic levels of free cellular ammonia.

POSSIBLE PRACTICAL SIGNIFICANCE OF ALTERED NITROGEN METABOLISM DURING STRESS

The stress-induced ammonia response of plants may have practical implications. Stress, albeit environmental, physiological, or mechanical, is not always detrimental to commercial agriculture. In fact, in an excellent review article, Grierson et al. [96] listed various stress conditions that are utilized to the benefit of agriculture, for example, pruning and bending in deciduous fruit, viroid inoculation for tree size control in *Citrus* species, and cold storage and modified-atmosphere storage of fruit for longer shelf life. Rabe [97] lists a number of advantageous and problematical side effects of stress in fruit culture. These include, among others, the use of dormancy-breaking chemicals for an even blossom, fruit thinning by various chemicals for fruit size improvement, and water-induced and low temperature–induced flowering response in various species. A few practical aspects are discussed in more detail.

Luxurious Nutrition Regimens

Most, or all, plants do not regulate N uptake. This is consistent with the fact that land plants evolved under conditions in which N was limited. Thus, there was little selection pressure for regulating nitrogen uptake or reduction [91]. In modern agriculture, the tendency is to stimulate vegetative growth, at least in the initial years after establishment of perennials, by applying excess N to the extent that the anabolic processes of the plant may not adequately cope with it.

Predisposition to Disease

The relative cost of ammonia-based versus nitrate-based fertilizers makes the former a much more attractive source of N. There is evidence, however, that the continual or excessive use of ammonium-based fertilizers can be a predisposing factor in increasing disease susceptibility. For instance, most root and cortical diseases are stimulated by N applied in the form of ammonium compared with nitrate [98]. Ammonium nutrition increases the incidence of *Fusarium* [99] and *Phytophthora* [100] rots in citrus. Furthermore, the deleterious effects of any environmentally induced stress condition are probably aggravated by high exogenous levels of ammonia, as was demonstrated by Lewis et al. [101] in both wheat and maize, in which the ammonium-grown plants were much more sensitive to salinity toxicity than nitrate-grown plants. Whether high exogenous levels of ammonia and high endogenously generated ammonia levels are ultimately equally harmful to the plant is uncertain. The indications are that it does not matter how the excess is attained. The relative P dependence of citrus rootstocks [102] and hence differences in the rootstock ability to metabolize excess ammonia levels [39] parallel the susceptibility to infection by the citrus nematode [103]. There are also indications that citrus rootstock species with different levels of endogenous ammonia during P deficiency (high in rough lemon versus low in trifoliate) [39] are correspondingly susceptible to *Phytophthora* infection (rough lemon being more susceptible; personal observation).

Tolerance to Stress Conditions Between Selections in the Same Genus and Species

Different genera in the same family (e.g., *C. limon* and *Poncirus trifoliata* in *Rutaceae*) have been shown to differ in their ability to cope with P deficiency [39]. This differential response was linked

to differences in N metabolism or leaf tissue ammonia levels. As just indicated, the correlation seems to be extended to differential disease susceptibilities. There may be other instances, other than in *Citrus*, in which this may be true. For instance, it would be of interest to determine how the mechanism of the differential tolerance of various tomato strains to P deficiency [104,105] is linked to differences in N-uptake characteristics and metabolism. Since cultivars, and selections within cultivars, have been shown to differ in terms of salinity tolerance, it is conceivable that the same differences may exist as far as the ability to detoxify the cell of excess levels of ammonia is concerned.

Manipulation of Flowering

Stress conditions are usually a prerequisite for flowering in many commercial species, including *Citrus*: water and low-temperature stress [106–111], girdling [108,112], restriction of root volume [113], and root pruning [80,108]. Some of these flower-inducing stress conditions have been demonstrated to increase the level of endogenous ammonia [86,87,106]. The water-deficit and low-temperature stress conditions were correlated with ammonia accumulation and flowering intensity in *Citrus* under greenhouse conditions [106]. Furthermore, the leaf ammonia content increased in a manner that paralleled the duration of the stress [114], establishing a cause-and-effect relationship between tree ammonia status and floral intensity. Raising the ammonia content of the trees artificially by foliar urea application of low-biuret urea increased leaf ammonia content and floral density without affecting the number of vegetative shoots produced. Utilizing these findings to economic benefit, Lovatt et al. [114] demonstrated yield increases in N-sufficient Washington navel orange trees in 3 years successively without a reduction in fruit size with winter applications of foliar urea. In our own field trials, we obtained positive yield responses to preblossom urea sprays in four different citrus cultivars [115]. Significant yield increases were obtained only in orchards with below optimum N status, however, suggesting that the urea sprays acted in supplementing the N nutrition of the tree. Edwards [116] reported similarly promising results with respect to increasing floral intensity with the application of ammonia, arginine, and polyamines on apple trees.

Stress Effect in Dormancy Release in Deciduous Fruit

Many deciduous fruit-growing areas traditionally do not have enough winter chilling to allow the natural release of buds from dormancy. Saure [117] reviewed this topic. Most of the chemical treatments being used are effective at concentrations very near the lethal point. Among these chemicals are the mineral oils, which alone or in combination with such chemicals as dinitroorthocresol (DNOC) or cyanamide (H_2CN_2) can effectively ''normalize'' bud break under conditions of inadequate winter chilling. Thiourea (TU) has also been shown to be effective as a rest-breaking chemical [118]. Interestingly, Terblanche et al. [119,120] found that a late autumn urea foliar spray increases the efficiency of the DNOC rest-breaking spray. The physiological basis for this is still not fully understood. Preliminary findings (unpublished data) point to a significant increase in the levels of a number of free amino acids during the early phase following the rest-breaking spray. We are currently devoting research efforts to this phenomenon.

NCC Analysis as an Early-Warning System

It has been demonstrated here that NCC and ammonia accumulation in response to environmental stress is correlated with general plant health. Although ammonia is known to be toxic to plants, an upper threshold tolerance value for leaf ammonia concentration has not yet been determined for any plant species. If this value were known, leaf ammonia content might provide a good indicator of plant health. Rabe and Lovatt [39] also suggested that leaf arginine could be used to monitor plant stress. Other possible compounds that could be determined for stress analysis include levels of total free amino acids or certain amines. Since these metabolic abnormalities occur before any

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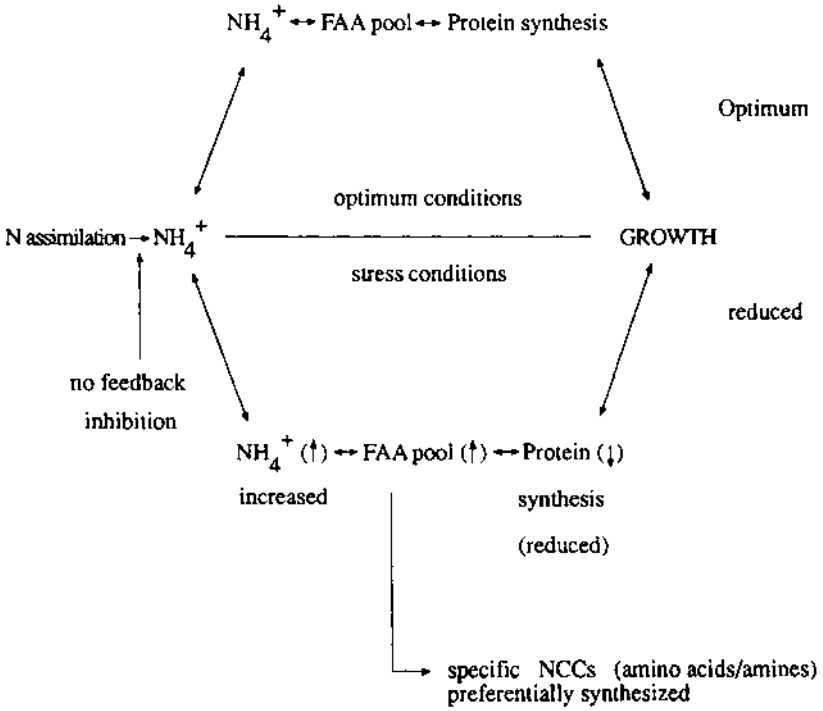

FIGURE 2 Schematic outline of the hypothesis.

visible symptoms of stress are evident, they may provide an early warning system on which to react before economic damage is suffered.

CONCLUSIONS

A schematic outline of the effect and consequences of optimal and nonoptimal conditions on N metabolism is presented in Figure 2. The lack of feedback inhibition on N uptake and reduction during periods of reduced growth or reduced protein synthesis causes NH_3-NH_4^+ accumulation and preferential synthesis of specific amino-containing compounds.

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Protein Synthesis by Plants Under Stressful Conditions

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INTRODUCTION

Environmental stresses present major challenges in our quest to achieve sustainable food production. The reactions of plants to environmental stresses are complex and involve many kinds of physiological and biochemical responses. Such reactions are initiated by plants growing in stressed environments to overcome, avoid, or neutralize the effects of stress. Tolerance or sensitivity toward a particular stressful condition depends on the genetic and biochemical make-up of the species. Much attention has been focused during recent years to evolve crop species with adaptibility built into their genetic and biochemical make-up toward various stressful environments.

Plants are unable to express their full genetic potential for production when subjected to stressful environments [1]. Various environmental stresses cause important modifications in gene expression in plants [2]. Such modifications may lead to the accumulation or depletion of certain metabolites, alterations in the behaviors of many enzymes, overall changes in protein synthesis, and, of particular interest, synthesis of new sets of proteins which are specific to the particular type of stress [3]. It has been shown that different environmental stresses induce the synthesis of new proteins in plants, which possibly provide evolutionary value to the plants for enhanced survival under adverse environmental situations. The synthesis of such stress-induced proteins has been well documented under salinity stress [4–10], osmotic stress [6,11–17], heat shock [2,18–21], low-temperature treatment [22–26], anaerobiosis [27–29], infection with pathogens [30–35], wounding [34–38], gaseous pollutants [39], and ultraviolet (UV) radiation [38–40].

The main idea underlying studies of stress-induced protein synthesis in plants is that the different sources of stress, their duration, and severity lead to differential expression of genetic information, resulting in changes in gene products, including mRNA and proteins. Such newly synthesized proteins are specific to the particular type of stress and possibly confer enhanced survival value to the plants [7]. In most cases, the stress-induced proteins have been identified by biochemical and molecular biology techniques from different organs of plants and are well characterized. Physicochemical parameters such as molecular weights and pI (isoelectric point) values of these proteins have been deduced [7,13,21,28], and in many cases data regarding association characteristics and

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amino acid sequences have also been reported [6,8,17,41]. Although these proteins are synthesized in plants when they are subjected to stress and can be revealed in tissues of plants adapted to stress, specific metabolic functions for most of these proteins have not been established as to how they confer adaptability toward stress [7,42]. Particularly, under anaerobic stress, the polypeptides which are synthesized have specific functions and belong to the enzymes of sugar phospate metabolism [27]. Heat-shock proteins, which are synthesized under heat stress, possibly assist in protein folding, protein-protein interactions, and the translocation of proteins across cellular compartments, and they have a possible role in protecting the organism from heat stress [20]. Similarly, the pathogenesis-related proteins do act in the defense of the plant and have a putative role in pathogen resistance [42]. Under salinity stress, it is suggested that the newly synthesized proteins, together with amino acids and soluble nitrogenous compounds, act as components of a salt-tolerance mechanism. These might function as compatible cytoplasmic solutes in osmotic adjustment in order to equalize the osmotic potential of the cytoplasm with the vacuoles in adverse conditions of salinity [43,44].

Studies related to the stress-induced synthesis of proteins have been performed using cultured plant cells [4,6,7,12], seedlings [10,18,23,45] excised plant organs [46], and intact plants [31,47–49]. Among these systems, cultured plant cells have proven to be superior to other systems, as they show uniform response and are under better controlled enviornmental parameters [7,50]. Cell cultures from tobacco, cowpea, potato, citrus, and many other plant species have been used to identify and characterize newly synthesized proteins under salinity, heat-shock, freezing, osmotic, and heavy metal stresses [2,6,7,8,50].

Besides the identification of stress-induced specific proteins, several investigators have tried to quantify the overall metabolic status of total and soluble proteins (including enzymes) of different metabolic pathways in stressed plant parts in order to evaluate the impact of stresses on various aspects of plant growth and metabolism [36,37,44,51,52]. Environmental stresses generally are detrimental to plant growth, adversely affect the metabolism of plants, and cause an imbalance in the level of protein as a result of their effects on the synthesis and hydrolysis of proteins [52–55]. In salt- and water-stressed plant parts, the protein content decreases owing to the decreased rate of protein synthesis and the increased rate of proteolysis [16,52,56]. In seeds germinating under salinity or moisture stress, however, an increase in the protein level is observed. This increase can best be explained by the fact that in germinating seeds, stress causes decreased proteolysis in endosperms resulting in the slower depletion of reserve proteins. This reflects an apparent increase in the endospermic protein level under stress, which is not a result of enhanced protein synthesis [52,56,57].

Stress tolerance is dependent on the genetic and biochemical characteristics of the species. Therefore, attempts have been made by certain investigators to differentiate stress-tolerant and stress-sensitive genotypes of crops on the basis of profiles or levels of soluble proteins, specific enzymes in germinating seeds, and growing plant parts [16,44,52,54]. The results of these attempts indicate that different levels of soluble proteins and many enzymes exist in the two sets of genotypes differing in stress tolerance.

Studies conducted so far indicate that stressful conditions adversely affect the protein metabolism in plants and that in all different types of environmental stresses, such as salinity, drought, heat, chilling, anaerobiosis, pathogenesis, wounding, heavy metal toxicity, and gaseous pollutants, new stress-specific proteins are synthesized. An overview of protein synthetic responses in plants and an alteration in the levels of key enzymes under various stresses is presented in Figure 1. This chapter presents the current knowledge about the effects of various environmental stresses on the overall aspects of protein synthesis in plants and the possible role of stress-specific proteins in confering an enhanced survival value to the plants against various environmental stress situations.

STRESSED ENVIRONMENTS AND PROTEIN SYNTHESIS

The major types of stress to which plants are exposed include salinity, drought, flood, heat, cold, anaerobiosis, infection by pathogens, metal toxicity, gaseous pollutants, and UV radiation. Plant

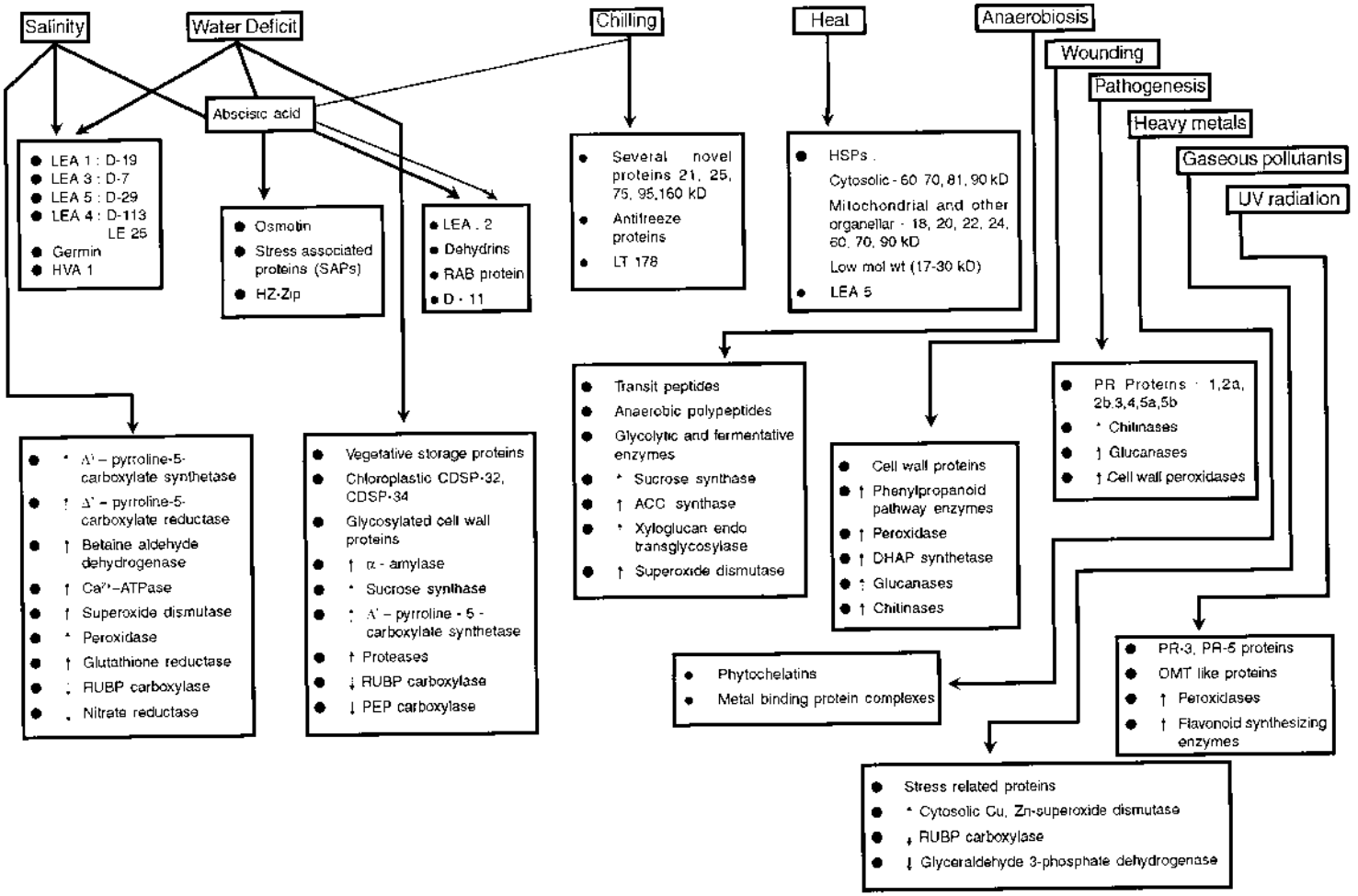

FIGURE 1 An overview of stress-induced protein synthetic responses in plants. Different stresses induce the synthesis of various groups of proteins and cause either elevation (↑) or decline (↓) in the levels of enzymes. Some of the responses of salinity, water deficit, and chilling are common and are mediated via elevated levels of abscisic acid. For details, see text.

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metabolism and, more specifically, protein synthesis are adversely affected under these conditions. The effect of stress depends on the developmental stage of the plant and genotypes of the plant species as well as the intensity and duration of the stress. In this chapter, recent progress toward understanding the impact of different types of environmental stress on protein synthesis is summarized.

Salinity

Soil salinity is a major environmental stress that drastically affects crop productivity. Salinity poses a severe threat for the cultivation of crops in arid and semiarid agricultural lands. Because of the continuous build-up of salinity in the soil, millions of hectares of usable lands have now become unsuitable for cultivation. It is estimated that every year more than a million hectares of land are subjected to salinization. Soil salinity is thus threatening our civilization by persistently reducing the area for crop cultivation. Salinity not only causes great losses in crop yields but also has an impact on other economic, environmental, social, and political problems in the affected countries. Progress in developing salt-tolerant crop varieties has been very slow because of our incomplete knowledge of the mechanism of salt damage and the complex nature of salt tolerance. Even different varieties of a particular species may exhibit different tolerance behaviors. Salinity affects seed germination, plant growth, nutrient uptake, and metabolism owing to the osmotic inhibition of water availability, toxic effects of salt ions, and nutritional imbalance caused by such ions [58].

Salinity promotes the synthesis of salt stress–specific proteins [5–7,42], causes either decreases [49] or increases [57,59] in the level of total and/or soluble proteins, depending on the plant parts studied, and leads to increased activity-synthesis of many enzymes [10,52,60–63].

Salt-Induced Protein Synthesis

Plants growing in saline environments show distinct changes in the pattern of synthesis and accumulation of proteins. Most of the experiments to study the salinity-induced synthesis of proteins have been conducted using plants cell cultures. Cell cultures rather than whole-plant systems have proven to be more advantageous for such studies, because, in cell cultures, environmental parameters can be better controlled and the stress-tolerant cell lines generated can be readily selected and assayed for newly synthesized proteins.

Several investigators have shown the synthesis of new proteins in cultured plant cells when subjected to salinity stress [4,6,7,42,59]. The levels of proteins differ in salt-tolerant and salt-sensitive genotypes when they are subjected to salinity stress [44,54]. Although it is well established that salt tolerance and sensitivity depend on the genetic and biochemical composition of the species, it has been difficult so far to specify the exact genetic domain responsible for salt-adaptation expression leading to the synthesis of these proteins in salt-adapted plants. These specifically synthesized proteins under salt stress appear to have a role in providing tolerance or adaptation to the plants. However, the overall mechanism of how these proteins could provide adaptation is not clearly understood.

To understand the mechanism of salt resistance in cultured tobacco cells, Ericson and Alfinito [4] examined the protein patterns of NaCl-adapted as well as NaCl-nonadapted cell lines of tobacco (*Nicotiana tabacum* L.). Their results indicated that cells adapted to a medium containing NaCl showed two protein bands of 32 and 20 kDa in more abundance than unadapted cells. Further, in the salt-adapted cells, a unique protein of 26 kDa appeared that was specific for these cells and was not present in unadapted cells or cells growing without NaCl. These investigators suggested that the three proteins synthesized in salt-adapted cells might be involved in a salt-adaptation process.

According to Singh et al. [64], in cultured tobacco cells, the process of cellular adaptation to osmotic stress in a saline environment involves the specific alteration in gene expression of salt-adapted cells leading to the synthesis of several novel proteins, including the predominant 26-kDa protein. Since 26-kDa protein is specifically synthesized and accumulated in cells undergoing os-

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motic adjustment to salt or dessication stress, this protein has been named ''osmotin'' [6]. Osmotin is regarded as a unique protein associated with NaCl-adapted tobacco cells [6]. Interestingly, the synthesis of osmotin is not induced by osmotic shock but starts only when cells are adapted to NaCl or polyethylene glycol [64]. In salt-adapted cells, osmotin constitutes about 10–12% of the total protein in the cell [64]. It is believed that the role of osmotin is in providing osmotic adjustment to the cells either by facilitating the accumulation of solutes or by providing certain metabolic alterations in the cell, which may be helpful in osmotic adjustment[6].

Similar to tobacco cells, the synthesis of salt-induced proteins has also been shown in maize[65], barley [5,49], citrus [7,8], tomato [7,59], rice [9], and finger millet [66]. Barley plants, when subjected to short-term NaCl shock or a long-term NaCl treatment for a period of 8 days, show marked quantitative and qualitative changes in protein profiles compared with nonstressed plants [5,49]. Maize callus tissue [65] shows predominantly accumulation of a 26-kDa protein under saline stress. In potato plants, high salinity leads to the increased synthesis of 32- and 34-kDa proteins in the thylakoids [67]. A comparison of the protein profiles of nonadapted and NaCl-adapted cell lines of citrus and tomato indicates that, in citrus, the level of most proteins is suppressed, whereas, in tomato, it is enhanced under salt stress [7]. Tomato cell cultures when grown in a medium with 25 mM NaCl and proline synthesize extra polypeptides of 190-, 58-, 45-, and 26-kDa, and with the further increase of NaCl in the medium, a new 67-kDa protein is accumulated [59]. In tobacco cells, enhancement in the level of certain proteins and a decrease in the level of others is observed when cells are adapted to NaCl [6]. This indicates that salt-induced changes in proteins are species specific and that different proteins are associated with salt tolerance in different species.

In certain plant species, such as rice (*Oryza sativa* L.) and Shamouti orange (*Citrus sinensis* L. Osbeck), it has been shown that salt-sensitive and salt-tolerant genotypes have different patterns of protein profiles whether grown in the absence or the presence of NaCl. Salt-tolerant genotypes of rice possess a 28-kDa protein in shoots which is absent in salt-sensitive genotypes, and the level of this protein is further elevated when the rice seedlings are raised in saline medium [68]. This shows that the presence of the additional 28-kDa protein band is associated with salt-tolerance characteristics in rice. In salt-sensitive rice species, some of the major preexisting proteins disappear and certain new proteins appear with an increase in salinity. Ten-day-old seedlings of rice cv Nona bokra, Basmati, IR 28, and IR 29 when treated with 2% NaCl for 8 h show a synthesis of two new proteins of 27.0 and 25.5 kDa [9], whereas seedlings of certain rice cultivars when exposed to salinity stress accumulate 87- and 85-kDa stress-associated proteins [69]. Similar to rice, in citrus (*Citrus sinensis* L. Osbeck), a 25-kDa protein has been shown to be associated with salt tolerance [7]. This protein appears to be a constitutive protein in salt-tolerant citrus cells and is present whether cells are grown in the absence or the presence of NaCl. The level of this protein is enhanced when cells are grown in saline medium. Enhancement can be readily observed by growing the cells in the growth medium containing 1% NaCl.

Since the synthesis of constitutive proteins associated with salt tolerance in rice or citrus does not depend on the presence of salt in the growth medium, it appears that the salt-tolerance trait is stable in these species. Salt-tolerant lines in these species show synthesis of such constitutive proteins up to many generations when grown in either the presence or the absence of NaCl. Similar constitutive proteins have also been shown to be associated with sensitive or tolerant genotypes of finger millet. Among finger millet (*Eleusine coracana* Gaertn.) genotypes differing in tolerance to NaCl, a 200-mM NaCl treatment causes the synthesis of many stress-induced proteins with molecular weights of 70–72, 52, 37, 24, and 23 kDa in all genotypes. But in tolerant genotype GE-415, the synthesis of a 54-kDa protein occurs under NaCl treatment, which is not observed in the salt-sensitive genotype VL-481 [66]. This suggests that the synthesis of NaCl-induced proteins in finger millet is correlated with the differences in salt tolerance of the genotypes. In a species of citrus, either in cell suspension or when seedlings are grown in the presence of 0.2M NaCl, the steady-state level of a citrus-LEA5 (C-LEA5) protein increases [8].

In many plants species, certain hydrophilic proteins and their mRNAs have been reported to

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be synthesized de novo in response to salt stress. Some of these proteins and mRNAs are also inducible by water deficit or treatment with abscisic acid (ABA) [42]. Such proteins have been grouped in different classes based on DNA sequences of their genes and/or predicted functions of the proteins [70]. In most of the cases, the functions of the inducible proteins have not been clearly established, and predicted functions have been proposed based on deduced amino acid sequences [70]. Salinity imposition during the period of seed development following maturation leads to the synthesis of late embryogenesis–abundant (LEA) proteins in cotton, carrot, barley, and maize [71]. In vegetative organs of many other plant species, salinity-induced proteins have been identified which share significant amino acid sequences with the LEA proteins of cotton. Thus various groups of LEA proteins have been identified which have been classified based on notable structural domains predicted by amino acid sequences [70]. LEA group 1 proteins include the Em family of proteins which are devoid of cysteine and tryptophan. Such proteins have been identified in many monocots and dicots, and it is suggested that these proteins function in a water-binding capacity creating a protective aqueous environment [70]. LEA group 2 proteins include dehydrin, RAB (ABA-responsive), and D11 proteins which have characteristic lysine-rich regions and are also expressed owing to treatment with ABA [70]. LEA group 3 and group 5 proteins are represented by D7 and D29 from cotton, respectively, and contain repeated tracts of 11 amino acids [70]. Citrus cell suspensions grown in the presence of 0.2 M NaCl or leaves of citrus plants irrigated with NaCl accumulate a protein (C-LEA5) which has high similarity with the cotton LEA5 protein [8]. LEA group 4 protein represents D113 protein, the synthesis of which is induced in drying cotton seeds, and this protein has a homologue in tomato LE25 [70].

In addition to the inducible proteins which have predicted functions, there are many salt-induced proteins which have no specified functions. A protein, RD22, is induced early during seed development in *Arabidopsis* and has homology to an unidentified seed protein from *Vicia faba* [70]. In the roots of salt-stressed barley plants, a ''germin''-like protein has been identified [70]. Germin is a protein which accumulates during the early growth of wheat plants and has no specified function.

The most extensively studied proteins from many plant species that accumulate in response to dehydrative forces like salinity, water stress, and low temperature are dehydrins (LEA-D11 family), which are composed of several typical domains joined together in a few characteristic patterns with numerous minor permutations [72]. Although the fundamental biochemical mode of action of dehydrins has not been demonstrated, dehydrins regarded as are surfactants which are capable of inhibiting the coagulation of a range of macromolecules and thereby preserving structural integrity [72].

Transgenic rice plants expressing a LEA protein gene (HVA1) from barley showed accumulation of HVA1 protein in both roots and leaves, and such plants showed increased tolerance to salinity [73]. These observations suggest that LEA proteins play an important role in the protection of plants under salt-stress conditions, and that LEA genes hold considerable potential for use as molecular tools for genetic crop improvement toward salinity tolerance [73].

Protein Level in Salt-Stressed Plants

Protein synthesis in plants growing in saline environments is adversely affected. Salt stress results in a general decrease in protein synthesis with a loss of polyribosomes [42]. In germinating seeds, as well as during later growth stages of plants, salinity causes impairment in synthesis as well as degradation of proteins. To access the general impact of salt damage on plant growth and metabolism, various investigators have attempted to study the overall status of total proteins and soluble proteins and the pattern of protein synthesis in different parts of plants growing under salinity stress. Salinity in the majority of cases lowers the level of protein in salt-stressed plant parts as a result of the decreased synthesis of protein as well as the increased activities of protein-hydrolyzing enzymes. In certain cases, however, an increased protein level is noticed under salinization, possibly due to the increased synthesis of new salt-induced proteins or the decreased activities of proteolytic enzymes.

The process of protein synthesis has been shown to be salt sensitive, as observed in wheat germ and *Suaeda maritima* [43]. Under in vitro conditions, a protein-synthesizing system extracted

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from these species is more sensitive to Na^+, K^+, and Cl^- than under in vivo conditions, as revealed by amino acid incorporation data. A salt-tolerant species like *S. maritima* can maintain optimum growth at a high salt level of 500 mM NaCl, whereas the enzymes extracted from this plant are sensitive to a low level of NaCl (170 mM). This suggests that, under in vivo conditions, soluble enzymes as well as enzymes of protein-synthesizing mechanisms are comparatively less sensitive to NaCl than isolated enzymes under in vitro conditions. Although studying the effects of salts on protein synthesis in *S. maritima*, Hall and Flowers [74] observed that, in this species, protein synthesis is sensitive to salts and that amino acids incorporation decreases under in vitro conditions when KCl concentration in the medium exceeds 50 mM.

In various crop species, a decrease in the protein level in salt-stressed plant parts is attributed to a decrease in protein synthesis, the decreased availability of amino acids, and the denaturation of the enzymes involved in amino acids and protein synthesis [49,74,75]. In pea roots, sodium salts inhibit the synthesis as well as hydrolysis of basic proteins. Thus, there is a decrease in the protein level as observed in roots of pea plants growing under saline stress [75]. In chickpea (*Cicer arietinum* L.), one of the major legume crops for semiarid tropics, a salinity treatment with 100 mM NaCl in nutrient solution caused a marked decrease in the level of proteins in developing seeds when plants were raised in sand cultures [76].

When rice (*Oryza sativa* L.) seeds were germinated under increasing levels of NaCl salinity, a decrease in total as well as soluble protein level was observed in the embryoaxes [52]. A greater decrease in protein level was observed in the embryoaxes of salt-sensitive cultivars than tolerant cultivars under similar level of salinization. Dubey and Rani [52] observed that under 14 dS m^{-1} NaCl salinity, the soluble protein level of embryoaxes of salt-sensitive rice cultivars Ratna and Jaya was reduced to almost one-third compared with nonsalinized seeds at 120 h of germination. A moderate salinity level of 7 dS m^{-1} NaCl had virtually no effect on the change in total and soluble protein levels in embryoaxes of germinating seeds of salt-tolerant rice cultivars CSR-1 and CSR-3, whereas a higher salinity levels caused a marked decrease in the protein level in the embryoaxes of these cultivars [52]. In barley plants, imposition of NaCl stress leads to a decrease in the leaf protein content and induces marked quantitative and qualitative changes in the polypeptide profiles affecting mainly the proteins with approximately equal mobility [49].

Although salinity causes decreased protein synthesis and increased proteolysis in various plant species, in many cases increased protein levels are observed under salinization in germinating seeds [52], growing seedlings [44], and different plant parts [54]. In germinating seeds, endospermic protein hydrolysis is suppressed under salinization. When seeds of rice cultivars differing in salt tolerance are germinated under increasing levels of NaCl salinity, it has been observed that salt treatment suppresses protein depletion from the endosperms of all cultivars, with greater suppression in the salt-sensitive cultivars than in the salt-tolerant cultivars [52]. A lower salinity level of 7 dS m^{-1} NaCl has virtually no effect on the change in the endospermic total and soluble proteins compared with seeds germinating without NaCl, whereas a higher salinity level of 14 dS m^{-1} NaCl caused a marked suppression in endospermic protein depletion in the salt-sensitive rice cultivars Ratna and Jaya [52].

An apparent increase in the protein level in the endosperms of germinating seeds is observed with an increase in salinity. This can be explained as a result of decreased proteolysis caused by salinity leading to slower depletion of reserve proteins, not as a result of enhanced protein synthesis [52]. The NaCl salinity caused a delay in the breakdown of endospermic proteins as well as inhibition in translocation of hydrolyzed products from endosperms to growing embryoaxes [57]. The obvious implication is that inhibition of seedling growth under salinization can also be partly attributed to delayed mobilization of reserve proteins, because proteolysis is probably the primary, but essential, step toward synthesis of new proteins for seedlings growth [77].

When raised under increasing levels of NaCl salinity, rice seedlings, show an increased level of total as well as soluble proteins compared with nonstressed seedlings [44]. During a 5- to 20-day growth period, when two sets of rice cultivars differing in salt tolerance were examined for the metabolic status of proteins in roots and shoots, it was observed that at a salinity level of 14 dS m^{-1} NaCl salt, stressed seedlings of tolerant cultivars maintained higher levels of total as well as

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soluble proteins compared with the seedlings of the sensitive cultivars [44]. The increased soluble protein level in rice seedlings became more significant when the salinity level was raised from 7 to 14 dS M^{-1} NaCl [44].

Similar to rice, in cowpea (*Vigna unguiculata* L.) seedlings, pea (*Pisum sativum* L.), and *Cajanus Cajan* plants, as well as in soybean callus cultures, NaCl salinity caused an increase in the protein content [59,78–80]. The increased protein level under salinization as noted in these cases appears to be due to the increased synthesis of preexisting as well as certain new sets of proteins [44]. The increased synthesis of certain specific proteins has been noticed in plants subjected to saline stress, but whether this increased synthesis is responsible for a net increase in the total and soluble protein level of stressed plants remains to be investigated.

To understand the mechanism of salt tolerance in crops, various investigators have studied the metabolic status of proteins and amino acids in germinating seeds and seedlings using cultivars differing in salt tolerance [44,52–54]. Especially in rice (*O. Sativa* L.), a staple food crop for the majority of the world population, salt-tolerant cultivars are characterized by a higher value of protease-specific activity as well as a higher total and soluble protein content in germinating seed parts under control and salt treatments compared with sensitive cultivars [44]. Further, tolerant rice cultivars maintain a higher level of total as well as soluble proteins in salt-stressed seedlings compared with sensitive seedlings [44]. This shows the salt-tolerance ability to be associated with a possible higher protein level in rice, seemingly endogenous proteins that are either not found or are very poorly expressed in sensitive cultivars.

Using a salt-tolerant rice variety, Pokkali, and a salt-sensitive variety, Taichung N1, a cDNA clone *oslea 3*, encoding a group three LEA proteins, was identified that accumulated to higher levels in the salt-tolerant variety compared with the sensitive one on imposition of salt stress [53]. Further, this stress-induced protein and its mRNA declined less rapidly on sustained salt shock in tolerant cultivars than the sensitive ones. Such observations indicate that the differential regulation of protein expression is associated with varietal differences in salt-stress tolerance [53].

Soybean (*Glycine max*) cultivars differing in salt tolerance show different levels of proteins and amino acids when grown in the presence of NaCl [54]. Salt-tolerant soybean cultivars Clark and Forest accumulate higher levels of soluble proteins, whereas sensitive cultivar Kint shows a decrease in the soluble protein level when grown in saline soils [54]. Such observations indicate that, in rice and soybean plants, the salt-tolerance ability is associated with a higher level of proteins, which are seemingly endogenous proteins that are either not found or are very poorly expressed in sensitive cultivars on imposition of salinity.

Enzyme Levels in Salt-Stressed Plants

Salinity induces changes in the activities of proteolytic [52,56,57], amylolytic [57,81,82], nucleolytic [83], phosphorolytic [60,62,84–86], oxidative [63], antioxidant [87], photosynthetic [49], and nitrogen assimilatory enzymes [88], in germinating seeds and in growing plants. Salinity causes either an increase or a decrease in the activity of enzymes, depending on the nature of the enzymes, extent of stress, the plant parts studied and the genotypes of plant species differing in salt tolerance.

In endosperms of germinating rice seeds, salinity causes a decrease in the activities of hydrolytic enzymes, including α-amylase, protease, RNase, phosphatase, and phytase [52,57,81,83]. The decrease is more in salt-sensitive than in salt-tolerant varieties. In growing seedlings of rice, salinity enhances the activities of nucleases [83], proteases [56,83], peptidases [56], phosphatases [60,62,84], and oxidases [61]. Genotypes of rice species differing in salt tolerance maintain different levels of salinity-induced activities of enzymes.

Barley plants grown in presence of 200 mM NaCl show stimulation in β-amylase activity in leaves [82]. β-Amylases are regarded as stress-induced proteins in barley [82]. Certain enzymes involved in the synthesis of osmolytes show a marked increase under salinity stress [71]. The enzymes of proline biosynthesis, Δ^{-1}-pyrroline-5-carboxylate synthetase and Δ^{-1}-pyrroline-5-carboxylate reductase, the penultimate enzyme of betaine biosynthesis, betaine aldehyde dehydrogenase, and the enzyme of sorbitol biosynthesis, aldose reductase, show increased activity in many plants subjected to salt stress [10,71]. Plant genes that code for key enzymes involved in osmolyte biosynthesis

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have been isolated from barley, spinach, sugar beet, soybean, and rice plants [71]. Salt-tolerant varieties of rice show a higher level of expression of the enzyme Δ^{-1}-pyrroline-5-carboxylate synthetase and its mRNA compared with salt-sensitive varieties when grown in saline medium [10]. The enzymes involved in membrane transport, such as plasma membrane–ATPase in cotton seedlings [86] and Ca^{2+}-ATPase in tomato plants [71], show a higher level of activity under salinization. An increased turnover of the tonoplast H^+-ATPase has been observed in leaves of *Citrus sinensis* plants under salinity stress [85].

The activities of the oxidative enzymes polyphenol oxidase and indole-3-acetic acid oxidase increase in the seedlings of salt-tolerant as well as salt-sensitive rice cultivars under salinization, and the extent of the increase differs in the two sets of cultivars [63]. Similarly, phosphohydrolases show varying behavior under salinity in rice plants of differing salt tolerance.

The changes in the activity behavior of the enzymes acid phosphatase, alkaline phosphatase, and ATPase isolated from the chloroplasts of two sets of rice seedlings differing in salt tolerance, when grown under increasing levels of NaCl salinity, are shown in Table 1. As it is evident, acid phosphatase activity was more inhibited owing to salinity in salt-sensitive cultivars compared with the salt-tolerant ones, whereas the activity of alkaline phosphatase increased in the salt-sensitive seedlings but not in the salt-tolerant ones. Further, salinity caused enhancement of ATPase activity in both sets of rice seedlings, with greater enhancement in tolerant cultivars than the sensitive ones. The activity of the antioxidant enzymes superoxide dismutase, peroxidase, and glutathione reductase increase in callus cultures of *Citrus limon* [87], *Oryza sativa* [59], and *Medicago sativa* [89] grown in the presence of NaCl. In soybean callus cultures, an increase in glutathione reductase activity in response to NaCl constitutes an adaptive response of callus tissues to NaCl [59].

The level and activity of the key enzyme of photosynthesis in C_3 plants, ribulose-1,5-bisphosphate carboxylase, decrease in barley plants on imposition of NaCl stress [49]. The prime enzyme of nitrate assimilation, nitrate reductase (NR), has been extensively studied for its behavior in different plant species under salinization [58,88]. Salinity effects on NR activity are varied and depend on the type and extent of salinity as well as genotypes of the plants studied. In intact tissues of wheat, lentil (*Lens esculanta* Moench), mulberry (*Morus abla*), sorghum, and tobacco plants, NR activity decreases owing to NaCl salinity [88], whereas in rice plants the behavior of NR varies in genotypes differing in salt tolerance when raised under NaCl salinity [90]. The salt-sensitive rice cultivars Ratna and Jaya show a decrease in NR activity in the shoot and root, whereas salt-tolerant

TABLE 1 Salinity-Induced Alterations in the Activity Behavior of Phosphorolytic Enzymes in Chloropasts of 20-Day Grown Rice Plants.

Rice cultivars	NaCl treatment (dSm^{-1})	Acid phosphatase	Alkaline phosphatase	ATPase
	0	4.00	0.68	0.12
CSR-1 (T)	7	3.20	0.50	0.15
	14	3.00	0.45	0.22
	0	4.30	0.70	0.12
CSR-3 (T)	7	3.20	0.64	0.16
	14	3.00	0.48	0.26
	0	3.20	0.62	0.06
Ratna (S)	7	2.60	0.70	0.08
	14	1.40	0.76	0.11
	0	2.80	0.59	0.05
Jaya (S)	7	2.00	0.64	0.08
	14	1.20	0.68	0.14

T and S in parentheses indicate tolerant and sensitive rice cultivars, respectively. Enzyme units are expressed as μmol substrate hydrolyzed h^{-1} mg^{-1} protein.

cultivars CSR-1 and CSR-3 show increased activity of the enzyme in both of these tissues under salinization [90].

Isoenzyme profiles of many enzymes are influenced by salinity. In certain cases, some of the molecular forms of enzymes present in nonsalinized plants disappear in stressed plants, whereas in other cases certain new molecular forms of enzymes appear under salinization. In shoots of 15-day-old nonsalinized rice seedlings, four acid phosphatase isoenzymes were observed, whereas when seedlings were raised at a salinity level of 14 dS m^{-1} NaCl, only one isoenzyme remained detectable [84]. The decreased number of acid phosphatase isoenzymes at a higher level of salinization paralleled the decreased activity of the enzyme under such conditions [84].

In the young embryoaxes of germinating seeds, certain new molecular forms of acid phosphatases appear under salinization. When acid phosphatase isoforms from the embryoaxes of germinating seeds of the salt-sensitive rice cultivar Jaya and the salt-tolerant cultivar CSR-1 were compared at 48 and 96 h of germination under increasing levels of NaCl salinity, it was observed that certain new isoenzyme forms appeared in both sets of cultivars under salinization [60]. Further, a greater number of isoenzymes were observed in the embryoaxes of salt-tolerant rice varieties than salt-sensitive varieties under both controls as well as salt treatments [60]. Therefore, under salinization, certain isoforms of acid phosphatase are not synthesized, whereas the synthesis of certain new isoforms is induced depending on the plant parts and the genotypes studies. It has been shown that salt tolerance is associated with the presence of a large number of acid phosphatase isoenzymes [60].

The specific activities and patterns of peroxidase and superoxide dismutase isoenzymes are altered significantly in plants subjected to salinity stress. When rice seedlings were raised under an increasing level of NaCl salinity, certain new isoforms of peroxidases appeared, and the intensities of some of the preexisting isoenzymes increased, especially in the salt-sensitive varieties [61]. Different patterns of peroxidase isoenzymes were observed in the two sets of rice cultivars differing in salt tolerance. In 15-day-old seedlings of a salt-tolerant rice cv CSR-1, three isoenzymes were observed in roots and five in shoots, whereas in a salt-sensitive cv Ratna, six isoenzymes were observed in the roots as well as in the shoots [61]. Salt-tolerant embryonic callus cultures of lemon (*Citrus limon* L. Burm) exhibited an increase in the activity of antioxidant enzymes involved in oxygen metabolism with an increase of peroxidase activity and with the induction of a new superoxide dismutase isozyme [87]. These studies and other similar studies suggest that peroxidase and superoxide dismutase isoenzymes can serve as useful markers in the analysis of gene functions and metabolic regulations, including salt-tolerance characteristics [61].

Like acid phosphatases and peroxidases, isoenzyme profiles of ribonucleases [91] and α-amylases [81] are also influenced under salinization. The presence of different isoenzyme patterns of phosphatases, peroxidases superoxide dismutase, and ribonucleases in salt-sensitive and salt-tolerant genotypes of crops strengthens the view that salt tolerance or sensitivity depends on the genetic and biochemical make-up of the species. Also, specific molecular froms of the isoenzymic proteins, which appear to be constitutive proteins, are possibly associated with the salt tolerance or sensitivity characteristics. However, the mechanism of the expression of intrinsic isoenzyme proteins related with sensitivity or tolerance and of those isoenzymic proteins that specifically appear under salinization remains to be investigated.

Water Stress

Water is the Earth's most distinctive constituent and is an essential ingredient of all life. Its deficit is one of the most common environmental factors limiting crop productivity. Drought is a natural calamity and has devastating effects on crop yields. Crop plants are frequently subjected to water stress during the course of their lifetimes. However, certain stages, such as germination, seedling, and flowering, are the most critical for water stress damage. Stress imposed during these stages drastically affects crop yields. Water stress reduces plant growth and manifests several morphological and biochemical alterations in plants ultimately leading to massive loss in yield. A reduction in

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the efficiency of key processes including protein synthesis, photosynthesis, respiration, and nucleic acid synthesis are among the biochemical manifestations of water stress. Water stress inhibits protein synthesis, induces the synthesis of small sets of stress-specific proteins, promotes important modification in gene expression, causes activation or inhibition in activities of many enzymes, and leads to changes in the ultrastructures of tissues. A considerable amount of work has been done by various groups of investigators in the last few years to understand the mode of protein synthesis in plant parts under water-stressed environments [11,14,17,19,72,92,93], the level of proteins in stressed plants [94,95], and the activities of key enzymes influenced under water stress [10,96,97,98].

Water Stress–Induced Proteins

Water stress causes an alteration in gene expression in plants leading to an inhibition of protein synthesis as well as enhanced synthesis of certain stress-specific proteins. Quantitative and qualitative changes occur in the synthesis of proteins in plants in response to water deficit. It is well documented that, in various crops, water stress causes tissue- and organ-specific differential genomic expression which results in changes in the patterns of protein synthesis in cells [11,19,92]. Plants growing in a water-stressed environment or cells undergoing adaptation to water stress show both a decrease as well as an increase in small sets of cellular proteins. Many of these proteins which are specifically synthesized under water stress have been isolated and well characterized [6,72,93,99].

Mild to moderate water stress decreases the efficiency of protein synthesis in plants, but such plants recover and their protein synthesis returns to normal when stress is reversed or plants are rewatered [11]. Imposition of water stress alters the status of the protein-synthesizing complex polyribosomes in the tissue. The content of Polyribosomes decreases with one type of water stress, and the extent of such a decrease varies among different plant species and even in different organs of the same plant [11,100]. Studies in maize [11] and wheat [100] have indicated that increasing the level of water stress causes a decrease in the polyribosome level. Plant species which can survive under water stress show a greater capacity to produce polyribosomes in the tissues.

Various investigators have demonstrated the synthesis of water stress–specific proteins in different crops [6,14,17,19,72,92,93,99]. Many of these proteins also appear in response to the application of abscisic acid (ABA), suggesting that ABA is a signal in the stress response. Genes encoding these proteins have been isolated and studied using DNA probes. Like salinity stress, water stress–inducible proteins have been grouped in several families depending on the DNA sequences of genes, their expression characteristics, and their predicted functions.

Major families of water stress–induced proteins have been described as LEAs (late-embryogenesis abundant), RABs (responsive to ABA), dehydrins, and vegetative storage proteins [42]. LEA proteins have been further subdivided into several group: group 1 (D19 protein from cotton), group 2 (D11 from cotton), group 3 (D7 from cotton), group 5 (D29 from cotton). Many of these proteins are hydrophilic and are soluble on boiling, and are therefore expected to be located in the cytosol. It is predicted that most of these proteins are involved in protecting cellular structures and components from dehydration associated with water deficit.

Among proteins that accumulate in plants in response to dehydrative forces or water deficit, dehydrins have been the most commonly observed. Barley, maize, pea, and *Arabidopsis* plants show increased synthesis of dehydrins under osmotic stress [71]. Dehydrins are composed of several typical domains joined together in a few characteristic patterns with numerous minor permutations. Dehydrin polypeptides are made up of less than 100 to nearly 600 amino acid residues [72]. Although the fundamental biochemical mode of action of dehydrins has not been demonstrated, it is believed that dehydrins are surfactants and thereby they inhibit the coagulation of a range of macromolecules and preserve the structural integrity of the cell [72]. Genes encoding dehydrins also are ABA regulated. In dehyrated leaves of tomato, maize, and *Arabidopsis* plants, endogenous ABA levels increase with the simultaneous increase in dehydrins and its mRNA [70]. Dehydrins are localized primarily in the cytoplasm of root and shoot cells [70].

In certain plant species, synthesis of dehydrin-like proteins has been observed under osmotic

stress or under treatment with ABA. In *Stellaria longipes* the synthesis of a dehydrin like protein is induced as a result of treatment with ABA or under osmotic stress [17]. Sequence analysis of this protein indicates that it shares some similarity in structural features with dehydrins of other plants and also exhibits certain unique characteristics [17]. In castor bean, the synthesis of dehydrin-like proteins is tissue specific and is dependent on the physiological stage of the seed. Patterns of water deficit–induced dehydrin-related polypeptides in endosperms differ from those induced during late seed development [45]. In drought-stressed roots and shoots of *Lathyrus sativus*, dehydrin-like transcripts accumulate, which are also expressed in unstressed seedlings owing to ABA treatment [101]. A novel protein with 40-KDa molecular weight has been detected in pea plants under dessication. The deduced amino acid sequence of this protein indicates two lysine-rich blocks; however, the remainder of the sequence differs markedly from other pea dehydrins [13]. By analogy with heat-shock cognate proteins, this protein has been designated as dehydrin cognate [13].

Like dehydrins, the RAB and D11 (group 2) family of proteins are also ABA regulated and possess a characteristic lysine-rich region with consensus amino acid sequences repeated at least two times. Proteins of this family have been identified in many plant species, including maize, tomato, wheat, alfalfa *Arabidopsis*, rice, and castor [45,70,71,102]. LEA group 1 (D19) proteins, which are devoid of cysteine and tryptophan, have been detected in cotton, barley, and carrot under water deficit [71]. It is suggested that LEA group 1 proteins function in a water-binding capacity creating a protective aqueous environment [70]. LEA group 3 and group 5 proteins, which are represented by D7 and D29 proteins, respectively, from cotton contain a repeated tract of 11 amino acids. These proteins have been isolated from dessicating mature cotton embryos, chloroplasts of *Craterostigma plantagineum*, and citrus seedlings exposed to drought [8,70]. A citrus cell suspension in response to salt stress, leaves of citrus plants irrigated with NaCl, or seedlings exposed to drought lead to an osmotic stress–induced elevated level of LEA5 protein and its mRNA [8]. Another group of LEA proteins, group 4, is represented by D113 protein, which has a homologue in tomato and is expressed in drying cotton seeds [70,71].

Using a transgenic approach, it is suggested that LEA proteins play an important role in the protection of plants under water stress. Expression of the barley (*Hordeum vulgare* L.) LEA protein gene, *HVA1,* in rice cell suspension leads to a high level of accumulation of this protein, and such plants slow an increased tolerance to water deficit [73]. Osmotin, the 26-kDa protein which is synthesized and accumulated in cells undergoing osmotic adjustment to NaCl, also accumulates in cells undergoing osmotic adjustment to polyethylene glycol [6]. ABA, which is known to induce osmotic adjustment in cells, also induces the synthesis of osmotin. Osmotin synthesis is regulated by ABA, but its accumulation is dependent on the extent of water stress and the adjustibility of the cells to stress. Like dehydrins, osmotin also is the much extensively studied protein which accumulates under water and salinity stresses in several plant species like tobacco, triplex, tomato, and maize [71]. An osmotically regulated glycine- and threonine-rich protein was identified in rice by Mundy and Chua [102]. This protein is a product of an ABA-responsive gene *rab* 21. Osmotic stress imposed by polyethylene glycol or dessication leads to an increase in the level of ABA, and in turn the *rab* 21 gene is induced and expressed to synthesize this protein in rice tissues.

From rice cv *Tainchung* native 1, a 15-kDa protein that accumulates in the sheaths and roots of mature rice plants and seedlings when subjected to either osmotic stress or treated with ABA has been isolated and characterized [99]. Rice varieties show considerable differences in sensitivity to drought, however, in many of the varieties examined, water-deficit created as a result of PEG (polyethylene glycol) led the induced synthesis of one 26-kDa protein with a pI of 6 [16]. In certain varieties of rice, water stress causes accumulation of 87-and 85-kDa proteins, called stress-associated proteins (SAPs), that also accumulate under salinity and high and low temperatures [69].

A boiling-stable protein (BspA) has been shown to accumulate in shoots of *Populus popularis* plants under water stress [93]. In addition to BspA, plants also show accumulation of the water stress–related protein dehydrin (dsp-16) and sucrose synthase under water deficit [93]. In a highly drought-tolerant legume, cowpea (*Vigna unguiculata*), which shows about 160 times higher accumu-

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lation of ABA in drought-stressed conditions compared with unstressed plants, two cDNA clones, CPRD 8 and CPRD 22, which encode putative proteins that are related to old yellow enzyme and group 2 LEA proteins, respectively, were identified in drought-stressed plants [103]. However, in 10-h dehydrated cowpea plants, two additional cDNA clones, CPRD 12 and CPRD 46, were identified which encode putative proteins related to nonmetallo–short-chain alcohol dehydrogenase (CPRD 12) and chloroplastic lipoxygenase (CPRD 46). These genes are also induced under salinity stress [104].

Exposure of *Arabidopsis thaliana* to drought stress results in the accumulation of the RAB18 protein, and such plants develop enhanced freezing tolerance [15]. Progressive water deficit in whole *Solanum tuberosum* plants leads to about a 2.5-fold increase in leaf ABA content and the synthesis of two chloroplastic proteins of 32 and 34 kDa, named CDSP 32 and CDSP 34, which are synthesized in the stroma and in the thylakoids, respectively [67]. A 65-kDa protein with pI value of 5.2 has been shown to accumulate gradually in tomato leaves during water stress [105]. Quantification of this protein by gold labeling indicates that synthesis of this protein occurs in nuclei and chloroplasts as well as in some cytoplasmic regions of the cells in drought-stressed plants [105].

Certain additional families of proteins and their genes which are induced by water deficit have been identified in specific plant species. In *Arabidopsis*, 77.9- and 64.5-kDa hydrophilic proteins accumulate under water stress, and their genes, which are adjacent to each other in the genome, have been characterized from different laboratories [70]. Synthesis of these proteins also occurs with ABA application. Similarly, a family of genes and their products, glycine-rich proteins, which are hydrophilic and ABA responsive, have been identified in alfalfa plants under water stress [70]. However, no specific predictions about the functions of these *Arabidopsis* and alfalfa proteins have been made under stressful conditions.

A gene named ATHB-7, which belongs to a class of recently discovered homeobox genes found as yet only in plants, has been characterized in all organs of *Arabidopsis thaliana*. Expression of this gene and the synthesis of its proteins, called homeodomain-leucine zipper (HD-Zip) proteins, is induced severalfold under water deficit as well as by exogenous treatment with ABA [106]. It is suggested that ATHB-7 is transcriptionally regulated in an ABA-dependent manner and may act in a signal transduction pathway mediating a drought response [106]. Besides cytosolic and organellar proteins which are induced under water stress, in cell walls of bean (*Phaseolus vulgaris*) seedlings, two proteins, 36- and 33-kDa, have been identified that are glycosylated and accumulate when plants are subjected to the gradual loss of water [107].

Studies conducted by various investigators related to the effects of water stress on protein synthesis in many important crops suggest that water stress severely affects protein synthesis, alters gene expression and protein profiles in stressed tissues, and induces the synthesis of stress induced–specific proteins. Many of these proteins are hydrophilic and belong to specified families and have predicted functions in protecting the cells from water stress. Some of the stress-induced proteins appear to be tissue specific, whereas others appear not to be specific for any particular tissue or organ. Genetic expression studies reveal that among the stress-induced proteins which are well characterized, the majority are the product of ABA-responsive genes. How stress conditions signal an increased production of ABA, how ABA modulates the expression of these genes, and what is the functional role of stress-responsive proteins in dehydration tolerance, such as osmoprotectants, radical scavengers, protectants of subcellular organelles and macromolecules, or as regulatory proteins, remains yet to be investigated in detail.

Protein Level in Water-Stressed Plants

The levels of total as well as soluble proteins are altered in plants growing under water-stressed environments compared with plants growing under nonstressed conditions. Various workers have observed either a decrease [51,94,108,109,110] or an increase [94,95] in the levels of total or soluble proteins in different organs of plants subjected to water stress. The increased or decreased levels of proteins depend on the plant species and organ studied as well as the severity of the stress.

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Shah and Loomis [111] observed decreased contents of soluble and total proteins in sugar beet leaves from data recorded on a per gram of dry weight basis when the plants were subjected to progressive water stress. These investigators observed that the response to water stress was quick and could be reversed by rewatering the plants. This indicates that water-stress effects are reversible to a certain extent. According to Hsiao [108], the rapid response of plants under water stress and its quick reversibility by rewatering suggest that water stress affects protein synthesis mainly at the translation level. When Bermuda grass plants were subjected to increasing water stress, a decrease in the soluble protein level was observed [109]. In whole chloroplasts as well as chloroplast membrane fractions isolated from drought-resistant as well as drought-sensitive genotypes of water-stressed wheat plants, a decrease in the protein content was observed compared with nonstressed plants [112]. Mung bean seedlings differing in water stress tolerance, when raised under increasing water deficits, show a decrease in the protein level in the axis [94].

In *Lycopersicon chilense* plants as well as cell suspensions, water stress leads to the decreased synthesis of a proline-rich 12.6-kDa protein in the cell walls [110]. This is possibly attributed to the downregulation of its gene, designated PTGRP under dessication. In nodules of water-stressed (−2.03 MPa) pea (*Pisum sativum* L. cv Frilene) plants, about 30% decline in soluble protein level is observed compared with well-watered plants [51]. When rice varieties differing in water stress tolerance were examined for changes in the protein profiles in different organs due to water stress, it was observed that in the two cultivars, Sinaloa and IR 10120, the synthesis of several polypeptides decreased owing to PEG-induced water stress [16]. It is suggested that in rice, the extent of the decrease in the levels of proteins or changes in protein profiles in different organs due to water deficit are cultivar specific [16].

A decreased level of the total as well as the soluble protein contents in water-stressed tissue [94] appears to be due to more degradation of proteins as well as the overall inhibition in protein synthesis under water stress. It has been observed that water-stressed plant parts show a high protease activity compared with nonstressed plants [113]. The high activity of protease in water-stressed plants appears to be of adaptive significance, because it leads to the accumulation of free amino acids as a result of the degradation of proteins. Increased levels of free amino acids together with organic acids and quaternary ammonium compounds serve as compatible cytoplasmic solutes to maintain the osmotic balance between the cytoplasm and the vacuole under conditions of water stress [109].

Genotypes of crop cultivars differing in water-stress tolerance, when raised under increasing levels of water stress, show different levels of proteins as well as a specific activity of protease in the two sets of cultivars. Seedlings of drought-tolerant Mung bean genotypes show a higher protein content in embryoaxes as well as cotyledons compared with drought-sensitive genotypes when raised at a −10.0 bar moisture stress level [94]. Similarly, drought-resistant maize (*Zea mays* L.) cultivars show a high protease activity at higher levels of water stress, whereas inhibition in protease activity is noticed under higher water-stress levels in sensitive cultivars [113]. While comparing the total protein and free amino acid pool size in drought-resistant and drought-sensitive cultivars of *C. arietinum* and *Z. mays*, Rai et al. [95] observed that resistant plants are characterized by an increase over nonstressed plants in total protein and free amino acid levels.

Certain investigators have observed an increase in protein levels in plants subjected to water stress [94,95]. Genotypes of *C. arietinum* cultivars, differing in water stress tolerance, when raised under increasing osmotic potential levels, show increased protein levels in shoots compared with nonstressed plants [95]. A drought-resistant *Cicer arietinum* cv C-214 showed an increase of 60% protein over control at an osmotic potential of −3 atm, whereas a sensitive cultivar, G-130, showed a 15% increase over control under similar conditions of stress [95]. Similarly, when drought-resistant *Z. mays* cv Ageti-76 plants were grown under increasing osmotic potentials in the range of 1 to 10 atm, an increase in protein content was noticed, reaching 190% of control [95]. Similarly, in cotyledons of germinating mung beans under water stress, an increased protein level was noticed when they were compared with nonstressed germinating seeds [94]. These observations indicate that water stress has varying effects on the level of proteins in different crop species, and the stress-induced

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response depends on the species of crop examined, and it may vary even in different organs within the same species.

Enzyme Levels in Water-Stressed Plants

The normal metabolism of plants growing under water-stress conditions is adversely affected with a concomitant disturbance of the enzymatic constitution of the plants. Water stress lowers the level of many enzymes in the tissues [51,96,98,113–115]. The activities of certain enzymes increase as a result of water stress [10,70,97,108,113]. Nitrogen assimilation and photosynthetic efficiency are reduced in water-stressed plants mainly owing to the decreased activities of the key enzymes involved in these processes. Nitrate reductase (NR), the prime enzyme in the N-assimilation process, is markedly inhibited by water stress [108]. The effect of mild (−0.5 MPa) levels of water stress on the level of protein and the activities of enzymes NR, glutamine synthetase (GS), alanine aminotransferase (AlaAT), and aspartate aminotransferase (AspAT) in roots and shoots of 20-day grown followed by 24-h water-stressed rice plants is in Table 2. As it is evident from Table 2, water stress causes a drastic decline in the protein levels as well as in the activities of the enzymes of NO_3^- assimilation, NR and GS, whereas the key enzymes of amino acid metabolism, AlaAT and AspAT, show increased activity under a mild water stress level; however, under a moderate water stress level of −2 MPa, a pronounced inhibition in the enzyme activity is noticed. The nitrate reductase activity is directly associated with protein synthesis and plant growth, and both of these processes are adversely affected by water stress [116].

The photosynthetic apparatus is sensitive to dehydration. Water stress has a direct effect on carboxylating enzymes. The activities of the enzymes RuBP carboxylase and PEP carboxylase decreased in the leaves of plants subjected to water stress [114,117]. In sugar cane leaves, a decrease in the leaf water potential up to −0.37 and −0.85 MPa led to about a two to nine times decrease in the activities of RuBP carboxylase, PEP carboxylase, fructose- 1,6-bisphosphatase, NADP malic enzyme, and orthophosphate dikinase leading to an overall decreased rate of photosynthesis [114]. Water stress alters carbon partitioning in plant parts owing to an alteration in the activities of sugar-metabolizing enzymes. In leaves of sorghum plants, water stress reduces sucrose formation owing to an inhibition in the activities of fructose 1,6-bisphosphatase and sucrose phosphate synthase [115]. However, in potato tubers, moderate water stress leads to an activation of sucrose phosphate synthase and stimulation of sucrose synthesis [98]. More extreme water stress in potato tubers leads to a further alteration in carbon partitioning, because it inhibits the activities of one or more of the enzymes involved in the terminal reactions of starch synthesis [98].

Water stress leads to oxidative damage in plants by inducing the production of active oxygen species and decreasing the activities of the antioxidant enzymes catalase, peroxidase, and superoxide dismutase [51,96]. An examination of the involvement of activated oxygen in the drought-induced damage of pea nodules indicates that water stress (−2.03 MPa) caused a decrease in the activities of catalase (25%), ascorbate peroxidase (18%), dehydroascorbate reductase (15%), glutathione reductase (31%), and superoxide dismutase (30%) with a simultaneous decrease in the contents of ascorbate (59%), reduced glutathione (57%) and oxidized glutathione (38%) [51]. The overproduction of antioxidant enzymes provides an elegent approach to engineer plant species genetically for water-stress tolerance. Transformed alfalfa plants expressing Mn superoxide dismutase cDNA from *Nicotiana plumbaginifolia* have been shown to be more resistant to drought stress [118].

Levels of many enzymes increase under water stress. Many hydrolytic enzymes show an increased activity in water-stressed tissues. The α-amylase activity increased under water stress, which was responsible for increased starch hydrolysis in vivo, leading to increased levels of sugars and a decreased level of starch, as observed in water-stressed tissues [108]. Proteases have been shown to be induced under water stress. A thiol protease in pea and two cysteine proteinases in *Arabidopsis* have been identified which are induced under water deficit [70]. Certain hydrolytic as well as oxidative enzymes show different behaviors in the crop cultivars differing in water-stress tolerance. While investigating the behavior of drought-resistant and drought-sensitive *Z. mays* culti-

TABLE 2 Water-Stress Induced Decrease in Level of Protein and Alteration in Activity of Enzymes of N Metabolism in Roots (R) and shoots (S) of 20-Day Grown Rice Plants.

Rice cultivars	Water stress		Protein (mg g^{-1} fw)	Nitrate reductase (nmol NO_2^- min^{-1} mg^{-1} protein)	Glutamine synthetase (μmol-γ-glutamyl hydroxamate formed min^{-1} mg^{-1} protein)	Alanine aminotransferase (nmol pyrurate min^{-1} mg^{-1} protein)	Aspartate aminotransferase (nmol pyruvate min^{-1} mg^{-1} protein)
Ratna	0	(R)	13.00	7.80	0.56	18.00	42.00
		(S)	24.00	8.50	0.36	120.00	30.00
	−0.5 MPa	(R)	6.00	6.00	0.48	32.20	54.00
		(S)	18.00	7.50	0.20	141.00	67.50
	−2.0 MPa	(R)	2.50	1.80	0.36	7.00	18.00
		(S)	10.80	2.00	0.08	15.00	17.50
Jaya	0	(R)	6.00	9.80	0.82	30.00	38.20
		(S)	14.60	11.00	0.65	62.00	45.00
	−0.5 MPa	(R)	5.00	7.90	0.75	42.50	62.00
		(S)	9.20	8.00	0.38	112.50	75.00
	−2.0 MPa	(R)	3.50	1.05	0.30	5.40	22.50
		(S)	6.20	2.00	0.16	15.00	12.40

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vars for protease activity under water stress, Thakur and Thakur [113] observed an increasing trend in protease activity with an increasing osmotic potential in resistant cultivar Ageti-76, whereas in the sensitive cultivar Vijay, they observed a decreased protease activity under severe water stress. While studying the behaviors of certain hydrolytic and oxidative enzymes in the leaves of water-stressed rice plants of the two genotypes differing in stress tolerance, Goyal and Kochhar [119] observed that protease, ribonuclease, peroxidase, and IAA (indole acetic acid) oxidase activities were inhibited by water stress. However, the activity of ascorbic acid oxidase increased in the both sets of cultivars. Different responses for these enzymes were observed for the two sets of cultivars differing in stress tolerance.

In certain plant species, the increased synthesis of sucrose [93] and proline [58] occurs under water stress owing to a stress-induced increase in the activities of the enzymes synthesizing these metabolites. In *Populus popularis* plants, the accumulation of sucrose accompanied by the increasing activity of its synthetic enzyme sucrose synthase occurs under water deficit [93]. The activity of the enzyme Δ^{-1}-pyrroline-5-carboxylate synthetase (P5CS), which is involved in the biosynthesis of proline, increases in rice seedlings under dehydration [10].

Different behaviors of certain oxidative enzymes have been observed depending on the different methods of creating water stress as well as the plant parts studied. The activities of IAA oxidase and peroxidase increased in etiolated water-stressed seedlings of winged bean (*Psophocarpus tetragonalobus* L.) and amaranthus (*Amaranthus caudatus*) plants, whereas the decreased activities of two enzymes were observed in water-stressed green seedlings [120]. The higher activities of oxidative enzymes under water stress is possibly due to a gradual shift of reductive metabolism to oxidative metabolism under these conditions. While studying oxidative processes in rice plants differing in water-stress tolerance, Lodh et al. [97] observed an increased activity of peroxidase in drought-tolerant cultivars Lalnakanda-41 and T(N) 1 × T.65 as well as drought-sensitive cultivar CO-13. These investigators observed an increased catalase activity in drought-sensitive rice cultivar CO-13 but not in the drought-tolerant cultivars. Polyphenol oxidase activity has been reported to increase in leaves and roots of drought-sensitive rice cultivar CO-13, whereas, in drought-tolerant cultivars Lalnakanda-41 and T(N) 1 × T.65, the activity of the enzyme decreased in the leaves as well as the roots under water stress [97]. These observations suggest that water stress leads to changes in the levels of various enzymes in stressed plant parts and that the effects of stress depend on the properties of enzymes, severity of the stress, and organs of the plants studied.

Heat Stress

High-temperature or heat stress adversely affects plant growth and yield in many areas of the world. Some plants can survive when the temperature exceeds even 20°C above ambient, whereas in most of the field crops, temperatures above 40°C cause heat injury, severely limit photosynthesis, and alter protein metabolism by causing protein breakdown, protein denaturation, enzyme inactivation, and other effects.

When plants are subjected to heat treatments beyond optimum growth temperatures, the normal protein synthesis declines owing to a coordinate loss of translational efficiency of most mRNAs and the enhanced synthesis of a small set of proteins known as heat-shock proteins (HSPs) occurs. It is believed that heat tolerance in plants is associated with the synthesis of HSPs, which protect plants from otherwise nonpermissive temperatures and provide them with an endogenous protection system for thermotolerance. The phenomenon of heat-shock response (HSR) is conserved among all biological organisms. Although HSPs provide the molecular basis for thermotolerance, but whether they act directly in signal transduction or induce the synthesis of secondary agents involved in protection is not yet clear. The synthesis of HSPs occurs in diverse plant species when they are exposed to temperatures 10–15°C above growing temperatures [18–21,48,121–123].

Crop plants, such as maize, soybean, cowpea, and wheat, start synthesizing HSPs in the tissues with a rise in tissue temperature beyond 32–33°C [2]. The induction of HSP synthesis parallels the increase in temperature. It has been observed that exposure of plants to higher temperatures of heat

shock leads to the stability as well as the rapid induction of specific mRNAs related to specific HSPs [124].

Synthesis of Heat-Shock Proteins

The synthesis of HSPs occurs in plant cell cultures undergoing thermoadaptation or intact plants subjected to heat stress [2]. It has been shown that not only heat stress but other conditions, such as treatment with arsenite, heavy metals, ethanol [2], ABA, water stress, and wounding [19],induce some of the HSP mRNAs and lead to the expression of HSPs in plants. This shows that the synthesis of HSPs can be induced even in the absence of heat stress and high temperature protection can be provided without prior heat shock.

Cytoplasmic distribution and subcellular localization of HSPs indicate that they remain either specifically associated with various subcellular organelles, such as nuclei, chloroplasts, mitochondria, plasma membrane, or as cytoplasmic aggregates distinct from ribosome granules [2,18]. When the tissue temperature exceeds 32–33°C, HSPs are typically seen. The appearance of these proteins has been positively correlated with enhanced thermotolerance, and, as well, it also provides a certain level of cross protection to other kinds of stresses [42].

In carrot cells, heat-shock treatment causes an inhibition of protein synthesis with the simultaneous appearance of new proteins [125]. In carrot cells, patterns of these newly synthesized proteins become different depending on the growth stages of cells and culture conditions. It was shown by Kanakus et al. [122] that tobacco cell suspensions synthesize HSPs in different phases of the growth cycle. When maize tissues are exposed to heat shock, they show the induction of heat-shock protein mRNA and synthesis of a set of 10 HSPs [19,121]. Different tissues of the same plant, as well as different developmental stages of the tissues, show different pattern of HSPs [47].

In field-grown cotton (*Gossypium hirsutum* L.) plants, when the temperature reaches 40°C for a few weeks, synthesis of eight unique polypeptides of 100, 94, 89, 75, 60, 58, 37 and 21 kDa occurs [47]. These polypeptides accumulate in dryland plants that have a canopy temperature of 40°C and are absent in irrigated plants that have a 30°C canopy temperature. A group of 11 newly synthesized polypeptides accumulated in laboratory-grown heat-shocked cotton plants, as revealed by autoradiography of radiolabeled polypeptides. Of these 11 polypeptides, 8 appear to be similar to those of heat-shocked field-grown cotton plants [47]. These results suggest that dryland crops synthesize HSPs in substantial levels in response to high temperatures.

Many desert succulent plants have been shown to accumulate HSPs when day and night air temperatures are raised from 30 and 20°C to 50 and 40°C [48]. The pattern of accumulation of HSPs is species specific in these plants; however, a unique 25- to 27-kDa protein accumulated in all species examined, which appeared to be associated with thermotolerance in these plants [48]. Elevation of the culture temperature to 32°C for 8 h leads to irreversible de novo synthesis of a number of HSPs of a 70-kDa class: HSP68 and HSP70 in *Brassica napus* [20]. Five-day-old rice seedlings, when subjected to a temperature stress of 45°C for 1–2 h, synthesized and accumulated a 104-kDa polypeptide, which constituted about 0.4% of the total soluble protein fraction [123].

Among the wide range of HSPs which accumulate under heat shock, some are specifically associated with organelles, including the nucleus, nucleolus, chloroplast, mitochondria, and plasma membrane. Certain other HSPs are found to be associated with ribosomes or they remain as aggregates in the cytoplasm [18]. In pigeon pea (*Cajanus Cajan*) plants, heat-shock proteins of 18, 20, 22 and 24-kDa are found to be associated with mitochondrial and membrane fractions, whereas the 60-, 70-, and 81-kDa proteins are found in the soluble fraction [126]. In mitochondria of pea plants, a novel 22-kDa protein accumulates in the matrix when normal growth temperature is shifted from 25°C to 40°C [127].

Types of Heat-Shock Proteins

Heat shock induces the synthesis of a wide range of HSPs in plants. A general system of classification for these proteins is based on their molecular weights and their localization in the cell. Some of

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the common HSPs synthesized in plants include HSP70, HSP60, low molecular weight HSPs, and high molecular weight HSPs. Many HSPs, like HSP60, HSP70, and HSP90, are present as constitutive proteins in the cytoplasm as well as other organelles like mitochondria and chloroplasts of plants in nonstressed conditions and their level increases under heat shock. Studies indicate that HSPs function in a fashion similar to molecular ''chaperons'' and assist the self-assembly of nascent polypeptides into their correctly folded tertiary structures and also prevent the formation of an aggregation of nonfunctional proteins resulting from heat denaturation [42]. Especially small HSPs which range in size from 17 to 30 kDa and are encoded by six nuclear gene families, accumulate to high levels in response to heat stress and bind partially denatured proteins, preventing irreversible protein inactivation and aggregation, and thus contribute to the development of thermotolerance [21]. According to Harrington et al. [128], HSPs have a possible function in signal transduction involving protein kinases and heat shock–induced calmodulin-binding proteins.

Chilling

Chilling is one of the most severe constraints limiting crop productivity. Low environmental temperatures lead to chilling injury in plants and result in the loss of plasma membrane integrity, irreversible and proportional loss of proteins from the cell, and ultimately the death of the cell [46,129]. According to Levitt [129], freezing-induced dehydration within the cell leads to aggregation of proteins owing to the formation of disulfide bonds as well as the denaturation of soluble proteins. Synthesis of many key enzyme-proteins decreases when plants are exposed to low temperatures [24,25], and synthesis of certain specific proteins is induced [26].

Many enzymic proteins, especially of those carbon assimilation, are extremely sensitive to chilling. The key photosynthetic enzyme of C3 plants, Rubisco, which constitutes about 60% of soluble proteins, undergoes changes in structure, conformation, and properties at low temperatures [24,46]. In *Zoysia japonica* plants, a drastic decline in the level of C_4-cycle enzymes phosphoenolpyruvate carboxylase (PEPC) and phosphoenolpyruvate carboxykinase (PCK) takes place during exposure to low temperature. In *Lycopersicon esculentum* and *Zea mays* plants, chilling stress results in an irreversible loss of Rubisco activity and stromal fructose-1,6-bisphosphatase activity [24]. In the conifer *Pinus sylvestris*, the contents of D1 protein of the photosystemII (PSII) reaction center and of the PSII light-harvesting complex (LHCII) proteins decline under low-temperature stress [130]. Similarly, the —SH-rich enzyme glutathione reductase becomes partially inactivated by freezing [131]. It is suggested that the activity loss of many enzymes on chilling is as a result of a modification of sulfhydryl groups or other side chains of the protein [24].

In many plant species, chilling injury leads to build-up of reactive oxygen species, oxidation of proteins, and decline in the activities of catalase, ascorbate peroxidase, and glutathione reductase [132]. The tolerance of rice cultivars to chilling injury is closely linked to the cold stability of catalase and ascorbate peroxidase [132].

Cold Acclimation

When plants are exposed to low nonfreezing temperatures for a few hours or day, certain new sets of proteins are synthesized and these plants develop the capacity to adapt to a subsequent chilling or freezing temperatures. Such a mechanism of adaptation is known as cold acclimation (CA). Generally temperatures from 4 to 15°C are considered to be chilling, whereas a temperature below 4°C, is considered to be freezing [42]. CA results in altered gene expression leading to synthesis of specific proteins and certain enzymes which are responsible for the development of freezing tolerance [26,133,134]. Several preexisting proteins abundant in the tissues of plants grown under normal temperature decline on exposure to low temperatures. However, many new transcripts and polypeptides are synthesized [22,26,135], which appear to play a major role in acclimation of plants to freezing temperatures [26].

Plants differ in their capacity to tolerate low temperatures. In many crop plants, freezing

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tolerance can be induced by exposure to low nonfreezing temperatures. Freezing-tolerant or cold-acclimated plants possess new proteins which are not present in normal or nonacclimatized plants. Uemura and Yoshida [136] while studying cold acclimation in winter rye (*Secale cereale* L.) seedlings, observed that more than 20 proteins disappeared in the plasma membrane during acclimation, and the concentration of 11 proteins increased, whereas 26 new proteins were synthesized.

In young rapeseed *Brassica napus* seedlings, a 48-h exposure to nonchilling temperatures induces important changes in gene expression. The synthesis of specific polypeptides is increased with a concomitant increase in respective mRNA levels, whereas the synthesis of six polypeptiedes is suppressed with degradation of their corresponding mRNAs [22]. Transcripts of a gene encoding a putative cell wall plasma membrance linker proline-rich protein has been isolated by Goodwin et al. [137] form *Brassica napus* leaves, which are specifically expressed in leaves on exposure to low temperature. This indicates that an increase in the level of specific mRNA transcripts and their corresponding proteins is correlated with improved freezing tolerance [22]. The CA of rapeseed seedlings leads to a decreased level of mRNA for the Rubisco small subunit as well as the reduced synthesis of this protein [22]. In cold-sensitive rice (*Oryza sativa* L.) plants, CA leads to suppression as well as induction in gene expression resulting in a decreased level of certain proteins and the increased synthesis of other specific proteins as well as corresponding mRNAs [23]. Hahn and Walbot [23], while studying the effect of cold treatment on the pattern of protein synthesis in rice leaves, detected several novel proteins of 95, 75, 25, and 21 kDa that were synthesized during 1–7 day of 11 and 6°C cold treatment. These proteins were cold specific; other stresses, such as water stress, salinity, and acid treatment, could not induce the synthesis of such proteins.

In freezing tolerant cereal plants, such as rice, wheat, and barley, antifreeze proteins are synthesized during CA, which play significant role in increasing freezing tolerance [26,134]. Six antifreeze proteins which have the unique ability to absorb onto the surface of ice and inhibit its growth have been isolated from the apoplast of winter rye leaves where ice forms at subzero temperatures [26]. Among the rye antifreeze proteins, two are endoglucanase-like, two chitinase-like, and two thaumatin-like proteins [26]. The accumulation of antifreeze proteins is not a general response to all plants, but it is a specific response that is important in the freezing tolerance in certain plants.

In certain plants, such as citrus [138] and some herbaceous species [139], a very high molecular weight protein has been identified that is specifically synthesized under CA. Durham et al. [138], while comparing polypeptide patterns resulting from in vitro translations of total RNA isolated from cold-acclimatized and non–cold-acclimatized leaf tissues of cold-sensitive *Citrus grandis* plants, observed a 160-kDa polypeptide in cold-acclimatized leaves that was not present in non–cold acclimatized citrus leaves. This 160-kDa unique polypeptide has also been detected in cold-acclimatized spinach and sweet orange, *C. sinensis* [139].

In *Arabidopsis thaliana*, two glycine-rich proteins, MSACIA and MSACIB, accumulate during CA. Timing and localization of the expression of these two proteins are different and the differential expression involves both transcriptional and posttranscriptional events [140]. Comparisons among different cultivars of *A. thaliana* suggest that low freezing tolerance is associated with the failure to accumulate these proteins. Mantyla et al. [15] have shown the accumulation of ABA-responsive RAB18 and LT178 proteins in *A. thaliana* plants under CA. In floral buds of a woody perennial blueberry (*Vaccinium*), three different dehydrin-like lysine-rich proteins of 65, 60, and 14 kDa accumulated in response to chilling [141]. Accumulation of high levels of dehydrin transcripts has also been observed in field-grown freezing-tolerant bromegrass (*Bromus inermis* L.) and rye (*Secale cereale*) plants [142]. Field-acclimated plants accumulating high levels of dehydrin transcripts have been regarded as being more freeze tolerant [142].

There does not appear to be any uniform pattern of protein synthesis among various plant species during CA. This implies that CA-induced proteins are not highly conserved as heat-shock proteins. A characteristic feature of CA-induced proteins is that some of the synthesized proteins are transient, whereas others are stable, the synthesis of which continues for weeks [135].

The inheritance of freezing tolerance appears to be a multigenic phenomena, and the precise function of the proteins encoded by these genes is not fully known. Both transcriptional and posttranscriptional controls have been shown to be involved in the expression of these genes [143].

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Abscisic Acid and CA

It has been observed that exogenous ABA induces freezing tolerance in many plant species [133,144], although the physiological basis of this phenomena is poorly understood. In certain plants, an increase in the endogenous ABA level is observed following CA [42,144]. Plantlets of potato (*Solanum commersonii*) stem culture, when treated with ABA for 14 days, develop cold tolerance with the concomitant induction of 30 polypeptides [144]. Several specific translatable mRNA populations and their in vitro translation products have been identified following ABA treatment of potato plantlets [142]. It is suggested that ABA alters gene expression leading to the development of cold hardiness [145] by the synthesis of certain specific polypeptides that are similar to some of the polypeptides synthesized during CA [15,144]. The ABA has been shown to induce the synthesis of certain polypeptides that are not synthesized in CA tissues [144]. A comparative study of CA-induced proteins and ABA-induced proteins suggests that both CA and ABA induce the synthesis of specific and certain common proteins. This also suggests that the full development of cold tolerance requires the synthesis of complete sets of CA-induced proteins, because certain genes, in addition to those responsive to ABA, are involved in the development of maximum freezing tolerance [133].

Anaerobic Stress

Anaerobic stress is generally caused by excessively wet soil or flooding conditions. Anaerobiosis affects plant metabolism as a result of a low oxygen concentration in the rooting medium. Plants adapting to anaerobic stress switch from oxidative to fermentative carbohydrate metabolism [27]. Under anaerobic stress, normal protein synthesis is suppressed, associated with the loss of polysomes, and gene expression alters leading to synthesis of specific sets of novel polypeptides commonly known as transition polypeptides (TPs) and anaerobic polypeptides (ANPs). Repression of preexisting aerobic proteins and the synthesis of new proteins appear to be the immediate biochemical response of anaerobiosis [145]. Most of the studies related to protein synthesis under anaerobic conditions have been performed in maize [146], rice, [27] and *Arabidopsis* [147]. Transient polypeptides are translated primarily during the first 5 h of anoxia, and they are stable and last long after their synthesis declines, whereas ANPs appear approximately after 90 min of anoxia, and their synthesis continues for several days until cell death [42].

In maize, an anaerobic response causes de novo synthesis of about 20 ANPs [146]. Because of an anaerobic conditions in maize, initially the rapid synthesis of four 33-kDa transition polyeptides takes place, whereas after 90 minutes of anoxia, the selective synthesis of an additional 20 polypeptides occurs [28,146], which represent about 70% of the total proteins synthesized during anaerobiosis [146]. Anaerobic stress–induced proteins are different from heat-shock proteins except for a few that are common to both types of stresses [28].

Most of the ANPs are apparently involved in maintaining ATP levels in the cells. Many of these are enzymes involved in glycolysis or fermentative processes such as alcohol dehydrogenase (ADH), lactate dehydrogenase (LDH), aldolase, enolase, glucose-phosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase, pyruvate decarboxylase, and sucrose synthase [28]. Among these enzymes, ADH is the best characterized. In several tissues of maize examined, *ADH* gene expression is maximal with anoxia [42]. Similarly, in *A. thaliana*, two *ADH* genes exist; one set is strongly induced by low oxygen stress mainly in roots, whereas the other set is expressed constitutively in both roots and leaves [147]. In maize seedlings during several days of hypoxic induction, LDH activity increases up to 3.5-fold. This increased activity is the result of increased protein levels, which can be correlated with the induction of 2 *ldh* transcripts of 1.3 and 1.7 kb [29].

Ricard et al. [27] observed a significant increase in the level of sucrose synthase with a concomitant increase in its mRNA level in rice seedlings subjected to anaerobiosis. Unlike maize, only one sucrose synthase protein exists in rice. Its synthesis is enhanced with a concomitant increase in mRNA levels under anaerobiosis [27] that indicates that its level of control is possibly transcriptional.

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Two enzymes, which have different functions than ANPs, have been identified, the level of which increases in response to hypoxia. These are 1-aminocarboxylate-1-cyclopropane synthase (ACC synthase), which catalyzes the rate-limiting step in the synthesis of ethylene, and the other is xyloglucan endotransglycosylase, which is possibly involved in aerenchyma formation during flooding [28,42].

In maize seedlings, it has been observed that treatment with ABA increases tolerance to anaerobic conditions [148]. Such an induction of tolerance is partly attributed to the synthesis of new proteins. It was shown by Hwang and Van Toai [148] that cycloheximide, when added together with ABA, reduced the survival rate of maize seedlings. However, ABA-induced tolerance appears to be species specific, because results similar to those with maize are not observed in other crops.

Pathogenesis-Related Proteins

When plants are infected with pathogens, such as bacteria, viruses, and fungi, certain novel proteins are synthesized. These host-coded proteins, which are induced by a wide range of pathogens, are commonly known as pathogenesis-related (PR) proteins. Many of the PR proteins are also induced by abnormal concentrations of plant hormones, or they are due to the presence of pollutants such as heavy metals [38]. Since these proteins are synthesized during infection, they appear to have a possible role in inducing resistance against further infection by the pathogen [33].

The PR proteins form a heterogeneous family of plant proteins and have been grouped into different classes: 1, 2a, 2b, 3, 4, 5a, and 5b [42]. However, the functions of classes: 1, 4, and 5b PR proteins are not fully known, but it is believed that they are involved in the defense of the plant. Class 5a PR proteins have a possible role in pathogen resistance, whereas classes 2 (a and b) and 3 have been extensively studied and identified as β-1,3 glucanases and chitinases, respectively [42]. Class 5a PR proteins include thaumatin-like proteins. Osmotin, a 26-kDa protein, initially described in tobacco suspension cultures under salinity stress, belongs to the family of class 5a PR proteins, and it has antifungal activity [42].

Chitinases and β-1,3-glucanases are the best characterized PR proteins. Chitinases hydrolyze β-1,4-acetyl glucosamine linkages of chitin polymers, which are primary constituents of fungal cell walls; whereas glucanases hydrolyze β-1,3-glucan residues present in fungal cell walls [42]. The overexpression of chitinases and/or β-1,3-glucanases in transgenic plants provides considerable protection against fungal pathogens. Chitinases show an increased level in many plant species, including *Arabidopsis* [149], tobacco, wheat [150], pepper [151], tomato [152], and *Phaseolus vulgaris* [153], in response to infection by fungal pathogens or viruses. Three classes of plant chitinases—I, II, and III—have been described based on their basic or acidic properties as well as their localization within or outside the cell [149]. Chitinases I and II, which are basic and acidic proteins, respectively, are expressed in an organ-specific and age-dependent manner in uninfected plants. Class III chitinases are mainly extracellular cucumber chitinases which are induced and accumulate in the extracellular space after pathogen attack [149]. Chitinases have potent antifungal activity [150]. Four isoenzymes of chitinase (26, 27, 30, and 32 kDa) are induced in tomato plants on infection by the fungus *Alternaria solani* [152]. It is suggested that a higher constitutive level of chitinase and β-1,3-glucanase and the induction pattern of a 30-kDa chitinase isoenzyme in early blight–resistant breeding lines is related to genetically inherited resistance of tomato to *A. solani* [152].

Two class 1 PR proteins, designated as acidic PR-1 protein (PR1a1) and basic PR-1 protein (PR1b1), which are low molecular weight proteins and are encoded by two closely related genes, have been characterized in tomato plants [33]. The expression of these two proteins is also induced by salicylic acid and ethylene. In transgenic tobacco plants infected with tobacco mosaic virus (TMV), the PR1b1 gene is strongly activated locally in tissues undergoing an hypersensitive response but not systemically in uninoculated tissues [33]. In the primary leaves of *Phaseolus vulgaris* plants following infection with southern bean mosaic virus (SBMV), 10 acidic and 8 basic PR proteins have been identified, which include 4, 17-kDa serologically related, acidic proteins of unknown functions; 2 chitinases, 1 acidic (29 kDa) and 1 basic (32 kDa) possessing antifungal activities; and 4 (21, 28, 29 and 36 kDa) serologically related, acidic glucanases [154].

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Different isoforms of β-1,3-glucanases have been characterized in the infected tissues. Infection of groundnut leaves with the early leaf spot pathogen, *Cercospora arachidicola*, leads to a marked increase in the extracellular β-1,3-glucanase activity with the synthesis of its three isoforms [155]. In pepper (*Capsicum annum*) plants, it has been suggested that the glucanase activity is involved in the mechanism of resistance to cucumber mosaic virus in tolerant cultivars [151]. Osmotin-like proteins, which are class 5a PR proteins, have been shown to be encoded by at least six members of a multigene family in *Solanum commersonii* after infection by *Phytophthora infestans*. The expression patterns of two osmotin-like proteins in *Solanum commersonii* plants suggests their dual function in osmotic stress and plant pathogen defense [156].

Some of the PR proteins are present in healthy tissues and are differentially expressed by signals involved in flowering and reproduction. This implies that they are involved in the normal physiological processes of the plants in addition to plant defense [149]. According to certain investigators, salicylic acid is involved in the signal transduction pathway leading to a resistance to pathogen infection and the synthesis of PR proteins [140]. The salicylic acid level increases in plants following pathogenic attack [149]. In barley leaves, salicylic acid treatment induces the accumulation of two PR proteins and one salicylic acid–specific protein [157]. In tobacco plants, salicylic acid acts as an endogenous signal for the expression of acidic PR-1 proteins [158]. However, using transgenic tobacco plants accumulating high levels of soluble sugars due to the cytosolic expression of an inorganic pyrophosphatase from *Escherichia coli*, the possible role of soluble sugars in the induction of PR proteins has been suggested [32,41]. Such an induction appeared to be salicylic acid independent in the source leaves of tobacco plants [32]. According to Malamy et al. [159], multiple pathways exist that lead to defense response in plants, one of which appears to be independent of salicylic acid. More evidence is required to address the signal transduction pathways leading to the synthesis of PR proteins and the function of PR proteins in plant defense.

Wounding

Wounded plants manifest increases in the activities of many enzymes and the levels of proteins [34,35,160]. Enzymes and proteins that show an increased level in response to wounding include enzymes of the phenylopropanoid pathway, peroxidase DHAP synthetase, glycine-rich and hydroxyproline-rich cell wall proteins, protease inhibitors, and 1-aminocyclopropane-1-carboxylate synthase [160,161]. It is believed that some of these enzymes and proteins are involved in a lignification process and thus form a wound periderm to limit pathogenic attack [161]. Both glucanase and chitinase activities are induced in the roots and stems of chickpea (*Cicer arietinum* L.) plants in response to wounding [34].

In tobacco crown gall turmor tissues a 16-kDa glycine-rich hydrophobic polypeptide has been characterized, which is a cell wall protein and is induced by mechanical wounding [162]. This polypeptide is involved in the wound-healing process in tobacco plants by modifying the cell wall composition [162]. In tomato plants, several systemic wound-response proteins (swarps) have been described [35,160]. Mehta et al. [160] noticed the appearance of several novel proteins of 80.0, 63.0, 33.0, 28.5, 25.5, and 29.0 kDa and a decrease in the level of a 15-kDa protein as a result of wounding in tomato fruit tissues. These investigators noticed a marked difference in the mRNA populations after wounding. A full-length cDNA encoding an aspartic protease (LeAspP) was cloned from a tomato leaf cDNA library, the mRNA of which was shown to be systemically induced by wounding [35]. These observations suggest that wounding stress leads to differential protein accumulation and altered gene expression in tissues.

Metal Toxicity

Industrialization has led to the increased introduction of several heavy metals like Cd, Pb, Zn, Cu, and Hg in the soil environment. High levels of heavy metals in the soil adversely affect plant growth, cause the induction or inhibition of enzymes, and induce the synthesis of metal-binding cysteine-rich polypeptides called phytochelatins. Phytochelatins have a primary structure of $(\gamma\text{-Glu-Cys})_n$-

Gly or $(\gamma\text{-Glu-Cys})_n$-β-Ala, when n = 2–11, and have apparent function in the sequestration of metal ions within the plant.

Cadmium, which is a major environmental pollutant, inhibits the activities of many enzymes owing to its interaction with —SH groups of enzymes [36,37]. In rice seedlings raised under 500 μM Cd, an inhibition in the activities and a decrease in the synthesis of ribounclease and acid phosphatase isoforms have been observed [37,163].

When grown in the presence of Cd or Cu, many plants synthesize phytochelatins. However, several proteins with higher molecular weights than phytochelatins have also been reported in plants, which are induced by heavy metals [55]. A 18-kDa Cd-binding protein complex has been isolated by Shah and Dubey [55] from root tissues of rice plants grown in the presence of cadmium. This protein has four —SH groups per molecule and helps in the sequestration of Cd ions in rice tissues. Lupin roots exposed to lead, copper, or nitrite ions show an increased accumulation of a 16-kDa polypeptide, which appears to be a cytosolic Cu:Zn-superoxide dismutase [164]. From mercuric chloride–treated maize leaves transcriptionally activated cDNA clones have been isolated which represent various known proteins, such as glycine-rich proteins, PR proteins, chaperons, and membrane proteins [165]. A novel metallothionein like protein, the expression of which is regulated by metal ions, osmoticum or ABA, has been isolated from Douglas fir trees during embryogenesis [166]. These observations suggest that some of the heavy metal–induced proteins also are induced by other abiotic stresses.

Gaseous Pollutants

Ozone (O_3), sulfur dioxide (SO_2), and nitric oxide (NO_2) are considered as the major air pollutants. These molecules generate activated oxygen species, inhibit the synthesis of many proteins, and induce the activities of some antioxidant enzymes [40,167–170].

Ozone fumigation causes a decrease in the steady-state mRNA levels of genes encoding the small subunit of Rubisco, chlorophyll *a*/*b*–binding protein, and a 10-kDa protein of the water-evolving complex of PSII [169]. Similarly, in potato plants, ozone accelerates senescence with a decline in Rubisco small-subunit mRNA as well as a decline in transcripts of glyceraldehyde-3-phosphate dehydrogenase [167]. Plants grown in sites with high industrial pollution levels exhibit significantly low concentrations of soluble proteins [170].

Many antioxidant enzymes show an increased level when plants are exposed to ozone. Cytosolic Cu:Zn–superoxide dismutase is the best characterized enzyme, which shows increased activity with the simultaneous synthesis of new isoforms, in plants subjected to O_3 exposure [40,169]. In *Arabidopsis thaliana*, O_3 exposure enhances the activities of superoxide dismutase, peroxidase, glutathione reductase, and ascorbate peroxidase and modifies the substrate affinity of both glutathione reductase and ascorbate peroxidase [40,169]. However, in the chloroplasts of ozone-exposed plants, a decline in the levels of Fe–superoxide dismutase and glutathione reductase has been observed [169]. An ozone-induced transcript has been characterized in *A. thaliana* that encodes a 8.6-kDa basic protein which represents a novel stress-related protein [168]. It is suggested that major classes of ozone-induced proteins include antioxidant enzymes and a number of stress-related proteins associated with other biotic and abiotic stresses, and that ozone-induced responses are caused in part by the activation of a salicylic acid–dependent signaling pathway [168].

UV Radiation

Owing to the depletion of stratospheric ozone, in the future, the influx of solar UV-B radiation (280–320 mm) will tend to increase. UV-B radiation inhibits the growth of plants, causes inhibition in protein synthesis [171], and induces the activities of peroxidase-related enzymes [40] and the enzymes of flavonoid biosynthetic pathway [172]. Leaf protein biosynthesis is rapidly inhibited by UV-B radiation [172].

The chloroplast appears to the main target of UV-B radiation damage. Very early events of

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UV-B damage include the decrease of mRNA transcripts for the photosynthetic complexes and other chloroplast proteins [171]. In pea leaves, exposure to UV-B radiation for 7 days causes a rapid inhibition in protein synthesis and a reduction in mRNA transcripts for the chlorophyll *a*/*b*–binding protein [173]. Genes encoding defense-related enzymes, for example, of the flavonoid biosynthetic pathway, are rapidly upregulated on UV-B exposure. The activity of flavonoid-synthesizing enzymes, especially phenylalanine ammonia-lyase, 4-coumarate:CoA ligase, chalcone synthase, and UDP-apiose synthase, are induced in a coordinate way with UV-B treatment [172]. Peroxidase-related antioxidant enzymes are induced under UV-B exposure [40]. In *Arabidopsis thaliana*, UV-B exposure preferentially enhanced guaicol-peroxidases, ascorbate peroxidase, and peroxidases specific to coniferyl alcohol and modified the substract affinity of ascorbate peroxidase [40]. In certain plants, the synthesis of some novel proteins has been reported under UV-B irradiation [38,165,174]. UV-B exposure in sunflower leaf disks causes the induction of PR3 and PR5 proteins [38], whereas expression of a membrane channel protein and PR proteins is induced in maize [165]. The accumulation of an atypical transcript encoding a 42.3-kDa polypeptide, and showing sequence similarity to O-methyltransferases (OMTs) from different plant species has been observed in barley leaves after UV-B treatment [174]. It is suggested that the response to increased levels of UV-B radiation is dependent on the developmental stage of the tissue and involves complex changes in gene expression [173].

CONCLUSIONS

In general, environmental stresses, including salinity, drought, heat, chilling, anaerobiosis, heavy metals, gaseous pollutants, and UV radiations cause an alteration in gene expression in plants leading to the induction of specific genes and an increased abundance of their translatable mRNAs and proteins. As a result, the increased synthesis of certain novel proteins occurs in stressed plants with a concomitant decrease in the level of certain preexisting proteins. These stress-specific proteins appear to endow plants with the capacity to adapt to a stressful environment by physiological and biochemical adjustments. Most of these stresses induce the synthesis of proteins specific for the particular stress. However, certain proteins are common and can be synthesized under more than one type of stress. For example, cold, drought, and salinity stresses cause the expression of some common proteins that can also be induced by treatment of normal tissues with abscisic acid. Many of the genes which are activated under stressed conditions have been isolated and sequenced. Most of the stress-induced proteins have been isolated and well characterized for their physicochemical properties and their amino acid sequences have been determined.

Although stress-specific proteins are thought to lend a protective role to the tissues against the deleterious effects of stress, the exact physiological functions of many of these proteins are not very clear. Further, experiments are necessary to determine the functions of these proteins. Proteins induced under salinity or water stress are believed to act as osmoprotectants, as regulatory proteins, or as enzymes of biosynthetic pathways for osmolytes. However, the functions of many proteins are still unknown. The HSPs are believed to perform many essential functions in normal as well as stressed cells. The molecular mechanisms underlying these processes and the role of HSPs in protecting heat-stressed tissues remain to be elucidated. Specific proteins are synthesized during cold treatment that improve freezing tolerance in crops, but the functions of these proteins in the acquisition of cold tolerance remain to be established.

Further studies are required to define the detailed functional roles of many of these proteins, to specify the signal transduction pathways, and the mechanisms by which expression of stress-specific genes are controlled. Such studies will enlighten the mechanisms of stress tolerance in crops at the genetic level, and based on the gene-technological tools, it will be possible to introduce single or multiple genes associated with stress tolerance from a tolerant to a sensitive plant in order to produce plants with enhanced stress tolerance. Nucleotide probes specific for these genes can similarly be utilized for the selection of stress-tolerant varieties of crops.

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Heat-Shock Proteins and Temperature Stress

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INTRODUCTION

Plants growing in natural environments may encounter various stresses, such as high radiation stress, water stress, and high and low temperature stresses, during all or part of their growth and development. As plants cannot move to evade stresses and therefore have to endure any given environmental changes, they must have evolved mechanisms to adapt to these stresses.

In this chapter, we summarize the recent progress made toward understanding of the role of heat-shock proteins (HSPs) in terms of thermotolerance of plants and crops. A number of excellent reviews have been published, and the reader should consult for more detailed information and discussions concerning individual plant HSPs and for molecular ''chaperone'' functions [1–4] and for the regulations of plant HSP genes [5,6].

HEAT STRESS AND HEAT-SHOCK PROTEINS

Cellular Changes Induced by Heat Stress

Heat induces a wide variety of changes in cellular structures and metabolic processes. When the magnitude and duration of the heat stress exceeds a threshold, cells are irreversibly damaged and die.

In higher eukaryotes, heat shock perturbs many aspects of normal cellular physiology. Parcell and Lindquist (see Ref. 7 and references therein) described a number of changes and damages that heat produces. These are disruption of the cytoskeleton and microtubules, fragmentation of the Golgi apparatus, an increase in the number of lysosomes, swelling of mitochondria, a decrease in respiration and oxidative phosphorylation, disruption of normal protein synthesis, disappearance of polysomes, disruption of the splicing of mRNA precursors, cessation of pre-rRNA processing and a decline in transcription by RNA polymerase I, perturbation in DNA synthesis, inhibition in chromatin assembly, and a number of changes in cell membranes. Higher plants are no exception in terms

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of all these heat-induced perturbations. Some observations to support this view are rapid degradation of polysomes into monoribosomes and subunits when an excised soybean hypocotyl was incubated at 40°C [8], repression of most normal protein synthesis and initiation of transcription/translation of a set of proteins [8–10], disruption of pre-rRNA processing and ribosome assembly in tomato cell cultures [11], uncoupling of respiration from oxidative phosphorylation in soybean mitochondria [12], and disruption of the splicing of *Arabidopsis* HSP81-1, 81-2 mRNA precursors [13], and other structural changes [14,15].

Among these changes, the synthesis of a set of proteins known as HSPs which is a ubiquitous phenomenon in all prokaryotes and eukaryotes appears to be particularly important for the protection of cells from thermal damage, as will be described in the following sections.

Correlation Between the Level of HSP Accumulation and Thermotolerance

The importance of the HSP induction in cellular thermotolerance comes from the apparent correlation between the level of HSP accumulation and thermotolerance.

When whole organisms or cultured cells are given short treatments at moderately elevated temperature, their resistance to be killed by extreme heat increases dramatically [7]. For example, when yeast cells grown at 25°C are pretreated at 37°C, they can survive at 50°C 1000-fold better than nonpretreated cells [7]. This increase in thermotolerance is observed in virtually every organism studied [7].

Such tolerance-inducing treatments generally also induce the synthesis of HSPs [7]. This induction is rapid and intense, suggesting that it should be an important emergency response. The extent of the HSP induction appears to be correlated with the dose of heat stress subjected to an organism. As shown in Figure 1, the expression of HSP (GroEL, an HSP60 homologue) increases proportionally to the temperature increase within a certain range of temperatures (H. Nakamoto, unpublished results). The temperature of maximum HSP synthesis varies among species and is positively correlated with the optimal growth temperature of each species [1].

Besides a short and rapid increase in temperature, a treatment that has often been used for an experiment in a laboratory but may not have relevance to plants in the field, a gradual increase in temperature also confers thermotolerance in plants [10]. When the temperature was gradually increased at the rate of 3°C/h, soybean seedlings responded differently to the temperature shift from those given a sudden heat shock [10]. There was an upward shift of several degrees in the temperature at which maximum protein synthesis occurred. A gradual increase in temperature into the heat-shock range (40–43°C) enabled cells to survive (as measured by staining with Evans blue) when they were subsequently exposed to a lethal temperature (52°C). This gradual increase in temperature effectively elicited the synthesis of HSPs as well. Similar observations have also been made in other plant species [16].

A treatment at a nonpermissive temperature for a permissive period followed by a treatment under unstressed conditions also induces HSPs and results in thermotolerance. Lin et al. [17] showed that a brief (10-min) pulse treatment at a nonpermissive temperature (45°C) followed by a 28°C incubation for 2–4 h made dark-grown soybean seedlings tolerant to a subsequent exposure at 45°C for 2 h at the same degree of effectiveness with a preconditioning at 40°C for more than 1 h. They measured the thermoprotection by monitoring both seedling growth and protein synthesis ($[^3H]$leucine incorporation). This brief heat treatment was not long enough to kill the seedlings, although prolonged incubation (1–2 h) at 45°C resulted in death. During the preincubation either at 40 or at 28°C after a shift from a brief ''pulse'' treatment at 45°C, a set of HSPs were produced as the major newly synthesized proteins [17]. HSPs, whose genes are activated by heat shock and accumulate during exposure to a high yet permissive temperature or normal temperatures, seem to be involved in protecting vital cellular functions and structures at these otherwise nonpermissive temperatures.

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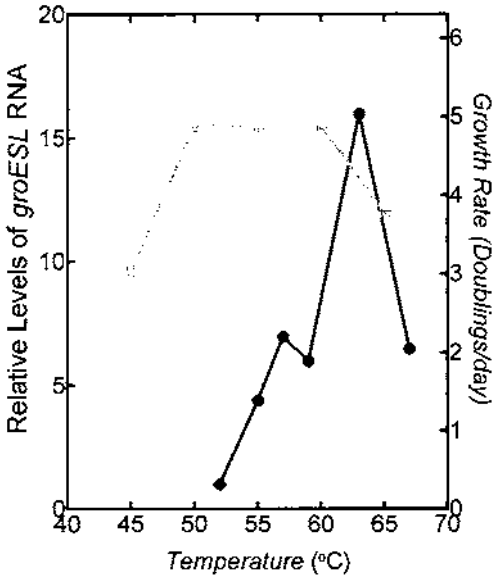

FIGURE 1 The influence of temperature on growth rate and accumulation of *groESL* mRNA in the thermophilic cyanobacterium *Synechococcus vulcanus* (H. Nakamoto, unpublished results). The growth rate is expressed as the reciprocal of days required for optical density at 730 nm of a liquid culture to double. The level of mRNA accumulation was determined by northern blot analysis of total RNA obtained from cells after shifting a culture (grown at 50°C) from 50°C to a different temperature and incubating for 30 min at that temperature. A specific radiolabeled probe for *Synechococcus vulcanus groEL* was used for the northern blot analysis [88], and the hybridization signals were quantified with a BAS1000 Mac bioimaging analyzer (Fuji Film, Tokyo).

A heat treatment for acclimation as shown above could also have some other effects than the induction of HSPs that might contribute to the induced thermotolerance as well. Several pieces of evidence, however, have indicated otherwise and further support the HSPs' major role in thermotolerance; a treatment of soybean seedlings with arsenite (50 μM for 3 h) at a regular temperature induced HSPs and the seedlings acquired thermotolerance [17]; and transgenic *Arabidopsis*, whose heat-shock transcription factor was genetically engineered to synthesize HSPs constitutively acquired thermotolerance without previous heat treatment [18]. These are just a few of many examples in prokaryotic and eukaryotic organisms to suggest that the acquisition of thermotolerance is correlated with de novo synthesis and accumulation of HSPs.

Plant species adapted to temperate environments, including crop plants such as soybean, pea, maize, and wheat, begin to synthesize HSPs when the tissue temperatures exceed 32–33°C [1] Therefore, it is likely that many crop plants reach heat-shock temperatures in the field, especially during summer and/or under nonirrigated conditions. Foliage temperatures increase as a consequence of the decline in water available for transpirational cooling. Under full sunlight, the leaf temperatures typically go up to 10–15°C above the surrounding air temperatures and often exceed 40°C (see Ref. 10 and references therein). Thus, the above-stated heat-shock response, that is, increased HSP synthesis, appears to have quite a relevance to plants in the field in coping with heat stress.

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HSP Families

Several classes of HSPs have been described in eukaryotes, including plants. They are designated by their approximate molecular masses in kilodaltons as HSP100, HSP90, HSP70, HSP60, 15- to 30-kDa low molecular mass HSPs (also called low molecular weight HSPs or small HSPs; the term *small HSP* will be used hereafter), and others [1–3]. A unique feature of the heat-shock response in higher plants is that small HSPs, ranging in size from 15 to 30 kDa, dominate the protein-synthesis profile of many plants during heat stress and accumulate in abundance [1–3]. Notably, plants typically produce up to 30 distinct small HSPs in response to heat stress, with each HSP belonging to one of six nuclear gene families [1,2]. Other eukaryotes have far fewer genes for small HSPs; for example, only one single gene in mammals.

A comparison of the major HSPs in different organisms has shown that they are in general highly homologous among eukaryotes. In most cases, homologous proteins have been identified in prokaryotes as well. For example, HSP60 homologues are found in all extant species, including eubacteria, archaebacteria, and eukaryotes. The global alignment of HSP60 sequences indicates that a large number of positions in the sequences are highly conserved among all species [19]. The pairwise amino acid identity matrix of HSP60 sequences indicates that the minimum identity that is observed between any two HSP60 sequences is 42% over their entire length [19]. Surprisingly, the *Escherichia coli* homologue shows 50.5% amino acid identity and 68.4% similarity with that of humans. The HSP70s are also a highly conserved protein family ~50% amino acid identity among all species characterized [7]. Therefore, at least some HSPs are highly conserved across a wide variety of organisms not only in the way they are induced by heat but also in their primary structures. This suggests that HSPs play extremely important and common roles in the whole biological world.

HSPs Are General ''Stress Proteins''

Heat is not the only treatment that leads to elevated expression of HSPs. The expression of some HSPs in different organisms has been shown to be affected by a number of chemicals: arsenite [17,20,21]; sodium fluoride [20]; amino acid analogues, such as azetidine-2-carboxylic acid [22]; 2,4-dinitrophenol [20]; canavanine [20]; malonic acid [23]; methomyl, an insecticide-nematicide [24]; plant hormones such as abscisic acid, 2,4-dichlorophenoxyacetic acid, and kinetin [20,25]; and heavy metals such as cadmium, cobalt, copper, nickel, and silver [20,21]. It is also induced by some physical treatment: anaerobiosis [20], high concentrations of salts such as KCl [20,26], low water potential and dehydration [20,26,27], a shift from dark to light [28], and even low-temperature stress [26,29,30].

It should be noted that those stresses listed above do not necessarily induce all the HSPs alike; each HSP species may respond differently to different types of stress. This suggests that the same induction mechanisms may not be involved for different HSPs. For example, small HSP genes in sunflower are regulated differentially and mRNAs from one of the two small HSP genes of sunflower accumulated equally in response to heat shock, abscisic acid treatment, and mild water stress [31]. In contrast, mRNAs from another gene accumulated only in response to heat shock but not to other treatments. The expression of the ERD1 gene which encodes a ClpA,B–like protein in *Arabidopsis* was strongly induced by dehydration of plants but was affected neither by heat or cold stress, treatment with heavy metals, nor plant hormones [27]. This indicates that ERD1 is regulated differently from other usual heat-induced HSPs. These results suggest that each HSP species may have evolved differently to acquire specialized function(s) in response to specific type(s) of stresses in order to cope with various hostile environments.

Pre–heat treatments elicit resistance not just to high temperatures but also to other stresses [7]. In addition, exposure to other stresses elicits protection not just against higher doses of those particular stresses but also against high temperatures as well [7]. This remarkable phenomena can

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be easily understood if we assume the involvement of HSPs in many forms of stress and the heat-shock response to be representative of more general stress responses.

Importance of HSPs Extends Beyond Their Potential Role in Protection from High-Temperature and Other Stresses

Not all, but some, HSPs are expressed under unstressed conditions. All major HSPs, at least in eukaryotes, are multigene families having more than one member in the families. Each member may not respond to a heat shock the same way. For example, one of the members of *Arabidopsis* HSP90 family is constitutively expressed and its expression is slightly enhanced by elevated temperatures, whereas another one is strongly induced by heat but stays in a very low level at normal temperatures [13]. Some HSPs are produced at particular stages of the cell cycle or during development in the absence of stress [1,3].

In fact, some HSPs appear to have an essential cellular function. Genetic analysis revealed that *E. coli* mutants that lacked the heat-shock sigma factor σ^{32} were extremely temperature sensitive and poorly grew even at temperatures lower than or equal to 20°C [32]. It has been known that σ^{32} is required for the heat-induced transcription of most of the heat-shock genes in *E. coli* [33,34]. Individual HSPs such as *E. coli* HSP60 (GroEL) [35] and yeast HSP90 [36] have been shown to be essential for viability at all temperatures.

A body of evidence has accumulated that at least some HSPs, such as HSP60 and HSP70, are involved in general and essential cellular functions; for example, protein folding and subunit assembly, protein translocation across membranes, and intracellular protein breakdown [37,38]. Given the ubiquitous need for the protein folding which is performed by a set of HSPs as molecular chaperones, as will be discussed later in this chapter, HSPs are being increasingly implicated as important players in virtually all aspects of cellular activities. In his review, Yahara [39] stated the importance of HSPs as, ''it is evident that cellular proteins are taken care of by stress proteins *from the cradle to the grave*''.

Genetic Evidence for Involvement of an Individual HSP in Thermotolerance

In addition to the above-stated results that correlate HSP accumulation with thermotolerance, genetic evidence also supports that HSPs can confer heat tolerance to an organism in vivo. One of the genetic approaches to prove a specific contribution of HSPs in thermotolerance is the selection, construction, and phenotype analysis of mutants and transgenic organisms in which expression(s) of a specific HSP(s) is either completely inactivated, partially repressed, or overinduced. If it is not easy or impossible to select or construct such a mutant, a mutant lacking a specific HSP, which has been successfully constructed with a certain species, is used to examine whether a homologous gene from a particular organism of interest can complement the mutant's phenotype.

Mutants of *E. coli*, yeast, and *Drosophila*, which produce no or a reduced amount of HSP70, showed defects in either growth, survival, or development at high temperatures (see Ref. 7 and references therein). Genetic analyses of heat-inducible family members of HSP100 from yeast (HSP104) and *E. coli* (ClpB) also showed that they are required for thermotolerance [40,41]. In addition to these studies with mutants and transgenic organisms expressing no or reduced levels of a specific HSP, studies on cells overexpressing a specific HSP also indicated that the acquisition of thermotolerance is attributed to HSPs. Overexpression of HSPs such as HSP90 [42], HSP70 [43], and small HSPs [44,45] rendered cells heat tolerant.

In higher plants, studies with mutants or transgenic plant to test critically the in vivo role of plant HSPs have still been limited. At present, there have been only two published papers which studied the role of HSPs with transgenic plants [47,48].

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There are two types (cpn60α and cpn60β) of cpn60 (HSP60 homologue) present in chloroplasts [46]. They are also known as ribulose bisphosphate carboxylase-oxygenase (Rubisco)–binding proteins which are thought to be required for the assembly of the active Rubisco enzyme complex in vivo. Antisense cpn60β, transgenic tobacco plants with reduced levels of cpn60β showed drastic phenotype alterations, including slow growth, delayed flowering, stunting, and leaf chlorosis [47]. Several antisense plants failed to survive to maturity and, in another case, segregate in a 2:1 ratio and do not produce homozygous viable progeny. These results indicate that cpn60β is essential for viability. Unexpectedly, however, these transgenic plants accumulated Rubisco with specific activities equal to or even higher than that of controls. Thus, the result has challenged the prevailing function of cpn60 in the assembly of Rubisco. Details for the function of cpn60 will be discussed in a following section.

One of the main difficulties in characterizing the function of a plant small HSP is the genomic complexity of the small HSP gene family, which, in plants, encompasses a large number of well-conserved members, as described above. Osteryoung et al. [48], chose a small HSP of chloroplasts, HSP21, to generate transgenic *Arabidopsis* plants that either underexpress or constitutively overexpress that particular small HSP, because this chloroplast small HSP is known to be encoded by a single or a few genes in higher plants [49]. The overexpressing plants accumulated considerably more HSP21 in the absence of heat stress than the heat-stressed wild-type plants did. The HSP21 could be detected in leaves, stems, roots, and flowers. Since the overexpressed protein comigrated with mature, authentic HSP21, it is likely that the transit peptide had been cleaved and the protein was properly targeted to the chloroplasts. However, no obvious differences in any readily observable parameter of growth and development were noted between the wild-type sibling and the overexpressing plants.

These papers by Zabaleta et al. [47] and Osteryoung et al. [48] did not describe the thermotolerance of the resulted transgenic plants. Thus, to date, there is no direct evidence to confirm the in vivo role of an individual plant HSP in thermotolerance. However, complementation tests and overexpressions of a specific plant HSP in a hybrid system have shown there is an important role in thermotolerance in higher plants as follows: A gene encoding a HSP100 homologue was isolated from *Arabidopsis* [50]. The *Arabidopsis* homologue is 43% identical to the yeast HSP104. The expression of the gene was heat inducible, which is similar to that of the yeast homologue. When the plant homologue was expressed in yeast, it complemented the thermotolerance defect caused by a deletion of the HSP104 gene [50]. Similar results with a soybean HSP100 homologue were also obtained by Lee et al. [51]. The ability to protect yeast from severe heat stress strongly suggests that the plant HSP100 homologues have important roles in thermotolerance in higher plants as well.

In another case, a gene encoding the rice 16.9-kDa small HSP (class I small HSP), Oshsp 16.9, was introduced into *E. coli* using the pGEX-2T expression vector [52]. *E coli* cells transformed with a recombinant plasmid containing a glutathione S-transferase Oshsp16.9 fusion protein (after isopropyl β-D-thiogalactopyranoside induction) demonstrated thermotolerance at 47.5°C, a treatment lethal to the cells transformed with the pGEX-2T vector (the control). The result suggests that a plant class I small HSP provides thermotolerance to plants as well as to prokaryotic cells.

So far, there has been no report on the production of null mutations of HSPs in higher plants. Usually, in higher plants, it is not easy to inactivate a specific gene by a genetic method such as gene targeting. Another difficulty in characterizing the function of a plant HSP is that plant HSPs have multiple members within one HSP family. It would be difficult to define a specific phenotype for a certain gene product with its null mutants if there are other genes present which are homologous with that particular gene and some of their functions are interchangeable.

Cyanobacteria, like other organisms, synthesize a diverse range of HSPs on exposure to high temperatures [53]. Chloroplasts are thought to originate from a cyanobacterium that became associated with an originally nonphotosynthetic host cell. Cyanobacteria have proven to be valuable model organisms for studying photosynthesis, since they have a photosynthetic apparatus functionally and structurally similar to those of higher plants. Hence, information on the functions of HSPs obtained with cyanobacteria will be useful for understanding the functions of HSPs in chloroplasts. Importantly, cyanobacteria can readily be manipulated genetically [54].

Disruption of a single copy gene, *clpB* from *Synechococcus* sp. PCC 7942, which encodes a HSP100 homologue in the mesophilic cyanobacterium, reduces the cell's ability to develop thermotolerance in the cell's survival and photosynthetic activity by at least fourfold, but it has no effect on the survival of cells during sudden, severe heat shocks [55]. These results clearly show that induction of ClpB at high temperatures is vital for sustained thermotolerance.

The *htpG* gene that encodes HtpG, a homologue of HSP90, have been isolated from *Synechococcus* sp. PCC 7942 (N. Tanaka and H. Nakamoto, manuscript in preparation). Transcripts of *Synechococcus htpG*, which does not have any other homologous gene in the genomic DNA, increased 20-fold within 15 min on heat shock. Stable mutant strains in which the gene was interrupted by gene targeting were generated. Even under unstressed conditions (30°C), the photoautotrophic growth of the mutant was significantly inhibited. At higher temperatures (i.e., 45°C), the mutant could not grow at all, whereas the wild type could still grow. Our results with the photosynthetic prokaryote have shown a striking dissimilarity with the *E. coli* homologue. Deletion of the *E. coli htpG* has a slight growth disadvantage at normal temperatures and produces a subtle reduction in growth at high temperatures [56]. As described above, yeast HSP90 is essential for viability at normal temperatures, and increasing concentrations of HSP90 are required for survival at higher temperatures [36]. Thus, *Synechococcus htpG* appears to be functionally more similar to eukaryotic homologues, although it has a prokaryotic origin.

As described above, the antisense *Arabidopsis* which expressed a reduced amount of a chloroplast small HSP did not show a notable phenotype. Recently, a single copy gene, *hsp16.6*, encoding a small HSP homologue in the mesophilic cyanobacterium *Synechocystis* sp. PCC 6803 has been inactivated (H. Fukuzawa, H. Kosaka, and K. Ohyama, unpublished results). The mutant was sensitive to high temperatures and could not develop ''acquired thermotolerance.'' Characterization of the photosynthetic activities of the mutant at high temperatures may provide an important insight for the function of the small HSPs of chloroplasts in higher plants.

Plant HSPs Can Confer Thermotolerance to Organelle Functions and Proteins

Recently, direct evidence has been presented to show that the chloroplast small HSP protects photosystem II (PSII) electron transport during heat stress [57]. PSII is known to be a highly thermolabile step in the photosynthetic electron transport system [58]. However, isolated thylakoid membranes from preheated (at 43°C for 6 h) tomato plants showed an approximately twofold increase in PSII electron transport activities at a high temperature (47°C) as compared with those from nonheated control plants. This acclimation effect by the heat treatment was completely abolished by treatment with antibodies against the chloroplast small HSP. Furthermore, a purified chloroplast HSP preparation added to thylakoid membranes prepared from unstressed plants that did not contain small HSP protected PSII at the high temperature. Previously, 22- and 25-kDa chloroplast HSPs were shown to be bound to the thylakoid membranes on heat shock [59]. These results indicate that the chloroplast small HSP protects thermolabile PSII. The location(s) to which the small HSP is associated as well as the mechanism for the protection is not known. In contrast to HSP60 and HSP70, which are expressed even at normal temperatures, chloroplast small HSPs are specifically induced at elevated temperatures. This suggests that the small HSP is particularly important for the thermoprotection of photosynthetic activities.

The 70–100% ammonium sulfate fraction of the postribosomal supernatant of heat-shocked soybean seedlings enriched with all of the HSPs showed a significant ability to tolerate heat denaturation of the control postribosomal supernatant of non–heat-shocked seedlings [60]. Heated at 55°C, some 50% of the control proteins, which were normally denatured and precipitated out after heat treatment, were protected and kept in a soluble form for at least 1 h when the fraction was added. A similar assay to examine the thermoprotection of rice soluble proteins from heat denaturation was performed by using a purified recombinant rice 16.9-kDa heat-shock protein, a class I small

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HSP, instead of using the ammonium sulfate fraction [61]. The recombinant protein protected soluble proteins from heat denaturation more highly than the HSP-enriched ammonium sulfate fraction did [61]. Thus, one possible function of HSPs, especially plant small HSPs, may be to stabilize cellular proteins in their soluble form. This is one of the functions of molecular chaperones, as described below.

Localization of HSPs

In eukaryotes, HSPs are found in essentially every cellular compartment [3]. This may not be surprising if we consider that major HSPs are molecular chaperones, as will be discussed below. For example, HSP70 and HSP60 (Cpn60) are localized in chloroplasts and mitochondria; some members of HSP100 (Clp proteins), HSP90, and HSP70 are in cytoplasm; and small HSPs are in cytoplasm, mitochondria, chloroplast, and endoplasmic reticulum.

Remarkably, several HSPs, originally localized (most likely) in the cytoplasm, become selectively localized and associated with organelle fractions such as nuclei, mitochondria, and ribosomes on heat shock, as shown in soybean seedlings [17]. In contrast to heat treatment, proteins induced by arsenite treatment are not selectively localized with organelle fractions at 28°C [17]. HSPs associated with the ribosomal and nuclei fractions were primarily HSPs with the molecular mass of 15–18 kDa. Also, 69- to 70-kDa HSPs are associated with the fraction though much less in amount. Additional HSPs with sizes ranging from 22 to 24 kDa were found associated with the mitochondrial fraction [17]. It has been shown that the association of small HSPs (15–18 kDa) with mitochondria is particularly important for the thermotolerance of the oxidative phosphorylation [12].

THE MOLECULAR MECHANISMS: HOW DO HSPs FUNCTION?

Accumulating evidence indicates that through stabilization of proteins in a particular state of folding, HSP90, HSP70, and HSP60 facilitate a wide diversity of important processes, including protein folding, transport of proteins across membranes, assembly of oligomeric proteins, and modulation of receptor activities [1]. All of these functions require the alteration or maintenance of specific polypeptide conformations. Based on these activities, HSP90, HSP70, and HSP60 have been termed ''molecular chaperones'' [1]. Furthermore, HSP100 and small HSPs have recently been shown to act as a type of molecular chaperone.

What are the mechanisms to protect cells from stresses? So far, at least two general roles of HSPs have been suggested for helping cells to cope with stress-induced damage to proteins [37]. Some HSPs, for example, some members of HSP100 (Clp) family, can promote degradation of abnormal proteins, whereas others can reactivate stress-damaged proteins and functions as molecular chaperones to prevent the aggregation or promote the proper refolding of denatured proteins. Some of the recent progress on these matters, especially HSP60 and HSP70, including the structural information, will be summarized below together with some historical background.

HSP60 Is the First Molecular Chaperone to Be Found

This family of HSP60 proteins include the GroEL of prokaryotes and cpn60 of chloroplasts and mitochondria. The first HSP60 to be reported was GroEL of *E. coli*, which was identified together with GroES as a gene product of an operon on the *E. coli* genome. Mutants that lacked the gene *groEL* could not form mature phages [62]. A purified preparation was first reported as an oversized ATPase complex, and was later identified as the GroEL itself from its amino acid sequence [63]. In a meantime, the so-called Rubisco-binding protein (RBP), known to be necessary for the assembly of the functional Rubisco complex, was found [64]. In 1988, when an amino acid sequence of RBP was determined, it became obvious that RBP was highly homologous with GroEL. At this point,

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the name *chaperonin* was coined to describe ''one family of highly sequence-related proteins acting as molecular chaperones'' [65]. The term *molecular chaperone* was originally used by Laskey et al. [66] to describe the properties of nucleoplasmin, an acidic nuclear protein necessary to assemble correctly nucleosome cores out of DNA (acidic) and histone (basic). Nucleoplasmin looked quite unusual in its role. It binds to the histones only transiently and is not incorporated into the final assembly. As it does not change itself throughout this operation, the activity seems ''catalytic.'' Yet it should not be called an enzyme, because there is no chemical reaction involved.

The term *chaperonin* is presently used for HSP60 and its homologues, whereas other proteins of this nature are called molecular chaperones. The chaperones are molecular analogues of the human chaperone—an assistant accompanying a debutante. The traditional role of the human chaperone is to prevent improper interactions among people at a party, ''without either providing the steric information necessary for their correct interaction or being present during their subsequent married life—but often appearing at divorce and remarriage'' [67]. Soon after, a homologue was found in mitochondria as a stress-induced protein, HSP60 [68]. The basis of the currently popular mechanism of chaperonin action was founded when Lorimer and coworkers reassembled the Rubisco complex in vitro by using chaperonin and ATP [69].

HSP60 Forms Two Heptamer Rings

Presently, there is a consensus that two heptamer rings stack each other and form a tetradecamer from the GroEL monomer (~60 kDa) subunit. GroES, sometimes called co-chaperonin, is in the form of a single heptamer ring, and plays an important role in the chaperone action. ATP is necessary for successful refolding of once denatured proteins. X-ray analyses have revealed 3-dimensional structures of these complexes [70,71].

Chaperonin Works with HSP10 and Needs ATP

One of the recent proposals as to the action of a chaperonin is summarized in Figure 2 (based on Ref. 72). A denatured or half-denatured polypeptide (so-called ''molten globule'') enters in a hole of one of the GroEL rings (steps A and B). A small ring of the GroES heptamer binds to this side like a lid (step C, lower). The peptide is now folded to become a native form inside the central cavity (step D). The release of a matured protein by removing the GroES lid requires energy input from ATP hydrolysis (step E). Alternatively, the polypeptide leaves the GroEL ring unproductively without being repaired and seeks another chaperonin to repeat the cycle (step C, upper).

The mechanism depicted here is an oversimplified version. The reader should refer to more details that have been discussed particularly in terms of the binding sites of ATP/ADP and a substrate polypeptide [73]. The structures of the rings are quite flexible and overall behaviors of those components should be very dynamic. It should be noted, however, that this scheme is by no means definitive. The reader may refer to an excellent summary on this matter by Ellis [67].

Chloroplast cpn60

As stated in the previous section, chloroplast chaperonin (ch-cpn60) was first discovered as the Rubisco-binding protein (RBP). There are two isomers of ch-cpn60, α and β, in almost stoichiometrical amounts, whose nuclear encoded precursors are synthesized outside the chloroplast and imported into it. The proteins are constitutively expressed and the levels increase only slightly during heat shock. The two isoforms are only 50% identical in terms of amino acid sequence. Although these subunits form two heptamer rings, as in the case of GroEL of *E. coli*, little is known about whether the ring is a hetero-oligomer or homo-oligomer.

An antisense study has shown that the ch-cpn60β may not be essential for the assembly of the Rubisco complex, as described in the preceding section, although ch-cpn60α may substitute for the function of ch-cpn60β. It is now considered that the ch-cpn60 facilitates the folding, not assem-

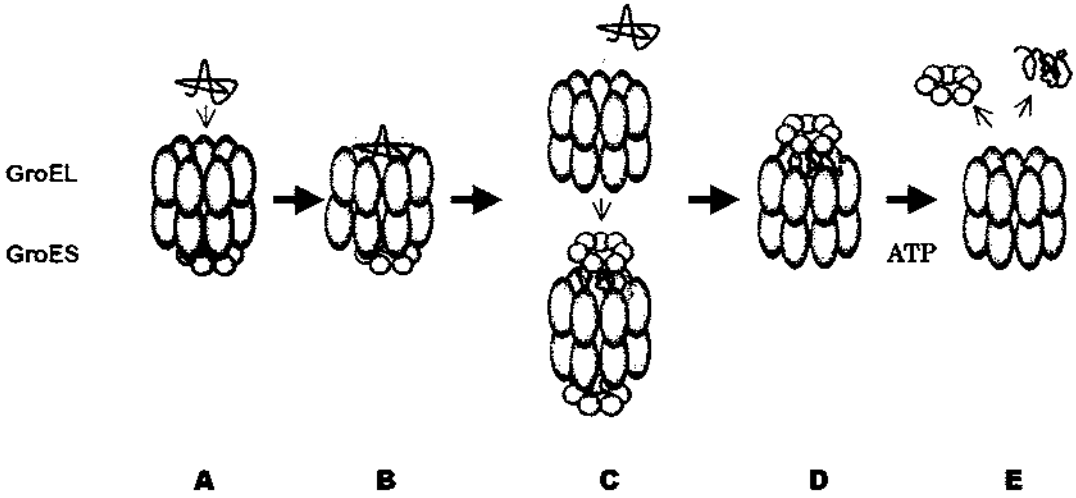

FIGURE 2 A simplified scheme to show how a GroEL/GroES chaperonin system works. For details, see text.

bly, of Rubisco large subunits [69]. A possibility remains that they might also take part in the folding of the small subunits [74,75]. On the other hand, there is no evidence at present that ch-cpn60 directly mediates the formation of the quaternary structure of the Rubisco complex.

Chloroplast cpn10 Is Rather ''cpn21''

Chloroplast cpn10 was originally isolated as a protein that formed a stable complex with GroEL [76]. Remarkably, the molecular weight was almost twice as that of GroES, and it had an amino acid sequence with two complete GroES-like sequences fused head-to-tail to form a single protein. Despite these unusual characteristics, these subunits form toroidal structures reminiscent of GroES under the electron microscope [77].

This unique 21-kDa cpn is widely found throughout the plant kingdom and in photosynthetic eukaryotes [77,78]. One should not confuse this chloroplast ''GroES'' with HSP21 described in the previous section, because the latter is a member of the small HSPs, which are a completely different type of HSP.

Folding Mechanisms by GroEL and chl-cpn60 Are Similar

Studies with purified proteins revealed a similar mechanism of protein folding in the case of chl-cpn60 and chl-cpn10 with that of GroEL and GroES [46,76–78]. Recent experiments showed that the chl-cpn60 functions equally well with bacterial, mitochondrial, or chloroplast cpn10 [78]. This means that the unique binary chloroplast protein is not obligatory for the chl-cpn60–mediated folding. In contrast to GroEL and mammalian and yeast mitochondrial cpn60s, the folding reaction mediated by chl-cpn60 does not require K^+ ions [78].

As in the case of GroEL, chl-cpn60 has a rather broad substrate specificity and forms a stable complex with a variety of proteins besides Rubisco, notably with those imported into chloroplasts

[75,79–81]. These are the Rieske FeS protein [80], ferredoxin $NADP^+$ reductase [81], and the multisubunit coupling factor CF_1 core complex [82].

Cyanobacterial HSP60

Cyanobacteria are of interest, as plant chloroplasts are thought to have evolved from endosymbiotic cyanobacteria, as described above. A few cases of GroELs have been reported so far from cyanobacteria [83–88]. These reports are all about genes encoding GroELs (*groEL*) except for [85], and little is known about the activity of expressed proteins. Recent investigations in our laboratory have revealed that GroELs from a thermophilic cyanobacterium, *Synechococcus vulcanus*, may exist in vivo at least partially in the form of monomer rather than in the tetradecameric form that is ubiquitous among other prokaryotes and organelles (T. Hiyama et al., manuscript in preparation). There is some indication that a monomeric form is also present in mesophilic cyanobacteria (H. Hayashi, personal communication). We found that this monomer had chaperone activities similar to those of the small HSPs described in the previous section; that is, ATP-independent protein folding, protection against heat denaturation, and prevention from aggregation (H. Nikaido and T. Hiyama, manuscript in preparation). A preliminary survey indicated some possibility that this monomeric form may be present in vivo in other prokaryotes. Although there is little doubt about the proposed mechanism of protein folding mediated by the HSP60 tetradecamer, the heptameric ring of HSP10 and ATP, and the majority of the case should be explained by the scheme outlined in Figure 2, it may not be an excessively wild speculation that this type of non–energy-consuming chaperone's actions are also important, particularly in a more hostile environment at temperatures higher than usual where much more vigorous chaperoning is needed.

TCP-1/CCT/TriC and Archaebacterial cpn60

Distantly related chaperonin-like proteins are present in the eukaryotic cytosol, and they are variously called TCP-1, CCT, or TriC [89]. In plants, however, very little is known about this type of cytosolic chaperonin and protein folding in plants, although several DNA sequences encoding proteins with some homology to the TCP-1 of the mammalian cytosol have been reported [90,91]. In terms of sequence homology, these cytosolic chaperonins are more related to archaebacterial HSP60s, which are highly heat inducible, whereas TCP-1 is not. They are now categorized as group II cpn60, as opposed to group I, to which other eubacterial and organellar types belong [92]. Judging from electron microscopic observations, a recent report proposed that the archaebacterial chaperonin forms the cytoskeleton [93].

HSP70 (DnaK)

HSP70, sometimes called HSC70 (for heat-shock cognate), or better known as DnaK, is present in abundance in the cytosol of virtually all eukaryotic cells. Some members of the HSP70 family are strongly induced by heat [94,95]. This HSP constitutes a family of highly conserved proteins [96]. According to a tertiary structure solved for bovine brain HSP70 [97], HSP70 consists of an N-terminal ATPase domain (~45 kDa) and a C-terminal peptide-binding domain (~25 kDa). Although there may be certain preferences in the sequence pattern like sequences with seven to eight amino acid residues of hydrophobic and/or basic rather than acidic nature [98–102], the overall substrate specificity is low, although not as low as that of HSP60 families.

DnaJ (HSP40) and GrpE Are Necessary for DnaK (HSP70) Actions

The chaperone machinery involving DnaK has been proposed to be progressive and structured models with other proteins like DnaJ and GrpE, which are also heat-shock proteins. One example is

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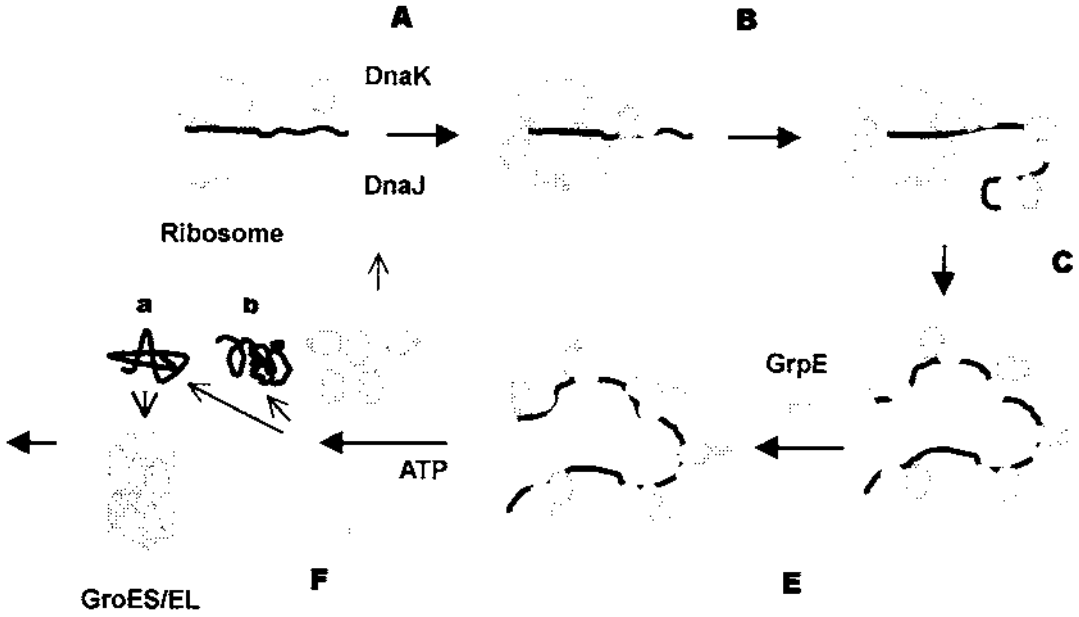

FIGURE 3 A simplified scheme of a DnaK/DnaJ/GrpE protein-folding system. For details, see text.

shown in Figure 3 (based on Ref. 103). To a nascent polypeptide just synthesized on a ribosome or to a denatured protein, DnaK and/or DnaJ bind (step A–B). As the polypeptide chain grows, more DnaK and DnaJ attach to the chain, protecting it from incorrect folding and/or aggregation (step C). Free from the ribosome, another factor, GrpE, comes on to the scene assisting the release of DnaK and DnaJ (step E). By this time, the peptide has either been correctly folded (*b*) or become an intermediary form (*a*, so-called ''molten globule''). The latter enters the next step where the GroEL and GroES machinery takes care of the final maturation stage (step F).

Plant Cytosolic HSP70s Are Encoded by Multiple Genes

A number of genes encoding plant cytosolic HSP70s have been reported (see a review in Ref. 3). A single plant species often has multiple genes, all of which encode proteins with more than 90% homology. Chloroplast counterparts are more of the bacterial type in terms of sequence [81]. It should be mentioned it as been implied that HSP70 has a role in importing proteins synthesized in the cytosol into organelles like chloroplasts. The relevance of this activity to thermotolerance remains to be elucidated.

Other Molecular Chaperones

Recent biochemical analysis of both HSP90 and small HPSs has revealed that they may act as molecular chaperones involved in protein folding and unfolding events [104]. Although small HSPs are one of the least conserved HSPs, they can be identified based on their similar hydropathy profiles and homology to the α-crystalline, an abundant protein in eye lenses [105]. The small HSPs from many different organisms are found in high molecular weight complexes in vivo, ranging from 200 to 800 kDa, that appear to be homo-oligomers [2,3]. Recently, it has been shown that α-crystalline, mammalian, and plant small HSPs possess molecular chaperone activity in vitro [106–108]. Small

HSPs are able to promote refolding of chemically denatured proteins in an ATP-independent manner in contrast to the ATP-dependent molecular chaperones of HSP60 and HSP70. Furthermore, they can prevent heat-induced protein aggregation and facilitate reactivation of heat-inactivated model substrates. HSP90 also act as an ATP-independent molecular chaperone [104]. Recent work on members of the HSP100 (Clp) family, which are ATP-dependent proteases, suggests that they may act as chaperones [105]. Detailed descriptions of these HSPs are found elsewhere [2,3].

CONCLUSIONS

Molecular chaperones are a diverse group of proteins that share the property for the binding of substrate proteins that are in unstable, nonnative structural states. Recent research indicates that many, if not all, cellular proteins interact with chaperones during their lifetime. Different chaperone systems are required for synthesis, targeting, maturation, and degradation of proteins in all cellular compartments. Thus, these diverse sets of proteins affect an exceptionally broad array of cellular processes required for both normal cell function and survival under stress conditions.

An overwhelming volume of evidence supports the assumption that HSPs are some of the most important entities to provide heat tolerance to plants. Most of the HSPs function as molecular chaperones, most of which have been shown to be essential for cellular functions at normal temperatures. They must become even more indispensable at higher temperatures where probabilities of denaturation, incorrect folding, and aggregation of cellular proteins are much higher.

The life on the Earth may have started originally under extremely hostile conditions, particularly very high temperatures. Thus, all primitive cells could have been thermotolerant in the beginning. In the course of the evolutionary development, cellular functions had to become more and more elaborate. As the temperature was gradually cooling down, cells might have undergone structural changes needed for functional sophistication in proteins and other molecules at the cost of losing heat tolerance. Thus, most of the present-day organisms are rather heat sensitive, because some, if not all, of the proteins and other macromolecular structures have become heat labile. In order to protect themselves from an occasional rise in temperature, the HSP, which is one of the most ancient proteins, judging from their ubiquitous presence, might have been preserved to the present, and evolved into one of the essential families of cellular proteins as molecular chaperones.

It is rather obvious that heat tolerance is by no means solely due to the activities of heat-shock proteins and molecular chaperones. Genetic manipulation to introduce a heat-tolerant HSP system alone may not suffice in an attempt to provide in a crop plant such traits as heat tolerance and resistance to dehydration, salt, and other stresses. Nevertheless, an understanding of heat shock proteins and molecular chaperones are essential for future development of such a crop plant strain.

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Growth, Respiration Rate, and Efficiency Responses to Temperature

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CLIMATIC TEMPERATURE

Seasonal and Diurnal Temperature Changes

Temperature on Earth changes along latitudinal and altitudinal clines and in most localities also with season. Diurnal and seasonal changes are larger for continental and high-altitude climates and areas with low relative humidity than for areas near large bodies of water, in lowland tropics, or with high humidity. Plant life exists across the whole range of atmospheric temperatures (−89–+58°C). However, most plants are especifically adapted to, and grow over, a limited range of temperatures [1]. Factors such as toxins, water, and mineral availability may also interact with temperature to determine growth. For instance, if annual precipitation occurs mainly as snow in the winter, plants may grow at cooler temperatures than in regions with abundant spring and summer moisture.

Many species have a growth season of only a few weeks during the year. Others grow whenever conditions are suitable. For some, growth may occur in the spring and others in summer or fall. Some plants may have more than one growth period during the year. Although other factors are important, climatic temperature is often dominant in determining conditions for the growth of a particular species and thus its distribution worldwide [2,3].

Temperature Effects on Growth

Growth is an irreversible increase in volume and structural biomass involving cell division, cell enlargement, maturation, and specialization to form tissues and organs. The rate of growth is propor-

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tional to the product of the rate of catabolic activity and the efficiency for conversion of photosynthate to structural biomass. Growth is influenced by several environmental factors, none more important than temperature.

The pattern of daily and seasonal temperatures and the effects on growth rates are complex. Plots of mean daily temperature and mean kinetic temperature over a year for a particular locality produce curves as in Figure 1. Because the relation between the growth rate and temperature is logarithmic, the growth rate is proportional to the mean kinetic temperature and not to the average temperature. Superimposed daily temperature extremes illustrate the range of temperatures and the constantly changing thermal environment encountered by plants.

Climatic temperature affects plants in three ways. First, seasonal temperature changes require that the timing of plant life-cycle events be appropriate for survival and reproduction. For example, in temperate climates, germinating too early can be fatal because of too cold spring temperatures, whereas germinating too late can be fatal if high temperatures occur before the plant is well established. Temperature can also affect flowering and seed set in similar ways. The growth and elongation of perennials, such as conifers, is often limited to those short periods of time when conditions are appropriate. These effects of seasonal temperature changes have been discussed elsewhere (1) and are not treated further here.

Second, extreme temperatures can limit plant survival and reproduction, and because this effect can be modeled in a simple yes/no way, it has been the most widely described in the literature. In this extreme temperature model, the growth range and season are set by the periods when the temperature extremes do not exceed the stability limits of the species. These temperature limits, both high and low, are commonly known approximately for many species, particularly crop plants.

Third, short-term temperature fluctuations within the extreme limits, typically diurnal, also affect plant growth, and thus the ability of a plant to compete or to produce well agronomically. Figure 1 shows that even when mean daily temperatures are within the range for active plant growth, diurnal changes may cause the growth rate to be very small during much of the day. Diurnal temperature variations thus have large effects on plant growth [4].

Because temperature is one of the strongest determinants of the plant growth rate, nearly all texts on plant physiology devote a section to the responses of plants to temperature. Typically, plant growth has been described as ''slow at low temperatures, then accelerated until a certain temperature is attained, above which growth becomes slower, and when a certain temperature is exceeded, growth ceases. The temperature at which growth rate is most rapid varies greatly with species'' [5]. Details

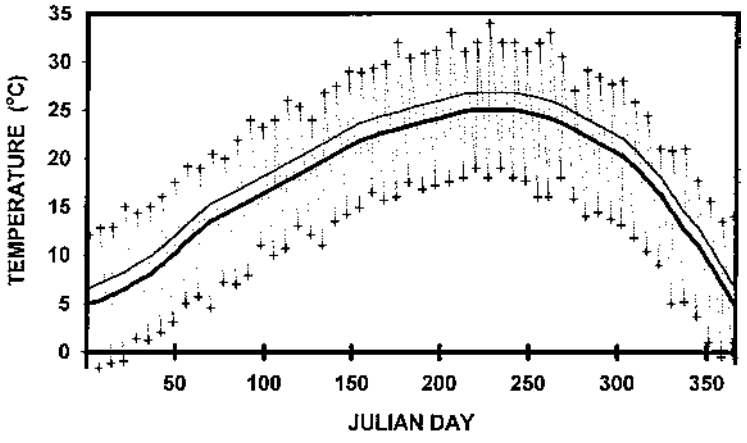

FIGURE 1 Mean daily (−), mean kinetic (−), and mean daily high and low temperatures (+, plotted weekly) at Davis, California.

of temperature effects on growth rates beyond such generalized descriptions have been difficult to obtain. Curves of the plant response to temperature [4,5] are commonly constructed by interpolation among a limited number of data points (typically less than 10). Consequently, the differences in temperature responses between and within plant species are often not well defined [6]. A quantitative understanding of the plant growth dependence on temperature is essential for the rapid selection of cultivars to optimize growth in different climates, to understand the physiological responses to climate change, and to identify and quantify the effects of climate.

Consideration of growth as the result of a concerted set of chemical reactions with known temperature dependences makes it readily apparent how small differences in enzyme activity among plants can give rise to large overall growth rate differences. Assume, for example differences arising between two plants because of a small mutational change in an enzyme that determines the growth rate. Mutation can give one of three outcomes: (1) either the enzyme activity (and consequently plant growth) is unchanged by the mutation, or (2) enzyme activity is destroyed, in which case the plant will likely fail to grow, or (3) the activity is altered but the enzyme is still functional. The last of these possibilities is the only one of interest here. A change in the catalytic rate indicates a change in the activation energy of the enzyme-catalyzed reaction; that is, a change in the temperature coefficient. Thus, the temperature dependences of the relative growth rates of mutant and nonmutant plants must differ. Because reaction rates change exponentially with temperature, the growth rate difference between the native and mutant plants is related to the difference between two exponentials, and small changes in temperature will have large effects on the relative growth rates. Although a small change in the rate of an enzyme-catalyzed reaction in a homeotherm may have little effect on growth, plants that encounter a wide range of constantly changing growth temperatures may show a large diversity of responses from small differences in enzyme activity.

Besides depending on the magnitude of the temperature, the effects of temperature on growth and metabolism are often also time dependent. A well-known example of this phenomenon is the response of chilling-sensitive plants to temperatures above freezing but below about 15°C. Temperatures near the upper end of this range damage tissues, but very slowly, whereas temperatures near freezing damage tissues very rapidly [7]. Figure 2 illustrates this with time-temperature surfaces for the metabolic heat rate for tomato tissue at high and low temperatures [8]. These surfaces describe the immediate metabolic response to temperature change; growth responses follow similar curves. The intermediate time response of plants to changing environmental conditions is by acclimation; that is, phenotypic plasticity. The process of acclimation to a stress is known as hardening [5]. In an even longer time frame, natural selection among populations may lead to adaptation. Both acclimation and adaptation are means for achieving tolerance to a particular stress.

Responses of plants to environmental stress, particularly temperature stress, have been the object of much study [9]. Since plants generally grow best under minimal stress, an easily and rapidly measured quantitative physiological predictor of plant growth (and response to stress) is highly desirable.

A quantitative mechanistic model [10] has recently made it possible to connect the temperature effects on growth to the better-known effects of temperature on metabolism. Previously, many of the effects of diurnal temperature variation on growth were ascribed to the differential effects of soil and air temperatures on different organs of the plant; for example, keeping the roots cool [11] and thus conserving carbon.

MECHANISM OF ACTION OF TEMPERATURE

Temperature and Reaction Rates

It is helpful to recall that temperature is not a measure of the amount or concentration of a substance or of total energy but is a measure of molecular motion; that is, the molecular kinetic energy within the system. Thus, the rates of all elementary reactions increase exponentially with increasing temperature. Metabolism is a combination of many elementary reactions, most of which have rates con-

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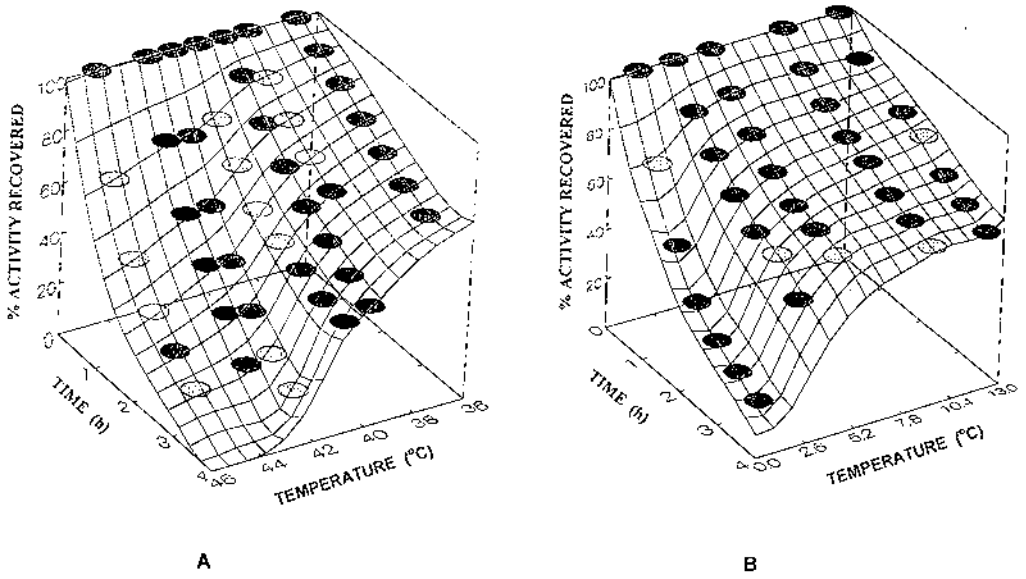

FIGURE 2 Response-surface plot for tomato cells. Activity recovered at 24°C is presented as a function of both exposure time and stress temperature. (a) Cells exposed to high temperatures. (b) Cells exposed to low temperatures. (From Ref. 8.)

trolled by enzyme activity. The rate of an enzyme-catalyzed reaction is typically regulated either by the number of active copies of the enzyme or by a temperature-dependent chemical equilibrium; for example, substrate, activator, or inhibitory binding. Therefore, the overall rate of reactions along a metabolic path may either increase or decrease with increasing temperature. Whether a reaction rate is controlled by kinetics (number of copies) or by a chemical equilibrium is not distinguishable from the functional form of the temperature dependence, however, because the Arrhenius equation for the temperature dependence of rate constants and the van't Hoff equation for temperature dependence of equilibrium constants are identical exponential functions of the reciprocal absolute temperature. In both cases, the rates of biological processes change exponentially with temperature. This has been experimentally verified for a wide variety of biological processes [12].

The most general means for describing the temperature dependence of a rate is thus the Arrhenius temperature coefficient, μ, as defined in Equation (1).

$$R = A \exp(-\mu/T) \tag{1}$$

where R is the rate, A and μ are empirical constants, and T is the Kelvin temperature. In linear form, Equation (1) becomes

$$\ln R = \ln A - \frac{\mu}{T} \tag{2}$$

and μ is readily obtained as the slope of a plot of $\ln R$ versus $1/T$. The use of Q_{10} values to describe temperature dependence should be discouraged, because these have no fundamental connection to the molecular description of matter, are not comparable unless measured at the same temperatures, cannot be used directly for extrapolation, and can be misleading. Note also that μ is constant (i.e.,

Equation [2] is linear) only over limited temperature ranges, and that the value of μ may be influenced by conditions other than temperature.

Relation Between Growth and Metabolism

Using direct measurements of plant growth rates to monitor and characterize plant responses to temperature change has many difficulties. Experiments conducted in the field are slow, expensive, difficult to reproduce, and often lack precise control over temperature and other conditions. Experiments to test multiple temperature conditions in chambers are limited by time and the number of chambers available. Thus, measurements over broad temperature ranges with multivariate, controlled environmental conditions are rare. In addition, growth rate measurements provide little information on the fundamental physiological responses that limit growth and therefore contribute little toward understanding the physiological basis of temperature responses. As a consequence, many efforts have turned toward defining the rates of various physiological processes other than growth as functions of temperature. If such are to be of value, they must ultimately be related to the growth and development rates at various temperatures.

Because metabolism is required for growth, it is clear that some relation must exist between the metabolic rates of plants and their growth rates. A major problem has been the identification of which metabolic reactions to examine; that is, which reaction is growth-rate determining under a given set of growth conditions. Moreover, simply measuring reaction rates that may correlate with the growth rate does not yield adequate information for understanding the effects of temperature on growth. A model must be developed that explains the widely differing growth and metabolic responses to temperature change among species and among ecotypes within a single species. The model must explain, by direct cause and effect functions, relations among changing physiological activities, growth rate changes, and temperature.

Most attempts at using physiological measurements to characterize plant growth rates and responses to temperature have centered on photosynthesis, but some have looked at respiration, because it is the source of energy for incorporating stored carbon into the structural biomass. Others have looked at the rates of nutrient uptake or incorporation. All of these processes are essential for growth at all temperatures, but which is appropriate for the study of growth rate responses to temperature? Certainly, photosynthesis is growth-rate determining for plants growing in extremely low light, just as any nutrient may become growth-rate limiting when present in low concentrations. Under conditions at which all required nutrients and photosynthate are available in ample quantities, the rate of incorporation of these materials into the biomass (i.e., respiration-driven biosynthesis) becomes growth-rate limiting. As growth conditions change, the identity of the rate-limiting process can also change.

One approach to define the rate-limiting process is to examine the variation in each of the putative growth rate–limiting reactions (or subset of reactions) with changing temperature. A parallel response should exist between temperature effects on the rate-determining process and the growth rate. However, although a parallel response to stress between the growth rate and a physiological reaction is necessary to prove cause and effect, it is not sufficient to prove causation. In contrast, a lack of a direct relationship between the stress response of a physiological reaction and growth is sufficient to eliminate that physiological reaction as the rate-limiting process for growth. Thus, if the temperature dependence of a process, such as photosynthesis, is not the same as the temperature dependence of growth, then that process can be ruled out as growth-rate determining under the conditions studied.

Photosynthesis-Based Descriptions of Plant Growth Rates

Photosynthesis supports nearly all life on Earth and has long been considered by many workers to be the growth-rate determining process for plants. Certainly, growth can proceed no faster than

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carbon can be assimilated through photosynthesis. However, other factors such as mineral deficiency or lack of water can limit growth even when the rate of photosynthate supply is adequate. Thus, it is not surprising that although many studies have tried, none has established a general relation between rates of photosynthesis and growth [13,14]. (Note: the relation sought is not the tautological relation between integrated net photosynthesis and integrated growth [15,16].)

Observations of direct correlations between the plant growth rate and the photosynthetic rates are rare except in limiting light conditions. More commonly, no correlation is found and sometimes even a negative correlation is found between the growth rate and the photosynthesis rate. In some cases when photosynthesis has been stressed to lower rates by as much as 50%, there is no corresponding change in the plant growth rate [17]. Finally, the temperature dependences of photosynthetic rates have not been shown to be directly correlated with the temperature dependences of growth rates. As noted above, the lack of such a correlation between a physiological parameter and the plant growth rate response to temperature eliminates the possibility of a direct cause and effect relationship.

Variations in light-harvesting systems and the carbon fixation path generally result in differences in growth rates among C_3, C_4, and Crassulacean Acid Metabolism (CAM) plants. Many investigators have tried to establish correlations between the rates of photosynthesis and the growth rates among these photosynthetic types [14]. Generally, these studies sought to establish a relation between the rates of CO_2 uptake in the light and plant growth rates [18]. None has been successful in establishing a relation.

Using another approach, Farquhar et al. [19] demonstrated that as C_3 plants close their stomates in response to drought stress, transpiration water loss decreases more rapidly than CO_2 uptake. Because some plants are more effective at this than others, water-use efficiency is related to carbon isotopic fractionation [20]. Relative growth can then be predicted because of the inverse relation between growth and water-use efficiency. The method uses leaves that are collected in the field, dried, combusted, and the CO_2 analyzed in an isotope ratio mass spectrometer. Although cumbersome and expensive, the technique is now widely used to predict relative plant growth rates. Its use is limited to field-grown C_3 plants under water stress.

Another photosynthetic property that has been examined for its relation to growth is photosystem II fluorescence. Fluorescence competes in the deactivation of excited chlorophyll [21], and the variable to maximal photosystem II fluorescence ratio (F_v/F_m) has been used as a measure of damage caused by free radicals formed in response to temperature and other stresses. Fluorescence has not proved to be useful as a predictor of plant growth rates [22].

Respiration-Based Descriptions of Growth Rates

Burke and Upchurch [23] have shown a relation between the temperature dependence of K_m for several plant enzymes and the temperature dependence of growth rates. The kinetic parameters are altered by plant-growth temperature in patterns consistent with these enzymes having a determining role in growth. Correlative responses are thus established for these enzymes, but cause and effect relations between the growth rate and the kinetics of the reactions catalyzed by these enzymes are not clear.

Several lines of evidence imply that the respiratory activity should be proportional to the growth rate, because rates of catabolism are controlled by anabolic demand. The sites of control are phosphofructokinase for glycolysis and isocitric dehydrogenase for the Krebs cycle [24]. Pasteur observed that yeast cells produced as much or more CO_2 in the absence of oxygen as in air; however, more yeast cells per mole of CO_2 were produced in air. More substrate had to be converted to CO_2 to obtain sufficient energy for growth. Similarly, in the presence of uncouplers that destroy the proton gradient across the inner mitochondrial membrane, CO_2 production and O_2 consumption rates increase. Furthermore, mitochondria isolated from rapidly growing tissues exhibit respiratory control, responding with an increased O_2 consumption to the addition of ADP.

Respiration rates have been correlated with growth rates in many experiments [25], but the

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question remains: Can measurements of respiration as a function of temperature provide quantitative growth rate information? The respiration rates of plants are readily measured in three ways: the rate of CO_2 production, the rate of oxygen consumption, and the respiratory heat rate. These three measures do not provide the same information and their temperature dependences are not necessarily the same. R_{CO_2} and R_{O_2} are measures of the mass flow through respiratory catabolism, whereas the heat rate (q) is a measure of the rate of energy loss. Because heat production in plants is primarily from oxidative reactions in the respiratory processes, heat-rate measurements are predominantly a measure of the rate of oxidative reactions of the respiratory pathway [26]. Contrary to common belief, the metabolic heat loss has little direct relation to the amounts of alternative pathway activity [27].

In plants, the dark-respiration rate has been shown to be both negatively correlated and positively correlated with growth [5,25]. Thornley [28] proposed a model based on partitioning respiration between growth and maintenance to describe the relation between plant growth and respiration, but the parameters in this model are impossible to measure or define in a meaningful way. These compartment models assume that the energy produced by respiration can be subdivided into two or more compartments which are amenable to calculation from measurements of R_{CO_2} or R_{O_2}. However, the calculation of both the growth respiration and the maintenance respiration requires assumptions that cannot be tested or measurements that cannot be made. The fundamental problem with this approach is that R_{CO_2} and R_{O_2} measure only the rate of mass flow, whereas the energy relation shown to be necessary by Pasteur's experiments must be guessed. A more recent approach uses simultaneous measurements of mass flow (as R_{CO_2}) and energy flow (as q) to predict the relative values of the specific growth rate [10], thus avoiding the assumptions inherent in compartment models.

Respiratory Heat Rate as a Function of Temperature

The rate of metabolic heat production can be measured directly on small whole plants or plant tissue sections by isothermal or temperature-scanning calorimetry [29,30]. The measurement of the metabolic heat rate as a function of temperature allows detailed examination of respiration rate differences among species or genotypes within species.

Isothermal heat-rate measurements at successive temperatures allow development of curves defining the plant metabolic heat-rate response to temperature (Fig. 3) [26,31,32]. Moreover, the

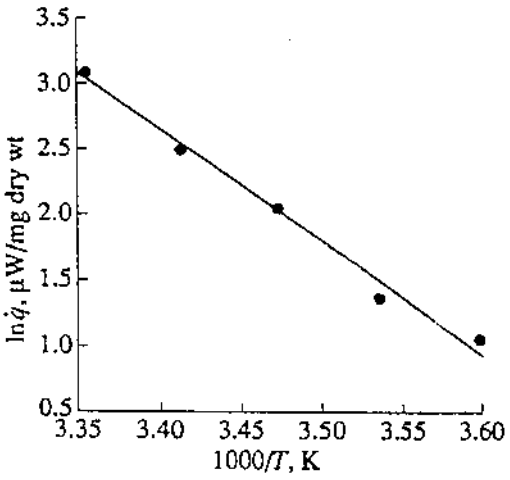

FIGURE 3 Representative Arrhenius plot for metabolic heat rate for maize shoot tissue. (From Ref. 31.)

decrease in metabolic heat rate with time at a series of temperatures can be combined to yield a time-temperature response, as shown in Figure 2 [8]. In comparison with scanning calorimetry, thermograms from isothermal calorimetry are less well defined along the temperature axis, because they are not continuous, but they are more accurate on the heat-rate axis, because the uncertainty is smaller. Isothermal calorimetry can also be used to measure R_{CO_2} [33].

In experiments lasting only a few hours [34] differential temperature-scanning calorimetry makes it possible to measure metabolic heat rates as a continuous function of temperature over the entire temperature range encountered by growing plants. The data obtained describe general patterns of respiration responses to temperature. The shapes of thermograms and key temperatures indicating metabolic activity changes are species dependent and define the response of plant tissue to a temperature increase. Figure 4 shows key features of thermograms collected over about 5 h with continuous temperature scanning. The major features of these curves that are useful for species or intraspecies comparisons are indicated by labels ''A'' through ''D.'' The segment of the curve from (A–B) is an exponential increase in the metabolic rate as temperature increases, in accord with the Arrhenius equation. At temperatures above B, metabolic rates no longer increase exponentially; instead the slope decreases with temperature. Thus, B is an inflection point and is termed the low shoulder temperature (T_{ls}). A second point with significance is T_{max}, the temperature at which the maximum heat rate is achieved (indicated by C in Fig. 4). The shape of the curve around the maximum is characterized by ΔT_w, the distance on the temperature axis from 80% T_{max} on the ascending curve to T_{max}. The decrease, in the metabolic activity with increasing temperature above T_{max} is irreversible on the time scale of these studies. Most plant tissues exhibit an exothermic reaction (probably oxidation of unsaturated lipids) at high temperature (D).

Figure 5 shows thermograms for several plant species, indicating the range of interspecies

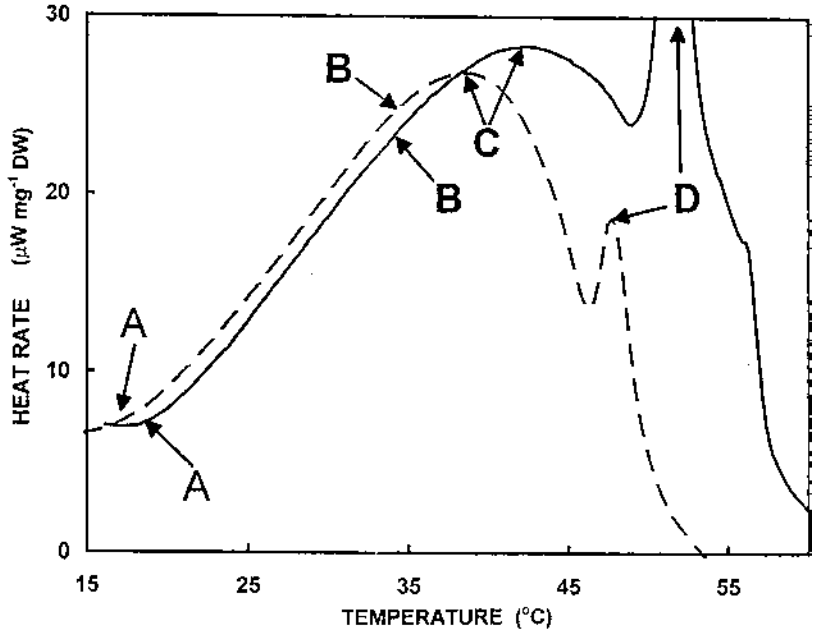

Figure 4 Continuous temperature-scanning thermograms showing metabolic heat rates versus temperature for meristem tissue from *Callistemon citrinus* collected from the same plant in mid May (- - -) and in mid July (——). Points indicated by A, B, C, and D are discussed in the text. (From S. Douglass, personal communication.)

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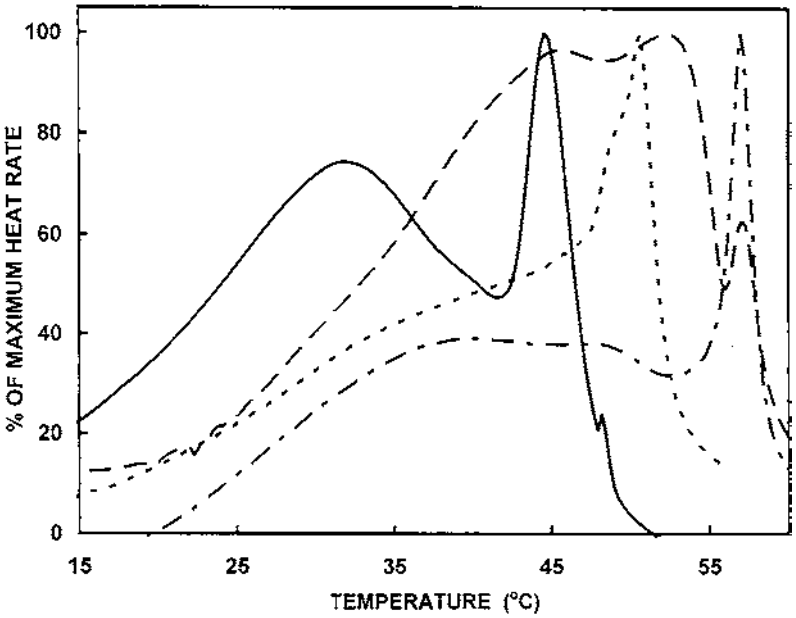

FIGURE 5 Continuous temperature-scanning thermograms for tissues from *Eremocarpus setigerus* (——), *Betula pendula* (– – –), *Mentha spicata* (- - -), and *Lantana camara* (– - –), (From S. Douglass, personal communication.)

responses observed. Although the thermograms in Figure 5 have several similarities, the large differences in temperatures of key events and in the detailed shapes of the curves show that there is no ''typical'' curve describing the effects of temperature on plant metabolism. All the samples tested have in common a nearly exponential rate of increase through the temperature range commonly encountered during growth, although the slope can vary a great deal between species and accessions [35]. The exponential rise is followed by a temperature range in which a species-specific array of responses to a further temperature increase is noted. Beyond these generalizations, the exact shapes of the curves, values of T_{ls}, T_{max}, and ΔT_w, temperatures of rapid inactivation of metabolism above T_{max}, and the temperature of the exothermic peak at high temperature all vary with species. In addition, metabolic heat rates can vary with developmental stage, growth location, and/or season.

Thermograms are highly reproducible for repeat measurements on individual plants when the scanning conditions and the tissue sample size, location, and age are all carefully controlled. Scanning rates must be carefully controlled among experiments, which is also for good reproducibility [34]. Both the peak temperatures observed in the thermograms and curve shapes can vary with the temperature-scan rate. Increasing scan rates generally result in higher values of T_{max}, because inactivation of metabolism at high temperature is a function of both time and temperature [8]. Intraspecies differences also exist among the thermograms of accessions of a single species that are adapted for growth in different climates (Fig. 6). The temperature coefficient, μ, and T_{max}, T_{ls}, and T_{hs} all differ systematically for plants collected from across such a growth range [35]. The correlation between temperature parameters obtained from the curves (i.e., T_{ls}, T_{max}, and ΔT_w) for different accessions and climatic temperature at the native growth site demonstrate that metabolism adapts to the climate to optimize growth, survival, and reproduction of the species.

In summary, scanning calorimetry allows rapid and continuous assessment of the respiratory rate as a function of temperature, which in turn offers important insights into climates suitable for successful growth of a particular genotype. Thermograms of metabolic heat rate versus temperature

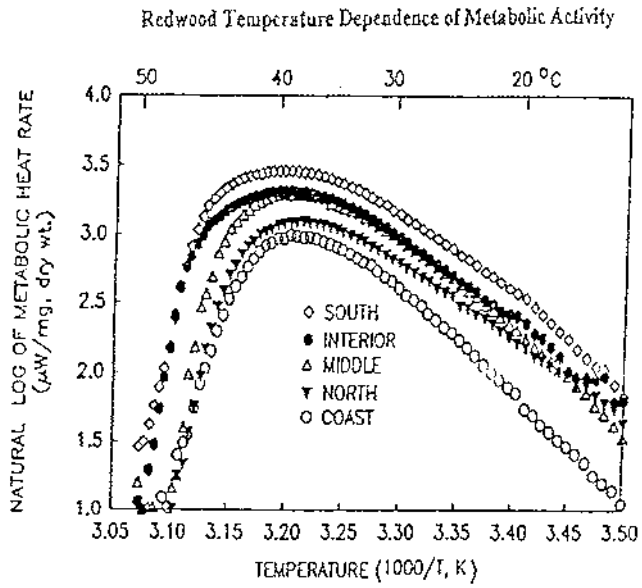

FIGURE 6 Continuous temperature-scanning thermograms for meristem tissue from *Sequoia sempervirens* accessions. (After Ref. 35.)

can clearly define (a) temperature limits to robust respiratory metabolism and therefore to growth, (b) differences in temperature responses within and among species, and (c) temperatures at which changes in metabolic pathways occur. The differences observed among the thermograms of species reflect a wide range of responses to a temperature change and provide information that should ultimately be related to the growth climates.

Acclimation of plant respiratory metabolism to a climatic or seasonal temperature change occurs over much longer time periods than the experimental times typically employed in calorimetric experiments. Thus, measurements of metabolic heat rates across a range of temperatures indicate short-term responses to temperature and reflect plant responses to short-term, continuously changing temperatures like those experienced daily during growth (e.g., see Fig. 1). Evidence for acclimation of metabolic heat rates to growth at different temperatures must be obtained from examination of tissues collected throughout the season or across a temperature cline.

A Respiration-Based Model Describing the Temperature Dependence of Growth

The temperature dependence of the rate and efficiency of respiratory and biosynthetic metabolism provides insight into the processes controlling temperature effects on growth. Plant growth may be limited by the rate of acquisition of carbon or of other resources, or by the rate of processing of those resources into the structural biomass. (Note that photosynthate which is used for energy production and anabolic processes is not part of the *structural* biomass). As previously stated, the photosynthesis rate will be proportional to the growth rate only if carbon is the limiting resource. In general, the rate of growth will be equal to the rate of acquisition of any resource only when the resource is growth-rate limiting. In contrast, it is always true that the rate of growth is proportional to the rate of processing of substrate into a new biomass multiplied by the efficiency of the process.

The general relation between the respiration and growth rates is easily derived by inspection of the chemical reaction describing aerobic growth (Equation [3]).

$$C_{\text{substrate}} + (xO_2 + \text{N, P, K, etc.}) \rightarrow \varepsilon C_{\text{biomass}} + (1 - \varepsilon)CO_2 \quad (3)$$

where ε is the substrate carbon conversion efficiency. (The symbol ε is used here to avoid confusion with various earlier symbols and definitions of efficiency.) The quantitative relation between the specific growth rate (R_{SG} in moles of carbon per time per mass), specific respiration rate (R_{CO_2} in moles/time per mass), and substrate carbon conversion efficiency of a plant or tissue is given by Equation (4).

$$R_{SG} = R_{CO_2}\left[\frac{\varepsilon}{1 - \varepsilon}\right] \quad (4)$$

where $[\varepsilon/(1 - \varepsilon)]$ derives from the ratio of coefficients in Equation (3). Equation 4 accurately describes the plant growth rate no matter what the nature of the growth-limiting factor; that is, whether the growth is limited by photosynthate, nitrogen, some other nutrient, the metabolic rate, or other factors, but it is only useful if ε can be readily determined.

Beginning at low temperatures, R_{CO_2}increases with increasing temperature until some physical change occurs or a limitation on the rate of transport or nutrient acquisition occurs. Above this temperature, R_{CO_2} usually decreases rapidly with further temperature increases. The substrate carbon conversion efficiency, ε, may go through a maximum or continuously decrease with increasing temperature across the range of growth temperatures; that is, from 0 to 50°C. Increasing temperature through this range in a period of a few hours or less thus causes the growth rate to increase, go through a maximum, and then decrease; the exact function depending on how various metabolic pathways respond to temperature. Because other environmental stresses also affect metabolic pathways, the temperature response is affected by other stresses. Whether this interaction leads to cotolerance or synergism of the stresses depends on how the metabolism is affected.

The dependence of ε on time-temperature can be measured by relating ε to the metabolic heat rate (q) and the respiratory CO_2 rate (R_{CO_2}), as shown in Equation (5) [10].

$$\frac{q}{R_{CO_2}} = 455\left(\frac{1 - \gamma_p}{4}\right) - \left[\frac{\varepsilon}{1 - \varepsilon}\right]\Delta H_B \quad (5)$$

where γ_p is the average oxidation state of the substrate carbon for respiration and biosynthesis, ΔH_B is the enthalpy change for conversion of substrate carbon to biomass carbon, but including all other elements, and the constant 455 kJ/mol of O_2 is from Thornton's rule [10]. Thus, if γ_p and ΔH_B do not change, changes in the ratio q/R_{CO_2} measure the changes in ε with temperature or time-temperature.

The temperature dependence of growth rates is thus a function of the temperature dependences of the metabolic rate and the metabolic efficiency, both of which change continuously with temperature. The ultimate cause of high-and low-temperature growth limits is not primarily membrane phase transitions or enzyme denaturation, as has been commonly supposed, but loss of substrate carbon conversion efficiency; that is plant cells lose the ability to produce energy at a rate sufficient to maintain the cellular structure. Structural changes may then occur as a consequence of the lack of energy, not as a cause.

As described earlier, variation of the plant metabolic rate and pathways with temperature determines how plant growth rates vary with temperature, since, aside from morphological differences, the ability of a plant to grow and survive in a given environment is defined by metabolism. If the growth rate is expressed as the rate of storage of chemical energy in the structural biomass with the substrate as the reference energy state, then the growth rate can be expressed as the difference between the rate of energy produced by respiration and the rate of energy lost to the surroundings. Equation (6) is a form of Equation (4), obtained by combining Equations (4) and (5), and assuming the substrate carbon compound is carbohydrate with γ_p equal to zero.

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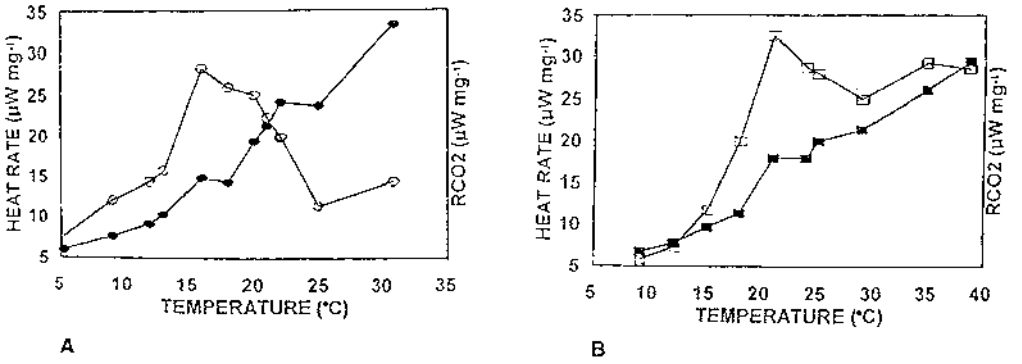

FIGURE 7 Values of isothermal heat rates (●) and 455 R_{CO_2} (○) at various test temperatures. (a) Cabbage leaf tissue. (b) Tomato leaf tissue. (From Ref. 32.)

$$R_{SG}\Delta H_B = 455R_{CO_2} - q \tag{6}$$

Equation (6) states that the growth rate is proportional to the difference between the rate of energy loss, that is, the heat rate (q), and the rate of energy generation, that is, a constant times the CO_2 rate. Measured temperature dependences of R_{CO_2} and q and Equation (6) can be used to predict the growth rate as a function of temperature. Further, the ratio q/R_{CO_2} provides easily measured, relative values of the substrate carbon conversion efficiency, ε, as indicated by Equation (5). The absolute value of q/R_{CO_2} also provides information on the oxidation state of the substrate carbon; that is, γ_p.

Relating Growth to Environmental Temperature with a Respiratory Model

Tomato and cabbage are representative warm- and cool-climate species that are used here to illustrate broad differences in the growth and respiration responses among Temperate Zone plants. Similar differences are expected both within and among other species, because their respiration characteristics reflect adaptation strategies for fitting into available thermal niches. Methods outlined here can provide temperature response profiles identifying appropriate growth temperature ranges for other cultivars of tomato and cabbage or for other species [32]. Figures 7 and 8 show the metabolic heat

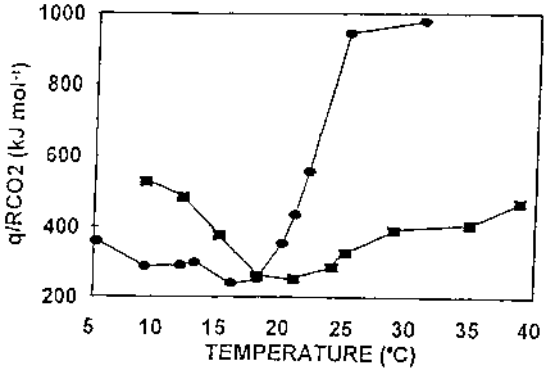

FIGURE 8 Values of q/R_{CO_2} for tomato (■) and cabbage (●) at various temperatures calculated from the data of Figure 7. (After Ref. 32.)

rates, the CO_2 production rates, and the ratio q/R_{CO_2} measured at different temperatures for tomato and cabbage leaf tissue [32]. For each species, q and R_{CO_2} have distinctly different temperature responses. The values of q increase approximately exponentially over the temperature range measured, but the CO_2 rates show a marked discontinuity in the rate of increase with temperature. The responses also differ between the species. For cabbage, a maximum in R_{CO_2} is observed between 15 and 16°C. Tomato has a maximum R_{CO_2} between 21 and 22°C.

For cabbage, plots of q and $455R_{CO_2}$ intersect below 0°C and again at 21°C (see Fig. 7). At temperatures where $q > 455R_{CO_2}$, q/R_{CO_2} becomes large, indicating a negative substrate carbon conversion efficiency or a change in substrate. From 7 to 20°C, q/R_{CO_2} is small, has a minimum near 16°C (large ε), and then increases rapidly (decreasing ε and/or change in γ_p from 0 to negative values) as the temperature is increased above 16°C (see Fig. 8). $R_{SG}\Delta H_B$ is positive but small near 0°C, reaches a maximum near 16°C, and then decreases and becomes negative above 21°C, where $455R_{CO_2} > q$ (see Equations [5] and [6]) (Fig. 9). Thus, these data predict that cabbage will grow slowly at temperatures near freezing, grow at increasing rates up to 16°C, and then at decreasing rates up to a temperature near 21°C, where growth stops, energy gradients are lost, and tissue damage begins.

The curves for tomato differ greatly from those of cabbage. For tomato, the q and $455R_{CO_2}$ versus temperature curves intersect near 12°C and again near 38°C (see Fig. 7). Below 12°C and above 38°C, q is greater than $455R_{CO_2}$ and no growth is expected. Values of q/R_{CO_2} for tomato are high (small or negative ε) at temperatures below 12°C. As the temperature increases in the range 12–20°C, $455R_{CO_2}$ becomes increasingly greater than q so that q/R_{CO_2} decreases, indicating an increasing substrate carbon conversion efficiency (see Fig. 8). From 20 to 35°C, q/R_{CO_2} increases and calculated values of $R_{SG}\Delta H_B$ indicate that growth becomes less efficient as 35°C is approached. Growth stops, that is, $R_{SG}\Delta H_B$ becomes negative, above 35°C (see Fig. 9).

Observed growth rate measurements on tomato as a function of temperature are consistent with the calculated values of $R_{SG}\Delta H_B$. The classic studies of Went [4] established 10°C as a critical low temperature for the growth of common tomato cultivars and defined conditions of decreased growth rates above 30°C. Many subsequent studies have confirmed the temperature range from 10 to 12°C as a region below which precipitous decreases in growth rates are noted for tomato and other chilling-sensitive plants [7]. Also, our studies with tomato cell cultures show diminishing

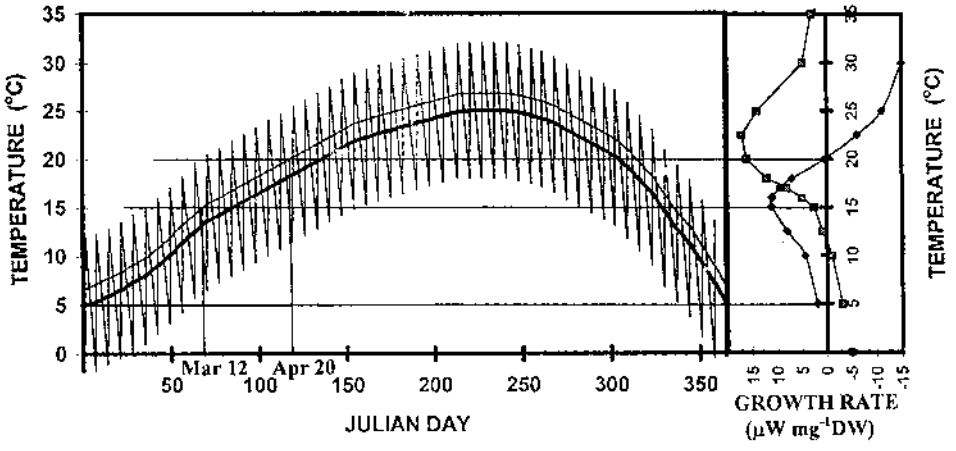

FIGURE 9 Growth rates ($R_{SG}\Delta H_B$, see Equation [6]) calculated from measured q and R_{CO_2} values (see Figs. 7 and 8) for cabbage (◆) and tomato (■) keyed to climatic temperatures at Davis, California. (From Ref. 32.)

metabolic rates above about 30°C and a rapid decrease above 35°C (see Fig. 2) [8]. Growth rate versus temperature data on cabbage is less abundant, but again the known general pattern of the cabbage growth rate response to temperature is described by the respiration parameters. Cabbage and other cold-climate crops such as wheat have significant growth rates near zero, grow well at temperatures below about 20°C, and grow slowly or experience tissue damage when temperature is high. Recent studies on wheat elongation show a maximum rate around 20°C with a sharp downturn by 25°C [36].

The data on tomato and cabbage [32] presented here leads to a novel rationale for growth rate maxima and growth rate changes observed at temperature extremes. The growth rate increases with temperature only so long as the product $R_{CO_2}[\varepsilon/(1-\varepsilon)]$ increases with temperature (see Equation [4]. The growth rate decreases with increasing temperature when efficiency decreases faster than R_{CO_2} increases. Because the temperature responses of q and R_{CO_2} are different, the substrate carbon conversion efficiency (ε, see Equation [5]) must change continuously with temperature and can exhibit either an increase or decrease with increasing temperature.

The temperature responses of q and R_{CO_2} observed for cabbage and tomato cause their specific growth rates to increase, go through a maximum, and decrease with increasing temperature. Their growth behavior as a function of temperature thus can be explained without postulating a reversible or irreversible inactivation of the enzyme activities or the membrane phase changes. Thermal damage may occur but is a consequence of the energy imbalance rather than direct thermal inactivation of enzymes or membranes. Many efforts have been made to identify a thermally induced event such as a lipid membrane phase transition as the ultimate cause of observed high- and low-temperature tissue damage [37]. However, the crossing of the curves for $455R_{CO_2}$ and q at about 12°C for tomato (see Fig. 7) show that chilling sensitivity does not require a physical event but can be accounted for by an inability of respiration to supply sufficient energy to maintain structures in the tissue.

Figure 9 shows that tomato achieves nearly double the specific growth rate of cabbage (assuming equal ΔH_B), but tomato grows rapidly in a narrower temperature range than does cabbage. Plants adapted to cool climates usually show a growth pattern similar to cabbage; that is, they grow slowly at a fairly constant rate over a wide range of low temperatures. We hypothesize such an adaptation is an advantage in temperate, arctic, and alpine climates with large diurnal temperature fluctuations. To achieve a relatively constant growth rate across a wide range of temperatures requires minimizing the difference in the temperature dependences of q and R_{CO_2}. Plants from climates with small diurnal temperature fluctuations are expected to have higher temperature dependences of q and R_{CO_2} and greater differences in the temperature dependences of these parameters. Thus, the temperature dependence of the respiratory metabolism is related to the ability of a plant to survive and reproduce within a given temperature environment.

The vertical lines in Figure 9 relate the growth-rate responses calculated from respiration-rate measurements to calendar dates through climatic temperatures; that is, seasonal, diurnal fluctuations, and mean kinetic temperature. The line at 20°C connects the high-temperature growth limit of a particular cabbage cultivar to seasonal times when it will not grow well at this location (Davis, California). Figure 9 shows that by day 115 (April 25), the mean kinetic temperature exceeds the high-temperature limit and does not decrease below this limit until after day 310 (November 6). At this location, this cabbage cultivar is predicted to grow well during the period from mid November to mid April, with maximum growth rates at about March 12 and November 27. The optimum growth period for this tomato cultivar is predicted to be from about day 150 (May 30) to about day 300 (October 27) when the mean kinetic temperature is near optimum and daily minimums do not exceed the low limit. Figure 9 thus demonstrates how measurements of q and R_{CO_2} over a range of temperatures can be used to predict the growth of a plant in a particular climate.

Terminology and Mechanism of Action of Temperature Stress

Terminology previously used to describe the effects of the temperature on plant growth was derived as a parallel to the effects of nutrient concentrations on plant growth; that is, a low-temperature

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threshold comparable to a low-concentration threshold below which growth is limited, a range of temperatures or concentration sufficient to support maximum growth, and a high-temperature threshold or a threshold concentration beyond which stress was exhibited. But temperature is not an extensive property related to the amount or concentration of a material, but it is an intensive property related to molecular kinetic energy. Concepts of threshold ''stress temperatures'' or ''unstressed growth temperature ranges'' are invalidated by the data in Figures 7–9. Because there is no temperature range in which the changing temperature does not affect the growth rate, there is no such thing as an ''unstressed temperature range.'' The term *temperature stress* should be limited to qualitative comparisons only at temperature extremes, and a more proper term would be *time-temperature stress*.

Lipid phase changes and protein denaturation have been invoked to explain the tissue damage from both chilling and high temperature [37]. However, evidence that such phase changes occur in intact tissues at temperatures and conditions relevant to plant inactivation is circumstantial; no phase change has been directly observed in intact plant tissue [34,38,39]. Phase transitions (which in this context includes ''melting'' of protein tertiary structures and phase separations as well as lipid phase transitions) can occur at a discrete temperature in pure substances or can occur across a broad range of temperatures in both pure substances and in mixtures. First-order phase transitions occurring at a discrete temperature must have $\Delta H > 0$ for melting and are detectable by temperature-scanning calorimetry, but higher-order transitions occur across a range of temperatures, have $\Delta H = 0$, and are not readily detected by temperature-scanning calorimetry [38,39].

It is clear that changes in unsaturation do correlate with changes in cold tolerance. For example, Nishida and Murata [40] prepared mutants with altered lipids and demonstrated changes in cold tolerance. What is not clear is the implication that the changes are due to effects on, for example, structure and phase transitions. Such changes in the lipid composition must have very important effects on the metabolic rates, μ values, and/or efficiencies.

Another proposed explanation for observed temperature responses is a change in a rate-limiting metabolic process [12,38,39]. Such a change in kinetics produces a change in the slope of an Arrhenius plot of ($\ln R$) versus $1/T$ if the activation energies of the rate-limiting processes are different. Changes in the slope of Arrhenius plots are frequently observed but difficult to interpret. It is difficult unambiguously to ascribe the kinetic data to phase transitions or structural changes.

Although structural changes may be associated with plant responses to a temperature change, a change in the structure need not be the ultimate cause of the response or the damage eventually observed as a consequence of the temperature change. Cells are compartmentalized and concentration gradients of ions and molecules exist across the membranes of the compartments. These gradients, both of concentration and of electrical potential, are the immediate energy source used to drive biosynthesis in the cell. Biosynthesis creates not just new molecules from substrate but also new structures as well. Thus, energy gradients are necessary both to create and to maintain structures. Respiration is the source of these energy gradients. In any circumstance in which respiration is unable to replenish the gradient as rapidly as it decays, the gradients will be lost and loss of structure will follow. Thus, we conclude energy gradients cannot exist without structure and structure cannot exist without energy gradients. At any temperature where respiration is either too slow or too inefficient to provide energy at a rate sufficient to maintain the energy gradients in at least a steady state, the gradients will diminish until the structure can no longer be maintained and the system will then begin to lose structures within the cell. If the process continues too long, the cell will be unable to repair itself and death ensues.

TEMPERATURE AND ECOLOGY

Respiration and Plant Distribution

Respiratory rates and efficiencies for plants from the same species but from different climatic regions in the growth range have quantitatively different temperature dependences [31,35,41,42]. Evolutionary adaptations to permit growth and reproduction in different regions are necessary for species

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survival [1–3]. For example, some plants are adapted to warm and other to cold climates, some to a narrow range, and others to a broad range of temperatures. Different responses to temperature are seen within as well as among species. Plant metabolic activities in tropical areas must be optimized for warm, comparatively invariant climates, whereas metabolism in arctic plants must be matched to a cooler and broader range of rapidly changing ambient temperatures.

Consequences of Differences in Temperature Dependences of Heat Rates and CO_2 Rates

Biosynthesis (anabolism) is closely coupled to and dependent on respiratory catabolism. R_{CO_2} is thus proportional to the rate at which energy is made available to drive growth. q Measures the rate of energy loss from the plant or tissue. The difference between the rates of energy production and loss is the rate of addition of chemical energy to the photosynthate to produce the structural biomass by biosynthetic reactions; that is, a measure of the growth rate. When the rate of energy production during respiration is greater than q, the growth rate is positive; that is $[455(1 - \gamma_P/4)](R_{CO_2})$ is greater than q, or when $455R_{CO_2} > q$ if $\gamma_P = 1$ (see Ref. 10). When the difference between 455 R_{CO_2} and q becomes zero, growth stops, and under these conditions, the plant must use energy reserves to survive. If the difference becomes negative, the tissues are breaking down through spontaneous, exothermic reactions. The difference between q and $455(1 - \gamma_P/4)R_{CO_2}$ changes with temperature, because the temperature dependence of q (μ_q) and the temperature dependence of R_{CO_2} (μ_{CO2}) are not the same (see Equations [1] and [2] for a definition of μ). Therefore, plots of $455R_{CO_2}$ and q plotted against temperature on the same axes must cross. These curves cross in temperature ranges commonly encountered by plants growing in their native climatic temperature ranges [31,32,42]. For plants adapted to growth in a warm, stable climate, $455R_{CO_2}$ is greater than q between a low temperature of about 10°C and a high temperature of about 35°C; that is, growth only occurs between these temperatures (e.g., see data for tomato in Fig. 9). For plants adapted to cold, highly variable climates, $(455R_{CO_2} - q)$ is positive at low temperature and goes to zero above a temperature in the range 20–30°C (see data for cabbage in Fig. 9). Such plants grow well below, but not above, the crossover temperature.

Because $(455R_{CO_2} - q)$ is small near the crossover temperature, plants will have slow growth rates in this temperature range. Farther from the crossover temperature, growth rates will be greater. The change in the growth rate with temperature thus depends on the relative and absolute values of μ_q and μ_{CO_2}. When μ_q and μ_{CO_2} are greatly different, growth rates will change rapidly with temperature. When they are similar, growth rates will change only slowly with temperature. These patterns of the growth-rate response to temperature are linked to survival within a given climate and therefore to plant distribution.

Perennial plants grown in their native location and in common gardens have μ_q values related to their climate of origin [35]. Plants from low-elevation and low-latitude sites have lower μ_q values than plants from high elevation and high latitude. μ_q Appears to vary linearly with an additive function of latitude and elevation. Consideration of elevation and latitude as a surrogate for climatic temperature suggests that plants from cool and highly variable temperature sites have low μ_q, whereas those from warmer, more constant temperature regions have higher μ_q. μ_{CO_2} Appears to be lower for plants from cooler climates with more extreme temperature fluctuations than for plants from warmer, more thermally constant environments.

Plant Distribution and μ Values

Jeffree and Jeffree [2,3] have performed a data analysis that clearly illustrates the importance of extreme temperatures in determining plant distributions. They prepared plots with the mean temperature of the coldest month as the x axis and the mean temperature of the warmest month as the y axis for locations including the entire known distribution of a species. The occurrence of the species at each combination of mean high and mean low temperatures was indicated on the graph and an

ellipse drawn to include 77% of the total samples. Maximum and minimum high temperatures and maximum and minimum low temperatures for species survival at the 77% level were obtained from inspection of ellipses which enclose the ''temperate space'' allowing growth, survival, and reproduction of the species. In this analysis, annual temperature variation was assumed to be the key determinant of plant distributions.

As an aid in visualizing the effects of temperature on plant distributions, Jeffree and Jeffree [3] also produced a figure showing the possible vector directions of changes in seasonal temperature and the relation of these changes to climatic temperature patterns (presented in modified form as Fig. 10). The temperature patterns at various locations were described using the terms *oceanic* to indicate regions with little annual temperature fluctuation and *continental* for regions with large temperature fluctuations. Regions at higher elevations and high latitudes generally have the large temperature fluctuations implied by continental. Regions of low elevation and low latitude are more oceanic. Vector directions presented in the Jeffree and Jeffree plot indicate the consequences of various patterns of climatic temperature.

Figure 10 is a modified form of the Jeffree and Jeffree drawing showing the relation between the vector direction plot and the physiological parameters μ_q and μ_{CO_2}. Although the plots of Jeffree and Jeffree are based only on yearly temperature fluctuations, diurnal fluctuations in temperature, particularly during the growing season, are also extremely important to plant growth, survival, and reproduction. There is a generally positive correlation, but not a one-to-one relation, between sea-

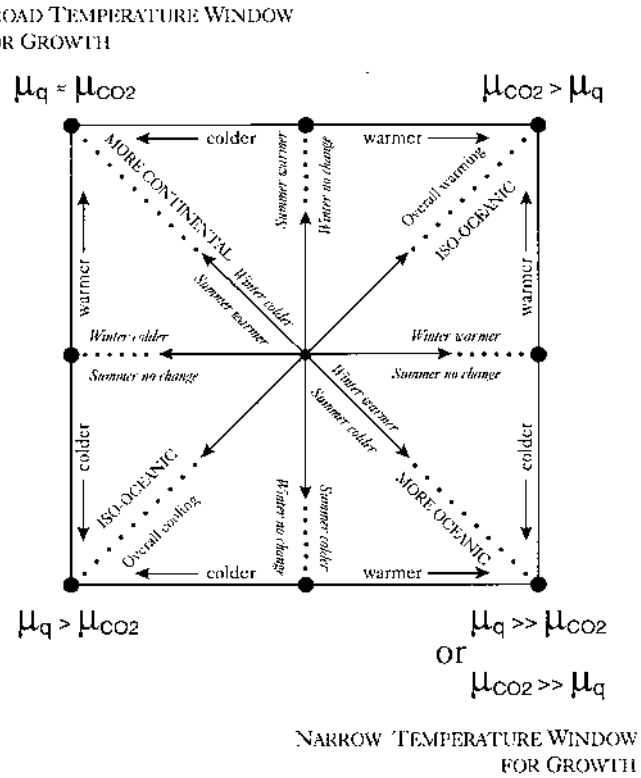

FIGURE 10 The influence of climatic thermal dependences on metabolism. Modified from Jeffree and Jeffree [3] to include the relation between growth climate and temperature coefficients for q and R_{CO_2}.

sonal fluctuations and diurnal fluctuations. Thus, conclusions based on seasonal differences must be carefully considered in finer terms of day-to-day and day-to-night fluctuations during the growing season. This is important when considering responses to a climatic temperature change at both the species and the individual plant level or when considering moving plants from one location to another. Physiological parameters provide a better reflection of the temperature responses of growth during specific periods when growth is occurring and emphasize potential growth and competition in the realm of the ''potential niche'' size rather than the ''realized niche'' size determined by ecological and historical limitations and observed in studies of natural distribution [43].

Although Figure 10 shows the relations among μ_q, μ_{CO_2}, and oceanic or continental, it does not uniquely identify μ values best suited for each temperature zone. Note, for example, that where the figure indicates constant temperature, more oceanic regions may be suitable for plants with either $\mu_{CO_2} \gg \mu_q$ or with $\mu_q \gg \mu_{CO_2}$ but does not identify which of these options is better for warmer or cooler oceanic climates. A more complete representation is needed to be able to define the growth conditions for which plants with μ_{CO_2} greater or less than μ_q are favored [30].

Figure 11 shows changes in the growth rate with temperature as a function of the ratio of μ_q/μ_{CO_2}. When $\mu_q > \mu_{CO_2}$, specific growth rates are significant near 0°C, increase slowly with increasing temperature, and then decrease rather abruptly as the temperature is increased further. The larger the ratio of μ_q/μ_{CO_2}, the better the plants are adapted for growth at low temperature, the narrower the range of temperature allowing growth, and the lower the high-temperature limit for growth. Growth rates for such plants are positive below temperatures where the ratio $\mu_q/\mu_{CO_2} = 1$. These plants are best suited to the nearly constant temperatures of cool, oceanic-type climates. When $\mu_q < \mu_{CO_2}$, the plant growth rate is zero or very slow at low temperatures and increases rapidly as the temperature is increased. Smaller ratios of μ_q/μ_{CO_2} indicate adaptation to higher temperatures, narrower ranges of temperature allowing growth, and a higher low-temperature limit for growth. Growth rates for such plants are positive above temperatures where the ratio $\mu_q/\mu_{CO_2} = 1$. These plants are best suited to the nearly constant temperatures of warm, oceanic-type climates. When values of μ_q and μ_{CO_2} are nearly equal, plants are adapted to a broad range of temperatures and grow relatively slowly over the entire range of temperatures. Such plants are best suited for survival

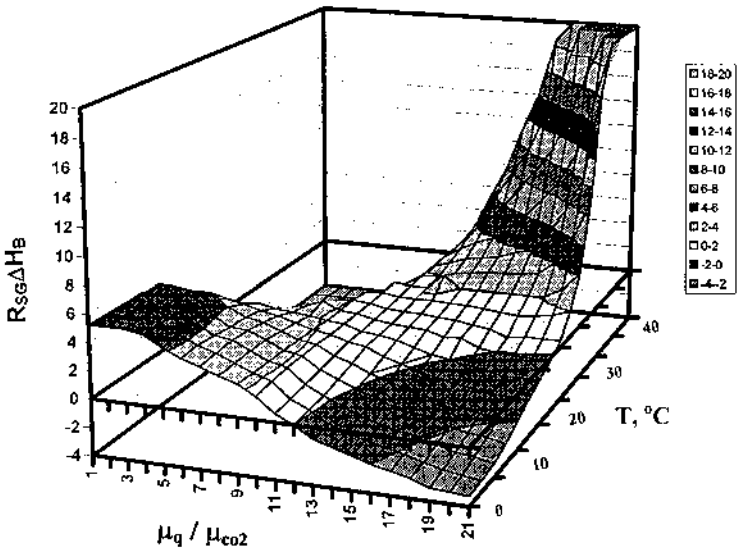

FIGURE 11 Plot showing the relation of growth rate (vertical axis) to temperature and the ratio of the temperature dependences of q and R_{CO_2} (i.e., μ_q/μ_{CO_2}).

in continental-type climates with large and rapid temperature fluctuations. They also can potentially grow in narrow temperature zones but will commonly be out-competed in such niches, because narrowly adapted species will grow more rapidly.

Figure 11 illustrates the relation between the temperature limits of growth described by Jeffree and Jeffree [2,3], seasonal and annual temperature fluctuations, and plant physiological parameters. With Figures 10 and 11 and the established physiological relationship, it is possible to understand the relations among the metabolic rates, efficiency, plant growth rate, and plant growth range. Plants adapted to narrow temperature windows, either cold or warm, can have very high metabolic efficiencies (large values of $[455R_{CO_2} - q]$) and, therefore, rapid growth over a narrow temperature range. This is done at the risk of severe damage in the form of the loss of energy to maintain potential gradients if the temperature moves outside the allowed narrow limits.

Plants that are adapted to broad temperature ranges can maintain relatively small positive values for $(455R_{CO_2} - q)$ over the entire range. They do so at the expense of being able to have large values in any given narrow temperature range. Thus, such plants are at a competitive disadvantage within niches having oceanic climate character.

The experimental observations of differences in μ values for the energy-producing and energy-using metabolic pathways of plant metabolism force the conclusions that energy-use efficiency must change with temperature and that the pattern of change describes the ability of a plant to grow in a given climate. Plant distribution is uniquely explained in terms of the temperature dependence of metabolic efficiencies. Figure 11 focuses only on the ratio of μ values. In addition, the absolute values of the rates, not just their ratios, impacts growth rates. Individual plants with identical ratios of μ values can have different growth rates while having the same responses to temperature change.

Certainly, additional factors enter into the determination of plant distribution. For example, the morphological features of plants allow buffering of the plant responses to temperature, water fluxes, salt, and other stresses. Dormancy considerations also define when a plant will grow and therefore what temperatures it must face during its growing season. Still, even with all of the known plant adaptations developed to aid plant survival, the plant growth and metabolism must respond to an ambient temperature and its daily and seasonal fluctuations with the general pattern illustrated in Figure 11. The metabolic and energy-based considerations illustrated here will contribute strongly to all plants (as well as other poikilotherms) in any environment.

The above conclusions about the relation between temperature coefficients of respiration and plant distribution are readily extended to consider growth seasons for plants. Figure 9 shows the relation of temperature throughout a growing season, among growth rate, and species or cultivar. This figure extends the discussion of seasonal temperature associated with Figure 1 to show how the mean, temperature, mean kinetic temperature, and daily high and low temperatures are related to the growth-temperature–response curves for specific cultivars of two annuals, tomato and cabbage.

Cabbage, a cool-climate species, has relatively low μ values and $\mu_q > \mu_{CO_2}$. As discussed earlier and illustrated in Figure 9, cabbage grows slowly at 5°C, increases the growth rate up to about 15°C, and stops, growing above about 21°C. In January at Davis, California, 5°C is near the mean temperature. The mean kinetic temperature on this date is 6–7°C, and the mean daily high is near 12°C. Thus, cabbage will grow for a significant portion of the time in here, even during early January. By March 12, the mean kinetic temperature is about 15°C and growth of cabbage is optimum. Daily temperature fluctuations do not go below a range allowable for growth and rarely exceed the high-temperature limits for active growth of cabbage. However, by April 20, the mean kinetic temperature limits the active growth of cabbage. By April 20, the mean kinetic temperature begins to exceed 21°C and cabbage encounters a temperature condition at which there is no growth. Survival during these periods depends on the use of stored energy reserves. This pattern quite accurately reflects the seasonal growth of cabbage at this location.

Tomato, a warm-climate plant, also has $\mu_q > \mu_{CO_2}$, but Figure 9 shows that growth for the cultivar examined does not proceed rapidly below about 12–15°C. The values of the mean kinetic temperature in Davis do not reach this level until the first or second week in March. From this time on, tomato can grow for a fraction of the day, but much better growth rates are achieved when the

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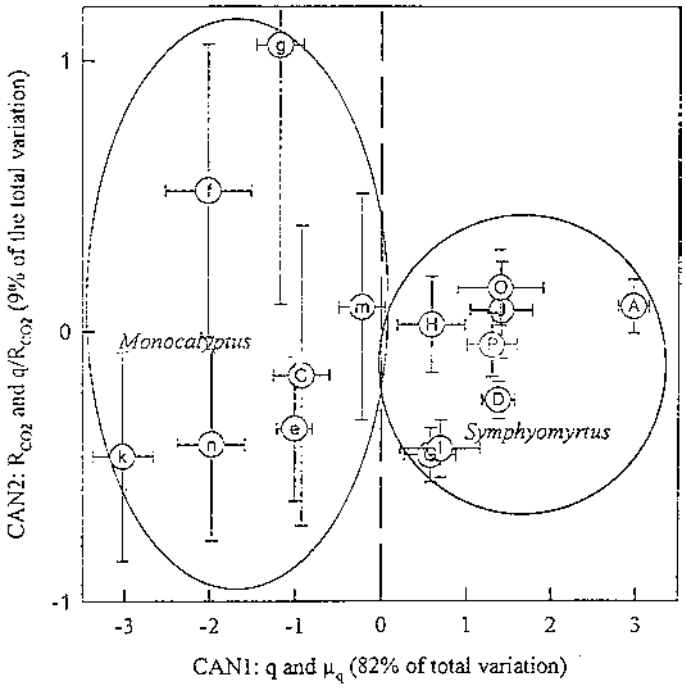

FIGURE 12 Subgenera of *Eucalyptus* species separated by respiration traits. Subgenus *Symphyomyrtus* species are represented by upper-case letters and subgenus *Monocalyptus* species by lower-case letters. Species of *Eucalyptus* included: *E. brookerana* (A); *E. cypellocarpa* (C); *E. dalrympleana* (D); *E. delegatensis* (e); *E. fastigata* (f); *E. fraxinoides* (g); *E. maidenii* (H); *E. glaucescens* (I); *E. nitens* (J); *E. obliqua* (k); *E. ovata* (L); *E. radiata* (m); *E. regnans* (n); *E. rubida* (O); and *E. smithii* (P). Mean estimated canonical variables, CAN1 and CAN2 for species respiration traits are presented. Error bars are the standard errors of the means of CAN1 and CAN2. The dashed vertical line emphasizes the separation of subgenera on the basis of respiratory traits. (After Ref. 44.)

mean kinetic temperature is near 20–25°C. This does not occur until early to mid May. Daily high-temperature fluctuations during mid to late summer in Davis extended into ranges that exceed the optimal values for the growth rate but seldom reach values that exceed the zero growth limit. The growth of this tomato cultivar is well suited to the Davis climate over the season from mid May until the end of August, because the mean kinetic temperature is in a near-optimum temperature range and the temperature extremes generally fall within the allowable range for tomato growth.

Other cultivars of cabbage and tomato may have different patterns of growth-rate responses to temperature. The examples used in this discussion to illustrate detailed patterns of the growth-rate response to temperature have focused largely on a single cultivar or genotype. However, intra- as well as interspecies heterogeneity exist with respect to temperature. An envelope drawn to include $R_{SG}\Delta H_B$ versus temperature curves for the entire population of tomato genotypes would be much broader than the tomato curves of Figure 9, as each plant would have its unique pattern of response to temperature. The broad envelope is indicative of the temperature range for growth and survival of the species.

Patterns of the growth-rate response to temperature and the respiratory parameters defining the growth rate are heritable, and values of respiratory properties are related to plant origin (or climate). Canonical analysis of the respiratory and growth parameters show distinct separations of

species for the two major *Eucalyptus* subgenera based on this respiration rates and their temperature dependence [44] (Fig. 12). A relation between respiration and the limiting temperature has been observed for growth among ecotypes within coast redwood (see Fig. 5). Examination of the respiration rate versus temperature for redwoods collected from locations across the species range, but grown in a common garden, shows high-temperature limits of respiration for each population reflect the climatic temperature at the site of origin [35]. Respiration in plants from northern sites with cooler summer temperatures were inactivated at lower temperatures than those from warmer southern and interior sites. Direct evidence of heritability also comes from recent studies (G.F. Moran, personal communication,) showing defined loci for q, R_{CO_2}, μ_q, μ_{CO_2}, and R_{SG} on quantitative trait loci gene maps of *Eucalyptus nitens*.

Plant distribution studies have been used to develop equations to define seed and plant transfer rules for vigor and survivability [45–48]. Rehfeldt [49] and Sorensen and Webber [50] indicate that ''300 m of elevation is generally proposed as the critical limit of safe transfer of western conifers.'' Although this appears to be a good general estimate, some individuals within a species at a given area may survive a 400- to 500-m increase in elevation quite well but only a 100-m decrease, whereas others may tolerate a larger elevation decrease than an increase. The distribution of individual plant tolerances within a population also determines the consequences of a climatic change on the species. The ability to recognize and quantify the variability in individual tolerances by rapid measurements of q and R_{CO_2} will be valuable for the selection of plants capable of undergoing desired growth responses to meet patterns of climatic change or for transport into new environments.

CONCLUSIONS

Plant growth and distribution are a function of temperature; temperature being one of the key environmental variables that limits survival, growth, and reproduction of plants. The life cycle, morphology, and metabolism of a plant must be matched to the seasonal and diurnal cycles of temperature, and the plant must be able to withstand or avoid unseasonal extremes of temperature in the growth environment. Just as plants have adapted their morphology, they have adapted their metabolism to optimize growth, survival, and reproduction at the average, seasonal, and diurnal temperatures of their environment.

Plant growth is determined by metabolism. Metabolic rates and pathways change with temperature. The plant-growth rate may be limited by a limiting nutrient such as carbon or nitrogen or by the rate at which the plant can process substrates into the structural biomass. The growth rate under any condition must be proportional to the rate of catabolic respiration times the efficiency of substrate carbon conversion. Growth-rate responses to temperature can thus be predicted from measurements of the metabolic heat rate and the respiratory CO_2 rate as functions of temperature.

Temperature limits to growth, both high and low, can be predicted from measurements of the metabolic heat rate and the respiratory CO_2 rate as functions of temperature. Thus, damage from chilling and too high temperatures is initiated by the loss of energy gradients across membranes within the cell. Loss of structure then follows the loss of energy gradients generated by respiration.

The metabolic heat rate and the respiratory CO_2 rate have different temperature dependences in the same sample of tissue. The metabolic heat rate typically increases exponentially with temperature until high-temperature tissue damage occurs. The CO_2 rate typically increases exponentially at low temperature, goes through a maximum, and then begins to decrease before temperatures high enough to cause tissue damage occur. Thus, both the metabolic (i.e., respiration-driven biosynthesis) rate and the substrate carbon conversion efficiency (which is related to the ratio of the metabolic heat rate to the CO_2 rate) change with temperature and the growth rate is closely related to the temperature dependences of the heat and CO_2 rates.

The relation of the growth rate to the temperature dependences of the heat and CO_2 rates can be used to understand how plants adapt their metabolism to optimize their chances for survival, growth, and reproduction at the climatic temperature conditions where they grow. Describing the

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temperature dependences of the metabolic heat rate and CO_2 rates by their Arrhenius temperature coefficients, that is, μ_q and μ_{CO_2}, respectively, allows definitive statements of the correlation between the metabolism and climate.

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19

Effect of Low Temperatures on the Structure of Plant Cells

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INTRODUCTION

Cell ontogeny is considered to be a chain of structural and functional processes that represent changes in cell development from structurally simple to the highly specialized cell types. Plant cell development encompasses three types of processes: (a) new cells are produced by division in meristems, (b) cell growth and enlargement, and (c) cell differentiation into its specialized state.

Cell development is continuous process that is governed by internal and external factors carried out in a certain environment. The pathway of cell ontogeny is changed when the natural environment is distinctly modified. Many processes are interrupted and others only occur in response to particular circumstances. Structural and morphological changes of the cells are the result of the expression of the cell genome under the influence of external factors.

Among the external factors that greatly affect cell development is temperature. A favorable temperature has a positive effect on structural and physiological processes of plant cells. When the temperature is increased or decreased, a harmful effect on the plant cells can be observed.

Different plants distinctly react to temperature fluctuations. Plant sensitivity to the temperature depends on the plant's origin and phylogeny. The effect of temperature on cell ontogeny has been extensively studied and there are reviews elsewhere concerning this topic [1,2].

In this chapter, we have tried to submit the results regarding the effect of low temperatures on the structure of the plant cell. Mutual comparison of existing results and their generalization is not easy, because variable plant species in different ontogenetic phases have been used in the observations. In spite of these difficulties, the plants sensitively react to the low temperatures by changes of their metabolism by adaptive reactions, and therefore its quite tempting to find out how the cell architecture is changed under these conditions.

The temperatures on the Earth's surface are very different, changing during the seasons as well

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as during the day and night. Despite these differences, plants grow almost everywhere. However, to be able to survive the unfavorable temperatures, plants have to adapt to this oscillation in temperature. Plants are divided into three groups on the basis of their sensibility to the temperature [1]:

1. *Chilling-sensitive plants*: These are seriously injured by the temperatures above zero (usually below 15°C).
2. *Chilling-resistant plants*: These are able to tolerate low temperatures but are seriously injured when ice starts to form in their tissues.
3. *Frost-resistant plants*: These are able to tolerate exposure to very low temperatures (−50–−100°C even when immersed in liquid nitrogen).

Most perennial plants growing in the temperate regions undergo a ''hardening'' process in the autumn of each year to prepare for overwintering. In most agricultural areas, unseasonal frost can occur throughout much of the growing season. During periods of active growth, most crop species do not tolerate freezing. Depending on the minimum temperature and the duration of the frost, plants may be partially damaged or killed, resulting in lower yield and quality at harvest or even complete crop failure. Most winter crops, however, have the ability to develop freezing tolerance when exposed to hardening conditions.

Each plant is characterized by a certain genetically fixed level of resistance to low temperatures, which reduces its metabolic activity. This level of resistance (or survival capacity) can vary among individual plants and species. Low temperatures act as a stress factor that has a strong impact on the growth, reproduction, and distribution of plants. The ability of plants to survive and grow depends on different ecological and physiological mechanisms [1–6].

Since plant metabolism is, in fact, a set of chemical reactions, the effect of cold stress (both chilling and freezing) strongly influences metabolic processes in the cells. These metabolic changes are accompanied by structural alterations of the cells.

CHILLING

Chilling injury can be observed on many plants of tropical and subtropical origin when they are exposed to low, but nonfreezing temperatures, in their chilling range, which is usually from 25 to 10°C [7]. For plants of temperate origin, the chilling temperatures usually range from 15 to 0°C. The chilling effect is manifested by physiological and cytological changes. Depending on the time and temperatures, the cytological changes can be either reversible or irreversible. However, the chilling-sensitive plants are also able to adapt to the chilling if they are hardened a certain amount of time at temperatures slightly above their critical temperatures.

Many light and electron microscopic studies have shown different structural changes of the cells in chilling-sensitive plants after their exposure to a long period of chilling stress [8–11].

Cell Membranes

The cellular membranes are those cell compartments, where the primary events of chilling stress occur [12]. An increase in the permeability of the plasmalemma and leakage of organic and inorganic substances is considered to be the first symptom of cell injury [13]. Light- and electron microscopic observations of tomato cotyledons growing at 5°C for 3 days have revealed the loss of cell turgor, vacuolization of the cytoplasm, swelling, and desintegration of cell organelles [14]. More detailed ultrastructural time-course studies have shown injury of the plasmalemma after 20–24 h. Desintegration of the plasmalemma can be observed after prolonged cold treatment or at lower temperatures [15,16].

During plasmolysis of hardened and nonhardened cells of rape and alfalfa plants, the plasmalemma is pressed against the tonoplast and deleted into the vacuole as sac-like intrusions [17].

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The similar sac-like invaginations of the tonoplast into the vacuole during the hardening of potato leaves at 5°C can be seen [18].

Chilling of the roots of the tropical plant *Episcia reptans* results in tonoplast discontinuity within 1 h at 5°C and 3 h at 10°C [19]. Two types of crystalline deposits (cytoplasmic and tonoplast-associated) are seen in root cells after chilling stress. Since similar deposits also have been observed in the epidermal, mesophyl, and vascular cells of *Episcia reptans* leaves [20] and on the tonoplasts of potato cotyledons [16], and these deposits closely follow tonoplast disruption, it can be supposed that these deposits probably serve as an indication of cell injury in the plants with increased time of exposure. Although the injury of a majority of the membranes after a short period of chilling is usually reversible, injury of the tonoplast is irreversible [21]. Irreversible injury of the tonoplast may govern the ability of plants to survive rewarming [22].

Frequently, as a result of chilling stress or hardening at low, above-zero temperatures, lipid bodies accumulate in the cytoplasm or in close association with the plasmalemma [23–26].

Plastids and Mitochondria

Swelling of plastid membranes and mitochondria is very a common symptom of chilling temperatures. The harmful effect of these temperatures is mostly time dependent. Chloroplasts from the leaves of *Episcia reptans* chilled for 6 h at 5°C have an irregular and less organized membrane system and fewer plastoglobules. An increase in the exposure time results in both swelling of the chloroplast thylakoids and in a decrease in the size and number of starch grains [20]. After 4 h of exposure at 5°C, injured chloroplasts desintegrated thylakoids can be seen [14]. Full grana desintegration and increasing of the number and size of the plastoglobules can be observed in hardened cucumber leaves after 11 days of chilling. Hardening of potato leaves for 10 days at 5°C causes dilation of the thylakoids and the disappearanse of starch grains [18]. The chilling stress induces the reduction of starch grains and thylakoids in winter wheat and in maize [24,27]. When *Ephedra* cells are cultivated after 15 days at 2°C, the plastids together with the mitochondria are organized into groups. Plastid grana are innumerous and plastids very often contain membrane-free stroma [28].

During transition of poplar xylem ray cells from summer to winter conditions (chilling at 0°C), the amyloplasts are without starch and thylakoid dilation has been observed [29]. Contrary to this result, the long-term hardening of young seedlings of Norway spruce at 3°C increases the content of the starch grains in plastids and the thylakoids are not distinctly dilated. Such plastids possess numerous plastoglobules [23]. The similar accumulation of starch after 8 days of hardening at (5/2°C day/night) has been recorded in chloroplasts of hardy *Solanum acaule* but not in the chloroplasts of less hardy *S. tuberosum* [30].

After the 1-day exposure of *Ephedra* cells to 2°C, the mitochondria have less dilated cristae and their matrix is transparent [28]. Swollen mitochondria with reduced cristae have been observed in chilled onion cells [31], in maize root cortex (Fig. 1) [11], and in both the root and leaves cells of *Episcia reptans* [19,20]. Owing to the mitochondrial swelling in chilled tissues, their volume is doubled in comparison with the mitochondria from the plants grown at a favorable temperature [20]. The first visible symptoms of injury of the mitochondria have been recognized after exposure of tomato cotyledons at temperature 5°C for 4 h [16]. These mitochondria possess a reduced number of cristae and discontinuities in their envelope. Structural alterations of the mitochondria have been seen in the microsporocytes and tapetum of *Rhoeo discolor* exposed to temperatures of 4–5°C for 4 days [32].

No visible changes in the mitochondria have been detected in xylem ray cells of poplar trees at a temperature of 0°C for 14 days [29].

Endoplasmic Reticulum and Dictyosomes

The endoplasmic reticulum of plant cells seems to be very sensitive to cold. After exposure of plants to cold, an extensive dilation and vesiculation of the smooth endoplasmic reticulum cisternae can

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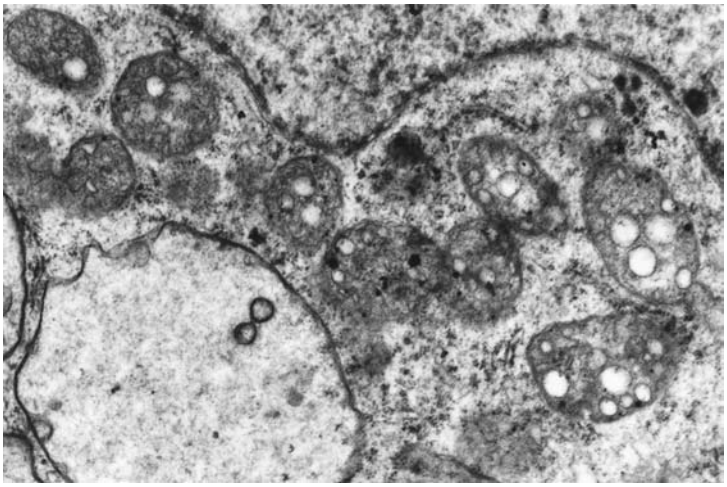

Figure 1 Mitochondria with dilated cristae from maize root cortex cells at 5°C (× 24 000) (From Ref. 11.)

be observed quite clearly, and the profiles of the rough endoplasmic reticulum almost completely disappear. These dilated vesicular endoplasmic reticulum cisternae probably serve as accumulation sites of cryoprotective substances [29].

Prolonged exposure of *Cornus stolonifera* callus cells to 0°C for 12 h results in partial dilation followed by microvesiculation of the rough endoplasmic reticulum and releasing of the ribosomes from the membranes. Vacuolization of the smooth endoplasmic reticulum is visible after 24 h of chilling [21]. Dilation of the rough endoplasmic reticulum without ribosomes has been observed in cooled microsporocytes [32]. The vesicles originating from the dilated rough endoplasmic reticulum without ribosomes have autolytic functions in chilled cells [14,15]. It might be suggested that the transformation of the rough endoplasmic reticulum into vacuolated smooth endoplasmic reticulum represents an early stage of chilling [21].

A strict correlation has been found between the temperature (between 30–5°C) and the volume of the endoplasmic reticulum labyrinths irrespective of the sampling at different hours of the day in five different plant species. The endoplasmic reticulum labyrinth that was extended at 20°C had disappeared completely with the drop in temperature to 5°C [33]. In fact, since the volume and form of the endoplasmic reticulum system in mature leaves fluctuates diurnally in response to environmental factors such as temperature and light, we could suggest a full reversibility of the ultrastructural changes. This shows that the endoplasmic reticulum system is very dynamic; it is probably the most dynamic structure in plant cells [34].

Dictyosemes are cell organelles that also respond to chilling stress by swelling. The swollen dictyosome cisternae occur in tomato cotyledons after 4 h of chilling at 5°C [16] or after 24 h at 0°C in *Cornus stolonifera* cells [21]. Longer exposure to a chilling temperature causes desintegration of the dictyosomes [21,35].

Nucleus

The nucleus of plant cells also sensitively reacts to unfavorable temperatures. Numerous studies of the effect of chilling temperatures from 0 to 4°C on the functional and structural behavior of nuclei in pollen mother and tapetal cells of *Rhoeo discolor* have been done. A short treatment of nuclei with cold does not cause any important changes in the morphology of the nuclei or in DNA synthesis [36]. However, a longer cold treatment considerably reduces both DNA and RNA synthesis [37].

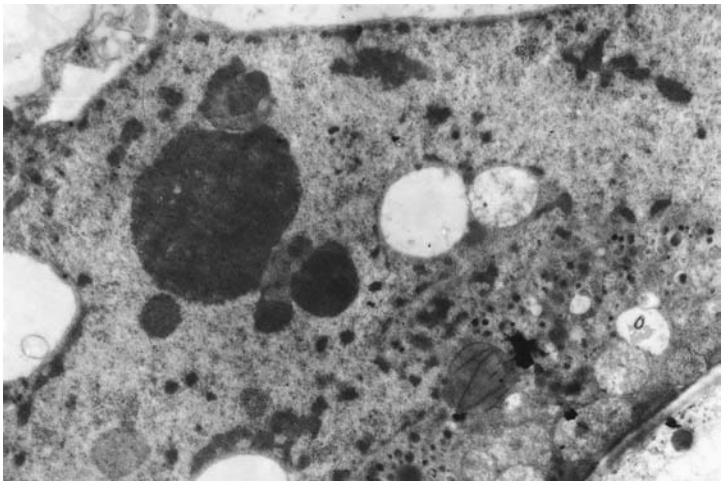

FIGURE 2 Nucleus with a nucleolus, pronounced nucleolus organizer region and nuclear bodies in the nucleoplasm from maize root cortex cells at 5°C (× 18 000). (From Ref. 42.)

Modification of the nuclear structure of plant cells has been observed by both light microscopy and electron microscopy. Lobed nuclei in *Ephedra* cells have been observed after long-time (15 days) exposure to 2°C [28]. The nuclei of other plants respond to a longer exposure to low temperatures by swelling and modification of the nuclear envelope [16,32] and chromatin coagulation [38–40]. Following cold stress at 5°C for 3 days, the nuclei in the root cells of maize contain rather dispersed chromatin and nuclear bodies often occur in the nucleoplasm, and the nucleolar organizer regions are pronounced (Fig. 2) [41,42].

Full nuclear disruption is observed in *Cornus stolonifera* cells and in very sensitive *Episcia reptans* cells after 2 days at 5°C [19,21]. In tomato cotyledons, irreversible injury of nuclei is seen after 20–24 h of chilling [16]. After exposure of wheat cells to the chilling temperatures, the fibrillar zone of the nucleolus is more abundant and the granular zone becomes diffused [39,43]. A high amount fibrillar components can result in the formation of nucleolus-like bodies in the cytoplasm [44] or in the nucleoplasm [11,27,42].

Besides the nuclear structure, the mitotic activity in plant cells also is strongly influenced by low temperatures. A decrease in the temperature from the optimum value to the minimum value (about 1°C) is accompanied by a progressive slowdown of the mitotic cycle as well as of the duration of mitosis. At the temperature of 3°C, for instance, the mitotic cycle in *Vicia faba* root cells may be 22 times longer than at 25°C [45].

Cytoskeleton

According to some investigators, the alterations that occur in the cellular membranes of plants at low temperature can be only a secondary response to the chilling stress. The primary response may be a breakdown of the cytoskeleton. It has been suggested that chilling stress has a direct effect on the microtubules [46], which form a major component of the cytoskeleton, because they have been found to depolymerase during cold treatment [47,48]. Depolymerization of the cortical microtubules at chilling temperatures (0–4°C) has been repeatedly observed in several chilling-sensitive species of higher plants [47] and in various cell types, including the root cells of maize [49], the guard cells of onion [50], the suspension culture cells of maize [51], and protoplasts isolated from tobacco [52].

In experiments with cucumber cotyledons, it has been found that treatment with antimicrotu-

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bular drugs makes the chilling worse, whereas treatment with abscisic acid protects cotyledons from drug effect and chilling injury [53].

There is a connection between chilling of the cytoskeleton and the inhibition of cytoplasmic streaming. The chilling temperatures can influence the equilibrium of Ca^{2+} and ATP, which is connected with F-actin activity [8,9,54]. Actin filaments have also been found to be involved in cold-induced conformational changes and the reorganization of the endoplasmic reticulum [33,35]. These results indicate that low temperatures (4–0°C) most likely influence either the interaction of the force-generating system, probably myosin, with actin filaments or the force-generating mechanism of the actomyosin-driven intracellular movement, but they do not affect actin-filament integrity [55].

The effect of low, nonfreezing temperatures on the plants also is visible at anatomical and morphological levels. These aspects are connected mainly with the adaptation reactions of less chilling-sensitive and cold-resistant plants (like winter cereals) to growth at low temperatures. Such anatomical and morphological changes like altered stomatal frequency [56], decreased epidermal cell size [57], increased mesophyll cell size, and suberinization [56,58] are associated with acclimation of plants to nonfreezing temperatures.

From the above-mentioned results, it is obvious that the extensive and variable structural reactions of cell compartments are the response to chilling temperatures. According to Wang's scheme of the responses of sensitive plants to the chilling-stress alteration of cellular structures are only one of many secondary responses [18]. The primary response is an alteration in the cell membranes—a physical phase transition of membranes from the liquid-crystalline to the solid gel state [12].

FREEZING

Generally, freezing in plants consists of the conversion of liquids in cells to a solid state, which is accompanied by loss of heat. Two types of freezing occur in plant cells and tissues: (a) vitrification—solidification of the cellular content into a noncrystalline (amorphous) state and (b) crystallization—arrangement of liquid molecules into orderly structures [59]. Vitrification of liquids in cells is a result of rapid freezing (more than 3°C/min) of plant tissues to a very low temperature. It is enhanced by hardening of plants at low temperatures. Although vitrification does not occur in nature, it is of great interest to researchers, because it enables plants to survive temperatures close to absolute zero [3].

On the other hand, crystallization (or ice formation) is a very common phenomenon in nature. The crystallization of ice may occur either within or outside the cells, but the process depends on the speed of cooling. The formation of ice inside the cells may occur by both internal nucleation or by penetration of external ice crystals into the cells [60]. In both cases, this type of freezing, also called intracellular, is lethal because of the immediate disruption of the cells. Only in the case of cells that exhibit deep supercooling may there be an exception to this rule [61,62]. Plant cells can also survive intracellular ice formation when the ice crystals that form by freezing are very fine, cooling is extremely rapid, and these crystals melt before they reach a harmful size [63].

There are three types of intracellular ice formation in the epidermal cells of onion plants at high-speed of cooling [64]: (a) Ice formation spreads from cell to cell through the plasmodesmata Freezing from cell to cell is observable on the *Tradescantia* staminal hair cells [65] and in mesophyll cells of Norway spruce during the winter frosts [66]. (b) Less frequently, ice can be formed in the cell walls adjacent to the intercellular spaces. Ice arises first in the plasmolyte between the cell wall and cytoplasm and then rapidly in the cytoplasm. (c) Intracellular ice originates spontaneously from centers of nucleation within the cytoplasm and later in the surrounding plasmolyte.

If the speed of cooling is slow enough (in nature, the cooling rate seldom exceeds 1°C/h), the liquids in the cells freeze extracellularly, causing cell dehydratation of cytoplasmic solutes and a reduction in cell volume and surface area, all factors which can potentially damage the cells irreversibly [5]. Ice formation for most plant tissues begins on the surface of the cell walls, in water

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transport elements, or on external surfaces [62,67]. Although the cooling is slow and the plasmalemma remains intact, ice formation will be confined outside of cells [68].

There are two major strategies allowing plants to survive freezing stress: freezing tolerance and freezing avoidance [2]. Tissues displaying freezing tolerance respond to freezing stress by the loss of cellular water to extracellular ice, resulting in collapse of the cell. As a consequence, an increased concentration of the cell sap and a lowered freezing point will occur. In plants displaying second-strategy–freezing avoidance, tissues exhibit deep supercooling, in which cellular water is isolated from the dehydrative and nucleating effects of extracellular ice [69].

The formation of ice in tissues and the appearance of frozen plant cells is well documented, mainly in studies employing light microscopy [3,65]. Descriptions of frozen cells at the electron microscopic level also have been done [70,71].

Cell Membranes

As already mentioned [68], functionally intact cell membranes are an effective barrier to the propagation of ice; however, this barrier may vary depending on the temperature or cold hardening [72,73]. Although the mechanisms involved in plant cold acclimation and frost injury are extraordinarily complicated, the freezing and thawing of cellular water have been found to be basic elements of freezing injury in plant tissues [60]. It has been established that the cellular membranes are more susceptible to freezing damage than soluble enzymes. The plasma membrane seems to be the most susceptible and, therefore, it has been identified as the major site of lethal injury [74].

Leakage of ions from thawed tissues is a common phenomenon of freezing injury. The leakage is usually considered the consequence of the loss of membrane semipermeability or membrane rupture by freezing injury. However, observations on onion epidermal cells have shown that freezing injury does not result in membrane rupture or complete loss of semipermeability. These results indicate that freezing injury is due to a specific alteration in the membrane semipermeability to K^+, and secondary effect is protoplasmic swelling [75].

There are numerous studies dealing with the physiological and biochemical changes occurring in membranes during the freezing and cold hardening processes, respectively [6,74–80] but observations regarding alterations in the cellular membranes are rather insufficient [63,70,81–83].

Isolated plant protoplasts make an excellent model system to study destabilization of the plasma membrane after freezing stress. The using of protoplasts has shown that destabilization manifests in various ways: by intracellular ice formation, by loss of osmotic responsiveness, or by expansion-induced lysis [73]. If cellular membranes are the site of freezing injury, then cellular alterations during cold acclimation that allow the cells to survive freezing also will appear in membranes [77].

Cold acclimation involves chemical and structural alterations of the plasma membrane to resist freeze dehydration, mechanical stress, molecular packing, and other events caused by extracellular freezing. Cytological changes associated with an abrupt increase in hardiness occur at 0 or $-3°C$ within 7–10 days. However, these cytological changes may be indirect.

From studying the reaction of the plasmalemma to freezing, it is obvious that this cell membrane is very sensitive to this type of stress. Observations on *Robinia pseudoacacia* have revealed a seasonal transition in the plasmalemma from a physical state of relative smoothness and regularity in summer to a highly folded state in winter. It is considered that a highly folded membrane state would facilitate water flow and alleviate the stresses of contraction and expansion during freeze-thaw cycles [84]. However, the plasma membrane of the cortical cells of mulberry twigs in winter is relatively smooth, and highly folded states have not been observed. Only after cold acclimation in October at 0 for 20 days or $-3°C$ for 7 days, when hardiness increased from $-15°C$ to $-70°C$, was the plasma membrane highly folded and microvesicles with a double lipid layer membrane appeared in the peripheral cytoplasm. These microvesicles originate from the endoplasmic reticulum. A very similar ultrastructure has been observed in the cold-acclimated cells collected at the end of autumn. In April, at a decreased hardiness of $-15°C$, the plasma membrane is already smooth and regular. When these dehardened cells are rehardened at 0°C for 10–15 days, the hardiness increases,

the plasma membrane becomes folded, and microvesicles reappear near the periphery of the cytoplasm. From these results, it appears that a highly folded state of the plasma membrane and the formation of numerous microvesicles represent a transition associated with higher freezing tolerance rather representing a special membrane structure characteristic for extremely hardy cells in the winter state [85].

Formation of osmiophilic regions associated with the plasmalemma has also been observed. Substantial regions of the plasmalemma bilayer are transformed into either amorphous, osmiophilic or densely packed regions or into multilayered structures with high surface curvatures [86,87]. Deep invaginations of the plasmalemma and formation of electron-dense deposits outside the plasmalemma in the xylem parenchymal cells occur in peach and oak trees in a frozen state at −10°C [69]. We have observed similar changes of plasmalemma in the mesophyll cells of silver fir [88] and numerous electron-dense lipid bodies associated with the plasmalemma in the mesophyll cells of Norway spruce during winter when the frost resistance of these species is very high [66]. Augmentation of lipidic globules and their localization in the cytoplasm along the plasmalemma apparently results from the changes in the lipidic part of membranes during the freezing treatment [89].

It has been found [72,90] that osmotic shrinkage of protoplasts isolated from *Secale cereale* results in an irreversible decrease of the surface area of the plasmalemma concurrent with the formation of endocytotic vesicles. The present observations of in-turning of the plasmalemma together with vesicles in the cytoplasm may lend support to the idea the reduction of the plasmalemma surface area and the reduction of the volume of the protoplast through dehydration occur as initial responses to slow freezing [91].

Most microscopic investigations report changes in the plasmalemma, and sometimes the tonoplast, which can be correlated with the deletion of the membrane into vesicles as the cell volume is reduced by dehydration [17,72,90,91].

An increase in the intramembranous particles and plasmalemma invaginations has occurred in the more frost-resistant *Chloromonas* cells, whereas in the frost-sensitive *Chlamydomonas cells*, they are absent [92]. A higher frequency of osmiophilic globules in acclimated (−25−−30°C) isolated protoplasts of *S. cereale* have been found than in nonacclimated (−3−−5°C) protoplasts. Osmiophilic regions observed under transmission electron microscopy corresponded to the extrusions of the surface of acclimated protoplasts observed under scanning electron microscopy (EM) [73].

Scanning EM observations on apple parenchyma cells have revealed similar copula-shaped protrusions on the surface membranes. The protrusions are associated with the fibrillar formations of exoplasm. It is clear that mechanical breaks of the membrane may arise on the plasmalemma near protrusions under stress conditions of freezing. It can be supposed that plasmalemma instability zones are formed under freezing stress connected with protoplast compression under dehydratation, whereas protrusions themselves consist of structural lipids of higher unsaturation. Intracellular processes leading to the membrane stabilization are evidently related to condensation of polyphenols, which makes cell resistance under stress conditions at super-low temperatures essentially higher [93].

If plants are nonacclimated or the freezing stress is very severe, disruption of the plasmalemma and cell organelles and the collapse of the cell wall with the protoplast can occur [26,91,94]. On the basis of present results, the plasmalemma is the most injured membrane during the freezing-thawing process [95,96]. If the plasmalemma is considerably damaged, its protective function against quick dehydratation of cells or penetration of ice into cells can be replaced by parallel layering of the endoplasmic reticulum [95].

Endoplasmic Reticulum

The endoplasmic reticulum is a structurally and functionally highly dynamic part of the endomembrane system of plant cells. The response of the endoplasmic reticulum is immediate at the low temperatures, which is accompanied by shift of its structure and space organization in the cells.

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One of the specific features of wintering plants is the absence of rough endoplasmic reticulum (ER) in the cells. This type of endoplasmic reticulum, observed in the cortical cells of apple during the growing season, at freezing temperatures in winter becomes sparse and replaced by vesicular endoplasmic reticulum [97]. The cells enriched with numerous tubular and vesicular smooth endoplasmic reticulum cisternae have also been observed in ray parenchyma cells of poplar. These smooth endoplasmic reticulum cisternae are the most characteristic components of cells in the winter stage, and they are suspected to be the site of sugar accumulation [98]. In the cells that survive freezing temperatures by a deep supercooling mechanism, the presence of tubular endoplasmic reticulum is a feature of dehydratation tolerance [99].

The ultrastructural study of such extremely cold hardy cells as cortical parenchyma cells of mulberry collected in winter has shown that initiation of freezing at 5°C results in the formation of multiplex lamellae that completely cover the area in the vicinity of the plasmalemma. The multiplex lamellae are produced by fusion of preexisting vesicular ER via a reticular network. The complete multiplex lamellae are composed of a parallel array of sheet-like ER cisternae. The formation of multiplex lamellae on the initiation of freezing is largely dependent on seasonality in close association with the development of freezing tolerance [100].

Parallel layering of endoplasmic reticulum sheets has been found in various plant tissues. The phenomenon has sometimes been associated with different types of stress, for example, water deficiency [101], freezing [95], and anaerobic conditions [102,103].

Examples of stacked endoplasmic reticulum were also found in the dormant buds in potato and in several other species, mainly trees, species, such as *Betula* [104], *Sorbus, Querous, Fraxinus* [104,105], *Rhododendron* [106], and *Salix* [107]. The stacking of endoplasmic reticulum disappears in spring in association with the breaking of dormancy. In a study of dormant *Tilia* buds, using freeze-fractured material, no concentric layering of the endoplasmic reticulum was observed, but an extensive network of endoplasmic reticulum close to the plasmalemma was found [108].

The groups of stacked endoplasmic reticulum cisternae have been observed in cells of wheat seedlings at −10°C, whereas at −30°C, endoplasmic reticulum has been present in the form of numerous vesicles and sacs [109]. The presence of numerous vesicles and cisternae of smooth endoplasmic reticulum close to the cell wall is considered to be a characteristic feature of frost-resistant cells [70,84,95,110]. The occurrence of the concentric type of rough endoplasmic reticulum in frozen cells is an adaptive mechanism protecting ribosomes against injury by low temperature [35].

In most cases, the stacking of the endoplasmic reticulum is reversible. The stacking observed in dormant buds is possibly a consequence of water stress in buds during winter conditions, although an effect of low temperature [35,83,111] or anaerobic conditions [102,103] cannot be excluded.

A striking increasing of the number of cisterna-like cytoplasmic membranes has been observed after ice encasement of winter wheat seedlings. The imposition of an ice cover results in the proliferation of the endoplasmic reticulum membrane system in the cells and in the formation of concentric whorls of membranes that often enclose cytoplasmic organelles. It has been suggested that these membranes are the consequence of the reorganization of preexisting membrane components, possibly vesicular endoplasmic reticulum [112,113].

Many studies on numerous plant species have shown that the formation of parallel and concentric layering of endoplasmic reticulum cisternae can by induced by different types of stress, and therefore it might be suggested that these configurations are a manifestation of an adaptive mechanism protecting the cells and of repairing processes within stress-damaged cells [112].

The endoplasmic reticulum is considered to be the locus of membrane biosynthesis and is intimately involved the turnover of plasma membrane components. Thus, the endoplasmic reticulum plays an important role in membrane transformation during cold acclimation.

Vacuole

The vacuolization of the cytoplasm is a very important phenomenon, and it is often described as a structural reaction of cells to freezing. Reversible splitting of the large central vacuole into many

smaller ones has been observed in many plants; namely, woody species. At the beginning of cold acclimation of peach stem tissue, the cells have their typical architecture—a large central vacuole and a thin band of peripheral cytoplasm—but with continuing cold acclimation, distribution of the cytoplasm gradually becomes more homogeneous—the nucleus is located centrally and many small vacuoles appear in the case [69]. Splitting of the central vacuole has been recorded in the mesophyll cells of *Pinus cembra* and *Picea excelsa* after the first autumn frosts [114], and in the phloem cells of *Metasequoia glyptostroboides* [115], and in the mesophyll cells of Norway spruce and silver fir during winter [66,88].

A dense and extensive cytoplasm containing numerous small vacuoles is characteristic for winter-hardy cells. Cell vacuoles contain a variety of lytic enzymes, such as protease, acid phosphatase, ribonuclease, carboxypeptidase, aminopeptidase, invertase, hydrolase, and ATPase. Autophagic activity of the vacuoles after severe cold injury has been observed in many plant cells, which results in the digestion of cytoplasmic structures and the reorganization of distinct cytoplasmic organelles. The release of protein-toxic vacuolar substances results in frost injury of spruce needles due to loss of cell compartmentation and concomitant flooding of the cell interior [115]. The functional stability of the tonoplast, therefore, can play an important role in the frost resistance of spruce needles.

Seasonal changes in the vacuole from winter to spring in mulberry cortical cells consist of an engulfment of the tonoplast, fusion and inflation of small vacuoles, and coalescence into larger vacuoles [84]. Similar findings have been seen in the mesophyll cells of both Norway spruce and silver fir [66,88] and in the leaves of the evergreen species *Aucuba japonica* and *Prunus laurocerasus* [26]. With the fall in temperature the central vacuole splits into smaller ones forming small vacuoles in the cytoplasm. This phenomenon occurs, at temperatures below zero. Decay of the central vacuole to small vacuoles is an adapting mechanism of the plants to low temperatures in autumn and in winter. It is a feature of hardening of the plants against low temperatures and is accompanied by the loss of water from the cells. This process of adaptation is reversible. With increasing temperatures in spring, the central vacuole is differentiated again by the fusion of small vacuoles. It appears that such intracellular digestion from winter to spring plays an important role in the adaptation of plants to changing environmental conditions [116].

On the other hand, although the splitting of the central vacuole is also observed in other woody trees (e.g., *Sambucus* and *Betula*), at temperatures of −30–−50°C, this phenomenon can be considered the consequence of a decrease in frost resistance [95,110]. A decrease of frost resistance in the cells of *Robinia pseudoacacia* also results in the degradation of cell membranes, including the tonoplast [117].

Vesiculation of the tonoplast into the vacuole may represent a similar mechanism (like vesiculation of the plasmalemma) for the reduction of the surface area of the tonoplast and the volume of the vacuole. Observations using osmotically manipulated isolated cells of *Brassica napus* and *Secale cereale* support this assumption [17].

Chloroplasts

The response of chloroplasts to low-temperature stress depends on the temperature and hardening capacity of a particular species. Numerous data from some extremely hardy conifers and from a few moderately frost-resistant herbaceous plants indicate variable changes in the chloroplast membranes in different species [118]. For instance, coniferous species tolerate temperatures at around −40°C (and lower), whereas moderately frost-resistant plants such as winter annual herbs and grasses are killed at −10–−15°C.

It is often assumed that the chloroplasts are the cell organelles most sensitive to low temperatures [35]. Observations of three grass species have been shown that the transition from 25°C to low-temperature conditions (10°C, 0°C, −5°C) causes swelling of chloroplasts in mesophyll cells at 0°C. Dilatation of thylakoids has occurred at −5°C. Similar structural changes of chloroplasts and the disappearance of starch grains were observed in the mesophyll cells of *Sorghum* and *Paspalum* [119].

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During the growing season, the chloroplasts in the mesophyll cells are of oval shape and are placed in the cytoplasm along the cell wall. In the winter when the temperature goes down, the chloroplast architecture is altered, but to what extent depends on the plant species and its physiological state. The onset of the hardening process of winter rape leaves grown under field conditions in autumn (above-zero temperatures) causes some modifications in the cell membranes; however, the chloroplasts still have their typical oval shape, the stroma is filled with ribosomes, starch grains, and tiny osmiophilic globules, and a well-developed membrane system can be observed. In winter (temperatures −5–−10°C), the chloroplasts are clumped into groups, the thylakoids and grana are swollen, and the chloroplast envelope also is damaged. There are no starch grains in the stroma, but many osmiophilic globules are present. After rewarming during the spring, the structural recovery of the chloroplasts can be observed [96].

The comparison of three varieties of winter wheat with different levels of frost resistance has shown that, after the first stage of hardening, all investigated varieties have chloroplasts with well-developed membranes. During the second stage of hardening at −16°C, loss of starch and swelling of the thylakoid membranes are seen. At the same time, in the case of plants with lower frost resistance, the chloroplast membranes and also other organelles are injured lethally [116]. The chloroplasts of resistant wheat varieties change their shape during the hardening and retain their individuality, but during winter, they are clumped together [120]. On the other hand, cold acclimation of rye does not induce any substantial changes in the structure of chloroplasts.

A 1-day exposure of nonacclimated hybrid wheat plants to a temperature of −4°C does not induce morphological changes in chloroplasts, whereas a 1-day exposure to a temperature of −8°C results in the degradation of the chloroplasts and their gathering into groups [81].

According to the position of chloroplasts in the cells during freezing stress, plants can be divided into two groups: (a) the chloroplasts retain their integrity but migrate from a summer position near the cell wall to a crowded position in the cell center and (b) the chloroplasts agglutinate, lose their integrity, and merge with each other to become a continuous mass from which the chloroplasts separate again when spring approaches [3].

Hardening of winter wheat causes the preservation of the chloroplast structure and membranes during the freezing period and recovery of their functional activity after thawing [121]. These adaptative alterations in the chloroplast membranes during hardening are probably the result of changes in their lipid composition [122].

The correlation of the chloroplast ultrastructure and membrane lipid composition to the different degrees of frost resistance in leaves of ''moderately hardy'' spinach, ''very hardy'' ivy, and ''extremely hardy'' spruce have shown characteristic differences with respect to changes in the lipid composition and chloroplast structure during adaptation to subzero temperatures [123]. In spinach leaves, there is no increase in the total lipids, whereas the membrane lipid content in ivy leaves and spruce needles increases considerably. A striking shift from saturated to unsaturated fatty acids can be detected in ivy and spruce chloroplasts. This increase in the lipid content is related to the increase in the chloroplast envelope surface resulting from the formation of many protrusions and invaginations that occur during cold hardening [123,124].

Much attention has been focused on the seasonal changes of the chloroplasts in woody plants, especially in evergreen species. The studies have been on evergreen broadleaf woody plants, of which frost resistance is considered to be that of between the above-mentioned moderately hardy plants and the extremely frost-resistant conifers [118]. Unlike later studies, in earlier observations, the substantial changes in the chloroplast structure in the leaves of broadleaf evergreen woody species that occurred during the year were found [125,126].

Contrary to these results, the observations on broadleaf evergreen woody species such as *Aucuba japonica* [127], *Prunus laurocerasus* [128], *Skimmia japonica* [129], and *Mahonia aquifolium* (unpublished observations) have revealed remarkable changes in the chloroplast structure occur during the year. In summer, the chloroplasts are oval shaped, they are placed along the cell walls, and their inner architecture is the same as in other higher plants [130]. At this season of the year, this position of the chloroplasts and the presence of starch granules in them are typical.

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In autumn, the originally lens-shaped chloroplasts of *Aucuba* and *Skimmia* become globular and move gradually from the cell wall to the center of the cell. The chloroplasts of *Prunus* and *Mahonia*, which are more frost resistant than *Aucuba* and *Skimmia*, are still positioned at the cell wall. At this season of year, no starch grains have been observed in the chloroplasts of the plants studied [131].

In winter, the chloroplasts of *Prunus* are still distributed along the cell wall, whereas the chloroplasts of *Skimmia* and *Aucuba* create irregular formations in a different part of the cell. The well-developed membrane system with the signs of slight dilatation can be observed. The membrane system of the chloroplasts is often located in one part of chloroplast, leaving only membrane-free stroma in the other part (Fig. 3). In the chloroplast stroma, a small group of plastoglobules are present and no starch grains are visible.

A characteristic feature of the mesophyll cells of all these species in the spring is the presence of a large number of starch grains in the chloroplasts. Because of this, the plastid shape becomes irregular. These irregularly shaped plastids represent an atypical stage within the plastid ontogenesis chloroamyloplasts [130]. The increased presence of starch in the plastids during the spring is not only typical of broadleaf species. The same large content of starch also is observed in the cells of *Abies alba* and *Picea abies* [23,88]. Reports have confirmed that the plasticity of the chloroplast membrane system enables the cell to overcome unfavorable conditions during the winter.

Mistletoe is well-known semiparasitic plant in which its leaves exhibit a high level of frost resistance. We have not found any striking differences in the ultrastructure of *Viscum* chloroplasts in the winter and summer. In the winter, green leaves possess chloroplasts of oval to elongated shape. The plastid envelope is smooth with no signs of plastid protrusions. The thylakoid system of the chloroplasts is composed of numerous grana and stroma lamellae. Both the stroma lamellae and marginal thylakoids of grana show signs of slight dilation. The membrane system remains well differentiated even at a temperature as low as 7°C. The chloroplasts are regularly sheathed by membranes of the endoplasmic reticulum during the summer, but in the winter, these membranes are fragmented into vesicles of variable shape and size (Fig. 4) [132].

Extensive studies of seasonally and experimentally induced changes in the chloroplast ultrastructure have been done on conifers; for example, on Norway spruce needles [133] and pine needles [134]. The chloroplasts of conifers respond to cold acclimation and freezing by extensive changes in their architecture and localization in the cells. Generally, the chloroplasts of conifers respond to

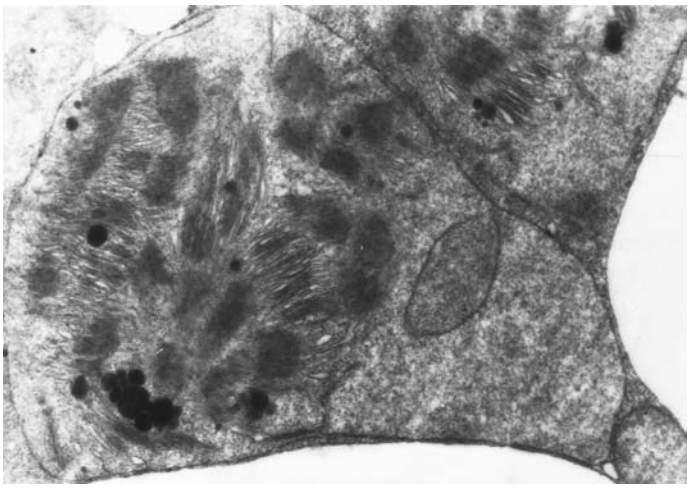

FIGURE 3 Chloroplast of *Aucuba japonica* in winter with membrane free stroma and slight thylakoid dilation. (×22 000). (From Ref. 127.)

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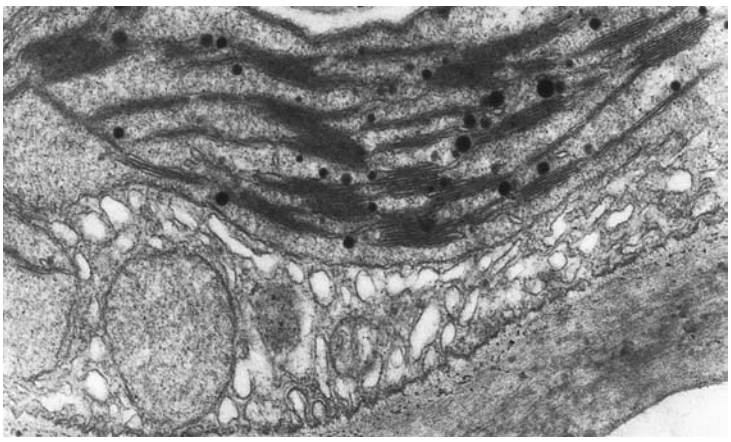

FIGURE 4 Mistletoe chloroplast with an extensive fragmentation of endoplasmic reticulum into vesicles at −7°C (×19 000). (From Ref. 132.)

low temperatures mainly by a reduction of the starch content, with an increase in the number of osmiophilic globules and of membrane-free-stroma, and by swelling of the chloroplasts and their aggregation in one part of the cell [134].

When the needles of Norway spruce are both artificially and seasonally frozen, the thylakoids are dilated and the starch disappears. The chloroplasts are tightly pressed together at one side of the cell or they move toward the cell center around the nucleus [23,66,133,135]. Similar studies have been done on white spruce [126], balsam fir [136], and Scotch pine [135].

The significant presence of numerous starch grains, the thylakoid system has been recorded in the chloroplasts of all investigated evergreen species in the spring when the chloroplasts are again less frost resistant. This, so-called ''spring starch'' serves as a source of energy for the growth processes in this period [127,128,133]. On the other hand, the disappearance of the starch from the chloroplasts during cold acclimation is a typical reaction of both broadleaf and coniferous evergreen species [126,133], as well as of the cells cultivated in vitro on a decrease in temperature [23,137]. The hydrolysis of starch is one of the basic physiological mechanisms for the increase in the frost resistance of plants [138].

Nucleus

Although the nucleus, because of its regulation of cell metabolism is considered to be the cell organelle most resistant to the nonlethal effect of low temperature [139], there are not many studies concerning its structural response to freezing stress and to the process of developing frost resistance. The nuclei in the cells of black locust bark become more dense during cold acclimation in the autumn [140]. The nuclei of the acclimated cells in the shoot apex of rhododendron plants are ovoid and contain relatively large nucleoli and little heterochromatin, or they are irregularly shaped with small protruding lobes or nucleoplasmic extensions [106]. In the cortical cells of apple [97] at the stage of cold acclimation, each nucleus contains relatively lower amounts of heterochromatin and is located in the central part of the cell.

The cooling of tobacco cells to −10°C induces the formation of numerous small vacuoles in the nucleus [141]. Similar vacuolization of nuclei also have been observed in the cells of wheat leaves at −4°C after 8 days of freezing, whereas in the nuclei exposed to −12°C, vacuoles already occur after 1 day [81].

The second step of wheat hardening at −16°C results in folding of the nuclear membrane

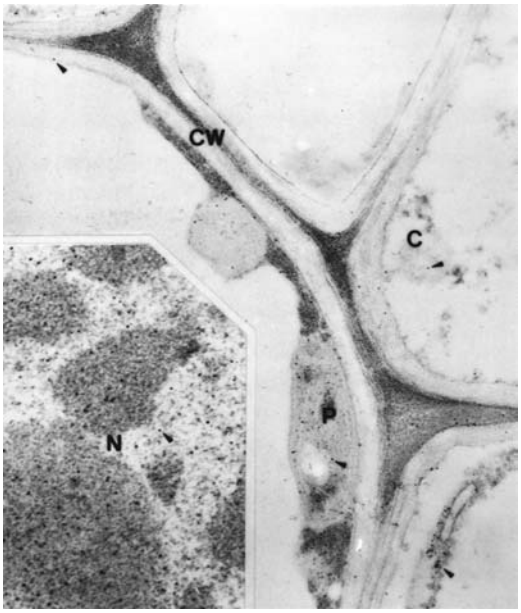

FIGURE 5 Epitops of abscisic acid in the nucleus (inset) in the pea epicotyl tissue from the seedlings grown for 2 days at 25°C and then 7 days at 2°C. (×34 000) (From ref. 144.)

and condensation of the chromatin [116]. Heterochromatin condensation seems to be a common reaction of the nuclei to a freezing temperature both in perennial grasses [81,82,142] and in woody plants [66,143]. In the mesophyll cells of spruce, in addition to nuclei with condensed heterochromatin, large nucleoli and a changed nuclear membrane also occur during winter at −10°C–−15°C [66]. During the winter, the nuclei move to the central portion of the cells and are surrounded by aggregations of the swollen chloroplasts. The movement of nuclei toward a central position in the cortical cells of apple during cold acclimation also has been observed. The gathering of plastids around the nucleus begins in late November when the temperature drops to −20°C [97]. The aggregation of the chloroplasts around the nucleus is a phenomenon associated with the winter metabolism of the cell and is a characteristic feature of the most frost-resistant species [125].

Owing to its regulatory role in plant metabolism the nucleus also is involved in the process of hardening, and it is known that abscisic acid considerably affects frost hardening. Using an electron microscopic immunohistochemistry method in which gold-conjugated antibodies recognized abscisic acid (ABA) has proved the presence of ABA epitopes in the nuclei in pea tissues during cold acclimation (Fig. 5) [144]. This finding supports the idea that hardening is governed by nucleus through abscisic acid activation. However, there is no comparison between the accumulation of abscisic acid and the structural changes (i.e., the occurrence of heterochromatin) in the nucleus of pea tissues [97].

Mitochondria

The mitochondria are the primary sites of intermediary metabolism in the cell, and therefore they are an excellent means to study of the plant response to the changes in the environment [145]. The alterations of the mitochondrial membranes directly influence the process of cell respiration.

Decreasing the temperature in the environment is accompanied by a decrease in the number of mitochondrial cristae. The mitochondria in the cortical cells of woody species have well-devel-

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oped cristae and an electron-dense matrix at favorable temperature conditions. The fall in temperature in winter results in the reduction of the cristae and the matrix becomes electron transparent [85,95,110]. Swollen mitochondria and a reduction in the number of the cristae or their atypical orientation in the cell have been found in the dormant buds of *Salix* in early winter [107] and in the shoot apex cells of *Rhododendron* [106].

The reaction of herbaceous plants to freezing stress is similar to that seen in woody species. The mesophyll cells of winter rape in October at a temperature −6°C possess mitochondria with a reduced number of cristae and low electron density of the matrix. In December, when the cells are highly injured (about 80%), the mitochondria are hardly visible because of their changed structure in the strongly vacuolated cytoplasm. However, after 48 h recovery of the conditions the swollen mitochondria are able to rebuild their membrane system [96].

Well-developed mitochondria are present in rye mesophyll cells at 5°C, but in the cells of both cold-acclimated and cold-nonacclimated rye plants slowly frozen to −12°C, the mitochondrial cristae are strongly disorganized [91,146]. No significant differences in the respiration of the mitochondria in the extracellularly frozen cells of both acclimated and nonacclimated rye seedlings have been detected [147]. It can be concluded that the mitochondria in situ retain normal function even after the cells have been killed by extracellular freezing. However, reports have shown that the mitochondria of rye leaf cells frozen in situ are much more susceptible to frost injury than the chloroplasts [91,146].

Dictyosomes

The dictyosomes are the cell organelles which are metabolically very active in such cell functions as protein sorting and membrane formation. Abundant dictyosomes, usually composed of four to seven cisternae with numerous vesicles originated from their ends, are a common feature in the cells of different species not only during growth season but also during cold acclimation [97,106,148].

The presence of dictyosomes in poplar cortical cells in September is common. The number of dictyosomes decreases with the fall in temperature in October, and their level continues to the decreased on until the next spring [149]. Since some vesicles generated from dictyosomes are confined to the surface of protein-lipid bodies, dictyosomes might be involved in their formation as well as other organelles. During cold hardening, the cells of *Arabidopsis thaliana* contain more microvesicles that are either associated with the dictyosome cisternae or located in their vicinity. The dictyosomes probably take part in the structural and conformational modification of the plasmalemma [150]. In mulberry parenchyma cells, the dictyosomes secrete numerous vesicles and some of them are located beneath the plasmalemma during slow freezing at −5°C [100]. It may be possible that these secretory vesicles might participate in the formation of the multiplex lamellae that are very often found during slow freezing in mulberry cells.

In spite of the frozen state of tissues, the dictyosomes in the cells of woody plants can be occasionally identified in their original form. There is evidence [97] that in mid November, when the cortical cells of apple survive freezing at a temperature −20°C, the dictyosomes are still active and they produce vesicles. In late January, when the cells are hardy to a temperature of −30°C, dictyosomes can be observed, but they are not active. Similar alterations in the dictyosome ultrastructure and their localization in the cell have been observed in the cortical parenchyma cells of mulberry twigs [85], in the xylem ray parenchyma cells of *Prunus persica* [69], and in bark tissue of *Robinia pseudoacacia* [84].

Cytoskeleton

The cytoskeletal components take part in many structural and functional processes in the plant cell; for example, cytoplasmic streaming, secretory and transport processes, cell division, cell polarity, and stability of the cell size. For this reason, cytoskeletal elements may play an important role in the cell response to different stresses, including low-temperature stress. The altered stability of the

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cytoskeletal elements at low temperatures has been recognized in different plant species. Cold-induced depolymerization of microtubules at temperatures below 0°C has been observed; for example, in the cells of onion [48], cotton [151], spinach [152], garlic and winter wheat [153], and rye [154,155].

The existence of both cold-labile and cold-stable microtubules in the root cells of onion has been reported [48]. It is suggested that the cold stability of microtubules is related to the cold hardiness of the plant [153]. However, although the plant cells respond to cold hardening by altered stability of the microtubules, the lack of consensus regarding a positive correlation between cold acclimation and the cold stability of microtubules still prevails [152–154].

The effect of cold acclimation on cortical microtubule stability during freezing has been studied in cold-acclimated and cold-nonacclimated rye leaves [155]. The experiments have shown that unchanged microtubule arrays are still present in cold-acclimated leaf cells after a −4°C temperature treatment, whereas microtubules are shorter and less abundant in the leaf cells of nonacclimated plants and in the root cells of both cold-acclimated and cold-nonacclimated plants. After a −10°C temperature treatment, the cortical microtubules are almost totally depolymerized in both types of root cells and in the leaf cells of nonacclimated plants, whereas cold-acclimated leaf cells constantly have abundant cortical microtubule arrays. Semiquantitative analyses of the cortical microtubules of protoplasts have confirmed the findings with intact leaf cells. These experiments have shown that the cortical microtubules of nonacclimated leaf cells are cold labile and that cold acclimation induces cold-stable microtubules in leaf cells as well as in protoplasts (Fig. 6) isolated from cold-acclimated leaves [155].

A similar cold stability of microtubules has been found in cold-acclimated garlic and winter wheat cells [153]. The above results suggest that the cells need to have enough long cortical microtubules to keep their plasmalemma intact and responsive to the osmotic changes caused by subzero temperatures. Under these stressful conditions, the microtubules may serve as a necessary support for the plasmalemma [155].

It has been found that, besides the microtubules, the actin filaments play a role in the cell response to cold treatment. The crucial role of microtubules and/or microfilaments in the movement

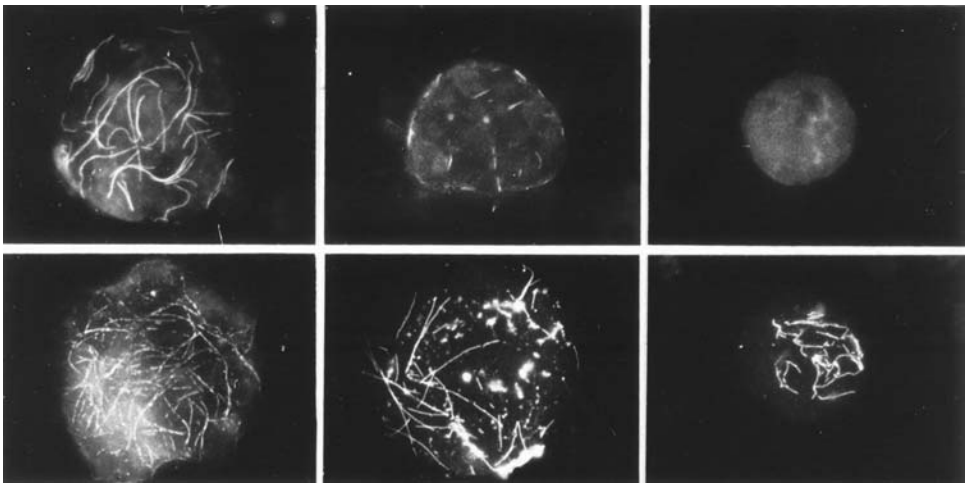

FIGURE 6 Responses of microtubules of isolated leaf protoplasts to freezing visualized by indirect immunofluorescence with anti-α-tubulin (1:100). Upper line (from left to the right) nonacclimated protoplasts: control protoplast, after freezing to −4°C and after freezing to −10°C. Lower line (from left to the right) cold acclimated protoplasts: control protoplast, after −4°C treatment and after −10°C treatment (×800). (From Ref. 155.)

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and reconstruction of the endoplasmic reticulum on the freezing has been reported in the cortical parenchyma cells of mulberry [100]. A contraction of the endoplasmic reticulum tubule (functional state) to a central rod (nonfunctional state) in the plasmodesmata during cold treatment is caused by changes in the actin-myosin filaments [33]. The partial disruption of actin filaments can accompany or promote freezing tolerance of carrot cell suspensions during preservation at extremely low temperatures [156].

CONCLUSIONS

From the presented results, it is obvious that low-temperature stress considerably affects the structure of plant cells. The structural response of the cells is variable and is determined by external (strength and duration of stress) and internal (plant species, ontogenetic phase of the plant, type of the tissue, and genetically determined level of resistance) factors. Therefore, it is difficult to decide which cell compartment plays the primary or the most important role in the cell responses to both chilling and freezing stresses.

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Carotenoids and Stress in Higher Plants and Algae

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INTRODUCTION

Alterations in the light environment, ambient temperature, and mineral and water supply to plants and algae may affect their viability. The rapidity and efficiency of the metabolic responses of plants differ among plant species or even among cultivars and their aptitude to adapt to unfavorable conditions is a major factor for their survival. The photosynthetic apparatus is one of the most important targets of stress in chlorophyllous organisms. Indeed, most of the metabolic responses induced by stress conditions (e.g., stomata closure) have consequences on the aptitude of the plant to maintain an efficient light energy conversion. The photosynthetic pigments, especially carotenoids which exhibit both light-harvesting and photoprotective functions, are affected by a large variety of stresses. In this chapter, we present a short discussion about the diversity of carotenoid responses to stress and focus on their photoprotective participation in the stress response through the so-called xanthophyll cycle and on the production of economically important secondary carotenoids by microalgae as a consequence of stress.

PROPERTIES OF CAROTENOIDS IN PHOTOSYNTHETIC ORGANISMS

Carotenoids are C40-polyisoprenic compounds characterized by a large number of conjugated double bonds ($n > 7$). Over 600 carotenoids have been identified to date and several new ones are reported annually. Conjugated double bonds allow carotenoids to absorb light in the near ultraviolet

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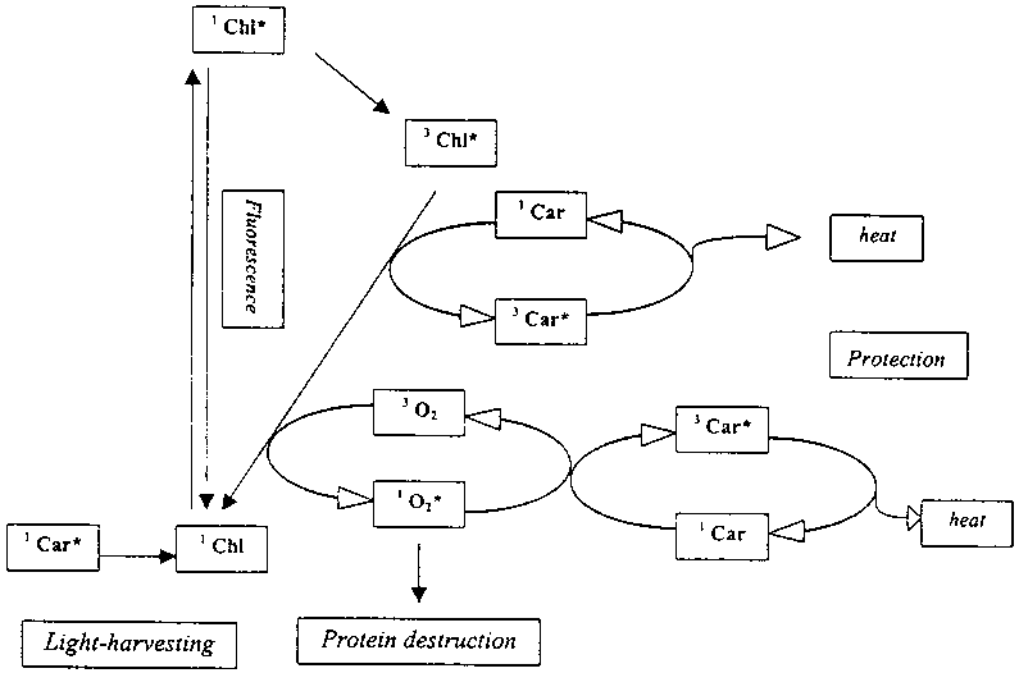

FIGURE 1 Schematic representations of light-harvesting and photoprotection roles of carotenoids in the photosynthetic apparatus. An efficient singlet-singlet energy transfer occurs in antenna complexes from the carotenoid excited state to chlorophyll. Singlet excited chlorophyll can give rise to the longer-lived triplet species. Carotenoids can protect either by quenching ^{3}Chl* before it can interact with oxygen or by quenching $^1O_2^*$ that can be formed.

(UV) as well as in the visible region. In photosynthetic organisms, carotenoids are bound, together with chlorophylls, to proteins and participate to light harvesting (Fig. 1). This very efficient singlet-singlet energy transfer from carotenoids (Car) to chlorophyll (Chl) needs a precise arrangement of pigment molecules in the light-harvesting complexes. Carotenoids are also recognized to be essential for the survival of illuminated plants, since their numerous conjugated double bonds are able to quench the Chl triplet state and also scavenge singlet oxygen and the other reactive oxygen species which are abundantly produced during photoinhibition (Fig. 1). This photoprotective function is generally achieved via triplet-triplet energy transfer.

DIVERSITY OF STRESS EFFECTS ON CAROTENOIDS

Various environmental stress factors (e.g., nutrient deficiency, excess light, drought, chilling) are known to have consequences on the photosynthetic apparatus and photoinhibition may be often observed. This happens when the rates of transfer of energy from the antennae to the photochemical reaction centers are in excess of the rates of transfer of excitation energy to the transducers [1]. This means that even low light levels may become excessive if present in combination with chilling and then may result in photoinhibition in crops or in algae. The consequences at the carotenoid level of any stress may be multiple, and they have been reviewed previously [2]. Among these stresses, the *photoisomerization* of carotenoids may be noted. Besides large amounts of all-*trans*-β-carotene, a low proportion of Z-isomers, essentially 9-13′-*cis*-, 13-*cis*-, and 15-*cis*-β-carotene, have been detected in reaction centers of spinach [3]. On illumination, a decrease of 13-*cis*- and

15-*cis*-β-carotene in photosystem I (PSI) reaction centers of the cyanobacteria *Synechococcus vulcanus* has been noted [4]. *High-light plants* also differ from low-light plants in their pigment composition [5]: smaller physiological photosynthetic unit, lower xanthophyll:β-carotene ratio and higher photosynthetic rates at high quantum flux densities. Similar differences may be observed between the leaves on the south- and north-facing sides of a tree [6]. Exposure to photoinhibitory conditions may lead to *photobleaching* of carotenoids and chlorophylls, the extent of carotenoid photooxidation, with β-carotene being the highest among them [7]. β-Carotene 5,6-epoxide may be produced at the expense of β-carotene destruction when photooxidative damage occurs [2,8].

Acclimatization to *cold stress* has been largely studied in conifers of the boreal zone which encounter a considerable combined stress of low temperature and high light during winter when photosynthetic dissipation of energy is blocked. The winter-stressed Scots pine (*Pinus silvestris*) has been shown to accumulate substantial amounts of xanthophylls, essentially zeaxanthin, in a large complex maintained in a highly quenched state that dissipates excitation energy nonradiatively [9]. Despite a greater β-carotene content in the PSI reaction center which may act as a sink for excess excitation energy, photoinhibition of photosynthesis remains a significant component of the winter-stress effects that Scots pine encounters during the winter. Since pine enters a dormant state during the fall, photoinhibition may be of significant importance as a mechanism for controlled dissipation of energy. In winter rye (*Secale cereale*), on the contrary, cold acclimation induces increased resistance to photoinhibition which appears to be beneficial for the maintenance of active growth and development at low temperatures in this species [10].

Drought is a harmful phenomenon for plants which may induce irreversible damage or death in case of severe stress. Little attention has been paid to the drought effect on pigments. In their analysis of drought effects in bean plants (*Phaseolus vulgaris*), D'Arcy-Lameta et al. [11] revealed a decrease of both chlorophylls and of α- and β-carotenes together with xanthophylls. A different pattern in the distribution and relative enrichment in the xanthophylls was due to the appearance of antheraxanthin and zeaxanthin as observed in drought-stressed *Nerium oleander* [12] and *Gossypium hirsutum* [13]. In contrast to the observations of Barry et al. [14] in barley, no xanthophyll esters could be detected.

Atmospheric pollutants, including several photooxidants such as O_3, SO_2, NO_x, have caused severe destruction of northern European forests (see Ref. 2) with degradation of photosynthetic pigments. A *mineral supply deficiency* also may have important consequences on the pigment composition of higher plants. The best-documented deficiency concerns the iron supply to plants [15]. In response to iron deficiency, pea plants have the peculiarity to exhibit increasing stages of chlorosis from bottom leaves to the apical leaves and thus to offer the possibility to follow, by comparison of the leaves, the effects of increasing iron depletion. The most chlorotic leaves showed a reduction of 97% of their pigment content [16]. The Chla/Chlb ratio reached six times that of the control and the carotenoids/chlorophylls ratio was nine times higher. The main carotenoids were violaxanthin, antheraxanthin, and zeaxanthin, which represented up to 36% of the total pigment content of the yellow leaves. Iron deficiency favored a dramatic decrease of light harvesting chlorophyll protein (LHCP) complexes and a relative enrichment in PSII reaction center complexes (Cpa).

Some xanthophylls have been shown to be concerned in most of the various stress conditions we have surveyed. They are implicated in the nonphotochemical quenching of chlorophyll fluorescence in plants and algae and thus participate in an important photoprotective process known as the xanthophyll cycle. On the other hand, under environmental stress conditions, some algae accumulate economically important carotenoid species such as β-carotene and astaxanthin. We will particularly focus on these two aspects of carotenoids and stress.

XANTHOPHYLL CYCLE

Discovery and Description

In their natural environment, plants are confronted with reconciling an excessive energy supply with the demands of the photosynthetic carbon reduction cycle for the products of electron transport,

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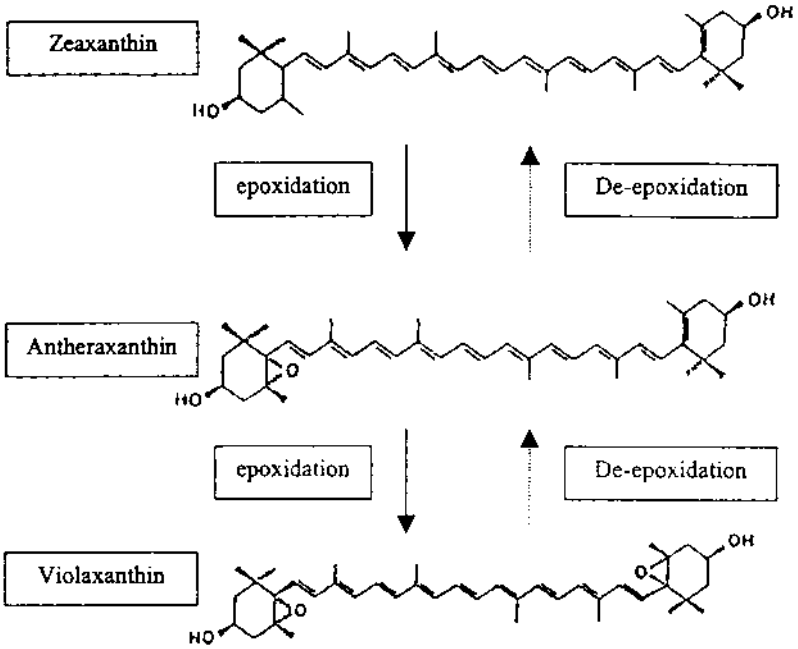

FIGURE 2 The violaxanthin cycle. Under stress conditions, violaxanthin is deepoxidized into antheraxanthin and zeaxanthin and after suppression of the stress its amount is slowly restored by the addition of 5,6- and 5′, 6′-epoxy groups.

ATP and NADPH. When light absorption by photosynthetic pigments exceeds both the capacity to use the photosynthetic NADPH and ATP for carbohydrate synthesis and the capacity of energy-dissipation mechanisms, photosynthesis is progressively inhibited (i.e., photoinhibition phenomenon) and pigment composition starts to change mainly through the xanthophyll cycle [17–20].

Sapozhnikov et al. [21] were the first to report a dramatic decrease of the amount of violaxanthin in various leaves under high-light intensities. Yamamoto et al. [22] related this violaxanthin decrease to an increase of both antheraxanthin and zeaxanthin. Later on, Hager [23] and Yamamoto et al. [24] demonstrated that the violaxanthin pool is reconstituted from zeaxanthin in darkness or under low-light conditions, confirming the pigment interconversions in a cycle called the xanthophyll cycle (Fig. 2).

The xanthophyll cycle has not only been evidenced in higher plants, ferns, mosses, and lichens (see Ref. 25 for a review) but also in algae [26] in which various effects are observed owing to their pigment composition. Both Chlorophyta (green algae) and Phaeophyta (brown algae) present a cycle similar to that found in higher plants. In some Rhodophyta (red algae) which are devoid of violaxanthin, a partial cycle limited to the conversion of antheraxanthin-zeaxanthin often may be observed [27]. Sometimes zeaxanthin is the only pigment of the cycle to be present and no pigment interconversion happens: Cyanobacteria such as *Spirulina* are very rich in zeaxanthin, up to 40% of their carotenoid content, but they are totally devoid of any xanthophyll cycle. In other algae, as for instance *Cryptomonas rufescens* (Cryptophycea), all the pigments of the xanthophyll cycle are absent.

Besides the extremely frequent presence of the violaxanthin cycle, another xanthophyll cycle has been discovered in algal classes such as the diatoms (Bacillariophyceae) by Hager and Stransky [26]. In this cycle diadinoxanthin, a monoepoxide compound with an alkaline bond in the 7,8 position is deepoxidized into diatoxanthin (Fig. 3). Thus, it appears that the xanthophyll cycles developed

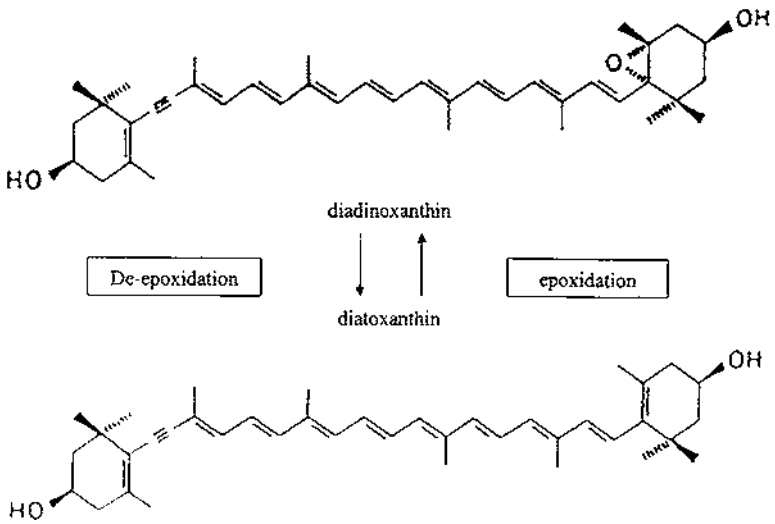

FIGURE 3 The diadinoxanthin cycle. Under stress conditions, diadinoxanthin may be de-epoxidized into diatoxanthin and after suppression of the stress epoxidation restores its amount.

and exhibited some diversity among algae and that the violaxanthin cycle has been maintained during evolution to higher plants.

Operation of Xanthophyll Cycle and Photoprotection

In greening tobacco leaves, where photoprotection needs are important, higher levels of xanthophyll cycle pigments than those of mature leaves could be detected [28]. Similarly, an increased resistance to photoinhibition could be correlated to higher contents in the xanthophyll cycle pool of pigments in *Chlorella* [29]. In two Pheophyceae, *Pelvetia canaliculata* and *Laminaria saccharina*, the amounts of zeaxanthin accumulated, via the xanthophyll cycle operation, after a light stress are very different on a chlorophyll *a* basis (respectively, 11.0:100 and 2.9:100). Such a difference has been shown to be one of the main factors responsible for the specific distributions of these two species at opposite levels on the seashore [30]. Thus, as well in higher plants as in algae, a better phototolerance appears to be frequently associated with an increase of the xanthophyll cycle pool of pigments.

Molecular Location of Xanthophyll Cycle

In order to investigate the role that the xanthophyll cycle might play in photosynthesis, it is important to be precise about its location and its site of action. It is likely that, in vivo, more than 80% of the carotenoids involved in the xanthophyll cycle are bound to proteins, but these have not been yet identified precisely in higher plants. This is mainly due to the fact that pigments are loosely bound to proteins and are easily detached during sample preparations. Since no violaxanthin was found associated with the core antenna proteins, it was suggested that the xanthophyll cycle takes place on the light-harvesting complex (LHC) proteins. This was confirmed by violaxanthin extraction from pigment-protein complexes obtained from grana and stroma membranes [31–34].

The first attempts to localize the precise site of pigment photoconversion in PSII submembrane fractions were realized by Bassi et al. [35]. These authors suggested that the xanthophyll interconversions were restricted to the minor LHCII complexes (CP24, CP26, CP29). Each minor complex binds one violaxanthin per monomer. On the contrary, Horton's group [36] showed that, owing to

the much higher amount of pigments associated with the main LHCII complexes, more than 50% of zeaxanthin may be part of LHCIIb, and then the xanthophyll cycle appears to be operative in all LHCII complexes. This is confirmed by the observation of equivalent deepoxidation states (Z + 0.5 A)/(V + A + Z) in LHCIIb, c, and d. Nevertheless, the ratio of xanthophyll cycle carotenoids/Chl is much higher in the minor complexes, which support an important role for these xanthophylls in the control of energy dissipation through the ''high-energy state quenching QE'' in these complexes. Nevertheless, a pool of violaxanthin was demonstrated to be transformed into zeaxanthin in the total absence of LHCII proteins in the *chlorina f2* mutant of barley [37]. These authors, as well as Adamska [38], have suggested a possible association of these xanthophylls to ELIPs (early light–induced proteins). On the contrary, Yahns and Krause (see Ref. 39) proposed that, when the antennae are reduced (intermittent light–grown plants), a part of the xanthophylls could be free in the thylakoid membranes.

Brown algae present a higher violaxanthin to the chlorophyll *a* ratio than higher plants. In these algae, a pure LHC fraction may be prepared [40] which accounts for more than 50% of the total pigment content and contains, besides chlorophyll *a*, high amounts of chlorophyll *c*, fucoxanthin, and violaxanthin. In *Pelvetia canaliculata*, this main LHC has been separated into two fractions by isoelectric focusing [41]. The first fraction was enriched in chlorophyll *c* and fucoxanthin and was totally devoid of violaxanthin. Since it exhibited efficient energy transfer to chlorophyll *a*, it was concluded that it could be specialized in light harvesting. The second fraction was highly enriched in violaxanthin, and the xanthophyll cycle was found to be operative in this fraction on light stress

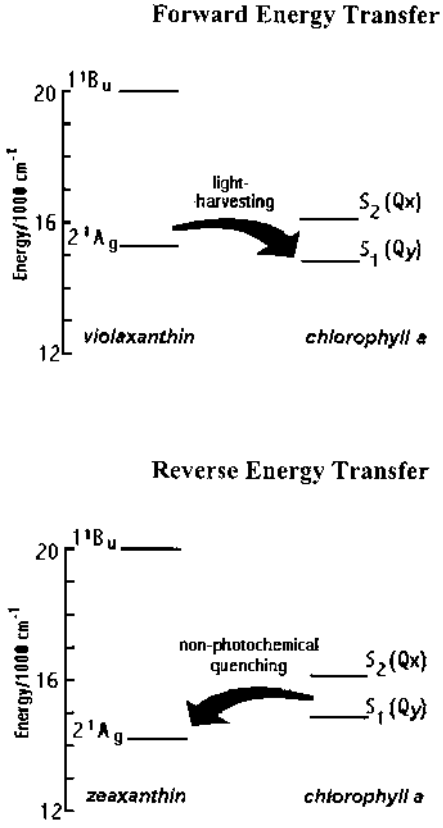

FIGURE 4 The molecular gear shift model showing S1 energy levels of violaxanthin and zeaxanthin relative to Chla. (Redrawn from Refs. 59 and 61.)

suggesting an efficient photoprotective function. Such a specialization of the chlorophyll-protein complexes in photoprotection or in energy supply is an original feature which has not yet been observed in other organisms.

In any stress condition, part of the violaxanthin is not deepoxidized. It may reach 20–30% of the total violaxanthin content [42–44]. This fraction could have a structural role as revealed by LHCII reconstitution experiences in vitro (see Ref. 39 for a review).

Enzymes Involved in the Xanthophyll Cycle

Two different enzymes have been identified as being involved in the xanthophyll cycle: violaxanthin deepoxidase and zeaxanthine epoxidase. Both are associated with photosynthetic membranes, with violaxanthin deepoxidase and zeaxanthine epoxidase being localized at the lumen and stromal side of the photosynthetic membranes, respectively [45,46]

Violaxanthin Deepoxidase

The enzyme violaxanthin deepoxidase is free in the lumen at neutral pH. During a strong illumination, the lumen pH decreases, since proton import into the lumen is very active in these conditions. This pH shift triggers conformational modifications allowing the enzyme to bind the membrane [43]. The optimum pH for enzyme activity is around 5.2 [47].

Violaxanthin deepoxidase enzyme has been isolated and even purified in an active state [48–50]. The isolated enzyme has an apparent molecular weight of 43 kDa [50–52] and is very specific for all-*trans*-3-hydroxy-5,6-epoxy-carotenoids presenting a 3S,5R,6S conformation [49]. Full enzyme activity does not require cofactors other than ascorbate [42,50] and lipids, such as monogactosyldiacylglycerol (MGDG), which is the main lipid found in chloroplast membranes [51,52]. The enzymatic activity is strongly inhibited at low temperature [53,54] and also by the addition of DTT (dithiotreitol), which strongly suggests that disulfur bridges are required for full activity [55].

Zeaxanthine Epoxidase

The enzyme zeaxanthine epoxidase was isolated but never purified [46]. Recent results suggest that this enzyme could be a flavoprotein [56,57]. Its optimun pH is around 7.5. Full activity requires oxygen and NADPH [58]. The NADPH requirement explains why the back transformation of zeaxanthin to violaxanthin is quicker under a weak illumination than in complete darkness.

Hypothesis for Xanthophyll Cycle–Induced Photoprotection

When light absorption by photosynthetic pigments exceeds both the possibility for its utilization by the transducers and the capacity of energy dissipation mechanisms, violaxanthin is progressively converted to antheraxanthin and zeaxanthin. A linear relationship has been found between the amount of zeaxanthin produced and the capacity for energy dissipation. Several mechanisms have been proposed to explain the photoprotective properties of the zeaxanthin molecules formed through the xanthophyll cycle operation.

- Zeaxanthin has a higher number of conjugated double bonds than violaxanthin (11 instead of 9). These two additional conjugated double bonds lower the energy of its lowest singlet state (2^1Ag) which in turn can directly accept energy from the first chlorophyll excited state (^{1}chl) via a singlet-singlet energy-transfer process [59–61]. The excess of energy on the carotenoid is then dissipated as heat (Fig. 4).
- Zeaxanthin could also indirectly participate in the deviation of excess light energy as heat [52,62,63]. The acidification of the thylakoid lumen (associated with the electron transfer at the plastoquinone level) brings about protonation of the LHC apoprotein and LHCII aggregates [64]. Ruban et al. [65,66] have shown in vitro that aggregation of LHCII by acidification is accompanied by a quenching of chlorophyll fluorescence. Such an aggrega-

tion may be stimulated by the addition of zeaxanthin and inhibited by violaxanthin [66]. Zeaxanthin, which is much more hydrophobic than violaxanthin, was shown to induce LHCII aggregation even in a hydrophobic medium [67]. Zeaxanthin accumulation via the xanthophyll cycle operation could participate in the induction of LHCII aggregation and to the resulting chlorophyll fluorescence quenching and to the increase of energy dissipation as heat (Fig. 5). The main features of these carotenoid-mediated alterations to the LHCII organization have been discussed recently [61].

It was also proposed that zeaxanthin epoxidation is a way to remove active oxygen species formed during a stress [68,69]. The violaxanthin formed could be again deepoxidated. In such a case, the epoxidation could be nonenzymatic [69].

A light-induced decrease in membrane fluidity occurs in thylakoid membranes when violaxanthin is allowed to convert to zeaxanthin under stress, and thus the thermostability of the thylakoid membranes increases. The LHCII prepared from illuminated leaves was shown to be poorer in xanthophyll cycle pigments than the LHCII prepared from dark-adapted leaves [70]. A diffusive displacement of zeaxanthin from the pigment protein complexes to the surrounding lipid domain [18,71] has been suggested to increase membrane rigidification. On the other hand, the thylakoid membranes which contain a very high proportion of unsaturated fatty acids are a good target for lipid peroxidations. Recently, it was demonstrated that photoinduced peroxidative damage in leaves is highly increased if the violaxanthin cycle operation is inhibited by the addition of DTT [72]. Thus, besides its direct photo-

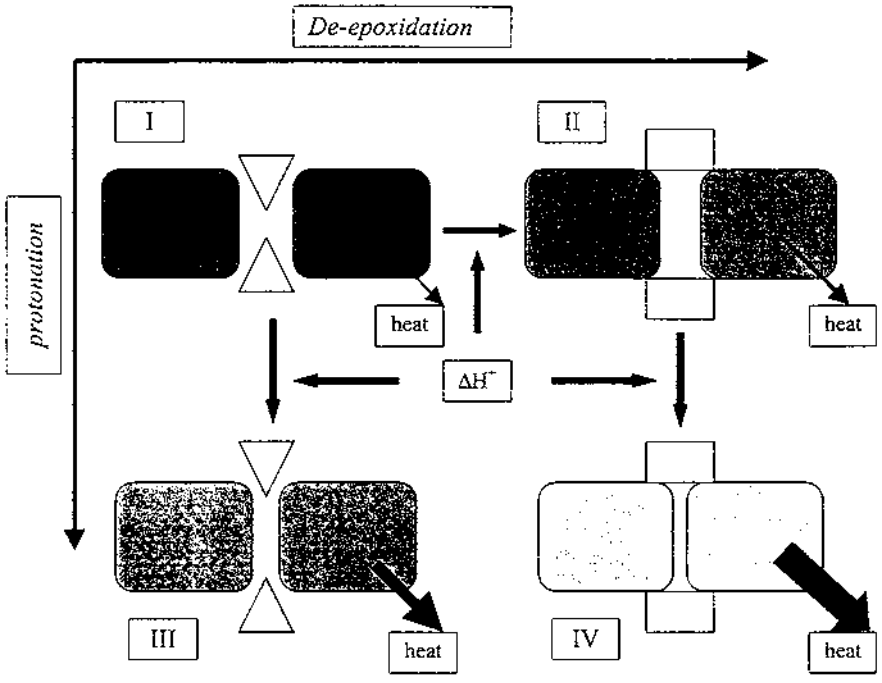

FIGURE 5 LHCII model for the control of energy dissipation as heat by structural changes brought about by deepoxidation and protonation. Four states are shown: I unprotonated, binds violaxanthin (△) and unquenched; II unprotonated, binds zeaxanthin (□), slightly quenched; III protonated, binds violaxanthin, quenched; IV protonated, binds zeaxanthin, highly quenched. (Redrawn from Refs. 63 and 61.)

protective effect through excess energy dissipation improvement, the xanthophyll cycle could also increase the resistance of the photosynthetic membranes toward stress-induced peroxidations.

SECONDARY CAROTENOID PRODUCTION BY MICROALGAE UNDER STRESS

Under unfavorable conditions (e.g., nitrogen depletion, high photon flux densities, extreme temperatures), algae synthesize and accumulate several secondary carotenoid species. Secondary carotenoids accumulate out of the thylakoid membranes and their biosynthesis regulation is not similar to that of photosynthetic carotenoids. The most frequent carotenoids which accumulate are β-carotene and/or its derivatives echinenone, 4′-hydroxyechinenone, canthaxanthin, and astaxanthin. These carotenoids sometimes accumulate inside of the chloroplast in the interthylakoidal space. Volvocales of the genus *Dunaliella* (e.g., *D. salina* var. *bardawil*) are the only photosynthetic organisms which accumulate β-carotene in intraplastidial lipid globules. These globules are essentially constituted of polar lipids and of a small amount of nonpolar membrane lipids [73]. Some other organisms such as the Ulvophyceae sp. *Trentepohlia aurea* [74] or the halotolerant cyanobacterium *Aphanothece* sp. [75] may also accumulate high amounts of β-carotene. Generally, secondary carotenoids accumulate out of the plastids. The extraplastidic pigments which are found under adverse nutritional conditions in the green alga *Protosiphon botryoides* are located in lipid globules which contain a high proportion of proteins. These carotenoproteins appear to originate from chloroplast-degenerating structures and to accumulate into the cytoplasmic globules during encystment of the alga. They contain mainly lutein and canthaxanthin [76].

Several other algae produce extraplastidic ketocarotenoids when exposed to stress conditions (see Ref. 77 for a review). Among them we may quote *Haematococcus pluvialis* and *Chlamydomonas nivalis*, the aplanospores of which cause the color of ''blood rain'' and ''blood snow'' [78]. Several strains of *Scenedesmus* and *Chlorella* accumulate echinenone and astaxanthin at the expense of chloroplasts carotenoids. High amounts of echinenone and canthaxanthin accumulate in colonies of the hydrocarbon fuel–producing palmellaceae *Botryococcus Braunii* under the effect of nitrogen deficiency [79]. These secondary carotenoids synthesized by algae under the effect of stress have economical importance: β-carotene as provitamin A in human food or as antioxidant and astaxanthin as an additive to fish feed that is responsible for the pink color of the flesh in Salmonideae [80]. For now, *Dunaliella salina* var. *bardawil* and *Haematococcus pluvialis* are the most frequently studied microalgae, and we will discuss the studies which have been devoted to them.

Response of *Dunaliella salina* to Stress

The biochemical and physiological response of the halotolerant green alga *D. salina* var. *bardawil* to conditions of stress has been reviewed by Cowan et al. [81]. This alga responds to hypersalinity stress by increased glycerol concentration in the cytoplasm as a product of photosynthesis rather than a synthesis of starch in the chloroplast [82]. Glycerol functions as a solute to adjust the cellular water potential to the osmotic concentration of the surrounding medium. Glycerol synthesis appears to be regulated by the intracellular concentration in orthophosphate (Pi) which plays a key role in the regulation of carbon metabolism. According to these investigators [82], cell shrinkage in hypersaline conditions results in an increased (Pi) in the cytoplasm which drives the Pi/triose phosphate translocator located in the inner chloroplast membrane to transport Pi into the chloroplast and triose phosphate out to the cytoplasm where it finally gives glycerol. The high glycerol 3P amounts needed for this antiport system are obtained in the plastids through starch degradation (phosphofructokinase) and via the photosynthetic Calvin cycle operation. Part of this carbon flux also triggers isoprenoid biosynthesis and especially the resulting β-carotene accumulation that is commonly observed [73,83]. Levels of β-carotene approaching 14% of cell dry weight have been reported [73,84].

All *Dunaliella* species are not able to accumulate β-carotene in stress conditions (e.g., *D. prava*). Massyuk and Radchenko [85] have reported canthaxanthin accumulation in a *Dunaliella* species under stress. *D. salina* var. *bardawil*, which may accumulate very high levels of β-carotene, is grown for a commercial purpose. When synthetic β-carotene has a >99% all-*trans* configuration, β-carotene accumulated by *Dunaliella* is a mixture of 9-*cis*-β-carotene and of all-*trans* molecules, the relative proportions of which depend on light intensity: when the ratio of 9-*cis* to all-*trans* was 0.2:1 in *D. salina* grown in low light, it could reach 1.5:1.0 under high light [86,87].

Carotenoids that accumulate and function in the chloroplast are biosynthesized within that organelle [88]. Xanthophylls such as zeaxanthin, antheraxanthin, and violaxanthin are elaborated from β-carotene through the introduction of hydroxy groups at C3 and C3′ and epoxy groups at C5-C6 and C5′-C6′. When the cells experience osmotic and light stresses, the xanthophyll cycle operates in the opposite way and thus violaxanthin and antheraxanthin are deepoxidized to zeaxanthin. Such a high level of zeaxanthin could retard the conversion of β-carotene to zeaxanthin and contribute to the accumulation of β-carotene [81].

A modification of abscisic acid (ABA) balance seems to participate in the regulation of the cellular responses to stress conditions in *Dunaliella*. The levels of this hormone are known to rise and fall dramatically in several tissues and organisms in response to environmental changes. Increased levels of ABA have thus been noticed in *Dunaliella* when the organism is exposed to a salinity stress [89] before the accumulation of β-carotene. The ABA is synthesized from all-*trans*-violaxanthin via 9′-*cis* neoxanthin which is cleaved to form xanthoxin and then converted in ABA via ABA aldehyde [90,91]. Thus, the operation of the xanthophyll cycle, which suppresses a large proportion of violaxanthin, could contribute to limit ABA biosynthesis: Low amounts of ABA have been measured in β-carotene–accumulating and zeaxanthin-enriched salt-stressed *Dunaliella* [81]. Cellular volume changes in response to osmotic stress alter the cytoplasmic pH and would be responsible for a redistribution of intracellular ABA which results in an inhibition of the plasma membrane H^+-ATPase and in an activation of the Na^+/H^+ antiporter [92]. The ABA activation of phospholipase C results in the hydrolysis of inositol-containing phospholipids such as phosphatidylinositol 4,5-bisphosphate (PIP2), which is present in plasma membranes, to inositol triphosphate (IP3) and diacylglycerol (DG). The IP3 acts as a second messenger in the cytoplasm in mobilizing intracellular calcium and the DG, in concert with the elevated concentration of calcium, activates a protein kinase [93]. The effect of ABA on Ca^{2+} channels could also contribute to increase cytoplasmic Ca^{2+} concentration by a Ca^{2+} influx, and the metabolic response seems to be promoted through Ca^{2+}-calmodulin regulation and protein kinase activity.

The accumulation of β-carotene appears to be an economically important feature of the response of *Dunaliella* to stress. This happens when it is grown under high light intensity, high salt concentration, extreme temperatures, or nitrate deficiency [73]. β-Carotene accumulates in lipid droplets essentially composed of neutral lipids together with small amounts of nonpolar membrane lipids, proteins, or carbohydrates [73]. These lipids enhance the stability of the secondary carotenoids which accumulate [94]. β-Carotene accumulation has been considered by Borowitzka et al. [95] to be a carbon sink for *Dunaliella*. Increased ATP and triose phosphate levels in the chloroplasts would induce isoprenoid biosynthesis at the expense of photosynthetically fixed carbon rather than carbohydrate synthesis.

When exposed to stress, the production of ABA precedes β-carotene accumulation. The new production of enzymes for β-carotene synthesis could depend on ABA control as shown by the suppression of both accumulations in the presence of inhibitors of chloroplast protein synthesis [96,97]. On the other hand, the operation of the xanthophyll cycle (zeaxanthin accumulation) seems to be associated with the transduction and translocation of a nuclear gene, *cbr* [98], coding for a thylakoidal peptide (19 kDa). These investigators suggest that this pigment-protein complex (Cbr-zeaxanthin) could be a modification of LHCIIb which could participate in the protection of the photosynthetic system against excess light, as was suggested for ELIPs in higher plants [38]. The accumulation of β-carotene has also been considered by Ben-Amotz et al. [87] to act to protect *Dunaliella* against high irradiations in the blue region of the spectrum. This hypothesis is explained

by the decrease of photoinhibition resistance induced by the addition of the phytoene desaturase inhibitor norflurazon [99] in *Dunaliella salina* var. *bardawil* which is then depleted in β-carotene.

Astaxanthin Production by *Haematococcus pluvialis*

A few microorganisms have the capacity to accumulate astaxanthin: *Chlamydomonas nivalis* [100], *Euglena rubida* [101], *Acetabularia mediterranea* [101], *Protosiphon botryoides* [76], and the fungi *Phaffia rhodozyma* [102] for instance.

Most studies on astaxanthin accumulation were conducted in *Haematococcus pluvialis*, a freshwater monocellular green alga (Volvocale) very common in ponds and puddles, which may sometimes be tinted red by its astaxanthin-rich akinetes [103]. It may also cause the red color of so-called blood rain. The various conditions inducing the accumulation of astaxanthin and its mono- and bi-esters are nitrogen deficiency, excess light, drought, or extreme temperatures [104]. *Haematococcus* may grow within a wide range of temperatures [105], and when environmental conditions are suitable, it appears as a biflagellated chlorophyllous cell with an eyespot in the chloroplast which is responsible of phototactic movements [106,107]. Under stress conditions, the motile cells lose their flagellae and give rise to motionless spherical cells (akinetes). Astaxanthin accumulates in the cytoplasm; up to 8% total fresh weight [108,109] after growth stops [110]. Kakizono et al. [111] have shown that an increase of the C:N ratio causes encystment, the associated astaxanthin accumulation, and important metabolic changes which give rise to resting spores with a thick cell wall. On return to suitable environmental conditions, the red akinetes give rise to oval chlorophyllous cells which become sensitive to stress [112].

Astaxanthin synthesized by *Haematococcus* constitutes more than 99% by the 3S,3′S optical isomer. The cytoplasmic droplets containing astaxanthin and its esters are located at the periphery of the cell or around the nucleus. These red droplets could constitute a screen absorbing excess light radiations, mostly in the blue and UV region, and reduce light absorption by the pigment-protein complexes. Such a limitation of photoinhibition and photodamage has been proposed for astaxanthin accumulated by Antarctic red snow algae (*Chlamydomonas* spp.) by Bidigare et al. [103]. The esterification of astaxanthin with fatty acids is postulated by these investigators to represent a mechanism by which the chromophore can be concentrated within lipid globules to maximize its photoprotective efficiency. Astaxanthin could then play a similar photoprotective role as proposed for β-carotene in *Dunaliella* [109,113] even if these two pigments have different accumulation sites [112], respectively, in the cytoplasm and in the chloroplast.

The different steps of carotenoid biosynthesis to β-carotene are well known in higher plants and algae. Nevertheless, the precise biosynthetic pathway from β-carotene to astaxanthin has not been definitely established. Harker and Young [94] suggested the existence of two synthetic pathways via, respectively, echinenone, canthaxanthin, and adonirubin for the first one and via β-cryptoxanthin, zeaxanthin, and adonixanthin for the second. Chumpolkulwong et al. [114] have confirmed both possibilities and Harker and Hirschberg [115] have suggested in addition the possible participation of two other intermediates (3′-hydroxyechinenone and 3-hydroxyechinenone).

Lotan and Hirschberg (see Ref. 114) have recently cloned a *CrtO* gene from *Haematococcus* coding for the enzyme C-4-oxygenase. This enzyme allows the conversion of β-carotene to canthaxanthin. Nevertheless, it is not definitely established in which order in vivo the keto groups in C4 and C′4 and the hydroxy groups in C3 and C′3 are introduced by the products of *CrtO* and *CrtW* genes.

The synthesis of astaxanthin by green *Haematococcus* cells under the effect of a high light stress was followed by Rmiki et al. [116]. During a first period corresponding to the four earliest hours of stress, the amount of astaxanthin accumulated appeared to be exactly equivalent to the linear decrease of the β-carotene content of the cells suggesting pigment conversions. Low-temperature fluorescence emission spectra revealed a large decrease in the PSI emission band traducing the alteration of the PSI antennae which are known to be enriched in β-carotene [117]. An association of astaxanthin with PSI chlorophyll-protein complexes was previously proposed [118]. Free astaxan-

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thin may be detected during the first hour of stress, with its mono-esters appearing only during the second hour and its bi-esters after more than 2 h of light stress. A second reddening period which began 4 h after the onset of the light-stress was characterized by a net carotenoid biosynthesis corresponding to a small rise in the β-carotene content and especially a huge accumulation of astaxanthin and its esters. A depletion of the photosynthetic capacity of the cells happened corresponding to the bleaching of the cells described during encystment by Kobayashi et al. [119–121] Astaxanthin mono-esters represent the preponderant form at the begining of encystment and later on astaxanthin di-esters are more abundant in thick cell walled cysts [104].

CONCLUSIONS

Carotenoids are commonly concerned in environmental stress and participate efficiently to the plant response which is necessary to its survival strategy. In this regard, their photoprotective function appears to be of great importance. An essential function of carotenoids in photosynthesis is to prevent harmful photooxidative reactions related to the presence of oxygen. This is particularly important at the level of the reaction centers: One to two β-carotene molecules are known to be associated with each P680 in the PSII reaction center. Besides their contribution to improve light harvesting in the pigment-protein complexes, the xanthophylls may also participate efficiently in the regulation of nonphotochemical energy dissipation via the operation of the xanthophyll cycle which may be observed in most of stress conditions. In some algal species, the metabolic response to stress leads to the accumulation of economically important secondary carotenoids.

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Light Stress: Photoinhibition of Photosynthesis in Plants Under Natural Conditions

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INTRODUCTION

Since sunlight is the energy source for photosynthesis, its deficit inevitably limits photosynthesis. However, too much sunlight can induce photoinhibition of photosynthesis and even photodamage of the photosynthetic apparatus in plants, and thus it may become a stress factor (i.e., light stress).

Photoinhibition is a phenomenon of a light-induced decrease in photosynthetic activity when light energy received by the photosynthetic apparatus is in excess of what can be used by photosynthesis. It is mainly characterized by a decline in the photosynthetic efficiency, which is often expressed as the ratio of variable to maximal fluorescence, Fv/Fm, or the photosynthetic quantum yield of carbon fixation or oxygen evolution. According to the recovery time, photoinhibition can be distinguished into two main classes: dynamic and chronic. Dynamic photoinhibition is more rapidly recovered, and it is associated principally with some energy-dissipation processes, whereas chronic photoinhibition is more slowly recovered, and it is largely related to photodamage of the photosynthetic apparatus [1]. It is different from photooxidation or photobleaching characterized by a bulk loss of pigment [2]. It has been estimated that one-tenth of the potential carbon gain in willow shoots is lost owing to photoinhibition under conditions of optimal temperature [3].

Before 1950, there were only a few ecophysiological studies on this phenomenon, although reports on photoinhibition-like phenomena could be found as long ago as the middle of the 19th century [4]. Kok [5] first used the term *photoinhibition* in 1956. Since the 1980s studies on the mechanism of photoinhibition increased in number. In the past decade, it became an important topic in several international conferences on photosynthesis. Moreover, at least two books on photoinhibition have been published [6,7]. Nevertheless, its exact mechanism is not clear because of its complexity.

In the past, many studies on the mechanism of photoinhibition were carried out in laboratories

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with isolated material such as chloroplasts of plants grown in growth chambers. Such in vitro studies are absolutely necessary for revealing the molecular mechanism of photoinhibition. However, these studies are inadequate for understanding the essence of photoinhibition in vivo under natural conditions. Therefore, studies in vivo on photoinhibition in nature are more important. The amount of such studies has increased in recent years owing to the development of portable equipment suitable for photosynthetic measurement and chlorophyll fluorescence analysis in the field. In this chapter, the recent progress of photoinhibition research concerning photodamage, thermal energy dissipation, and protective systems of the photosynthetic apparatus is discussed with emphasis on those field studies where other stress factors were absent.

PHOTODAMAGE TO THE PHOTOSYNTHETIC APPARATUS

Most of the studies on the mechanism of photoinhibition, especially the earlier ones, were conducted with algal cells or isolated chloroplasts, thylakoids, or particles of photosystem II (PSII) reaction centers from higher plants grown in growth chambers under low light conditions and by using some extreme treatments, for example, very intense light of more than 3000 μmol photons $m^{-2}s^{-1}$ that never have been encountered in nature, to induce photoinhibition. Such photoinhibition is often a result of damage of the photosynthetic apparatus, mainly D1 protein loss [8–11].

It is well known that the primary site of damage during photoinhibition is mainly PSII. In such photodamage, there are two possible mechanisms: acceptor-side and donor-side mechanisms [12–15]. The acceptor-side mechanism occurs when the plastoquinone pool is fully reduced owing to the inhibition of photosynthetic carbon assimilation. In this case, the doubly reduced QA accumulates and then is protonated, forming QAH_2. Owing to the blocking of electron transport to QA, the radical pair $P680^+Pheo^-$ recombines, generating the triplet state of P680, $^3P680$. In the presence of oxygen, the $^3P680$ can readily react with oxygen to produce singlet oxygen, $^1O_2$, a strongly oxidizing species. $^1O_2$ can damage the proteins or pigments in its vicinity. Several histidines in the D1 protein, His190, His195, and His198, are speculated to be potential targets for $^1O_2$ formed around P680. Obviously, the mechanism is oxygen dependent. The donor-side mechanism occurs when water oxidation is inhibited. The lifetime of $P680^+$ increases, because the oxygen-evolution complex cannot rapidly provide electron to the reaction center. $P680^+$ is a strong oxidant that is able to extract electrons from substances surrounding it leading to the damage of both protein and pigment such as D1 protein, chlorophyll, and carotenoids. In contrast to the acceptor-side mechanism, this mechanism is not oxygen dependent; it also occurs in the absence of oxygen.

There has been work indicating that the acceptor-side mechanism may be the more common route of photodamage. The studies on donor-side photodamage are few, and most of them have been made with isolated thylakoid membranes or the particles of the PSII reaction center and have used various ways to inhibit the donor-side activity of PSII, for example, Tris-washing, hydroxylamine treatment or Cl^- depletion. Because the thylakoid luminal pH is expected to be around 5.0–5.5 during continuous illumination in vivo, based on their results that donor-side–induced photoinhibition became dominant below pH 4.5, Spetea et al. [16] have proposed that intact plants can probably avoid extensive donor-side–induced photodamage.

Although for nearly a decade photoinhibition has been almost synonymous with D1 protein loss, there has been no direct evidence of the photoinhibitory loss of the D1 protein of leaves in natural environments [17–19]. Perhaps the paradox originates from a lack of the link between laboratory and field studies. In other words, the conception about the mechanism of photoinhibition based on the laboratory studies in vitro may not fit the actual case of photoinhibition in nature.

LIGHT ENERGY DISSIPATION AS HEAT IN THE PHOTOSYNTHETIC APPARATUS

In field studies on the photosynthetic efficiency in plant leaves, we found that the apparent quantum yield of photosynthetic carbon fixation often displayed a significant midday decline in many C_3

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plants such as soybean and wheat on clear days. However, the midday decline did not occur on cloudy days. And shading could prevent it; namely, the photosynthetic efficiency in shaded leaves was higher than that in control leaves exposed to full sunlight. Considering these facts, it was deduced that photoinhibition may be a likely cause of the midday decline in the efficiency [20–22]. The deduction was supported by the experimental results from chlorophyll fluorescence analysis [23–25]. Similar to our finding, Ögren [26] reported that photoinhibition often occurred in willow leaves even if water and nutrients were sufficient and temperature was optimal. Through further study, we found that at noon on clear days, photoinhibition in sweet viburnum leaves was not accompanied by a significant loss of D1 protein [27]. A similar case was observed in wheat leaves [28]. Nevertheless, DTT (dithiothreitol), an inhibitor of deepoxidase involved in the xanthophyll cycle, introduced via the transpiratory stream resulted in a substantial loss of D1 protein in wheat leaves exposed to strong sunlight around noon [28]. It appears that under natural conditions with strong sunlight as the sole stress factor, photoinhibition is a result of the enhancement of some protective processes such as xanthophyll cycle–dependent energy dissipation rather than the damage to the photosynthetic apparatus.

Xanthophyll Cycle–Dependent Energy Dissipation

The light-dependent interconversions of the leaf xanthophylls were found about 40 years ago [29,30], but the xanthophyll cycle did not attract much attention until Demmig and coworkers [31] proposed a possible role for it in the protection of the photosynthetic apparatus against photodamage. There have been several papers reviewing the regulation and function of this cycle [18,32–38].

The xanthophyll cycle consists of the interconversions of three carotenoids. Under conditions of excessive light, a deepoxidation process operates from the diepoxide violaxanthin (V) via the monoepoxide antheraxanthin (A) to the epoxide-free zeaxanthin (Z), whereas under light-limited conditions, an epoxidation process occurs in the reverse direction. The two processes are catalyzed by two enzymes, deepoxidase and epoxidase, respectively. Zeaxanthin has two possible action patterns in the energy dissipation as heat. It interacts directly with the singlet excited state of chlorophyll [32] or it facilitates aggregation of light-harvesting complex II (LHCII) and, as a consequence, increases energy dissipation [39]. The key site of energy dissipation is thought to be the antenna systems within the photochemical apparatus under physiological conditions [35].

The remarkable characteristics of the xanthophyll cycle–dependent heat dissipation are the declines in photosynthetic quantum yield, PSII photochemical efficiency (Fv/Fm), and the initial fluorescence level (Fo), because light energy transfer to PSII reaction centers is reduced due to heat dissipation.

There have been many reports showing the diurnal variations in both the contents of xanthophyll cycle pigments and heat dissipation, the difference in both the pool size of the pigments and heat dissipation between sun and shade leaves, and the increase in the pool size and capacity of heat dissipation caused by environmental stresses such as low temperature, drought, and nutrient deficit [35,40].

Besides V, A, and Z derived from β-carotene, other xanthophylls such as lutein (Lut) and loroxanthin (Lor), both derived from α-carotene, may also be involved in thermal dissipation. Through genetic experiments using *Chlamydomonas*, a single-celled green alga, Niyogi et al. [41] have provided the first evidence that xanthophylls derived from both β-carotene and α-carotene play critical roles in the thermal dissipation of light energy. The generality of their results in the vascular plants remains to be demonstrated.

More recently, Demmig-Adams and Adams [42] reported that all increases in thermal energy dissipation were associated with increases in the zeaxanthin level in the leaves of the 24 plant species examined. It seems that these responses to excessive sunlight are not species specific, and the xanthophyll cycle–dependent energy dissipation acts as the predominant protective mechanism against photodamage to the photosynthetic apparatus in all plant species. However, the following results from our laboratory indicate that among several types of energy dissipation as heat, the

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xanthophyll cycle–dependent one is unlikely to be a predominant protective mechanism in soybean leaves. First, the initial fluorescence level, Fo, in soybean leaves always increased significantly after exposure to strong light. Second, in soybean leaves, DTT had little effect on the changes in Fv/Fm induced by strong light. Third, the number of inactive PSII reaction centers in soybean leaves increased under strong light and decreased in subsequent darkness. These results indicate that the changes in Fo and Fv/Fm in soybean leaves during strong light illumination and subsequent dark recovery are mainly due to the inactivation and reactivation of some PSII centers [43].

Energy Dissipation Dependent on PSII Reaction Center Inactivation

Since the concept of PSII heterogeneity was suggested in the 1970s [44], a hypothesis that the reversibly inactivated PSII reaction centers can protect the photosynthetic apparatus from photodamage has been put forward by some investigators [19,45–47].

There are two types of PSII reaction centers: the active and inactive. They are different from each other in both structure and function. The active centers are linked with a larger antenna, localized in the granal partitions, and can transport electrons to plastoquinone, whereas the inactive centers are linked with a smaller antenna, localized in the stromal lamellae, and cannot transport electrons to plastoquinone [44,48–51]. The inactive centers, the formation of which is a phase in the repair cycle for those damaged PSII centers, can transform into the activated form [52].

There are several different methods for the measurement of inactive centers. One is based on the amplitude of the electrochromic band shift at 518 nm (ΔA518) and is due to photochemical charge separation in the reaction centers under both in vitro and in vivo conditions [53]. The other method uses the amplitude of the initial fluorescence yield (Fo) increase in the induction dynamic curve as a measure of the relative concentration of the inactive centers based on the inability of them to transfer electrons from QA to QB [54]. In addition, the amount of inactive centers can be calculated from a change in the content of active centers caused by some treatments. Functional PSII reaction centers are conveniently assayed by repetitive flashes in leaf disks [55,56].

There have been several lines of evidence demonstrating the presence of physiologically inactive centers in leaves [51]. The reversible inactivation of PSII reaction centers is likely an effective mechanism protecting PSII from photodamage [57]. Anderson and Aro [46] proposed a hypothesis that under strong light an increase in the photoinhibited PSII reaction centers can act as a pathway of light energy dissipation protecting the functional centers against damage. Moreover, they have demonstrated that photoinactivation of PSII is a light-dosage effect depending on the number of photons absorbed rather than the rate of photon absorption. Hence, it will occur at all light levels from limiting to supersaturating light [58]. Recently, the hypothesis has been supported by our experimental results from soybean leaves grown in the field. Based on these results, it has been deduced that photoinhibition in soybean leaves may result from enhanced thermal dissipation by the inactivated PSII reaction centers rather than D1 protein loss or xanthophyll cycle–dependent heat dissipation [24]. The main reasons for this deduction are as follows.

First, the initial fluorescence level, Fo, always increased significantly, while Fv/Fm decreased in soybean leaves even under unsaturating light conditions without environmental stress. There are several explanations for the mechanism of Fo change. On the basis of the model, suggested by Kitajima and Butler [59], Demmig et al. [31] proposed that the damage to PSII reaction centers can lead to an increase in Fo, and an increase in heat dissipation can result in a decline in Fo. In some studies, therefore, an increase in Fo was taken to indicate photodamage [60,61]. Moreover, a good correlation between the rise in the Fo level and the loss of the D1 protein was observed from the moss samples grown under low light (60 μmol photons $m^{-2}s^{-1}$) and subsequently exposed to high light (1000 or 2000 μmol photons $m^{-2}s^{-1}$) for 1.0–4.5 h [62]. However, such explanations of the Fo rise to be due to PSII center damage are not always valid in every case. In some cases, the photosynthetic function in leaves could recover within a short time after photoinhibition had occurred and Fo increased significantly. Therefore, an increase in Fo can also be explained by the

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inactivation of PSII reaction centers [14,48,57,63–65]. Recently, it has been suggested that as well as the separation of the light-harvesting chlorophyll protein complex of PSII from the PSII core complexes, partially reversible inactivation of the PSII reaction centers at high temperature is the cause of the increase in the Fo level [66]. The reversible part of the Fo increase can also be ascribed to the formation of the reduced QA [67].

Second, after the removal of photoinhibitory conditions, the PSII photochemical efficiency, Fv/Fm, could recover in the dark at the same rate as under weak light of 30–100 μmol photons $m^{-2}s^{-1}$. This indicates that the photoinhibition observed by us in soybean leaves is not involved in net loss and resynthesis of D1 protein, because D1 protein synthesis is strictly light dependent [14,68].

Recently, new evidence supporting the above deduction has been obtained. Photoinhibition caused by strong light in leaves of soybean grown in a phytotron was accompanied by an increased Fo and an accumulation of inactive PSII reaction centers manifested by chlorophyll fluorescence analysis but no net loss of D1 protein (S.-S. Hong and D.-Q. Xu, unpublished data).

Unfortunately, the nature of the inactive centers and the mechanism whereby excessive light energy is dissipated as heat remain unclear, although there have been some studies related to the mechanism of PSII center inactivation. Some examples are as follows.

The light-induced inactivation of the PSII reaction centers can be resolved into a reversible conformational change (characterized by a rise in Fo and a decrease in Fm) followed by an irreversible modification of D1 protein [69] or three processes with different kinetics: the fast process ($t_{1/2}$ = 1–3 min), the slow process ($t_{1/2}$ = 15–40 min), and the very slow process ($t_{1/2}$ more than 100 min) [70]; or four intermediates: fast ($t_{1/2}$ = 30s), semistable ($t_{1/2}$ = 2 min.), stable ($t_{1/2}$ = 30 min), and nondecaying on the basis of their Fo fluorescence decay times [67].

PSII complexes can exist in dimeric and monomeric states. The more stable dimers are monomerized during photoinhibition, and phosphorylated D1, D2, and CP43 retard the photoinduced monomerization [71]. The less stable PSII monomers are located in the nonappressed granal margins, whereas the more stable PSII dimers are located in the appressed granal cores [72]. The cyanobacterium *Synechococcus* sp. pcc 7942 possesses two functionally distinct forms of the D1 protein: D1:1 and D1:2, and PSII centers containing D1:1 are less efficient and more susceptible to photoinhibition than those centers containing D1:2 [73]. Once D1 protein is phosphorylated, it is neither dephosphorylated nor degraded in the light [74].

These data are very useful but not sufficient for understanding the mechanisms of the PSII center inactivation and reactivation. In addition, dissociation of P680 from the light-harvesting antenna of PSII also can explain the apparent inactivation of PSII reaction centers [75]. Moreover, there have been some reports in which an increase in Fo caused by heat treatment is linked to the supposed disconnection of PSII reaction centers from the antenna complexes [76]. It appears that the relationship between the disconnection and the reversible inactivation of PSII reaction centers is worth studying.

Energy Dissipation Associated with Cyclic Electron Flow Around PSII

Cytochrome b_{559} is always present in isolated PSII reaction centers, but its function remains unclear. It has been proposed that PSII cyclic electron transport involving Cytochrome b_{559} may act as a mechanism to protect the reaction center from photodamage [77–80]. This is an attractive hypothesis, but it is yet to be demonstrated experimentally. The report that pyocyanine stimulated light-dependent Cytochrome b_{559} reduction and alleviated photoinhibition in spinach thylakoids [81] is in favor of the hypothesis. Critchley and Russel [57] have suggested that the inactive centers could dissipate excessive light energy as heat through a cyclic electron flow around PSII.

More recently, Prasil et al. [82] proposed a model about the relationship between PSII function and cyclic electron flow around PSII. According to this model, the redox state of the plastoquinone (PQ) pool is a determinant of the extent of the coupling between the noncyclic electron flow from

PSII via quinone acceptors to PSI and water oxidation. Under weak light, the PQ pool is mostly oxidized and the noncyclic electron flow is fully coupled to the oxygen evolution; under strong light, low-oxygen concentration, or low-temperature conditions, the PQ pool becomes mostly reduced and a cyclic electron flow via Cytochrome b_{559} in the high-potential form around PSII operates in parallel to the noncyclic one.

ΔpH-Dependent Energy Dissipation

As electrons are transferred directionally on the membrane, a proton gradient across the thylakoid membrane, namely, ΔpH or high-energy state, is developed during illumination. This ΔpH is not only necessary for ATP formation but also an important regulatory factor in the energetic metabolism of chloroplasts. Fork et al. [83] proposed that such a high-energy state is a protective mechanism of the photosynthetic apparatus. It was demonstrated that the removal of the high-energy state by an uncoupler could exacerbate the photodamage in chloroplasts [84] and leaves [85]. More recently, using the experiments of the cumulative exposure to strong light for a short time, Shen et al. [86] showed that such a proton gradient across the thylakoid membrane played a protective role for PSII when other protective mechanisms did not operate effectively during the induction of leaf photosynthesis.

An increase in the proton gradient across the thylakoid membrane is a fast response to excessive light energy. It is not only an effective protective mechanism itself but also an essential prerequisite for the operation of other thermal dissipation processes mentioned above. At least the activity of violaxanthin deepoxidase is controlled by the luminal pH [38].

The thermal dissipation processes mentioned above play important protective roles against photodamage to the photosynthetic apparatus when light is in excess of photosynthetic demand. It was estimated that in leaves of irrigated cotton exposed to full sunlight, about 25% of the excitation energy was used for CO_2 fixation, an additional 19% was consumed in photorespiration and the remainder, 56%, was dissipated via thermal dissipation [87].

PROTECTIVE STRATEGIES OF THE PHOTOSYNTHETIC APPARATUS AGAINST PHOTODAMAGE

Higher plants always live in an environment where light intensity often changes substantially. Unlike animals, they cannot escape from unfavorable environments. Therefore, plants have developed some strategies for both increasing light absorption under light-limited conditions and avoiding photodamage to the photosynthetic apparatus under excessive light. Besides those thermal dissipation processes mentioned above, there are several protective strategies against photodamage to the photosynthetic apparatus, which are as follows.

Decreasing Light Absorption

When the received light energy is in excess of the photosynthetic requirement, plants may reduce light absorption by light-avoiding movements of leaves and/or chloroplasts or by an increase in leaf reflectance through hair cover or an accumulation of salt crystals on the leaf surface [88].

Reducing Light Energy Distribution to PSII

When a transition to state 2 occurs, light energy received by PSII decreases, thus alleviating the pressure of excessive light energy on PSII. It was considered that state transition might make a significant contribution to the total nonphotochemical dissipation of light energy in leaves even under conditions of saturating light [89]. However, different opinions exist regarding the role of state transition under high light [90]. Our study showed that the transition to state 2 induced by a stream of weak red light could alleviate photoinhibition caused by strong light in wheat leaves [91],

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but in leaves of wheat, sweet viburnum, and soybean grown in the field, of the three components (fast, middle, and slow) of the nonphotochemical quenching of chlorophyll fluorescence, the middle one related to the state transition had the smallest quenching coefficient under strong midday sunlight [T. Hong and D.-Q. Xu, unpublished data] indicating that state transition is not likely an important protective mechanism.

Increasing Photosynthetic Utilization of Light Energy

After plants are transferred from a low-light environment to a high-light one for several days, the contents of both the components of the photosynthetic electron transport chain and Rubisco in leaves increase resulting in an increased photosynthetic capacity [92].

Enhancing Energy Consumption by Metabolic Processes

Since photorespiration was discovered, its physiological function has been an open question. Osmond [93] suggested that photorespiration in C_3 plants can be effective in preventing photoinhibition under strong light with no CO_2, because it can dissipate excessive light energy. Since this suggestion is based on experiments performed under strong light and in the absence of CO_2, naturally the question appears whether photorespiration can also play a role in preventing photoinhibition in normal air. We observed that after exposure of a cotton leaf to strong light for 3 h, photoinhibition was enhanced on suppression of photorespiration by low O_2 (2%), indicating that photorespiration can indeed alleviate photoinhibition under strong light with normal CO_2 [94]. However, these results can not be fully explained by the suggestion of Osmond, because under low O_2, increased photosynthesis can use the excessive light energy induced by inhibition of photorespiration. Namely, only from the point of view of energy utilization, one would not expect that a low O_2 treatment may lead to an enhanced photoinhibition. Considering the suggestion by Sharkey et al. [95] that a deficient Pi supply may limit photosynthesis, and the suggestion by Gao et al. [96] that photorespiration has a function of maintaining the internal level of Pi in the chloroplast, it is reasonable to speculate that photorespiration can alleviate photoinhibition by enhancing the recycling of Pi in the photosynthetic process of chloroplasts. This speculation is supported by our experimental results of Pi content determination and Pi feeding. Under strong light and low O_2 conditions, the net photosynthetic rate gradually declined. As is consistent with this, the Pi content decreased more in the leaf segment in low-O_2 air than that in normal air. However, the difference in the decline of the net photosynthetic rate between the low-O_2 treatment and control (normal air) disappeared after Pi feeding [94].

From the carbon metabolic pathways of photosynthesis and photorespiration, it is clear that with respect to Pi release photorespiration is a faster way than starch or sucrose synthesis. This is because Pi release in photorespiration is performed only through a reaction from phosphoglycolate to glycolate, whereas that in starch or sucrose synthesis needs to pass through many reactions from phosphoglycerate to starch or sucrose. Therefore, photorespiration can considerably accelerate the Pi turnover during photosynthesis.

More recently, Park et al. [97] presented evidence that the electron transport to oxygen via the twin processes of photorespiration and the Mehler reaction mitigates the photoinactivation of PSII in vivo, and photorespiration is as effective as the Mehler reaction in protecting PSII against light stress. Interestingly, dark respiration also plays a role in the protection of the photosynthetic apparatus, as shown by a report that inhibition of dark respiration by azide increases the susceptibility of the cynobacterium *Anacystis nidulans* to photoinhibition and slows down the recovery from photoinhibition [98].

Raising Capacity of the Scavenging of Active Oxygen

Even under optimal conditions, some metabolic processes (e.g., the Mehler reaction) in plants can produce active oxygen species which have severe destructive effects on the photosynthetic apparatus.

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However, the active oxygen–induced damage to the photosynthetic apparatus does not occur under normal conditions owing to the effective operation of an antioxidative defense system, which can scavenge active oxygen species at an adequate rate in chloroplasts. Recently, Foyer et al. [99] have reviewed in detail the mechanisms of generating and scavenging of active oxygen in photooxidative stress.

The antioxidative defense system in plants is composed of both nonenzymatic and enzymatic constituents. The enzymatic antioxidative components include superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (AsPOD) as well as those enzymes involved in the synthesis and regeneration of the small-molecule antioxidants, such as dehydroascorbate reductase and glutathione reductase (GR). O_2^- forms H_2O_2 and O_2 by catalysis of SOD. H_2O_2 can interact with ascorbate in a reaction catalyzed by AsPOD to produce H_2O or be destroyed by CAT. Xu et al. [100] found that in soybean leaves the activities of these enzymes displayed significant diurnal variations with increases around noon on clear days. The nonenzymatic antioxidants are some small molecules such as ascorbate, glutathione, tocopherol, and flavonoids as well as carotenoids. They can interact with active oxygen species to scavenge them. For instance, the carotenoids may change $^1O_2$ into normal oxygen.

Accelerating Reparation of Damaged Parts

The D1 protein of the PSII reaction center has the highest turnover rate among thylakoid membrane proteins encoded by chloroplast genomes. For the damage and repair of PSII reaction center, Aro et al. [52] have proposed a complex cycle involving several key stages: (a) the reversible inactivation of PSII center, (b) the formation of nonfunctional center with a damaged D1, (c) the migration of the nonfunctional center from the granal region to stromal lamellae, (d) the removal of damaged D1 from the nonfunctional center followed by its degradation, (e) the insertion of a newly synthesized D1 into the nonfunctional center, and (f) the migration of the complex with a new D1 back to the granal region and the recovery of photochemical competence.

The fact that photoinhibition in leaves of wheat grown in the field was not accompanied by a net loss of D1 protein [28] does not imply the nonoccurrence of D1 degradation during photoinhibition. It is likely that under strong light D1 protein is constantly degraded and synthesized forming a dynamic equilibrium with no net loss of the D1 protein. Just owing to the effective operation of the protective system mentioned above, no net loss of the D1 protein was observed during photoinhibition under natural conditions [28]. During photoinhibition caused by some extreme conditions, the loss of the D1 protein is often unavoidable, because those experimental plants grown under low light have such low capacities for photosynthesis and protection of the photosynthetic apparatus from photodamage that they cannot adapt to a sudden strong light, or because the isolated thylakoids [101] or PSII reaction centers [11,102] used in experiments have lost most components of their protective systems.

In addition, specific light-stress proteins can be considered to be part of these protective strategies. These proteins are stable under light-stress conditions but are rapidly degraded during recovery at low light. Their function may be related to transient binding of chlorophylls, which may subsequently be reused for insertion into newly synthesized proteins [103].

RELATIONSHIP BETWEEN PHOTOINHIBITION AND PHOTODAMAGE

It is often thought that photoinhibition is a result of net loss of the D1 protein, so it occurs only when the rate of damage to the D1 protein exceeds the rate of its repair [9,71,104–106]. However, some experimental results from plants grown in cabinets [107–111] and grown in the field [27,28] have clearly indicated that photoinhibition is not necessarily accompanied by a net loss of the D1 protein. Furthermore, under anaerobic conditions, the restoration from light-induced PSII electron

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transport inhibition of isolated spinach thylakoids without de novo protein synthesis has been reported [112]. Obviously, one should not simply equate photoinhibition with photodamage. The cause of photoinhibition is not always photodamage. Nevertheless, there are some different viewpoints about the relationship. For instance, Tyystjarvi and Aro [113] have defined photoinhibition as the light-dependent irreversible inactivation of PSII reaction center activity, which can be restored only via the degradation and synthesis of the D1 protein. Moreover, they consider that photoinhibition is as common as light, not restricted to stress conditions and high light, but occurs in vivo under all light intensities, and that the repair mechanism is normally rapid enough to prevent the symptoms of photoinhibition from appearing under optimal growth conditions.

Since the photosynthetic function could rapidly recover within minutes or hours after photoinhibitory conditions were removed, Krause [63] and Öquist [114] proposed that photoinhibition can be viewed as a controlled protective mechanism that serves to dissipate excessive energy and to minimize damage to the photosynthetic apparatus. Furthermore, Björkman et al. [115] reported that the observed responses of mangrove leaves to excessive light may reflect a regulatory and protective response rather than a damage to the reaction center complex of PSII. Therefore, photoinhibition in vivo should be viewed as the capacity of plants to adjust photosynthetically to the prevailing environmental conditions rather than a process which inevitably results in damage or injury to plants [116]. Critchley and Russell [57] proposed that what has been considered previously as photodamage may in fact be the mechanisms of reversible downregulation, and actual damage to the photosynthetic apparatus by light may be an event restricted to extreme environmental conditions, including those used in laboratories. Meanwhile, Björkman and Demmig-Adams [88] guessed that photoinhibitory damage is uncommon in natural plant stands, and responses that in the past were thought to be indicative of photoinhibitory damage in many cases now appear to be reflections of the operation of protective processes. Our studies also indicate that under natural conditions without other environmental stress, photoinhibition is mainly a reflection of the enhancement of the thermal energy-dissipation processes [24,28]. Similarly, photoinhibition also occurs in rain forest understory plants when they are exposed to saturating sunflecks, and it may most likely be the result of rapid heat dissipation, as no indication of photodamage has been seen [117]. In a word, photoinhibition of PSII in vivo is often a protective strategy rather than a damaging process [58].

Under natural conditions, photodamage to the photosynthetic apparatus is often a result of the combined effects of light and other severe environmental stress such as low temperature, drought, or nutrient deficit. For instance, under low temperature, especially frost conditions, low temperature not only inhibits photosynthesis leading to an excess of light energy but also hinders both the protective systems and repair process from operating effectively inducing photodamage to the photosynthetic apparatus, because the rate of damage exceeds the rate of repair. Therefore, a pronounced photooxidative bleaching of chloroplast pigments was often observed in winter [118].

CONCLUSIONS

Light stress often causes photoinhibition of photosynthesis in the upper layer leaves of plant canopies. In the last two decades, photoinhibition has been an important subject in photosynthesis research. However, the studies carried out under natural conditions have been few until now. From the available data, it appears that under natural conditions without any environmental stress other than light, photoinhibition is only a reflection of enhanced operation of thermal energy dissipation rather than a result of photodamage to the photosynthetic apparatus. In plants, there are many protective strategies against photodamage to the photosynthetic apparatus under light stress. The irreversible photodamage is unavoidable only under extreme conditions, including natural and artificial conditions, which hinder seriously the operations of both photosynthesis and protective systems. For understanding the molecular mechanism(s) of photoinhibition in nature, there are some topics to be studied such as the mechanism of reversible inactivation of PSII reaction centers, the relative contribution of each type of thermal energy dissipations in protecting the photosynthetic apparatus

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against photodamage, and the physiological and biochemical bases of interspecific and intraspecific differences in resistance to photodamage.

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22

Photooxidative Stress in Higher Plants

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INTRODUCTION

Plants have a high capacity to convert light energy to chemical energy under optimal conditions. Several conditions, like low concentration of carbon dioxide, low or high temperatures, and water deficit can cause a decrease of the energy-transfer efficiency from light to carbon dioxide, resulting in an excess of light energy. Plants have mechanisms to dissipate the energy excess as heat, but high-light excess and/or failure of these mechanisms allows the generation of reactive molecules: triplet excited chlorophyll and active oxygen species (AOS) [1].

The generation of active oxygen species occurs even under optimal conditions, because they are normal metabolic products of many processes [2]. Plants have several mechanisms to suppress the production of active molecules [1]. The failure of the scavenging systems or even high excess of photons leads to an excess production of active oxygen species [1]. Such a light-dependent AOS generation is referred to as a photooxidative stress [3].

PHOTOSYNTHETIC APPARATUS UNDER LIGHT STRESS

Light-Induced Production of Active Oxygen Species

The biologically important oxygen species are the singlet oxygen ($^1O_2$), the superoxide radical anion ($O_2^{\bullet -}$), the hydrogen peroxide (H_2O_2), and the hydroxyl radical ($OH^{\bullet}$).

The generation of singlet oxygen will be described later. Active oxygen species are produced by the reduction of molecular oxygen in a series of one-electron transfers [1]. The superoxide radical anion is formed by one-electron reduction of a ground-state oxygen. Mehler [4] originally proposed that the stromal side of photosystem I (PSI) is the major site of superoxide production. Later it was shown that there are two sites of oxygen reduction on the reducing side of PSI [1,5,6].

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The reduced ferredoxin (Fd) is a major $O_2^{\bullet -}$–generating component [7]. The apparent K_m value in physiological conditions is about 60 μM [1].

$$2\ O_2 + 2\ Fd_{red} \rightarrow 2\ O_2^{\bullet -} + 2\ Fd_{ox}$$

The $O_2^{\bullet -}$ reduction can occur in the aprotic interior of thylakoid membranes, where the solubility of oxygen is much higher than in water. The sites of reduction are the Fe-S centers X and A/B. The K_m for oxygen reduction in this centers is 10 μM, but it increases severalfold with an increase in light intensity or in damaged membranes [1,3]. However, there is evidence that under physiological conditions, superoxide may donate electrons to the electron donors of PSI, so there might be a superoxide-mediated cyclic electron flow around PSI [1].

Oxygen can be reduced by PSII during photooxidation [8]. Superoxide radicals can receive an additional electron, undergo protonation, and produce hydrogene peroxide. Most of the hydrogen peroxide in chloroplasts arises in the disproportioning of superoxide. This reaction occurs spontaneously, but its rate is greatly increased by the thylakoid-bound and stromal superoxide dismutases [1].

$$O_2^{\bullet -} + O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2$$

or from the reduction of superoxide by ascorbate, manganese ions, or ferredoxin [1].

$$O_2^{\bullet -} + AH_2 + H^+ \rightarrow H_2O_2 + AH$$

The last reaction has a minor contribution as compared with the enzyme-catalyzed reaction [9]. Additionally, H_2O_2 may be generated in glyoxysomes as well as peroxisomes by glycolate oxidase during photorespiration or by acyl-CoA oxidase via β-oxidation and the glyoxylate cycle [10,11].

Hydrogen peroxide can be converted into a strongly oxidizing hydroxyl radical ($OH^{\bullet}$) by one-electron transfer. The main source of ($OH^{\bullet}$) is the decomposition of hydrogen peroxide in the Fe-catalyzed Haber-Waiss reaction; that is, a superoxide-assisted Fenton reaction [12]. In this reaction, trace amounts of Fe^{3+} are reduced by $O_2^{\bullet -}$ to produce Fe^{2+} which subsequently reacts with H_2O_2 to form $OH^{\bullet}$.

$$Fe^{3+} + O_2^{\bullet -} \rightarrow Fe^{2+} + O_2$$
$$Fe^{2+} + H_2O_2 \rightarrow OH^{\bullet} + OH + Fe^{3+} \text{ (Fenton reaction)}$$

Although superoxide and hydrogen peroxide are able to migrate a considerable distance in membranes before reacting with other molecules, hydroxyl radicals are extremely reactive, so they can only diffuse a few molecular diameters before reacting [9,13].

Photoinhibition of Photosynthesis

Photoinhibition of photosynthesis is generally used to denote a decrease in the photosynthetic activity when plants are exposed to a high-light intensity that exceeds the capacity of the dark reactions (electron transport and carbon metabolism) or the ability of the light-harvesting system to dissipate light energy not used for photosynthetic functions. Powles [14] considers the photoinhibition as a first stage of high light–induced damages related to the reduction of photosynthetic capacity. The second stage, which he refers to as a pigment photooxidation, occurs after a long-term exposure of plants to strong light and concerns the bleaching of the antenna pigments. The latter process requires light and oxygen [15]. Since the photoinhibition represents the other side of the Blackman light-response curve, and simply equates with photosynthesis under conditions of photon excess (at excessive photosynthetically active photon flux density [PPFD]), the catena of processes which participate in photoinhibition is the same as in photosynthesis [16]. On basis of the relaxation times of biophysical, biochemical, physiological, and ecological processes included in the catena, two main classes

of photoinhibition are distinguished—dynamic photoinhibition, usually predominant in sun plants, and more slowly relaxing chronic photoinhibition, predominant in shade plants [16].

PSII is primarily affected by photoinhibition [14]. Two mechanisms of photoinactivation are involved which affect the acceptor side and donor side, respectively [17,18]. They are distinguished on the basis of differences in the primary site of electron transport malfunctioning, the subsequent D1 protein degradation, the light intensity, and the oxygen requirement of the process (Fig. 1).

Acceptor side–induced photoinhibition of PSII occurs under high-intensity irradiation that exceeds the saturation level of the photosynthetic electron transport [17]. Excess photon exposure causes nonphysiological overreduction of the first quinone electron acceptor in PSII. Sequential modifications happen at the level of the QA and/or QB acceptors [19]. These conditions lead to the recombination of the radical pair, $P680^{+}Pheo^{-}$ [20] and the production of the triplet state of P680-^{3}P680 [21]. Under aerobic conditions, these chlorophyll triplets may be quenched by oxygen and singlet oxygen $^{1}O_2$ thus produced [22,23]. Alternatively, oxygen may receive electrons and thus produce oxygen radicals [24]. It has been shown that the addition of singlet oxygen scavengers such as histidine [25–28], diazobicyclooctane [26,29], azid [23], or rutin [27] as well as free radical scavengers such as uric acid or propylgallate [30] provide partial photoprotection against the acceptor side–induced photoinhibition of PSII. All steps in this mechanism, prior to singlet oxygen formation, are reversible if plastoquinone is reoxiding.

There is still no consensus on the role of active oxygen in photoinhibitory damage. The singlet

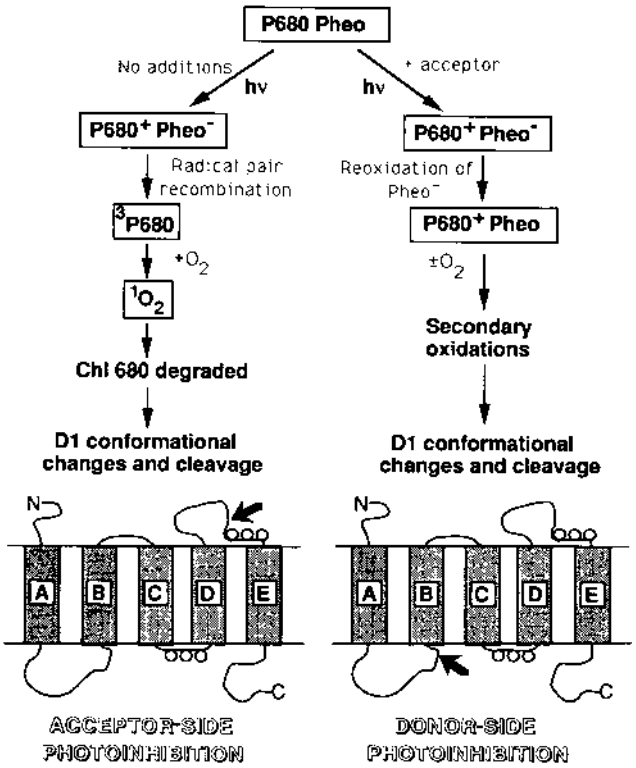

FIGURE 1 Scheme showing the two routes of damage due to acceptor- and donor-side photoinhibition, as identified from experiments conducted with isolated reaction centers of PSII. Putative cleavage regions are indicated with arrows on the folding diagrams for the D1 protein. (From Ref. 18.)

oxygen participates in the initiation of the following degradation of the reaction center protein D1 [31,32]. It is able to trigger D1 protein damage, probably by promoting a special conformational change, which makes the protein susceptible to proteolytic cleavage [33]. One possibility is that in complex in vivo systems, such as leaves and photosynthetic algae, the D1 protein may be cleaved by the direct action of active oxygen [26]. The major cleavage site on D1 is on the stromal side of the thylakoid membrane [33–37], and the characteristic degradation products of the D1 protein are 23-kDa N-terminal [33,36] and 10-kDa C-terminal fragments [36]. The D1 protein is the protein in PSII with the highest turnover rate [38,39]. This phenomenon might be linked to the requirement to repair PSII after it has been damaged by photoinhibitory light [40].

Donor side–induced photoinhibition of PSII occurs under both high- and low-light intensities when the capacity of the water-oxidizing complex to donate electrons to P680 is inactivated before irradiation by other stress factors such as low temperature [41,42]. The water-oxidizing complex is unable to keep up with the rate at which electrons are transferred from P680 toward acceptor-side components. This may lead to an increase in the lifetime of $P680^+$ with a high oxidizing potential [43]. This process would occur both in the presence and absence of oxygen. The $P680^+$ is able to extract an electron from its surrounding environment and subsequently destroy the proteins D1, chlorophylls, and β-carotenes associated with reaction center II or within the vicinity [44,45]. Cleavage sites on D1 are on the luminal side of the membrane [35,36,46], and hence the characteristic degradation products of the D1 protein are 9-kDa N-terminal and 24-kDa C-terminal fragments [46].

PSI is also affected, and recent studies indicate that the mechanism of oxygen-dependent light inactivation is similar to the acceptor side–induced photoinhibition in PSII. The recombination of the primary charge separation products in PSI leads to the formation of P700 in the triplet state and thus to the production of singlet oxygen [47]. Alternatively, in this photosystem, oxygen can be reduced by reduced ferredoxin to the superoxide radicals through the so-called Mehler reaction [4,7,47]. PSI has stability which is explained in terms of an effective control process associated with this system [48].

Under favorable natural conditions, mild photoinhibition frequently occurs even at light levels below the light saturation. There is an efficient recovery process operating which ensures that mild photoinhibition is restricted to clear and changeable days; photosynthetic efficiency is regained not later than the next morning in the absence of climatic stress [49].

The damage resulting from excess light absorption may also be induced by changes in other environmental factors other than light. Many types of stress causing a decrease in the rate of photosynthesis will lead to an increase in excess photon flux [50]. Plants which are exposed to severe stress have an increased susceptibility to photoinhibition with subsequent photooxidative damage. This is because stresses such as chilling temperature [51,52], mineral deficiencies [53], or gaseous pollutants [54] disturb plant metabolism and often cause a decrease in the capacity for photosynthetic carbon assimilation. Although there are a number of recovery mechanisms for avoidance of photoinhibition, environmental stress may cause a decrease of the photosynthetic activity when the rate of damage exceeds the capacity of these mechanisms and exhausts the adaptation systems. Taken alone, photoinhibition, may serve as a drastic but very efficient strategy to cope with the photooxidative processes in an early stage by reducing the light-driven formation of highly active oxygen species [55].

Photooxidation Processes in Plants

During photosynthesis, in conditions of intensive illumination of the plant cell, the continuous production of oxygen is observed, which creates a potential hazard from the development of a variety of photooxidation processes, including photodestruction of the photosynthetic apparatus. Experimental data propose clear evidence of the oxygen-dependent oxidation character of a whole chain of photodestructive processes taking place in the chloroplasts [56–59].

The double nature of oxygen which on one hand is absolutely necessary for most living organ-

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isms on Earth, but on the other hand is a toxic element for all life forms is due to the structure of its molecule, chemical interactions, and biological functions. Oxygen in its basic or triplet state, $^3O_2$ (or O_2), possesses two single electrons with parallel spins at different orbitals. On excitation, the molecule of oxygen can be transferred into one of two possible singlet states. The first one, ($^1\Delta$), is found 22.5 kcal\mol higher than the basic one; and the second one, ($^1\Sigma$), 37.5 kcal\mol higher. In its first singlet state, oxygen is exclusively reactive owing to its excess of energy and its very long lifetime. In its second singlet state, its lifetime is very short. Chlorophyll is the main generator of the triplet state in the chloroplasts. Its photoexcitation results in the production of triplet molecules of the pigment ^{3}Chl [60–63], which can generate $^1O_2$ under normal conditions and especially well in the absence of carotenoids. Oxidation can also be due to other plant pigments: porphyriens and phlavins [64,65]. Accumulated in plant tissues, the active oxygen forms show a complex influence on the total plant metabolism by inhibition of photosynthesis, inactivation of key enzymes of the oxidation path of specific amino acids, damage of the cell membranes due to the oxidation of the membrane proteins and nonsaturated fatty acids, and finally all reactions lead to the disintegration of the plant tissues [66–69]. Regardless of the damaging role of highly reactive forms of oxygen, under ''ordinary'' circumstances, they are ''normal'' cell metabolites. Probably there is a critical balance between the generation and detoxification of reactive oxygen in the plant cell. During the course of evolution, the chloroplasts have elaborated their own endogenous defense systems which perform either detoxification of active oxygen or stringent control over its production [15,70,71].

ANTIOXIDATIVE SYSTEM

Nonenzymatic Defense

Photoprotective Role of Carotenoids

The essential function of carotenoids is photoprotection. The β-carotene protects the PSII reaction center against photooxidative damage via quenching of singlet oxygen or of the chlorophyll triplet state that sensitizes singlet oxygen formation [72]. It has been shown that β-carotene limits the destructive reactions by scavenging singlet oxygen rather than by trapping the chlorophyll triplet [25]. Chlorophyll triplets are unable to give their spin to β-carotene [22], because they are not situated sufficiently close to each other in the PSII reaction center. If carotenoids were close enough to quench ^{3}P680 efficiently, they would be competing with water as an electron donor to $P680^+$ and thus be efficiently oxidized and rapidly degraded. A likely explanation is that the oxidizing photochemistry in PSII prevents evolution of an efficient quenching system for chlorophyll triplets in the PSII reaction center [73].

$$\mathbf{h\nu}$$
$$\mathrm{Chl} \rightarrow {}^1\mathrm{Chl}^*$$
$$^1\mathrm{Chl}^* \rightarrow {}^3\mathrm{Chl}^*$$

⇓	⇓
$^3\mathrm{Chl}^* + \mathrm{Car} \rightarrow \mathrm{Chl} + {}^3\mathrm{Car}^*$	$^3\mathrm{Chl}^* + O_2 \rightarrow \mathrm{Chl} + {}^1O_2$
$^3\mathrm{Car}^* \rightarrow \mathrm{Car} + \mathrm{heat}$	$^1O_2 + \mathrm{Car} \rightarrow O_2 + {}^3\mathrm{Car}^*$
	$\mathrm{Car}^* \rightarrow \mathrm{Car} + \mathrm{heat}$
Quenching of chlorophyll triplet	Quenching of singlet oxygen

The β-carotene in the PSII reaction centers, together with Chl670, play a protective role and acts as a secondary electron donor to prevent donor-side photoinhibition [74]. Under conditions where their oxidation may be relatively infrequent, both of them could serve to protect against accumulation of $P680^+$. The reaction centers are only photoinactivated after the secondary donors are exhausted, presumably because $P680^+$ can now oxidize the protein matrix.

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Krieger and Weis [75] show that the reaction center quenching is caused by Ca^{2+} release from PSII, when the lumen pH falls below 6. The water-splitting complex is inhibited by Ca^{2+} and electron donation to the oxidized primary donor, $P680^+$, is decreased. The $P680^+$, per se, may be responsible for quenching. This type of quenching is redox sensitive and does not lead to a significant decrease in the F_0 level of chlorophyll *a* (chla) fluorescence.

Xanthophylls are involved in a second type of photoprotective process: regulation of absorbed light energy utilization in the PSII antenna of higher plants and some algae [76]. In this process, generally referred to as an energy- or ΔpH-dependent nonphotochemical chlorophyll quenching (q_E or NPQ), the excess of absorbed light energy in the PSII antenna is dissipated as heat [76]. Therefore, NPQ decreases the efficiency of PSII when the rates of electron transport and carbon metabolism reach saturation at high photon fluxes and favor antenna-based photon protection [16].

Two general types of molecular mechanisms have been proposed to account for NPQ. The first is based on a strong correlation between NPQ and the extent of violaxantin conversion to zeaxantin via the xantophyll cycle [76,77]. The mechanism for the role of zeaxanthin in NPQ has been studied in detail by Owens and coworkers [78]. The participation of the different carotenoids in a definite type of energy transfer depends on their excited state singlet (S_1 and S_2) and triplet energy levels. That a singlet-singlet energy transfer from carotenoids (violaxanthin) to chla originates from the carotenoid S_1 state has been concluded from studies of the light-harvesting function [72].

The NPQ is associated with the conversion of violaxanthin to zeaxanthin that consists of deepoxidation of the epoxide groups forming cyclohexenyl rings, and this results in an increase in the number of conjugated carbon-carbon double bonds from 9 to 11. The key process in this mechanism of NPQ is the conversion of violaxanthin to zeaxanthin that lowers the energy of the carotenoid S_1 state to approximately the same level as the chla S_1 state and introduces a potential new pathway for reversible singlet-singlet energy transfer from chla to zeaxanthin [79] (Fig. 2). Because the total rate of radiative and nonradiative transitions from S_1 to the ground state is more than 2 orders of magnitude larger for carotenoids than for chla-typically 10–40 ps for carotenoids and 5 ns for chla [78], weak quenching centers in the antenna are produced.

$$\mathrm{Car} \xrightarrow{h\nu} {}^1\mathrm{Car}^*$$
$${}^1\mathrm{Car}^* + \mathrm{Chl} \rightarrow \mathrm{Car} + {}^1\mathrm{Chl}^*$$

Singlet-singlet energy transfer from carotenoids to chlorophyll—light-harvesting function

$$\mathrm{Chl} \xrightarrow{h\nu} {}^1\mathrm{Chl}^*$$
$${}^1\mathrm{Chl}^* + \mathrm{Car} \rightarrow \mathrm{Chl} + {}^1\mathrm{Car}^*$$

Singlet-singlet energy transfer from chlorophyll to carotenoids—NPQ via xantophyll cycle

An alternative mechanism associates the increase in NPQ with changes in the aggregation of the light-harvesting complex II (LHCII) in vivo [80]. In the light-harvesting chla/b–binding proteins, chlorophyll molecules, although at close distance, are separated from each other by xanthophyll molecules. These strong antiquenchers prevent close chl-chl interaction and quenching [81,82] and do not interfere with optimal energy transfer. The key process in this way of NPQ is protonation-promoted changes in the protein structure leading to a xanthophyll/chl aggregation and allowing the direct quenching of singlet chl by carotenoids and energy dissipation (Fig. 3). The studies of the dependence of xanthophyll's aggregation on the increasing polarity of ethanol/H_2O mixture show that zeaxanthin has the strongest tendency to aggregate [83].

State transitions can adjust the excitation ratio of PSI and PSII by changing the cross section of antenna chlorophyll for the respective reaction centers depending on the environmental light spectrum [84].

The yield of NPQ is regulated by the size of the pH gradient across the thylakoid membrane [76]. When the lumen pH falls, protonation of specific amino acids on the luminal side of LHCII in the vicinity of zeaxanthin increases the energy transfer between chla and zeaxanthin by modulating

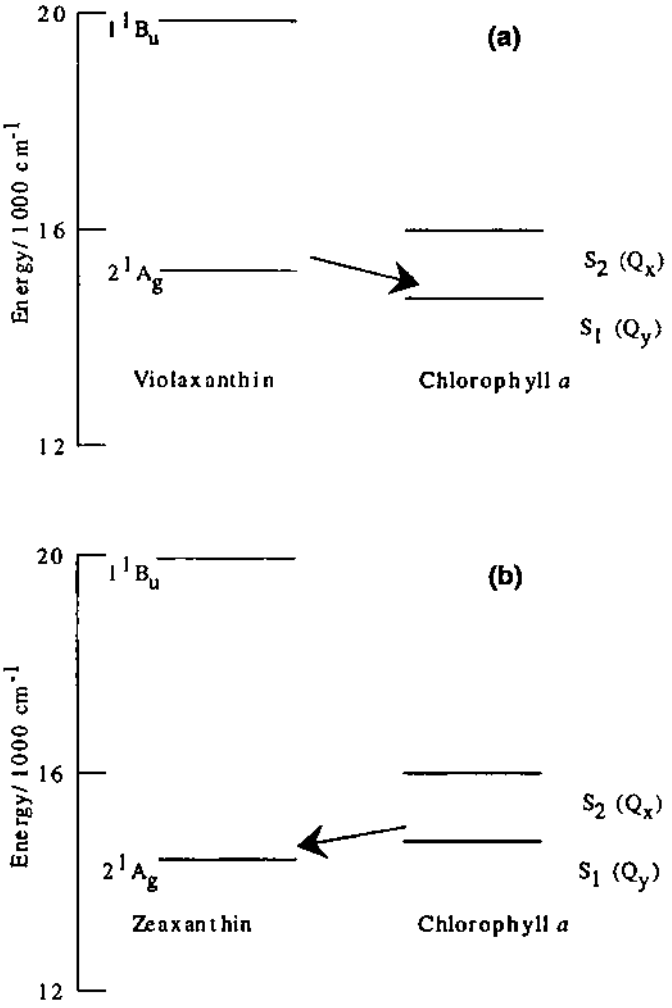

FIGURE 2 Process of (a) light-harvesting by violaxanthin (i.e., forward energy transfer from carotenoid to chlorophyll) and (b) nonphotochemical quenching by zeaxanthin (i.e., reverse energy transfer from chlorophyll to carotenoid). (From Ref. 79.)

the chla-zeaxanthin spectral overlap or the dipol strength of the zeaxanthin $S_1 \rightarrow S_0$ transition [78,85,86]. The low pH could also increase the activity of the violaxanthin deepoxidase located on the luminal face of the thylakoid membrane [87]. At the same time, the differences in ΔpH may also determine the capacity for NPQ by the level of chl/xanthophyll aggregation [88].

Thus, the relationship between the NPQ, xantophyll cycle, LHCII aggregation state, and ΔpH could be highly dependent on the experimental conditions and on the physiology of the sample. Under conditions where NPQ becomes saturated, photoinhibition develops [89].

Photooxidative stress can be also produced by the irradiation of plants in which the biosynthesis of colored carotenoids has been blocked by treatment with bleaching herbicides such as Norflurazon (SAN-9789) [90]. In green plants or dark-grown plants irradiated with high-intensity light, the effect of Norflurazon could be connected to the photoprotective role of carotenoids [91,92] for chlorophyllide-sensitized photodestruction or to their structural role [93,94].

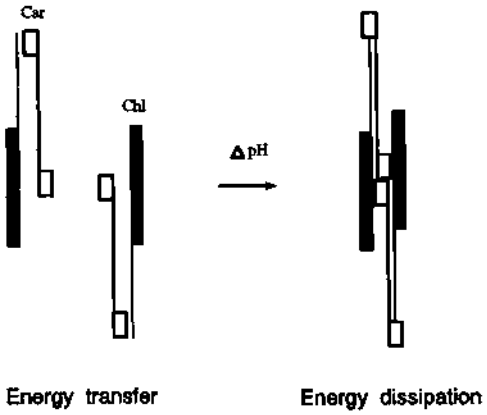

FIGURE 3 A model to explain how quenching may arise from specific interactions between xanthophylls and chlorophylls in LHCII. In the relaxed state, pigment-pigment distances are extended and prevent quenching, but on protonation of amino acid residues, pigment aggregates are formed giving rise to energy dissipation and accounting for absorption changes associated with nonphotochemical quenching. (From Ref. 88.)

On the other hand, our findings show that the total Chl content is higher during the first hours of irradiation (weak red or moderate white light) of Norflurazon-treated dark-grown wheat plants compared with the nontreated plants which is followed by a decrease in chlorophyll due to progressive photodamage (Fig. 4) [95]. The flash irradiation or a brief irradiation with weak light of carotenoid-deficient dark-grown plants causes phototransformation of a greater number of originally accumulated prolochlorophyllide (Pchlide) molecules to chlorophyllide (Chlide), and in that way the chl

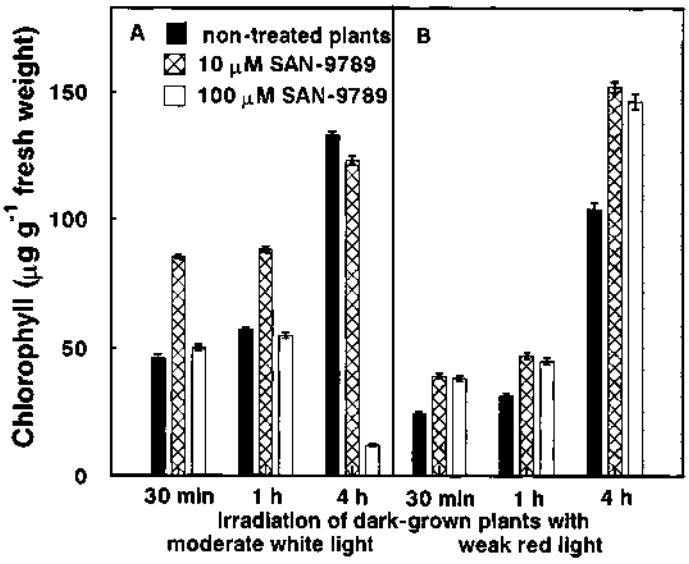

FIGURE 4 Chlorophyll accumulation after irradiation of young dark-grown Norflurazon-treated wheat plants with moderate white (A) or weak red (B) light.

synthesis that stops in darkness can continue more efficiently [96]. These results confirm the statements of Koski and coworkers [97] that carotenoids depress the phototransformation of protochlorophyll to chl in normal etiolated leaves, because they absorb blue light very strongly, and that the lower efficiency of the photochemical action in normal seedlings compared with albino ones can be attributed to the competitive absorption of blue light by carotenoid pigments.

According to the hypothesis of Reinbothe and coworkers [98], an oxygen-insensitive Pchlide oxidoreductase protein (POR) is formed that tightly bounds the protochlorophyllide and used light and NADPH for the reduction of protochlorophyllide to chlorin. In both gymnosperms and angiosperms, POR is active during the transition from dark to light [99] when protochlorophyllide and chlorin must be shielded from interacting with O_2 in the atmosphere. During the early stages of the dark-light transition of etiolated plants, while the newly formed chlorophyllide is still bound to POR, the photoprotection of the Chlide against the O_2 occurs even at a carotenoid deficiency.

High Light–Dependent Protein Synthesis

The early light–inducible proteins (ELIPs) are found in etiolated plants exposed to light, and being expressed in the first hours of the greening process [100,101]. In the greening of dark-grown plants, ELIP transcription is induced by red light, so it is controlled by the phytochrom receptors [102]. Recently, ELIPs were detected in mature green pea plants with strong light-promoted photoinhibition [103,104]. In mature light-grown plants, ELIP transcription is under the control of a criptochrom receptor [103,105], which is induced specifically by strong blue or ultraviolet A (UVA) light.

Sequencing of ELIP cDNA clones of pea [106,107] and barley [101,108] shows that ELIPs are related to the light-harvesting chla/b (cab) gene family. They are synthesized in the cytosol as precursors, imported into the chloroplast, and inserted into the thylakoid membranes after processing [100,101], predominantly in the fraction enriched in PSII complexes [109].

Because ELIPs are induced and stable under light-stress conditions but disappear during the recovery process, it may be inferred that they take part in one of the protective mechanisms against the light damage. There are many experimental results concerning the physiological role of ELIPs, but to a certain extent they are contradictory. Nevertheless, it is possible that ELIPs may act as a carrier or a transient structural component of the LHCII responsible for carotenoid location and/or their protective activity in the reaction center-antenna complexes during light stress [76,110,111]. The other possibility for ELIPs is that they bind the free Chl, generated by the degradation of Chl-protein complexes, such as the PSII reaction center or LHCII during light stress [104,108,112].

Under high-light conditions, there can be overexpression of genes for the synthesis of the D1 protein replacing the photodamaged protein [113] and for scavenging enzymes against photoinhibition [3]. At the same time, high-light stress could limit the expression of the light-harvesting proteins [104] in order to reduce the antenna size for minimum absorption of light quanta.

Other Protective Mechanisms for Avoidance of Photoinhibition

The PSII cyclic electron transport involving cytochrome b_{559} can also serve as a mechanism to protect the reaction centers from damage caused by light with high intensity [43,114,115]. According to the model of Barber and De Las Rivas [116], the cytochrome b_{559}, which exists in two forms, may also be involved in a regulation of photoinhibition. The high-potential form protects against donor-side photoinhibition by acting as an electron donor to the oxidizing side of PSII. The low-potential form protects against acceptor-side photoinhibition by acting as an electron receptor from reduced pheophytin.

The cyclic electron flow around PSI can adjust the ratio of NADPH to ATP production depending on the requirement of the stromal biosynthetic reactions, which also promotes the photon-utilizing capacity as a whole [117–119]. Photorespiration supplies carbon dioxide and other indirect photon energy acceptors (ADP, NADP) for leaf cells by consuming photosynthetic products resulting in an increase in the photon-utilizing capacity [120].

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Enzymatic Defense

Plants generate active oxygen species even under nonstressful conditions [2]. They have developed protective systems to suppress the production of active molecules and for removal of AOS [1]. These protective systems can be grouped into two categories: enzymatic and nonenzymatic defenses [121]. The enzymatic defense system involves a series of enzymes for detoxification of AOS. The plants usually respond to stress conditions by an increase in the activity of certain protective enzymes, as is the case with Norflurazon-treated plants (Fig. 5).

The investigations of this area start with the discovery by McCord and Fridovich [122] that a copper-containing protein from bovine serum can dismutate two superoxide anion radicals to hydrogen reoxide and molecular oxygen. McCord and Fridovich called the enzyme superoxide dismutase (SOD). The SOD localized in chloroplasts, together with the ascorbate-specific peroxidase (APX), monodehydroascorbate reductase (MDAR), dehydroascorbat reductase (DHAR), and glutathion reductase (GR), can fully reduce the toxic superoxide radicals to water [121]. According to this scheme, SOD catalyzes the dismutation of superoxide into hydrogen peroxide and oxygen at diffusion controlled rates. The ascorbate peroxidase, both stromal and thylakoid-bound forms, reduces H_2O_2 to water via monovalent oxidation of ascorbate to monodehydroascorbate (MDA) [123]. The ascorbate is regenerated by the ascorbate-glutathione cycle using electrons from PSI and including several parallel reactions:

The MDA produced by the thylakoid-bound APX is reduced by ferredoxin [123].

The stromal MDAR catalyzes the reduction of MDA by NADPH. The MDA can dissociate into ascorbate and dehydroascorbate and stromal DAR, using glutathione as an electron donor, reduces dehydroascorbate to ascorbate. Glutathione is regenerated by GR with NADPH as an electron donor [9].

Superoxide Dismutases

Superoxide dismutases (EC 1.15.1.1) are metal-containing enzymes that catalyze the dismutation reaction of two superoxide anions to oxygen and hydrogen peroxide [122,124]. On the basis of their metal cofactors, three different isoforms can be distinguished: the copper and zinc (Cu/Zn) form, manganese (Mn) form, and iron (Fe) form.

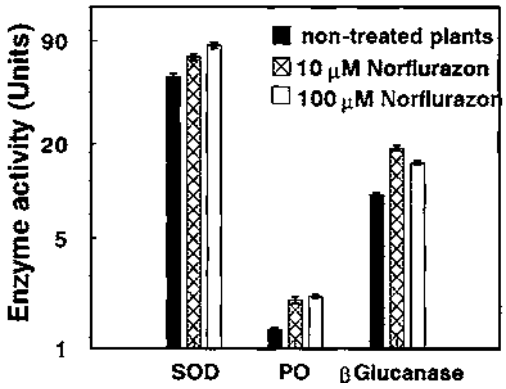

FIGURE 5 Enhanced activity of certain protective enzymes, superoxide dismutase (SOD), peroxidase (PO), and β-(1,3) glucanase in young light-grown wheat plants treated with Norflurazon (unpublished data).

Mn- and Fe-SOD are structurally related: In some bacteria, the metal cofactors can be exchanged without loss of activity. The Cu/Zn-SOD is structurally unrelated to other SODs [125].

Plants usually contain all three SOD forms [126]. The Mn-SOD is typical for mitochondria and the cytosol contains Cu/Zn-SOD [126]. Chloroplasts contain generally Cu/Zn-SOD. A chloroplastic Fe-SOD has been recently found in many plant families [55,126,127]. Plastidic Cu/Zn-SOD and Fe-SOD are expressed differentially in *Nicotiana plumbaginifolia* and they probably complement each other in action [128]. The preferential expression of Cu/Zn-SOD or Fe-SOD can depend on the plant species, tissue, stage of development, or environmental conditions [55].

The subcellular localization of SOD and superoxide radical production often coincides. Since superoxide radicals are mainly produced at the electron transport chains of chloroplast, of mitochondria, and in the endoplasmatic reticulum, most of SOD activity is localized in these compartments [2].

Mn-SOD and Cu/Zn-SOD have also been found in peroxisomes of pea leaves and watermelon (*Citrullus vulgaris* Schard). It is suggested that Cu/Zn-SOD occurs in the matrix, whereas Mn-SOD is bound at the external side of peroxisomal membrane [129]. There is also evidence of the existence of SOD in the extracellular space [130].

The expression analysis in several plants show that *Sod* genes are differentially regulated throughout development and respond in a different way to environmental stress [131]:

- In *N. plumbaginifolia*, expression of cytosolic Cu/Zn-SOD (*SodCc*) mRNA is enhanced most by heat shock, chilling, or paraquat treatment in the dark in contrast to Fe-SOD (SodB), in which expression is induced mostly by chilling and paraquat treatment in the light [132].
- The expression in tomato of both the choroplastic and cytosolic Cu/Zn-SOD genes (*SodCp* and *SodCc*) is stimulated by paraquat treatment and light, but only the cytosolic isoform shows induction of expression after exposure to drought [133].
- Different members of the maize Mn-SOD gene family (*SodA*) are differentially expressed during the development and pattern of Mn-SOD mRNA accumulation. Accumulation generally depends on mitochondrial activity during plant growth [134].

The large increase of SOD transcription on stress treatment generally correlates with a much more moderate increase in SOD activity. Possibly stress causes a more rapid turnover of SOD proteins, thereby necessitating activation of gene expression to maintain SOD levels [131].

Different stress conditions might lead to a different extent of oxidative stress in the various subcellular compartments, necessitating the expression of those *Sod* genes encoding the SOD isoform(s) needed to protect a particular compartment. The nature of the expression signals is still unclear [131,132]. However, the opportunity to increase the stress tolerance of crops via overexpression of superoxide dismutase and other antioxidant enzymes is a rather tempting issue and recently a large number of laboratories are working in that direction.

A chimeric gene, containing a full length cDNA of chloroplast Cu/Zn-SOD under the control of the Rubisco ssu promotor from *Petunia*, is overexpressed in tobacco and tomato. Fifteen independent transformants of tobacco have been obtained, which according to the data express from total leaf extracts 30- to 50-fold more SOD [135]. Some experiments show 30-fold increase of chloroplastic SOD activity, but this percentage decreases with leaf age [136]. However, such a massive overexpression does not protect CO_2 assimilation, the PSII reaction center or the light-harvesting pigments against methyl viologen [135] or against ozone [137], and chilling-induced photoinhibition [135]. In contrast, overproduction of Mn-SOD in tobacco chloroplasts increases the resistance against paraquat [138].

A chimeric gene consisting of the coding sequence for chloroplast Fe-SOD from *Arabidopsis thaliana* coupled to the chloroplast-targeting sequence from pea Rubisco ssu is expressed in tobacco [139]. Expression of the transgenic Fe-SOD protects both the plasmalemma and PSII against superoxide generated during illumination of leaf disks impregnated with methyl viologen [139]. In contrast, the overproduction of mitochondrial Mn-SOD form *Nicotiana plumbaginifolia* in the tobacco

cv SK1 chloroplasts protects only plasmalemma but not PSII against methyl viologen [136]. The possible reason might be the different membrane affinities of the transgenic Fe-SOD and Mn-SOD [139].

Ascorbate Peroxidase

The ascorbate peroxidase (APX, EC 1.11.1.7) belongs to the group of heme peroxidases. The enzyme catalyzes the oxidation of ascorbate by hydrogen peroxide. Similar to other heme peroxidases, APX is oxidized by hydrogen peroxide to the intermediate compound I. Reduction to the oxyferryl heme and the occurrence of tryptophan radicals near the heme show an analogy to the cytochrome *c* peroxidases [1,140]. Compound I then univalently oxidizes ascorbate forming compound II and the monodehydroascorbate radical (MDA). The interaction of compound II with another ascorbate molecule produces a second MDA [1].

$$2 \text{ Ascorbate} + H_2O_2 \rightarrow 2 \text{ MDA} + 2H_2O$$

Based on their subcellular localization, the four types of APX are the chloroplast stromal soluble form (sAPX), chloroplast thylakoid-bound form (tAPX), cytosolic form (cAPX), and glyoxisome membrane form (gmAPX) [1,141–143]. APX is inactivated in the absence of an electron donor, especially in the case for sAPX and tAPX, whose half-inactivation time is only 15 s [123]. The inactivation is caused by a rapid degradation of the intermediate compound I. Since the stroma thylakoids are enriched in PSI complexes, the tAPX is bound close to the sites of superoxide production [1]. This means that the generated H_2O_2 is scavenged primarily by tAPX at the thylakoid level, whereas the sAPX represents a second level of defense against hydrogen peroxide which escaped the tAPX [144].

The comparison of the molecular properties shows a high degree of homology in the amino acid sequence of the N-terminal of tAPX and sAPX but not with the same region of cAPX [144]. The molecular mass of tAPX is 10 kDa higher than that of cAPX and sAPX. The additional size of tAPX might contribute to its binding to the thylakoid membranes [1,144]. The gmAPX is recognized as 31-kDa polypeptide with a single, membrane-spanning region near the C-terminal [142].

Genes encoding the four types of APX have already been cloned [131,142]. Apx gene expression is rapidly induced by various stress conditions, such as paraquat, ethylene, drought, and heat shock. It would suggest an important role for APX in stress tolerance [145]. A marked discrepancy is found between the increase in Apx steady-state transcript abundance, which is relatively large, and the increase in APX protein activities, which is relatively small. It appears that the high level of Apx transcript observed in response to stress is restricted from interaction with polysomes [146,147]. It has been shown that a three-fold increase in cAPX activity and APX mRNA occurs in transgenic tobacco plants with overexpressed chloroplast Cu/Zn-SOD. Similar data have been reported about chloroplast APX activity in transgenic tobacco overexpressing Fe-SOD [136]. It has been concluded on the basis of these results that the increased level of the gene product in one part of the antioxidant pathway can affect other enzymes in the enzyme defense system [147].

Enzyme Systems Involved in Regeneration of Ascorbate

For the operation of hydrogen peroxide–scavenging system in chloroplasts, the regeneration of ascorbate from monodehydroascorbate (MDA) and/or dehydroascorbate (DHA) is indispensable [1]. As it is mentioned above, the MDA produced by the tAPX is reduced by ferredoxin [148].

The reduction of the stromal MDA is catalyzed by a FAD enzyme called MDA-reductase (MDAR, EC 1.6.5.4). The enzyme operates with NAD(P)H as a electron donor. The NAD(P)H reduces the enzyme FAD to $FADH_2$ and then the enzyme donates electrons to MDA through two one-electron transfers with FAD semiquinone as an intermediate [1].

When MDA is not reduced to ascorbate either by reduced ferredoxine or by MDAR, DHA will be produced by disproportion of two molecules of MDA to ascorbate and DHA. The regeneration of

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DHA to ascorbate is catalyzed by the thiol enzyme DHA-reductase (DHAR). The DHAR is localized in the chloroplast stroma and operates with glutathione as an electron donor. Since the predominant amount of MDA is directly reduced to ascorbate, the contribution of DHAR and glutathione is a minor part of the regeneration of ascorbate [1].

Recently, a full-length cDNA clone encoding MDAR was isolated from cucumber and pea [149,150]. The DHAR is purified from spinach chloroplast. It has been found that the amino-terminal sequence of DHAR is similar to the Kunitz-type trypsine inhibitors [151]. The DHAR and soybean trypsine inhibitor are both capable of reducing dehydroascorbate when in reduced form and acquiring trypsine-inhibiting activity in the oxidized form [151].

Glutathione (γ-glutamyl-cysteinyl glycine, GSH) is the major low molecular weight thiol compound in most plants. Many of the important metabolic regulatory and antioxidative roles of glutathione result in its oxidation to glutathione disulfide. For most of its functions, glutathione must be in the reduced form [152]. The reduction of GSH is carried out by the flavoprotein oxidoreductase glutathione reductase (GR, EC 1.6.4.2). The GR operates with NADPH as an electron donor [9].

Glutathione reductase has been studied in a wide range of plants. Most of the GR activity in plants cells is present in the chloroplasts [152]. It has also been found that in pea leaves 77% of total GR activity is localized in the chloroplasts, only 3% in the mitohondria, and the rest is cytosolic [153]. In pea leaves, GR is a protein with a molecular weight of 55 kDa. However, eight different isoforms have been detected by two-dimensional isoelectric electrophoresis. It is not possible to conclude from the biochemical data whether GR isoforms represent different gene production or arise as a result of posttranscriptional or posttranslational modification [152].

The full-length cDNA encoding GR from pea and tobacco has been isolated and extensively studied. The pea and tobacco GR cDNA shares 78% homology at the nucleotide level. Both plant GR sequences contain an amino-terminal extension, which is probably a chloroplast transit peptide. In addition, they have similar C-terminal extensions rich in alanine, lisine, and serine, which suggests that this domain probably has a function as an additional signal sequence [152].

The GR activity is also influenced by various environmental factors known to increase oxy radical formations like gaseous pollutants, extreme temperatures, senescence, and pathogen attack [152]. The combination of high light with magnesium deficiency, which would be expected to cause photooxidative damage in the chloroplasts, increases the GR activity by seven times [154].

There have also been several experiments done with GR overexpression in transgenic plants. The GR from *Escherichia coli* is overexpressed in the cytosol of tobacco [155,156] and in the chloroplasts [157], with the level of GR activities of transgenic plants being between 1.4 and 4.0 times higher. It has been found that GR overexpression does not protect CO_2 assimilation after gaseous fumigation, but a decrease of sensitivity to methyl viologen has been found [155,157].

Catalase and Other Enzyme Systems Involved in Plant Oxidative Defense

The overexpression of GR could increase the reduction of the glutathione pool under stress conditions. The existing data suggest that glutathione may have a pivotal role in the defense against oxidative stress [158].

Glutathione can serve as a substrate for glutathione-S-transferase. This enzyme can detoxify the products of lipid peroxidation and xenobiotics. It also shows peroxidase activity [159,160].

One recently proposed mode of action is the role of GHA as a substrate of glutathione peroxidase (GPX, EC 1.11.1.9). The glutathione peroxidases are a family of multiple isoenzymes which catalyzes the reduction of H_2O_2, organic hyperoxides, and lipid hyperoxides by reduced glutathione and thus help to protect cells against oxidative damages [161,162]. They are known as an antioxidative defense enzymes in animals, but there are indications that they also exist in plants. Genes with significant sequence homology to one member of the animal GPX family, namely, phospholipid hyperoxide glutathione peroxidase, have been isolated from several plants. So far, only one protein,

Cit-SAP, a product of the citrus *csa* gene, which is induced by salt stress, has been isolated and characterized. This protein differs from the animal enzyme in its rate of enzymatic activity and in containing cysteine instead of selenocysteine. The physiological role of Cit-SAP and its homologues in other plants is not yet known [163].

The plants eliminate H_2O_2 by catalases and peroxidases. The metalloenzyme catalase is one of the most efficient proteins in the redox reaction:

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$$

At present, three differentially regulated catalyses have been revealed. In *Nicotiana plumbaginifolia* the *Cat1* gene product plays a role in the removal of photorespiratory H_2O_2. The expression pattern of the *Cat3* gene suggests that the encoded protein has a role in scavenging of H_2O_2 generated by the β-oxidation of fatty acids in the glyoxisomes. The *Cat2* product most likely has a specific role in protecting the cell from H_2O_2 produced during the oxidative stress [131]. Exposure of plants to ozone, sulfur dioxide, and UVB radiation all lead to a rapid decline in *Cat1* steady-state transcript levels and a concomitant rapid increase in *Cat2* transcript levels [164]. The control mechanisms are still unclear, but a number of evidence supports the idea that the balance between catalyase expression and hydrogen peroxide production play a central role in the tolerance of plants to different types of stress [131].

PHOTODYNAMIC HERBICIDES

Long before Rebeiz [165] announced the discovery of a new class of herbicides, called photodynamic, or ''laser,'' herbicides, it was known that exogenous δ-aminolevulinic acid (ALA) causes accumulation of large amounts of chlorophyll precursors in the dark [166]. The action of the photodynamic herbicides is due to the accumulation of protoporphirin IX (Proto), Mg-protoporphyrin(ester) (Mg-Proto[E]), and protochlorophyllide (Pchlide) in exclusively large amounts in plants kept in darkness. Light is a specific factor which determines the rate of damage of the plants treated with photodynamic herbicides in darkness. After exposure to high-light intensities, such plants exhibit oxidation-destruction processes due to the photosensibilization caused by porphyrins, usually resulting in the death of the plant [165,167–175]. It is well known that porphyrins act as effective sensibilizors of the photooxidation processes leading to damage of living systems due to the action of light in the presence of oxygen [176–182]. First Granic [176] and later other researchers [183,184] have also shown that the irradiation of etiolated plants which have accumulated exclusively large amounts of chlorophyll precursors from exogenous ALA leads to the death of the plant cells.

Some diphenyl ethers can also act as photodynamic herbicides [185–189], since they cause an accumulation of exclusively high levels of Proto in plant tissues [168,187–191], which is most probably the mechanism of their photodestructive action. As a whole, the tetrapirol-dependent photodynamic herbicides are compounds which force green plants to accumulate unnecessary amounts of metabolite precursors of the biosynthetic pathways of heme and chlorophyll. In light, the accumulated tetrapirols photosensibilize the formation of singlet oxygen, and the latter kills the treated plants by the oxidation of their cell membranes.

The compounds which can induce the formation of porphyrins [192] in the treated plant tissues are usually called modulators of the chlorophyll biosynthesis [165]. Tetrapirol-dependent photodynamic herbicides are usually composed of ALA, the precursor of all tetrapirols in plant and animal tissues, and one of the modulators of the chlorophyll biosynthesis such as, for example, 2,2′-dipyridyl and 10-phenantroline. The ALA and the modulator act simultaneously. The amino acid is the precursor of damaging tetrapirols, whereas the modulator increases their effect [165,171]. If plants are treated with ALA in the beginning of the dark phase, in dark there is an accumulation of porphyrins, photodynamically active protochlorophyllide and other photoactive compounds. These precursors are not part of the light-collecting complex and are not able to transform the excitation to the

reactive centers of photosynthesis [171]. During the next light period, the formed tetrapirols act as effective photosensibilizators leading in their turn to the death of the sensitive plants [165,193]. Oxidation-destructive changes take place in the plant cell. Transformed into the excited triplet state, the accumulated porphyrins interact with oxygen and form oxygen-containing products with a destructive effect [185].

Our investigations in this respect show that the photodynamic light stress is caused by the tetrapyrrole precursors of Chl synthesis. One of the most important precursors in this respect may be Pchlide. There are data which suggest that the accumulation of superoptimal amounts of the monovinil- and divinil-Pchlide forms are the most important cause of cell destruction in certain plant types [165,194,195]. Other precursors, for example, Proto-IX, also could be accumulated under the influence of photodynamic combinations [182,190]. Our investigations show an accumulation of both those precursors (Figs. 6 and 7), although in different amounts in the monocotyledons and dicotyledons. The proposed mechanisms of action of the precursors [196] suggest that there might be no direct connection between the amount of the accumulated precursors and the photodynamic effect. The photodestructive effect of the precursors strongly depends on the plant type (monocotyledons or dicotyledons) and not entirely on the Pchlide and Proto levels (Figs. 6 and 7). At the same time, the species-dependent variations in the accumulation of Proto, as well as the differences between the plant tolerances, are the critical factors in defining the photodynamic effect in plants [179]. This applies particularly to the changes in the Proto content which may be in direct correlation with the photoherbicide effect in the sensitive plants.

In treatment with acifluoren, the photoherbicide action correlates with the Proto level in cucumber leaves [190]. However, it is known that the Proto is not photostable in vivo and it is photooxidized by singlet oxygen which it generates in the presence of molecular oxygen and light. Proto photodestruction is faster in vivo, because molecular oxygen is dissolved easily in the lipids of the membranes, than in water, and Proto is a lipophilic molecule [196]. In addition, the singlet oxygen initiates a rapid build-up of lipid peroxides, which leads to a sequence of peroxidase reactions which might play a role in the oxidative photodestruction of Proto in vivo [196]. This means that the

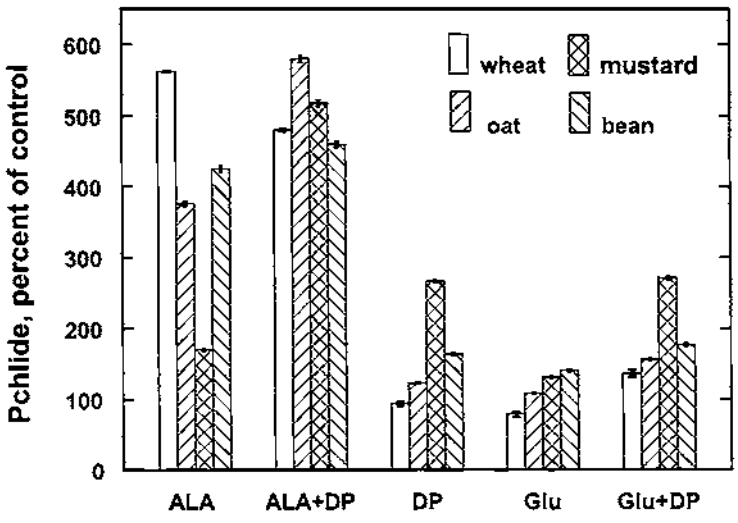

Figure 6 Effect of 2,2′-dipyridyl (2,2′-DP), glutamic acid (Glu), and δ-aminolevulinic acid (ALA) and their combinations on the protochlorophyllide (Pchlide) content in green plants darkened for 17 h after treatment.

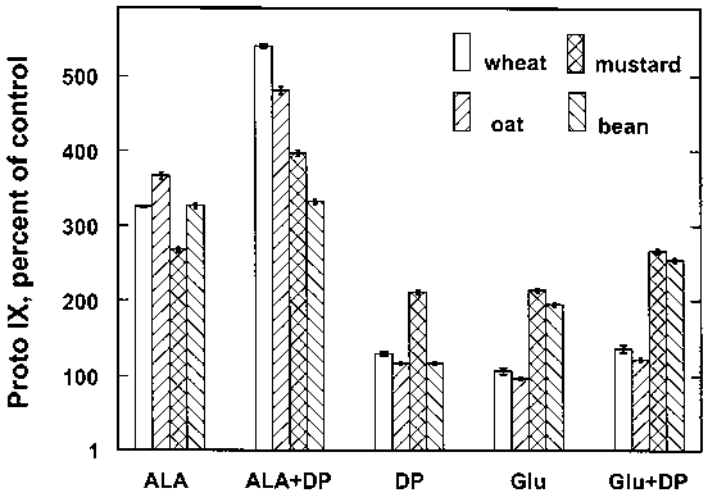

FIGURE 7 Influence of 2,2′-dipyridyl (2,2′-DP), glutamic acid (Glu), and δ-aminolevulinic acid (ALA) and their combinations on the protoporphyrine IX (Proto) content in green plants darkened for 17 h after treatment.

photodestruction is not necessarily always connected with the Proto level (Fig. 6 and Table 1). This might be the reason for the higher resistance of some monocotyledonous plants to the Chl precursors which have accumulated in darkness. However, there is no visual photodynamic damage in monocotyledonous plants (oat and wheat) and no visible destruction of the plants. Despite the differences in the precursor levels (see Figs. 6 and 7) in the treated plants, they do not differ from the controls neither in their appearance nor in their leaf color. This difference might be a consequence of the biochemical heterogeneity of the Chl biosynthetic pathway, as well as the different ways of greening of different plant groups. The experiments support the finding of Rebeiz et al. [197] that show despite the increased levels of Pchlide and Proto, there are no visible signs of destruction in monocotyledonous plants (wheat and oat). This is most probably due to the fact that these plants belong to the greening group D MV/L DV [171]. The accumulation of tetrapyrroles in the treated plants may be considered necessary, but not the only condition for the specific photodynamic sensitivity and

TABLE 1 Photodynamic Herbicidal Damage (%) of Various Plant Species to δ-Aminolevulinic Acid, glutamic Acid, and 2,2′-Dipyridyl

Early precursors and modulator of Chl synthesis	Photodynamic damage of the leaves (%)			
	wheat	oat	mustard	bean
Control	0	0	0	0
δ-Aminolevulinic acid	0	0	70	5
δ-Aminolevulinic acid and 2,2′-dipyridyl	0	0	100	30
2,2′-dipyridyl	0	0	70	10
Glutamic acid	0	0	100	15
Glutamic acid and 2,2′-dipyridyl	0	0	100	20

Destruction was determined visually according to Ref. 165.

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photodynamic damages of the different plant species [171,198]. The level of this destruction depends most probably on the amount of accumulated tetrapyrroles, the greening group of the plants, as well as the chemical nature of the synthesized precursors. For example, it is known that mustard plants belong to the D DV/L DV greening group [197]. Under the treatment of such a plant with ALA and 2,2′-DP, the plants are forced to synthesize the wrong tetrapyrrole type (MV) in darkness [197], which makes them sensitive and leads to their destruction.

Concerning the phytolized pigments, all the investigated species mainly accumulate higher amounts of Chlb and less Chla (Fig. 8a–d). This result is rather surprising, since it is well known that Chlb is synthesized through a biosynthetic pathway from Chla [199]. There are also some contradictory data for greening plants showing a higher sensitivity of Chlb if compared with Chla [200]. A negative correlation between the level of the precursors, especially Proto IX, and the chlorophylls, is found in other plant species like *Amaranthus retroflexus* and *Abutilon theophrasti* Medic. that have been treated with acifluoren [190].

The protein content is relatively stable in all investigated plants. This could mean that the destruction of plants is a specific photodynamic effect and it is not a result of an inhibition of protein synthesis or a toxic effect on other processes connected with the protein synthesis. It might also influence the Chla\b–binding proteins (LHCP), presumably through the inhibited transport of the

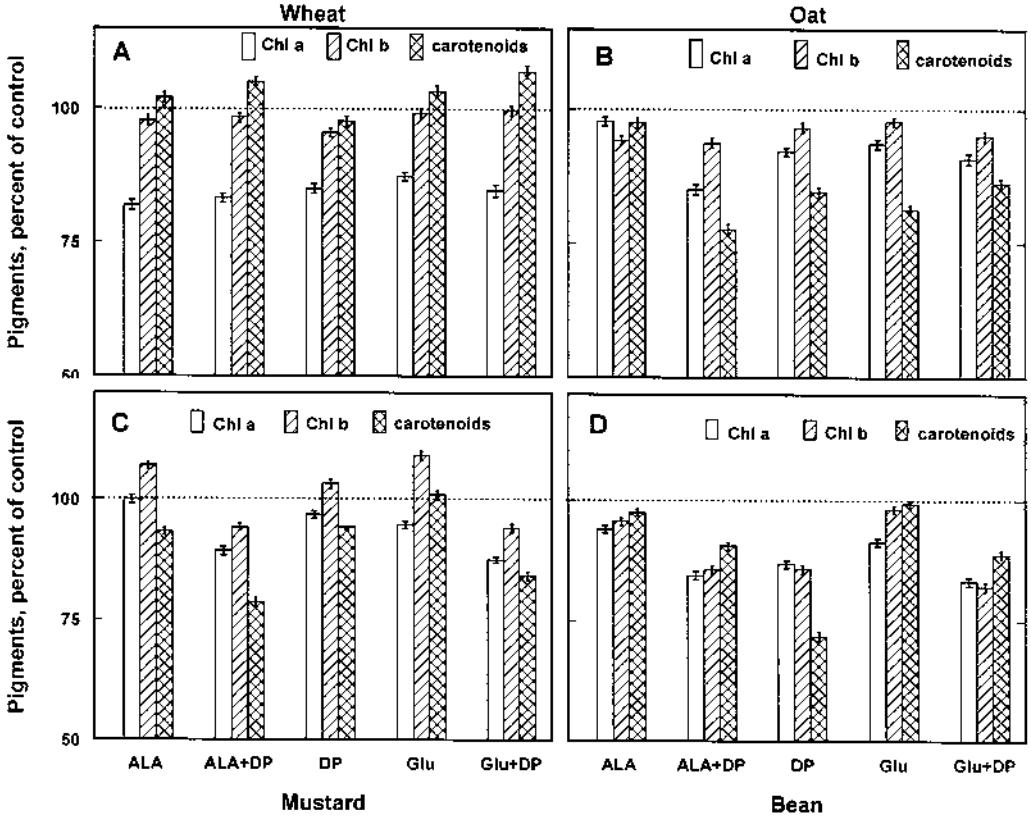

FIGURE 8 Changes in the content of the chlorophyll *a* (Chla), chlorophyll *b*(Chlb), and carotenoids (Car) in green plants of (a) wheat, (b) oat, (c) mustard, and (d) bean plants darkened for 17 h after treatment.

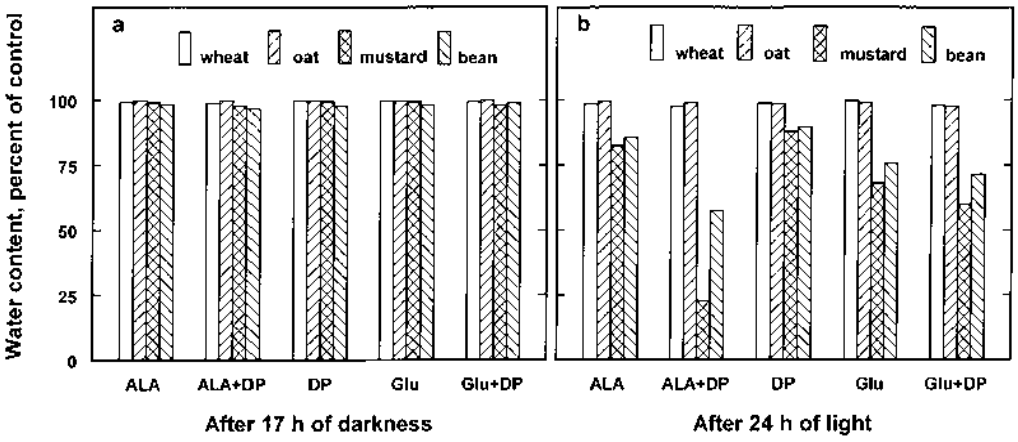

FIGURE 9 Changes in the water content of the leaves of the investigated plants immediately after the dark incubation (a) and after irradiation for 24 h (b).

protein in the plastids [201]. The Glu and 2,2′-DP does not influence the total protein amount which makes the combinations we used a nontoxic photoherbicide.

The changes in the water content in the treated plants (Fig. 9a,b) correlate very closely with the well-known effect of the combination ALA and 2,2′-DP, which is associated with the membrane's destruction and damaging of the selective permeability of the plant cell [165,194].

Our investigations characterize the dicotyledonous plants bean and mustard as being highly sensitive to the treatment with substances that cause the accumulation of Chl precursors, such ALA and Glu, in combination with 2,2′-DP [202]. In comparison, wheat and oat are more resistant, which might also be connected with the different permeabilities of the leaves owing to the differences in their morphological structures. The biochemical mechanism of the different sensitivity is mostly associated with the diverse rate of tetrapyrrole destruction in the cell, as well as with the specific acceleration of the synthesis of monovinyl and divinyl tetrapyrroles induced by different exogenous substances. Generally, concerning the photodamaging effect, the influence of a combination of Glu and 2,2′-DP was relatively close to the action of the better-known effect of ALA and 2,2′-DP but was a little less active when they were compared.

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23

Photosynthetic Pigment Metabolism in Plants During Stress

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INTRODUCTION

When we started to write this chapter on photosynthetic pigment degradations and transformations under stress, we had to face up to an essential question: What is the definition of a stress? After consulting a dictionary and several textbooks on plant physiology, we decided to adopt the following definition: ''A stress is an adverse force or influence that tends to inhibit normal systems from functioning'' [1]. Complementary precisions have been brought by Lichtenthaler [2] on the stress concept in plants.

Hendry et al. [3] estimated that around 10^9 tons of chlorophyll (Chl) are broken down annually worldwide. This estimation took into account degradations occurring during autumn, those resulting from human activities, and those involved in daily pigment variations.

As the daily pigment turnover concerns a small proportion of molecules at a defined moment, investigations are especially difficult in green leaves. For this reason, experiments on pigment catabolism were principally conducted on senescent tissues (e.g., leaf and cotyledon during seed degreening and fruit ripening) where the totality of the pigments is transformed. Pigment transformations occurring in these tissues can be considered as part of the normal developmental sequence, but in respect to the stress definition adopted, they can also be regarded as the result of various simultaneous stresses (e.g., temperature, nutrient, light).

In the first part of this chapter, we present the main steps of the catabolism pathways of Chl and carotenoids as established with senescent tissues. In the second part, we describe pigment modifications occurring as a response to various stresses. Owing to the limitation of space, only an overview of these changes is given here.

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CATABOLIC PATHWAYS

Chlorophylls

Since Hendry's review in 1987 [3], entitled ''The chlorophyll degradation: a biological enigma,'' significant progress has been reported (reviewed in Ref. 4). Although a general scenario for the Chl degradation pathway has been proposed [4], the existence of some intermediates remains only speculative. Moreover, it cannot be excluded that several pathways are involved in Chl degradation or (co)exist depending on the plant species and the stress conditions. It also remains to be determined whether Chl degradation intermediates occurring during leaf yellowing and seed degreening are similar to that involved during daily Chl turnover.

The first steps of Chl degradation pathway consist of dephytylation and demetallation to give their chlorophyllides (Chlide) and pheophorbides (Pheoide), respectively [8–9] (Fig. 1).* The order of the reactions was reported to vary from organ to organ and also from species to species [8]. Enzymes removing the phytol moiety and the central Mg atom from Chl have been described. The former is called chlorophyllase (EC 3.1.1.14; reviewed in Ref. 10) and the latter Mg-dechelatase [11].

In *C. protothecoides* and *Chenopodium album*, Pheoide is 13^2-demethylated prior to the tetrapyrrole ring cleavage [12–13], whereas in *Brassica napus*, the reaction occurs later [14] (Fig. 2). A pheophorbidase catalyzing this reaction has been recently isolated from *C. album* leaves [14]. The subsequent decarboxylation of the 13^2-carboxy-PPheoide to pyropheophorbide (PPheoide, 13^2-demethoxycarbonyl-PPheoide) is a nonenzymatic step which was observed under *C. protothecoides* culture acidification. PPheoide production can also result from a preparation artifact [12–13]. Then the 13^2-methylcarboxy- or 13^2-carboxy-PPheoide ring is oxidized between C4 and C5 by one oxygen molecule through the catalytic action of a monooxygenase without any carbon loss [15]. This contrasts with heme oxidation which requires three oxygen molecules with loss of one carbon [4]. In higher plants, the monooxygenase activity requires reduced ferredoxin as a reductant [16]. A putative mechanism for the reaction has been proposed by Gossauer and Engel [4]. The chlorophyllase and the monooxygenase were found associated with the chloroplast and gerontoplast envelopes [17,18]. To be comprehensive, we must add that several other enzymatic activities have been reported to be involved in the oxygen-dependent Chl breakdown (e.g., peroxidase [19] lipoxidase [20], Chl oxidase [21]). However, their products are not related to those obtained with monooxygenase, which have been identified as the common structural motif in the Chl-degradation product pathways.**

Thus, in the green unicellular alga *C. protothecoides* and under the monooxygenase activity, (13^2-carboxy)-PPheoide is transformed to (13^2-carboxy)-10,22-dihydro-4,5-dioxo-4,5-seco-PPheoide, which is excreted in the culture medium. There either it isomerizes to (13^2-carboxy)-*15(E)*-10,22-dihydro-4,5-dioxo-4,5-seco-PPheoide or it is transformed to a yellow pigment (13^2-carboxylate)-10,15,22,24-tetrahydro-4,5-dioxo-4,5-seco-PPheoide [12] (Fig. 3) which probably constitutes the endproduct of chlorophyll degradation in this organism. In *B. napus* cotyledons and in barley leaves, Pheoide is transformed by the mononooxygenase to 13^2-methylcarboxyl-10,20-dihydro-4,5-seco-4,5-dioxo-22H-PPheoide (also called *Bn*-FCC-2) [6]. The 13^2-methylcarboxyl-10,20-dihydro-4,5-dioxo-4,5-seco-22H-PPheoide isolated in *C. protothecoides* would be an intermediate

* Catabolites from Chla and Chlb have been found in *Chlorella protothecoides* [5]. The Chlb catabolite intermediates reported are similar to that of Chla except that the 7-methyl residue of Chla is replaced by a 7-formyl residue in Chlb. Consequently, here Chl means Chla and Chlb for *Chlorella* and only Chla in the other cases, since in higher plants, only catabolites derivating from Chla have been described [6]. Interestingly, it was recently reported that Chlb can be enzymatically transformed to Chla [7] in higher plants.

** Until recently, both Chlide *a* esterification by geranylgeraniol pyrophosphate and removal of the phytol moeity were ascribed to chlorophyllase. Although enzymes responsible for these two activities are not yet purified, it seems more likely that the two reactions are catalyzed by two different enzymes.

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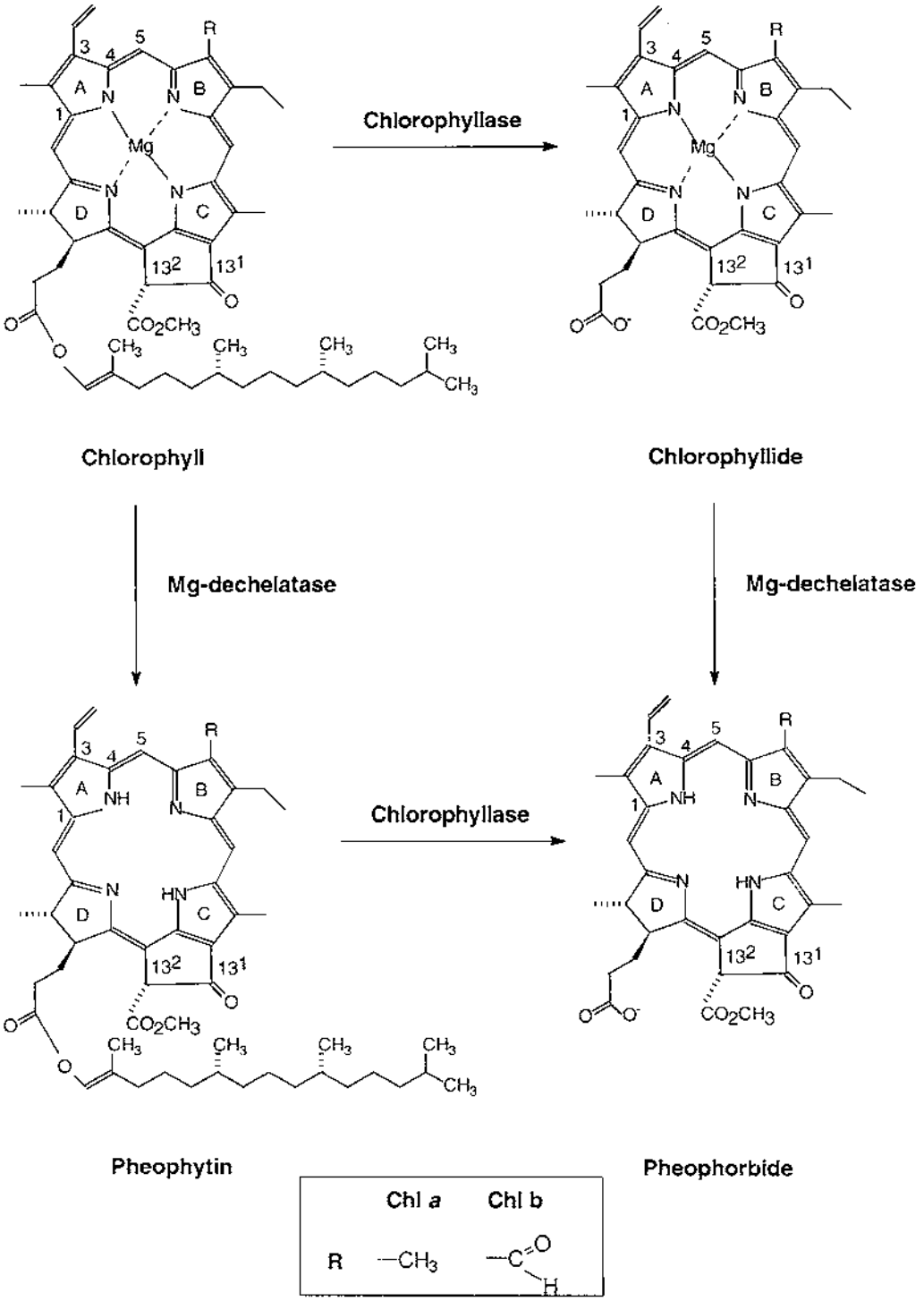

FIGURE 1 Reaction scheme of Chl transformation to Pheoide.

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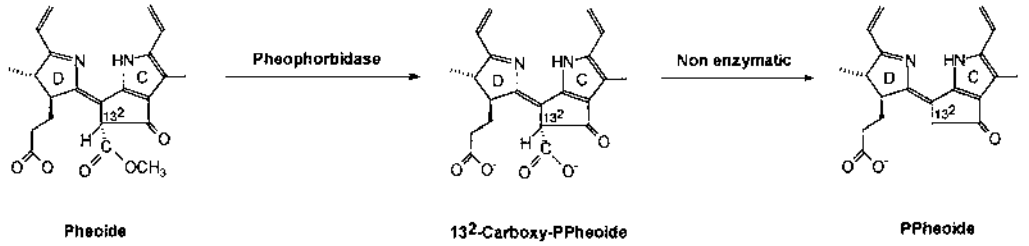

FIGURE 2 Reaction scheme of Pheoide to carboxy-PPheoide and PPheoide.

(not shown in Fig. 4). *Bn*-FCC-2 is a nongreen pigment [9] whose formation is light-dependent and inhibited by 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), an inhibitor of photosynthetic electron transport. This finding strongly suggests that *Bn*-FCC-2 formation utilizes photosynthetic ATP inside chloroplasts [22]. The light-dependent *Bn*-FCC-2 formation can be mimicked if exogenous ATP is added to chloroplasts in darkness [22]. The 8-ethyl residue is then hydroxylated and subsequently becomes the tautomerization of the 13^2-methylcarboxyl-10,20-dihydro-4,5-seco-4,5-dioxo-8-hydroxyethyl-22H,24H-PPheoide to 13^2-methylcarboxylate-10,20-dihydro-4,5-seco-4,5-dioxo-8-hydroxyethyl-22H,24H-PPheoide (structure not shown on Fig. 4) [23,24]. These reactions are probably used to help the exportation of this compound to the mesophyll cell vacuoles [25] where it is accumulated. Then the intermediate undergoes an ultimate transformation consisting of the hydrolysis of the 13^2-methylcarboxyl group to a 13^2-carboxyl (Fig. 4, right). The final product is a pink pigment denoted *Bn*-NCC-3 [14]. A similar compound *Hv-NCC-1* (formerly called RP14) has been isolated from senescent barley leaves [26]. It differs by the presence of a 3-malonyl residue instead of the 3-vinyl residue found in *Bn*-NCC-3 [23,26]. It is interesting to note that both *Bn*-NCC-3 and *Hv*-NCC-1 are similar to the endproduct of the Chl catabolism found in *C. prothecoides*.

The reactions reported above have been established using senescent organisms. As we mentioned previously in the Introduction section, there are several reports indicating that the Chl content varies daily (Table 1). These data point out that in vivo, Chl stability greatly varies with the plant developmental stage. The older the leaf, the more stable the Chl. Sironval [28] measured daily Chl content oscillations in *Fragaria* (strawberry) plants. The amplitude of the oscillations appeared to be dependent of the leaf developmental stage and on the day length. We found that Chl degradation is much stronger in 2-day-old bean leaves than in 10-day-old leaves during the dark period (8 h) following the first illumination (16 h). In these conditions, Chlb is found more sensitive than Chl *a* [30]. Although the high-performance liquid chromatography (HPLC) method used was able to separate both pheophorbide and pheophytin [31–32], no evidence for their accumulation was observed suggesting that the degradation reactions are fast. Data reported by Shioi et al. [33–34] show that both Mg-dechelatase and chlorophyllase are present in the chloroplasts.

Carotenoids

During the autumnal senescence, carotenoids are less degradated than chlorophylls, thus giving yellow to red leaf coloration (however, some exceptions exist [35]). During senescence, the levels of most xanthophylls (i.e., lutein, neoxanthin, violaxanthin, and antheraxanthin) were found to decrease [36–37]. Those which remain were found to be acylated in many species [37]. Other derivatives, that is, xanthophyll epoxides and esters, have also been observed. The latter constitutes the major carotenoid pool when the leaves turn completely yellow [35]. They are progressively accumulated in plastoglobuli [38], which may serve to increase their relative stability with respect to oxidation. The pattern of carotenoid degradation varies from species to species [39]. During the senescence process, β-carotene and violaxanthin appear to be the least and the most sensitive carotenoids, respectively. Enzymes probably operate during some of the senescence events (e.g., a peroxidase

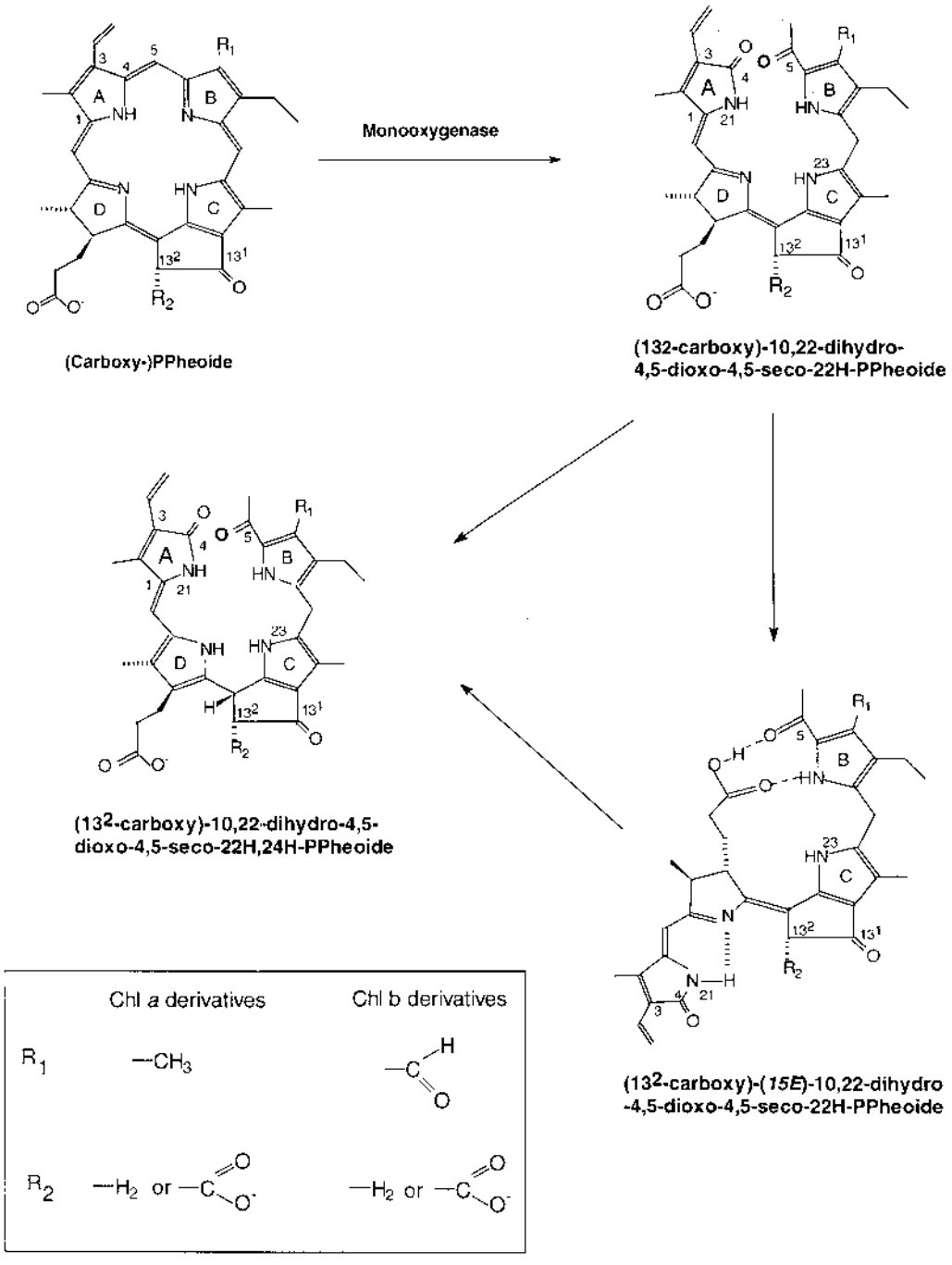

FIGURE 3 Catabolism reactions of carboxy-PPheoide in *C. protothecoides*.

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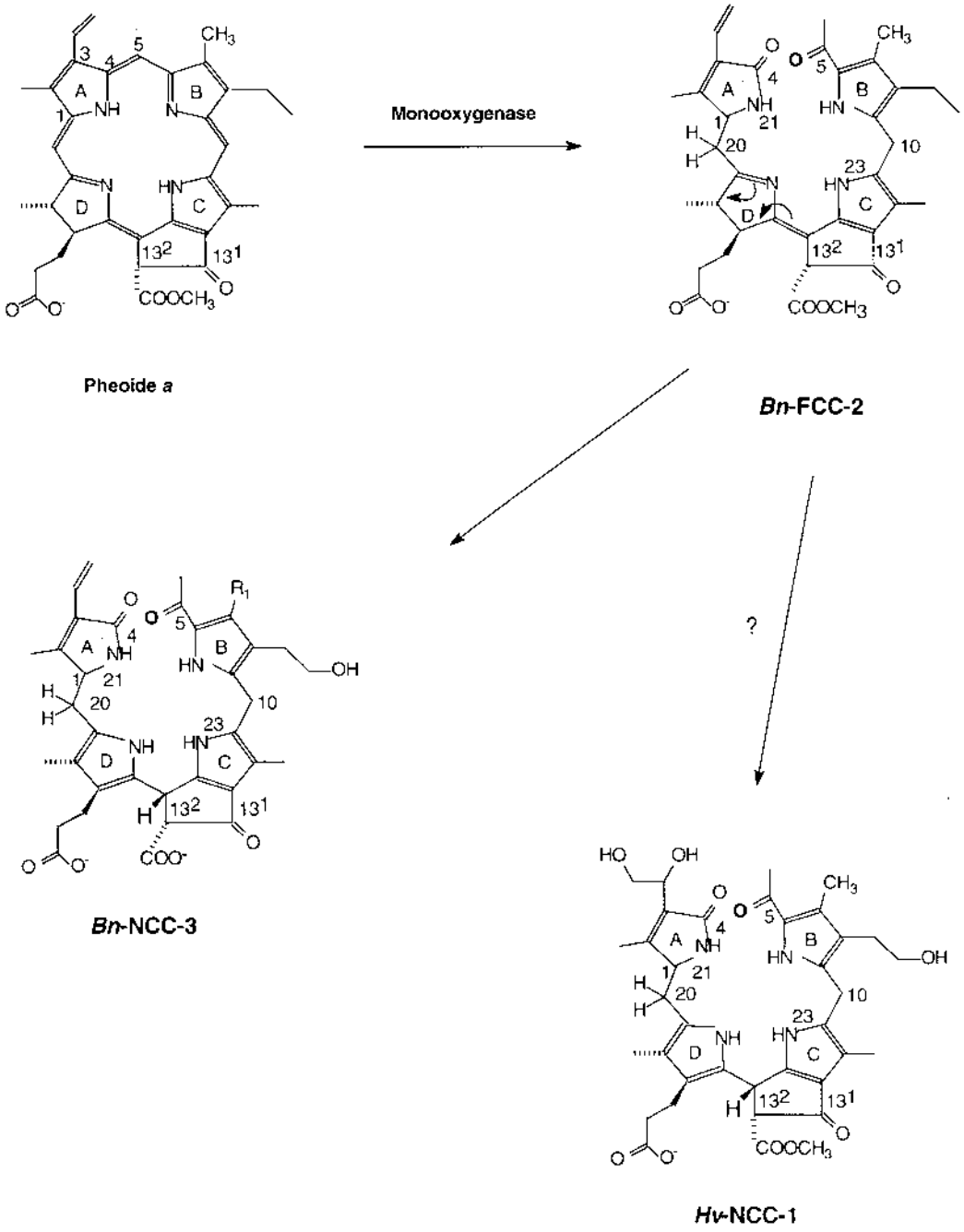

FIGURE 4 Catabolism reactions of Pheoide in higher plants.

TABLE 1 Literature Data About the Chl Turnover Rate as Determined in Various Plants and Different Developmental Stages

Material	Pigment type	Half-life time (h)	Reference
Soya leaf	Chl *a* and Chl *b*	24	27
Young soybean leaf	Chl *a*	108	28
Young soybean leaf	Chl *b*	93.6	28
Barley leaf greened for 24 h	Chl *a* and Chl *b*	16.4	29
Barley leaf greened for 48 h	Chl *a* and Chl *b*	58.3	29

activity) [40]. Although the regulation of carotenoid degradation is not established, it is interesting to note that phytochrome could delay this process [41].

PIGMENT MODIFICATIONS UNDER STRESS CONDITIONS

Light-Intensity and Oxidative Damage

When light absorption by photosynthetic pigment exceeds both the capacity to use the photosynthetic NADPH and ATP for carbohydrate synthesis and the capacity of energy dissipation mechanisms, photosynthesis is progressively inhibited (i.e., photoinhibition phenomenon). As a consequence, the pigment composition starts to change mainly through the xanthophyll cycle. If these adverse conditions are prolonged, pigment destructions through photooxidation occur (i.e., energy transfer from the lowest excited triplet state of Chl to molecular oxygen in its triplet ground state resulting in the formation of singlet oxygen). The formation of a strong cation radical ($P680^+$) has an oxidizing potential high enough to oxidize Chl and β-carotene associated with the reaction center II (reviewed in Ref. 42). Since NADPH and ATP are used in enzymatic reactions whose rates are primarily a function of temperature, light-damage can happen even at low-light intensities [43]. Chla is more sensitive to photooxidation than Chlb, since the latter transfers excitation energy to the former. In contrast to what was observed with senescence, β-carotene is the most sensitive chloroplast pigment to photooxidation [44]. Several mechanisms helping plants to fight against adverse effects of light and implying pigment transformations have been shown. Most of them are involved in the modification of excessive energy dissipation, whereas others imply Chl binding to particular proteins (e.g., early light–inducible proteins: ELIPs).

Dissipation of Excessive Energy

Carotenoids are recognized to be essential for the survival of illuminated plants, since their numerous conjugated double bonds (>9) are able to quench the Chl triplet state and also scavenge singlet oxygen and the other reactive oxygen species which can photooxidize Chl (reviewed in Ref. 45). Plant mutants devoid of carotenoids cannot survive in light [46]. It was suggested that β-carotene-5,6-epoxide formation was the result of β-carotene oxidation by light [47], since its production is low in the dark and progressively rises during the day [48].

Another photoprotecting mechanism involving xanthophyll interconversions has been described in many species (reviewed in Ref. 49). In green plants (including green algae), the xanthophyll cycle is a light-induced deepoxidation pathway from zeaxanthin to violaxanthin via antheraxanthin triggered by overacidification of the inner thylakoid space during the light stress [50] (Fig. 5A). The reaction also requires O_2 and a reducing power [51]. Zeaxanthin seems to exert its photoprotective function by direct singlet-singlet energy transfer from the excited singlet Chl state to that of zeaxanthin, followed by loss of the excitation energy by heat. Another hypothesis proposes an indirect action of zeaxanthin via alteration of the properties of the thylakoid membrane [52]. In

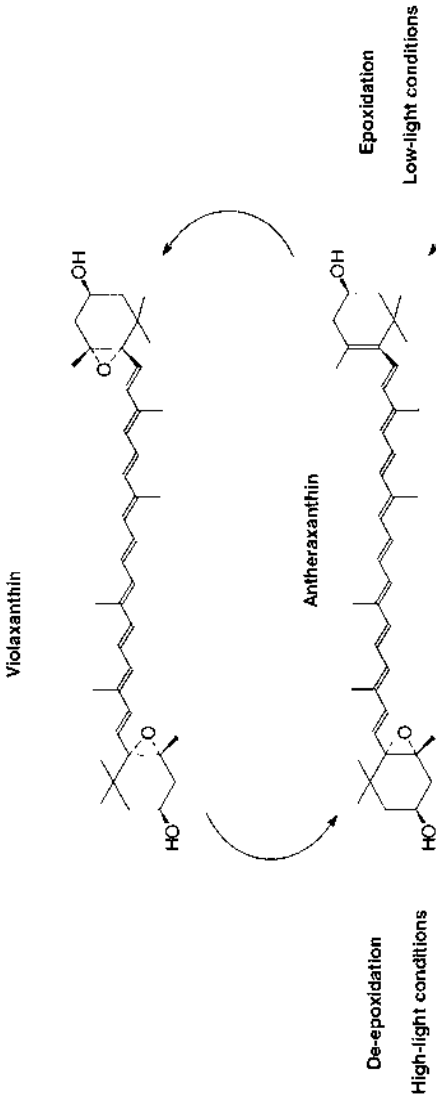

FIGURE 5 Xanthophyll cycle in (A) higher plants and in green algae and (B) in chromophytes.

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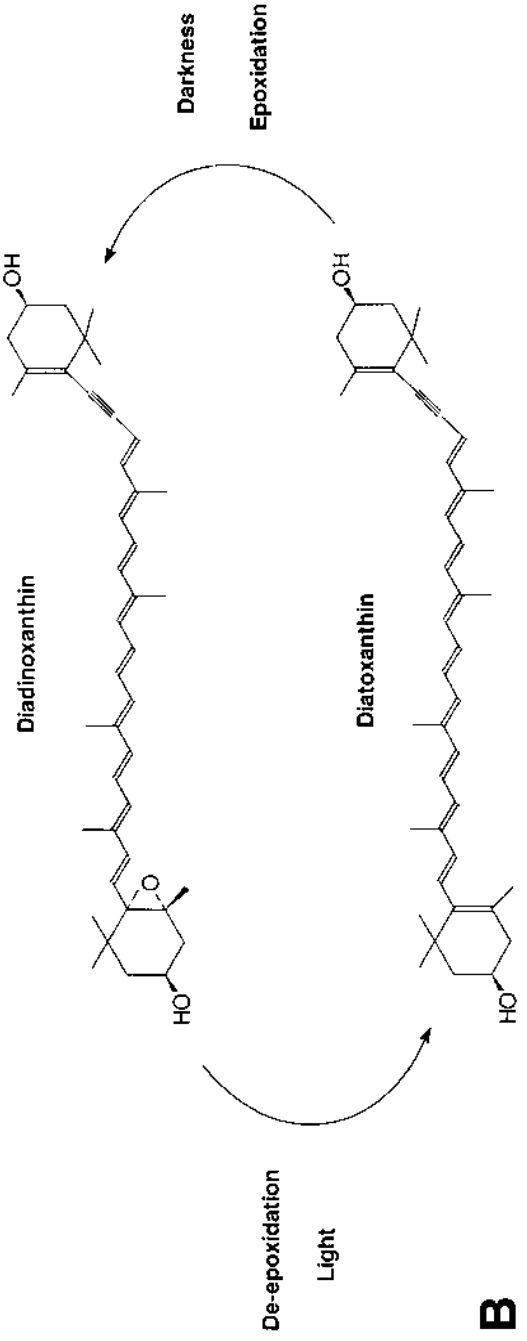

FIGURE 5 Continued.

Digitalis purpurea, the deepoxidation state of the cycle increases to a maximum at a growth-light intensity of 500 μmol m^{-2} s^{-1} and then slightly decreases with a further increase in growth-light intensity [53]. Other investigations showed that the xanthophyll cycle activity depends on light intensity and is strongly enhanced at fluence rates beyond those saturating photosynthetic CO_2 fixation [51].

Some rhodophytes (*Gracilaria gracilis* [Stackhouse] and *Gracilaria multipartita* [Clemente; Harvey]) have shown an atypical xanthophyll cycle [54], whereas others [*Gracilariopsis longissima* [S.G. Gmelin], *Ahnfeltiopsis concinna* [J. Ag.] Silva et DeCew, *Laurencia mcdermidiae* [J. Ag. Abbott], *Porphyridium cruentum*) are devoid of this pathway [54–56]. Chromophytes presented another xanthophyll cycle involving diadinoxanthin to diatoxanthin transformation [54,57] (see Fig. 5B). The xanthophyll cycle has not been demonstrated in cyanobacteria. However, in *Oscillatoria*, an increase of the photon flux density triggers a decrease of the Chla and phycocyanin levels and a relative increase of myxoxanthophyll and zeaxanthin corresponding to a decrease of β-carotene and echinenone [58].

Intertidal macroalgae are regularly stressed because of high-light intensity, desiccation, and temperature changes occurring during emersion. A morphological and pigmentary adaptation against too high photon flux densities was described for tropical intertidal rhodophytes [55]: *A. concinna* and *L. mcdermidiae* grow in dense turf allowing self-shading over small vertical distances and exhibit microscale pigmentary adjustments; the canopy of *A. concinna* appears yellow-orange, whereas the canopy of *L. mcdermidiae* appears green. Tissues from understory locations of both species appear red to purple-black. Actually, *A. concinna* reduces the levels of all phycobilins, whereas *L. mcdermidiae* maintains the phycocyanin and allophycocyanin levels while reducing phycoerythrin with exposure to high photosynthetic flux densities. Both turfs increase the levels of carotenoids and mycosporine-like amino acids [59], but only *L. mcdermidiae* increases absorbance of ultraviolet A (UVA) radiation.

During the first stages of chloroplast development, newly formed Chlide *a* is not protected by carotenoids and can be easily photooxidized [60]. Nevertheless, Chlide *a* shows a relative photoprotection against photodestruction when it is under the form of a Chlide-NADPH: protochlorophyllide oxidoreductase–NADPH complex [61] (reviewed in Ref. 62). It was shown that this complex can be transformed to a Chlide-NADPH : protochlorophyllide oxidoreductase–$NADP^+$ complex under illumination. This light reaction can be reversed in darkness [63]. A cycle between the two complexes thus exists (Fig. 6) and has been recently correlated to Chlide protection against photooxidation [64,65].

Early Light–Inducible Proteins

It was found that ELIPs accumulates under light stress condition correlates with the photoinactivation of photosystem II (PSII) degradation of reaction center protein D1 and changes in the level of pigments [66]. ELIP-related proteins, namely, cbr (for carotene biosynthesis–related), have been described in the green unicellular *Dunaliella bardawil* in response to light-stress proteins [67]. The mechanism of protection by ELIP is still unclear. Since Chl, which is not associated with protein-carotenoid complex, can be very harmful when sensitized by light absorption, it was proposed that ELIPs may be involved in the transient binding of pigments under light-stress conditions. Alternatively, ELIPs may have a function of free Chl scavengers and be involved in the binding of released Chl and thus ensure protection against free radical formation through Chl sensitization.

Accumulation of Specific Pigments

A limited number of algae (e.g., *Dunaliella, Haematococcus*) present the peculiar property of accumulating secondary carotenoid (mainly β-carotene, canthaxanthin, and astaxanthin) as a response to a light stress (e.g., see Refs. 68 and 69; reviewed in Ref. 70). Little is known about the regulation of these syntheses. We found that at the beginning of the stress, green *H. pluvialis* cells synthesize astaxanthin at the expense of β-carotene present in the PSI [69]. The bulk of astaxanthin requires

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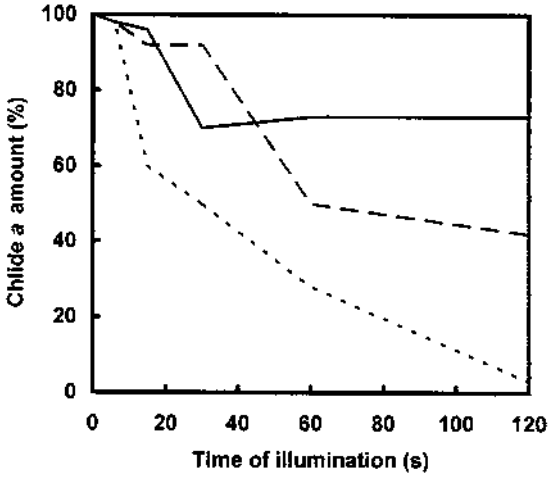

FIGURE 6 Time course of Chlide a degradation under different light-intensities. Chlide a amounts are expressed as the percentage of the absorbance measured at 678 nm in vivo before the illumination. Light intensities: 21 μE m^{-2} s^{-1} (—), 105 μE m^{-2} s^{-1} (––); 210 μE m^{-2} s^{-1} (----). (Adapted from Ref. 30.)

β-carotene resynthesis. Although the postulated role of secondary carotenoid accumulation is photoprotection, the molecules or cellular structure(s) which would benefit from this protection remain to be determined.

The peculiar property of carotenoid accumulation in higher plants is not common. When stressed by high irradiance and drought, *Aloe vera* accumulates the xanthophyll rhodoxanthin [71].

Chilling/Freezing Stress

As noted previously, photoinhibition can occur at low temperature even at low-light intensities. The result is that pigment degradation by photooxidation is more pronounced at higher temperature, especially in high-light conditions. This situation is worsened by the impairment of zeaxanthin formation at low temperature [72]. In contrast, it was reported that exposure of canola fields to sublethal freezing conditions (−5°C) during seed development inhibits seed degreening, possibly due to the inactivity of the peroxidase that degrades Chl [19]. Pigment degradation is more pronounced in chilling-sensitive *Cucumis sativa* and maize than in the chilling-tolerant *Pisum sativum* L. [72,73].

Salinity Changes

As a general rule, salt stress induces the modification of the carotenoid composition and Chl degradation in salt-sensitive plants, with the latter being partly due to a chlorophyllase activity enhancement [74]. Chl degradation mainly affects Chla. In contrast, an increase in the Chl content has been observed for tolerant species (reviewed in Ref. 75).

Variation of seawater salinity from 40‰ to 30 or 25‰ triggers bleaching of scleractinian corals [76]. This change in color is mainly due to a decrease in both the number of zooxanthella in each polyp and in the total amount of pigments in each zooxanthellae (i.e., Chla, $Chlc_2$, peridinin, diadinoxanthin, and dinoxanthin).

Herbicides

Herbicides which inhibit photosynthetic electron transport (e.g., DCMU) and redox-active herbicides (e.g., paraquat) are known to allow generation of active oxygen species (e.g., superoxide) which

are able to induce extensive leaf chlorosis and necrosis [77]. Moreover, DCMU and monuron both inhibit the deepoxidation of violaxanthin [70].

Photodynamic herbicides (e.g., diphenyl ether–type) inhibit the Chl biosynthetic pathways at the level of protoporphyrinogen oxidase (EC 1.3.3.4) [78]. Protoporphyrinogen IX is then accumulated and partly exported out of the chloroplast where it is autooxidized to protoporphyrin IX [79]. Protoporphyrinogen IX exportation is understood as a mechanism associated with the removal of a potential oxygen-sensitizer which can damage the membranes and their components [80]. In effect, singlet oxygen has a lifetime much longer in nonaqueous solution (20–25 μs) than in aqueous medium like the cytoplasm (3–4 μs) [81,82]. The exportation activity appeared to be very weak in dark-grown leaves [83]. The production of oxygen-sensitizer tetrapyrroles also occurs in plants fed with δ-aminolevulinic acid, the tetrapyrrole precursor, except that other Chl precursors are accumulated [84] (reviewed in Ref. 62). After a prolonged treatment with photodynamic herbicides, large amounts of xanthophyll acyl esters are produced. The production of these esters is not light dependent and could result from lipid peroxidation [70].

Other herbicides (e.g., diflufenican, aminotrizole) interfere with the carotenoid biosynthetic pathway. For instance, they inhibit the desaturase and the cyclization reactions resulting in the accumulation of carotenoid precursors (e.g., phytoene) lacking cyclic structures and extended conjugated double bonds [85]. These carotenoids are unable to bind photosynthetic protein and consequently to photoprotect Chl molecules.

Atmospheric Pollutants

O_3, NO_2, and SO_2 generally trigger the photooxidative destruction of both Chl and carotenoids [86]. However, the data remain puzzling and sometimes contradictory (reviewed in Ref. 70). Long-term exposure of spruce trees to low levels of SO_2 or $SO_2 + O_3$, but not of O_3 or acidic precipitation (pH 4), causes a decrease in the Chl content of the needles [87]. The phytol release from Chl increases with the ozone amounts applied to *Picea abies* [88]. Interestingly, low concentrations of O_3 may serve to promote the epoxidation of violaxanthin to zeaxanthin [89]. Plants actually have no defense system protecting their pigments against atmospheric pollutants.

Nutrients and Heavy Metals

As a general rule, nutrient deficiency induces plant senescence, the appearance of necrosis, and chlorosis (reviewed in Ref. 90). As a consequence of these phenomenons, pigment composition is altered. For example, a potassium deficiency lowers the carotenoid content in sunflower leaves [91]. In several cases, it was reported that nutrient deficiency triggers an increase of the xanthophyll cycle activity [91,92]. The Chla/b ratio is found to be higher in iron-deficient leaves than in the control [93].

Little is known about the influence of nutrient abundance on photosynthetic pigments. In contrast, the excess of a metal on photosynthesis is quite well documented. For instance, cadmium was found greatly to reduce the Chl content in *Chlamydomonas* cells, whereas manganese has only a slight effect [94] (B. Schoefs and M. Bertrand, unpublished results). Recently, a study was performed on maize in order to determine the copper toxicity sites. Copper was found to interfere with the primary photochemistry, principally at the PSII level, and with the light-adaptation processes allowing light-harvesting migration from PSII to PSI, decreasing the light-adaptation capacity [95]. In these conditions, pigment destruction would be favored. In vitro experiments carried out with submersed plants have even shown that the Chl magnesium could be substituted by mercury, copper, cadmium, nickel, zinc, or lead [96].

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Photoinhibition of Photosystem II in Leaves: Stress, Acclimation, or Regulatory Response?

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INTRODUCTION

Light Stress in Leaves

Stress is defined in *Chambers Science and Technology Dictionary* (W&R Chambers, Ltd. and Cambridge University Press, Cambridge, 1988) as follows: ''Excessive and aversive environmental factors that produce physiological responses in the individual.'' A maximum sunlight irradiance of approximately 2200 μmol m^{-2} s^{-1} is considered excessive for most plants [1]. This is because irradiance-driven photosynthesis in many C_3 species saturates at about half that photon flux. In such species, the quantum efficiency and often the maximum rate of photosynthesis are reduced when exposed to irradiances above the saturation level [2].

Interestingly, photosynthesis in C_4 plants, whose bundle sheath chloroplasts operate in higher than ambient CO_2, saturates at >2000 μmol m^{-2} s^{-1}. This suggests that the electron transport capacity can be very high indeed, and in C_3 species, it may exceed the rates required for carbon fixation [3]. It is also significant that these very high electron transport rates in some types of C_4 bundle sheath chloroplasts are achieved with little or no granal structure or photosystem II (PSII). In these chloroplasts, ATP is generated by rapid cyclic electron transport around photosystem I (PSI). More intriguing, leaves with chloroplasts that have very large granal stacks and lots of PSII have only very low rates of photosynthesis. High uncoupled rates of electron transport measured in vitro also suggest that the electron transport capacity can exceed the carbon fixation rate and capacity. All this points to the possibility that PSII actually controls the rate of electron transport.

Evolution of Energy Use and Dissipation

Oxygenic photosynthesis probably evolved in much lower light intensities and higher atmospheric CO_2 concentrations, so that originally the photosystems became optimized for light absorption.

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When species moved onto the land, moderate light intensities were combined with high CO_2 levels, and further increases in absorption capacity may have been made to increase the photosynthetic rate. The means of dealing with ever-increasing light intensities and O_2 levels and decreasing CO_2 concentrations as the atmosphere changed seem to involve O_2 radical scavenging systems, reflective leaf surfaces, and multiple cell (chloroplast) layers in individual leaves and multiple leaf layers in canopies. Furthermore, although very efficient absorption properties were maintained, highly effective dissipation processes and the C_4 (CO_2-concentrating) mechanism were added to deal with exciton pressure in excess of the carbon-fixing capacity in leaves exposed to maximum light intensities [3].

Although carbon fixation and electron transport capacity colimit the photosynthetic rate [4], in reality, C_3 photosynthesis is often limited by carbon-fixation activity. This limitation must exert a feedback regulation on the operation of the electron transport chain reducing the electron flow rate during periods of low CO_2 availability and increasing the energy dissipation rate. Therefore, photosystems reflect in their protein architecture, pigment organization, and arrangement in the thylakoid membrane all the requirements for efficient light absorption and maximum electron flow as well as effective energy dissipation. The two apparently conflicting properties have to be built into the same type of photosystem, or the photosystem has to change with fluctuating circumstances (i.e., become heterogeneous). PSII fulfills these requirements. PSII certainly is heterogeneous, since it has active and inactive forms. Several questions about what PSII heterogeneity actually means and what role it plays have yet to be answered. The questions can be summarized as follows:

How is multiple functionality of PSII achieved?
Is photoinhibition a stress response or is it the dissipation (downregulated) state of chloroplasts that have reached their maximum rate of CO_2 fixation?
Is photoinhibition a manifestation of PSII photodamage?
Is PSII especially sensitive to stress or particularly adaptable?
Does PSII effectively regulate the rate of electron transport?

Photoinhibition

Photoinhibition (also referred to as high-light stress), which has received much attention and research over the past two decades, has focused on the effects of excess irradiance on photosynthetic organisms. In many instances, irradiance is an exacerbating factor concomitant with other abiotic or biotic stresses. Photoinhibition is manifest in chlorophyll fluorescence changes, which, when measured at ambient temperatures, are attributable almost entirely to PSII. Therefore, PSII functioning measured as a reduction in chlorophyll fluorescence has become synonymous with an estimate of the degree of stress experienced by the plant. In fact, PSII fluorescence changes are considered the earliest and most sensitive stress monitors available. Parameters such as the dark-adapted Fv/Fm ratio, the fluorescence yield in the light, $\Delta F/Fm'$, or a fluorescence-quenching parameter, qN, are all used increasingly to identify and diagnose a stress response [5]. Measurement of these parameters is sometimes accompanied by an analysis of the underlying biochemical/physiological processes in attempts to understand the mechanism of photoinhibition and the nature of the response causing the fluorescence change.

Photoinhibition has historically been conceptualized as injury or damage to PSII [6], since it causes a reduction in the quantum efficiency and often, but by no means always, a decrease in the maximum rate of photosynthesis. It was then, and still is, inconceivable to most scientists that a reduction in activity, especially photosynthetic activity, should be a good thing for the plant. Photoinhibition has, therefore, always been considered to be deleterious to plants. In some instances, it did indeed reduce productivity [7].

D1 Protein Turnover

When the D1 protein was identified as one of the two reaction center proteins of PSII [8], its high turnover rate was immediately explained: photon absorption and charge separation processes in

oxygenic organisms damage the D1 protein and its renewal is therefore required [9]. Rapid D1 protein turnover was neatly accounted for in this way and photoinhibition simply explained. The lack of even a correlation, let alone evidence for a causal relationship, between the degree or the severity of photoinhibition and the rate or the extent of D1 turnover has not stood in the way of this paradigm becoming widely accepted. The D1 damage/repair model of photoinhibition, the fatal flaw of PSII, although highly complex biochemically [10], is conceptually simple and therefore appealing.

In this chapter, a different view of photoinhibition and the role of D1 turnover is presented. Experimental evidence will be provided which shows that photoinhibition is not directly linked to D1 turnover, and that D1 turnover does not cause photoinhibition. It will be suggested instead that D1 turnover is a light-activated, largely constitutive activity that is switched on by reduction of QB and the generation of high assimilatory power rather than by damage-induced D1 degradation. It will be argued that photoinhibition is synonymous with a significant proportion of PSII being converted to a dissipative function so that quantum efficiency, and sometimes maximum light and CO_2 saturated rates of photosynthesis, are reduced. Some of the questions posed above will be answered. Photoinhibition clearly occurs independently of the rate of D1 turnover unless this turnover is artificially blocked. The maximum rates of D1 turnover are measured when no photoinhibition is apparent. Whether and how DI turnover influences photoinhibition and/or the photosynthesis rate remains to be elucidated.

RELATIONSHIP BETWEEN D1 TURNOVER AND PHOTOSYNTHESIS

In recent years, many studies have shown that D1 turnover proceeds at maximum rates when the photochemical efficiency and the light-saturated rate of photosynthesis are maximal and when there is no evidence of photoinhibition [11]. Indeed, the maximum rates of D1 turnover can be measured at light intensities below saturation [12,13]. Other factors appear to influence D1 turnover more than light intensity per se.

It was also demonstrated that photoinhibition does not cause a net loss of D1 protein in vivo [14] or in vitro [15] unless chloroplast protein synthesis inhibitors are employed [16]. The argument that damaged D1 protein becomes phosphorylated, arresting its susceptibility to degradation, is not very convincing. Even more importantly, only a proportion of PSII functions at times of maximum photosynthesis [17]. It follows that only a proportion of D1 protein has to be functional at any one time. The function the thylakoid membrane system has to perform is this: The photosystems, especially PSII, have to be built and arranged in such a way that excitation energy is maximally absorbed and optimally channeled to those that are functional when photons are rare. At low-light intensity, excitation energy must go to QB reducing active PSII to achieve maximum or near maximum quantum efficiency. When photons are in excess, dissipative centers are formed and excitation energy is channeled to them as well as the active centers. If large proportions of dissipative centers persist in low-light intensity, the quantum yield is reduced until the balance is restored. For both scenarios and both types of PSII, the D1 protein has to be in place and functional. Whether D1 is different in dissipative, inactive, and active centers that function differently remains to be established.

Centers in the process of reconstruction obviously do not normally absorb many photons or participate in electron transport or energy dissipation. Minimal absorption may be ascertained by their location in the thylakoid membrane, by light-harvesting complex II (LHCII) disconnection, CP43 displacement from the core [18], and monomerization [18,19].

Experimental Evidence: Varying Irradiance

In sun and shade leaves of *Schefflera arboricola*, D1 turnover was measured as degradation (disappearance) of radioactively prelabeled D1 protein [12]. The palisade parenchyma and spongy meso-

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phyll tissues were separated because of a strong light gradient in these leaves. D1 turnover rates in the spongy mesophyll tissue of sun leaves when illuminated with limiting or saturating irradiance (90 or 300 μmol m^{-2} s^{-1}, respectively), which caused absolutely no change in Fv/Fm ratios, were virtually identical (Fig. 1A). Similarly, high D1 turnover rates were measured in the palisade tissue from sun and shade leaves exposed to 24 h of excess irradiance (1500 μmol m^{-2} s^{-1}), whereas PSII photochemical capacity, estimated from dark-adapted Fv/Fm ratios, decreased at very different rates (Fig. 1B). There was generally no correlation between D1 turnover rates and the degree of photoinhibition. Moreover, D1 turnover rates seemed remarkably similar under very different experimental illumination as well as previous growth conditions. Only in shade leaves was there a small difference between the treatment light intensities.

Similar results were obtained in studies of high- and low-light–grown pea leaves [20,21].

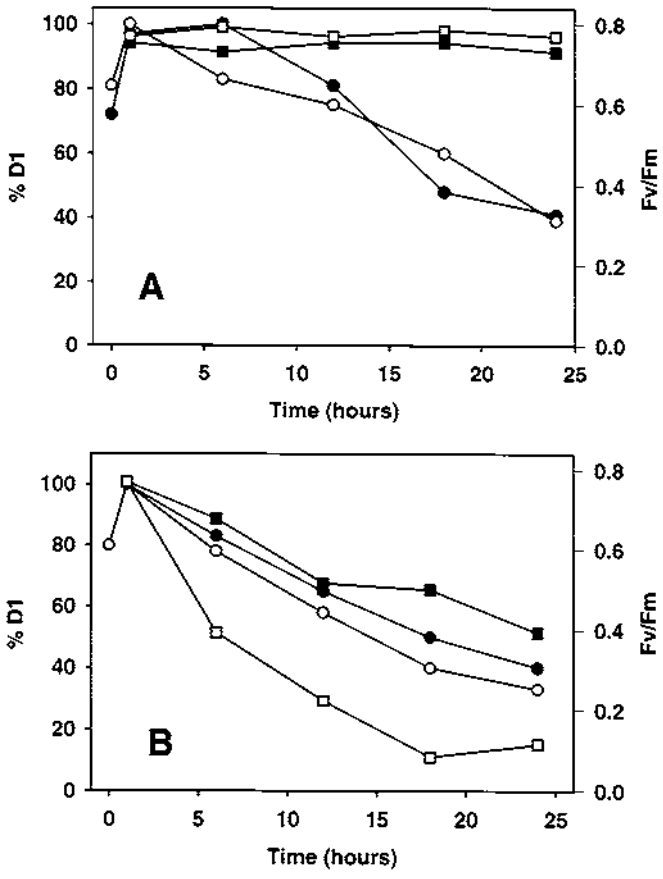

FIGURE 1 (A) Degradation of prelabeled D1 protein (○●) and the Fv/Fm ratio (□■) as a function of exposure time to excess irradiance. Thylakoids and D1 protein were isolated from palisade parenchyma cells of *Schefflera arboricola* leaves grown in the sun (●■) or the shade (○□). (B) Degradation of prelabeled D1 protein (○●) and the Fv/Fm ratio (□■) as a function of exposure time to saturating (●■ or limiting (○□) irradiance. Thylakoids and D1 protein were isolated from spongy mesophyll cells of *Schefflera arboricola* leaves grown in the sun.

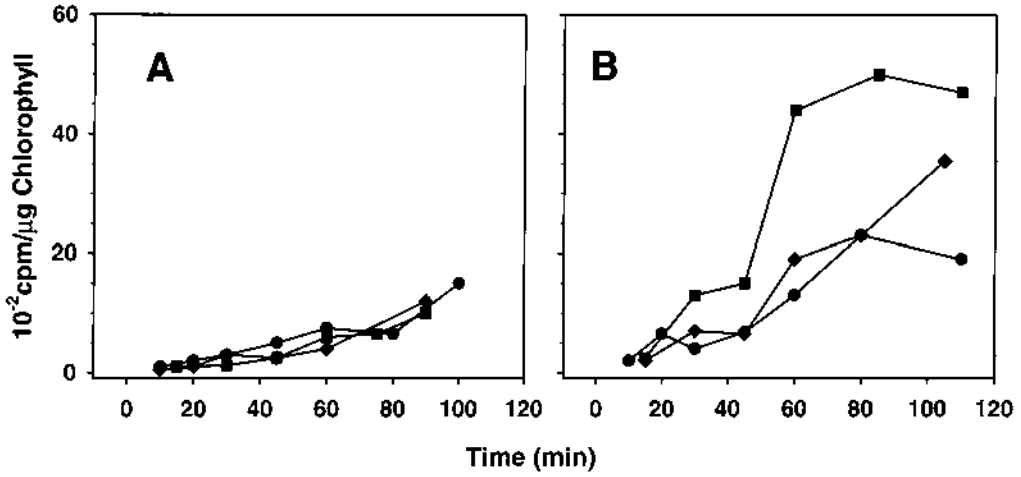

FIGURE 2 Accumulation of radioactive label in the D1 protein of *Pisum sativum* leaves grown in (A) high light (1200 μmol m^{-2} s^{-1}) or (B) low light (90 μmol m^{-2} s^{-1}) and exposed during labeling to limiting (90 μmol m^{-2} s^{-1}, ■), or saturating (350 μmol m^{-2} s^{-1}, ◆), or excess (1500 μmol m^{-2} s^{-1} ●) irradiance.

There was also no difference between treatments in high-light–grown leaves (Fig. 2A), but there was some variation in D1 turnover with irradiance in low-light plants (Fig. 2B). In the low-light–grown leaves, D1 turnover rates were maximal in limiting light conditions, a little lower in saturating light, and somewhat inhibited in excess irradiance (Fig. 2B), which are quite inconsistent with a causal relationship of photoinhibition and D1 turnover but confirms the correlation between high rates of photosynthesis and maximum D1 turnover activity.

These data clearly contradict the results obtained in other laboratories, although the different experiments are not strictly comparable: Aro and colleagues [22] suggest that the rate constant of photoinhibition is directly proportional to the light intensity and that there is a kinetic agreement between D1 protein degradation and the inactivation of PSII. However, this is only ever observed in leaves in which chloroplast protein synthesis, and hence D1 turnover, is prevented by treatment with lincomycin or chloramphenicol. Under such conditions, both D1 protein degradation and photoinhibition are light-intensity dependent and appear to be linked. What these experiments do show is that light-dependent D1 protein degradation can proceed without concomitant protein synthesis, and that photoinhibition is eventually more severe when D1 synthesis is blocked.

Degradation of D1 protein in extremely low light has also been shown to occur independent of protein synthesis activity [23]. This degradation was suggested to be due to recombination of QB^- and $S_{2,3}$ states creating damage to the protein, thereby causing its degradation. These experiments were also carried out in the presence of chloroplast protein synthesis inhibitors.

In both sets of experiments, D1 protein detection and quantification was performed by Western blotting. This method may not quantitate the D1 protein accurately (B. Morrison and C. Critchley, in preparation). In a comprehensive study on *Arabidopsis thaliana* leaves, Russell and collaborators [13] showed that there was no correlation between D1 turnover and any other activity or parameter measured. It was revealing that the two methods used to identify and quantitate the D1 protein following the exposure of leaves to different irradiances, that is, Western blotting and ^{14}C-DCMU binding, did not agree with each other. They also did not correspond with the number of functional PSII present (Fig. 3). Of the two quantitative measures, antibody-detectable D1 protein correlated somewhat better with amounts of functional (O_2 evolving) PSII than did ^{14}C-DCMU binding.

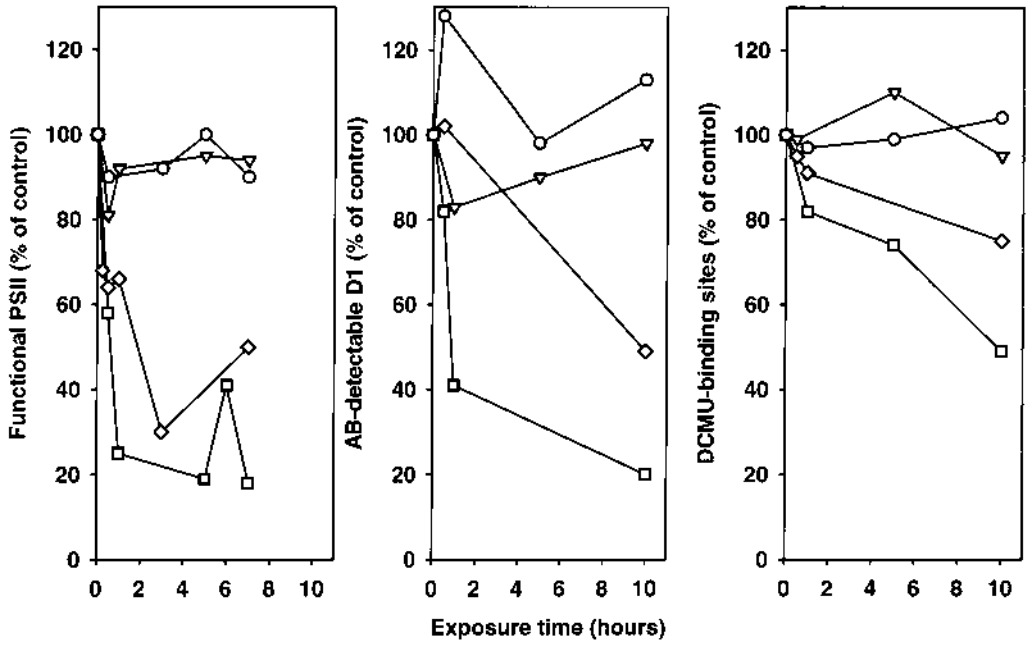

FIGURE 3 Changes in the amounts of (A) functional PSII, (B) antibody detectable D1 protein, and (C) DCMU binding sites in leaves of *Arabidopsis thaliana* grown in low light as a function of exposure to limiting (250 μmol m^{-2} s^{-1}, ○), saturating (420 μmol m^{-2} s^{-1}, ▽), mildly photoinhibitory (1350 μmol m^{-2} s^{-1}, ◇), or strongly photoinhibitory (2200 μmol m^{-2} s^{-1}, □) irradiance.

Experimental Evidence: Varying CO_2 Concentration

CO_2 availability is a major constraint on the photosynthetic rate and many plants respond positively to an increase in the CO_2 concentration during long-term growth or short-term change. Under controlled conditions and supplied with sufficient nutrients, *Schefflera arboricola* had increased saturated rates of CO_2 fixation in elevated CO_2 both in high and low irradiance (Table 1). When light and CO_2 concentrations were experimentally altered, the most significant change in the rate of D1 protein turnover was observed when the CO_2 concentration was changed. In fact, the rates of D1 protein turnover were linearly related to the photosynthetic rate, but not at all correlated with the

TABLE 1 Changes in the Rates of D1 Turnover in Leaves Grown in High-Light and Ambient or Elevated CO_2 Concentrations as a Consequence of Experimentally Changing the CO_2 Concentration for 9 h

	% of Prelabeled D1 protein degradation (turnover)	
	Ambient CO_2 grown	Elevated CO_2 grown
Ambient CO_2 treatment	6.8 (±1.6)	7.2 (±2.6)
Elevated CO_2 treatment	15.0 (±1.5)	19.9 (±4.8)

degree of photoinhibition (A.W. Russell et al., submitted for publication). These experiments clearly demonstrated that a temporary stimulation of photosynthesis by elevated atmospheric CO_2 caused an increase in D1 turnover despite a decrease in the electron pressure on PSII.

Experimental Evidence: Varying Maximum Rates of Photosynthesis

Altering the maximum rates of photosynthesis in identical irradiance and nonphotoinhibitory conditions should confirm the dependence of D1 turnover on the rate of photosynthesis. Experiments with wheat grown in small pots at elevated temperature or under N deficiency but in identical light conditions were performed. Although on a leaf area basis the photosynthetic rates were identical in all treatments and control leaves (maximum light and CO_2-saturated rate 20 μmol O_2 m^{-2} s^{-1}), they differed significantly between controls and treatments on the basis of the amount of chlorophyll. The rates of the N-deficient and elevated temperature leaves were higher, whereas those grown in small pots were much lower than control rates. The D1 turnover rates, measured as the rate of radioactive labeling of the protein (also on a chlorophyll basis) matched these rates very well (Fig. 4). There was no evidence of photoinhibition in any of the control or treated leaves.

ROLE OF PHOTOINHIBITION: EFFECTIVE DISSIPATION OF EXCESS ENERGY

Many plants recover fully from daily photoinhibition events but others seem to maintain a level of dissipative capacity that is partially retained overnight [24]. In both types of leaves, D1 turnover

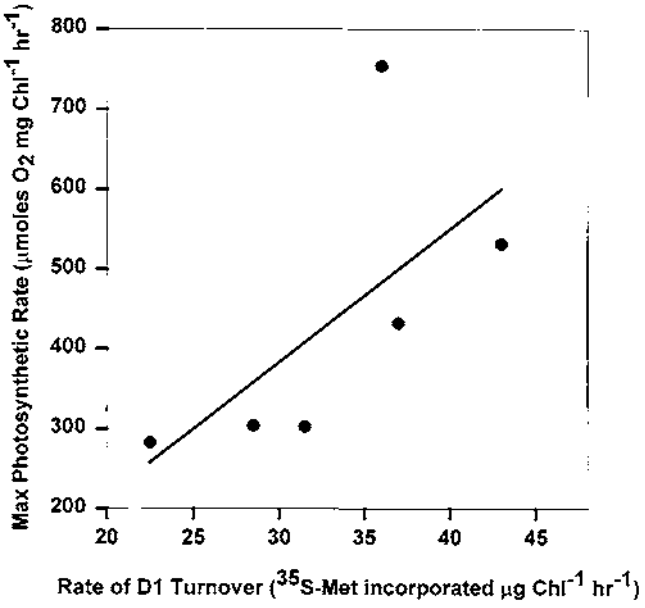

FIGURE 4 Relationship between the maximum photosynthetic rate (light and CO_2 saturated) and the rate of D1 protein turnover calculated from ^{35}S-methionine incorporation of various *Triticum aestivum* leaves grown under control, elevated temperature, small-pot, and N-deficient conditions. Growth irradiance was approximately 1500 μmol m^{-2} s^{-1}; irradiance during labeling was 800 μmol m^{-2} s^{-1}.

proceeds at high rates. This suggests two possibilities. Dissipative centers are prevented from turning over D1 protein (perhaps by phosphorylation) and remain associated with active centers, always sharing excitation energy and reducing quantum efficiency [25]. On the other hand, both dissipative and active centers undergo D1 turnover, becoming inactive during that time [26]. This question can only be resolved when inactive, active, and dissipative centers have been isolated and biochemically identified.

Although no inactive or active centers have been isolated and biochemically identified, they are known to be present in the chloroplasts [27,28]. The structural differences between them may be very subtle, because they may involve only small conformational changes in the D1 protein of the type suggested by Bracht and Trebst [29]. Attempts to isolate such centers from leaves and separate them biochemically may fail for two reasons: multiple cell layers in leaves causing a light gradient and heterogeneity among the chloroplasts and the inability to separate these cell layers.

The answer to the first and second questions posed above may be that: multiple functionality of PSII is achieved by changing populations of active, inactive, and dissipative centers present in leaves at any one time, providing for optimum quantum efficiency as well as effective dissipation, and depending on irradiance and other environmental conditions. This heterogeneity may be achieved by interconversion of the various forms. Photoinhibition is an adaptive response during which PSII is modified and downregulated. Modification and turnover of the D1 protein may play a role in this.

ROLE OF D1 TURNOVER: LIGHT ACTIVATION AND CONTROL OF PSII ELECTRON TRANSPORT RATE

Since there is no convincing or compelling evidence that D1 turnover is a response to PSII photodamage in vivo, the possibility of a positive role for this turnover in the chloroplast must be considered. In view of the light requirement for D1 turnover, this positive role may lie in activating the requisite number of PSIIs for the adequate rates of electron transport to be achieved. We know that electron transport rates initially have to be very high to satisfy the requirements for enzyme activation, nitrogen metabolism, and the generation of assimilatory power. This may be achieved by D1 turnover causing dimerization, thereby activating PSII. D1 turnover may also play a role in the formation of supercomplexes, enzymes catalyzing dark and light reactions associated with nonappressed membranes in the chloroplast [30]. Süss and Sainis [31] provide evidence for such supercomplexes and have termed them photosynthesomes. These supercomplexes may channel substrates and cofactors effectively by being associated with several active electron transport chains. They may also concentrate CO_2 to some extent.

PSII photodamage only occurs when photoinhibitory adaptation is no longer sufficient. PSII is especially adaptable because of its heterogeneity which is unlike any of the other electron transport complexes. PSII heterogeneity thereby controls the quantum efficiency and electron transport rate.

CONCLUSIONS

For many years, photoinhibition and D1 turnover have been studied in conjunction and have, therefore, become inextricably linked. This is very unfortunate because of the failure to approach the two processes separately. We have provided much evidence, some of it summarized in this chapter, demonstrating that the two are not necessarily linked and may occur quite independently of each other. That evidence has been largely ignored in the literature.

Both processes are highly complex and regulated. D1 turnover activates and regulates the electron transport rate and perhaps facilitates the formation of supercomplexes. Photoinhibition ensures the dissipation of excess energy. Whether D1 turnover influences the extent of the dissipative state or whether dissipative centers undergo D1 turnover remains to be established.

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Oxidative Damage to Proteins in Plants

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INTRODUCTION

Although oxidative modification of protein and oxidative stress have been studied for many years, it is only recently that molecular analysis of protein damage has been generally considered. As complete genomes become sequenced, our experiments can expand to consider the whole variety of gene products that make up the proteome. For example, where previously a general assay of protein oxidation may have revealed some manifestation of protein damage, future definitive analysis will seek the identity of all of the modified proteins as well as their sites of modification. In this way, an overall picture of sensitivity will emerge highlighting potential targets for genetic manipulation. Maybe the structures of key enzymes can be altered to lower susceptibility or the expression of protective enzymes can be upregulated to boost antioxidant capacity. Furthermore, it will be necessary to tie our understanding of vulnerability to the mechanisms that recognize damage and initiate replacement. There are likely to be cases where susceptibility cannot be avoided, thereby requiring acceleration of turnover and reassembly processes for effective improvement of performance under conditions of oxidative stress.

OXIDATIVE MODIFICATION OF PEPTIDYL AMINO ACID RESIDUES

Specific Modifications Required for Function

Disulfide Formation

Disulfide cross links are formed via oxidation of a pair of peptidyl cysteine thiols. This occurs spontaneously in the presence of oxygen but may require the assistance of enzymes, such as protein-disulfide isomerase, for the formation of the correct disulfide [1,2]. Specific disulfides may be required for correct folding and consequent enzymatic activity. Free thiols are common and play a variety of roles in catalysis, metal binding, and regulation. Reversible disulfide formation is be-

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ing found to be a common means of linking redox poise to enzymatic activity and gene expression [3–6] as well as modulating physiological responses such as phloem fiber cross linking in response to wounding [7]. In summary, the correct management of thiol/disulfide is essential to many aspects of plant health and may be upset if oxidative stress leads to irreversible oxidation of cysteine residues (see section on Nonspecific Modifications/Damage below).

Other Modifications

A novel oxidative modification is found at the active site of the enzyme arylsulfatase. The original cysteine residue is altered to a serine semialdehyde, 2-amino, 3-oxopropionic acid [8]. Humans unable to perform the modification lack arylsulfatase activity and suffer from multiple-sulfatase deficiencies. The observation of the same modification in arylsulfatase from *Volvox carteri* was used as an argument for its ubiquitous presence in eukaryotes [9]. Workers using the enzyme as a reporter [10] should be aware of the requirement for modification of the arylsulfatase precursor by other gene products.

Nonspecific Modifications/Damage

Reversible Modifications

Reversible oxidative modifications to proteins are of considerable importance. They raise the possibility of a scavenging role for proteins in the protection from oxidative damage [11,12] and introduce the potential for the presence of feedback mechanisms connected to appropriate pathways of metabolism and gene expression [13–15].

Methionine

A common oxidative modification to plant proteins is that of methionine to its sulfoxide (MetO) [16,17]. The amino-acyl thioester is an effective scavenger of active oxygen species (AOS) provided it is exposed to the aqueous environment. Buried methionyl side chains may be highly resistant to oxidation by virtue of inaccessibility.

The formation of sulfoxide on proteins can be highly inhibitory, especially if an active-site functionality is modified [18]. More subtle effects may arise owing to disturbance of tertiary structure [19]. The quaternary structure may also be perturbed on modification of methionyl residues, with implications for regulation; the T-state of hemoglobin, for example, is completely destabilized by oxidation of met55 of the β-subunit [20].

Peptidyl methionine sulfoxide can be readily converted back to the native thioether by the enzyme peptidyl methionine sulfoxide reductase (MSR) provided that the enzyme is expressed in the local tissue and can interact favorably with the sulfoxide. A modified buried sulfoxide might not be accessible to reduction unless the protein was unfolded or became degraded. Multiple turnovers of MSR require that it be regenerated via thioredoxin, thioredoxin reductase, and NADPH [21]. Deficiency in any of the components necessary for MSR turnover can be predicted to result in accumulation of MetO. The enzyme MSR is assumed to be ubiquitous [21] and has been detected in a number of plants. The enzyme has been cloned and its expression confirmed in *Brassica napus* [22]. Yeast cells, where expression of MSR has been knocked out, are sensitive to oxidative stress and peptidyl methionine sulfoxides accumulate confirming the essential role of this enzyme as an antioxidant in vivo [23]. Oxidation of methionine to the sulfone is less frequent but irreversible.

Cysteine

Proteins containing free thiols have the potential to be readily oxidized to mixed disulfides in the presence of oxygen (S-thiolation). Partners may include other proteins or more usually small free thiols such as glutathione, γ-glutamylcysteine, and cysteine. Thus, a decrease in free thiol [24,25] can be a useful measure of protein oxidation. Such a technique detects oxidation in vertebrate cell

lines as well as thiol regeneration after insult [26], and it was suggested this reversible modification might serve a protective function. Protein disulfide isomerase may play a role in reversal [27]. Free thiols can be modified in reactions with AOS to the progressively more oxidized sulfenic (RSOH), sulfinic (RSO_2H), and sulfonic acids (RSO_3H). Sulfenic acid can react with a free thiol to generate a disulfide providing a pathway for reversibility, but the latter higher oxidation states are effectively irreversible. Thus, it makes considerable sense for plants to tie up free thiols as disulfides during oxidative stress. The disulfide itself is not completely resistant and maybe cleaved oxidatively generating the more highly oxidized species above.

Irreversible Modifications

All of the amino acid residues of proteins are susceptible to oxidative modification, and the vast majority of these covalent changes are irreversible. Side chain modifications, backbone cleavage, and cross linking are all possible [28,29]. The protein becomes permanently modified raising the possibility for aberrant behavior. Thus, it is thought that oxidized proteins are generally targeted for proteolysis and degradation [29,30] leaving the deficit to be replenished by protein synthesis. Systems for recognition and removal of oxidized protein are ubiquitous owing to the unavoidable consequences of ionizing radiation. Damage may result from direct absorbance of radiation by protein or via attack by hydroxyl radical generated by radiolytic cleavage of water even in the absence of oxygen. The extreme reactivity of the latter species precludes complete protection via scavengers. Under normal conditions, oxidized protein is removed efficiently, however, overload of the system leads to accumulation of damaged molecules [31]. The different AOS have been described in detail elsewhere [32–34] and will be referred to in specific examples. Irreversible modification can also result from the reactivity of other products of oxidative damage. For example, malondialdehyde, which is a by-product of lipid peroxidation, can modify peptidyl amino acid residues contributing to protein damage.

EXPERIMENTAL APPROACHES FOR ANALYSIS OF OXIDATIVE DAMAGE

Analytical Biochemistry

Assays

Until recently, most studies of protein oxidation involved the use of assays to measure some general feature associated with protein oxidation. Assays of protein carbonyl [35], free thiol [25], or methionine sulfoxide [36] have all been used. Although these assays have provided important indications of cellular oxidative stress, their nonspecific nature provides no indication as to the identity of the damaged proteins.

Molecular Characterization Using Mass Spectrometry

Once the goal of the experiment progresses to the identification of the targets of oxidative damage, the criteria required of the methodology become much more rigorously defined. Proteins must be separated from the biological milieu and unambiguously identified prior to localization and mapping of the sites of sensitivity. Definitive studies of protein oxidation will rely increasingly on mass spectrometric analysis of potential targets.

The accuracy of mass measurements that may now be achieved, around 0.01% with a quadrupole machine, is sufficient to observe the first and subsequent oxidations (usually + 16 Da) on protein molecules up to 50 kDa and beyond. Electrospray-ionization (ESI) is the technique of choice because of the superior resolution that is usually observed. Thus, the first oxidation adduct (+16 Da) may be resolved next to the unmodified protein. Matrix-assisted laser desorption ionization combined with time of flight detection (MALDI-TOF) can achieve favorable accuracy but poorer

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Table 1 Techniques for Analysis of Molecular Protein Damage

Method	Performance[a,b]	Limits of detection (with respect to oxidative modification)
SDS-PAGE	Accuracy 2–10%; poor resolution	Major molecular weight changes. Gross changes in polypeptide mobility, cross linking/polymerization, backbone cleavage.
MALDI-TOF	Accuracy to 0.01%; moderate resolution	Subtle molecular weight changes provided all species are similarly modified. Detects the alteration of mass of the most dominant species in a mixture.
Electrospray-ionization (ESI-MS)	Accuracy $< 0.01\%$; good resolution	Detects appearance of low-abundance (<10 %) adducts compared with major species. Resolves earliest site of oxidation.

[a] Accuracy: % error = (measured mass − actual mass) × 100%/actual mass.
[b] Resolution: ability to detect small amounts of a species of mass close to the most abundant species in a mixture.

resolution. Thus, information is lost, because less abundant adducts are obscured by the most abundant form. In the case of increasing oxidation, this will manifest itself as a measurable increase in mass concomitant with broadening of the peak. This situation is favorable compared with sodium dodecylsulfate–polyacrylamide electrophresis (SDS-PAGE), which only reveals more extensive changes in mass that alter electrophoretic mobility (Table 1).

In a sample of mixed proteins, it is usually necessary to separate the individual components prior to mass spectrometry (MS). MALDI-TOF has a reasonable capacity to deal with a protein mixture, but ESI requires highly purified material, particularly when low-abundance adducts must be observed. Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) is commonly used to separate a complex mixture of proteins in a biological sample and several groups have applied MS to identify and characterize individual polypeptides after 2D-PAGE [37,38]. However, there are severe drawbacks to using this approach for studies of protein oxidation. The exposure of proteins to SDS-PAGE and the extraction procedures required to recover protein for MS analysis routinely lead to oxidation of methionine and acrylamide adducts of cysteine as well as other modifications even in samples that have not experienced an oxidative insult. Furthermore, proteins are usually fragmented by in-gel digestion prior to extraction, since smaller peptides are more easily recovered. The peptide maps that are subsequently obtained by MS allow identification of the protein by comparison to fragments predicted from genome data, but they are frequently incomplete, especially in the case of membrane-bound proteins [38]. Thus, a potential for lost information arises when modified peptides are not recovered. Consequently, it is desirable to obtain mass spectra of the intact proteins from oxidized samples to compare with controls. In this way, the entire molecule can be screened for the earliest signs of oxidative modification. Protocols have been presented for the recovery of intact proteins from SDS gels [39], but routine problems of low yield, especially in the case of larger proteins, persist. Fortunately, attractive alternative methodologies are available to

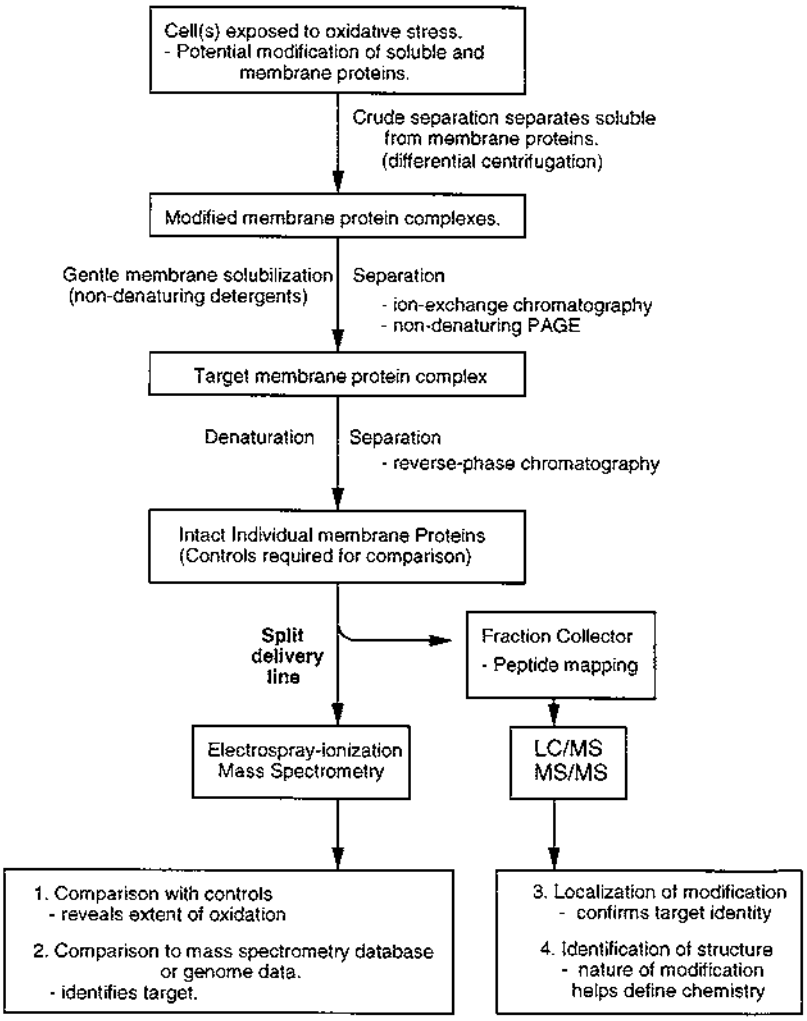

FIGURE 1 Global analysis of the earliest stages of protein oxidation. A flow chart is shown that summarizes an approach that is designed to reveal the identities of the oxidized proteins and their sites of modification. Central to the technique is the use of electrospray-ionization mass spectrometry (ESI-MS). The accuracy and resolution that ESI-MS provides ensure that the earliest stable covalent modifications accompanying oxidative damage to protein can be detected. The scheme that is shown is specifically for the analysis of intrinsic membrane proteins, which have recently yielded to ESI-MS analysis. (From Ref. 40.)

achieve the overall MS goal without the use of SDS-PAGE. Figure 1 depicts a flow chart for the analysis of a sample of oxidatively damaged membrane protein.

High-performance liquid chromatography (HPLC) is considerably more compatible with MS than SDS-PAGE and reverse-phase separations can be directly coupled to ESI (LC/MS). Since buffers and solvents are routinely degassed, the potential for in vitro oxidation is dramatically reduced. A single reverse-phase separation can provide excellent resolution, but it must be combined

with another method to provide the separating power of 2D-PAGE. Ion-exchange chromatography is an excellent candidate (see Fig. 1) to achieve separation in the first dimension prior to multiple reverse-phase LC/MS runs. Unfortunately, these runs are usually performed one at a time, so that 2D-HPLC is more time consuming than 2D-PAGE. After LC/MS has been used to find proteins that appear to be oxidized, it is necessary to identify them and localize the sites of oxidation. By splitting the fluid line that runs from LC to MS (see Fig. 1), it is possible to collect fractions and perform the MS analysis simultaneously. Thus, fractions are available for subsequent enzymatic or chemical cleavage. Cyanogen bromide treatment is especially favored owing to the compatibility of the reaction conditions with solubilization of intrinsic membrane proteins [40,41] as well as the potential to map sites of methionine oxidation in conjunction with mass spectrometry [42].

The oxidation products of methionine resist CNBr treatment providing a facile technique for their localization [43,44]. The loss of the CNBr site leads to the disappearance of two smaller peptides concomitant with the appearance of a larger one derived from the smaller two connected by the modified methionine residue [42]. By using mass spectrometry, it is possible to identify these peptides by mass providing a rigorous test of chemical identity that further distinguishes sulfoxide from sulfone. Occasional CNBr cleavage failure has been noted and used as the grounds not to use CNBr mapping for identifying sites of methionine oxidation [45]. The use of MS effectively overcomes this argument, since oxidized sites of cleavage inhibition are distinguished from those at which failure was due to unmodified methionine which is converted to homoserine during CNBr treatment. Mass-based CNBr mapping represents a powerful means with which to characterize methionine oxidation [42]. Of course, there are other modifications that can accompany oxidative damage. Such modifications would not remove CNBr sites, but would lead to increases in mass of peptides containing oxidized residues. The sites of modification must then be identified by other means. A powerful means of achieving this goal is to use tandem MS. A peptide is selected in the vacuum of the first mass spectrometer and then is directed into a second chamber containing inert gas molecules where collision-induced dissociation (CID) leads to limited fragmentation prior to final analysis of the peptide ion fragments in the second mass spectrometer [46,47]. In a successful experiment, deconvolution of the different fragment ions provides a description of the chemical structure of the peptide revealing its sequence and modification site.

A recent variant of ESI termed ''nanospray'' can be useful for MS and MS/MS analyses of small quantities ($<$1 pmol) of peptide [48]. However, care must be taken during studies of protein oxidation, since it is reported that low-flow electrospray ionization can quite effectively oxidize methionine residues [49]. Controls involving nonoxidized material must be incorporated at all stages of the analysis.

In summary, mass spectrometry of intact proteins is used to identify those that are oxidized. Further analyses involving the extensive use of MS are then used to identify sites of modification. In this way, it is possible directly to monitor the earliest events in oxidation and identify the sites most susceptible to damage.

There are limitations to the application of MS. The approach relies on measurable change of mass, and therefore ''silent'' modifications may go unnoticed. Fortunately, most stable oxidative modifications involve the addition of oxygen and can therefore be observed. Disulfide formation (loss of 2 Da) is unlikely to be detected by analysis of the intact protein, and studies involving this specific type of oxidative modification must use different strategies to detect their formation. Mass-based titration of free thiols with thiol reagents could provide a convenient way to approach such a problem using MS of intact proteins before and after oxidative treatment.

In Vitro Oxidation

Various agents can be used to generate AOS in the test tube allowing in vitro oxidation of plant and other proteins. Such experiments may be performed for comparison with damage occurring in vivo to test the nature of the damaging species in vivo. Studies of the sensitivity of important targets also are performed and may be used to probe structure and function. Any chemical system that

generates AOS or other reactive species can be used to modify proteins. Commonly used reagents are peroxide or cloramine T for methionine oxidation to sulfoxide. Tryptophan and cysteine residues may also be oxidized [21]. Fe(II)/peroxide systems may be useful for hydroxyl radical generation, and illumination of certain dyes such as Rose Bengal may be used to generate singlet oxygen [21,33] with both systems oxidizing a wider range of amino acids. Extreme care must be taken when extrapolating the behavior of AOS from the test tube to the cell.

Reverse Genetics

Reverse genetics provide a powerful tool for the analysis of protein oxidation in plants and other systems. The relative abundance of plant transformation systems has enabled several groups to examine gene function in vivo by targeted mutagenesis. Such studies range from site-directed mutagenesis studies that alter a single specific amino-acyl side chain (with potentially dramatic changes in the level of expression) to studies that delete genes. A useful approach involves the upregulation or downregulation of the expression of a gene such that the overall level of accumulation of a specific protein is changed providing the chance to observe the importance of that protein and the ability of others to be upregulated in the light of deficit. Examples of these techniques follow.

Site-Directed Mutagensis

In this example, the electron transport pathway through the photosystem II (PSII) membrane pigment–protein complex was manipulated such that the supply of electrons for reduction of the photooxidized primary donor $P680^+$ was limited. Under these conditions, it may be postulated that protein is oxidized to provide electrons leading to covalent modification after reaction with oxygen or other species. Transformation of the chloroplast genome of the green alga *Chlamydomonas reinhardtii* was used to engineer site-directed mutants of the D1 polypeptide PSII subunit [50]. Where aspartate 170 had been replaced by histidine (D170H), asparagine (D170N), or threonine (D170T), normal levels of the reaction center accumulated, although the ability to evolve oxygen was inhibited by 50, 95, or 100%, respectively. The proline (D170P) mutant only expressed PSII at half the level of the others and also was fully inhibited. Biophysical measurements were consistent with a specific defect in the electron donation to the reaction center, but measurements of oxygen flash yields in D170H showed that those reaction centers capable of oxygen evolution did so normally. Apparently, the alterations inhibit the initial binding of manganese as the functional chloroplast oxygen-evolving complex (OEC) is assembled. Thus, the mutants amplify the problem faced by wild-type PSII, namely, the photosensitivity associated with a fully assembled reaction center lacking a donor, awaiting assembly of the OEC. If proteins are damaged before the OEC is assembled, the complex must be removed and the damaged components replaced contributing to the drain that the photodamage/repair cycle imposes on photosynthetic production. It is predicted that protein damage due to donor-side defects will be exaggerated in the mutants allowing identification of the particular areas of modification by HPLC and mass spectrometry, as described above (see section on molecular characterization using mass spectrometry) 2 [51]. Studies like this will contribute to our understanding of the structure and function of PSII and will provide insight into the weaknesses of the current design. Recently, the secondary donor to the reaction center TyrZ was altered to phenylalanine leading to the accumulation of $P680^+$ [52]. Pigments were bleached under these circumstances, although unfortunately the damage to proteins was not assessed in parallel.

Deletion Mutagenesis

The PSII complex is made up of many different proteins and the product of the chloroplast *psb*I gene is known to be intimately associated with the reaction center core [53]. To investigate the role of this low molecular weight membrane protein, expression of the gene was disrupted by insertional mutagenesis effectively deleting it. An *aad*A expression cassette that confers resistance to spectinomycin was introduced through biolistic transformation of *Chlamydomonas reinhardtii* [54]. The

mutants could grow photoautotrophically in dim light, but their sensitivity to high light implies superphotosensitivity of PSII. Exposure of low-light–grown cells to high light and molecular examination of the resulting damage will reveal specific details of the role of this protein in photoprotection of PSII.

Inhibition of Expression

Some of the most revealing studies arise when the expression of a gene is limited rather than eliminated. Transformation of tobacco plants allowed the production of plants containing about 10% of the wild-type level of catalase [55]. Just like the psbI mutants, the plants grew well under low-light conditions. High-light stress, however, leads to the development of white necrotic lesions on the leaves. Although accumulation of H_2O_2 was not detected, leaf necrosis was accompanied by the accumulation of oxidized glutathione and a fourfold decrease in ascorbate suggesting that protective mechanisms were seriously compromised. Increased ascorbate peroxidase and glutathione peroxidase levels indicate that alternative pathways for relief of oxidative stress could be upregulated. Indeed, the elevated expression of ascorbate peroxidase and glutathione reductase has been noted after photoinhibitory insult to *Arabidopsis* [56]. High-light–induced damage to the tobacco plants was prevented under elevated CO_2 illustrating the contribution that photorespiration makes toward oxidative stress.

Elevation of Expression

A common approach to the study of oxidative stress involves the elevation of the amount of a target enzyme followed by tests to investigate whether the resistance to insult is improved. Tobacco plants overexpressing chloroplast Cu-Zn superoxide dismutase were especially resistant to photoinhibition under chilling stress implying a role for superoxide in damage under these conditions [57]. Overexpression of glutathione reductase in poplar chloroplasts generated plants with an increased resistance to photoinhibition apparently due to increased levels of the scavengers glutathione and ascorbate [58]. The expression of mannitol via the introduction of bacterial genes to tobacco chloroplasts increased resistance to oxidative stress by supplementing endogenous antioxidants [59]. Progress toward generating crop plants with an increased stress tolerance is encouraging [60], and it should be possible to diminish oxidative damage to proteins provided they are accessible to scavengers and the aqueous milieu.

Acclimation and Synergy

The expression of many of the components involved in the protection from oxidative stress varies with the acclimation state of the plant. Thus, it is impossible to consider oxidative stress without careful consideration of growth conditions and the recent history of the plant. Furthermore, it is becoming apparent that many factors may contribute to oxidative stress with certain combinations showing considerable synergy. It will be necessary to consider the acclimatory state of plants and the synergistic nature of stress factors in future studies of protein damage.

Nonintrusive Imaging

A long-term goal is the development of systems that allow the remote observation of protein damage without any disturbance of the cell or whole plant. Electron spin resonance was used to monitor the loss of free thiol during heart ischemia [61] providing an early example of this type of work. Other approaches might involve the identification of specific magnetic resonance signals for mapping experiments using magnetic resonance imaging (MRI) or the development of specific probes compatible with positron emission tomography (PET).

Structural Biology

The interpretation of detailed molecular studies on protein oxidation is greatly aided if a high-resolution structure has been determined. Structural information is especially useful when the mecha-

nisms of the reactions are to be considered. By using x-ray crystallography, it was possible to characterize two sites of metal-catalyzed protein oxidation on the enzyme glutamine synthetase [62]. The first oxidation modified the active site, thereby inactivating the enzyme, whereas the second inhibited substrate binding and loosened the structure presumably enhancing its protease sensitivity and turnover [62].

ENDOGENOUS OXIDATIVE STRESS

Wherever AOS are generated there is the potential for protein damage to occur. Damage is usually minimized through the use of enzymes that convert reactive to less reactive species and antioxidants that ''scavenge'' AOS becoming modified themselves. All cellular components are susceptible to damage if given the chance to interact with AOS, and the problems confronting lipids and DNA have become widely appreciated. Damage to proteins, however, may be widespread even at the early stages of oxidative stress. In this section, the chloroplast is considered, because the origins and nature of protein damage are most widely studied, although still poorly understood.

Case Study; PSII

Chloroplasts face special problems associated with oxidative stress. Several AOS are generated as by-products of the photosynthetic electron transport machinery, but the organelle cannot be made anaerobic without removal of the water-splitting activity of PSII. Thus, nucleic acids, proteins, pigments, and lipids are potentially exposed to AOS. The lipids of the thylakoid have highly unsaturated fatty-acyl chains making them especially susceptible to the peroxidation chain reaction. Highly developed scavenging systems provide effective protection mechanisms, but may be swamped under certain circumstances leading to oxidative damage. Regions with limited access to antioxidants such as the hydrophobic cores of the numerous thylakoid membrane proteins remain especially susceptible to AOS, particularly those that are generated internally. The generation and management of AOS in the chloroplast has been extensively reviewed [32–34]. The focus here will be on molecular protein damage to PSII, since this complex is apparently the site most susceptible to photooxidative damage under normal circumstances.

A number of different manifestations of protein damage within PSII have been identified. Cleavage of the polypeptide backbone was first observed for the rapidly turning over D1 polypeptide, and a 23.5-kDa N-terminal breakdown product was identified that was apparently derived from the N-terminal [63]. More recent studies have equated this fragment with damage derived on the acceptor-side of the reaction center [64]. A 24-kDa fragment derived from the C-terminal of the D1 polypeptide arises under conditions of ''donor-side inhibition'' when the OEC is inactive but electron acceptors are present [65]. Both fragments can be observed after isolation of PSII from photoinhibited pea leaves implying that both mechanisms are significant in vivo [66]. Covalent cross linking of proteins has also been detected after photoinhibitory treatments. Evidence for the formation of a dityrosine cross link within D1 was presented [67], but this modification has not been generally reported. Under moderate illumination, the N-terminal of the α-subunit of cytochrome b_{559} can become linked to D1 in the region 239–244 [68], generating a 41-kDa adduct. Cross linking of D1 to other components, including the CP43 polypeptide, has also been reported [69]. Methionine sulfoxide was detected in low molecular weight polypeptides of PSII after the photoinhibitory treatment of the reaction centers in vitro [17] demonstrating that more subtle oxidative modifications also take place. Most studies have relied on SDS-PAGE for the detection of modifications, so it is unlikely that minor changes in the molecular mass are detected. Thus, it is not clear whether the observed phenomena are related to the earliest events in oxidative damage or whether they are simply the first *observable* events. As described above (see section on molecular characterization using mass spectrometry), MS will be the tool of choice for future studies of PSII damage. Now that the D1 and D2 proteins can be weighed with accuracy exceeding 0.01% [40], it will be possible to identify

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material showing the characteristic + 16-Da mass shift accompanying the first addition of oxygen to the structure.

Damage to PSII proteins is likely to be caused by AOS which are generated within the complex itself. The potential for PSII to generate singlet oxygen is now well established [70]. Charge recombination in the reaction center ($P680^+$, $Pheo^-$) leads to generation of the P680 chlorophyll triplet that is quenched by oxygen, thereby exciting it to the singlet state [71]. The reactivity of this species has been suggested to underlie the sensitivity of PSII to photodamage [72,73] that demands the turnover of reaction center polypeptides and the repetitive reassembly of the complex [74]. Since singlet oxygen is generated within the hydrophobic core of the complex, it is assumed that local pigments and amino-acyl residues are the sites of damage [71] explaining the necessity for disassembly. It is further conceivable that the pigments and protein around the donor side of PSII are directly oxidized by $P680^+$, particularly if the secondary donor tyrosine Z is also oxidized [51]. Since photoinactivation is faster in the absence of oxygen [75], it could be argued that the production of singlet oxygen is a protective measure. The lifetime of singlet oxygen (μs–ms) may provide enough time for diffusion to a local strategically placed quencher, thus preventing direct oxidation of local protein or chlorophyll by $P680^+$. Carotenoids in the reaction center can probably quench singlet oxygen [76], although they may be oxidized themselves in the process [77–79]. The position of the carotenoids is critical, highlighting the need for a high-resolution structural determination of the reaction center. Unfortunately, the complex has not yet produced highly ordered crystals, which may be due to the heterogeneity displayed by the reaction center subunits [40]. Examination of the sites of damage under conditions of singlet oxygen production will provide important insights into the role of this AOS in vivo.

Superoxide is produced at the acceptor side of PSII via the direct reduction of molecular oxygen by the first quinone acceptor QA^- [80]. Evidence was presented that the high-potential form of cytochrome b_{559}, an intrinsic part of the reaction center itself, is a superoxide dismutase [80]. Thus, photoproduction of hydrogen peroxide at the acceptor side of PSII is observed, although its physiological concentrations are kept low by catalase. During photoinhibitory stress, it is possible that the production of peroxide might exceed the capacity for its removal leading to potential protein damage by this species. If cytochrome b_{559} were converted to its low potential form, the more reactive superoxide might also accumulate as superoxide dismutase activity was lost. Some light-induced modifications to PSII apparently still occur under anaerobic conditions. It could be that these rely on hydroxyl radical species which may be generated in the absence of oxygen by, for example, radiolytic cleavage of water. The role of the bound metal ions of PSII, manganese, and iron may be of significance in this respect.

ENVIRONMENTAL STRESS

Many environmental stresses may be linked to oxidative stress. Pollutants such as ozone contribute directly as an AOS, whereas others such as the oxides of nitrogen may generate reactive nitrogen species (RNS). Reactive chlorine and other halogen-containing species also are predicted to contribute to the protein damage within a similar framework as oxidative stress [81]. Although moderate levels of pollutants may be tolerated under favorable conditions, it is likely that adverse effects will be observed under combinations of multiple stresses. Molecular analysis (see section on Experimental approaches for analysis of Oxidative Damage above) will help to reveal the specific role of environmental pollutants in the exacerbation of oxidative stress. Recent studies with ozone reveal the susceptibility of methionine and aromatic residues to oxidation and the dependence of the modification rate with the tertiary and quaternary structures of the protein [82].

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26

Plant Responses to Air Pollution and Heavy Metal Stresses

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INTRODUCTION

The term *stress* was introduced into the scientific field by the American physiologist Cannon. His student, physician, and biologist, Selye, introduced the general theory of stress to explain the physiological response of an organism to impacts from both external and internal environments. The effect of stress on plants has been defined by Larcher [1,2] as the exposure of plants to extremely unfavorable conditions. Thus, it is not necessarily a threat to the life of a plant, but it alerts the alarm response in the plant organism (defense and adaptation reactions). The problem of stress and its effect on plants has been studied in detail by Levitt [3].

In general, stress factors were divided into natural and anthropic. Stolina [4] has distinguished predispositive, specific, and incidental stressors. From the time effect point of view, stress has been differentiated as short time, medium time, and permanent [5]. Tesche [6] considered the intensity of the stress effect as being important. The plant response to natural stress factors, such as water and nutrient deficiency, has been described in many published reports [7–10]. However, in connection with the widespread forest decline in Europe, it has been shown that natural and antropic stressors act together (especially emissions) [11–17].

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EMISSIONS AND HEAVY METALS

In recent decades, plants have been intensively affected by the anthropic permanent acting stressors (emissions). This was compounded by the synergic effects of natural stress factors (extreme temperatures, drought). These factors have led to the widespread deterioration in forest health in Central Europe and North America [18,19]. Although the causes of this phenomenon have not been sufficiently explained, it is generally assumed that the forest decline is due to stress from emissions. Thirteen basic hypotheses have been written to explain the cause of the forest decline in Europe; two of which, ''stress'' [20] and ''ecostress'' [21], explain the forest decline on the basis of the stress theory.

According to the effect of emissions on plants, three basic categories have been distinguished: (a) ''disturbance,'' typical stage of excitatory metabolism without external visually observable symptoms; (b) ''injury,'' involving latent (physiological) injury with no visual symptoms or injury with visual symptoms (chlorosis, necrosis); and (c) dying out or death of plants [22].

Ecophysiological, Biochemical, Anatomical, and Productional Characteristics of Beech (*Fagus sylvatica* L.) Leaves from Regions with Varying Degrees of Emission Impact

Air pollutants influence the physiological processes in woody plants much earlier than in the case of depigmentation or necrosis. Air pollution penetrates into the assimilation organs through the stomata or a damaged cuticle. It also has a negative effect on the photosynthetic activity of woody plants; for example biochemical processes (enzyme activity and chloroplast ultrastructure) as well as chlorophyll content. However, the most frequent statement heard is that pollution induces a decrease in the photosynthetic [e.g., see Refs. 23–27] and transpiration rates [28–30]. In addition, structural and functional changes at the cellular level induce anatomical and morphological changes in tissues and leaves.

In this section of the chapter, the comparison of physiological, anatomical, and productional characteristics of sunlit and shady beech leaves growing under various emission impacts are reported. The results follow up our earlier reports [31–34].

Ecophysiological, biochemical, anatomical, and productional characteristics were estimated on the leaves of branches (about 1.5 m long), which were cut from undergrown and subdominant individuals (shady leaves) or from the dominant tree (sunlit leaves). Three beech experimental sites, situated in Central Slovakia (central Europe), with varying levels of emission impact, distance from emission source (aluminium factory in Žiar nad Hronom), and states of health, were chosen for a comparison study. These were the ecologicoexperimental permanent site, Kremnické vrchy—control site (no emission impact); permanent research site, Jalná—variant 1 (slight emission impact); and research monitoring site, Žiar nad Hronom—variant 2 (strong emission impact). The characteristics of the experimental sites are given in Table 1. The physiological characteristics were measured under both laboratory and outdoor conditions (for details see Ref. 35).

It was found that values in the mean daily net photosynthetic rate (P_N) measured outdoors showed a higher variability (Table 2) caused by variable microclimatic conditions (especially irradiance) during measurement. The mean daily P_N was higher in sunlit leaves than in the leaves of undergrown individuals or in shady leaves. Leaves of the undergrown individuals from variant 2 had approximately a five-times higher P_N than had the shady leaves. From comparison of the sites, it is obvious that the mean daily P_N has a decreasing tendency from the control site to variant 1 and variant 2. The statistical evaluation of the above-mentioned differences is presented in Table 3. However, differences in irradiance values (E_e) were not always statistically significant. These findings confirmed the reality that the leaf photosynthetic activity was influenced by other factors than the light conditions, which was the most important factor for carbon dioxide uptake. We surmise

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TABLE 1 Basic Characteristics of the Experimental Sites

Characteristic	Experimental site		
	control	variant 1	variant 2
Location	Kremické vrchy	Štiavnické vrchy	Štiavnické vrchy
Exposition	W	W	NNW
Altitude (m a.s.l.)	470–490	610	470
Average age (years)	85	69	60
Stocking	0.8–0.9	0.8	0.7
Parent rock	Dark pyroxenic andesites, tuffs, sandstones	andesites	rhyolite agglomerates
Soil type	Cambisol	Cambisol	Cambisol
Forest type group	Fagetum pauper in feriora	Querceto-Fagetum	Fagetum pauper
Average annual temperature (°C)	6.8	6.2	7.6
Average annual precipitation (mm/year)	857	850	750
Distance from emission source (km)	18	7	1.5
Average defoliation of leaves (%)	7.3	15.2	36.7

Control (C): Ecologicoexperimental permanent site Kremnické vrchy (no emission impact from aluminium factory in Žiar nad Hronom).
Variant 1 (V1): Permanent research site Jalná (slight emission impact from aluminium factory).
Variant 2 (V2): Research monitoring site Žiar nad Hronom (strong emission impact from aluminium factory).
Source: From Ref. 35.

TABLE 2 Values of Net Photosynthetic Rate (P_N) in Beech Leaves and Irradiance (E_e) During Photosynthesis Measurements

Site	Leaf type	P_N (mg CO_2 $m^{-2}s^{-1}$)		E_e (Wm^{-2} PhAR)	
		$\bar{x}$	c_v	$\bar{x}$	c_v
Control	Sunlit	0.2501	41.3	193	56.0
	Shady	0.1363	80.5	207	54.1
	Undergrown Individuals	0.2227	42.9	104	91.3
Variant 1	Sunlit	0.2862	36.1	184	8.2
	Shady	0.1184	88.7	204	50.0
	Shady	0.0365	181.6	188	58.0
Variant 2	Undergrown Individuals	0.1913	54.9	105	63.8

$\bar{x}$, mean; c_v, coefficient of variance (%).
Source: From Ref. 35.

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Table 3 Statistical Significance of Differences in Net Photosynthetic Rate (P_N) and Irradiance (E_e) Among Leaf Types According to Site and Among Sites According to Leaf Type

	P_N			E_e		
Site	S-T	T-P	S-P	S-T	T-P	S-P
Control	*	N	N	N	N	N
Variant 1	***	—	—	N	—	—
Variant 2	—	*	—	—	N	—
	P_N			E_e		
Leaf type	C-V1	V1-V2	C-V2	C-V1	V1-V2	C-V2
Sunlit	N	—	—	N	—	—
Shady	N	*	*	N	N	N
Undergrown individuals	—	—	N	—	—	N

S, sunlit leaves; T, shady leaves; P, leaves of undergrown individuals; N, statistically not significant differences; $P = .95$ (*); $P = .99$ (**); $P = .999$ (***); C, V1, V2, see Table 1.
Source: From Ref. 35.

that in both physiological characteristics, photosynthesis and the function of the stomata apparatus, the decisive factor was the different degree of emission impact.

The mean daily values of the transpiration rate (TR) measured simultaneously with the P_N measurements, are presented in Table 4. The TR values for both leaf types increased from the control site to variant 2. These findings are in agreement with the tendency found for the water loss curves (Figs. 1 and 2). We suppose that in the case of variant 1 and variant 2 in comparison with the control site, the regulatory system for water loss through the stomata as well as physical water loss through the cuticle was disturbed. Significant differences between leaf types were found in all sites studied (Table 5). The water-loss rate in relationship to the transpiration coefficient (TC) rose from the control site to variant 2. The tendency of the TC to increase in relation to the different degree of emission impact was similar to that found for the TR. The only difference was that the TC values of sunlit leaves were lower in comparison with shady leaves, whereas the tendency for the TR was

Table 4 Mean Daily Values of Transpiration Rate (TR) Measured Simultaneously with the Net Photosynthetic Rate (P_N) and the Values of Transpiration Coefficient (TC)

		TR ($mg\ H_2O\ m^{-2}s^{-1}$)		TC ($mg\ H_2O\ mg^{-1}CO_2$)
Site	Leaf type	$\bar{x}$	c_v	$\bar{x}$
Control	Sunlit	7.63	34.8	30.51
	Shady	4.83	37.5	35.44
Variant 1	Sunlit	13.38	22.4	46.75
	Shady	6.71	37.0	56.67
Variant 2	Shady	6.89	36.8	188.77

For explanation, see Table 1.
Source: From Ref. 35.

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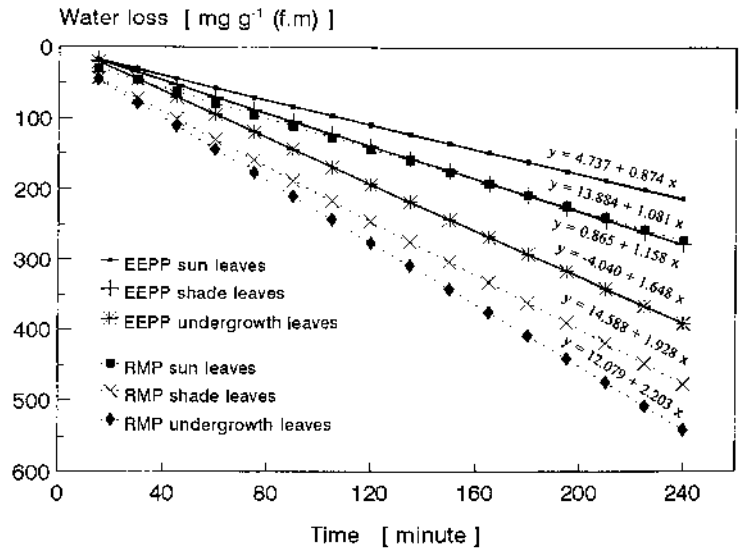

FIGURE 1 Water-loss curves for various types of leaves according to the test sites in 1989. Control, ecologicoexperimental permanent site Kremnické vrchy; variant 2, research monitoring site Žiar nad Hronom (under strong emissions impact of fluorine type). (From Ref. 35.)

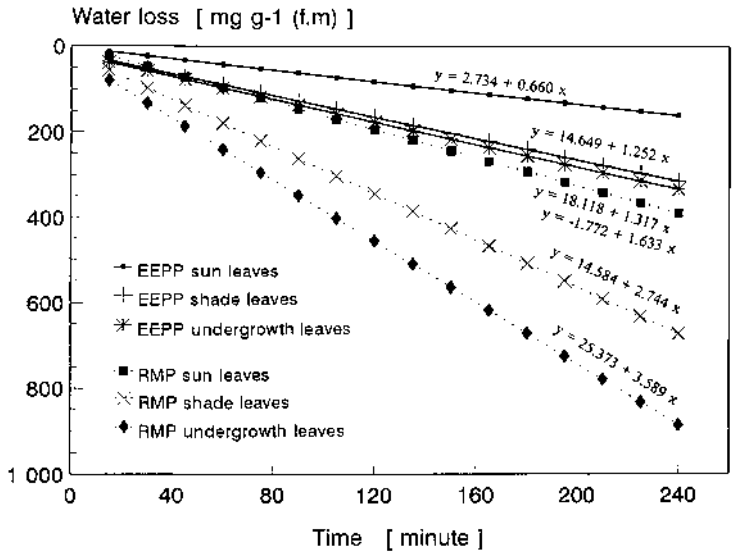

FIGURE 2 Water-loss curves for various types of leaves according to the test sites in 1990. Control, ecologicoexperimental permanent site Kremnické vrchy; variant 2, research monitoring site Žiar nad Hronom (under strong emissions impact of fluorine type). (From Ref. 35.)

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Table 5 Statistical Significance of Differences in Transpiration Rate, (TR) (mg H_2O $m^{-2}s^{-1}$) Between Leaf Types and Sites

Site-leaf type	Control-S	Control-T	Variant 1-S	Variant 1-T	Variant 2-T
Control-S	—	**	**	**	N
Control-T		—	N	*	*
Variant 1-S			—	**	**
Variant 1-T				—	N
Variant 2-T					—

For explanation, see Tables 1 and 3.
Source: From Ref. 35.

opposite. From the dynamics of the TC values we can state that the cost of water production increased depending on the degree of the emission load, which means that the leaves from sites with a higher degree of emission impact needed for assimilation of 1 mg CO_2 a higher amount of water for transpiration than leaves from the sites with a lower degree of emission impact. Figures 1 and 2 show that water-loss rate of various leaf types as well as various sites studied using the method of water-loss curves. The water loss was more intensive in the leaves of undergrown individuals than in shady or sunlit leaves (Table 6). On comparing leaf types from the various research sites, the water loss was significantly higher in the case of trees from variant 2 than that in trees from the control site (Table 7). The relationship between the water loss rate and the time was linear, with very high values of correlation coefficient (Table 6; see Figs. 1 and 2).

On analyzing the stomata apparatus, it was found that the smallest stomata were in the leaves of undergrown individuals, but the highest values of stomata density (SD) were estimated in the sunlit leaves of adult trees. At variant 2, it was observed that the leaves of undergrown individuals had higher values of SD than the shady leaves (Table 8). Comparing only the shady leaves, a decreasing tendency for all quantitative parameters of stomata apparatus (SD, SL, SW) was apparent from the control site to variant 1 and variant 2. The shady leaves had at the same time the highest SD variability of all sites studied. The quantitative analysis confirmed that significant differences existed not only between different leaf types but also between sites with different emission impact (Table 9).

Table 6 Linear Relationship Between Time of Test (Minutes) and Water-Loss Rate, v (mg g^{-1} fresh mass min^{-1})

		Correlation coefficient			
Site	Leaf type	1989	1990	1989	1990
Control	Sunlit	0.939	0.911	0.87	0.66
	Shady	0.971	0.982	1.16	1.25
	Undergrown Individuals	0.977	0.995	1.65	1.32
Variant 2	Sunlit	0.959	0.988	1.08	1.63
	Shady Individuals	0.989	0.974	1.93	2.74
	Undergrown Individuals	0.990	0.988	2.20	3.59

For explanation, see Table 1.
Source: From Ref. 35.

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TABLE 7 Statistical Significance of Differences in Water Loss Rate (v) Among Control Site (Kremnické vrchy) and Variant 2 (Žiar nad Hronom) According to Type and Among Leaf Types According to Site in 1989–1990

	Year			1989			1990		
Leaf type	1989	1990	Site	S-T	T-P	S-P	S-T	T-P	S-P
Sunlit	N	***	Control	N	*	***	***	N	***
Shady	***	***							
Undergrown individuals	*	***	Variant 2	***	N	***	**	N	***

For explanation, see Tables 1 and 3.
Source: From Ref. 35.

Chlorophyll a, b, and $a + b$ contents (expressed per leaf area) as well as the chlorophyll a:b ratio were investigated (see Table 8). The sunlit leaves from variant 1 had the highest values of chlorophyll content and the leaves of undergrown individuals from variant 2 showed the lowest chlorophyll levels. The differences in leaf type as well as in research sites were significant (Table 10). The finding that in shady leaves the chlorophyll content increased, whereas the emission impact increased seems unusual. However, the opposite tendency was observed in the leaves of undergrown individuals. This can possibly be explained when one remembers that saplings at variant 2 had grown in quite different light conditions than the trees at the control site (crown overlighting in consequence of the tree defoliation (see section on Responses in Tree Foliation below and Table 1).

In general, the sunlit leaves of the Control site had the highest values in leaf area (A), and the lowest values for these productional parameters occurred in the leaves of undergrown individuals from the same site; that is, the control site (see Table 8). It was found (variants 1 and variant 2) that the shady leaves always possessed a larger leaf area than the sunlit leaves. The opposite tendency was observed at the control site. In all research sites, the leaves of undergrown individuals had the smallest leaf area. The leaf area of the sunlit leaves decreased from the control site to variant 1 and variant 2, and the leaf area of the shady leaves decreased from variant 1, the Control site to variant 2. In the case of each research area studied, the values of the specific leaf area (SLA) increased from the sunlit and the shady leaves up to the leaves of undergrown individuals (see Table 8). Similarly, as for both the A and SLA of the sunlit leaves and the leaves of undergrown individuals, the values of these parameters (A and SLA) decreased from the control site, variant 1 to variant 2. As follows from the analysis, the values of the specific leaf mass (SLM) in comparison with the values of SLA decreased in the order: sunlit and shady leaves and leaves of undergrown individuals (see Table 8). Comparing the research sites, the SLM values of the sunlit leaves and the leaves of undergrown individuals increased from the control site through variant 1 to variant 2. In the case of shady leaves, the SLM values were relatively steady. Statistical evaluation of A values is stated in Table 11. Analysis of the productional parameters showed that leaf areas in variant 2 were the smallest; however, differences between sunlit and shady leaves were not conspicuous, which was in agreement with the high degree of tree defoliation (see Table 1). This is documented by the fact that these shady leaves do not have a typical shady character as in the case of leaves coming from healthy stands of the Control site where the differences between the leaf types were significant.

Our results confirmed the findings of other investigators [13,36] that the photosynthetic rate is negatively influenced by the air pollutants. In our experience, the leaf damage of trees growing on the forest stand at the Control site was caused by the synergism of air pollution and soil drought (long-term period in summer months without rain). This is borne out by the water-loss curves which characterized leaf (and/or plant) capacity for water maintenance. We also observed cuticle transpiration, which is the most suitable parameter for assessment of water-maintenance capacity. Water-

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TABLE 8 Anatomical, Biochemical, and Productional Characteristics of Sunlit and Shady Leaves, Respectively, and Leaves of Undergrown Beech Individuals

Characteristics		Sunlit leaves			Shady leaves			Leaves of undergrown individuals	
		control	variant 1	variant 2	control	variant 1	variant 2	control	variant 2
Stomata density, SD (No. mm^{-2})	$\bar{x}$	171	189	—	135	129	122	103	133
	c_v	11.8	9.8	—	15.5	11.5	16.2	14.4	13.5
Stomata length, SL (μm)	$\bar{x}$	27.4	27.7	—	28.2	26.7	26.1	25.7	24.9
	c_v	9.4	8.5	—	8.6	6.8	6.7	6.1	5.8
Stomata width, SW (μm)	$\bar{x}$	25.9	26.0	—	25.7	25.6	25.2	24.9	23.9
	c_v	6.0	5.4	—	5.5	4.6	3.9	4.6	5.4
Chlorophyll content, Chl (mg dm^{-2})	$\bar{x}$	3.004	3.849	—	2.214	2.337	2.297	2.295	1.681
Chla	c_v	7.2	9.8	—	3.4	2.9	1.8	4.4	3.6
Chlb	$\bar{x}$	1.000	1.315	—	0.834	0.834	0.774	0.923	0.592
	c_v	7.6	17.8	—	2.0	11.5	6.7	7.5	10.5
Chl(a + b)	$\bar{x}$	4.003	5.164	—	3.048	3.216	3.071	3.218	2.273
	c_v	6.9	11.8	—	3.0	5.2	2.9	5.3	0.5
Chlorophyll *a*:*b* ratio (Chla:b)	$\bar{x}$	0.010	2.977	—	2.654	2.683	2.978	2.493	2.884
	c_v	5.4	9.5	—	1.5	8.1	5.4	3.03	15.2
Leaf area, A (cm^2)	$\bar{x}$	25.89	19.08	17.66	19.53	22.03	18.65	11.23	13.13
	c_v	30.6	35.9	36.1	35.7	38.0	37.6	46.0	28.0
Specific leaf area,SLA ($dm^2\ g^{-1}$)	$\bar{x}$	1.82	1.42	1.13	2.77	3.19	3.59	4.58	3.98
Specific leaf mass,SLM (g dm^{-2})	$\bar{x}$	0.55	0.70	0.89	0.36	0.31	0.29	0.22	0.25

For explanation, see Tables 1 and 2.
Source: From Ref. 35.

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TABLE 9 Statistical Significance of Differences in Stomata Density (SD), Stomata Length (SL), and Stomata Width (SW) Among Leaf Types According to Site and Among Sites According to Leaf Type

	SL			SW			SD		
Site	S-T	T-P	S-P	S-T	T-P	S-P	S-T	T-P	S-P
Control	*	***	***	N	***	***	***	***	***
Variant 1	**	—	—	*	—	—	***	—	—
Variant 2	—	***	—	—	***	—	—	***	—
	SL			SW			SD		
Leaf type	C-V1	V1-V2	C-V2	C-V1	V1-V2	C-V2	C-V1	V1-V2	C-V2
Sunlit	N	—	—	N	—	—	***	—	—
Shady	***	*	***	N	*	**	*	**	***
Undergrown	—	—	***	—	—	***	—	—	***

For explanation, see Tables 1 and 3.
Source: From Ref. 35.

loss values in this phase are equivalent to the water-loss rate while the plants are stressed by soil drought or plant wilting [28].

Significant differences in the photosynthetic rate between all research sites studied (for both leaf types) were caused by long-term drought (June and July) during the growing season. The most conspicuous negative effect was in the case of trees growing under intensive emission impact (variant 2).

On the basis of our findings, it is possible to conclude that the leaves of trees growing at the Control site had better water-maintenance capacity (or higher resistance to drought) than the leaves of trees growing at variant 2 where this mechanism was disturbed. Furthermore, the highest water-maintenance capacity was observed in sunlit leaves and the lowest in the leaves of undergrown trees. The shady leaves showed a higher water-loss rate than the sunlit leaves (control site and variant 2) which resulted from the different anatomical structure of the leaves as well as (in the case of variant 2) from the greater damage of the subdominant trees in comparison with dominant trees. Similar results were found by others [37,38] using a fumigation chamber for the investigation of F and SO_2 effects. The investigator observed that the fluorine effect caused a decline of the regulatory ability of the stomata resulting in a gradually decreasing stomata and an increasing cuticle transpiration [38]. It is important to emphasize that the transpiration rate in forest trees growing under emission impact depends on tree species, pollutant concentration, and duration of the effect as well as on other ecological factors (e.g., air and soil humidity, air and soil temperature, irradiance, wind). In particular soil drought can be an important predisposing factor and, on the other hand, the water-maintenance capacity may be greatly reduced by emission impact (e.g., see Ref. 39). Our results confirmed these findings.

The stomatal apparatus together with the whole leaf anatomical structure reflect the relatively favorable environmental conditions under which they have grown. In general, the sunlit leaves in comparison with the shady leaves had higher values of SD but the stomata were smaller. This was found in forest trees not only in the present paper but also in the others (e.g., see Refs. 33, 34, 40, and 41). However, absolute values of the stomata number per mm^2 found in our experiments were a little lower than those published in above-cited literature, which could be due to the different age and light conditions or variations in this quantitative leaf parameter [42,43]. Gellini et al. [44] observed in the leaves of damaged beech trees higher values of SD than in the leaves of the control tree. Similarly, Getko and Sergejčik [45] confirmed the effect of air pollutants (compounds of sulfur)

Table 10 Statistical Significance of Differences in Chlorophyll Content (Chl*a*, *b*, *a* + *b*) and Chlorophyll *a*:*b* Ratio Among Leaf Types According to Site and Among Sites According to Leaf Type

	Chl a			Chl b			Chla + b			Chl a:b		
Site	S-T	T-P	S-P	S-T	T-P	S-P	S-T	T-P	S-P	S-T	T-P	S-P
Control	**	N	*	*	N	N	**	N	*	*	N	*
Variant 1	**	—	—	N	—	—	*	—	—	N	—	—
Variant 2	—	***	—	—	*	—	—	***	—	—	N	—
	Chla			Chlb			Chla + b			Chla:b		
Leaf type	C-V1	V1-V2	C-V2	C-V1	V1-V2	C-V2	C-V1	V1-V2	C-V2	C-V1	V1-V2	C-V2
Sunlit	N	—	—	N	—	—	N	—	—	N	—	—
Shady	N	N	N	N	N	N	N	N	N	N	N	N
Undergrown individuals	—	—	**	—	—	**	—	—	**	—	—	N

For explanation, see Tables 1 and 3.
Source: From Ref. 35.

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Table 11 Statistical Significance of Differences in Leaf Area (A) Among Leaf Types According to Site and Among Sites According to Leaf Type

	A		
Site	S-T	T-P	S-P
Control	***	***	***
Variant 1	**	—	—
Variant 2	N	***	***
	A		
Leaf type	C-V1	V1-V2	C-V2
Sunlit	***	N	***
Shady	*	**	N
Undergrown individuals	—	—	**

For explanation, see Tables 1 and 3.
Source: From Ref. 35.

on both the number and size of the stomata in three woody species. This phenomenon is probably caused by an emission impact resulting in stress similar to that caused by drought. Therefore, the leaves adapt anatomically, which means that leaves acquire a xerophilous character (they had higher values of SD). Similarly, undergrown beech individuals in variant 2 showed higher values of SD than the shady leaves of adult trees. This could be explained by injury of the forest stand (decrease in the stocking and crown canopy and a high degree of defoliation). Therefore, the undergrown trees did not receive sufficient shade and the leaves did not exhibit so-called typical shady leaves. This was confirmed by the occurrence of undergrown trees only on overlighted sites. Masarovičová and Minarčic [32] also found that the beech seedlings had leaves with an anatomical structure of an intermediate type; that is, their anatomical structure was between the typical sunlit and shady leaf types. To study the effect of pollutants [46,47] or heavy metals [48] on the chlorophyll content, the leaf anatomical structure should be considered.

Despite the fact that both the leaf growth and the leaf development are determined by internal (genetic) factors, the specific leaf area and the specific leaf mass may be considered to be suitable and sensitive physiological parameters of adaptation to environmental conditions (cf. Ref. 34). Our results show slightly lower values which were probably caused by the natural leaf variability and plant age as well as by the date of sampling. The importance of the correct time for sampling was also confirmed by Gratani et al. [49]. The fact that at variant 2 the sunlit and shady leaves were found with the lowest leaf area was also reported by other investigators [50,51]. Moreover, the sunlit leaves of trees from variant 2 showed a xerophilous character (hard and thin leaves) so their values of SLM were also higher. These results confirmed that the sunlit leaves in comparison with shady leaves were more intensively affected by pollutants, which had been previously observed by Štefančík et al. [52] at the same forest stand.

The growth and leaf photosynthetic activity were also investigated in pollution-damaged oak saplings [27]. Shoots of damaged *Quercus dalechampii* Ten. saplings were shorter and growth lagged behind by more than a week compared with the control shoots. The photosynthetic activity in leaves of damaged trees was significantly reduced. Yet the leaf dark respiration rate was higher in damaged saplings. Changes in both the growth and leaf photosynthetic activities may also be used as a sensitive diagnostic parameter in ascertaining the negative effects of abiotic and/or biotic environmental factors.

Effect of Heavy Metals on Growth and Chlorophyll Content of Some Wild Herbs

The toxic effect of heavy metals is obvious at different levels of the structure and function of the plants: reduction in growth and productivity, changes in membrane structure, metabolic processes, or water and ion uptake. Plants have developed specific mechanisms to enable them to grow and reproduce under contaminated conditions. One of these mechanisms is the presence of barriers such as the production of calose and the efflux of malate and citrate into root surroundings or the production of slime on the root surface (e.g., see Refs. 53–55). Other mechanisms comprise the ability of certain plants to absorb and then immobilize ion uptake by specific proteins (''stress-proteins'' and ''chaperons'') such as phytochelatins and metallothioneins [56–58]. It is accepted [59] that the plant response to metal-enriched soils may involve (a) metal exclusion where there may be either a reduced uptake or restricted transport from root to shoot, (b) a passive uptake where metal concentrations in aerial plant tissues reflect soil concentrations (indicator behavior), or (c) accumulation whereby metals are concentrated in both the root and shoot dry mass after internal complexation and detoxication. It has always been presumed that metal hyperaccumulation must be a mechanism of metal tolerance in which a potentially toxic metal is complexed, translocated, and then compartmentalized in the plant tissues; often being stored as a water-soluble organic acid complex in leaf cell vacuoles. However, metallophytes have evolved several different strategies of physiological response to high soil metal concentrations (e.g., exclusion), in which hyperaccumulation is both an extreme and rare response in serpentine, calamine, and copper/cobalt floras [60]. Tolerance of the plants to heavy metals is either genetically fixed or acquired through adaptation processes. Species of genus. *Agrostis*, *Deschampsia*, or *Silene* produced ecotypes with a tolerance for higher metal concentration (especially Cu, Pb, Zn) in the substrate (e.g., see Refs. 61 and 62). These findings have a practical application in the recultivation of contaminated and degraded soils.

The results of a study on the effect of different Cu concentrations on the growth and chlorophyll content in some wild herb species of tolerant and sensitive populations are presented here. Seeds of *Agrostis stolonifera* L., *Melandrium rubrum* L., and *Rumex acetosela* L. were sampled from the following localities:

Piesky pri Starých horách (region Banská Bystrica, Central Slovakia, tolerant population): piles of soil contaminated by heavy metals.
Staré hory and Borinka (Little Carpathians, SW Slovakia, sensitive population): region with noncontaminated soils.

Soil samples were taken from the same localities as the seeds. Plants grown from seeds were cultivated under controlled conditions in growth chambers (for details see Ref. 63). Concentrations of Cu, Pb, Mn, and Cd in leaves and roots were determined. For plants from tolerant populations, a higher Cu concentration was found in both leaves (1.9 times) and roots (4.2 times) than in plants from the sensitive population. The concentration of Mn was lower in the roots and shoots of plants from tolerant populations (Table 12).

Both the root length and height of the plants decreased steadily with an increasing Cu concentration. For plants from sensitive populations, the greatest decline in the root length occurred at 0.3, 0.6, and 4.0 μM Cu concentrations. For plants from tolerant populations, the decrease in the root length was moderate and most significant at 0.6 and 4.0 μM Cu concentrations. Comparing sensitive and tolerant populations, the most sensitive of all studied species to Cu concentrations was *Agrostis stolonifera* (Table 13). The highest values of index of tolerance, IT (root length of the plant treated with Cu/root length of the plant without Cu and multiplied by 100) were found in all studied species from the tolerant population (Table 14). Pigment analysis confirmed that all chlorophyll components (Chla, Chlb, Chla+b) decreased in *Agrostis stolonifera* leaves of both the sensitive and tolerant populations under Cu treatment. The greatest decrease was found in the Chlb content, which was significantly manifested in the higher values of the Chla:Chlb ratio (Table 15).

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TABLE 12 Content of Cu, Pb, Mn, and Cd in the Root and Aboveground Part of *Agrostis stolonifera* Plants Cultivated in Piles of Contaminated Soil

Content of metal and population	Aboveground part of plants	Root
Cu (mg kg^{-1})		
Sensitive	933	2607
Tolerant	1764	10977
Pb (mg kg^{-1})		
Sensitive	14	16
Tolerant	12	16
Mn (mg kg^{-1})		
Sensitive	125	215
Tolerant	113	170
Cd (mg kg^{-1})		
Sensitive	0.6	0.7
Tolerant	0.6	1.0

Polymetallic ores were mined in the locality of Piesky pri Starých horách from the time of the Middle Ages to the end of the last century. Owing to the mining and processing of metallic ore piles were occupied by the wild herb with herb species (*Agrostis stolonifera* L., *Melandrium rubrum* L., and *Rumex acetosela* L.). Their expansion was limited by deficiencies in humus and nutrients, low soil humidity, and a high content of metals (mainly Cu). One of the first species to be observed was *Agrostis stolonifera*; at present, *Melandrium rubrum* is the dominant species with the highest cover [61,64]. Similarly, Hunter et al. [65] and Karataglis [66] found that species of *Agrostis* are among plants growing in soils contaminated by Cu.

Concentration of 12,700 mg Cu and 70 mg Pb kg^{-1} of soil were estimated at the sites studied. The control samples from Borinka contained only 20–80 mg Cu and 15–50 mg Pb kg^{-1} of soil. Comparable piles of contaminated soil in Europe are found in North Wales at Parys Mountain and Trelogan: 4,360 mg Cu kg^{-1} and 11,900 mg Pb kg^{-1} of soil [66]; Merseyside (NW England): 11,000 mg Cu kg^{-1} of soil [65]; Prescot near Liverpool: maximal concentration 53,000 mg Cu kg^{-1} of soil [67].

The ability of plant populations to tolerate intoxicated substrate depends on their physiological and biochemical adaptation to actual ecological conditions. The basis of this adaptation is ''avoidance'' (barrier against ion uptake) or ''tolerance'' (accumulation and immobilization of ion uptake) (e.g., see Refs. 68 and 69). Our results confirm tolerance as being the basis of plant adaptation.

The metal components also significantly influenced growth processes. Some investigators [61,70,71] found a reduction in biomass production of 40%. The species *Agrostis stolonifera* and *Rumex acetosela* had the lowest height of the aboveground part of the plants on the site studied.

As roots are in primary contact with metal ions, the root growth is influenced faster than shoots. Therefore, Wilkins [72] as well as Macnair [73] suggested the index of tolerance (IT) as the most sensitive parameter for testing root growth processes. It was found that the inhibition of the root growth by Cu ions was greater than in the shoot growth and the reduction of the root length was faster for sensitive plant populations (e.g., see Ref. 74). Our results confirm that all Cu concentrations caused a decrease in the root length and height in tolerant and sensitive plant populations. However, the length reduction was faster and the IT was lower for the plants of sensitive population. The higher IT values for plants in contaminated soil (tolerant population) were also found by others [68,75,76].

The chlorophyll content is one of the most investigated physiological (but not specific) charac-

Table 13 Root Length (mm) of *Agrostis stolonifera, Melandrium rubrum*, and *Rumex acetosela* Plants from Sensitive and Tolerant Populations Cultivated Under Different Cu Concentrations

Species and population	Control 0.0	Cu concentration (μM)				
		0.3	0.6	4.0	8.0	12.0
Agrostis stolonifera						
Sensitive	31.90 ± 0.08	6.38 ± 0.02**	6.65 ± 0.08**	2.26 ± 0.01**	1.52 ± 0.01**	1.77 ± 0.01**
Tolerant	27.70 ± 0.11	26.30 ± 0.13	19.40 ± 0.08	4.30 ± 0.02**	2.50 ± 0.01**	2.70 ± 0.01**
Merlandrium rubrum						
Sensitive	34.00 ± 1.29	—	23.45 ± 0.99	18.75 ± 0.88*	11.80 ± 0.61**	7.85 ± 0.54**
Tolerant	25.50 ± 1.21	—	19.90 ± 1.09	17.40 ± 0.70*	12.55 ± 0.57**	10.40 ± 0.44**
Rumex acetosela						
Sensitive	30.50 ± 1.17	—	27.70 ± 0.97	12.80 ± 0.46**	9.35 ± 0.40**	11.55 ± 0.42**
Tolerant	26.50 ± 0.95	—	22.45 ± 0.78	11.10 ± 0.52**	12.10 ± 0.45**	11.50 ± 0.38**

Significant differences between the control and Cu-treated plants are indicated by * ($P = .05$) or by ** ($P = .01$), respectively.

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Table 14 Mean Values of Index of Tolerance Found for *Agrostis stolonifera, Melandrium rubrum*, and *Rumex acetosela* Plants from Sensitive and Tolerant Populations Cultivated Under Different Cu Concentrations

Species and population	Cu concentration (μM)				
	0.3	0.6	4.0	8.0	12.0
Agrostis stolonifera					
Sensitive	20.3	18.4	7.1	4.3	5.5
Tolerant	96.0	78.8	16.0	8.9	10.3
Melandrium rubrum					
Sensitive	—	69.1	55.2	34.8	23.1
Tolerant		78.1	68.2	49.4	40.8
Rumex acetosela					
Sensitive	—	90.9	41.9	30.7	26.6
Tolerant		84.6	57.7	45.6	43.4

Table 15 Values of Chlorophyll Content (Chla, Chlb, Chla + b) and Chlorophyll *a*:*b* Ratio in Leaves of *Agrostis stolonifera* Plants from Sensitive and Tolerant Populations Cultivated in Control Conditions or with Cu Treatment

Population and Cu treatment	Soil	Pigments (mg g^{-1} [d/m])			
		Chla	Chlb	Chla + b	Chla:b ratio
Sensitive	+Cu	8.15 ± 0.69	3.35 ± 0.37	11.50 ± 0.94	2.93 ± 0.53
Sensitive	−Cu	9.97 ± 0.86	6.33 ± 1.08	16.30 ± 1.52	1.60 ± 1.12
Tolerant	+Cu	8.60 ± 0.38	4.53 ± 0.19	13.11 ± 0.57	1.90 ± 0.01
Tolerant	−Cu	11.26 ± 0.96	8.40 ± 0.79	19.66 ± 1.64	1.33 ± 0.94

teristics used for identification of physiological disturbances due to emission impact [35]. The most frequent finding is that both air pollutants [27,46,47] and heavy metals [48,77] cause a decrease in the total chlorophyll content. Our results also confirmed the decrease of the leaf chlorophyll content in the herbs studied, and they therefore agree quite well with the findings of other investigators [78–82]. Since the chlorophyll *b* content after the Cu treatment decreased faster than chlorophyll *a*, the values in the Chla:Chlb ratio were higher. However, the chla:chlb ratio reduction was lower for the plants of the tolerant population. A similar tendency was found by Masarovičová [77] in oak and by Stiborová et al. [78] in barley and maize leaves.

Our results confirm the presence of a different intraspecific tolerance of the population to heavy metals. These plants were able to adapt to high metal concentrations in the soil and to grow, reproduce, and cover anthropic substrates. Therefore, tolerant plant populations could be the key to solving problems concerning recultivation of contaminated and degraded soils.

Responses in Tree Foliation (Defoliation, Phenology)

Defoliation

Since the beginning of 1970s the novel phenomenon—forest damage caused by emissions—has become more obvious. Broad-leaved trees (especially beech) were considered to be more tolerant to emissions in comparison with conifers. However, the symptoms of damage also occurred in broad-

Table 16 Dynamics of Values of Mean Defoliation at Three Localities in Central Slovakia

Site	Distance from emission source (km)	No. of evaluated trees	Year								
			1988	1989	1990	1991	1992	1993	1994	1995	1996
EEPP	18	68	8.7	7.3	15.4	15.8	18.3	18.8	26.5	28.2	26.6
PRP	7	43[a]	—	5.9	13.3	10.4	20.4	16.8	23.6	21.8	18.8
RMP	2	78[b]	47.4	36.7	42.6	35.5	40.3	35.5	39.2	36.0	33.1

EEPP, Ecologico experimental permanent site Kremnické vrchy; PRP, permanent research site Jalná; RMP, Research monitoring site Žiar nad Hronom.
[a] From 1993 to 1996, 39 trees were evaluated.
[b] In 1991, 77 trees were evaluated; in 1992, 76 trees, in 1993 74 trees, and from 1994 to 1996, 72 trees, respectively.

leaved trees, including beech. This was the reason for an early yellowing and leaf fall, shortening of annual shoots, morphological lesions of branches and consequently of whole crowns, their sparsing, and necrosis of leaves and branches. The above-mentioned facts were first observed in Austria, Germany, and Switzerland and later in beech stands in Slovakia, especially in the localities near emission sources. This is an important fact, because European beech (*Fagus sylvatica* L.) is the most widespread tree species (approximately 30%) in Slovakia.

The same method (ICP, International Cooperative Program) [83] for assessment of the state of the health of forests has been used in Europe since 1986 and is based on two criteria: the discoloration and defoliation of assimilatory organs. The assessment is carried out every year in a network of permanent monitoring plots (PMP) in 34 European countries. It is interesting that data related to the extent of the defoliation of beech stands including dynamics are few apart from PMP [84–88]. Deterioration of the state of the health of forests has occurred throughout Europe. This was confirmed by the results of monitoring published in a report in 1995 (Forest Condition in Europe, 1995 [89]). According to this report, in the year 1988, defoliation of assimilatory organs was higher than 25% in only 10.3% of the total evaluated beech trees in 34 European countries. In 1995, it was already 26.1% (in Slovakia 24.4%). The mean defoliation of beech trees in Slovakia in the years 1987–1996 ranged from 17 to 23%. In the year 1991, an exception was recorded when the lowest defoliation (13%) was ascertained [90]. We compared the values of the mean defoliation of nine monitoring cycles of the assessment of the health state of beech trees at three localities in Central Slovakia. They are situated 2, 7, and 18 km (Table 16) from the emission source (aluminium factory).

Increased defoliation was most marked at the permanent research site (PRP), Jalná, and the ecologicoexperimental permanent site (EEPP) Kremnické vrchy, in 1990. This trend occurred not only in Slovakia [84,85,90] but virtually in every country in Europe (Forest Condition in Europe, 1995 [89]). The complex of synergistically affected factors is considered to be one cause of this unfavourable trend. Besides emissions, a significant role is played by changes in climatic parameters (long-term precipitation deficiency and extremely high temperatures during the growing season). This fact was evident at the end of the 1980s and the beginning of the 1990s. Broad-leaved trees responded to the above-mentioned factors by increasing the number of assimilatory apparatuses, which was apparent after several years of evaluation of the defoliation at PRP Jalná and EEPP Kremnické vrchy (see Table 16). In addition to a virtually continuous increase in the mean defoliation values at these sites, there were significant differences in the annual results (Table 17).

Markedly fewer cases of significant annual differences related to the mean values of defoliation were found during the extended period at the RMP Žiar nad Hronom. This could be explained by the fact that the forest stands are more adaptable to long-term negative changes in climatic parameters when subject to a strong emission impact. On the other hand, in stands growing under favorable ecological conditions, gradual but significant changes over a long period of time are con-

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TABLE 17 Significance of Difference in Mean Defoliation Values Between 1988–1996

		Year								
Site	Year	1988	1989	1990	1991	1992	1993	1994	1995	1996
	1988	—	N	**	**	**	**	**	**	**
	1989		—	**	**	**	**	**	**	**
	1990			—	N	*	**	**	**	**
	1991				—	*	*	**	**	**
EEPP	1992					—	N	**	**	**
	1993						—	**	**	**
	1994							—	N	N
	1995								—	N
	1996									—
	1989	—	—	**	**	**	**	**	**	**
	1990			—	N	**	*	**	**	**
	1991				—	**	**	**	**	**
PRP	1992					—	N	N	N	N
	1993						—	**	*	N
	1994							—	N	**
	1995								—	*
	1996									—
	1988	—	**	N	**	*	**	**	**	**
	1989		—	N	N	N	N	N	N	N
	1990			—	*	N	*	N	*	**
	1991				—	N	N	N	N	N
RMP	1992					—	N	N	N	*
	1993						—	N	N	N
	1994							—	N	*
	1995								—	N
	1996									—

N, statistically not significant difference ($P > .05$); *, statistically significant difference ($P < .05$); **, statistically significant difference ($P < .01$).

spicuously manifested in the foliation of trees. An example of this is the gradual negative changes in foliation at EEPP Kremnické vrchy and PRP Jalná. This was also confirmed by research into the biochemical, physiological, anatomical, and morphological parameters of beech leaves at the same experimental sites [35]. Differences in the mean values of defoliation between sites at different distances from the emission source as well as differences in nine monitoring cycles were also confirmed statistically (Table 18). However, it should be emphasized that the method of visual terrestrial assessment of tree foliation is a subjective method and there is the possibility that the observer may make a subjective error in evaluation and thus influence the accuracy of the results [91–94]. The assessment of defoliation is also influenced by other objective factors such as the number, size, and deformation of leaves (leaf curling), which may cause changes in crown transparency and overestimation of real defoliation. This was observed especially in the sunlit leaves of dominant trees [95,96].

The number of trees and the leaf area are closely related to many environmental factors, moreover, they change every year [97]. It was stated that a decrease in the leaf area is in relation to the frequency of emissions. Glavač [51] found that a decrease in the leaf area of beech leaves was not only related to the emission impact but also to soil type as well. Oswald and Ziegler [50] found that beech trees with mild to severe injuries had leaves about 50% smaller than healthy trees. The higher crown transparency is also partly caused by leaf-eating insects. During mass outbreaks

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Table 18 Significance of Differences in Mean Defoliation Values, Presented in Table 16, According to Site

	Site		
Year	EEPP-PRP	EEPP-RMP	PRP-RMP
1988	—	**	—
1989	N	**	**
1990	N	**	**
1991	**	**	**
1992	N	**	**
1993	N	**	**
1994	N	**	**
1995	**	**	**
1996	**	**	**

For explanation, see Tables 16 and 17.

of certain insect species (e.g., *Calliteara pudibunda*), the leaf area was markedly reduced. This could be estimated indirectly by excrement production (Table 19) [98]. The extremely high temperatures during the summer of 1992 led to a significant increase in crown transparency. This was apparent as early as the end of July and beginning of August with the shedding of green leaves. Thus higher values of defoliation occurred in that year than in previous years (see Table 16). A similar observation was reported by Schütt and Summerer [95] and by Wittig and Werner [99] in a Bavarian forest. They found that some individuals of European beech had lost more than 50% of the total amount of leaves as early as the beginning of September and the other were fully defoliated by the end of September.

The time of year an assessment of beech forest health is carried out also is very important and is closely related to the phenology of evaluated tree species [100,101]. According to the ICP method, broad-leaved trees should be evaluated after the middle of August and that is not always objective as it also depends on weather conditions during the growing season in the actual year, the recommended period for evaluation could influence the accuracy of the obtained results. In

Table 19 Dynamics of Excrement Production of *Calliteara pudibunda* in RMP Žiar nad Hronom in 1990–1992 Expressed in Kilograms (kg) (Dry Weight) ha^{-1}

	1990		1991		1992	
Sampling	Date	kg ha^{-1}	Date	kg ha^{-1}	Date	kg ha^{-1}
1	August 9	Negligible	August 9	0	August 19	231.2
2	September 6	dry	September 6	166.4	September 7	457.0
3	October 10	weight of	October 10	326.2	October 7	607.4
4	November 7	excrement	November 7	37.0	November 6	21.2
5	—	production	December 4	0	December 4	0
Total				529.6		1316.8

Source: From Ref. 98.

TABLE 20 Leaf Litter Expressed in Percentage of Total Litter in 1990–1994

	EEPP		RMP	
Sampling	Date	%	Date	%
1	August 21, 1990	0.38	August 9, 1990	2.60
	August 20, 1991	1.60	August 9, 1991	4.76
	August 20, 1992	2.58	August 19, 1992	10.09
	August 18, 1993	1.64	August 23, 1993	5.32
	August 16, 1994	2.13	August 15, 1994	5.19
2	September 07, 1990	0.63	September 6, 1990	8.82
	September 06, 1991	0.34	September 6, 1991	5.69
	September 07, 1992	28.84	September 7, 1992	31.97
	September 06, 1993	6.09	September 8, 1993	2.32
	September 05, 1994	0.50	September 5, 1994	0.81
3	October 9, 1990	8.28	October 10, 1990	20.75
	October 9, 1991	12.78	October 10, 1991	32.82
	October 7, 1992	17.81	October 7, 1992	30.76
	October 6, 1993	3.72	October 6, 1993	9.79
	October 7, 1994	4.09	October 7, 1994	11.33
4	November 6, 1990	90.71	November 7, 1990	67.83
	November 6, 1991	53.32	November 7, 1991	17.39
	November 6, 1992	16.78	November 6, 1992	11.94
	November 10, 1993	77.62	November 10, 1993	68.99
	November 7, 1994	43.38	November 7, 1994	66.29
5	December 3, 1991	17.47	December 4, 1991	15.49
	December 4, 1992	33.99	December 4, 1992	15.24
	December 7, 1993	10.93	December 7, 1993	13.58
	December 2, 1994	49.90	December 5, 1994	16.38
6	January 31, 1992	14.49	February 3, 1992	23.85

For explanation, see Table 16.
Source: From Ref. 98.

beech stands subject to a heavy emission impact, earlier leaf fall occurred than in stands with a relatively slight emission impact [102,103]. This was confirmed by our 5-year investigation (Table 20) [98].

The foliation of beech trees is also influenced by their fructification [97,100,104]. Some investigators [105–108] have emphasized that yellowing and defoliation of assimilatory organs are nonspecific symptoms for the assessment of tree injuries subject to an emission impact. They argue that leaf yellowing could be the result, not only of emmission impact but also of nutrient deficiency in the soil and/or needles [19,109], drought, and high temperatures during the summer months [110].

Phenology

It is known that differences in the phenological manifestations of individuals such as bud breaking and leaf ageing exist in every population of a particular tree species. Since the 1970s a decrease in interest in phenological investigations has been noted. In this period, the unfavorable effects of emissions on forest stands started to be the most important problem in forestry. The ''novel forest damage'' started a new forest phenological approach in Europe. This was confirmed by the investigation of the air-pollution effect on the shift of tree phenophases, time of vegetative period, lag time

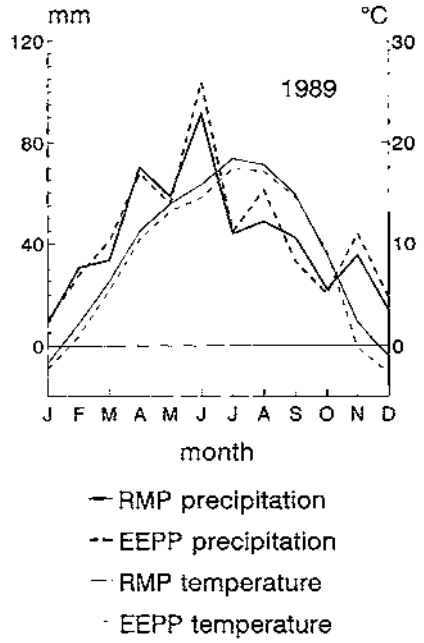

FIGURE 3 Climadiagrams constructed according to the data from meteorological stations in Sliač (Kremnické vrchy) and Žiar nad Hronom in 1989. RMP, research monitoring site Žiar nad Hronom; EEPP, ecologicoexperimental permanent site Kremnické vrchy. (From Ref. 103.)

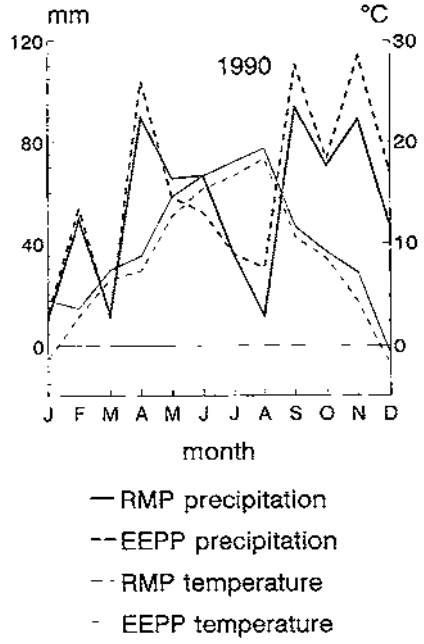

FIGURE 4 Climadiagrams constructed according to the data from meteorological stations in Sliač (Kremnické vrchy) and Žiar nad Hronom in 1990. RMP, research monitoring site Žiar nad Hronom; EEPP, ecologicoexperimental permanent site Kremnické vr
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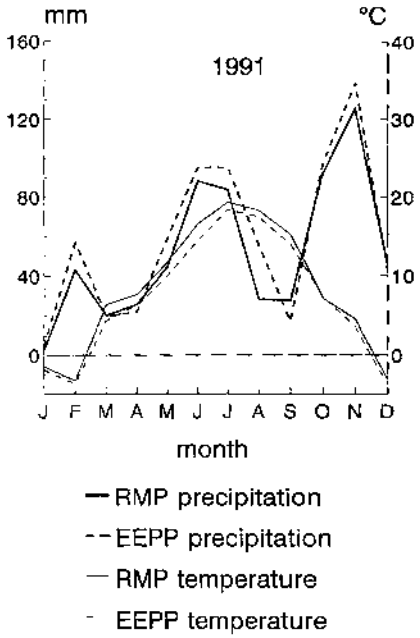

FIGURE 5 Climadiagrams constructed according to the data from meteorological stations in Sliač (Kremnické vrchy) and Žiar nad Hronom in 1991. RMP, research monitoring site Žiar nad Hronom; EEPP, ecologicoexperimental permanent site Kremnické vrchy. (From Ref. 103.)

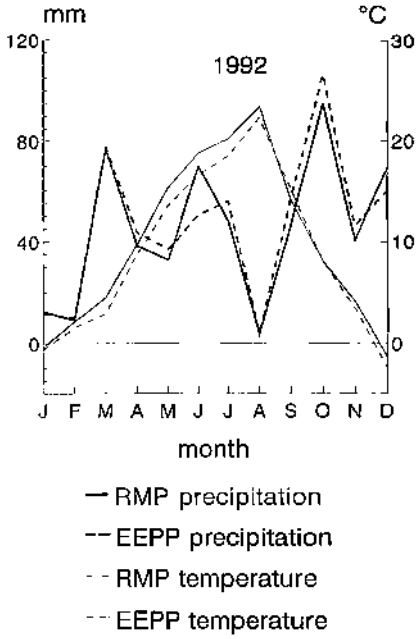

FIGURE 6 Climadiagrams constructed according to the data from meteorological stations in Sliač (Kremnické vrchy) and Žiar nad Hronom in 1992. RMP, research monitoring site Žiar nad Hronom; EEPP, ecologicoexperimental permanent site Kremnické vrchy. (From Ref. 103.)

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Table 21 Time Course of Spring Phenophases of Parent Stand (When More than 50% of Total Number of Observed Individuals of Parent Stand Have Reached the Given Phenophase) and Number of Days of Transition from One Phenophase to the Other or Total Days from Start (March 20) of Phenological Observation (data in parentheses)

		Bud-breaking phenophases (foliation)[a]						
Year	Site	1	2	3	4	5a	5b	5c
1989	RMP	—	3.4 (14/14)	9.4 (6/20)	15.4 (6/26)	19.4 (4/30)	26.4 (7/37)	5.5 (9/46)
	EEPP	—	3.4 (14/14)	7.4 (4/18)	15.4 (8/26)	19.4 (4/30)	23.4 (4/34)	1.5 (8/42)
1990	RMP	—	3.4 (14/14)	27.4 (24/38)	30.4 (3/41)	3.5 (3/44)	5.5 (2/46)	6.5 (1/47)
	EEPP	—	3.4 (14/14)	26.4 (23/37)	28.4 (2/39)	30.4 (2/41)	3.5 (3/44)	5.5 (2/46)
1991	RMP	3.4 (14/14)	11.4 (8/22)	28.4 (17/39)	3.5 (5/44)	4.5 (1/45)	9.5 (5/50)	12.5 (3/53)
	EEPP	3.4 (14/14)	13.4 (10/24)	28.4 (15/39)	2.5 (4/43)	4.5 (2/45)	9.5 (5/50)	12.5 (3/53)
1992	RMP	6.4 (17/17)	11.4 (5/22)	25.4 (14/36)	26.4 (1/37)	27.4 (1/38)	30.4 (3/41)	1.5 (1/42)
	EEPP	6.4 (17/17)	13.4 (7/24)	23.4 (10/34)	25.4 (2/36)	28.4 (3/39)	30.4 (2/41)	1.5 (1/42)

RMP, research monitoring site Žiar nad Hronom; EEPP, ecologicoexperimental permanent site Kremnické vrchy.

[a] 1, bud in winter stage; 2, growing bud; 3, bud green at the end; 4, outbreak bud; 5a, slightly foliated tree (foliation up to 1/3 of crown); 5b, average foliated tree (foliation up to 2/3 of crown); 5c, tree fully foliated.

Source: From Ref. 103.

of bud breaking, premature leaf ageing, and leaf fall [99,102,111]. Premature leaf yellowing and leaf fall in connection with the frequency of emission were established outdoors and also under laboratory conditions [112–114]. The shift in the autumn phenophases of beech was observed during our multiyear investigations [113] at the research sites EEPP Kremnické vrchy and RMP Žiar nad Hronom (see Table 1). Figures 3–6 show the climadiagrams for these research sites.

The 95 individuals in the beech parent stand at EEPP Kremnické vrchy and 78 individuals at the RMP Žiar nad Hronom were analyzed every year. Based on the data estimated outdoors, it was found that over 50% of the total number of analyzed individuals reached the given phenophase. The rate of the developmental phenophases was expressed as the number of days of transition from one phenophase to the next one and always to the corresponding date. The data on the time course and the start of partial phenophases were obtained using this methodical approach. Table 21 presents the time course of spring phenophases in the parent stand at both localities. The minimum differences between sites (1–3 days) were estimated. On the other hand, marked differences from year to year, especially in the date of the occurrence of the given phenophase, were found (Figs. 7 and 8).

A very interesting situation was observed in 1991, when the start of vegetation was the latest of the 4-year observations. Similarly, a ''late'' start of phenophases occurred in 1992, although the next phenophases showed a much faster course (mainly phenophases 3 and 5b). The reason for this phenomenon reflected a marked increase in the temperature values (average and maximum) at the end of April and/or the beginning of May. In our climatic conditions, bud breaking lasts from approximately April 20 to the middle of May and/or the full foliation of beech trees lasts from May

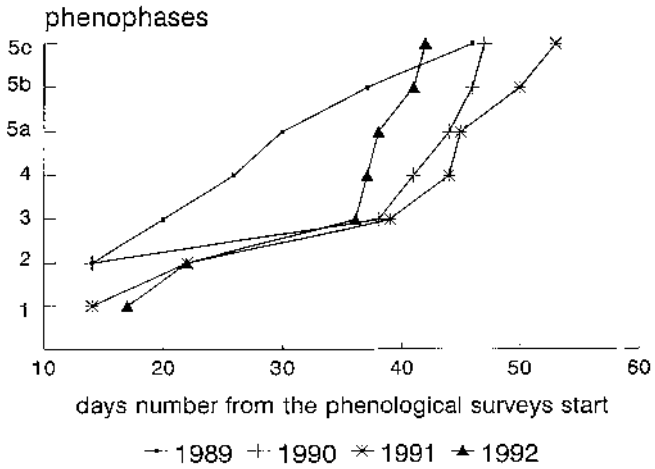

FIGURE 7 Time course of phenophases for the parent stand at the RMP Žiar nad Hronom (the time course in days on the x-axis is reckoned from the March 20 in every year). 1, bud in winter stage; 2, growing bud; 3, bud green on the end; 4, outbreak bud; 5a, slightly foliated tree (foliation up to 1/3 of crown); 5b, average foliated tree (foliation up to 2/3 of crown); 5c, tree fully foliated. (From Ref. 103.)

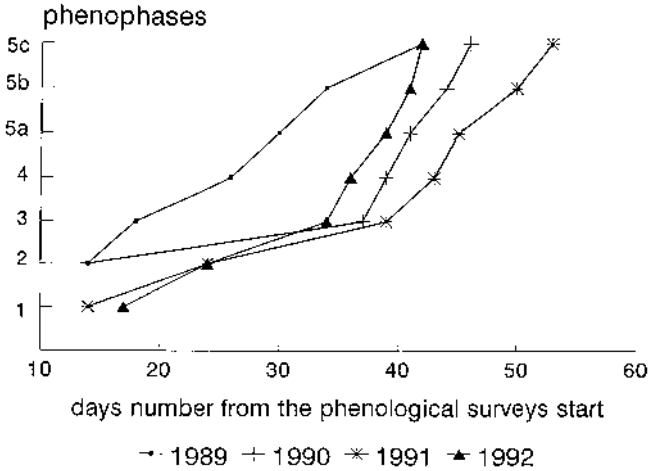

FIGURE 8 Time course of phenophases for the parent stand at the EEPP Kremnické vrchy (the time course in days on the x-axis is reckoned from the March 20 in every year). 1, bud in winter stage; 2, growing bud; 3, bud green on the end; 4, outbreak bud; 5a, slightly foliated tree (foliation up to 1/3 of crown); 5b, average foliated tree (foliation up to 2/3 of crown); 5c, tree fully foliated. (From Ref. 103.)

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Table 22 Percentage of Trees in Autumn Phenophases at RHP Žiar nad Hronom and EEPP Kremnické Vrchy and Average Defoliation in 1989–1992

Year	Site	Date	Defoliation (%)	Phenophase[a]					
				6	7	7a	7b	8a	8b
1989	RMP	Aug 21	37	80	20				
		Sept 1		56	44				
		Sept 15		3	97				
		Sept 29	50						
		Oct 30						42	58
	EEPP	Aug 23	8	100	0				
		Sept 13		49	51				
		Oct 2	14						
		Nov 1						19	81
1990	RMP	Aug 22	43	66	34				
		Sept 6		36	64				
		Sept 17	35	15	85				
		Sept 27	44						
		Oct 10	52						
		Oct 22				9	17	33	41
		Nov 7				—	—	21	79
	EEPP	Aug 20	16	100	0				
		Sept 18		84	16				
		Sept 28		23	77				
		Oct 9	16						
		Oct 23				6	15	28	51
		Nov 6				—	1	20	79
1991	RMP	Aug 21	35	84	16				
		Sept 6		23	77				
		Sept 18	37	9	91				
		Oct 1	54						
		Oct 14				15	48	20	17
		Oct 29				—	19	55	26
		Nov 11				—	—	63	37
	EEPP	Aug 20	16	100	0				
		Sept 19		67	33				
		Sept 30		5	95				
		Oct 15	16	0	100				
		Oct 30				19	31	44	6
		Nov 12				—	6	57	37
1992	RMP	Aug 19	39	76	24				
		Sept 16				—	26	26	48
		Sept 28				—	11	25	64
		Oct 15				—	8	21	71
		Oct 26				—	—	25	75
		Nov 6				—	—	10	90
	EEPP	Aug 17	18	99	1				
		Sept 17	31	99	1				
		Sept 29	47	7	93				
		Oct 12				46	37	11	—
		Oct 27				6	20	72	2
		Nov 12				—	—	27	73

RHP, research monitoring site; EEPP, ecologicoexperimental permanent site.

[a] 6, green (physiological adult) leaf; 7, yellow leaf; 7a, trees green enough (above 25% of green leaves); 7b, trees green partially (10–25% of green leaves); 8a, trees defoliated incompletely (more than 10% of yellow or dry leaves); 8b, trees fully defoliated.

Source: From Ref. 103.

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7 to the end of May [115]. During our 4-year observations, bud breaking occurred from the middle of April to May 3 depending on the temperature conditions at the beginning of the given year.

The time course of the autumn phenophases in parent stands at both localities is presented in Table 22. The comparison of the autumn phenophases over the 4 years showed that the course of yellowing and leaf fall was faster at RMP Žiar nad Hronom, where leaf yellowing became markedly evident in the first half of August. At RMP Žiar nad Hronom, there were few trees with green leaves (3–15%) in the middle of September, whereas at EEPP Kremnické vrchy, there were 49–99% on the same date. The above-mentioned results correspond with investigations of leaf-fall quantity at both sites as well (see Table 20).

Similarly to the spring phenophases, the autumn phenophases also showed differences in the years studied. From this aspect, the year 1992 is very interesting because of the extreme drought and the long heat wave (above 30°C) during the growing period, which resulted in even the fall of green leaves at the end of July and beginning of August. This effect was best seen at RMP Žiar nad Hronom, where on September 16, 1992, almost half the trees had become totally defoliated. Similar differences in the time and course of yellowing and leaf fall were found by Chalupa [115].

It could be concluded that the course of yellowing and leaf dropping depended on temperature and moisture (precipitation) conditions in a given year together with the synergic effect of air pollution [99,116].

CONCLUSIONS

The widespread contamination of our environment as a result of human activities over many centuries now presents us with the choice of undertaking a monumental clean-up task or leaving the worst problems and a degraded environment to the next generation. Plants are not yet widely used in the removal of organic or inorganic pollutants from the environment, but there is a large potential for this approach. Plants can concentrate metals in their roots and shoots to levels far exceeding those present in either soil or water. The value of metal-accumulating plants for environmental remediation is now being realized with the foundation of a new technology termed ''phytoremediation'' [117]. This is a new and promising approach to the difficult problem of remediating heavy metal–polluted soils. Fundamental to the environmental and economic success of phytoremediation is the existence of plants which hyperaccumulate metals. These are so-called metal hyperaccumulators or hyperaccumulator plants, of which about 400 taxa have been described so far from 35 families of angiosperms [60]. Apart from the interest in elucidating the underlying biochemical mechanism of metal tolerance, metal hyperaccumulators are attracting increasing attention because of their potential application in the decontamination of metal-polluted soils [118]. The use of the roots of terrestrial plants to remove organics or heavy metals from aqueous solutions may provide the foundation for a novel water-treatment technology. Phytoremediation, although still in its infancy, may one day become an established environmental clean-up technology [117].

Extensive biotransformation of pollutants by plants is known to occur, which includes methylation, conjugation, reduction/oxidation, and degradation. The fate of the biotransformation products ranges from incorporation into cellular components, deposition into specialized organs, to excretion and volatilization [119]. The further development of phytoremediation requires an integrated multidisciplinary effort which combines plant biology, soil chemistry, soil microbiology, and agricultural and environmental engineering. Plants that enhance the organic degradation or accumulate toxic metals can be grown and harvested economically leaving the soil or water with a greatly reduced level of toxic chemical contamination [118].

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Effect of Atmospheric Pollution, with Special Reference to Ozone, on Plants Under Normal and Saline Conditions

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INTRODUCTION

Air is a mixture of gases and is normally colorless, odorless, and tasteless. Its major constituent gases are nitrogen (78%) and oxygen (21%), with the remaining 1% comprises, Ar, CO_2, Ne, He, CH_4, Kr, H_2, CO, Xe, O_3, oxides of S and N, and water vapors [1]. It took about 4.5 billion years of evolution to produce this composition of air, which made this planet, a place capable of supporting life in its present form. Although *Homo sapiens* appeared late in the Earth's history, this species has been dominant in modifying its environment.

The effect of human activity on the global atmosphere has become increasingly evident during the last four decades. There has been a quantum jump in industrialization since the Industrial Revolution, urbanization, nuclear weapons testing, the increasing use of chemicals for optimum crop production to feed the ever-increasing population, especially in the developing countries, and the increasing demands made by humans as their standard of living rises have all contributed. As a consequence, the more eco-friendly composition of the atmosphere was disturbed because the air became polluted.

Air pollution is the contamination of the atmosphere by gaseous, liquid, or solid wastes or by-products that can endanger human or plant health or can attack material, reduce visibility, or produce undesirable odors [2]. It is one of our most serious environmental problems. According to conservative estimates, some 0.2 billion tons of such pollutants are released each year over the United States alone [3]. Many of these come directly from identifiable sources, for example, vehicu-

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lar exhaust, power generation, and industrial plants, whereas, others are formed through chemical reactions on certain precursors, for example, the production of ozone by the interaction of hydrocarbons, nitrogen oxides, and particulates under the influence of sunlight [4].

Pollutant concentrations are reduced by atmospheric mixing, which is dependent on factors like temperature, wind speed, and movement of high- and low-pressure systems. When a cold layer of air settles under a warm layer producing thermal inversion, atmospheric mixing is retarded and pollutants accumulate near the ground. This inversion may become sustained under a stationary high-pressure system coupled with low wind speeds. A period of only 3 days of poor atmospheric mixing in a high pollution area can lead to high concentrations of hazardous material and can result in severe injuries to all life forms there. For example, such an inversion phenomenon occurred in London in 1952 and 1962 and caused about 4000 and 700 human deaths, respectively.

The combustion of coal, oil, and gasoline accounts for much of the air pollutants. The pollutants thus produced in urban areas may drift to nearby rural areas and cause damage to vegetation there. Such a drift may occur at large distances from the source, as happens, when tall smoke chimneys boost the pollutants from factories higher into the atmosphere, thereby reducing their concentrations at the site of their production. These pollutants may be transported and gradually settle down far from their site of production. Sulfur dioxide (SO_2) and NO_2^- emissions from the Central and Eastern United States are, for instance, causing acid rain over New York State, New England, and eastern Canada. Similar situations have been observed in European countries, which are causing global environmental concern. In Sweden, for instance, according to one estimate, some 15000 lakes have become too acidic to support sensitive species of animals and plants. A large problem with acidic water is that it dissolves metals, both from soil and from water mains and pipes, resulting in higher levels of metal concentrations in drinking water and thereby posing a health hazard.

Research on air pollution damage to plants has been going on for a long time. This research has, however, gathered momentum since the early 1950s, and in addition to research papers, some detailed reviews and books have been published on this subject [5–10]. Thomas, whose paper in the *Annual Review of Plant Physiology* [9], is among the early ones on this topic, quotes some works from where, it is evident that the effect of mercury (Hg) vapors on plants was studied as early as 1797. With the advent of civilization, the oxides of sulfur, carbon, and later on of nitrogen have subsequently received much attention. These studies provided a better understanding of ''acid rains'' produced by sulfur compounds, the ''greenhouse effect,'' and warming of the atmosphere due to an increase in atmospheric CO_2 levels as a result of a worldwide increase in the burning of fossil fuels, coal, natural gas, and petroleum oil.

There has been a continuous increase in the levels of CO_2 in the upper atmosphere resulting in elevated temperatures. It is estimated that there has been an increase of 20% in the CO_2 content of the atmosphere raising the level from 280 parts per million prior to 1900 to the current level of 340 parts per million. As a consequence of the activities of the considerable increased in the human population, about 5.3 gigatons (1 gigaton is equal to 1000 million tons) of carbon dioxide is being added into the atmosphere annually [6], which can play a major role in the heating of the Earth's surface, because the heat which would normally dissipate into space is trapped by the CO_2 and deflected back to ground.

Assuming a rise in the use of fossil fuels by 2% each year, it is estimated that by the year 2000, the CO_2 level would increase to about 360–370 parts per million, and that would raise the Earth's temperature on an average by 1°C [6]. This would bring highly significant and far-reaching changes in the climates of the world. As the world warmed up, the seas would rise by 2.5 inches a decade on the average. They would generally swell by as much as 3 ft by the year 2100, affecting about 224×10^3 miles of the world's coastline [11]. In the absence of any precautionary measures, this would render some island countries uninhabitable, displace 10s of millions of people, seriously threaten low-lying urban areas, flood productive land, and contaminate freshwater supplies. This increase in the average temperature of the planet, therefore, has the potential of changing the overall patterns of food production globally.

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The ''ozone scare'' is a comparatively recent event, but it has gained worldwide significance owing to its impact on our ecology. In nature, a region of atmosphere from 20–50 km (12–30 miles) has an ozone concentration of as much as 10 ppm. This level of O_3 is dangerous for all life forms on Earth, but ozone layer in the upper atmosphere, actually protects all forms of life on Earth from the full force of the sun's ultraviolet radiation, which can cause skin cancers, sunburn, snow blindness, wrinkling of the skin, and cataracts, reduce the immune system response, and interfere with plant and oceanic phytoplankton growth.

Ozone is a primary product of the reaction between nitrogen oxides (NO, NO_2), hydrocarbons, and sunlight. Emissions from industries, vehicular exhausts, and refuse incineration are all processes that help to create favorable conditions (i.e. conditions which are favorable for formation of ozone) for the above reaction. Consequently, localized toxicity of ozone may occur. Ozone may, however, be destroyed as well by several chemical reactions. Scientists were therefore concerned when they discovered in 1970 that certain manmade chemicals, called chlorofluorocarbons (CFCs), used as refrigerants in different machineries and in aerosol spray, can pose a threat to the ozone layer. The excessive release of chlorine, Freon, and nitrogen oxides from various human activities, result in the reduction of ozone at high altitudes. Aerosols containing highly reactive chlorofluoromethanes also destroy the ozone layer. Other halocarbons and nitrous oxides may also act similarly.

Initially, it was thought that ozone depletion is on the increase all over the globe, but in 1985, it was revealed that a growing ozone hole concentrated over Antarctica, where 50% or more of ozone above this area was being depleted seasonally beginning in October of each year. This happens on account of heterogeneous reactions on ice particles, which facilitate the catalytic destruction of the ozone layer by chlorine. A 1986 *Newsweek* study predicted a loss of 5–9% of the ozone shield in the next 50 years, whereas a 2.5% decay means 15000 more deaths annually from skin cancers and related diseases. In 1985, 49 countries of the world hence agreed on the United Nations (UN) Convention to protect the ozone layer. This ''Montreal Protocol,'' which was renegotiated in 1990, calls for the gradual phase out of a certain amount of chlorofluorocarbons by the year 2000 and provides aid to developing countries in making this transition.

SALINITY AND PLANT GROWTH

In all arid and semiarid regions of the world, soil salinity is a major agricultural problem. Despite the advanced management technologies available today, salinization of millions of hectares of land continues to reduce crop production severely worldwide. The excess salts present in the root zone impair the growth of many field crops. Stunted growth, leaf-tip burning, and in some cases leaf necrosis, enhanced leaf senescence, and reduced yields are major visible symptoms of this type of injury and are accompanied by a loss in the selective absorption of essential plant nutrients, a decline in the transpiration rate, an altered water potential, and other biochemical changes in the tissue [e.g., see Refs. 12–15].

Plants require mineral nutrients from the root-substrate interface in their native soil environments. Under saline conditions, which are characterized by low-nutrient ion activities, nutritional disorders can develop and crop growth may be reduced. The nutrient availability and uptake by plants grown in saline environments are related to (a) the activity of the nutrient ion in the soil solution, which depends on pH, pE, concentration, and composition; (b) the concentration and ratios of accompanying elements that influence the uptake and transport of these nutrients by roots; and (c) numerous environmental factors.

An overwhelming amount of evidence from laboratory and field studies indicates that Na^+-dominated soils or solutions generally reduce the K^+ and Ca^{2+} uptake by plants and/or affect the internal distribution of these elements. Major ions can influence nutrient absorption by competition or by affecting ion selectivity of membranes. Salinity can also influence the mineral nutrition of plants by affecting the mobility of a nutrient element within the plant or by increasing the nutrient requirement for that element in the cells.

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Sodium and K uptake, and their distributions in plant parts, play key roles under saline conditions. Potassium has been established to play an important role in stomatal movement (in addition to being an essential macronutrient involved in many vital metabolic processes), and its importance needs no further elaboration. The cellular membranes are the main stress-sensitive sites in the cell. The increased resistance against this stress may be due to the protection of sensitive membranes, which is achieved by structural changes or synthesis of protective compounds. The connection between Na transport (a dominant phenomenon under saline conditions) properties and membrane constituents in a plant system has been reported. Lipids and proteins are the main building blocks of biomembranes and any change in them could be responsible for the sensitivity of a plant to certain potential stresses [16,17].

The demand of food and fiber for the growing world population requires the use of even marginal lands which, with time, are lost to secondary salinizations, a great hazard against which no country can consider itself immune today. On the other hand, the damaging effects of air pollutants are also worldwide and have been reported from the overpopulated cities of Bogota, Sao Paulo, Cologne, Copenhagen, London, Paris, Karachi, Ahmedabad, and Delhi and Baltimore, Los Angeles, New York, Chicago, Philadelphia, and San Francisco in the United States [3,18–21]. In terms of money, the estimates of damage to agricultural crops in California alone amounted to about $8 million annually for field and vegetable crops and about $18 million along the Atlantic seacoast where many highly populated cities of the continents of North and South America and Africa [9] are located.

Sulfur dioxide and ozone are produced mainly in urban areas. These gaseous pollutants produced by industries and the heavy vehicular traffic of cities drift to rural areas and have fallout effects causing injury to plants and resulting in crop losses [22,23]. The ecological implications of these pollutants are hence immense, as their harmful effects are not restricted to a limited area or site of production. Although, a considerable amount of work has been reported on the various aspects of the response of a variety of plants to different pollutants and salinity separately, their combined effects on plant growth and metabolism have remained comparatively less explored. The response of ozone to plant growth and development was reported to be considerably altered in the presence of SO_2 and nitrogen nutrition or low soil moisture [7]. Although carbon dioxide is another air pollutant of importance, it is also an integral part of the process of photosynthesis and has received attention in that context as well. This chapter attempts to highlight some of the effects of SO_2 and O_3 in combination with soil salinity.

EFFECT OF SULFUR DIOXIDE ON PLANT GROWTH

The important physiological processes of plant growth, photosynthesis, respiration, carbon allocation, and stomatal functions are known to be affected by air pollutants. A wide range in the sensitivity of plant growth both within and between species is evident from the literature for pollutants such as SO_2, O_3, N_2O, and HF. The study of the effects of pollutant mixtures on single plants and vegetation has now become a major area of research.

Air pollution by gaseous sulfur dixoide causes the development of soil acidity. The concentration at which atmospheric sulfur dioxide will damage plants have been extensively studied by Zahn [24]. Sulfur dioxide injury to vegetation and forest growth has been observed in the vicinity of several industrial operations, including metal ore concentrators and smelters, petroleum refineries, fossil fuel–burning power plants, and sulfuric acid manufacturing plants [25,26]. Investigations since 1970 in the United Kingdom have indicated that the SO_2 damage to pasture grasses is much more severe in the winter, not only as a result of the seasonal increases in the level of pollutants but also because plant resistance and regrowth are limited by unfavorable environmental conditions (low temperature and irradiance). This has been confirmed by Davies [27], who showed that the sensitivity of *Phleum pratense* plants depends on irradiance, but Davison and Bailey [28] have also demonstrated that fumigation of *Lolium perenne* plants with SO_2 can reduce their ability to tolerate freezing. It is likely that the rather mild effects of SO_2 fumigation on plants are a consequence of the low

S status of the soils used; and under these conditions, atmospheric SO_2 can act as a fertilizer stimulating rather than reducing growth [29].

With the use of SO_2 (0.068 ppm), a pollutant, visible injuries such as reduction in the rates of net photosynthesis and dry matter production and the disruption of water and ionic relations and of cell biochemistry have been observed in grass plants (*Dactylis glomerata, Poa pratensis, Lolium multiflorum, Phleum pratense*) [30]. Any environmental condition that favors stomatal opening increases the absorption of SO_2 and, therefore, injury [22]. SO_2 can cause stomatal opening even when the leaf is subject to water stress leading to increased SO_2 uptake and water loss. Biscoe et al. [31] found that stomatal resistance in the leaves of faba bean (*Vicia faba*) fell by 20% at 140 μg SO_2 m^{-3} and above.

Sulfur concentrations in the tissues of both saline and nonsaline wheat plants were increased greatly by SO_2 fumigation [32]. Increased S from absorbed SO_2 was mostly retained in the aboveground part of the plant, particularly in the leaves. Most of the absorbed SO_2 was oxidized into sulfate, with only a small increase in the concentration of organic sulfur by SO_2 fumigation. The small increase in the organic sulfur concentration may be mainly attributed to an enhanced glutathione content [33]. The NaCl salinity decreased the SO_4 concentration in the leaves of the nonfumigated and SO_2-fumigated plants [34]. Yeo et al. [35] observed that stomatal conductance and photosynthesis decreased in the old leaves of rice as salt accumulation increased over time. The SO_2 treatment of radish (*Raphanus sativus*) plants affected the plant nitrogen balance and subsequently decreased the ability of plants to respond to decreased nitrate availability by affecting resource partitioning to nitrate uptake and root growth [36].

There have been several studies on the interactions between SO_2 and soil salinity on plant growth responses [37,38]. The NaCl salinity might protect plants from SO_2 injury by increasing leaf stomatal resistance and decreasing SO_2 uptake [39]. However, in the long term, SO_2 fumigation increases the sulfur content in plants under salinity stress. Salinity and ozone have been implicated in stomatal closure. The closure of stomata may reduce yield. This closure will also limit the uptake of salt into the plant as a result of reduced transpiration.

Ozone has been implicated in damage to the membrane ion transport system in particular and membrane permeability in general [40]. Salinity and SO_2 have been found to be antagonistic in a number of crop species [41,42]. Long-term exposure to SO_2 can significantly increase the sulfur concentration in the shoot, and the SO_2 absorbed is mostly oxidized into sulfate [43]. Inhibitory effects increased with the duration of the fumigation period up to 8 h using 40 ppm SO_2 and 50–300 ppb ozone. At lower concentration, fumigation of tolerant and sensitive grafts of *Pinus strobus* (eastern white pine) with 50 ppb SO_2 and O_3, singly or in combination, gave no significant inhibition over a 1-week period [44]. A reduction in the stomatal conductance occurred in response to fumigation with ozone and sulfur dioxide [45]. The increased sulfate anion may increase the K^+ content and reduce the Cl^- contents in plants simultaneously exposed to NaCl salinity. This interaction may decrease the sensitivity of NaCl-treated plants to SO_2 pollution because of stomatal and/or mesophyll resistance and decreased SO_2 uptake. Therefore, SO_2 fumigation and NaCl salinity may antagonistically affect the uptake and accumulation of SO_2 and the salt in the plants.

Wheat (*Triticum aestivum* L.) plants were exposed to a factorial combination of two levels of salinity (control, and 50 mM NaCl) and three levels of SO_2 (10, 231, and 441 nL L^{-1}) in fumigation chambers for 4 h/day^{-1} for up to 42 days. These studies revealed that SO_2 fumigation significantly increased the sulfur concentration in the shoots but not in the roots. The NaCl salinity decreased the sulfate concentration in the leaves. There was an antagonistic interaction between SO_2 fumigation and NaCl salinity on the concentration of sulfate in the leaves. It was also observed that SO_2 fumigation, salinity, and their combinations affected the concentrations of Cl^-, K^+, Na^+, Ca^{2+}, and Mg^{2+} in the plant tissues. The antagonistic interaction between SO_2 fumigation and NaCl salinity on the SO_2 uptake and salt accumulation in the leaves may be responsible for the observed effect on plant growth [46].

The pigment concentration in leaves is an important parameter for determining the photosynthetic efficiency of plants. The chlorophyll content, an index of the photosynthetic potential of plants,

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is highly susceptible to pollutant action. The pigment interaction with pollutants leads to the destruction of photosynthetic leaf areas and the development of characteristic foliar symptoms. The greater effect of $O_3 + SO_2$ on the pigment concentration suggests their synergistic mode of action. Both O_3 and SO_2 individually are known to reduce the chlorophyll and carotenoid contents in crop plants. The decrease in the chlorophyll content associated with the development of injury symptoms in leaves may inhibit photosynthesis in O_3-and SO_2-treated plants.

Agrawal et al. [47] reported that exposure of rice plants to low concentrations of O_3 (0.08 ppm) and SO_2 (0.5 ppm), singly and in combination, showed foliar injury at different levels. The maximum leaf injury was noted in the case of O_3+SO_2 (0.04 + 0.25 ppm)– treated plants and the minimum in O_3-treated ones. Also, the reductions in chlorophyll *a* and *b* and the total chlorophyll and carotenoid contents in leaves exposed to O_3+SO_2 mixtures were higher than the reduction noted in the case of each individual pollutant. It has been suggested that O_3 by itself affects chlorophyll molecules or it impairs the synthesis of new molecules [48]. It can also affect both cellular and chloroplast structure and levels of chlorophyll in it [49]. The decrease in the carotenoid content in pollutant-treated leaves may be ascribed to the impaired synthesis of pigment. It has been observed that pollutant inactivate carotenoids. Ozone and SO_2 alone may cause the carotene contents to decrease [50]. The pigment reductions and foliar injury may lead to reduced photosynthate production and reduced plant growth.

EFFECT OF OZONE AND SOIL SALINITY ON PLANT GROWTH

Ozone has been proved to be one of the most important air pollutants affecting vegetations [51,52]. Several popular articles have been published concerning the plant responses to ozone and sulfur dioxide separately or in combination [53–57]. Other papers have mentioned that these two gases, both singly and in combination, disrupt various metabolic processes and consequently affect the growth and development of plants [58–60].

Bytnerowicz and Taylor [37] grew bean plants in half-strength Haogland solutions provided with three salinity levels of −40, −240, and −440 kPa and exposed four times to 390 $\mu g\ m^{-3}$ O_3, 520 $\mu gm^{-3}SO_2$, and 390 μgm^{-3} O_3 + 520 μgm^{-3} SO_2. The plants were fumigated with these treatments between 9 a.m. and 4 p.m. Plants fumigated with SO_2 alone showed no injury. Primary leaves of O_3–treated plants were injured more than those of plants fumigated with the combination of O_3 and SO_2. The amount of injury produced by O_3 and (O_3 + SO_2) mixtures decreased when the salinity of the solution increased. The plant growth was suppressed by increased salinity levels. The root growth of O_3 and (O_3 + SO_2)-treated plants was reduced at all salinity levels. Plants fumigated with SO_2 and (O_3 + SO_2) had a higher S content in roots than that of nonfumigated and O_3-treated plants. The highest S content in leaves was found in SO_2 -treated plants at the −40-kPa salinity level. The accumulation of Ca in leaves and of Mg in roots was lowest in plants fumigated with O_3 alone and mixtures of O_3 and SO_2 compared with control.

Maas et al. [61] found a similar decrease in Ca in leaves when bean plants were exposed to a greater dose of O_3. Plants fumigated with O_3 alone and (O_3 + SO_2) accumulated more K in the stems and leaves and more Fe in the roots and leaves compared with nonfumigated and SO_2-treated plants. Physiological explanations for the variation in plants' susceptibility to air pollutants and of the influence of environmental conditions on such a response were not completely understood. However, stomatal closure is considered to be an important factor in inhibiting the penetration of the pollutant. Maas et al. [61] have further stated that the salinity levels, achieved by the addition of NaCl and $CaCl_2$ to the nutrient solution, reduced the amount of visible O_3 injury symptoms. The evidence of membrane permeability involvement in susceptibility has also been presented by many researchers. Presumably, the reduction of injury by O_3 and (O_3 + SO_2) treatments was induced by stomatal closure, but the increase in stomatal resistance observed was much less evident than reported by Ting and Heath [10]. Mansfield [62] also stated that O_3 tends to cause stomatal closure.

Olszyk et al. [63] studied the interaction of ambient photochemical oxidants (primarily ozone,

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O_3) and salinity on the vegetation of alfalfa (*Medicago sativa* L.) in a field experiment having average electrical conductivities of 0.9, 3.4, and 6.3 dS m^{-1}, which resulted in mean saturated soil extract conductivities in the root zone of approximately 1.5, 5.8, and 8.1 dS m^{-1}, respectively. Plants were exposed in open-top chambers to filtered or unfiltered air at ambient O_3 concentrations. No overall interaction between O_3 and salinity occurred for alfalfa growth or yield. The only general effect of O_3 itself was to increase the percentage of empty nodes at three of the four harvests. The percentage of empty nodes due to ozone tended to decrease with increasing salinity. Salinity by itself was more detrimental to plants than O_3 and caused occasional decreases in dry weight and height. At the levels tested, salinity would affect plants more than O_3 in areas where both stresses occur.

Salinity reduced the O_3 effects on injury and yield for several crops, including alfalfa, pinto bean, and garden beet (*Beta vulgaris* L.) grown under laboratory conditions [64]. Bytnerowiz and Tyalor [37] found that the salinity of the solution decreased O_3 injury in snap bean (*Phaseolus vulgaris* L.). High salinity, however, had no effect on O_3-induced reductions in snap bean dry weight. In these salinity-air pollutant studies, the beneficial effects of salinity in reducing air pollutant effects were attributed to salinity-pollutant interactions in causing stomatal closure and, therefore, less pollutant uptake.

In most species, ozone stress reduces the root growth more than the shoot growth. The root growth of three grasses—orchard grass (*Dactylis glomerata*), rye grass (*Lolium prenne*), and canary grass (*Phalaris aquatica*)—was impaired more than the shoot growth by O_3 stress. The dry weight reductions were shown to be the result of the reduced net assimilation rate. At higher O_3 levels (greater than approximately 0.10 ppm), photosynthesis was drastically reduced and partitioning to all sinks fell, causing dramatic growth reductions in all organs. At these higher ozone concentrations, differential partitioning between organs is not as obvious as at lower concentrations [65].

The root growth in carrot (*Daucus carota*) decreased tremendously under O_3 stress, whereas the foliar growth actually increased [66]. Controlled fumigations of 0.19 and 0.25 ppm O_3 decreased the root weight 32–46%, whereas the leaf weight rose slightly. As the O_3 dose increased, the shoot weight of tall fescue (*Festuca arundinacea*) decreased and the root weight fell even more. Rising O_3 concentrations further reduced both the shoot and root weight and always decreased the root weight more.

It has been reported that senescence was enhanced in flag leaves of wheat by ozone, which was reflected in the time course of the oxidant stress biomarkers. The plant protein content decreased and the nonspecific peroxidase activity, ascorbic acid content, and malondialdehyde content increased only after the leaves had experienced high doses of ozone, whereas the ascorbic acid content levels were raised in ozone-treated plants after only a short exposure period. The catalase activity did not significantly respond to the ozone treatment [67]. Many crops such as rice [68], wheat [69], tobacco (*Nicotiana tabacum*), and legume plants [70] have been found to be more susceptible to elevated O_3 levels. Recently, in Germany, a 35% wheat yield reduction was observed. It is believed that if precautionary measures are not taken well ahead of time to reduce the anthropogenic emissions of photochemical oxidants, a gradual imbalance in the vegetation may occur and susceptible crop/grass species may become extinct in the long run.

Welfare et al. [71] reported that five varieties of rice (*Oryza sativa* L.) of varying salinity resistance were grown in nonsaline and in saline conditions with and without a repeated exposure to ozone at a concentration of 88 nmol mol^{-1} giving an AOT40 (cumulative exposure above 40 nmol mol^{-1}) of 3600 nmol mol^{-1} h. Salinity caused a substantial reduction in the shoot and root dry weights in all varieties, but the effect on the root growth was proportionately less than on the shoot growth, transpiration, and stomatal conductance. The potassium concentration in the leaves of all five varieties was reduced by salinity and by ozone in both saline and nonsaline treatments. Ozone reduced the Na concentration in plants grown at 50 mMol NaCl but had no effect on the Cl concentration. Ozone reduced the root dry weight, but the treatment used did not significantly affect the shoot dry weight. Both salinity and ozone reduced the plant height and carbon dioxide assimilation, transpiration, and stomatal conductance were all reduced by salinity and by ozone, and there was a close quantitative similarity between the effects of ozone and/or salinity on assimilation,

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stomatal conductance, and transpiration. There were some antagonistic effects, but there were additive effects of salinity and of ozone on the root dry weight, plant height, photosynthesis, transpiration, and stomatal conductance.

Nouchi et al. [72] reported a 50% decrease in the whole plant dry weight in rice plants that were continuously exposed to ozone at 100 nmol mol^{-1} for 6 weeks, although the effect on the dry weight of an exposure of 50 nmol mol^{-1} was not significant. Other studies have reported greater sensitivity of the roots than the shoots in response to moderate ozone pollution [73,74]. Ozone in the lower atmosphere originates predominantly from oxidation and photolysis of nitrogen oxides emitted from vehicular exhaust fumes in urban areas. The sensitivity of plants to atmospheric oxidants is conditioned by a number of environmental factors, including nutritional level, humidity, soil water stress, and root-medium aeration. Many of those vital factors affect the stomata aperature. Ozone, a major air pollutant, decreases the yield of some oxidant-sensitive crops more under nonsaline than saline conditions. Plant sensitivity to ozone is usually evaluated in terms of the visual indices of the foliar injury. This method has been proven to be satisfactory for general leaf crops such as lettuce (*Lactuca sativa* L.), spinach (*Spinacia oleracea* L.), alfalfa (*Medicago sativa* L.), and other forage crops and tobacco (*Nicotiana tabacum* L.). However, some studies now show that the ozone effects on crop yields may not be proportional to the leaf injury [75].

Salinity also is an environmental factor affecting the sensitivity of crop plants to atmospheric oxidants. This abberation has the tendency to make many crops grown in air-polluted regions appear to be more salt tolerant than they really are. The salinity-ozone interaction may be agronomically important in air-polluted areas. However, the increased ozone tolerance induced by salinity may be more than offset by the detrimental effects of salinity on the marketable yields of pinto bean and garden beet and other crops.

Oertli [76] studied the interaction of salinity and atmospheric oxidants. He grew sunflower (*Helianthus annuus* L.) plants in unfiltered air in different concentrations of nutrient solution and found the symptoms of oxidant damage to be greatest on plants growing in the more dilute solutions. However, Heck [77] raises the question of whether this is a salinity interaction or a nutritional interaction. Nonsaline treatments in outdoor experiments show more evidence of leaf damage than saline treatments. This raises a question as to whether the rising level of atmospheric oxidants causes an erroneous increase in the apparent salt tolerance of crops by selectively damaging the control treatments. Because plant sensitivity to ozone is known to depend on the physiological age of leaves and plants repeatedly exposed to ozone throughout their vegetative growth stage exhibit cumulative damage not only to a few leaves but also eventually to all leaves as they become susceptible to ozone injury.

The influence of ozone on pinto bean has been evaluated on a visible leaf damage percentage or an apparent photosynthesis basis. Hill and Littlefield [78] reported that fumigating 8-week-old pinto bean plants with 0.45 ppm of ozone for 1.5 h reduced the apparent photosynthesis rate to half the rate of the control. Likewise, Todd and Propst [79] found that photosynthesis of a single bean leaf exposed to 0.34 ppm of ozone for 11.5 h was half that of an ozone-free leaf.

The effect of ozone on the forage yield of alfalfa (*Medicago sativa* L.) at four controlled salinity levels (-40, -200, -400, and -600 kPa) and ozone levels of 10, 15, and 20 parts per hundred million (pphm) was studied in controlled climate chambers. It was observed that as salinity increased, ozone had less of an effect on yield. Alfalfa exposed to 20 pphm of ozone for 2 h daily yielded 25% more at -200 kPa osmotic potential than that at the nonsaline level, -40 kPa. The residual effect of ozone treatments reduced yield in the next cutting. Salinity at all levels or ozone at 20 pphm increased the water-use efficiency of alfalfa. Both ozone and salinity were required to increase the leaf diffusion resistance [80]. Their experimental findings further demonstrated the strong interactive effects of salinity and ozone on alfalfa.

In a later study, Hoffman et al. [81] studied the interaction of salinity (osmotic potentials of -0.4, -2.0, and -4.0 bars) and ozone (0, 0.15, 0.25, and 0.35 ppm) on the growth of pinto bean (*Phaseolus vulgaris* L.) in a controlled temperature light room with 2-h daily exposures to the treatments. They found that ozone at 0.15 ppm decreased the yield of nonsaline plants nearly 50%,

and at 0.25 ppm and higher, no significant yield was obtained. At −4.0 bars, the yield at 0.25 ppm was only reduced to half that of the ozone-free treatment. The results indicated no interaction between salinity and ozone below 0.15 ppm. Above 0.15 ppm, however, there was a large interaction. Plants grown in saline media show an increased resistance to ozone injury. Mass et al. [61] reported that definite tolerance thresholds exist for both the ozone concentration and the duration of exposure whether plants receive a single or more exposures as reported by Heck et al. [82].

Ogata and Maas [83] studied the interactive effects of the root media salinity and ambient ozone on injury, growth, and yield of garden beet (*Beta vulgaris* L.) under controlled environmental conditions. Plants were grown in nonsaline and saline nutrient solution cultures having osmotic potentials of −0.4, −4.4, and −8.4 bars, respectively, and were exposed to 5 weeks of 0.20 ppm ozone for 0–3 hs/day. They found that the growth of the nonsaline beet plants was not significantly affected by 0.20 ppm ozone until exposure times exceeded 1 h/day, although foliar injury in the form of a reddish purple stipple had developed on mature leaves. Longer ozone exposures produced severe leaf necrosis and reduced the growth of tops and storage and fibrous roots as much as 50, 40, and 67%, respectively. They further observed that, in contrast, foliar ozone injury on plants grown in saline media developed more slowly and the growth of both tops and roots were relatively unaffected by ozone exposure of up to 3 hs/day. Some reduction in the yield of storage roots did occur at −4.4 bars of osmotic potential when plants were exposed to ozone for 3 h/day. The interactive effects of salinity and ozone are apparent for both top and root growth. Like the findings of the pinto bean study [81], these results indicate that the beneficial effects of salinity in reducing ozone damage occur at salinity and ozone levels too high to allow economical beet production. The vegetative growth data, on the other hand, suggest that the interaction of these factors might be economically important for forage crops.

Ozone reduced photosynthesis in many plant species, for example, wheat [84], oats (*Avena sativa* L.) [85], common bean [86], and broad bean [87], although the degree of sensitivity varies between species. The reduction in photosynthesis and the breakdown of chlorophyll molecules are very similar to changes which occur during leaf senescence. Ozone appears to advance the onset of leaf senescence, which is accompanied by a reduced carbon fixation, the breakdown of the chloroplast envelope, the loss of associated proteins and chlorophyll [88], and a reduced ribulose bisphosphate carboxylase/oxygenase (Rubisco) activity [89].

The mechanism by which salinity (osmotic stress) increases the plant tolerance to ozone remains speculative. Essentially, any factor that increases water stress in the plant increases stomatal resistance and, presumably, reduces ozone diffusion into the leaves. Ting and Dugger [90] concluded from a comparative study of ozone-sensitive and ozone-resistant varieties of tobacco that differences in sensitivity were due in part to significant differences in the plant water potentials of the two varieties. Of course, other biochemical and physiological mechanisms may also be involved. For instance, salinity is known to increase the sugar content in some plants [91], and evidence indicates that high sugar levels are associated with an increased resistance of ozone injury to plants [5].

Miller et al. [92] have demonstrated that ozone and a water deficit can suppress photosynthesis, growth, and yield of crops, and both may alter the plant carbohydrate status. Ozone stress generally suppressed leaflet concentrations of total soluble carbohydrate (TSCs) and starch on most sampling dates in soybean (*Glycine max* L.). Impacts of a water deficit were less consistent, but starch concenrations usually increased when effects were significant. Interactions between the two stresses occurred infrequently, although water stress reduced the negative effects of O_3 on sucrose and TSCs when the data were analyzed over the season. The ozone treatment also slightly increased the proportion of sucrose compared with starch in the total nonstructural carbohydrate (TNC) pool.

There are several known mechanisms for alteration of the soluble sugar and starch content of plant leaves by O_3. The most obvious is suppression of photosynthesis by O_3, which is due in part to the reduction of chlorophyll and the decline in the activity and quantity of Rubisco. Ozone has also been shown to interfere with the translocation of soluble carbohydrates from the leaves which would tend to increase concentrations in those tissues. No indication was found that O_3 elevated carbohydrate concentrations in the leaflets. It is likely that the reduction of photosyntehsis

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by O_3 would more than counteract the reduced translocation resulting in lower levels of carbohydrates in leaves in most cases [93].

CONCLUSIONS

In the vicinity of urban and industrial areas, pollution of atmospheric air by vehicles and industries is a recurring and persistent environmental health hazard. Toxic gases such as carbon dioxide, carbon monoxide, sulfur dioxide, nitrogen oxides, unburnt hydrocarbons, particulate matter, and soot are generally found in abundant quantities and causing serious health hazards to humans and damage to crops and animals.

The global population growth also is considered to be one of the major driving forces of global climatic change. The effects of human activities on the global atmosphere have become increasingly evident during the last decades. As a result of the population explosion, accelerated urbanization, and continuous industrialization new environmental problems like those of greenhouse effects, ozone layer depletion, acid rains, and the increased use of pesticides are being created tremendously.

The ecological implications of pollutants are quite serious for crop growth and development. The gaseous pollutants such as sulfur dioxide, carbon dioxide, ozone, and nitrogen oxides produced by industries and the heavy vehicular traffic of cities drift to rural areas and have fallout effects causing injury to plants and resulting in crop losses. In addition, SO_2 and O_3 in combination with soil salinity have far-reaching effects on crop growth.

Air pollution by gaseous sulfur dioxide causes the development of soil acidity. Sulfur dioxide injury to vegetation and forest growth has been noted near industrial areas. Salinity and SO_2 have been found to be antagonistic in a number of crop species. Similarly, SO_2 in combination with ozone (O_3) has substantially reduced the crop growth and biochemical composition of plants.

Ozone in the lower atmosphere originates predominantly from oxidation and photolysis of nitrogen oxides emitted from vehicular exhaust fumes in urban areas. Ozone has been proven to be one of the most dangerous air pollutants affecting the physiology, growth, yield, and biochemical composition of many crops at present. Ozone and SO_2 are likely combinations in many areas. These two gases, both singly and in combination, disrupt various metabolic processes and consequently affect the growth and development of plants.

Similarly, salinity and O_3 combinations also have drastic effects on the growth of plants. However, salinity reduced O_3 effects on injury and yield for several crops. Reports indicated that the beneficial effects of salinity in reducing ozone injury does not appear to be of any economical importance in crop production.

There is a great risk of higher air pollution effluents due to the lack of effective control measures through catalyzers in the vehicles and filters in chimneys of the industries. There is an urgent need to survey the extent of air pollutants at the surface level, especially adjacent to areas of traffic and industries, and if it is beyond the critical level, we should formulate the structures (i.e., take necessary measures to reduce pollutant emission or introduce filters, etc.), so that air pollutant levels in the affected areas remain within the tolerance range in order to keep the environment pure and healthy for humans to live in.

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Herbicide-Mediated Changes in the Population and Activity of Root-Associated Microorganisms: A Potential Cause of Plant Stress

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INTRODUCTION

There has been a great deal of increase in the use of herbicides in the world during the past 30 years [1–3]. New and more effective herbicides are continuously being developed to replace the old ones. In the course of their development, herbicides are routinely screened for the absence of phytotoxicity to crops. However, little attention is given to the possibility that they may also be toxic to plant-associated microorganisms such as those that cause disease stress and those that protect crops against diseases [4]. Consequently, most herbicides are biologically active against microorganisms [5]. Some herbicides have been shown to cause changes in populations of some bacteria in the soil [6,7] and in the rhizosphere [8]. Herbicides are also known to cause changes in the incidence and severity of some types of pathogen-induced stress possibly by affecting plant pathogens [1,2,9–11].

In this chapter, we discuss the impact of herbicides on soil-residing plant pathogenic microorganisms that cause disease in plants and on root-associated beneficial microorganisms that suppress the activity of plant pathogens (biocontrol agents). We show that herbicide-mediated changes in the activity of plant pathogens and biocontrol agents may result in an increase or a decrease in pathogen-induced stress in plants.

We have been interested in the impact of herbicides on the activity of biocontrol microorganisms and on the intensity of pathogen-induced stress because (a) the herbicide concentration varies drastically in the soil, (b) microorganisms and plants are sensitive to changes in the levels of herbicides, and (c) unlike other soil factors (e.g., temperature, moisture), the impact of herbicides on the activity of biocontrol microorganisms has virtually been neglected by students of plant health.

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PLANT PATHOGENS AND THE DISEASES THEY CAUSE

Plants, like other organisms, have natural enemies that are capable of reducing their vigor and/or destroying them completely. These enemies are among both prokaryotic and eukaryotic groups, including plants, fungi, bacteria, viruses, nematodes, and a few other microorganisms. These pathogens attack plants at the aboveground and belowground levels. The damage to plants caused by insects is not traditionally considered to be a disease problem.

In a typical scenario, a pathogen is disseminated by wind, irrigation, or flood water and/or by insects and is deposited near a plant. Some pathogens, like nematodes and flagella-equipped bacteria and fungi, are attracted to the plant surfaces using chemotactic responses. Other pathogens are deposited passively on the plant surfaces. The first step in the infection process is penetration of the pathogen into plants through wounds and natural openings such as stomata. Some pathogens are capable of penetrating plant surfaces actively by enzymatically dissolving physical barriers. The penetration into the plant tissue often is followed by a rapid development and spread of the pathogen inside the plant.

The outcome of plant-pathogen interaction, that is, whether the pathogen succeeded or fails to cause disease, depends on a number of factors, including the virulence of the pathogen, the susceptibility of the plant, and the prevailing environmental factors [12]. All of the stages in disease development discussed earlier are sensitive to changes in the environmental conditions, including temperature, moisture, soil texture, pH, soil atmospheric composition, and the presence of chemicals, such as insecticides, fungicides, and herbicides in the soil. Environmental factors impact disease development by modulating the growth and the activity of pathogens, by interfering with the plant's ability to defend itself against pathogenic invasion, and by affecting the development and the activity of microorganisms that compete with pathogens for food resources and for microsites on and around plants.

PATHOGEN-INDUCED STRESS IN PLANTS

Plants often suffer from mild and acute forms of stress in response to pathogenic invasion. The stress is manifested by a variety of symptoms such as wilting as a consequence of water stress, chlorosis mainly due to chlorophyll breakdown, and necrosis as a consequence of the action of pathogen-produced toxins. Water stress can be induced by the destruction of the root systems, pathogen-induced change in the permeability of root and/or leaf cells, and by blocking of xylem elements. Pathogen-induced stress may also be manifested as abnormal growth and malformation due to hormonal imbalances. Most pathogens rob plants of vital nutrients creating nutritional stress. Some of these stress conditions may be reversed when environmental conditions are altered in ways that are no longer conducive to the growth and pathogenesis of pathogens. However, most plants suffering from acute forms of pathogen-induced stress often fail to recover.

BIOCONTROL OF PLANT DISEASES

Plant diseases are being controlled mainly by the use of chemicals (e.g., fungicides, nematicides, bactericides) and in some cases by cultural practices. Chemical approaches to managing plant diseases have in recent years been the subject of public concern, because of the harmful effect of chemicals on the environment, the effect on nontarget organisms, and the possible carcinogenicity of some chemicals. Other problems include the appearance of new races of pathogens that are resistant to chemicals, a gradual elimination and phasing out of some pesticides, and the reluctance of some chemical companies to develop and test new chemicals owing to the long registration process and escalating costs.

A promising new approach to controlling plant diseases is the use of biological agents and/

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or their products, which is an approach known as biocontrol [13]. Biocontrol is environmentally safe and in some cases is the only option available for protecting plants against diseases [4,13–15].

Unfortunately, the development of biocontrol strategies to combat plant diseases has been painfully slow. Many biocontrol agents perform consistently and efficiently under laboratory conditions but fail to do so in the field [4,13–15]. This is perhaps because the environmental conditions in the laboratory are often artificially selected to be conducive to the development and functioning of biocontrol agents [13]. The environmental conditions in the field, on the other hand, are too complex and exert an influence on the activity and performance of microbial biocontrol agents as well as disease incidence and disease development. Moreover, bacteria, fungi, and nematodes are highly sensitive to changes in environmental factors [16–20]. These observations are the basis for the generally accepted view that a better understanding of the impact of soil and environmental factors on biocontrol microorganisms is the prerequisite for transferring plant disease biocontrol strategies from the laboratory to the field [4,13,14,21]. Soil environmental factors that can potentially affect the intensity of stress induced by soil-residing pathogens, and biocontrol activity of microbial biocontrol agents in the field include moisture, temperature, pH, texture, organic content, atmospheric composition, and the presence of agricultural chemicals in the soil such as herbicides [12,18,22–25].

In this chapter, evidence is presented that preplant herbicides, which are being used heavily on a variety of crops throughout the world, may change the intensity of pathogen-induced stress (in the presence and absence of biocontrol agents) by influencing plants, pathogens, and/or microbial biocontrol agents. We have previously provided evidence that oxygen and carbon dioxide concentrations affect pathogen-induced stress levels by affecting the activity of biocontrol agents [18].

EFFECT OF HERBICIDES ON PATHOGEN-INDUCED STRESS

Herbicides are known to alter the incidence and severity of pathogen-induced stress in plants [1,2,5,11,26–33]. Altman and Campbell [1] showed that the incidence of sugar beet seedling death caused by *Rhizoctonia solani* increased significantly after application of cycolate to the field soil. Rovira and McDonald [11] showed that the application of chlorsulfuron to the field soil caused a significant increase in the level of disease stress to wheat and barley caused by *R. solani* and *Gauemannomyces graminis var. tritici*. The severity of stress in cereals due to infection by *Heterodera avenae* increased after application of trifluralin to the field soil [2]. Disease stress in wheat caused by *G. graminis var.* tritici infection [11] and in sugar beet caused by *R. solani* infection [1] increased after application of trifluralin, chlorsulfuron, and cycolate. Application of trifluralin and dinitramine to the field soil also increased *R. solani*–induced stress in cotton seedlings [26,31,32]. In contrast, the severity of *Fusarium oxysporum vasinfectum*–induced stress in cotton plants decreased following application of trifluralin, fluometuron, diuron, dalapon, and prometryn to the field soil, whereas the incidence of *R. solani*–induced cotton seedling death was not significantly affected by these herbicides [27].

We have studied the impact of three preplant herbicides, pendimethalin, prometryn, and trifluralin, on *R. solani*–induced stress in cotton seedlings in the microcosm and in the field in Arizona at two locations (Safford and Tucson). Plants attacked by this pathogen may be killed prior to or after emergence. In our microcosm experiments, preemergence and postemergence seedling death were increased in soils treated with two of the three herbicides. In a preemergence seedling death experiment in the microcosm, the stand count (number of emerged seedlings) in the soil treated with prometryn was significantly ($P < .05$) dropped by 21, 36, and 67% relative to the control 1, 2, and 3 weeks after sowing, respectively [24]. The stand count in the soil treated with pendimethalin and trifluralin were not significantly ($P > .05$) different from that in the control. In the postemergence seedling death experiment in the microcosm, the disease incidence in soils treated with pendimethalin and prometryn increased significantly ($P < .05$) by 64, 60, and 50% and by 64, 59, and 57%

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Table 1 Plant Stand for Soils Treated with Each Test Herbicide and Infested with *Rhizoctonia solani* Inoculum for the Safford Field Experiment

	Time (days after sowing)		
Treatment	15	25	50
R. solani only	166 (14) a	126 (12) a	105 (12) a
R. solani + pendimethalin	117 (19) b	77 (19) b	56 (11) b
R. solani + prometryn	121 (18) b	88 (16) b	64 (15) b
R. solani + trifluralin	163 (16) a	132 (13) a	112 (8) a

Stand is represented as mean (the average number of emerged seedlings in one plot or one replicate sown with 400 seeds). Each mean is an average of four values.
Means followed by the same letter in each column are not significantly different ($P > .05$) according to the Duncan multiple range test.
Figures in parentheses are standard deviation.

relative to the control 1, 2, and 3 weeks after inoculation, respectively. The disease incidence in the soil treated with trifluralin was not significantly ($P > .05$) different from the control [24].

The results of field experiments corresponded with those of microcosm experiments (Tables 1 and 2). The stand count in plots treated with pendimethalin and prometryn at Safford significantly ($P < .05$) decreased between 30 and 28%, 39 and 30%, and 47 and 39% for 15, 25, and 50 days after sowing, respectively (Table 1). The difference in the amount of disease in plots treated with trifluralin and in nontreated plots at Safford was not significant ($P > .05$) relative to the control. The stand count in plots treated with prometryn at Tucson significantly ($P < .05$) decreased by, 41, 49, and 54% relative to the control, 15, 25, and 50 days after sowing, respectively (Table 2). Pendimethalin and trifluralin did not cause significant changes in the stand count at the Tucson location (Table 2).

The reported impact of herbicides on the intensity of pathogen-induced stress is not always the same in different studies. For example, prometryn, which increased cotton seedling death in our studies, did not do so in a previous study [27]. Moreover, trifluralin, which has been reported to increase *R. solani*–induced cotton seedling death [28,30,32], did not affect the disease in our study and in a previous study [27]. The differential responses may be due to the differences in the soil

Table 2 Plant Stand for Soils Treated with Each Test Herbicide and Infested with *Rhizoctonia solani* Inoculum for the Tucson Field Experiment

	Time (days after sowing)		
Treatment	15	25	50
R. solani only	101 (13) a	87 (11) a	79 (18) a
R. solani + pendimethalin	91 (56) a	75 (43) ab	55 (12) ab
R. solani + prometryn	60 (28) b	44 (21) b	36 (15) b
R. solani + trifluralin	104 (24) a	97 (21) a	70 (11) a

Stand is represented as mean (the average number of emerged seedlings in one plot or one replicate sown with 400 seeds). Each mean is an average of four values.
Means followed by the same letter in each column are not significantly different ($P > .05$) according to the Duncan multiple range test.
Figures in parentheses are standard deviation.

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moisture, soil temperature, herbicide concentration, races of pathogens, plant varieties, composition of rhizosphere microflora, and rate of herbicide inactivation in various experiments. The development of tolerance to herbicides by pathogens as a result of a long-term herbicide use may also be a contributing factor.

EFFECT OF HERBICIDES ON POPULATION OF BIOCONTROL BACTERIA IN THE RHIZOSPHERE

To assess the impact of herbicides on the activity of biocontrol bacteria, it is necessary to examine the impact of herbicides on rhizosphere populations of biocontrol bacteria. This is because the ability of these bacteria to develop in the rhizosphere of target plants is a prerequisite for their biocontrol activity [34]. Any soil factors which can potentially interfere with the ability of biocontrol bacteria to develop in the rhizosphere is expected to affect their biocontrol activity as well. However, despite its importance and as far as we have been able to determine, the impact of herbicides on the population of biocontrol bacteria in the rhizosphere has not been studied except for our study (to be discussed below).

We studied the potential impact of three widely used herbicides, pendimethalin, prometryn, and trifluralin, on populations of five plant disease–suppressing bacterial isolates (three isolates of *Pseudomonas fluorescens* and two isolates of *Burkholderia cepacia*) in the rhizosphere of cotton seedlings [23]. All isolates are efficient cotton root colonizers and are capable of suppressing pathogen-induced stress. All five isolates were used in microcosm experiments and one isolate (D1) was tested in the field.

In microcosm experiments, the population sizes of most of the bacterial isolates in the rhizosphere of cotton seedlings in soils treated with each of the three herbicides were significantly ($P < .05$) lower than those in the untreated soils 2 weeks after sowing [23].

The ability of all three test herbicides to reduce isolate D1 population in the rhizosphere declined with time over a 4-week period of monitoring [23]. The population of the bacterium recovered from roots in the herbicide-treated soils were significantly ($P < .05$) lower than those recovered from controls after 1 and 2 weeks but were equivalent to the controls 3 and 4 weeks after sowing [23].

The results of the field experiments were similar to those of the microcosm experiments (Tables 3 and 4). Pendimethalin and prometryn caused a significant ($P < .05$) decrease in the D1 population in the rhizosphere 15 and 25 days after sowing at the Safford location (Table 3). Trifluralin

TABLE 3 Population Sizes ($\times 10^6$ cfu g^{-1} Root) of *Burkholderia cepacia* (Isolate D1) in the Rhizosphere of Cotton Seedlings Grown in Soils Treated or Not Treated with Pendimethalin, Prometryn, or Trifluralin 15, 25, and 50 Days After Sowing in the Safford Field Experiment

	Time (days after sowing)		
Treatment	15	25	50
Control (no herbicide)	5.3 (2.5) a	3.0 (2.2) a	2.2 (1.8) a
Pendimethalin	2.6 (1.8) b	1.4 (1.3) b	1.7 (1.1) a
Prometryn	2.4 (1.1) b	1.8 (1.3) b	1.8 (1.4) a
Trifluralin	4.1 (2.2) a	2.4 (1.9) ab	1.9 (1.3)a

Each figure is an average of four values obtained in one experiment with four replicates.
Figures followed by the same letter in each column are not significantly different ($P > .05$) according to the Duncan multiple range test.
Figures in parentheses are standard deviation.

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TABLE 4 Population Sizes ($\times 10^6$ cfu g^{-1} Root) of *Burkholderia cepacia* (Isolate D1) in the Rhizosphere of Cotton Seedlings Grown in Soils Treated or Not Treated with Pendimethalin, prometryn, or Trifluralin 15, 25, and 50 Days After Sowing in the Tucson Field Experiment

	Time (days after sowing)		
Treatment	15	25	50
Control (no herbicide)	7.2 (4.2) a	2.8 (2.0) a	1.0 (0.8) a
Pendimethalin	3.0 (0.9) b	1.3 (0.8) b	0.8 (0.5) a
Prometryn	2.0 (1.5) b	1.1 (0.9) b	0.7 (0.5) a
Trifluralin	5.6 (2.9) a	1.6 (1.0) b	1.0 (0.6) a

Each figure is an average of four values obtained in one experiment with four replicates.
Figures followed by the same letter in each column are not significantly different ($P > .05$) according to the Duncan multiple range test.
Figures in parentheses are standard deviation.

had no significant effect on the D1 population at this location (Table 3). Pendimethalin and prometryn caused a significant ($P < .05$) decrease in the D1 population in the rhizosphere 15 and 25 days after sowing but not 50 days at the Tucson location (Table 4). The trifluralin-induced decline in the D1 population was significant only 25 days after sowing (Table 4). Isolate D1, which was originally recovered from cotton plants in the field, may have developed tolerance to trifluralin as a result of the continuous exposure to this herbicide in the field.

EFFECT OF HERBICIDES ON BIOCONTROL ACTIVITY OF BACTERIA IN THE RHIZOSPHERE

Our earlier finding that the soil atmsopheric composition can modulate the biocontrol activity of selected bacteria [18] spurred us to examine the impact of other soil factors on the activity of biocontrol bacteria in the rhizosphere. We therefore examined the impact of three widely used herbicides, pendimethalin, prometryn, and trifluralin, on the efficacy of isolate D1 (a biocontrol bacterium) to reduce the severity of *R. solani*–induced seedling death. Isolate D1 is capable of reducing the incidence of *R. solani*–induced seedling death in the field [35]. In both field and microcosm experiments, the efficacy of isolate D1 was reduced in the presence of two of the three test herbicides. In the Safford field experiment, isolate D1 reduced seedling death severity significantly ($P < .05$) compared with the control (not treated with D1) 15, 25, and 50 days after sowing only in non–herbicide-treated plots and in plots treated with trifluralin and not in plots treated with pendimethalin and prometryn (Table 5). In a Tucson field experiment, the biocontrol bacterium (isolate D1) significantly reduced cotton seedling death in plots not treated with herbicides and in those treated with trifluralin compared with the control (not treated with D1) 15, 25, and 50 days after sowing (Table 6). Pendimethalin and prometryn both significantly ($P < 0.05$) decreased the efficacy of isolate D1 in reducing cotton seedling death 25 and 50 days after sowing in a Tucson experiment (Table 6).

In contrast to our findings, the herbicides pendimethalin and metribuzin have been reported to enhance the biocontrol activity of *Streptomyces corchorusii* and *S. mutabilis* in greenhouse tests [36].

MECHANISM OF HERBICIDE-MEDIATED CHANGE IN PATHOGEN-INDUCED STRESS

The mechanism of the observed herbicide-mediated change in the intensity of pathogen-induced stress is not known. The phenomenon may be due to the effect of herbicides on the plant, on the

TABLE 5 Stand Count (Number of Emerged Seedlings) in Soils Treated with Each Test Herbicide and Inoculated with *Rhizoctonia solani* and/or Biocontrol Bacterium (*Burkholderia cepacia*, Isolate D1) 15, 25, and 50 Days After Sowing in the Safford Field Experiment

	Time (days after sowing)		
Treatment	15	25	50
R. solani alone	166 (15) b	126 (12) b	105 (12) b
R. solani + D1	217 (23) a	193 (19) a	184 (15) a
R. solani + D1 + pendimethalin	153 (53) b	121 (50) b	98 (40) b
R. solani + D1 + prometryn	163 (13) b	127 (15) b	85 (26) b
R. solani + D1 + trifluralin	202 (23) a	186 (20) a	161 (23) a

Each figure represents the average number of emerged seedlings in one plot (one replicate) sowed with 400 seeds. Each figure is average of four values obtained in one experiment with four replicates.
Figures in parentheses are standard deviation.
Figures followed by the same letter in each column are not significantly different ($P > .05$) according to the Duncan multiple range test.

TABLE 6 Stand Count (Number of Emerged Seedlings) in Soils Treated with Each Test Herbicide and Inoculated with *Rhizoctonia solain* and/or Biocontrol Bacterium (*Burkholderia cepacia*, Isolate D1) 15, 25, and 50 Days After Sowing in the Tucson Field Experiment

	Time (days after sowing)		
Treatment	15	25	50
R. solani alone	101 (13) b	87 (11) b	79 (18) b
R. solani + D1	171 (29) a	164 (29) a	158 (25) a
R. solani + D1 + pendimethalin	117 (33) b	107 (32) b	102 (32) b
R. solani + D1 + prometryn	168 (51) a	121 (25) ab	110 (31) b
R. solani + D1 + trifluralin	180 (17) a	157 (19) a	151 (21) a

Each figure represents the average number of emerged seedlings in one plot (one replicate) sowed with 400 seeds. Each figure is average of four values obtained in one experiment with four replicates.
Figures in parentheses are standard deviation.
Figures followed by the same letter in each column are not significantly different ($P > .05$) according to the Duncan multiple range test.

pathogen, on the activity of indigenous microbial competitors, and/or on the interactions among these entities.

Herbicides may cause changes in the plant root physiology such as root exudation [2,37]. These changes, in turn, may alter microbial community structures in the rhizosphere in ways which may encourage or discourage the development of competitors of an introduced biocontrol bacterium. Such changes may enhance or depress the activity of biocontrol bacteria. Herbicides may alter the intensity of pathogen-induced stress by changing the plant's resistance levels to pathogens. Starratt and Lazarovits [38] showed that the application of dinitroaniline herbicides induced resistance in tomato seedlings to the pathogen *Fusarium oxysporum* f. sp. *lycopersici*. Herbicides also may cause changes in crop plants which may influence the outcome of plant-pathogen interactions [1,39–41]. Herbicides have been reported to cause alterations in the growth, lignin-containing substances,

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β-glucoside [41], and waxy layer on leaves [39,40] and in the release of glucose from roots [1,42]. Although the height, biomass, and root densities of cotton seedlings grown in soils treated with pendimethalin or prometryn were generally lower than those of control (untreated soil) in our studies, the differences were not statistically significant ($P > .05$) indicating that the physical characteristics of cotton seedlings were not affected by test herbicides [25].

The observed herbicide-mediated change in the intensity of pathogen-induced stress may be due to the effect of herbicides on the pathogen [1,26,31,43]. Such an effect may be stimulatory or inhibitory. For example, in the *R. solani*–sugar beet combination, the herbicide cycolate may interfere with the growth of the fungus and at the same time may enhance root exudates [1]. In such cases, the impact of the herbicide on the intensity of pathogen-induced stress is determined by the balance of stimulatory and inhibitory effects [1]. In our study, the growth of *R. solani* in vitro was not significantly affected by pendimethalin, prometryn, or trifluralin [25].

Herbicide-mediated alterations in pathogen-induced stress may also be due to the effect of herbicides on indigenous microbial antagonists of pathogens [23,25,36,44]. The observed absence of soilborne diseases in some fields in the presence of susceptible hosts and virulent pathogens is most likely due to the presence of indigenous microbial antagonists of the pathogen [13]. As pointed out earlier, we found that the herbicides, pendimethalin, prometryn, and trifluralin, decreased the populations of some biocontrol bacteria in the rhizosphere of cotton [23].

Finally, herbicides may change the intensity of pathogen-induced stress by interfering with the activity of fungicides used to curb the pathogenic activity of pathogens [26,30,45,46]. Application of fluchlovalin and alachlor to the soil altered the effectiveness of fungicides to control the condition in cowpea known as damping-off [45]. Application of nurflurazon, pendimethalin, fluometuron, prometryn, fomesafen, and oxyfluorfen to the field soil significantly reduced the efficacy of the fungicides tolclofos-methyl, pencycuron, carboxin, flutonalit, metalaxyl, and chloroneb against cotton seedling diseases [30]. In contrast, the antifungal activity of the fungicides captan and mounsrin was shown to be increased in the presence of herbicides, paraquat, and simazine [47].

Although plants, pathogens, and antagonistic microorganisms are perhaps the primary target of herbicides, the possibility of other herbicide-mediated changes cannot be overlooked. One such possibility is an alteration of the microclimate as a consequence of the removal of weeds, as suggested by Heitefuss [39]. We agree with Altman and Campbell [1] that no single factor may be solely responsible for the observed herbicidal effect on the outcome of plant-pathogen interactions.

MECHANISM OF HERBICIDE-MEDIATED CHANGE IN BIOCONTROL ACTIVITY OF BACTERIA

The observed herbicides' interference with the biocontrol activity of isolate D1 [25] and *Streptomyces* sp. [36] is most likely due to the effect of herbicides on the biocontrol agents. The sensitivity of microorganisms to herbicides has been demonstrated [6,17,23]. The results of our preliminary studies also have shown that the growth of isolate D1 in a liquid medium was reduced by 48, 44, and 32% 24 h after exposure to pendimethalin, prometryn, or trifluralin, respectively. The herbicide-mediated change in the performance of biocontrol agents may also be a consequence of a herbicide-induced change in the pathogen and the plant which was discussed earlier. For example, the increased activity of a pathogen (in terms of growth and aggressiveness) in the presence of a herbicide may tip the balance in favor of the pathogen reducing the effectiveness of the biocontrol agent. Herbicides may also provide a favorable environment for some indigenous competitors of the biocontrol agent. Herbicide-induced changes in plant may cause changes in the microbial community structures encouraging or discouraging biocontrol activity. Another possibility is the herbicide-mediated shift in the quality and the quantity of the root exudate and/or border cells in plants [48] which can alter the microbial community structure. Finally, herbicides may affect cross communication among microorganisms and plant roots causing drastic changes in the activity of the introduced biocontrol

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agents and their indigenous competitors. Cross communication among microorganisms and between plants and microorganisms has been demonstrated [49,50].

CONCLUSION

The results of studies presented here clearly show that herbicides which are being used extensively throughout the world (a) may alter the severity of pathogen-induced stress, (b) may affect the efficacy of biocontrol agents used to curb pathogen-induced stress. Herbicides may vary in effectiveness, as microorganisms are differentially sensitive to herbicides. In our study, trifluralin did not cause any significant change in the incidence of cotton seedling death both in the microcosm and in the field experiments. Rhizosphere-associated microorganisms, including *R. solani*, may have developed some levels of tolerance to this herbicide owing to its widespread and long-term use in cotton fields.

The results presented have important implications for disease management. This is particularly true for seedling diseases in which plants are vulnerable to attack by pathogens when most herbicides may still be present in the soil at biologically active levels. The selection of an herbicide must be done cautiously in areas where plant diseases are important. Ideally, herbicides available for a particular crop need to be screened for their effect on pathogen-induced stress and on the biocontrol activity of the selected biocontrol agents. Since bacterial isolates are differentially sensitive to herbicide, it may also be possible to first select an herbicide and then to choose a biocontrol agent whose activity is not adversely affected by the presence of the selected herbicide. It may also be possible to construct bacterial isolates with increased tolerance to herbicides.

The sensitivity of some microorganisms, including biocontrol-active *Burkholderia cepacia*, to herbicides [7,17,23] and the herbicides' ability to increase the incidence of some diseases [1,2,11,36,51] provide additional support in favor of the concept of the integrated pest management (IPM) strategy. This strategy encourages crop specialists to base their decision regarding the use of a pesticide not only on the effectiveness of the pesticide against the target pest but also on its potential impact on all crop pests in the region. The selection of an ideal pesticide (one which is not harmful to the crop, to the non-target microorganisms, and to the beneficial insects) is difficult because of the number and diversity of pests involved in any one crop in any region. For example, in addition to insects and weeds, cotton seedlings are damaged by a number of soilborne pathogens besides *R. solani*. Despite these problems, IPM strategies need to be developed for specific crops in specific regions. The development of IPM requires a knowledge of the impact of a selected pesticide not only on its intended target but also on plants as well as on beneficial and harmful microorganisms and insects. Application of the IPM strategy also requires major changes in agricultural development policies and institutions [52].

Although many herbicides are readily biodegraded within a week, others may remain active for up to 2 months following application to the soil [2]. We found that the ability of all three test herbicides to reduce the biocontrol bacterium (isolate D1) populations in the rhizosphere decreased with time perhaps owing to degradation of the herbicides. However, all three herbicides tested in the Safford field experiment were biologically active up to 25 days after sowing. The persistence of herbicides in the soil depends on several factors, including soil moisture, temperature, pH, organic matter content, clay content, and the chemical structure of the herbicides [2].

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Photodynamic Herbicides Affecting Structure and Function of the Photosynthetic Apparatus

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INTRODUCTION

For centuries humans have endeavored to increase the productivity of crop plants. Plant productivity is closely related to photosynthesis. Most crops achieve less than 50% of their photosynthetic potential owing to limiting factors such as, for example, shading, water supply, or air pollution [1]. Therefore, one of the main goals to improve bioproductivity is to increase the transformation of solar energy to chemical energy and to improve the quality of plants and their resistance to unfavorable environmental factors [2]. With the advent of genetic engineering, one can believe that crop productivity can be improved using methods of biotechnology. One of these methods is to introduce biotechnologically improved plants of better quality to modify crop plants for higher photosynthesis [3].

The application of herbicides to control weed growth is another very common and important agricultural practice. The demand for rapid biodegradable and highly effective herbicides that are nontoxic to animals and humans opens ways for the production of new herbicide-resistant crops [3] and for the development of new highly selective groups of herbicides.

In searching for highly selective and environmentally safe herbicides in the 1980s, Rebeiz and coworkers [4] used compounds which induce the accumulation of chlorophyll (Chl) precursors in the darkness and cause photooxidative damage of plants during subsequent illumination. These investigators named this group of compounds photodynamic herbicides.

Since photodynamic herbicides interfere with the biosynthesis of chlorophyll and cause photodynamic destruction in the light, we decided to analyze in more detail the influence of these compounds on the structure and development of the photodynamic apparatus.

We expected that photodynamic herbicides could change the normal development of the photosynthetic apparatus and to cause some destruction of the photosynthetic and cellular membranes in already developed chloroplasts.

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CONCEPT OF PHOTODYNAMIC HERBICIDES

Rebeiz and coworkers [4] had an idea to combine the following phenomena: (a) the capability of accumulating chlorophyll (Chl) precursors in the darkness induced by δ-aminolevulinic acid (ALA) or complexing agents (e.g., 2,2′-bipyridyl (2,2′-B), (b) the fact that tissues which accumulate tetrapyrrole intermediates become very sensitive to light, (c) the species-dependent biosynthetic heterogeneity of the Chl biosynthetic pathway.

Higher plants treated during the dark period with ALA or with complexing agents such as 1,10-phenanthroline (Phe), 2,2′-B, and 8-hydroxyquinoline (8-H) accumulate large amounts of Chl precursors [5–9]. Aromatic heterocyclic components that bind iron inhibit heme biosynthesis and lead to inhibition of the tetrapyrrole pathway at the level of ALA formation or at the later stages. The complexing of iron could also inhibit the enzymatic processes of the Chl biosynthesis [10,11]. One of these mechanisms, or a combination of them, leads to the accumulation of Chl precursors in dark-grown plants. During subsequent illumination, the accumulated tetrapyrrole intermediates act as photodynamic sensitizers. As mentioned previously, compounds which act via this mechanism were named photodynamic herbicides by Rebeiz and coworkers [4]. The photodynamic effects are manifested by bleaching of leaves, a severe loss of turgidity of affected plant tissues, and finally by death of susceptible plants [4]. These effects were mainly due to the accumulation of protochlorophyllide (Pchlide) [4]; or, as was established later, they are mainly due to the accumulation of protoporphyrin IX, Mg-protoporphyrin, and its monomethyl ester rather than of Pchlide [6].

It is believed that the action of photodynamic herbicides is based on a photooxidative mechanism: Accumulated porphyrins photosensitize the formation of singlet oxygen, which in a free radical chain reaction oxidizes the lipoprotein components of cellular membranes [12,13].

Rebeiz and coworkers [4] found that various plant species respond differently to ALA and 2,2′-B treatment exhibiting a very pronounced organ-, age-, and species-dependent selectivity [4] (Fig. 1a–c). The first group of plants, represented by some dicotyledonous weeds such as mustard, lambsquarter, common purslane, and red-root pigweed, accumulated a significant amount of tetrapyrroles in the leaves and stems, and they were subject to severe photodynamic damages after exposure to light (Fig. 1a). The second group of dicotyledonous plants, such as soybean, kidney bean, and cotton, accumulated a significant amount of tetrapyrroles in the leaf tissues but not in the stems. The leaves exhibited severe photodynamic damage and died within a few hours, although the stems remained unaffected. These plants usually recovered from photooxidative damage and produced new leaves (Fig. 1b). The third group of plants, monocotyledonous agricultural plants (corn, barley, oat, or wheat), responded differently to ALA and 2,2′-B treatment. They accumulated a significant amount of tetrapyrroles, but the photodynamic damage was either imperceptible or consisted of small necrotic regions only. These plants (Fig. 1c) developed into healthy ones.

The selectivity of the photodynamic herbicide action seems to be based on (a) different capabilities of tetrapyrrole accumulation by various plant tissues (e.g., see Fig. 1a–c), (b) uneven distribution of various Chl biosynthetic routes, and (c) simultaneous application of different modulators [14–16].

Regarding the second point, there are differences among various angiosperms in Chlorophyll *a* (Chla) biosynthesis. Most of Chla is formed via two parallel biosynthetic routes: divinyl (DV) and monovinyl (MV) [17]. However, the endproducts in higher plants are MV not DV Chla and Chlb except probably for the lethal maize mutant which forms only DV Chla and Chlb [18]. Depending on which Chla biosynthetic pathway predominates during the dark and light phases of the photoperiodic growth, higher plants have been classified into three groups: dark DV light DV, accumulating mainly DV Pchlide a at night and mainly DV Chla during the day (e.g., cucumber, mustard, and representatives of more primitive plant groups: algae, bryophytes, ferns, and gymnosperms); dark MV light MV, accumulating mainly MV Pchlide a at night and MV Chla during the day (e.g., Johnson grass) and the largest groups of plants; dark MV light DV, accumulating mainly MV Pchlide a at night and mainly DV Chla during the day (e.g., corn, wheat, soybean). This phenomena is species dependent and has evolutionary significance [19].

Regarding the third point, some chemicals can modulate the Chl biosynthetic pathway by

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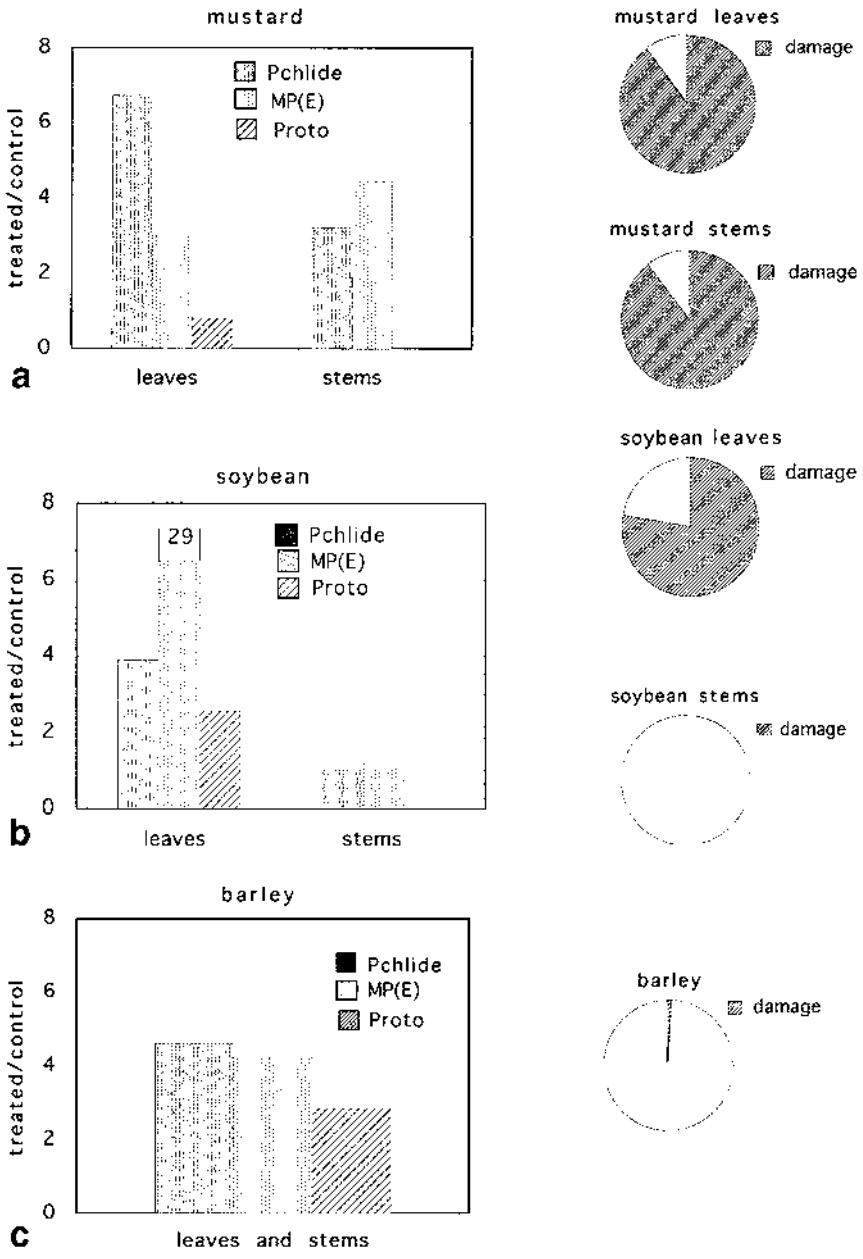

FIGURE 1 Three types of photodynamic herbicidal response of various plants species to ALA + 2,2′-B treatment and subsequent irradiation. The Type I response is represented by mustard (a), type II by soybean (b), and type III by barley (c). (Based on Refs. 4 and 14.)

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forcing the treated plants to accumulate a type of MV or DV tetrapyrrole that is not produced in the natural Chl biosynthetic routes of this plant species and thus cause a strong photodynamic effect [14,15,20].

It was recently shown that evolution under domestication as manifested in selection for higher plant yields has favored, in certain cases, the MV route and not the DV route [21]. Recent results also showed that the treatment of plants with a low concentration of ALA, the main precursor of all tetrapyrroles including Chl, enhanced the accumulation of MV Pchlide in darkness, especially in dark DV plant species such as cucumber and velvetleaf [21].

INFLUENCE OF PHOTODYNAMIC HERBICIDES ON CHLOROPLAST DEVELOPMENT IN ETIOLATED AND SUBSEQUENTLY ILLUMINATED PLANTS

Plants in Toto

First detailed analysis of a reaction of a single plant species, cress (*Lepidium sativum*), to photodynamic herbicides, with special attention to pigment accumulation and the ultrastructure of plastids during the greening process, was made by Kittsteiner and coworkers [7,8]. Etiolated cress seedlings fed with low (2–5 mM) doses of metal chelators (Phe, 2,2′-B, 8-H) or ALA to accumulate Chl precursors and subsequently irradiated had damage to the upper part of the hypocotyl. The place of photooxidative damage did not coincide with the area of the highest porphyrin accumulation detected by fluorescence microscopy [7,8].

Pea (*Pisum sativum* L.) was another plant species analyzed after treatment with photodynamic herbicides and subsequent illumination. In etiolated pea seedlings, the strongest photooxidative damage was caused by 2 mM Phe and by 2–5 mM 2,2′-B. In the case of pea seedlings, the place of damage was the upper part of the epicotyl [22,23].

According to Rebeiz and coworkers [4], compounds such as ALA and 2,2′-B do not affect monocotyledonous plants. Therefore, it was interesting to find out whether Phe, a photodynamic herbicide, causes photooxidative damage in maize (*Zea mays* L.), a monocotyledonous plant, and whether it inhibits the normal development of chloroplasts during maize plant ontogenesis. Etiolated and then irradiated maize plants were resistant to 2–5 mM of Phe with respect to morphology (A.M., unpublished results).

Pigment Content

In cress, the large accumulation of Pchlide in the dark and its disappearance after 2 h of illumination was reported [7,8]. The rate of greening (after 2 and 12 h) was strongly reduced in cress seedlings. This applied to Chlb to the same extent as Chla but not to carotenoids (Car) [8]. A much stronger effect was achieved after application of complexing agents and subsequent irradiation than after ALA treatment [8]. Only trace amounts of Chla and Chlb were detected with 2,2′-B administration. In contrast to the strong synergistic effect of ALA and complexing agents described by Rebeiz and coworkers [4], the combination of ALA and 2,2′-B (2,2′-B caused the strongest inhibition effect) did not lead to increased inhibition of Chl accumulation. On the contrary, ALA reduced the effect of 2,2′-B [8]. Flash irradiation of cress cotyledons revealed that photoconversion of Pchlide to chlorophyllide (Chlide) was not influenced by chelator treatment. However, the esterification of Chlide was inhibited [8].

Pigment analysis of leaves taken from Phe- or 2,2′-B -treated and subsequently irradiated pea plants indicated an inhibition of both Chla and Chlb accumulation (Fig. 2) similar to cress plants 8,22,23]. After the treatment with the highest dose (3 mM) of 2,2′-B and subsequent 6 h of illumination, the Chla + Chlb content was only 7% of that in control plants (Fig. 2) [23]. The Car level

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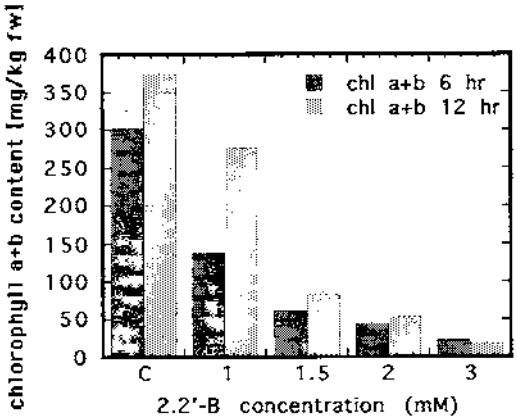

FIGURE 2 Chlorophyll a + b content of control, 1.0, 1.5, 2.0, or 3.0 mM of 2,2′-B-treated pea seedlings, etiolated, and subsequently illuminated for 6 or 12 h. Mean + SE. (From Ref. 23.)

was lower in 2,2′-B–treated pea seedlings and subsequent 6 and 12 h of illumination. It is not consistent with the unchanged level of Car in cress plants [8,23].

Mesophyll Cell Ultrastructure

Dark incubation of etiolated plants with ALA, Phe, 2,2′-B, or 8-H in low doses (1.5–5 mM) did not significantly change the ultrastructure of cells of cress cotyledons [8] nor of pea leaves pretreated with Phe (Fig. 3a) or 2,2′-B [22,23]. The plastids of pea cells treated with Phe had normal paracrystalline prolamellar bodies (PBs) and normal arrangements of prothylakoids. The ultrastructure of the mesophyll cells of maize leaves was not changed after pretreatment with Phe compared with control plants (A.M. unpublished results).

Two to 6 h of irradiation of etiolated plants pretreated with ALA or complexing agents was sufficient to cause photooxidative damage in the cotyledon cells of cress seedlings as well as in the mesophyll cells of pea seedlings, but it was not long enough to change markedly the ultrastructure [8,22,23]. The transformation of PBs and the formation of thylakoids were very similar in treated and control plants both in cress and pea. In pea, the thylakoid length increased in the same way in control and Phe-treated plants as in dark-treated plants.

The first ultrastructural change on illumination in both the Phe- or 2,2′-B–treated pea mesophyll cells was a strong dilation of endoplasmic reticulum (ER) cisternae which persisted during prolonged irradiation (see Fig. 3b,e–g); [22,23]. Phe-dark treatment and subsequent irradiation also caused structural changes of the mitochondria in cress and pea cells (see Fig. 3c); [8,22,23]. Most of mitochondria became vacuolized. In pea cells, they contained dark deposits mostly in crystalline form after treatment with Phe (see Fig. 3c) [22].

In control plants, extension of the irradiation time up to 12 h caused the development of grana with two to seven thylakoids in the plastids of the mesophyll pea cells (see Figs. 3d and 4a) and with three to five thylakoids in cress plastids [8,22,23]. The treatments of pea seedlings with a very low 2,2′-B concentration (1.5 mM) did not cause a strong inhibition of the grana formation (Fig. 4b); however, after 12 h of illumination, the number of thylakoids in the grana was on the average smaller than in control ones (Fig. 4b). The ultrastructure of plants treated with 3–5 mM of Phe or 2,2′-B irradiated for 12 h differed significantly from control plants. Plastids from such plants *did not form grana* (see Figs. 3e,g and 4c). These treatments, however, did not significantly influence

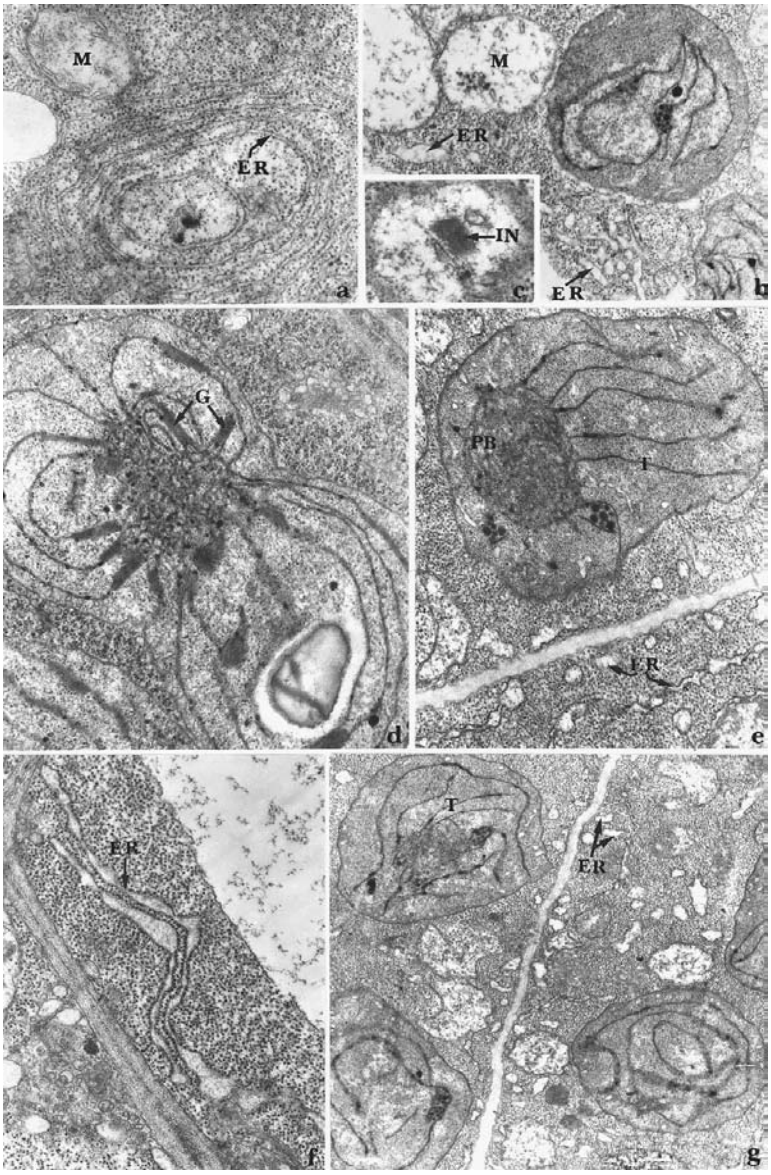

FIGURE 3 Portions of mesophyll cells of dark-grown pea seedlings treated with 2 mM Phe in darkness (a), or treated with 2 mM Phe in darkness and illuminated for 2 h (b,c), or untreated and illuminated for 12 h (d), or treated with 2 mM Phe in darkness and illuminated for 12 h (e–g). ER, endoplasmic recticulum; G, granum; IN, crystalline inclusion within the mitochondrial matrix; M, mitochondrium; PB, prolamellar body; T, thylakoid. a, d, and f×38,000; b and g ×19,500; c ×50,500; e ×29,500. (Based on Ref. 22.)

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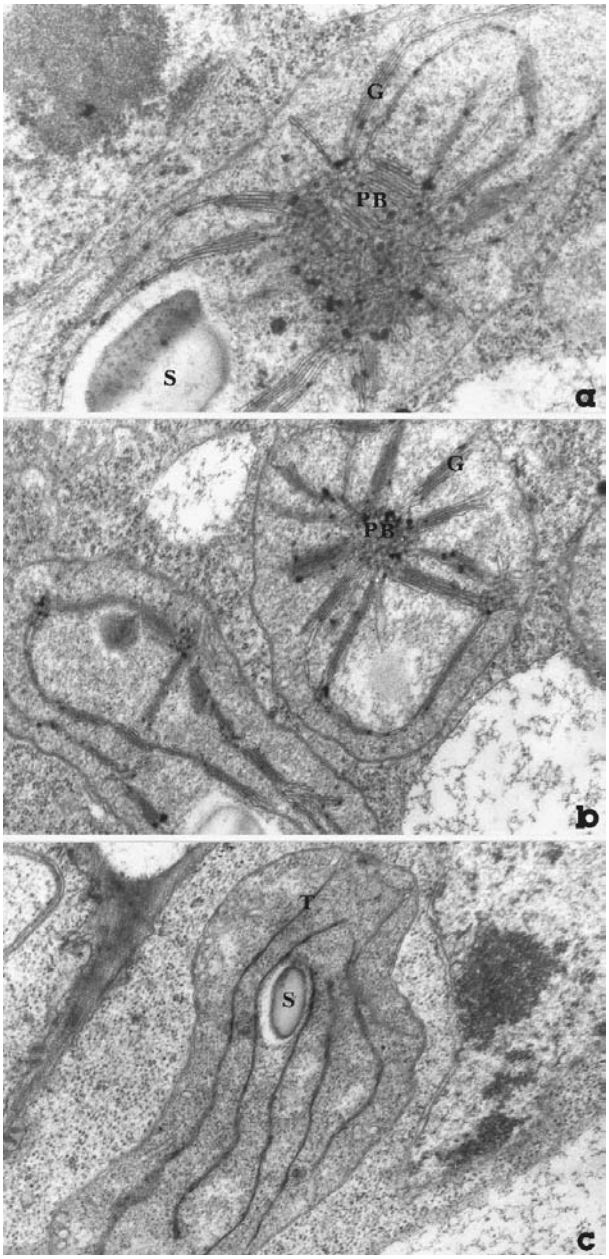

FIGURE 4 Portion of mesophyll cells of control pea seedlings grown in darkness and illuminated for 12 h (a), portions of mesophyll cells of pea seedlings treated with 1.5 mM of 2,2′-B in darkness and illuminated for 12 h (b), or treated with 5 mM of 2,2′-B in darkness and illuminated for 12 h (c). G, granum; PB, prolamellar body; S, starch grain; T, thylakoid. All figures ×33,000. (Based on Ref. 23.)

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the development of single thylakoids. The total length of the thylakoids in treated plants increased after 12 h of irradiation but did not reach the level of control plants [22,23].

Although maize plants in toto were resistant to a low (5 mM) dose of Phe, treatment with Phe in this concentration inhibited the greening and grana formation after exposure to light. Similar to cress and pea plastids, in the case of maize plastids, Phe treatment did not influence the normal transformation of the prolamellar bodies nor formation of single thylakoids on illumination (A.M., unpublished results). Higher Phe concentrations (10–20 mM applied mainly to register the sites of damage) followed by exposure to light caused not only total inhibition of greening but also dilation of the thylakoids, swelling of plastids, and finally total destruction of the plastid structure (A.M., unpublished results).

Conclusions

In cress and pea plants treated with complexing agents and subsequently irradiated, the ultrastructural changes such as the inhibition of grana formation and the dilation of ER cisternae together with the inhibition of Chl accumulation may be due to the inhibition of the transport of certain proteins to the plastids, diminished accumulation of chlorophyll proteins (e.g., light-harvesting complex proteins [LHCP]), and a decrease in the activity of the chlorophyll synthetase. A decreased LHCP level and an inhibition of chlorophyllide esterification has been described in Kittsteiner et al. [7].

EFFECT OF PHOTODYNAMIC HERBICIDES ON THE ULTRASTRUCTURE OF CHLOROPLASTS, PIGMENT CONTENT, AND CO_2 EXCHANGE IN GREEN PLANTS

Plants In Toto

It was interesting to determine if and how photodynamic herbicides induce changes in the structure of chloroplasts in green plants with an already developed photosynthetic apparatus.

The first experiments were done on pea plants, which are sensitive to Phe when applied on etiolated and subsequently illuminated plants [24]. Treatment of green pea plants with Phe in experimental conditions corresponding to a natural field environment would determine if pea is resistant to this photodynamic herbicide. In toto pea plants treated with 2 mM Phe were resistant to low doses (2 mM) of Phe during 9 h of illumination as opposed to the 10- and 20-mM Phe-treated and subsequently illuminated plants. During 9 h of irradiation, both Phe 10 and 20 plants were strongly faded and lost about 40% of their water content [24].

Experiments on the effect of another photodynamic herbicide,-2,2′-B, on green pea seedlings showed similar effects to those in pea plants. Pea seedlings in toto were resistant to a low dose (5 mM) of 2,2′-B, however, 10 and 30 mM 2,2′-B and illumination caused the loss of turgor of treated plants and photooxidative perturbations [25].

Similarly green maize seedlings were resistant to a low dose (5 mM) of Phe with respect to morphology, however, 10 and 20 mM Phe and illumination caused the loss of turgor of treated plants (A.M., unpublished results).

Pigment Content

The influence of photodynamic herbicides on the structure and function of the photosynthetic apparatus in already green pea plants, especially on the pigment content, chlorophyll fluorescence, CO_2 exchange, and the ultrastructure of mesophyll cells, was also examined [24,25]. It was interesting to know if Phe influences or destroys differentiated chloroplasts with well-developed photosynthetic membranes and thereby affects photosynthesis. Since we did not expect low doses (2–5 mM) of Phe or 2,2′-B to destroy chloroplasts, we also applied higher (10–30 mM) doses to determine the sites of damage [24,25].

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The changes in the pigment content, both the Chl and Car in 2,2′-B–treated and subsequently illuminated green plants corresponded to the reaction of plants in toto with such treatment. This means that, in plants treated with 5 mM 2,2′-B, the Chl content remained unchanged during 9 h of illumination (Fig. 5a) and the Car content did not decrease much on longer illumination (Fig. 5b); however, in 10- and 30-mM 2,2′-B–treated plants, these values decreased by about 30% during the 9-hr illumination period (Fig. 5a,b) [25].

In 2- to 20-mM Phe-treated pea plants, the Chl content and the Chla/b ratio did not differ from these values in control plants and did not change significantly during 9 h of illumination [24].

Chlorophyll Fluorescence and Photosynthesis

The results obtained on the Chl fluorescence measurements and on the net photosynthesis rate (P_N) indicated dysfunction of the photosynthetic apparatus already caused by the lowest (5 mM) 2,2′-B concentration and subsequent illumination. Rapid fluorescence already changed after 20 min of light treatment, which indicated that the function of photosystem II (PSII) was strongly affected by 2,2′-B and that the action of 2,2′-B probably began during the dark period. Thus, PSII is a primary target of the 2,2′-B action [25]. The P_N of 5 mM 2,2′-B–treated plants was almost half that of control plants, already after 1 h of illumination and decreased additionally by about 20% during the next 5 h (see Fig. 5c). A dramatic (more than fivefold) decrease of P_N took place after 1 h of illumination in plants treated with 10 or 30 mM of 2,2′-B; after 3–4 h of illumination, the P_N in these plants was essentially zero (see Fig. 5c) [25].

In Phe-treated plants, the P_N was similar in control and 2-mM Phe-treated plants, but it was extremely low after treatment with 10 and 20 mM Phe [24]. At the beginning of the experiment with CO_2 exchange, this value in 10-mM Phe-treated plants was only 22% of that of control plants, and during 9 h of irradiation it decreased to 8%. The inhibition of photosynthesis was not due to the decreased Chl content. The Chla and Chlb content did not differ in control and Phe plants and did not change significantly during the whole experiment [24].

Mesophyll Cell Ultrastructure

Similar to plant in toto, the ultrastructure of the mesophyll cell was the same in control and 2- to 20-mM Phe-treated or 5- to 30-mM 2,2′-B–treated pea and maize plants if the plants were not transfered, to light after administration of the herbicide [24,25] (A.M., unpublished results).

Both pea and maize seedlings treated with low Phe or 2,2′-B doses and subsequently illuminated had a comparable ultrastructure of the mesopyll cells and chloroplasts, in particular, with that of control plants (Figs. 6a, c and 7a); [24,25] (A.M., unpublished results).

However, 10- and 20-mM Phe treatment or 10- and 30-mM 2,2′-B treatment followed by irradiation caused not only a severe loss of turgidity and the inhibition of photosynthesis but also changes in the cell structure. The difference between control and 2,2′-B–or Phe-treated plants became visible after 4 h of irradiation and intensified throughout 9 h of irradiation. This difference was mostly manifested in thylakoid swelling (see Figs. 6b, d and 7c), in dilation of the ER cisternae (see Fig. 7b), in degeneration of the internal mitochondrial membrane, and even in the disruption of the chloroplast envelope (see Figs. 6d and 7c) [24,25]. In green plants, like in etiolated ones, 2,2′-B or Phe treatment and subsequent irradiation did not much affect the formation of new thylakoids, although it destroyed the existing ones. During 6 h of illumination, the length of granal thylakoids and the total length of thylakoids per 1 mm^2 of a plastid section, as well as the number of grana, the number of thylakoids per granum (eight on average) increased to the same extent in control as in Phe-treated plants [24]. However, extended irradiation (between 6 and 9 h) in Phe-treated plants inhibited this development as compared with the control plants [24].

The number and size of starch grains in the chloroplasts of control pea plants continued to increase throughout 9 h of illumination. Finally, especially in Phe-treated pea seedlings followed by 9 h of illumination, grana and intergranal thylakoids were squeezed between large starch grains

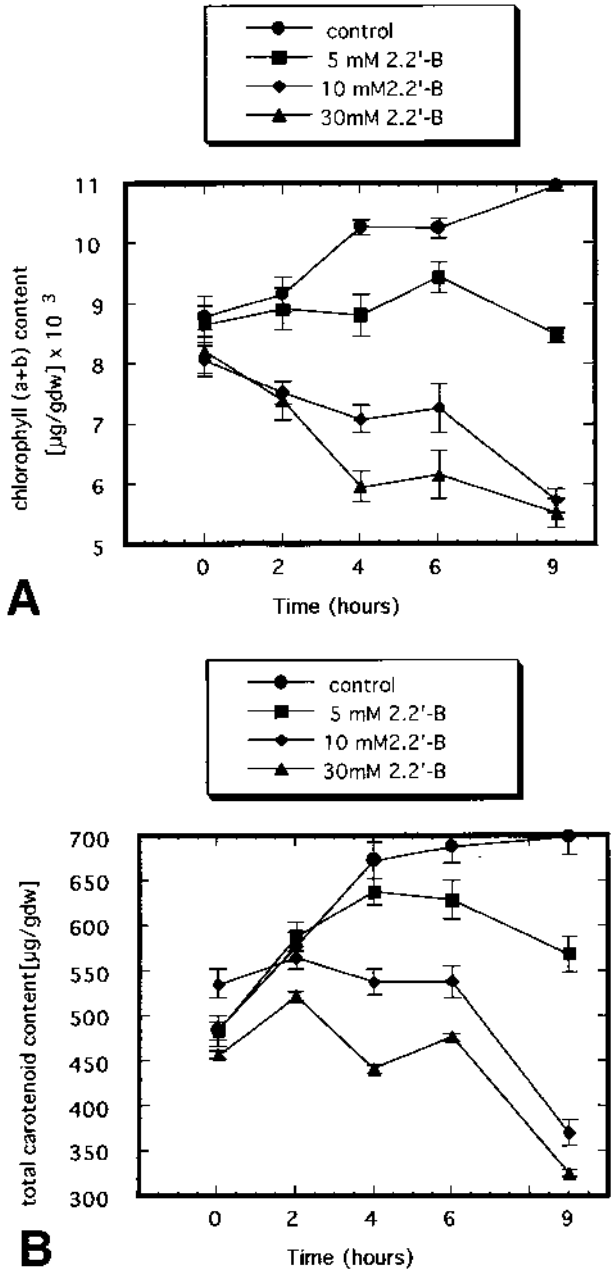

FIGURE 5 (a) Effect of 2,2′-B (5–30 mM) and subsequent illumination on chlorophyll *a* + *b* content in pea seedlings. Mean + SE. (b) Effect of 2,2′-B (5–30 mM) and subsequent illumination on carotenoid content in pea seedlings. Mean + SE. (c) Effect of 2,2′-B (5–30 mM) and subsequent illumination on net photosynthesis rate in pea seedlings. Mean + SE. (From Ref. 25.)

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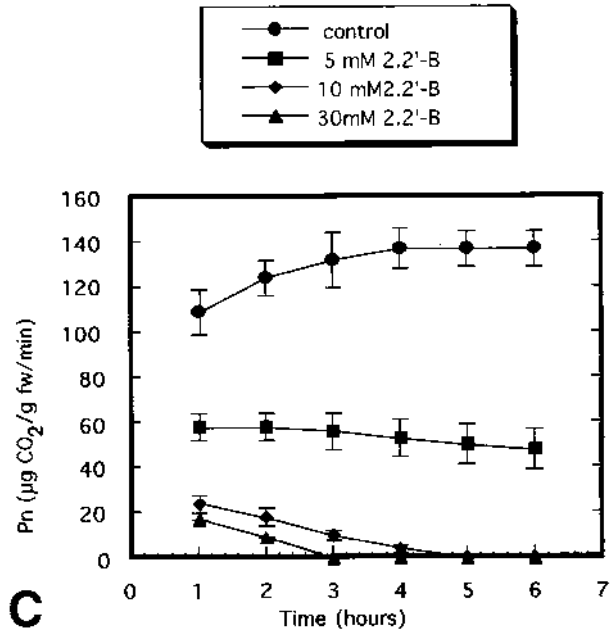

or pushed toward the chloroplast envelope (see Fig. 6c). As opposed to the chloroplasts of control plants, starch grains were not seen in any chloroplasts of 2,2′-B or Phe-treated plants after a long period of illumination (see Figs. 6b,d and 7c) [24,25].

There was a difference in the changes in the ultrastructure after high doses of Phe or 2,2′-B and illumination. During the first 4 h of illumination, 20 mM Phe induced stronger ultrastructural changes than did 10 mM Phe. After a longer illumination period, there was no significant difference between the intensity of the ultrastructural changes in Phe 10 and Phe 20 plants [24]. However, 30 mM of 2,2′-B induced stronger than 10-mM 2,2′-B changes during the whole experiment leading finally to strong destruction and deformation of the chloroplasts [25].

The 10 and 20 mM Phe applied to maize seedlings followed by exposure to light caused the strong destruction of chloroplasts and whole mesopyll cells comparable with the reaction of pea chloroplasts on 30 mM 2,2′-B and illumination (A.M., unpublished results).

We tried to find some links between the rapid inhibition of photosynthesis and the ultrastructural changes. Since the 2,2′-B or Phe dark treatment did not influence the ultrastructure of the mesophyll cells, the inhibition of the photosynthetic processes during the first minutes of illumination could not be the result of structural changes. Thus, increasing the structural and functional alteration on illumination might have the same basis [24,25].

The chelating properties of Phe and 2,2′-B could be related to its inhibition of photosynthesis as well as with ultrastructural changes. Phe and 2,2′-B are strong chelators and easily form complexes with iron. However, complexing agents also can bond other ions, for example, bivalent ones, and thus cause membrane depolarization leading to a change of its permeability. The amount of Mg^{2+} ions might be reduced among to the chelating properties of 2,2′-B or Phe. Thus, immediately after the exposure to light, there is no sufficient amount of Mg^{2+} for carboxylase activation and thus photosynthesis is inhibited [26].

A similar interpretation is possible for ultrastructural changes on illumination. Swelling of ER cisternae, thylakoid swelling, and disruption of the chloroplast envelope should be a consequence of an increase in the space between the membranes which might be due to changes of membrane permeability based on the chelating properties of Phe. The changes of membrane permeability could

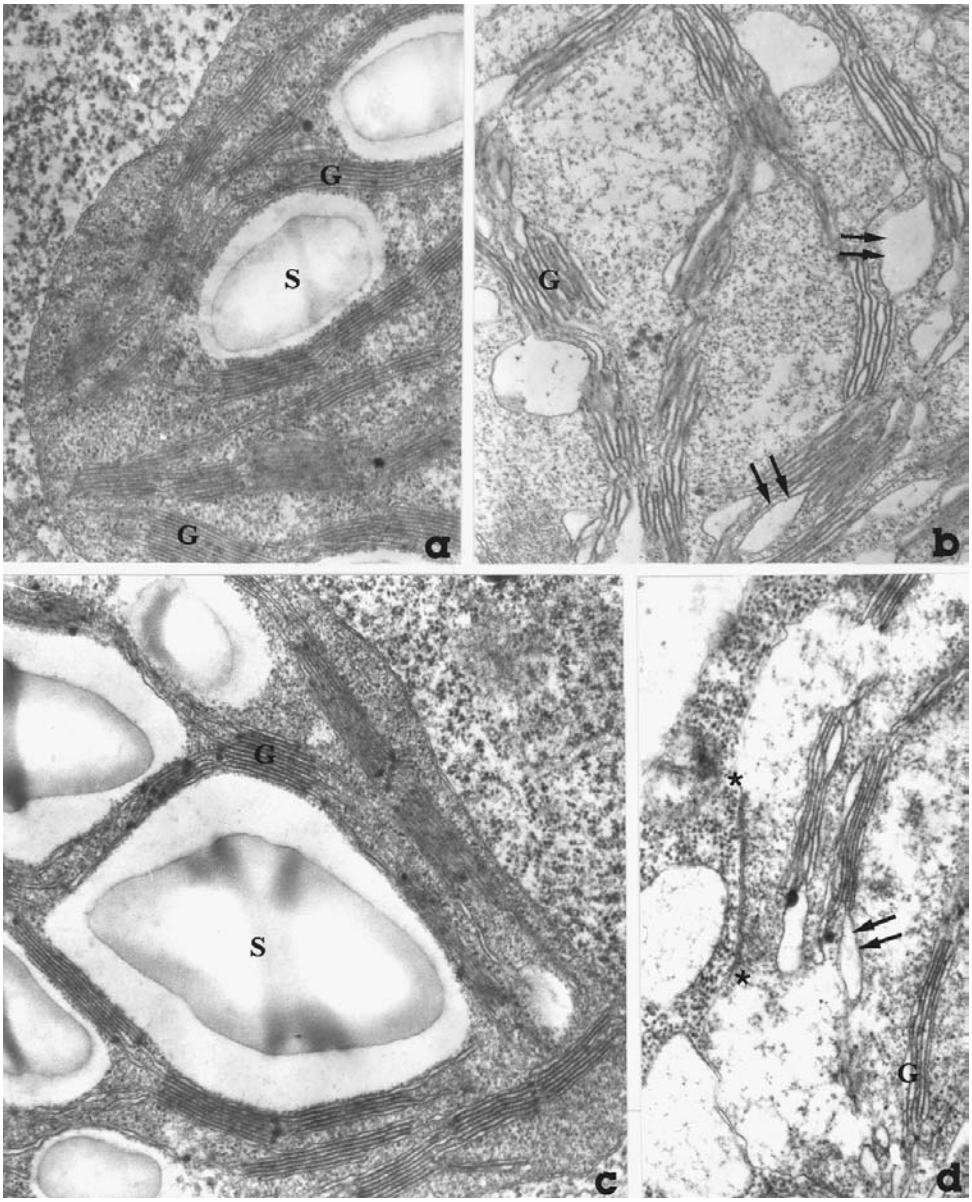

FIGURE 6 Portions of mesophyll cells of green pea seedlings, untreated with Phe, fixed after the 13-h dark period, followed by 6 (a) or 9 h (c) of illumination, or treated with 10 mM Phe, kept for 13 h in darkness, and illuminated for 6 (b) or 9 h of irradiation (d). ER, endoplasmic reticulum; G, granum; M, mitochondrium; PB, prolamellar body; S, starch grain; T, thylakoid. Double arrow indicates swollen thylakoid; stars indicate broken membrane of chloroplast envelope. All figures ×40,000. (Based on Ref. 24.)

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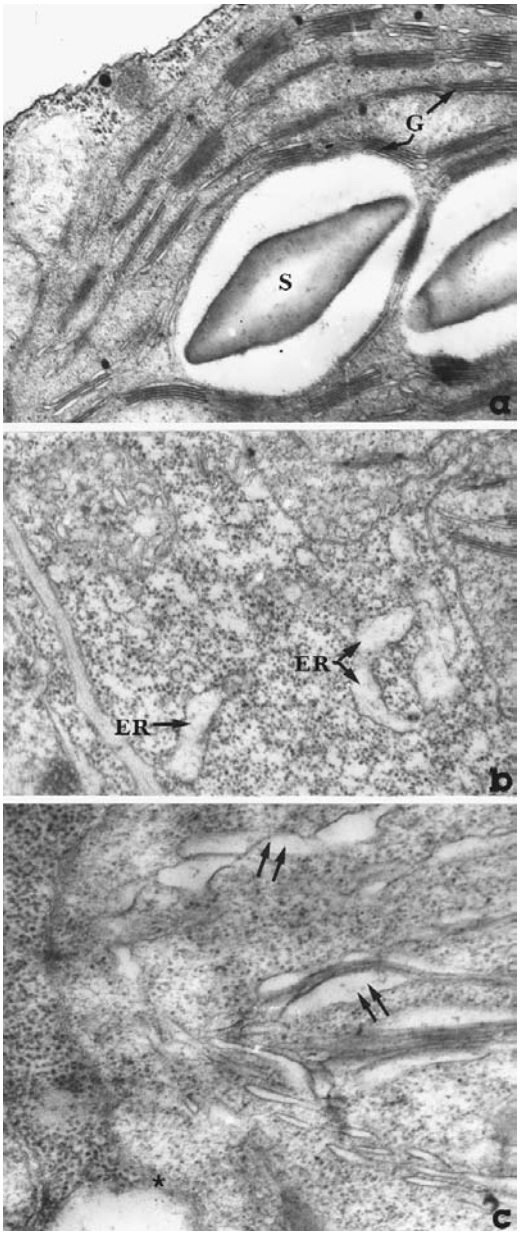

FIGURE 7 Portions of mesophyll cells of control green pea seedlings fixed after a 12-h dark period followed by 9 h of illumination (a) or of pea seedlings treated with 30 mM 2,2′-B and illuminated for 6 h (b,c). ER, endoplasmic reticulum; G, granum; S, starch grain. Double arrow indicates swollen thylakoid; star indicates broken membrane of chloroplast envelope. a ×35,000; b ×40,000; c ×50,000. (Based on Ref. 25.)

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explain swelling of the ER cisternae and mitochondria [24,25]. Similar changes of the ER and mitochondria were previously described in etiolated Phe-treated plants [22].

Conclusions

The results of applying a low concentration (2 mM) of Phe to green pea and maize plants grown in normal field conditions indicated that these plant species have a high resistance to this herbicide. From application of higher Phe concentrations to pea and maize plants, sites of damage in the cell, particularly the destruction of cell membranes, were shown. The results of applying a low concentration (5 mM) of 2,2′-B to green pea plants indicated that they were resistant to this dose of 2,2′-B with respect to the morphology, pigment level, and structure of the mesophyll cells. However, dysfunction of PSII as well as P_N was already observed with low (5 mM) 2,2′-B treatment. Higher 2,2′-B doses and illumination caused photooxidative perturbations at the structural and functional level in the chloroplasts.

The destruction caused by Phe and 2,2′-B and subsequent illumination, especially the damage caused by longer illumination, might be caused by a standard photoinhibition mechanism, as proposed by Rebeiz and coworkers [4]. However, we have presented some other possible interpretations of the effect of Phe on the structure and function of the photosynthetic apparatus. It is difficult to determine without further experiments which mechanism is the leading one.

FINAL CONCLUSIONS

Photodynamic herbicides represent a group of compounds which induce the accumulation of chlorophyll precursors in darkness and cause photooxidative damage to plants during subsequent illumination. Photodynamic herbicides, particularly 2,2′-bipyridyl and 1, 10-phenanthroline in low concentrations inhibit grana formation, chlorophyll, and carotenoid accumulation, cause a strong dilation of the endoplasmic reticulum cisternae and delays the transformation of prolamellar bodies in etiolated pea and maize seedlings that are subsequently illuminated. The reasons for the reduced greening process are related to the chelating properties of 2,2′-B, a standard photooxidative mechanism, and/or LHCP accumulation within the ER cisternae.

Applications of low and higher 2,2′-B or Phe concentrations on green pea and maize plants revealed that these plants were resistant to low doses with respect to the morphology, pigment level, and structure of the mesophyll cells, but high doses with illumination caused the loss of turgor and photooxidative damages seen at the ultrastructural level and a decrease of the pigment contents.

Both Phe and 2,2′-B caused two types of reactions. The rapid reaction occurs directly after exposure to light. It is probably effected by processes which took place during the dark period and is monitored by changes of Chl fluorescence and of the photosynthetic activity rate. The slow reaction consists of destructive changes in the mesophyll cell ultrastructure: dilation of the thylakoid membranes and ER cisternae, disruption of the chloroplast envelope, and a decrease in the pigment content. Slow reactions are induced by light and require a longer illumination time. We consider PSII as a primary target of photodynamic herbicide action. Other dysfunctions are the result of the PSII damage; however, they also are induced by light and are based on the mechanism of photosensitizing of porphyrins.

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Molecular Responses to Water-Deficit Stress in Woody Plants

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Slater, Iowa

INTRODUCTION

The establishment and productivity of forest trees are drastically affected by various environmental stresses [1] of which water deficit is the principal stress [2]. Similarly, water availability is a primary factor affecting the growth and survival of shrubs in desert areas of the world. Water is very significant to trees and shrubs because of its biological roles as a solvent and as a transport medium, as an electron donor in biochemical reactions, and as an evaporative coolant [3]. Water balance is very often impaired by external water-deficit stress (WDS) conditions which affect trees and shrubs in all populations across all geographical and climatic areas. Whether it is episodic or perennial, WDS impairs growth and development and renders woody plants susceptible to secondary, normally sublethal insults. In the extreme case, WDS will destroy large areas of forest and shrublands; thus contributing to land erosion and to desertification. Of the three main climatic factors, moisture, temperature, and wind, moisture is the most variable and definitive for the formation and well-being of shrublands [4].

Water-deficit stress is a common cause of seedling mortality in both naturally regenerated and planted stands of loblolly pine (*Pinus taeda* L.) in the United States [5]. Dry weights of four-winged saltbush (*Atriplex canescens*) more than 2 years after planting are directly correlated with

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moisture availability at three sites in west Texas [6]. Williston [7] notes that over a 16-year period, as much as 60% of the first-year mortality in pine plantations is due to water deficit. Extensive research has been expended toward the production of genotypes that are morphologically targeted for severe drought-prone areas [8] and contain a genetically determined drought tolerance [9]. In established stands, WDS accounts for 80% of the variation in annual ring width of conifers in humid temperate climates [10] and up to 90% in semiarid regions [11].

Before visual damage is evident owing to WDS, there is a preceding alteration in the woody plant metabolism and gene expression [12–16]. Changes in gene expression are fundamental to both short- and long-term WDS responses. In numerous plant species, the imposition of stress results in the production of new proteins or the elevated synthesis of certain others [17–23] which may prevent cellular damage in various tissues [24–28] and detrimental changes in plasma membrane properties [29,30]. As with most plants, an osmotic adjustment in response to WDS occurs in woody plants, including both pine [31–34] and saltbush [35] and solutes such as proline [31,32] and sucrose [22] accumulate. With a prolonged WDS, a reduction in the tissue elasticity also can result [34,35]. These changes in the tissue properties and solute accumulation are a result of an altered gene expression, which has become the focus of research in many plant species, including woody crops. This chapter focuses on the molecular events associated with the tissue and metabolic changes in trees and shrubs. Numerous reports have been published on the effects of WDS on the molecular responses in many herbaceous crop plants [3,36,37]. The results of these investigations have been included and discussed when they provide more understanding of the general mechanisms and models of the molecular responses which can be applied to woody plants. Our goal is to provide more understanding of how woody plants respond to WDS at the molecular level which will allow researchers genetically to improve these important perennials and eventually overcome the severe economic losses associated with WDS. First, a generalized conceptual model of WDS perception and gene induction is discussed; second, woody plant physiological responses and tissue changes during WDS are presented; and finally, individual gene isolations and characterizations and gene products from several woody plant species are described.

PERCEPTION OF WATER-DEFICIT STRESS AND INDUCTION OF GENE EXPRESSION

Responses to WDS begin with its perception at the cellular level (Fig. 1). It is still not definitively known how a cell perceives WDS, but once it does, a complex cascade of events ensues [38]. Many of the steps of these events have not been elucidated, but one of the most significant observations in the WDS response is the increase in the levels of abscisic acid (ABA) [39–42].

Abscisic Acid Mediates the WDS Response

It has been shown that exogenously applied ABA can mimic many of the responses elicited by WDS; thus ABA is considered to be an essential mediator between WDS and the plant responses [42,43]. Transcription and translation are required for ABA biosynthesis during stress [44] indicating that proteins involved in the biosynthetic pathway must be synthesized in order for elevated levels of ABA to accumulate. The loss of the cell turgor and distortion of the cell membrane as a result of dehydration precede the increase of bulk ABA in the tissues, and the increase is correlated with an induced gene expression [45]. Both rapid and slow responses to ABA have been observed. Rapid responses include the early events of gene expression, including membrane-initiated signal transduction as occurring with guard cells, whereas slow responses include both the repression and expression of genes by ABA. The rapid response is not a prerequisite for the slow response, and the two responses may require different ABA receptor proteins [45].

Although genetic approaches using ABA-deficient and ABA-insensitive mutants (*abi*) have clearly indicated that ABA plays an important role in the WDS response [46,47], its specific mode of action, and its pathway leading to such a response remains unclear [36–38]. In a cascade of many

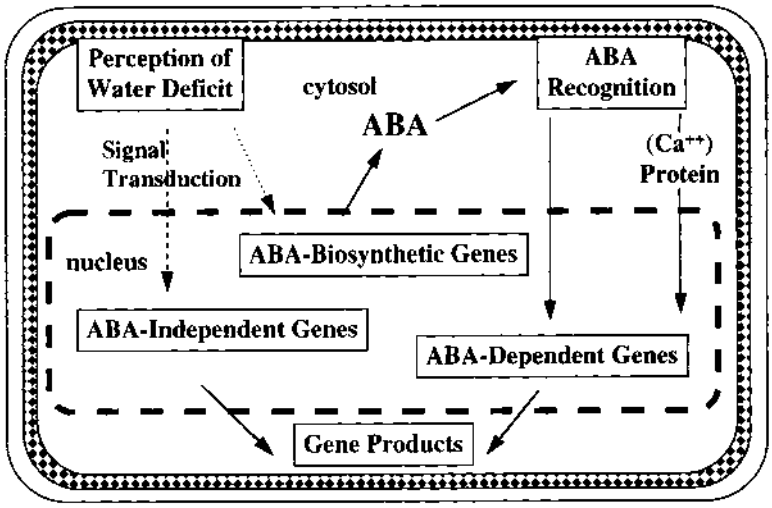

FIGURE 1 Generalized, conceptual model of cellular perception of water deficit stress and gene induction. (Modified from Ref. 38.)

events known to be involved in this response, the function of calcium as a second messenger has been established (see Fig. 1). Abscisic acid induces an increase in the cytoplasmic calcium in a variety of cell types, and, in guard cells, calcium acts as an intracellular second messenger in the ABA regulation of the stomatal aperture [48,49]. In *Arabidopsis*, the ABA-responsive *abil* gene mediates various responses, including the stomatal aperture, seed dormancy maintenance, and the inhibition of plant growth [46,47]. The *abil* gene codes for a phosphatase with novel calcium-binding site called EF-hands. These are protein domains each consisting of 29 amino acids arranged in a helix-loop-helix conformation which allows for the reversible binding of calcium. EF-hands appear to have the function of integrating ABA and calcium signals with phosphorylation events [46,47].

Gene Expression is Modulated by ABA via Transcription Factors

The transcriptional regulation of expressed genes (see Fig. 1) is accomplished by the sequence-specific interaction of *trans*-acting protein factors that bind regulatory *cis*-elements [50–53]. Many WDS-inducible/ABA-responsive genes contain conserved *cis*-acting sequences within the 5′ regulatory region to which transcription factors bind directly or indirectly in order to regulate gene expression. These sequences are usually identified either by exodeletion or mutation analysis [54–56]. The presence of short elements called ABA-responsive elements (ABREs) in the 5′ upstream region of the transcription initiation sites of the *em* gene of wheat [50] and in genes of several other species [55–57] have been reported. In wheat, these elements bind to the cloned transcription factor, EmBP1, and therefore regulate expression. Most of the transcription factors involved in stress responses belong to the bZip protein class [58,59] and can be induced by ABA [60]. In addition to ABREs, AS-1 and E8 *cis*-acting elements in the 5′ upstream sequence of the osmotin gene have been reported as well as enhancer-like and negative regulatory elements [55].

In the barley ABA-responsive gene, *hva22*, ABREs are necessary but not sufficient for the ABA response [56]. However, an ABRE complexed with a novel coupling element confers a high level of ABA responsiveness. In contrast, genes have been characterized whose expression is induced by ABA, but they lack the ABRE factor in their promoters [21]. Another novel 9-bp *cis*-acting element called DRE (dehydration-responsive element) has been isolated from *Arabidopsis* [61,62].

Although not specifically related to WDS, it has also been shown that not only the 5′ upstream region but also the 3′ untranslated region and/or a leader intron are also important in conferring both the temporal and spatial expression of genes [63]. Furthermore, expression enhancers in the introns [64] and in the 3′ untranslated region have a significant effect on mRNA stability [65].

Calcium-Binding Proteins Operate in the ABA-Signaling Cascade

Calcium modulates many physiological processes as a second messenger in a number of signal transduction pathways [66–69]. Calcium is involved in such processes as mitosis, cell elongation, cytoplasmic streaming, polarized cell growth, enzyme secretion, turgor regulation, hormone action, gravitropism, and enzymatic activities [67,68]. Proteins that have calcium-binding capabilities include calmodulin, troponin C, myosin regulatory light chain, calcineurin, sarcoplasmic calcium–binding protein, calpain small and large chains, aequorin, parvalbumin, and α-actinin [70]. Many of these have calcium-binding domains called EF-hands each consisting of 29 amino acids arranged in a helix-loop-helix conformation which allow for the reversible binding of calcium. Calcium binding to the protein stimulates a change in conformation resulting in a change in activity [71].

There appear to be plasma membrane receptors for ABA (see Fig. 1) that act as intermediates between ABA and calcium ion accumulation [72–74]. An increase in the cytosolic calcium ion content has been observed in response to inositol triphosphate treatments [75], one of the early intermediates in the signal transduction pathway. Calcium ions, acting as secondary signal transducers, have been implicated in the activation/deactivation of certain proteins by phosphorylation/dephosphorylation mediated by calmodulin [70]. These proteins lead to the transcription of specific genes followed by translation of new proteins [73]. The ABI1 phosphatase of *arabidopsis* with calcium-binding domains appears to be involved in phosphorylation events initiated by ABA and calcium [46,47]. Frandsen et al. [76] have isolated a calcium-binding protein gene from rice that is responsive to both ABA and osmotic stress. Dias [13] and Padmanabhan [77] have identified a WDS-ABA–responsive pine gene encoding a calcium-binding protein.

CELLULAR AND PHYSIOLOGICAL RESPONSES TO WATER-DEFICIT STRESS

Different orders of complexity in the WDS response pathway exist [3]. Low-complex mechanisms are WDS-response changes involving a single biochemical pathway [3]. They include mechanisms such as compatible solute production, ion uptake and partitioning, and facilitated water uptake [55]. These mechanisms of low complexity could be involved in physiological processes such as cellular protection and osmotic adjustment [36].

High-complexity mechanisms of response to WDS involve changes of many biochemical pathways [3]. These pathways are modified during stress to protect major processes such as photosynthesis and respiration and to preserve structures such as the cytoskeleton and the cell wall [78]. Another example of a high-complexity mechanism is the change in DNA content (79,80).

Cellular Protection

A number of genes whose translational products may be involved in cellular protection have been identified in several plant species. The functions of these genes are mostly predicted from the amino acid sequence of the proteins they encode. They encode hydrophillic proteins which possess a specific amino acid composition as well as conserved repeats of specific amino acids. The predicted functions of these proteins are the sequestration of ions, protection of other proteins or membranes, renaturation of unfolded proteins, or molecular chaperoning. Prominent examples of these types of genes are *lea* in cotton [26,81], *rab* from rice [25] and maize [82], *em* from wheat [83], dehydrin

from barley [27], *asr* from tomato [84–86], *rd22* [87] and *erd* [88] from arabidopsis, *pum* in alfalfa [89], and *le* in tomato [90]. The *lp3* gene, similar to the *asr* gene from tomato, has been identified in loblolly pine [91]. In tomato, ASR2 is localized in the nucleus [86] and is induced by WDS, ABA, and ethylene in ripening fruits [92]. The BspA protein from poplar has been suggested to have a protective role during WDS [93].

Osmotic Adjustment

In both herbaceous and woody species, it has been well demonstrated that they respond to WDS conditions by lowering their osmotic potential through the accumulation of osmolytes in the cytoplasm. This accumulation has been demonstrated in a large number of plants and appears to be related to turgor maintenance. An increase in solutes such as sucrose [22,23] and proline [32–34] has been observed in woody plants, and genes involved in the biosynthesis of WDS-induced osmolytes have been cloned [3]. A few genes encoding membrane channel proteins, called membrane intrinsic proteins (MIPs), are induced in response to a loss in turgor [94–98] and are hypothesized to form water-specific ion or solute channels. During stress, these proteins are predicted to modulate the movement of water or solutes from the vacuole to the cytosol, altering either the water content or the osmotic potential of the cytosol.

In yeast, an enhanced production and accumulation of intracellular glycerol occurs in response to a high external osmolarity [99]. The gene *gpdl*, which encodes the enzyme glycerol 3-phosphate dehydrogenase, is involved in glycerol biosynthesis [100], and its expression is regulated by the *high-osmolarity glycerol* (HOG) signal transduction pathway, which is defined by *pbs2* and *hog1* genes belonging to the MAP (mitogen-activated protein) kinase and MAP kinase groups, respectively [101]. A phosphorylation of HOG1, resulting from the expression of *hog1*, dependent on the protein product (PBS2) produced by the expression of *pbs2*, is necessary for the response to high external osmolarity [102]. Another gene, *spc1*, encoding a MAP kinase which is closely related to the *hog1* and mammalian p38 kinases, is activated by several forms of stress and regulates the expression of both *gpd1* and *tps1*, the latter of which codes for trehalose-6-phosphate synthase. The activation of *spc1* itself requires phosphorylation by another MAP kinase called WIS1. This SPC1 MAP kinase signal transduction pathway links to the G2/M control of the cell cycle with changes in the extracellular environment such as high osmolarity and nutrient depletion [103].

Modifying the Cell Wall

Shoot tissue elasticity is reduced in water-deficit–stressed pine seedlings [104], suggesting that structural changes in the cell walls have been induced. WDS-induced genes whose products have apparent cell wall functions in loblolly pine encode for proteins such as O-methyl transferase and S-adenosylmethionine (SAM) synthetase, enzymes which are involved in lignin biosynthesis as well as encoding copper-binding and glycine-rich proteins (GRPs) [14] which have presumed structural roles in the cell walls.

GRPs are characterized by their repetitive primary structure which contains up to 70% glycine arranged in short repeat units. Genes encoding GRPs have been isolated from a variety of plants, including tomato, arabidopsis, petunia, tobacco, carrot, pumpkin, bean, maize [105], pine [13], and saltbush [16]. Many GRP clones predict a common feature of an amino-terminal signal peptide suggesting that they are localized in the cell wall; however, cytoplasmic GRPs have also been identified. Generally speaking, those found in the cell wall are thought to be developmentally regulated, whereas the cytoplasmic GRPs are regulated by stress conditions such as ABA and WDS [105]. However, an exception to this may be the putative cell wall protein from pine [13] whereby the GRP is induced by WDS. GRPs are most often associated with vascular tissues, including the protoxylem and metaxylem, primary and secondary xylem, vessel elements, phloem, and fibers. Secondary structure prediction of GRPs indicate that they exist as β-pleated sheets with a varying number of antiparallel strands (Fig. 2), a structure that could provide elasticity as well as tensile

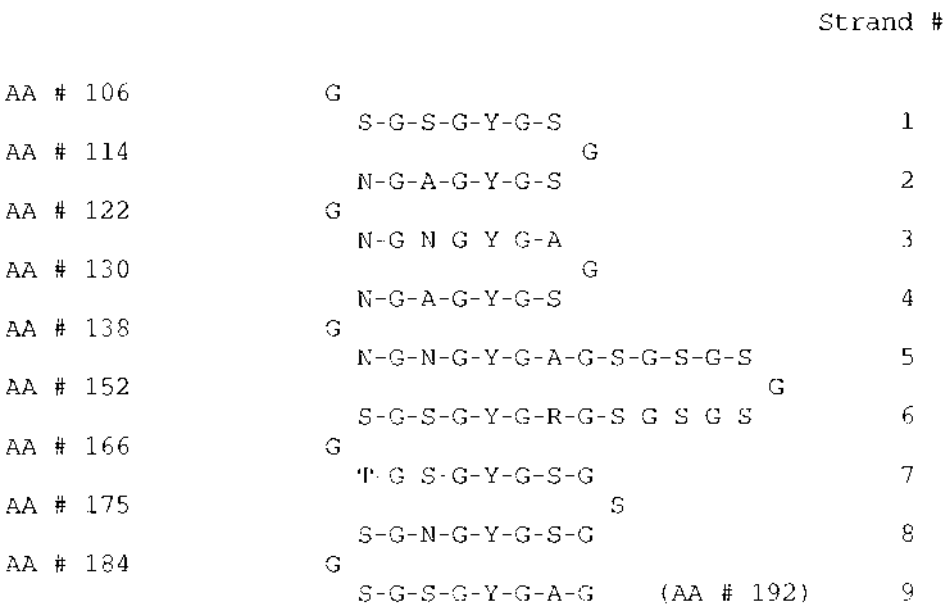

FIGURE 2 The proposed anti-parallel strand structure of the LP5 protein from loblolly pine [13].

strength during vascular development [105]. It has been suggested that GRPs provide a ''nucleation site'' for lignin biosynthesis [106] or a link with other cell wall proteins [105]. Non–cell wall cytoplasmic GRPs may have a role in WDS tolerance [105].

GENES INDUCED BY WATER-DEFICIT STRESS IN PINE

Molecular events leading to the WDS response have been characterized in several woody plants. In most cases, the putative functions for those genes that have identified are predicted based on their homology with genes from herbaceous species, but, in a few cases such as in pines, the genes have been studied in some detail with further characterization of their roles in response to WDS. Genes and gene products from pine are discussed in the following sections.

cDNA Clones from Pine

The synthesis of new proteins in *Pinus taeda* during WDS indicates that gene induction has occurred [19]. To identify these genes, a cDNA library was constructed from *P. taeda* roots which were stressed to a plant water potential of −1.5 MPa. The cDNAs were differentially hybridized to messages present in the roots of water-deficit–stressed and irrigated control trees. Several DNA clones were isolated. These genes were induced in *P. taeda* subjected to a progressive WDS which mimicked that of the natural environment (Fig. 3). When sequenced and characterized, the clones revealed a high homology with different WDS-inducible genes in other plants [14].

The protein encoded by *lp2* [14] shows a high identity with SAM synthetase from a number of plants [107–109]. This enzyme catalyzes the biosynthesis of SAM from methionine and ATP [110]. SAM is a cofactor in numerous biochemical reactions, acting as a methyl donor to proteins,

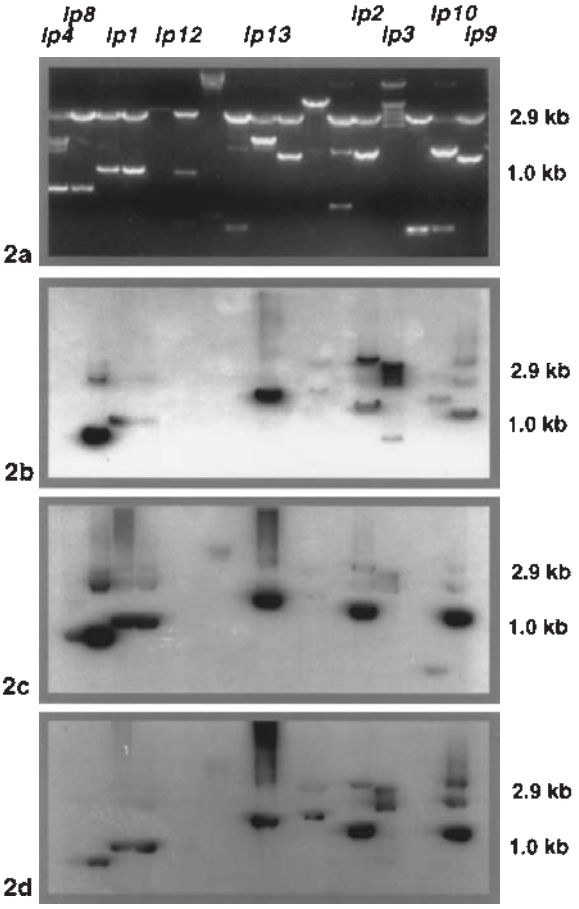

FIGURE 3 Plasmid screening for water-deficit stress (WDS)–responsive clones from loblolly pine [13]. (a) Ethidium bromide–stained gel. (b–d) Autoradiograms of the membranes hybridized with the indicated probe. The size of the DNA fragments is given on the right.

lipids, polysaccharides, nucleic acids [110], and intermediates in lignin [111] and ethylene [112] synthesis.

The *lp3* clone is preferentially expressed during WDS in roots and has very low to nonexistent expression in stems and needles [77]. The expression is also modified by ABA [77]. There are at least four members of the *lp3* gene family, and these members have distinct expression patterns in different tissues under WDS conditions. The predicted protein encoded by *lp3* is highly hydrophilic, and it shows a very high homology with clones from *Lycopersicon* [85,86,92], *Solanum* [113], *Oryza* (nonpublished results) and *Citrus* (nonpublished results). The homology is more than 89% at the C-terminal region, and it is variable in the N-terminal region in all the homologues. The *lp3* clone has a putative nuclear-targeting signal in the C-terminal region similar to its homologues. The protein counterpart of LP3 in tomato, ASR2, is localized in the nucleus [86] and is induced in leaves by ABA and WDS and in fruits during ripening. The expression of *lp3* homologues in other species varies.

Picton et al. [92] observed that the levels of the tomato ERT16b protein, homologous with the LP3 protein, increased during the latter stages of fruit ripening, but the message was not present

in the leaf before and after wounding. In a fruit-ripening, mutant, *rin*, very high levels of ERT16b expression were observed when fruit treated with ethylene were compared with air-stored control fruits. Interestingly, there were no transcripts detected in the leaf. In *Solanum chacoense*, DS2 was induced to very high levels in response to WDS, but it was constitutive to ABA application [113]. In response to wounding, no transcript was detected either in control or wounded leaves. Interestingly, this transcript also was not present in roots, tubers, or flowers. Since *lp3* is homologous with these genes with pleiotropic effects having a consensus nuclear-targeted signal, it could be that *lp3* codes for a transcription factor acting with ABA in the pathway of events induced by WDS.

Clone *lp4* codes for a protein that is similar to stellacyanin, a copper-binding glycoprotein from the Japanese lacquer tree [114] and to copper-containing glycoproteins from cucumber [115] and *Arabidopsis* [116]. *lp5* is preferentially expressed during WDS in roots, and its expression is very low to nonexistent in stems and needles. Genomic Southern analysis indicates that *lp5* belongs to a small multigene family [13]. The *lp5* cDNA codes for a novel putative glycine and serine-rich (41% glycine and 20% serine) cell wall protein with a distinct hydrophobic signal peptide in the N-terminal region suggesting that this could be a cell wall targeted structural protein. The accumulation of glycine-rich proteins in stressed plants has been well documented [24,78,117]. LP5 has nine repeats of GGXYXGG which are postulated to form antiparallel strands forming a β-pleated sheet in the secondary structure (see Fig. 2) with side chains of bulky residues exposed on one side of the sheet [118,119]. The rigid tyrosine residues are in the center of the strand, whereas folding occurs at the flexible glycines except where serine is present (see Fig. 2). There is also the possibility of cross linking between LP5 and other proteins or components of the cell wall via the tyrosine residues in the β-pleated sheet structure. In this way, the putative LP5 protein appears to function in strengthening the cell wall during WDS conditions.

The transcripts of *lp8* were induced to very high levels in root tissues and in stems to a very small degree during WDS [77]. The expression of *lp8* is also modulated by ABA [77]. The predicted product of *lp8* contains a very high degree of homology to several calcium-binding proteins in various organisms. It has four possible calcium-binding sites called EF-hands, which agrees quite well with the consensus model from various species. Therefore, the apparent function of LP8 is to bind to the elevated cytosolic calcium level during WDS and modulate the expression of other genes. Genomic Southern analysis indicates that *lp8* belongs to a small multigene family. Clone *lp9* is induced in stems only under moderate WDS (−1.6 MPa), whereas its expression is downregulated in needles and is constitutive in roots. No significant homologies were observed with the sequences of *lp9* and *lp10* when compared with those from Genbank.

Promoter Analyses of Pine Genes

Genomic clones for *lp3*, *lp5* (a glycine-serine–rich cell wall protein homologue), and *lp8* (a calcium-binding protein), which are induced preferentially in roots during WDS, have been isolated. The genomic clones of both *lp3* (V. Padmanabhan, nonpublished) and *lp5* [13] have been sequenced and the upstream regions of both contain ABA-responsive elements at different positions [120], with *lp3* also having a root-responsive element (V. Padmanabhan, nonpublished results). No consensus sequence for ethylene responsiveness was found. Segments of the two promoters with different amounts of their upstream sequences fused in front of the *uidA* reporter gene encoding β-glucuronidase have been cloned. Preliminary experiments with *lp3* and *lp5* promoter-driven *uidA* expression in bombarded *Pinus elliottii* (slash pine) cell suspension culture cells indicate that both promoters are active. In transient expression analysis, both of these promoters were observed to be induced in response to mannitol (5.5%) which simulated WDS equivalent to −1.1 MPa (Fig. 4). The promoters have been cloned in plasmids pBI121 and p35SmGFP, inserted into *Agrobacterium*, and transferred into tobacco. *lp3* DNA driven by the 35S promoter in tandem with *uidA* driven by the *lp3* promoter have been inserted into *Agrobacterium* and also transferred into tobacco (R.J. Newton, M.A.D.L. Dias, and V. Padmanabhan, nonpublished results).

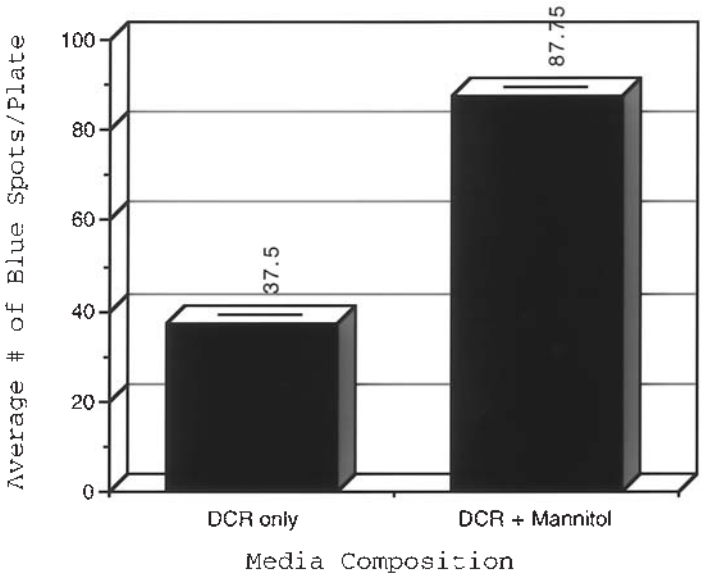

FIGURE 4 Average number of blue spots obtained indicating GUS activity driven by the loblolly pine *lp5* promoter. Expression in slash pine suspension cells subjected to osmotic stress with mannitol after biolistic transformation with *plp5*(p)GUS-SK [13].

GENES DOWNREGULATED BY WATER-DEFICIT STRESS IN LOBLOLLY PINE

Besides several upregulated genes, a few downregulated cDNAs such as *lp6* [121], *lp15*, and *lp20* also were isolated [77]. Even though the expression of *lp10* is downregulated under WDS, the transcripts of this gene are very abundant in roots. The translational *lp6* product bears a strong resemblance to class I chitinases [121]. However, LP*6* lacks the signal peptide, the cysteine-rich chitin-binding domain, and the glycine-proline–rich ''hinge'' region typical of class I chitinases. It is not known whether LP6 possesses chitinase activity. Benhamou and Asselin [122] have speculated that an alternate substrate for chitinases may be glycolipids in the cell walls of plants.

GENES INDUCED BY WATER-DEFICIT STRESS IN OTHER WOODY PLANTS

Saltbush (*Atriplex* spp.)

Through construction of a cDNA library [123], several clones of WDS-inducible genes have been isolated from saltbush, *Atriplex canescens* [15]. Two homologous cDNA clones (*p19-3* and *p27-3*) are nearly identical and encode for a small hydrophillic polypeptide of 95 amino acids (11 kDa) similar to one in rice, but whose function is yet to be determined [15]. Two additional clones, *p8-3* and *p23-3* (D. Villalon, nonpublished results), are similar to proteinase inhibitor I from potato [124]. Also in *A. canescens*, two other clones (OI2-2, OI14-3) putatively encode GRPs with cell wall–targeting signal peptides, which are induced by sulfur dioxide as well as WDS [16]. They have homologous amino acid alignments with ABA-inducible GRPs from alfalfa [89].

The saltbush GRPs induced by stress (ozone, sulfur dioxide, and WDS) also appear to be

arranged in β-pleated sheets. This predicted secondary structure has two specific conformations whereby tyrosine residues are exposed for cross linking with GRPs and other cell wall components, and whereby the spacing of the tyrosine residues determines the potential pore size of an intermolecularly cross-linked network. Genes encoding for GRPs are activated by a variety of stress conditions such as wounding, pathogen infection, WDS, heat, sulfur dioxide, and ozone [16,105]. A possible role for GRPs in these responses may be inferred from studies about cross linking of the extensin protein in the cell wall [125]. Wound-induced plant defense involves H_2O_2 oxidative cross linking and insolubilization of extensin which strengthens the cell wall in the initial stages of plant defense [125]. Similarly, the changes in the cell wall GRPs of both pine and saltbush could be associated with a decrease in the cell wall elasticity as induced by both ozone [126] and WDS [34].

A 59-KDa peptide, similar to the tobacco osmotin, has been detected in the cells of *A. nummularia* [127]. Two cDNA clones encoding osmotin-like proteins have been isolated, and the *pA9* clone is induced by NaCl [127]. Compared with glycophytes, the proteins of the halophytic *A. nummalaria* appear to be unique. Although these genes are similar to osmotin genes, they have different regulatory properties.

Cotton (*Gossypium* spp.)

Seeds of the woody cotton shrub have been the source of the late embryogenesis–abundant genes (*lea*) which encode for proteins which are expressed during the final stages of seed development at the onset of desiccation, and their expression dominates the mRNA population in dehydrated embryo tissues [71]. During dehydration, water loss leads to crystallization of cellular components resulting in cellular damage. LEA proteins can counteract these processes by maintaining the integrity of cellular structures in the absence of water. They are predominantly cytosolic proteins with hydrophilic residues, and their coiled nature is consistent with their proposed role of binding to water [37]. Some LEA proteins could be involved in the ''solvation'' of cytosolic structures during desiccation and/or in mitigating the damaging effects of an ever-increasing ionic strength in the desiccated cytosol [128], whereas others may have a regulatory role [129]. However, data which support these protective roles for LEA proteins during dehydration have not yet been obtained.

Poplar (*Populus*)

A novel 66-kDa protein (BspA) accumulates in the shoots of cultured aspen (*Populus tremula* L.) in response to gradual WDS [93]. Slash pine (*Pinus elliottii* Engelm.) callus cultures exposed to mannitol-induced water stress also express a 66-kDa protein [17]. BspA is the major WDS-responsive protein in aspen. It accumulates in response to ABA application as well as cold and osmotic stress, and it is boiling-stable. It accumulates as early as 1 h after initiation of the WDS treatment and decreases on rehydration. The N-terminal amino acid sequence shows a high homology with the wheat germins GF-2.8 and GF-3.8 [130]. Wheat germins also share a homology with spherulin, a putative cell wall protein in the slime mold, *Physarum polycephalum*, that increases during spherulation in response to osmotic stress [130]. An additional similarity was detected between BspA and a barley cold-responsive protein (pA086) [131] and a protein found in salt-stressed barley roots [132]. The wheat germins appear to be similar to the amino acid sequence of barley oxalate oxidase [133].

The differential accumulation of BspA in two *Populus* species may be related to their tolerance to WDS [23]. When subjected to WDS the detached leaves of *P. popularis* lost less water, had less ion leakage, and accumulated more BspA than did those of *P. tomentosa*. It is suggested that BspA is ''dehydrin-like'' and contributes to membrane stability. The dehydrins [134,135] are polypeptides which are highly hydrophilic, remain soluble after boiling [27], and are suggested to prevent cellular damage during desiccation by binding to macromolecular structures [136]. Dehydrin homologues of 43 and 31 kDa were induced in the shoots and roots of aspen [22], and they are similar to dsp-16, a dehydrin from the resurrection plant, *Craterostima plantagineum* [137].

Sucrose synthase and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) also accumulate in aspen shoots in response to WDS, and this is accompanied by increased sucrose and decreased glucose levels [23]. Sucrose synthase accumulates in stressed leaves of *P. popularis* along with a simultaneous increase in the glucose levels, whereas in stressed leaves of *P. tomentosa*, sucrose synthase is nondetectable and glucose levels decrease [23]. Sucrose synthase is a cytosolic enzyme involved in the breakdown of sucrose into fructose and UDP-glucose, and GAPDH is a key enzyme in glycolysis and gluconeogenesis pathways. Thus, both sucrose synthase and GAPDH may play a dominant role in sugar metabolism during WDS.

CONCLUSIONS

Many environmental stresses affect the establishment and productivity of trees and shrubs, and water deficit is the principal stress. Water has many roles in woody plants such as turgor maintenance, solute transport, donation of electrons, and cooling of tissues and organs by evaporation. The delicate water balance in trees and shrubs is very often impaired by external WDS conditions. It affects woody plant populations across all geographical and climatic areas, by impairing their growth and development. Extreme cases of WDS destroy large areas of forest and shrubland and contribute to land erosion and to desertification. An understanding of WDS responses is important for evaluating the warming effects of the global climatic changes because WDS is often associated with heat stress. Warmer climates could have consequential effects on tree growth via responses to WDS.

For the last decade, researchers have been investigating the molecular responses to WDS in woody plants. Many clones of genes that are either upregulated or downregulated by WDS have been identified. Those genes that have been studied further and partially characterized appear to be involved in modifications of the cell wall, gene activation, signal transduction, and protection against desiccation. We now have sufficient information, expertise, and knowledge to ascertain in a more definitive vein what the function and role of these genes are in response to WDS. Furthermore, by studying their regulation, we have the potential to identify promoter sequences as well as genes which in the future could be useful in the genetic engineering of trees and shrubs. However, at this point, even though specific WDS-responsive genes have been identified in a large number of plant species, for most of these genes, it is still not known what their specific roles are. In addition, these genes have not been shown to provide tolerance to WDS. These data will be needed before specific WDS-responsive genes can be considered for genetic engineering purposes.

Only a few investigations have focused on the responses in woody plants where the cell walls in wood formation and wood composition are of primary importance. Trees and shrubs, because of their demonstrated adaptation to very diverse environments and their perennial growth habit, may have unique and specialized responses involving gene expression which deserve further investigation. Furthermore, trees and shrubs have large, prolific root systems and other factors which provide for compensating responses to WDS, thus they respond in a very gradual manner, whereas many responses of herbaceous plants may be more sudden and abrupt and may involve a different cadre of genes compared with those induced by WDS in woody plants. In most of the investigations reported here, an experimental system which reflects a gradual, slow response to an increasing severity of WDS was used, and this simulates best what trees and shrubs experience in their native environment.

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DNA Content, Water Relations, and Environmental Stress in Gymnosperms

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INTRODUCTION

Most of the theory concerning the evolution of the genome size and the effects of the varying DNA content on the physiology, development, and adaptation of plants has been derived through the study of herbaceous angiosperms. Therefore, a brief introduction to these concepts is necessary before specifically addressing gymnosperms.

Extent of DNA Content Variation

The widespread variability in the genome size (DNA content) among eukaryotic organisms has been extensively documented [1–4]. Among angiosperms, the range in genome size varies several hundredfold from 2C = 0.1 to 254 pg [3]. The DNA content varies up to fortyfold and ninefold within families and among congeneric species, respectively. The DNA content variation among both plants and animals does not correlate with the evolutionary advancement or genetic complexity [5]. Furthermore, only a very small amount of DNA apparently has coding functions, and most of the eukaryotic genome is made up of repetitive sequences [6,7]. The repetitive sequences may be tan-

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demly arranged and/or dispersed throughout the genome [8–10]. Changes in the genome size primarily involve a variation in the amount of repeated DNA sequences [7,11,12].

Fluidity of the Plant Genome

The current model for genome evolution includes the concept that the plant genome is fluid and has repeatedly undergone amplification and deletion of DNA sequences over evolutionary time [9,13,14]. Repetitive sequences have diverged in base composition, and they apparently have undergone extensive translocation, reamplification, and deletion [9,13,14]. This cycle has apparently resulted in the massive variation in the genome size observed among plants [11,14]. The mechanisms for amplifying and/or deleting repetitive sequences are not understood. Proposed mechanisms include errors in the replication of DNA, saltatory amplification, unequal crossing over, and the actions of transposable elements [6,15–17].

Changes in the DNA content may be triggered by stresses imposed by the genetic and/or physical environment [18–20]. The ability to generate variability in response to stress has been proposed to be part of an evolutionary strategy for adaptation to a changing environment [19]. Examples of the stress-induced variation in the DNA content include the formation of megachromosomes in hybrids of *Nicotiana tabacum* with *N. otophora* and *N. plumbaginifolia* [21,22], the instability of the DNA content in hybrids of *Microseris* [23,24], fertilizer-induced changes in the DNA content in *Linum usitatissimum* [25], and the within-plant variation of the 2C DNA content in sunflower, *Helianthus annuus* [26]. In sunflower, the DNA amount is influenced by the photon flux density and the ratio of the red to the far red portion of the spectrum [27]. The reduction in the DNA content of plants growing under light that is rich in far red suggests that phytochrome may be involved. Since light reflected from vegetation is richer in far red light than unobstructed light, it was further proposed that the far red light–induced reduction in the DNA content in the sunflower may represent an adaptation for shade avoidance in competition with neighboring plants [27]. The extent of environmentally induced changes in the DNA content among plants remains to be determined. However, the examples studied so far indicate that a sizable fraction (up to at least 45%) of the plant genome is potentially unstable and subject to rapid content changes.

Most of the variation in the DNA content is associated with classes of repetitive DNAs [7] composed of retroelements; that is, several classes of mobile DNA that transpose through an RNA intermediate [28]. These elements comprise at least 50% of the maize genome, and they are arranged as nested elements in spaces between genes. Retroelements are highly abundant in plants with large genomes, and this suggests that they account for most of the great variation in the genome size [28]. Because retroelements all transpose without excision, their mobility always increases their copy number, and this would tend to increase the genome size [29]. The question still remains whether woody plants such as the pines, which have a large genome sizes, are moving toward the development of even larger genomes, or if there are active processes for removing the interspersed repetitive sequences. So far, no mechanism has been found for removing them [29], but data from sunflower [26,27] imply that mechanisms for removal of interspersed repetitive sequences must exist.

Nucleotypic Effects

Various hypotheses have been proposed to explain the widespread variation of the DNA content observed among eukaryotes, including several nonadaptive ones such as junk, selfish, or parasitic DNA sequences [30–32]. However, an alternative theory that has garnered much support considers the DNA content to be of adaptive significance through cellular and phenotypic effects owing to its mere bulk [33]. These effects are called the nucleotype. The nucleotype is determined by the total quantity of nuclear DNA, both genetic and nongenetic, and influences cellular and developmental parameters such as chromosome size, nuclear volume, cell volume, mitotic cycle time, and the duration of meiosis. It has been proposed that the nucleotype influences the phenotype so that,

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under some conditions, selection favors accumulation of nuclear DNA regardless of its nucleotide sequence, and under different conditions, the loss of sequences not necessary for survival may confer considerable adaptive advantage [1,33,34].

It is well established that chromosomes that have more DNA also are larger [35,36]. Strong positive correlations have been established between the DNA amount and the chromosome volume and mass [37,38] and between the DNA content and the total length of the synaptonemal complex [39]. Baetcke et al. [40] established a strong positive correlation (slope = +1) between the DNA content and the nuclear volume, and Price et al. [41] detected a similar relationship between the DNA content, nuclear volume, and cell volume. Price [42] presented the hypothesis that the DNA content determines the fundamental nuclear volume and cell volume which are in turn influenced by developmental, genetic, and environmental factors. Selection for the DNA content may, therefore, be partly through its effects on nuclear and cell size.

A relationship between the nuclear DNA content and the minimum mitotic cycle time is well established among diploid angiosperms [43–45]. Generally, the higher the DNA content, the longer is the mitotic cycle time. The average duration of the mitotic cycle increases at a rate of 0.38 h/pg DNA, and this increase is mainly attributed to a longer duration of the DNA synthesis [46,47]. Bennett [48,49] established a correlation between the duration of meiosis and the DNA content in diploid plants. The increase in the duration of meiosis with an increased DNA amount was attributed to similar increases in all meiotic stages.

DNA Content and Adaptation

Although there is no overall correlation of the genome size and phylogeny in plants, the distribution of the variability in the DNA content is not random. Evolutionary trends are apparent. Andulov [50] detected that plants of grass tribes and genera centered in the tropics, or those that grew only during warm seasons in temperate climates, had uniformly small- to medium-size chromosomes. Those growing in the more temperate northern regions tended to have larger chromosomes. Levin and Funderburg [38] concluded that genome sizes are generally larger in temperate compared with tropical herbs and that families indigenous to tropical and subtropical regions have substantially smaller genomes than those of temperate regions. Bennett [51] concluded from a study of cereal grain crops, cultivated pasture grasses, and legume pulse crops that humans had generally chosen species for cultivation that resulted in a distribution paralleling or exaggerating the natural tropical-temperate cline in the DNA content. A south to north cline of an increasing DNA content has been found for some conifers. Miksche [52,53] detected that the DNA contents of plants from northern populations of *Picea glauca* and *P. sitchensis* were greater than 50% larger than those of southern populations.

From a study of 271 species, Bennett [54] detected a relationship between the growth form of herbaceous angiosperms and the DNA amount. He observed that (a) annual species of both monocots and dicots have a significantly lower mean DNA content than perennials, (b) the range of the nuclear DNA amount is smaller among diploid annuals of both classes, (c) ephemeral annuals have a lower mean DNA content than nonephemeral annuals, and (d) among monocots the mean DNA amount of obligate perennials is greater than that of facultative perennials, and the mean values for facultative perennials and annuals are not significantly different. Bennett [54] proposed that the nuclear DNA content and the minimum generation time are correlated and the DNA content is causally correlated with the rate of development.

Grime and Mowforth [55] suggested that selection may operate on the genome size through a differential effect of temperature on cell division and cell expansion. They proposed that cell expansion is inhibited to a lesser degree than cell division at low temperature, and plants growing in cooler environments should have growth dominated by cell enlargement that would favor larger cells and a higher DNA content. Growth should be facilitated by more rapidly dividing cells with less DNA under warmer conditions where temperature is not adversely affecting the mitotic cycle time. Phenological data collected from British flora [55,56] support this hypothesis. From early

spring to midsummer in the Scheffield region, growth rates were significantly and negatively correlated with the genome size. In a species-rich grassland community in northern England, the DNA content and the rates of leaf elongation of grasses, sedges, forbs, and small herbs were studied in relationship to temperature and chronology [56]. The most rapid rates of leaf expansion occurred in species with relatively high DNA content during the cold early spring. The differences were not apparent under the warmer conditions of early summer.

Strong support for an adaptive nature of a variable DNA content comes from studies of the genome size, speciation, and adaptation in congeneric species and within species. The two most thoroughly studied examples come from the genera *Microseris* and *Zea*.

In the Microseridinae (Asteraceae) of western North America, there are correlations between the ecological adaptation and the DNA amount at the interspecific and intraspecific levels. The more primitive diploid species of *Microseris* occupy cooler and more mesic habitats than do the specialized annual species that are adapted to warmer, more xeric, and time-limited habitats [57]. The evolution of these diploid annuals from their putative perennial ancestor(s) resulted in massive diminution of the DNA sequences so that they have only about 40–60% of the DNA amount of the perennials [57–59].

The DNA amount varies over 20% within the annuals *M. bigelovii* [60] and *M. douglasii* [61]. In *M. bigelovii*, the lower DNA values were detected in plants of geographically disjunctive populations at the low-rainfall, southern extreme of the species range and at the northern high-rainfall extreme on thin soil over a barren rock outcrop. The higher DNA values were detected from plants of habitats at the center of the species range where intermediate amounts of precipitation occur. Price et al. [60] suggested that the low DNA content resulted from selection in stressful and/or time-limited habitats at the extremes of the ecological adaptation of the species.

In *M. douglasii*, from which over two dozen populations have been sampled, high DNA values are restricted to plants growing in more mesic sites, generally on well-developed soil [61]. At some sites that were studied more extensively, temporal changes in the DNA content were observed that correlate with environmental changes; that is, amount of precipitation and the length of time available to complete the life cycle [61,62].

The observed distribution of the DNA amounts among and within species of *Microseris* suggests an adaptive role for the DNA amount. Selection may act on the nucleotypic effects of the DNA content that influence the cell size, mitotic cycle time, and rate of development [1,33,63].

The DNA content varies among North American populations of *Zea mays* representing open-pollinated and inbred lines from Mexico to Nova Scotia [64,65]. The nuclear 4C DNA amount ranged from 9.8 pg in Gaspe Flint to 13.5 pg in Zapolate Chico, or about 38%. Rayburn et al. [65] detected a significant negative correlation between the DNA content and the maturity zones. Since these maturity zones are roughly latitudinal in nature, there exists a latitudinal cline from higher to lower DNA values in North American maize. Maize apparently originated in Mexico or Central America as an obligate cultivar. It was subsequently taken northward by humans until the cooler and shorter growing season restricted maturation. Rayburn et al. [65] suggested that the smaller genomes of maize lines grown at high latitudes resulted from selection by humans for earlier maturation, maximum plant size permitted within climatic constraints, and yield. These more rapidly developing plants may have been achieved in part through selection from a shorter mitotic cycle time and more rapid cell proliferation resulting from less nuclear DNA.

NUCLEAR DNA CONTENT IN GYMNOSPERMS

Survey of Gymnosperms

The DNA content of more than 80 gymnosperm species from 24 genera have been reported [52,53,66–80]. Although real differences occur among species, some of the variability among and within studies is due to the methods, standard, and tissue used. If not properly controlled, the problems historically associated with determining the DNA content from gymnosperm root-tip nuclei result in increased variability and imprecise measurements of the DNA content [69,79]. Gymno-

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sperm root tips fixed in nonadditive fixatives release tannins from the vacuoles which may interfere with the Feulgen reaction and result in reduced staining [81]. The recent improvement in instrumentation and the emphasis on techniques and careful preparation of tissues should minimize the procedural variability of the DNA values of the same species determined from different laboratories.

Methods

About 75% of the DNA content reported for gymnosperms was derived from Feulgen densitometry with 15% from laser flow cytometry and 10% from chemical extraction [80]. Chemical extraction was the only method for measuring the DNA content prior to the development of microdensitometric techniques. Scanning Feulgen microspectrophotometry is the most frequently used method for determining the relative DNA content. However, laser flow cytometry (LFC) is a recently established method for plants [82] that we have used to determine the DNA content of *Pinus* species [72]. Our results showed that the mean DNA content of samples determined by Feulgen densitometry was significantly linearly correlated with the mean value determined by laser flow cytometry ($y = 0.132 + 1.04 \times r = 0.987$ [72]). Flow cytometric determinations of the DNA content in loblolly pine by two independent laboratories were 21–22 pg/1C [72] and 44–45 pg/2C [73] indicating the reliability of the method.

Standards

Four kinds of standards were used for Feulgen densitometry and laser flow cytometry [80]: *Allium cepa* cv. Ailsa Craig (2C = 33.5 pg), *Zea mays* (L.) ssp. *mays*, inbred line Va35 (4C = 10.32 pg), *Hordeum vulgare* cv. Sultan (2C = 11.12 pg), and chicken erythrocytes (2C = 2.5–3.5 pg). Price et al. [83] have pointed out that plant nuclei instead of chicken erythrocytes should be used as standards for plants. Compared with the values obtained with other standards, plant material using *A. cepa* as a standard have values which are 8–52% smaller than those using chicken erythrocytes. Values obtained using *Z. mays* and *H. vulgare* as standards are 1.2–1.3 times larger than those using *A. cepa*. These differences could be caused by tissue differences as well as differences in methods. Also, the use of a very low DNA content standard is not preferable when measuring nuclei such as those of gymnosperms that have relatively large genomes.

Tissue

Meristematic tissue in the root tips has been mainly used for the DNA content determinations; however, nuclei from leaves or other tissues have sometimes been used. It is often difficult to squash the root tips of woody plants so that nuclei are clearly separated and free of debris. Further, if not fixed properly, the DNA contents of gymnosperms may be underestimated owing to interference of the Feulgen reaction by tannins [81,84]. Uniformly squashed and well-separated nuclei are essential for an accurate DNA determination by scanning densitometry. We have found that the megagametophyte of *Pinus* is soft and easy to squash and can be readily used for precise and accurate measurements of the DNA content by both Feulgen scanning densitometry and flow cytometry using propidium iodide [72].

CONIFER RETROTRANSPOSONS

Although gymnosperms have enormous genome sizes, very little is known about their genomic structure and composition. Reassociation kinetics have shown 75% of the *Pinus* genome to be repetitive DNA [85,86] which apparently contains retrotransposons. Retrotransposons that proliferate by reverse transcription of RNA intermediates are the major class of mobile genetic elements in plants [7,9,11,14]. Retrotransposon elements, IFG7 from *P. radiata* [87] and TPE1 from *P. elliottii* [88], are both highly amplified in their respective genomes. The TPE1 element is uniformly dispersed over all 12 chromosome pairs and appears to be inactive. It is conserved in several *Pinus* species

as well as *Picea*, *Taxodium*, and *Gingko* [88]. Retrotransposon amplification may be the means by which plant genome sizes increase, but the mechanisms by which they decrease are not known [29]. Two mechanisms for reduction, resistance to retroelement amplification, and restricting contact with retroelements have been suggested [29]. Suppression/methylation processes may also be associated with both mechanisms [89]. Although changes in the genome size have been noted in relation to environmental stress, the nature of the repetitive entities and the mechanisms involved have not been well characterized. One of the suggested roles for the amplified repetitive DNA is that of altering gene function [29].

CONIFER DNA CONTENT AND NUCLEOTYPIC DEVELOPMENT

The conifer DNA content correlates to nucleotypic parameters such as nuclear volume and total length of the haploid chromosome complement [90,91]. There also appears to be a direct proportionality between the DNA content and tracheid dimensions such as length and diameter in a ''western'' subset of *Pinus* species [92]. This can be extrapolated further with a consideration of the relationship between these two parameters and the indices of growth and development of individual trees. Bailey [93] found a correlation between tracheid size and stem diameter in conifers, and a similar relationship has been observed in *Pinus*. DNA content data have been related to published data sets of growth and development [94]. There appears to be a direct positive relationship between the DNA content and the duration of tree development [92]. A significant relationship between the DNA content and the seed volume has been observed in several *Pinus* species [66,72].

ENVIRONMENTAL CORRELATES AND CONIFER NUCLEAR DNA CONTENT

Chronic stresses have been important agents in the evolution of conifers [94]. For example, the effects of water-deficit stress (WDS) have a strong influence on the allocation of maternal resources (seed weight) and tree size [94]. The habitats of *Pinus* species cover widely differing ecological areas. Rehfeldt and Lester [95] have suggested that the particularly variable environments to which pioneer tree species are subjected have led to the evolution of genetic systems with a high degree of developmental homeostasis or a fairly constant physiological and morphological response to a range of environments. Such homeostasis has been postulated to be due in part to redundant DNA control systems of high DNA–content species or provenances which activate the same developmental gene systems in response to varied stimuli [67]. However, little is known about the functions of repetitive DNA and any roles it may have in gene regulation. Environmental conditions can greatly alter the nuclear DNA and the phenotype in some plant species, and these are heritable to subsequent generations [20,96,97]. However, in conifers, the relationship of intraspecific variation in the DNA content to the environment is still unresolved [53,66,71,75,78,98,99]. The intraspecific variation has been reported to vary from the lowest to highest by a factor of 1.5–2.2 in five conifer species [49,50,72,84], but the DNA content was uniform and constant in six *Pinus* species [66,69,98]. Furthermore, two provenances of *P. wallichiana* [66] from areas representing extremes in water availability showed no differences in their DNA content.

Latitude and Elevation

The intraspecific DNA content per cell was related to the latitude of distribution among sources of *Picea glauca* [52]. Western sources revealed an inverse relationship between DNA per cell and growth, whereas eastern sources did not. The DNA values of different seed source populations of four conifer species measured along latitudinal gradients showed that populations from high latitudes had more DNA per nucleus than those collected from lower latitudes [52,53,100,101]. For *Picea*

sitchensis, the DNA content increased with latitude, with the more southern provenances having nuclear volumes and DNA contents one-half that of the more northern provenances [53,90]. However, in two *Pinus* species, latitudinal differences in DNA content were not observed [76,98]. Intraspecific variation can also be associated with elevation. Transcending an increase in elevation from 600 to 1500 m in New England, there was a detected increase in the nuclear DNA from 47.5 to 50.7 pg in *Picea rubens* [71]. These data provide evidence for the effects of temperature on the DNA content as theorized [55,63], but the relationships to the tracheid size and conductivity have not been investigated.

In conifers, the DNA content correlates to nucleotypic parameters such as an increase in the nuclear volume and total length of the haploid chromosome complement [90,91]. From an interspecific perspective, gymnosperm species with smaller nuclear volumes and smaller DNA amounts per cell tend to display greater geographical distribution than species with larger amounts of DNA [68]. Plant species from northern latitudes tend to have larger nuclear volumes than plants of the same or closely related species from southern regions [90,102,103]. Cavalier-Smith [63] suggests that this reflects an increased selection for large tracheids and large nuclei in cambial cells in cooler climates with shorter growing seasons. Ohri and Koshoo [66] showed that there is an association between the very high DNA content of *P. gerardiana* with a temperate and xeric habitat. However, these relationships are not always demonstrated in other species. Populations of *Picea rubens*, with a high DNA content, appear less resistant to high elevational environmental stresses than those of *P. mariana*, with a low DNA content [71].

Temperature and Precipitation

Seasonal variations in temperature and precipitation are considered to be the most important climatic factors limiting the geographical distribution of a given species [104]. McCune [105] concluded that convergent evolution had occasionally occurred with *Pinus* species, but shared ancestry and evolutionary divergence produced by a diversity of environments were more important.

Grime and Mowforth [55] suggest that selection operates on the genome size through a differential effect of temperature on cell division and cell expansion, where at low temperature, cell expansion is inhibited to a lesser degree than cell division. According to their thesis, growth should be dominated by large cells and high DNA in cooler environments, whereas growth should be facilitated by more rapidly dividing cells with less DNA in warm conditions [55]. In addition to temperature, Grime and Mowforth [55] hypothesize that natural selection has operated on the nuclear DNA content through differential sensitivity of the cell growth to moisture availability. The cell volume and DNA content may thus be an important parameter in the plant adaptation to WDS environments [42].

Price et al. [106] and Cavalier-Smith [63] suggested that the evolution of xylem tissues has been impacted by the evolution of genome sizes. Angiosperms with vessel elements could maintain good sap flow with smaller cell volumes, whereas gymnosperms continued strong selection for large tracheids. Cavalier-Smith [63] argued further that this change in xylem construction with vessel elements made possible the evolution of herbaceous annual and ephemeral angiosperms with their small, rapidly dividing cells, small-volumed conducting cells, and low DNA content. On the other hand, gymnosperms with their strong selection for large tracheid size maintained a high DNA content and a slower rate of development which prevented them from evolving an annual growth habit [63]. The influence of temperature on the DNA content and cell volumes may be related to the conductive properties of the xylem [63]. In some conifer species, the DNA content has been reported to vary by a factor of two [107], and in many species, there is a continuous cline in the Northern Hemisphere from a low DNA content in the south to a high DNA content in the north [53]. Because the viscosity of water increases by a factor of two as the temperature drops from 25 to 0°C, hydraulic conductivity would decrease unless the diameter of the tracheids increases. In conifer wood, the cambial cells are already quite large [108,109], and the first xylem cells laid down during the cooler spring growth season with ample soil water available are large [110].

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Temperature

In addition to moisture, it is theorized that natural selection operates on the genome size through temperature influences on cell division and expansion. Grime and Mowforth [55] proposed that growth in cooler climates is dominated by large cells and high DNA. By regression analysis, we have considered the relationship between the DNA content and the minimum daily air temperature for each month of the year in the habitat for each of the 18 *Pinus* species, but no correlation was observed (data not shown). The absence of such a relationship may reflect the limiting effects of extremely cold climates on plant metabolism and growth. However, a significant relationship exists between the nuclear DNA for *Pinus* species [72] and the mean daily maximum air temperature of their habitats [111] if only the four summer months are considered [72]. This relationship indicates that *Pinus* species with a high DNA content are associated with cooler climates.

The inverse relationship between the DNA content and the habitat air temperature supports the hypothesis of Cavalier-Smith [63], who contends that there is selection for a large tracheid volume and for a large nuclei in cambial cells in cooler climates with shorter growing seasons. To address this hypothesis further, a measured *Pinus* DNA–content data base [72] has been related to several published data sets of tracheid dimensions [112–115]. The data for both diameter and length cluster the species in two major groups: group I with DNA content ranging from 21 to 25 pg and group II ranging from 26 to 30 pg. Group I comprises ''eastern pines'' (with the exception of *P. radiata* from a coastal, humid, and cool climate), whereas group II includes the ''western pines'' (with the exception of *P. strobus* from northern latitudes) which experience extremes of low precipitation and low temperatures [116].

Precipitation

The availability of the comprehensive data set obtained by Wakamiya et al. [72] allows one further to address the hypotheses relative to environmental influences on the DNA content. These DNA content data have been related to published precipitation data [117], and there is an inverse relationship between the DNA content of *Pinus* species and habitat annual minimum precipitation. Compared with mesic *Pinus* species where minimum precipitation is over 1400 mm, there is a 25% increase in the amount of DNA per cell in those species adapted to habitats with only 300 mm. *P. eldarica* has a high DNA content of 31 pg [72] and has been adapted to the semiarid climate in the southwestern United States [118]. These data indicate that a high DNA content is an adaptation to WDS environments among the species of *Pinus*. However, intraspecific variation between two *P. taeda* provenances from habitats of varying water availability was not evident [72]. It appears that there is a positive relationship between the DNA content and the tracheid size as long as cell expansion is not limited by low water availability (or perhaps extreme cold temperatures). That is, the relationship between the two variables is highly correlated with species from habitats of moderate to high-rainfall levels. For example, one of the group II species with a large DNA content (*P. lambertiana*, 29 pg, [76]) also has one of the largest tracheid sizes reported for commercial pines (diameter = 52 μm, length = 6 mm [112]). This strong correlation may be due to the fact that the habitat of this species has a high precipitation range of 840–1750 mm [116], and that large tracheids have resulted from large precursor cells expanded by water. Conversely, the high DNA content of one of the group II species, *P. monophylla* (30 pg [72]) associated with very small tracheids (diameter = 32 μm, length = 3 mm [112]), appears to be related to the low precipitation of its habitat (200–460 mm [116]). These cells may have the capacity to be larger, but were unable to expand because of limited water available for expansion. We postulate here that embolism and cavitation [119,120] were important factors which influence the tracheid volume in stressed environments. Nevertheless, it appears that the direct relationship between the DNA content and the tracheid volume as hypothesized by Price et al. [106] and Cavalier-Smith [63] is most demonstrated in *Pinus* species from habitats of moderate to high levels of precipitation, although more data are needed.

Because a direct relationship between the tracheid length and the diameter in *Pinus* is evident, it is reasonable to conclude that the DNA content is most likely proportional to the tracheid volume.

Furthermore, it is appropriate to consider the relationship between the tracheid volume and hydraulic conductivity (L_p), because the adaptive, evolutionary significance of the nuclear DNA content can be more adequately assessed if it is related to the physiological processes of water transport. The Hagen-Poiseuille equation [121] predicts that the volume of water moving in a unit time along a tracheid is proportional to the fourth power of its radius. Measurements of the xylem hydraulic conductivity (L_{px}) in *Pinus* stem axes of 17-month-old seedlings indicated that there is a direct relationship between the L_{px} and the DNA content [122], but this was not observed in 21-month old seedlings [123]. These contradicting observations were related to differences in (a) seedling age, (b) ages of the cell types analyzed, and (c) methodology associated with the determination of the cell dimensions [123]. However, *Pinus* species with large genome sizes had thick cell walls and small ratios of lumen radii to cell wall thickness in their conductive cells, and those species lost their turgor to tissue dehydration at water potentials lower than those of species with a small genome size [123]. These characteristics of WDS tolerance may be contributing factors in establishing the inverse relationship between the genome size in the genus *Pinus* and the lowest mean precipitation in their natural habitats [72].

In addition to the tracheid diameter, the hydraulic conductivity is also related to the number of tracheids per unit of the stem transverse area. In *Cyrtomium falcatum* (holly fern), the overall decrease in the hydraulic conductance was strongly influenced by a decrease in the number of tracheids and the diameters of the largest tracheids [124]. Estimates based on the Hagen-Poiseuille equation consistently overestimated conductance, but this constant relationship supports the assumption that the conduit number and diameter are the principal determinants [124]. In *Psilotum nudum* [125], decreases in both the tracheid number and the lumen diameter were directly related to decreases in the hydraulic conductance. A model based on the Hagen-Poiseuille relation indicated that calculated conductances were in agreement with those measured [124], but for most woods, actual conductivities are 20–100% of the theoretical conductivities [120]. Furthermore, the conductivity advantages acquired by increases in the diameter became smaller as the tracheid diameter increased [125].

A correlation between the conductivity and the xylem cell diameter has not always been found. The hydraulic conductivity was not strongly correlated with the vessel diameter in 10 tree species, nor was it correlated with stem density [126]. In addition, there were no significant differences in the plant hydraulic conductivities (K_{plant}) between *Pinus taeda* seedlings from coastal versus interior ecotypes [127]. The K_{plant} describes the conductivity of water movement from the soil to leaves [128,129]. Large tracheid diameters could also be a detriment in stressful environments of low temperature and water deficits owing to embolism [119,120,130]. However, one of the advantages of gymnosperm tracheids as opposed to angiosperm vessels appears to be their capacity to localize embolisms [131].

CONCLUSIONS

A quantitative variation in the nuclear DNA content is apparently important in plant evolution, including adaptation to stressful environments. Natural selection may operate on the genome size through nucleotypic effects on cellular parameters such as cell volume and mitotic cycle time, and through differential effects of temperature and moisture availability on cell division and expansion. Large differences in the DNA content have spatial and temporal phenotypic consequences which profoundly affect when, where, or how plants grow both in relation to their global distribution and within a local community [132]. The variation in the DNA content may have considerable adaptive significance with far-reaching ecological implications and clearly illustrates that further, widely based comparative studies relating these correlations to the physiological bases of adaptation would be informative. There is a need to address the hypothesis that a quantitative variation in the DNA content is important to plant evolution which includes adaptation to stressed environments.

Data gathered with woody perennial genera indicate that conifer DNA contents do not relate

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to the environment in the same way as those of annual herbaceous species. Unlike many perennial angiosperms, gymnosperms appear to have adapted to stressed environments by maintaining a large genome and large cells. Large cells are thought to enhance the hydraulic conductivity which then may be related to the DNA content, but the data are conflicting and depend on the age of the plants studied and the methodology used. *Pinus* species with large genome sized maintain their turgor at lower water potentials than do species with a small genome size when subjected to WDS. This property may contribute to the apparent tolerance to low precipitation environments observed with *Pinus* species with a high DNA content.

If there is a positive relationship between the tracheid structural and conductive properties and the genome size in conifers, this would provide credence to the theory that the nuclear DNA content is important in the evolution and natural selection of conifers. This relationship between the DNA content and a physiological parameter would be informative from an adaptive standpoint. It also would provide an additional explanation for the large genomes associated with conifers compared with most other plant species. Better understanding of these relationships would contribute to our knowledge as to how gymnosperms respond and adapt to changes in the environment.

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Osmoregulatory Role of Proline in Plants Exposed to Environmental Stresses

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INTRODUCTION

Plants face frequent periods of environmental stress that impairs their growth and reproductive capacity. Environmental parameters with deleterious effects include unfavorable climates, water stress, and inappropriate soils. Restrictions of plant growth cannot be attributed to one single process, because plant growth is the result of many integrated and regulated physiological and biochemical processes. To survive and maintain a minimal growth potential, plants must conform to these extreme environments entailing adaptive changes in metabolism and cell composition. Of the various mechanisms enabling plants to cope with water stress, the most common is the accumulation of intracellular solutes, such as sugars and free amino acids. The most frequent nitrogen-containing compounds that accumulate in plants subjected to environmental stresses are amides (glutamine and asparagine), amino acids (arginine, proline, citrulline, and ornithine), and polyamines (putrescine) [1]. The accumulation of proline on dehydration due to water deficit or increasing osmotic pressure is the most recent information concerning the osmoregulatory role of proline in environmentally stressed plants.

OSMOREGULATION OR OSMOTIC ADJUSTMENT

Water deficits have been shown to induce a lowering of the osmotic potential in crops as a means of maintaining their turgor [2]. This decline in the osmotic potential as a response to water deficit can be achieved by solute accumulation within the plant cell or by a decreased cell volume leading to an increased concentration of osmotic solutes as water leaves from the vacuole. These phenomena are described as osmoregulation and osmotic adjustment. *Osmoregulation* has been defined as ''the regulation of osmotic potential within a cell by the addition or removal of solutes form solution until the intracellular osmotic potential is approximately equal to the potential of the medium sur-

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rounding the cell'' [3]. *Osmotic adjustment* refers to the lowering of the osmotic potential due to the net accumulation of solutes in response to water deficits or salinity [3]. Osmotic adjustment is an important mechanism in drought tolerance, because it enables (a) a continuation of cell expansion [4,5]; (b) stomatal and photosynthetic adjustments [6]; (c) better plant growth; and (d) yield production [7]. The compounds involved in osmotic adjustment are mainly soluble sugars, potassium, organic acids, chloride, and free amino acids [8,9]. The degree of the osmoregulatory processes is affected by the rate of stress, stress preconditioning, the organ type and age, and the genetic variation between and within species [10]. It is accepted that the nontoxic compatible organic solutes accumulate in the cytoplasmic compartment of cells and inorganic ions toxic to metabolic processes are restricted to the vacuolar compartment. Considerable research has been conducted to characterize the accumulation of proline, a compound known to contribute to the osmotic adjstment and tolerance of plants exposed to unfavorable environmental conditions. How much of a role it plays is still controversial and is discussed in detail in the following sections.

PROLINE METABOLISM

Proline metabolism is a typical mechanism of the biochemical adaptation in living organisms subjected to stress conditions.

Proline Synthesis

Two pathways of proline biosynthesis are known in higher plants: the glutamate pathway and the ornithine pathway (II). These pathways seem to be identical for all organisms. Most evidence about the roles of particular enzymes in proline biosynthesis or catabolism has been obtained from microorganisms.

Proline Catabolism

The ability of plants to degrade proline through an oxidation process has been shown clearly [11]. The catabolism of proline involves the conversion to glutamic acid via pyrroline-5-carboxylate reductase and a subsequent metabolism of glutamate by the Krebs cycle reactions that release CO_2 as the endproduct. Oxidation of proline is catalyzed by proline oxidase and requires oxygen as an oxidant.

Endproduct Inhibition

Proline manifests a conspicuous ability to control its own biosynthesis. Exogenous application to plant tissues of an amount of proline sufficient to increase the endogenous pools enhanced the rate of proline oxidation as a result of a feedback inhibition process [12,13]. It is known that proline oxidase, one of the enzymes involved in proline degradation, can be induced by high concentrations of proline [14,15]. Feedback inhibition of proline synthesis does not occur under water-stress conditions.

Impact of Environmental Stresses on Proline

Extensive research in this area has revealed proline accumulation as a universal response of plants under stress. Proline accumulation due to water or salt stress results from a stimulated synthesis in the tissue, an inhibited oxidation, or an impaired incorporation of proline into proteins. Clearly, under such conditions, the mechanisms of feedback inhibition cannot function. Indeed, this was proven by Boggess et al. [12], who showed that proline does not inhibit its own synthesis in wilted barley and tobacco leaves. The increased synthesis of proline due to wilting seems to be related to the first step of this synthesis. On the other hand, water stress and salinization inhibit proline oxidation in

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TABLE 1 Accumulation of Proline in Water- or Salt-Stressed Tomato Leaves[a]

	Proline content ($\mu M\ g^{-1}$ fresh weight)	
Salinity level	high soil moisture	low soil moisture
Nonsalinized	8.83	13.27
Low salinity	15.64	15.77
High salinity	18.31	21.47

[a] Plants were irrigated with saline water (4 or 10 dS m^{-1}) or irrigation was withheld for 7 or 14 days.

the mitochondria and alter the permeability of the mitochondrial membranes [16–18]. Further, the incorporation of proline into protein is inhibited by water stress, thereby also leading to proline accumulation under stress conditions [19]. The step affected in protein synthesis is probably the translation step [20]; however, this process is not obligatory, because proline accumulation was observed when protein synthesis inhibitors, such as cycloheximide, were applied [21]. The state of the hormonal balance in plants is suggested to play a considerable role in the mediation of proline accumulation under water or salt stress [21]. The key role in the osmotic adaptation of plants has been ascribed to abscisic acid (ABA).

In conclusion, the reduction in turgor is accepted as the primary trigger of proline accumulation in plants subjected to conditions of drought and salinity. The loss of turgor activates a complex sequence of adaptive events correlated to the level of stress, plant tolerances, and plant growth.

PROLINE ACCUMULATION AS AN OSMOREGULATORY RESPONSE

The accumulation of proline seems to be associated with adaptation to temperature stress. Free proline concentrations increased both under high [22] or low temperatures [23,24], and proline could serve as a stress indicator in plants exposed to these unfavorable growth conditions. Heavy metals or herbicides are other environmental factors that induce proline accumulation in plants. *Vicia faba* plants grown in hydrocarbon-polluted soils accumulated high levels of proline [25]. High-Pb concentrations reduced sunflower plants' biomass but increased the concentration of free proline [26]. The leaf proline content also increased in pea plants [27] or *Vigna radiata* [28] grown in nutrient solutions containing high-Cd concentrations. Chlormequat given to soybean plants increased the leaf proline content [29]. The foliar application of paraquat and diquat to *Parthenium hysterophorus* resulted in a reduction of the proline level 48 h after application followed by an increase 72 h after spraying [30]. The foliar application of fomesan, imazaquin, metobromuron, and metolachlor to soybean plants resulted in reduced levels of proline and some other amino acids, probably because of a disturbed protein synthesis [31]. Chlorsulfuron, norflurazon, and tri-allate increased the proline content in pea and *Vicia faba* [32]. Increased levels of proline were also found in barley plants infested with aphids because of low water potentials [33]. It is suggested that the changes in proline could be considered as a protective/adaptive mechanism against expected injuries resulting from pollution or herbicides.

Proline accumulation in dehydrated plant tissues was first noted in 1954 by Kemble and MacPherson [34]. Further experiments showed that the de novo synthesis of proline was involved, as the amounts were greater than those released by proteolysis. Proline accumulation was observed in many species as a result of exposure to water or salt stresses. For example, withholding irrigation or irrigation with saline water increased the proline content in tomato leaves (Table 1). Several

TABLE 2 Proline Accumulation in Sugar Beet Disks as a Function of Exposure Time to Stress[a]

Osmotic potential (MPa)	Proline content ($\mu M\ g^{-1}$ fresh weight) after stress application (h)			
	1	2	6	18
−0.2	0.32	0.34	0.37	0.48
−0.4	0.34	0.32	0.41	0.89
−0.8	0.35	0.27	0.41	1.02
−1.2	0.30	0.33	0.43	1.93
−1.6	0.30	0.35	0.49	4.15

[a] Disks were floated on solutions of different osmotic potentials for several hours and then the proline content was determined. The osmotic potential of the solution was lowered by the addition of PEG (polyethylene glycol) 6000.

reports correlate this phenomenon with stress resistance indicating that a better performance and survival can be expected in species that accumulate proline. Much evidence is available to corroborate the proline action as a compatible solute regulating and reducing water losses from dehydrated cells. The increase in the proline content during water stress is inversely proportional to the initial proline concentrations in plant organs [35]. It was found that the generative parts of bean plants contained considerably lower concentrations of proline than the vegetative parts after withholding water. The accumulation of proline during tissue dehydration is time dependent (Table 2). Diurnal changes in the proline content were also reported [36]. The accumulated proline is lost rapidly as a result of recovery from water stress but not as an immediate response to salt removal from the media.

All of the points just mentioned are addressed in detail in the following sections.

Osmotic Adaptation of Bacteria and Algae

Generally, microbes respond to water potentials by accumulating intracellular compounds that are compatible with the cellular metabolic functions. There is a natural ranking or differing osmolyte preferences among species of bacteria [37]. *Azotobacter chroococcum* accumulates trehalose and glutamate, *Azospirillum brasilense* accumulates proline and glutamate, and *Klebsiella pneumoniae* accumulates proline and trehalose [38]. It was observed that osmotically stressed cells generally favor the shift from glutamate to trehalose or proline as the culture ages or as salt levels increase, because they provide better protection for long-term adaptation to the new growth conditions. The mechanism of osmotic adaptation in these organisms can be attributed to an enhanced osmolyte uptake from the medium or to an increased net osmolyte biosynthesis. Partial alleviation of salt stress was obtained by adding low concentrations of proline or betaine to the growth medium of *Thiobacillus ferroxidans* [39]. Similar results were obtained for *Rhizobium* species [40]. In response to increased salt concentrations, algae also synthesize osmoregulatory solutes to counterbalance the low water potential of the growth media. Proline plays a key role in the osmoregulatory mechanism of *Chlorella autotrophica* (a marine microalga) when exposed to high salinities [41]. In this alga, proline accumulation begins immediately after an osmotic shock without a lag phase. This alga is not dependent on protein synthesis and requires light, because photosynthesis supplies the required energy for proline biosynthesis.

Accumulation of Proline in Callus Cultures and Isolated Cells

Callus cultures of rice, adapted to grow under increasing levels of NaCl, accumulated considerable amounts of free proline compared with unadapted cells [42]. This trait was not lost when the cells were transferred through 10 passages in the absence of selection pressure and regrown on salt.

On the other hand, mature embryo-derived calli from rice cultivars differing in their salt tolerance also accumulated proline when exposed to NaCl, KCl, or mannitol [43]. This accumulation did not depend on the nature of the stressing agent or the stress intensity and did not appear to be involved in osmotic adjustment; therefore, it was considered to be a symptom of injury in the stressed rice calli and not an indicator of resistance. In an embryogenic callus culture of lemon, selected for resistance to salinity, the proline concentration significantly increased as compared with control cells [44]. The transfer of salt-tolerant cell cultures of alfalfa to NaCl-containing medium resulted in a 10-fold increase in proline concentrations [45]. In *Brassica napus*, both unselected and tolerant calli responded to water stress by osmotic adjustment and proline accumulation [46]. Increases in proline concentrations were approximately linear in tolerant calli, reaching a maximum of 175 dry weight and 520 μM in unselected calli. This accumulation was correlated with growth inhibition and negatively correlated with the culture age for tolerant calli. Callus cultures of *Medicago sativa* accumulated proline in response to NaCl [47]. This accumulation was enhanced by calcium and was positively correlated with salt tolerance. The salt tolerance of sugar beet calli was also accompanied by a significant increase in the proline concentration under conditions of high salinity [48]. The proline content in a callus culture of pearl millet grown in 1% NaCl increased more than 20-fold compared with nonsalinized controls [49]. Exposure of tobacco callus cultures to osmotic shock greatly enhanced the proline accumulation in proportion to the amount of absorbed sorbitol [50]. NaCl-resistant cell lines of tobacco increased the proline content within 5–10 h after transplantation to a selective medium. In the wild strain, the proline content remained unchanged over a 24-h period [51,52]. A correlation between the viability of cells in a saline environment and the proline content was observed [52]. The intracellular proline concentration was positively correlated with the osmotic potential indicating that proline was a component of the osmotic adjustment of the cells [53]. Glutamate was the main source of the newly produced proline, and the relative contribution of the catabolic pathway was small. Cell lines selected for resistance to salt stress responded to water stress by accumulating markedly more proline than the wild type [52]. This response was stable through at least eight generations and was fully reversible. Similar results were obtained with cultured cells of a salt marsh grass (*Distichlis spicata* L.) [54]. Most of the accumulated proline effective in osmoregulation was found in the cytoplasm. Therefore, it plays a minor role in the osmotic adjustment of the vacuole. Cells maintained a cytoplasmic proline concentration at least one order of magnitude greater than that of the vacuole. Proline accumulation was inhibited by cycloheximide but not by actinomycin D. This indicates that mRNA translation, not mRNA transcription, is required before proline production. Reports are also available on a preferential accumulation of proline in nontolerant cells, as opposed to tolerant cells, indicating a dependence on a salinity threshold [55].

Cultured cells of sorghum exposed to water stress by the addition of polyethylene glycol (PEG) to the growing media increased the proline content significantly [56]. The magnitude of this increase was not correlated with the drought tolerance of the individual varieties ruling out the role of proline as an osmoprotectant in sorghum, as well as in other cereal crops.

Proline Levels in Germinating Seeds

Most of research on proline as an osmoregulatory compound has been carried out on the vegetative parts of the plants. Little attention has been paid to the reproductive organs, especially seeds. Recently, information was released on the existence of osmotic adjustment of seeds under stress conditions. Salt stress increased proline accumulation in the cotyledons and roots of germinating ground-

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nut seeds [57]. Proline accumulated in the endosperm and radicles of germinating barley seeds with increasing NaCl concentrations in the growing media [58]. This proline probably originated from the degradation of stored protein in the endosperm. Under increasing levels of salinity, germinating seeds from salt-tolerant cultivars of rice contained higher levels of free amino acids than salt-sensitive cultivars [59]. In contrast, irrigation of Cajeme wheat with saline water resulted in a continuous decrease in free proline in the grains [60]. This decrease was associated with increased protein concentrations suggesting either that the rate of protein incorporation was accelerated under salinity or that free proline accumulation was stunted in other parts of the plant.

Osmotic Adjustment in Halophytes

Halophytes are plants capable of growing and reproducing in highly saline environments. These plants usually absorb large amounts of NaCl, which is believed to be sequestered in the cell vacuoles; otherwise, enzymatic activity would be impaired. Proline, known to accumulate in the cytoplasm to balance the osmotic potential of the accumulated salt in the vacuole, does not play a role in the osmotic regulation of halophytes. For example, in halophytic Chenopodiaceae, such as *Suaeda monoica*, *Atriplex spongiosa*, and *Arthrocnemum fructicosum*, proline accumulation was observed only at high inhibitory salinities, not at low salinities, promoting normal growth [61,62]. Similar results were obtained with the halophyte *Mesembryanthemum crystallinum* grown at 400 mM NaCl [63]. Proline accumulation preceded the shift of CAM in these plants but only under light [64]. The results suggested that, in *Mesembryanthemum*, proline has a bifunctional role in the acclimation to high salt stress: an osmoregulatory role in the light and as a substrate for dark respiration to supply energy to the compartmentation of ions into vacuoles in the dark. Proline concentrations in *Spartina* varied with N availability, although the higher accumulation could not alleviate the growth inhibition caused by the high salinity level [65].

Changes in Proline Concentration in Cultivated Crops Induced by Water and Salt Stresses

The short- or long-term exposure of crops to conditions of water or salt stress are very common. Besides diurnal variations in environmental factors, approximately 30% of the agricultural area is affected by drought or salinity. This entails an adaptation of crops to these deleterious conditions to survive. As mentioned previously, one way to achieve this goal is by undergoing osmotic adjustment through a net accumulation of organic compatible solutes, such as proline. Much research has been devoted to this issue, and many reports on proline accumulation in plants under stress conditions are available. In this section, the most recent knowledge about the response of cultivated crops to stress, with respect to proline accumulation, is presented.

Wheat

Progressive water stress imposed on wheat resulted in proline and glycinebetaine accumulation [66]. This was the case for water stress encountered in the field or in laboratory experiments, particularly when developing gradually rather than all at once [66,67]. The accumulated proline was completely metabolized on rewatering. As far as salt stress is concerned, free proline accumulation was related to the salt tolerance of the wheat cultivar as well as to the nature of the salinity imposed [68]. Thus, the free proline content in *Triticum aestivum* decreased with NaCl salinity but increased with $CaCl_2$ and $MgCl_2$, salinities [69,70]. On the other hand, other studies reported an increase in the proline content following increased levels of NaCl [71]. Cultivar Kharchia-65, considered a salt-tolerant genotype, showed a higher level of proline than other cultivars at all growth stages except the mid grain filling stage [72,73]. Irrespective of the kind and concentration of salts, the free proline accumulation in wheat leaves increased with the plant age and occurred during the day, peaking toward evening [69].

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Barley

The exposure of barley seedlings to mild or moderate concentrations of PEG for extended periods resulted in a proline increase in all tissues proportional to a reduction in water and osmotic potential [74,75]. Nevertheless, the findings showed that this increase was not the reason but the consequence of osmotic adjustment. As for wheat, when water stress was relieved, the proline concentration decreased [76]. On the other hand, the proline concentration in salt-shocked barley leaves remained high even after salt stress was relieved. As a result of salt stress, proline accumulated only when a threshold value was reached, and its concentration was directly proportional to the sodium concentration. Salinity increased proline synthesis from glutamate and decreased the rate of proline oxidation, a process known to take place in the mitochondria [77]. It was reported that the properties of mitochondria from NaCl-treated barley were modified [78], also contributing to the production of intermediary compounds that could be precursors of solutes such as proline. Correlations between the accumulation of abscisic acid (ABA) and proline were observed in water-stressed barley plants [79]. During water stress, plants lost their turgor inducing ABA production. When ABA levels exceeded a threshold level, they induced proline accumulation. In salt-stressed barley plants, turgor was maintained and therefore proline accumulation was induced without precursory ABA accumulation. Thus, the mechanisms that initiate proline accumulation in barley in response to water and salt stresses are probably different.

Sorghum

A correlation between a growth delay and proline accumulation was observed for sorghum seedlings exposed to salinity [80]. As in other plants, proline did not start to accumulate until a considerable amount of inorganic solutes was already present in the leaf cells, presumably in the vacuolar compartment [81]. The threshold value was 200 $\mu M\ K^+ + Na^+\ g^{-1}$ fresh weight, implying the involvement of proline in the osmotic adjustment of sorghum under severe conditions of stress. Water stress of sorghum plants, imposed by withholding irrigation [82] or by polyethylene glycol [83] induced proline accumulation in the leaves. Plant relief from water stress decreased its levels, although proline remained relatively high when compared with nonstressed controls.

Rice

The free proline content increased in 5-day-old rice seedlings subjected to salt treatments [84]. This was an increase of approximately 400% compared with nontreated controls and was more pronounced in the susceptible than in the resistant cultivar, although young leaves of all cultivars accumulated proline [85]. Similar results were obtained with a rice cultivar grown in sand cultures in the presence of NaCl [86], although in the latter case the salt-tolerant cultivars maintained higher concentrations of protein amino acids.

Thinopyrum, Agropyrum, and Pascopyrum

For *Thinopyrum, Agropyrum*, and *Pascopyrum*, an increase in the glycinebetaine and proline content is a common response to salinity [87]. Proline content was higher in older than in young, expanding leaves. The proline concentration increased in both *Agropyron* and *Pascopyron* as water stress increased [88]. It decreased in *Agropyron* but increased in *Pascopyron* as plant development advanced.

Pearl Millet

The proline content in pearl millet seedlings increased with increasing salt concentrations [89]. This was true for all salts tested: NaCl, KCl, Na_2SO_4, and K_2SO_4. The combination of sodium with chloride and sulfate was more effective in accumulating proline. It is suggested that the increased content of proline under conditions of chloride salinity may be due to an increased water deficit, whereas under conditions of sulfate salinity, plants may undergo complete osmotic adjustment.

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Maize

The free proline levels increased significantly in response to water stress, favoring osmotic adjustment in maize seedlings [90], and mainly in the primary root tips [91]. The exposure of seedlings to NaCl induced a lower accumulation of proline than the exposure to PEG [92]. This is probably because of a decreased necessity to perform osmotic adjustment. It also depends on the way the plants were exposed to salt stress: gradually, leading to salt acclimation, or in one step, leading to a salt shock [93].

Cotton

Cotton plants subjected to water stress by withholding irrigation accumulated free proline to about 100 times the concentration of well-watered controls [94]. A high water potential threshold of about 1.7 MPa was required before proline started to accumulate. Both leaves and roots accumulated proline [95]. Within 48 h after watering, concentrations dropped to the control level. It is possible that proline is capable of functioning as a source of respiratory energy and N during the rewatering phase.

Sunflower

In sunflower, the free proline levels increased in response to water stress, contributing to osmotic adjustment, but their absolute values were very low [96]. Considerable variations were detected among different genotypes. Similar to many other plants, accumulation levels were much lower in NaCl-treated sunflower plants.

Safflower

Salt-tolerant accessions of safflower grown in salinized sand cultures accumulated significantly more proline in their leaves than salt-sensitive accessions [97]. This suggested that the salt tolerance of safflower is associated with a high accumulation of free amino acids in the leaves.

Soybean

The proline accumulation in soybean plants exposed to NaCl concentrations, increasing up to 100 mM, was culture specific [98]. The proline levels were inversely correlated with the tolerability of the salinity stress. Moreover, their accumulation was associated with chlorosis and plant injury and was therefore of no protective value.

Pea

Exposure of pea plants to 120 mM NaCl in the nutrient solution increased the amount of free proline in the roots [99]. The proline content represented approximately 3% of the total content of free amino acids. In pea shoots, potassium mediates proline accumulation [100], since an excess of endogenous K or a low Na/K ratio favor this phenomenon.

Chickpea

Both chloride and sulfate salinities enhanced proline accumulation in chickpea leaves [101]. However, the proline content was higher under conditions of chloride than sulfate salinity. Salinity also favored proline accumulation in pod shells and seeds [102].

Indian Mustard

Salt-tolerant cultivars of Indian mustard accumulated almost 80% more proline than nonsalinized controls, but this increase was only 64% higher in salt-sensitive than in salt-tolerant cultivars [103].

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Perhaps the higher accumulation of proline observed in salt-tolerant plants contributed to a better resistance to severe salt shock by lowering cell osmotic potentials and by maintaining turgor.

Oilseed Rape

Nutrient, salt, and osmotic stresses caused proline accumulation in oilseed rape plants [104]. The highest proline accumulation resulting from nutrient stress was demonstrated in the leaves and stems grown under conditions of Ca deficiency. For example, the proline levels in the stems were 50 times higher then in controls. Potassium, P, and Fe deficiencies caused a much lower proline accumulation. Nitrogen deficiency did not stimulate proline accumulation. The function of proline in nutrient-stressed plants is not clear. It was suggested that proline is capable of preventing protein degradation and enzyme inactivation. Salt stress caused differential enhancement in the proline level in *Brassica juncea* L. seedlings and leaf tissues at different developmental stages [105] as a result of the activities of proline biosynthesis of enzymes. At the same time, the activity of the proline-degrading enzyme, proline oxidase, decreased under salt stress.

Alfalfa

Water stress imposed on alfalfa plants by withholding irrigation stimulated proline accumulation in leaves and nodules [106]. The threshold water potential triggering such an accumulation was higher in nodular tissues, suggesting that, under severe water stress, nodular metabolic enzymes and structural proteins may be protected by this process. The proline accumulation in plant tissues also can be considered a soluble N sink, because it coincided with a decline in soluble protein. Salt application to alfalfa plants yielded only a slight accumulation of proline [107].

Tomato

The proline content increased by 40% in tomato plants growing in salinized nutrient solutions [108]. Short-term exposure to salinity resulted in increased uptake rates of ions and proline production in the salt-tolerant wild type of tomato but not in the salt-sensitive domesticated plants [109]. During long-term salt exposure, both species were able to adjust osmotically and both exhibited decreases in proline levels. A specific effect of nitrate on proline accumulation at high salinities was observed by Heuer and Feigin [110]. Less resistant forms of three tomato species showed a greater increase in the free proline content under saline conditions. Tomato plants subjected to drought by withholding irrigation adjusted osmotically by accumulating reducing sugars and proline (111) indicating a glycophytic response involving a high energy cost.

Sugar Beet

Osmotic and drought-induced stresses of sugar beets resulted in a rapid increase in the proline content while the growth rate and fresh weight decreased [112]. Salt stress also induced proline accumulation, but the fresh weight remained unchanged at the beginning and increased with continuous incubation.

Potatoes

The proline content increased in the leaves and tubers concomitantly of potato plants with a rise in the osmotic potentials [113] indicating a possible role for proline in the plants' adaptation to salinity.

Teff

Salinity induced a 20-fold increase in the leaf proline concentrations in teff, probably because of tissue dehydration [114]. When teff plants were subjected to drought, proline increased sharply only below 28% soil saturation.

Lolium

Acclimation of *Lolium perenne* to drought and low temperatures increased concentrations of proline and amino acids [115].

Arabidopsis

In *Arabidopsis thaliana* plants subjected to low water potentials, the proline accumulation was significant as a result of a de novo synthesis [116]. Proline formed 17–26% of the total amino acid concentration in reproductive tissues but only 1–3% in vegetative ones.

Pistachio

A significant increase in the bulk pistachio leaf proline concentration was reported by Walker et al. [117] inplying its involvement in plant osmotic adjustment under salinity.

Eucalyptus

The proline content in the leaves of *Eucalyptus* plants varied according to the salinity level [118]. It increased under 300 mM saline stress but decreased under 600 mM stress and represented up to 25% of the total amino acids.

Aspen

Drought stress applied to three aspen (*Populus tremuloides*) clones caused both organ-specific and clone-specific changes in the amino acid concentrations, but proline was a minor constituent [119].

Conifers

Pinus sylvestris and *Larix sibirica* grown under drought conditions had a lower protein content but higher proline in their cambial zone or in the assimilating organs and tissues than normally irrigated trees [120]. In conifers, young shoots can be used as test subjects to indicate moisture deficit by the composition of their free amino acids.

Coffee

Water-stressed coffee plants accumulated proline mainly in mature leaves [121]. Its concentration was related to the osmotic potential at zero turgor and to the osmotic adjustment of the plants.

Mulberry

The accumulation of total amino acids and proline was observed in the roots and leaves of mulberry plants exposed to water stress [122]. The activity of the enzymes responsible for proline degradation, proline oxidase, and proline dehydrogenase were inhibited under stress conditions mainly in the roots.

Trigonella

Fenugreek plants (*Trigonella*) grown in nutrient solutions containing NaCl or PEG-4000 increased the proline content in their leaves (123).

Artemisia

No osmotic adjustment was found in *Artemisia*, a drought-tolerant shrub [124]. A small increase in proline was observed with rapid recovery following rewatering. It was concluded that, depending on cell elasticity, the differences in the osmotic potential of nonirrigated shrubs could be attributed entirely to changes in leaf water volumes.

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Jojoba

Free proline was accumulated in high amounts in leaves of NaCl-treated jojoba plants [125]. The proline content increased with an increase in salt concentration in the growth medium, which decreased rapidly after the plant was restored to optimal growth conditions. The level of proline that accumulated in salt-treated jojoba plants was of a magnitude similar to that found in salt-treated halophytes.

Flowers

Azadirachta indica (Neem) and *Melia azedarach* (Persian lilac) irrigated with high concentrations of saline water showed an increase in the moisture and proline contents [126]. Proline also accumulated in poplar cuttings subjected to NaCl or PEG [127]. The most damaged cuttings showed the highest accumulation of proline. The increase in free proline was proportional to the temperature rise. Water- or salt-stress induced proline accumulation of *Dodonaea* [128], probably as a mechanism of drought resistance or salt tolerance. *Melanthera biflora*, a moderately salt-tolerant plant, accumulated proline in response to salt or water stress, which was mainly due to a decrease in the fresh weight/dry weight ratio [129].

Transgenic Plants

Proline production and accumulation has been correlated with tolerance to water and salt stresses. Consequently, the overproduction of proline in plants may lead to an increased tolerance against abiotic stress. To test this hypothesis, genes encoding relevant enzymatic activity were transformed into different species, mainly tobacco. The mothbean Δ^1-pyrroline-5-carboxylate synthetase, a bifunctional enzyme able to catalyze the conversion of glutamate to Δ^1-pyrroline-5-carboxylate, which in turn is reduced to proline, was overexpressed in tobacco [130]. The transgenic plants produced a high level of the enzyme and synthesized 10- to 18-fold more proline than control plants and the osmotic potentials of their leaf sap was less negative under water-stressed conditions. Transformation of the gene *ipt* from *Agrobacterium tumefaciens*, encoding isopentenyl transferase, into tobacco also resulted in increased levels of proline [131].

Varietal Differences in Osmoregulation

Many reports on the variation in genotypic resistance to environmental stresses are available. This response differs between cultivars adapted to certain growth conditions or regions as well as within species more or less tolerant to drought or salinity. One of the physiological mechanisms of tolerance is related to the ability to accumulate proline in plant tissue. Major differences in osmotic adjustment through proline accumulation were found in crop cultivars despite identical water potentials, implying genetic variations. Wheat varieties subjected to either drought or salinity accumulated proline in their leaves, stems, and roots [132]. This process was more obvious in the more sensitive cultivar, which maintained a lower relative water content in its tissues [133]. Compared with other wheat varieties, a higher accumulation of proline was found in the salt-tolerant genotype [72]. A total of 20 barley genotypes differing in their resistance to salinity, as well as chickpea plant types, showed no correlation between tolerance and proline accumulation [134]. In the F_1 generation of barley, the accumulation of free proline was significantly lower than in their parents [135]. The F_1 pearl millet hybrids revealed a significant negative correlation between yield and proline accumulation at a specified salinity level. Varietal differences in concentrations of Na, K, and proline within forage sorghum were not correlated with their differences in salt tolerance [136]. Most of the research carried out with rice varieties subjected to salt stress showed higher levels of proline in tolerant than in susceptible genotypes [137–139], although contradictory results are also available [50,140]. Significant differences in the proline concentration between the root tips of two corn cultivars were observed, but not as a result of salinity or calcium application [141]. No relation between the proline content in the leaves or tubers of potatoes and their relative tolerance or susceptibility to salinity

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was found [86]. Two cultivars of black gram irrigated with saline water significantly increased the proline content in leaves [142]. However, the proline levels in the leaves were too low to play any significant osmoregulatory role in these cultivars. The leaf proline content was negatively correlated with salt tolerance in black gram. A total of 15 species of *Melalenca* tested for proline content after their exposure to stress conditions varied in response [143]. Accordingly, they could be classified into five groups based on the presence of proline analogues in addition to proline. In alfalfa plants, the most tolerant species exhibited the highest potential for proline accumulation in response to salinity [107,144] suggesting its involvement in the osmotic adjustment of salt-stressed alfalfa plants. The F_1 hybrids of wheatgrass (*Thinopyrum*) contained only trace amounts of proline, and this was also beyond a threshold of 200 μM Na + K g^{-1} fresh weight [145]. Proline was detected in the extracts of plants at dusk only under severe conditions of stress. As already mentioned, this threshold was also reported in sorghum and barley. Brassica somaclones, selected in vitro for salt tolerance, contained higher amounts of proline than the nonselected somaclones or the parent genotype [146]. The salt-tolerant somaclones had a stable genetic change for proline overproduction, which is possibly responsible for their survival when subjected to lethal salt levels.

In conclusion, the accumulation of proline in cultivars differing in their tolerance to stress is not universal and covers a wide spectrum of responses. Therefore, it is not always advisable to look for a specific selection criterion based on the proline content for purposes of selection and breeding.

Negative Response

Despite the abundance of reports on proline accumulation in plants growing under drought or salinity, a few others demonstrate a negative response as far as osmoregulation is concerned. For example, the contribution of proline to osmotic adjustment in legumes is of minor importance [147]. No significant accumulation of proline in the leaves of green gram seedlings [148] or maize plants [149] was observed when grown in sodium bicarbonate or sodium carbonate salts. Increased concentrations of chloride or sulfate salts or of PEG could not effectively stimulate proline accumulation in sugar cane leaves of a salt-sensitive variety [150]. Similarly, pigeon pea plants failed to accumulate proline at high-salinity levels [151]. The lack of this adaptive mechanism may explain the failure to develop salt tolerance in cultivated pigeon pea or sugar cane. In a more recent work, however, salt tolerance could be found in wild relatives of pigeon pea belonging to the genera *Atylosia*, *Dunbaria*, and *Rynchosia* but without correlation between salinity tolerance and proline accumulation [152]. In *Andropogon glomeratus*, a C_4 nonhalophytic salt marsh grass, proline played no role in osmotic adjustment, since very high levels of salinity are required to increase its concentrations [153]. One of the adaptive mechanisms suggested as being associated with osmotic adjustment is a restriction in cell expansion [154]. Extensin, a major plant cell wall glycoprotein in dicots, was found to be a hydroxyproline-rich glycoprotein [155]. Therefore, some work was carried out to determine changes in cell wall proteins induced by salt stress and expressed as changes in proline and hydroxyproline concentrations. No significant effect of stress on the proline and hyroxyproline contents was found in a purified cell wall fraction of sunflower [156]. Therefore, changes in the physicochemical properties of the cell wall accompanying osmotic adjustment appear to lie in other posttransitional modifications of extensin. Cell membrane stability of four *Arachis* cultivars exposed to PEG was tested in response to drought tolerance [157]. It was found that proline was not effective in controlling the physiological status of the cell membrane and its stability.

EXOGENOUS PROLINE APPLICATION

One approach to assess the metabolic consequences of proline accumulation in response to stress is to examine its exogenous application to whole organisms or tissues. Exogenously applied proline stimulated the growth of bacteria subjected to osmotic stress [158,159]. Proline has also been applied to higher plants to determine its ability to counteract the inhibitory effects of environmental stress, mainly water, or salt stresses. The addition of 100 mM proline to a Hoagland solution containing

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120 mM NaCl neutralized the effect of salinity on pea plants [99]. Incubation of *Commelina communis* epidermal tissue in proline inhibited stomatal opening [160]. Exogenous proline had no effect on the germination of tomato seeds under water or salt stress [161] or on the ribosome stability in the presence of 250 or 500 mM KCl [163], but it increased pollen germination when exposed to brief temperature stress [162]. The addition of 10 mM proline to cultured barley embryos increased shoot elongation under saline conditions [164]. This effect was attributed to the ability of proline to decrease the leaf salt load. Proline allowed an enhanced K/Na discrimination in transport to the shoots and a better salt exclusion from the shoots with retention in the roots. The callus lines of *Cicer arietinum* grown in a medium containing 100 mM NaCl and 10 mM proline increased their fresh and dry weights [165]. Higher concentrations of proline (50 and 100 mM) inhibited the growth of NaCl-stressed as well as NaCl-nonstressed callus cultures of mung bean [166]. Optimal concentrations of proline increased the cellular levels of K and decreased Na and Cl levels. The presence of 1 or 10 mM proline in media containing 100 or 200 mM NaCl had little effect on the growth of the salt-adapted callus of rice [167]. Some concentrations significantly increased the growth of salt-unadapted callus. Spraying cotton plants grown under conditions of low soil water potential with proline solutions counteracted the effects of stress, especially at moderate and high stresses [168]. Proline (10 mM) inhibited the growth of salt grass suspension cultures in the presence of 260 mM NaCl [169]. Exogenous [^{13}C]proline inhibited the normal biosynthesis of proline that would have occurred in suspensions grown at this salinity level. Plants growing in saline environments usually accumulate large amounts of NaCl in their tissue. Because Na and Cl are inhibitory to a large number of enzymes, their presence in the cytoplasm should be minimal. Evidence of the compartmentation of electrolytes between the cytosol and the vacuole is available. The necessary osmotic balance between the two compartments is achieved through the accumulation of organic solutes in the cytoplasm. Proline is one of these compatible solutes. Besides playing an osmotic role, it should protect enzymes against denaturation or inhibition of activity. This could be determined easily by adding exogenous proline to the assay media or to crude extracts. Contradictory reports for differing plant species are known. For example, full protection of PEP carboxylase against NaCl inhibition was obtained in two Poaceae species with a proline concentration between 200–800 mM, and proline behaved as a competitive inhibitor in Chenopodiaceae [170]. Similar results were obtained from the activities of NAD-malate dehydrogenase, glucose-6-phosphate dehydrogenase, NADP-isocitrate dehydrogenase, and glyceraldehyde phosphate dehydrogenase [171]. Almost full protection of enzyme activities was obtained when proline was added at a molar ratio of 2:1 (protectant to salt) simultaneously with the addition of salt to the reaction media. No protection was found when proline was added after a 1-h preincubation of enzyme extracts with high salt concentrations. Extracts from air-dried leaves recovered almost fully after more than 5 h of preincubation in 1 M proline. Exogenous proline supplied to radish seedlings reduced tissue Hg levels owing to the inhibition of Hg uptake [172].

The method of using exogenous proline proved that mechanism of feedback inhibition of proline synthesis exists in fully turgid plant tissues but not in stressed tissues [12]. At this point, enhanced proline oxidation also cannot be ignored. Moreover, this technique emphasized the role of proline as a compatible solute involved in the process of the osmotic adjustment of living organisms.

PROLINE CONTENT AS AN INDICATOR FOR BREEDING PROGRAMS

The existence in plants of quantitative variations in the physiological trait of proline accumulation in response to water or salt stresses has suggested its possible consideration as a selection criterion for breeding programs. This was indeed recommended for cereals growing in Mediterranean environments [173]. Research performed with 12 paddy genotypes showed a stimulation of proline accumulation in the leaves of plants exposed to salinity [137]. The salinity index of yield showed a significant positive association with proline accumulation, prompting the suggestion of this physio-

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logical trait as one of the promising indices for breeding salt-tolerant genotypes in rice. The magnitude of the proline response also was suggested for screening alfalfa plants for salt tolerance [144]. On the other hand, Ashraf [142] concluded that proline accumulation cannot be used as an indicator for salt tolerance in black gram and is thus unsuitable for breeding programs. The same is true for soybeans [174] and pearl millet [134]. It can be concluded that, in general, proline accumulation is specific to a genotype, and generalization over different varieties of a crop is not always possible.

CONCLUSIONS

The accumulation of proline in plants subjected to water or salt stress has been observed widely, although not universally. Several possible physiological functions have been ascribed to induced proline accumulation by water shortage. These functions include osmoregulation, a soluble N sink, a signal of senescence, and an indicator of plant resistance to stress. Proline may affect the solubility of various proteins, thus protecting them against denaturation under water-stressed conditions. An increase in the proline content may be associated with either enhanced biosynthesis, with stimulated proline oxidation, or an impaired protein synthesis. In general, proline concentrations are directly proportional to the salinity level or to the intensity of water stress. Genotype variations are very common; however, a positive correlation cannot always be found between the proline content and a plant's relative tolerance or susceptibility. Restoring plants to optimal growth conditions results in a rapid decline in the proline content. Additional studies are required to elucidate conclusively the role of proline in plant adaptation to stress.

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Glutathione and Its Central Role in Mitigating Plant Stress

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INTRODUCTION

Glutathione (GSH) is a tripeptide (γ-glutamylcysteinylglycine) composed of glutamate, cysteine, and glycine. Plants, like their animal and microbial counterparts, have evolved to rely on the unique properties of this chemical to protect themselves from a broad range of environmental stresses. The ability of GSH to protect plants from stress is dependent on the two chemical properties of the thiol group of cysteine. The thiol group can be oxidized and thus provides a source of reducing equivalents to buffer the plants from a number of oxidative stresses, including active oxygen species and air pollutants. In addition, the chemical reactivity of the thiol allows glutathione to complex with a range of organic and inorganic chemicals and thus protects plants from their potentially toxic effects. We will consider these two roles separately.

Role of GSH in Protection from Active Oxygen

Many environmental stresses cause plants to produce a family of reactive oxygen species (ROS), including superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxide radical ($^{\cdot}OH$) [1–5]. These reactive molecules are interconvertible and have the ability to react with and damage membranes, proteins, and nucleic acids. These reactive oxygen species can form when plants are exposed to radical-forming air pollutants, including ozone (O_3), peroxyacyl nitrates (PAN), SO_2, some halogenated hydrocarbons, NO, and NO_2. In addition, active oxygen can be generated when environmental stresses cause overreduction of the chloroplast or mitochondrial electron transport chains. Chloroplasts, for example, produce active oxygen under conditions of high illumination and low temperature [1–3]. Chilling conditions also appear to restrict the activity of the cytochrome portion of the mitochondrial electron transport chain and may result in H_2O_2 formation.

Glutathione is involved both directly and indirectly in quenching these free radicals [4–6]. The indirect reaction involves the ascorbate/GSH cycle where ascorbate is the ultimate electron donor for reduction of H_2O_2 to water and GSH is an intermediate electron carrier, as shown in

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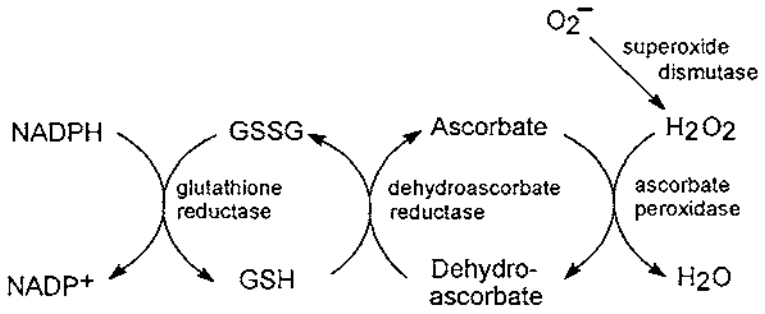

FIGURE 1 The ascorbate/glutathione cycle for quenching reactive oxygen species.

Figure 1 [6,7]. Ascorbate is also important in maintaining tocopherol (vitamin E) in the reduced state and, therefore, links GSH to the dominant free radical scavenger in membranes [8]. Direct reactions between GSH and ozone, PAN, and activated oxygen species have been documented and are implicated in GSH-based protection systems. It should be noted, however, there is substantial controversy about the role of GSH in protection from active oxygen and air pollutants [9], and some authors have even suggested that thiyl radicals produced by a single electron reactions with glutathione ($GS^{\bullet}$ and $GSSG^{\dot{-}}$ are a strong oxidant and reductant, respectively) might be involved in radical propagation and cell damage [10].

Role of GSH in Detoxification of Xenobiotics and Heavy Metals

Plants respond to a range of organic contaminants (herbicides are the best studied) by conjugating either contaminants or metabolites derived from them to GSH [11–13]. These chemicals, usually electrophilic alkylating agents, are then either stored or further metabolized into less toxic forms. The GSH conjugation reaction is catalyzed by one or more members of a family of proteins called glutathione S-transferases (GSTs). GSTs have been identified in a broad range of different plants and each plant probably has 5–10 different forms of the enzyme (isozymes) [14]. Each isoform will apparently conjugate a range of different organic compounds. GSTs are inducible. Exposure of the plant to an organic compound will result in a 3- to 10-fold increase in the level of the GST that conjugates that organic compound. Where investigated, the increase is transcriptionally controlled. A group of agrochemicals, Safeners, include chemicals that are capable of inducing GST isoforms that catalyze the formation of GSH conjugates with specific herbicides [14,15]. Treatment with Safeners, therefore, decreases the sensitivity of the plant to the herbicide (while presumably having little or no effect on weeds). It is assumed that these enzymes evolved to conjugate naturally occurring organics (e.g., hormones, cinnamic acid, anthocyanins) and thereby regulate their physiological effects or transport to the vacuole [11].

Perhaps one of the most fascinating aspects of the protection GSH affords plants is its ability to confer moderate levels of resistance to heavy metals, particularly cadmium and copper. Many heavy metals are toxic to plants. As a result, plants have a number of mechanisms for reducing heavy metal toxicity. These include limiting metal uptake, increasing metal efflux, and sequestering metals within the plants (usually in the vacuole) so that their interaction with enzymes is minimized [16]. These mechanisms are essential for plants to survive on land containing elevated levels of toxic metals. More recently, the ability of plants to concentrate some toxic metals has received renewed attention with the realization that it might be possible to use plants to remove metals from polluted soil. This process has been dubbed phytoremediation. The plants with the highest capacity

for bioaccumulation of metals are the hyperaccumulators. *Thlaspi caerulescens* has received the most study [17].

Metal sequestering within plant cells is usually carried out by organic or amino acids in the vacuole or by one of two groups of metal-binding, cysteine-rich polypeptides, metallothioneins and phytochelatins. Plants clearly contain metallothioneins [18–20] and overexpression of mammalian metallothionein genes in plants does confer some level of resistance to heavy metals [21]. Although the role of metallothionein in metal resistance in plants is still being debated, most scientists seem convinced that phytochelatins are important in conferring some level of metal (particularly cadmium and copper) resistance in plants [22,23].

Glutathione acts as a precursor for the synthesis of phytochelatins. Phytochelatins are composed of two or more repeating γ-glutamylcysteine units with a terminal glycine residue $(\gamma\text{-glu-cys})_n$ gly [22,23]. The structures of GSH and phytochelatins are shown in Figure 2. Plants synthesize phytochelatins in response to heavy metal (particularly cadmium) exposure (the mechanism of phytochelatin synthesis and the control of its synthesis is discussed later). Phytochelatins bind cadmium with a ratio of 1 Cd^{2+} per 2 SH groups [24,25]. The cadmium-phytochelatin complex is deposited within the vacuole. Work in Ow's laboratory has identified the phytochelatin transporter in the fission yeast *Schizosaccharomyces pombe* [26,27]. This transporter either works by itself to transport the cadmium-phytochelatin complex across the tonoplast or it transports the phytochelatin while a separate Cd^{2+}/H^+ counterport loads the vacuole with cadmium [26–28]. The complex would then form spontaneously within the vacuole. Experimental evidence seems to support the cytosolic formation of the cadmium-phytochelatin complex followed by its transport intact across the vacuole (Fig. 3). In rich medium or high cadmium concentrations, a larger molecular weight complex is formed that contains cadmium, phytochelatin, and sulfide. This complex has a Cd^{2+} thiol ratio of about 1:1 [24,29,30].

The role of phytochelatins in providing a level of resistance to cadmium was demonstrated initially with *S. pombe* and more recently with *Arabidopsis*. Hayashi's group [31] selected *S. pombe* mutants that were unable to grow in the presence of 0.5 mM Cd^{2+} (wild-type yeast grow normally at this Cd^{2+} concentration). Analysis of these mutants showed that they were blocked in the synthesis of GSH and phytochelatins. Howden et al. [32,33] have done the same experiments with *Arabidopsis*. Mutant plants were selected that were unable to grow in the presence of 0.09 mM $CdSO_4$. One set of plants, the *cad1* mutants, were missing the enzyme responsible for phytochelatin synthesis from GSH and the second group of plants, the *cad2* mutants, were deficient in one of the enzymes essential for GSH biosynthesis, γ-glutamylcysteine synthetase. Although a good deal of circumstantial evidence supports the role of phytochelatins in cadmium resistance in plants and fission yeast, the sensitivity of single-site mutants that are deficient in phytochelatins is the definitive evidence that these γ-glutamyl peptides are important in providing some level of resistance to cadmium. Chen and Goldsbrough [34] have provided information that strongly suggests that not only are mutants that are unable to make phytochelatins Cd^{2+} sensitive, but that overproduction of glutathione can create plants that are exceptionally Cd^{2+} tolerant. These investigators selected tomato tissue culture lines that could grow in elevated cadmium. Biochemical analysis revealed that the cadmium-tolerant lines had elevated γ-glutamylcysteine synthetase activity. This indicates that higher GSH and phytochelatin synthesis can result in increased cadmium resistance.

SYNTHESIS OF GSH AND PHYTOCHELATIONS

Mechanism of GSH and Phytochelatin Biosynthesis

Glutathione is synthesized by a two-step reaction beginning with the common amino acids, glutamate, cysteine, and glycine, and two moles of ATP (see Fig. 2). The first reaction is the formation of an amide bond between the γ-carboxyl group of glutamate and the α-amino group of cysteine to form γ-glutamylcysteine. This reaction requires Mg-ATP and is catalyzed by the enzyme γ-glutamylcysteine synthetase [35]. The cDNA for γ-glutamylcysteine synthetase (*gsh1*) was recently

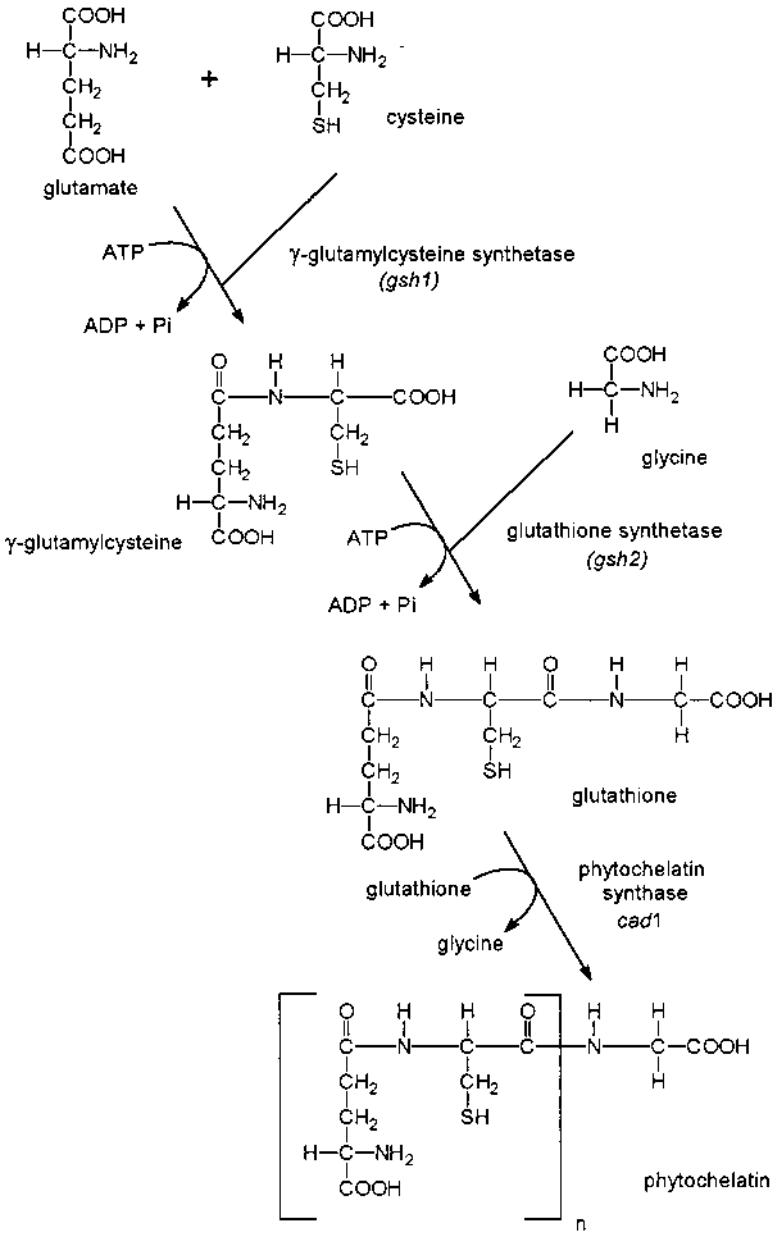

FIGURE 2 The biosynthetic pathway for glutathione and phytochelatins.

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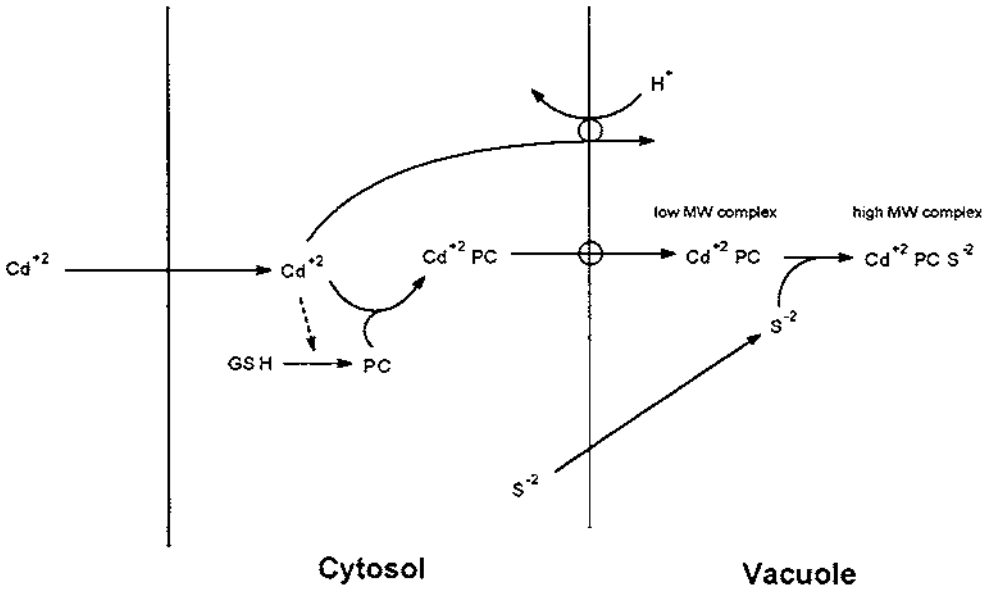

FIGURE 3 A model for Cd^{2+} uptake and sequestration in plant cells. Cadmium enters the cell and triggers phytochelatin (PC) synthesis. The phytochelatin binds the Cd^{2+} and this complex is transported into the vacuole. Additional Cd^{2+} enters the vacuole by a proton antiport. Sulfide and cadmium addition causes formation of a high molecular weight complex.

cloned by May and Leaver [36]. Glutathione is then synthesized by the formation of a second amide bond between the α-carboxyl of the cysteine residue and the α-amino group of glycine. This reaction also requires Mg-ATP and is catalyzed by the enzyme GSH synthetase. The cDNA [37,38] and gene [38] for this enzyme has recently been cloned from *Arabidopsis*. The *Arabidopsis gsh2* gene is capable of complementing *S. pombe* mutants that are cadmium sensitive owing to their inability to produce GSH synthetase [38]. The protein is also expressed in *Escherichia coli* and this system has been used to identify an essential flexible loop in the plant protein [39] as well as to clarify the role of the N-terminus region of the protein from higher eukaryotes [40] The GSH reductase (see Fig. 1) is needed to maintain GSH in the reduced form. Foyer et al. [41] have presented data suggesting that GSH is much more stable than GSSG and have presented preliminary data showing the effects of expressing bacterial *gsh1* and *gsh2* in poplar [41,42].

Phytochelatins are synthesized by the condensation of multiple GSH molecules to form a molecule with repeating γ-glutamylcysteine residues with a terminal glycine (see Fig. 2). The general structure is $(\gamma\text{-glu-cys})_n$ gly or $(\gamma\text{-EC})_nG$ and in plants n ranges from 2 to 8 with 3 to 5 being the most common. The enzyme that catalyzed this condensation is called γ-glutamylcysteine dipeptidyl transpeptidase or, more commonly, phytochelatin synthase [43,44]. The phytochelatins grow by the sequential addition of GSH molecules, $(\gamma\text{-EC})_nG + GSH \rightarrow (\gamma EC)_{n+1}G + G$.

The synthesis of phytochelatins is controlled posttranslationally. The activity of the enzyme is strictly dependent on the presence of heavy metals [43] with a preference order of Cd > Ag > Bi > Pb > Zn > Cu > Hg > Au (Al, Ca, Fe, Mg, Mn, Na, and K had no effect). Incubation of the enzyme with GSH causes no reaction. On addition of Cd^{2+}, the transpeptidase condenses two glutathione molecules to form $(\gamma\text{-EC})_2G$ followed by the sequential addition of GSH residues to form in sequence $(\gamma\text{-EC})_3G$ and $(\gamma\text{-EC})_4G$. As the phytochelatins accumulated in the reaction vessel,

they chelated the metal and the enzyme became inactive. The addition of more metal turned the phytochelatin synthase back on and phytochelatins continued to accumulate. The first level of control of phytochelatin synthesis is the direct activation of the enzyme phytochelatin synthase by heavy metals.

Control of GSH Synthesis

GSH synthesis is primarily controlled by feedback inhibition of γ-glutamylcysteine synthetase by glutathione [45]. Thus, the rate of GSH consumption controls its rate of formation. In addition, longer term controls are present where the plants can increase their overall capacity to produce GSH.

Exposing plants to cadmium causes a rapid drop in GSH levels. Several hours later, however, the plants develop an increased capacity for glutathione synthesis and the steady-state GSH levels return to near normal. Table 1 shows that when *Arabidopsis* plants are exposed to 50 μM Cd^{2+}, there is a rapid conversion of GSH to phytochelatins. Following 2 hs of Cd^{+2} exposure, there is a decrease in the GSH pool and an increase in the level of phytochelatins. After 24 hs of Cd^{2+} exposure, the level of GSH has returned to about 75% of its level in control tissues. Meanwhile, phytochelatin synthesis has continued at a high rate. After a 24-h exposure to cadmium, the total thiol pool (GSH and phytochelatins) increased about sevenfold in this experiment. Scheller et al. [46] have used their tomato suspension culture system to present more detailed kinetic analysis showing very similar results. The only way to explain the data in Table 1 and those of Scheller et al. [46] is that the rate of GSH synthesis is elevated, thus increasing the GSH pool at the same time as the rate of its conversion to phytochelatins is accelerated. Two mechanisms are possible. The enzyme γ-glutamylcysteine synthetase is inhibited by GSH in vitro. If this also is true in vivo, then the drop in the glutathione level that occurs with the activation of phytochelatin synthase would be expected to increase the activity level of γ-glutamylcysteine synthetase. This effect, however, should be fairly rapid and was insufficient to prevent the drop in the GSH levels during the first 2 hrs of Cd^{2+} exposure (see Table 1). The observation that the GSH levels do return within a day suggests that some additional control mechanisms are involved, probably at the level of the expression of the genes that control GSH biosynthesis.

Some published data provide support for the view that cadmium treatment increases the level of activity of the proteins for GSH biosynthesis and that this increase is controlled at the genetic level. Ruegsegger et al. [47] reported that Cd^{2+} treatment increased the level of GSH synthetase activity measured in vitro by three-to four-fold in peas. Schlunz (reported in Ref. 48) also has demonstrated a time-dependent increase in glutathione synthetase activity in vitro in peas following exposure to cadmium. Schneider (reported in Ref. 48) calculated that the rate of GSH synthetase

Table 1 Effect of 50 μM Cadmium on Glutathione and Phytochelatin Levels in Arabidopsis Plants Grown in Liquid Culture

Cd^{2+} Exposure (hs)	GSH Concentration (μmol/g)	Phytochelatins (μmol/g)
0	0.86	0.15
2 hr	0.05	1.40
24 hr	0.75	5.42

Arabidopsis seedlings in liquid shaker cultures were exposed to 50 μM $CdSO_4$ for 24 h before the entire seedlings were harvested and washed and GSH and phytochelatin levels determined.

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activity increased 8-fold and that γ-glutamylcysteine synthetase increased 10-fold when tobacco suspension culture cells were exposed to cadmium. Clearly Cd^{2+} increases the in vitro activity and probably the level of the proteins of GSH synthesis.

Other researchers have shown that the enzymes of GSH synthesis are also regulated by oxidative stress. Work by Smith [49] and Smith et al. [50] and May and Leaver [51] have shown that H_2O_2 can induce GSH synthesis. Smith's work showed that catalase inhibitors (or plants with mutations in the catalase gene) result in increased photorespiratory H_2O_2 concentrations. The elevated H_2O_2 concentrations result in increased GSH levels. In a catalase-deficient barley clone [49], the GSH level increased 5- to 10-fold when the plants were transferred to photorespiratory conditions. May and Leaver [51] inhibited catalase activity in an *Arabidopsis* suspension culture with aminothiazole. The increased H_2O_2 concentration caused a four-fold increase in the GSH level. The elevation in GSH protected the cells from oxidative damage caused by H_2O_2. Inhibition of GSH synthesis with buthionine sulfoximine (BSO, an inhibitor of γ-glutamylcysteine synthetase) allows the H_2O_2 concentration to rise and oxidative damage to the cell results. These observations [49,50,51] suggest that H_2O_2 induces GSH synthesis and that GSH protects the cells from oxidative damage.

Given this indirect evidence that the capacity for GSH synthesis was regulated in plants, we decided to address the possibility that exposure to metals would result in the increased expression of the genes needed for GSH biosynthesis. *Arabidopsis* seedlings grown in liquid culture were exposed to either 100 μM cadmium for up to 4 days or to varying concentrations of cadmium for 1 day. mRNA was isolated from the plants and probed with the cDNA clones for *gsh1* (γ-glutamylcysteine synthetase), *gsh2* (GSH synthetase), and *gr1* (cytosolic isoform of glutathione reductase). As shown in Figure 4, a 12-h exposure to 100 μM cadmium causes a substantial increase in the level of mRNA for all of the genes necessary for glutathione biosynthesis. These high mRNA levels were maintained for 96 h. The system is sensitive to low-cadmium concentrations with 1 μM Cd^{2+} causing a measurable increase in mRNA level. The amount of mRNA increased to about 100 μM cadmium. At 1000 μM Cd^{2+}, the level of mRNA dropped below the detection limit of the blot. This may represent a specific response to high-metal levels or a general toxic effect of the metal. Copper is the only other monovalent or divalent cation that causes similar increases. Run-on transcription experiments have demonstrated that this increase in the mRNA level results from an increase in the transcription rate. Clearly, the plants responded to cadmium (as well as copper) exposure by increasing the expression of the genes necessary for elevated rates of GSH synthesis.

It is interesting to speculate how plants perceive the exposure to heavy metals and what the signal transduction pathway is between cadmium (and possibly other environmental stresses) and the induction of the genes for GSH synthesis. A very generalized model is shown in Figure 5. Metal

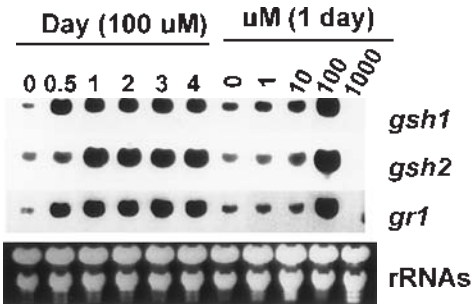

FIGURE 4 The effect of Cd^{2+} treatment on the level of mRNA for *gsh1*, *gsh2*, and *gr1*. *Arabidopsis* seedlings were grown in liquid shaker cultures and exposed to the indicated concentrations of $CdCl_2$ for the times shown. The seedlings were harvested, washed, and total mRNA extracted and analyzed by Northern blotting.

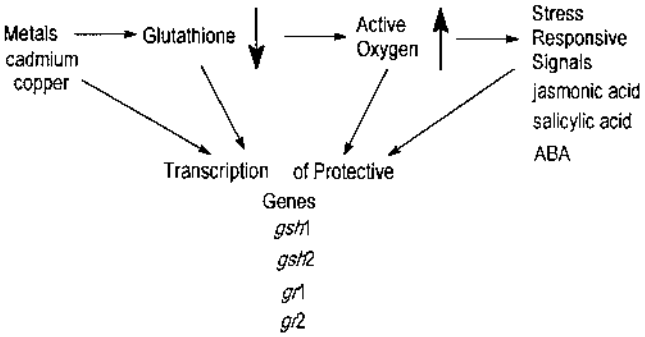

FIGURE 5 A model for the signal transduction pathway by which plants respond to cadmium and copper. Metal uptake causes a decrease in GSH levels which may result in an increase in reactive oxygen species and triggers the synthesis of signal molecules. One or more of these factors may trigger transcription of the genes for GSH synthesis.

treatments cause a decrease in the steady-state levels of GSH. This decrease in GSH causes an increase in the level of activated oxygen species as demonstrated in the case of H_2O_2 by May and Leaver [51]. Increased oxidative stress has been shown to elicit the synthesis of a number of stress-responsive chemicals, including jasmonic acid [52], salicylic acid, and abscisic acid. It is possible that one of these latter chemicals causes the activation of the genes needed for GSH biosynthesis. It could also be that the increase in active oxygen species or the decrease in glutathione induces these genes. It is even possible that the metals themselves induce gene expression directly in a manner that is directly analogous to the case with the metallothionein gene from bacteria [53] and mammals [54]. Given the central role of glutathione in mitigating a rnage of different environmental stresses, it will be essential to determine if these other stresses also alter GSH synthesis and how these responses are intergrated with those induced by metal exposure.

CONCLUSIONS

Glutathione seems to occupy a central role in protecting plants from a broad range of environmental stresses. The control of GSH biosynthesis is manifested in several steps. γ-Glutamylcysteine synthetase is feedback regulated by the concentration of GSH and works to maintain a steady-state level of this chemical. In addition, the expression of all genes essential in glutathione biosynthesis is regulated at the transcriptional level. The mechanisms by which environmental stresses control expression of these genes is unknown and the signal transduction pathway needs to be resolved.

ACKNOWLEDGMENTS

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Plant Hormones and Stress Phenomena

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INTRODUCTION

Theophrastus is quoted to have said, ''For growth and nourishment, the climate is one of the most important factors.'' There is an apt proverbial saying that it is the year which bears and not the field. Plants growing in nature and crops in an agricultural system complete their life cycle in environments that are, to a considerable degree, unfavorable for expressing their genetic potential for reproduction. For completing various stages of development, that is, from seed germination to seed production, plants/crops experience a constantly fluctuating environment. Physical and chemical systems respond in accordance with the pressure exerted on them, but the fundamental characteristics of the living system is to resist change, and thus they are self-regulating. Therefore, for their survival the sessile plants/crops, must be highly responsive and self-regulating to changes in their physicochemical environment. There is, however, flexibility in this regulation, which appears to be governed by Shelford's *law of tolerance* [1]. According to this law, as long as the increase in the factor enhances response, it is considered to be *deficient*, and when no further response is elicited, it is optimum. When the response starts declining, it is *inhibitory* or at the toxic level. It is thus obvious that plants are stressed at the levels of both *deficiency* and *inhibitory/toxicity*. But plants/crops seldom experience variation in a single environmental factor, and covariation of and interactions between them is a norm rather than an exception. Thus, plants/crops growing under limiting (biotic or abiotic) environments are ''stressed.'' Stress can therefore be defined as any change in the environment that decreases plant growth and reproduction below the genotype's potential until productivity becomes uneconomical and ultimately ceases. Thus, the term *stress* is measurable and meaningful to agriculture.

The estimation and impact of environmental stress on crop productivity varies with the source. It has been suggested that only 10% of the world's arable land may be classified as nonstressed, and about 20% is under mineral stress, 26% is drought stress, and 15% is freezing stress [2]. On the other hand, Boyer [3] has estimated that environmental stress limited the productivity of U.S. agriculture to 25% of the potential.

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Any environmental perturbance, regardless of its nature, that influences water and carbon balance unfavorably affects productivity. It is now generally accepted that drought, salinity, and temperature extremes disturb the water balance of plants. Besides water stress caused by salt acting as an osmoticum, a physical effect, salinity stress also causes ion toxicity by excess amounts of ion, particularly Na^+ and Cl^-, and in obtaining the required ions despite the predominance of other ions in the external media, a chemical effect. This classification is, however, artificial, because all the processes interact with each other. It has been suggested that in many plant/crop species, water stress causes an initial decrease in growth, whereas ion effects are responsible for a further reduction in plant growth. Therefore, plants exposed to saline environments need to adjust to three basic problems.

Most of the water in the hydrosphere is salty and much of the fresh water is frozen. Todd [4] estimated that oceans contain 97% of the planet's water, continents about 2.8%, and the atmosphere about 0.001%. About 77% of the water associated with land is found in ice caps and glaciers and about 22% is found in ground waters, much of which is uneconomical to retrieve. This leaves only a small percentage of readily manageable fresh water as a resource of the water supply.

Plants take up water from the soil to maintain cell turgidity and fix CO_2 from the atmosphere to provide, for example, food, fuel, fiber, drugs, and forest products, to humankind and animals. Stomates, representing a unique adaptation of terrestrial plants, play a key role in coupling leaf gas exchange to water availability. Plants transpire 100–300 times more water during the assimilation of CO_2 than is required for their growth and the production of a usable yield. It has been estimated that 600 kg of water is transpired to produce 1 kg of dry maize, and to produce 1 kg dry biomass, 225 kg of water is transpired [5]. It was further reported that to produce 1 kg of sucrose, sugar beet plants transpire 465 kg of water, and to produce 1 kg dried biomass, they transpire 230 kg of water [6]. Therefore, depending on the photosynthetic pathway, for every gram of C fixed, 250–600 g of water is lost through transpiration. Thus, a small fraction of 1% of the water that moved through the plant from the soil to the atmosphere became the part of the biomass. Therefore, optimum productivity is mainly dependent on a favorable balance between water and carbon.

The world's land surface occupies about 13.2×10^9 ha, of which 7×10^9 ha is arable, only 1.5×10^9 ha of which is cultivated [7]. Of the cultivated lands, about 0.34×10^9 ha (23%) is saline and another 0.56×10^9 ha (37%) is sodic. Salt-affected soils are not limited to semiarid and arid regions and cover nearly 10% of the total land surface in around 100 countries of the continents and subcontinents [8]. A salinity problem develops in a number of ways: seawater intrusion, saline irrigation and drainage waters, saline ground waters, brines from natural salt deposits or geological formations, brines from gas and oil fields, and saline and sodic soils. As elaborated by Tanji [9], the primary source of salts in waters and soils is the chemical weathering of earth materials; that is, minerals that are constituents of rocks and soils. Evaporative salinization, i.e., surface of evaporation of water and transpiration by plants, and dilution, e.g., snow melt waters, irrigation waters, rainfall, influence the concentration of dissolved mineral salts. Mineral solubility principally regulates the extent to which salts dissolve or accumulate.

Plants do modify their development in response to the above- or underground environmental stimuli, including gravity, light, water, salts, and pathogens. The modifications in their development frequently take the form of changes in the direction of growth. For example, as the salinity stress increases, it approaches a *threshold* above which the productivity starts declining until it becomes toxic (Fig. 1) [10]. In order to cope with the salinity stress, plants have devised strategies and they have accordingly been classified as *halophytes/mangroves* and *glycophytes*. Many halophytes are Na^+ includers, but they effectively keep this ion away from the growing cells. On the other hand, glycophytes (many of our agricultural crops) are Na^+ excluders (or K^+ includers), but variations do occur: *Spergularia marina* is a Na^+ includer at low salt levels and a Na^+ excluder at high salt levels[11]. Thus, the distinction between halophytes and glycophytes is relative one.

Being sessile, plants/crops respond to a stressful environment by a number of morphological and physiological changes which include the reduced growth of leaves, stems, and hairy roots;

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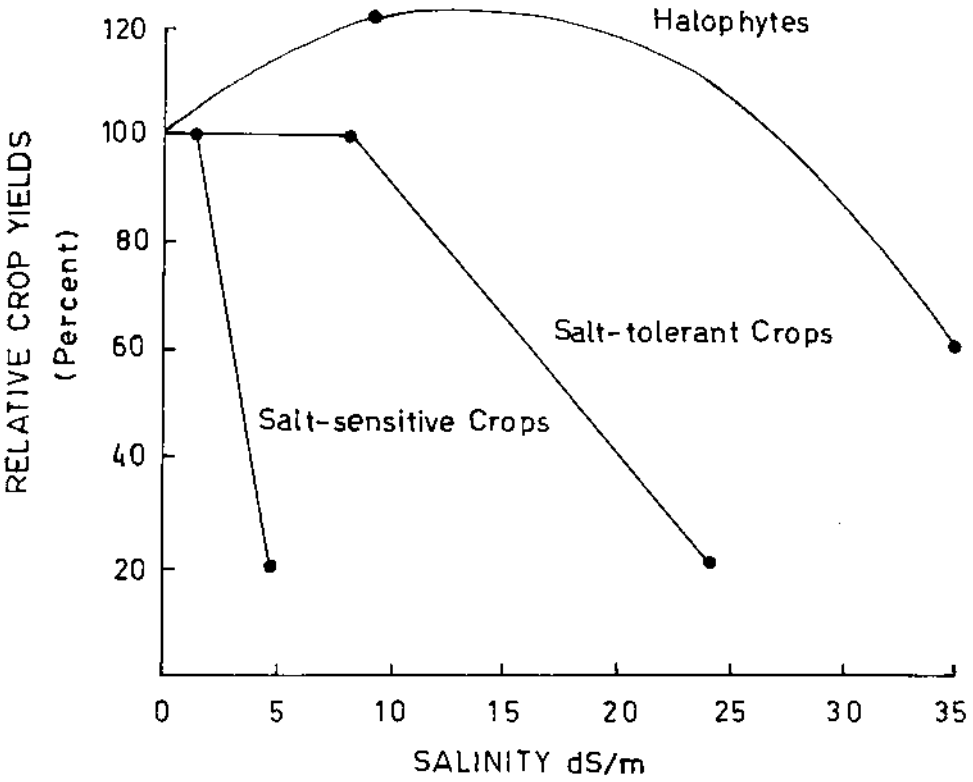

FIGURE 1 Growth response of plants and crops to salinity stress. (Reproduced from Ref. 10.)

stomatal closure; a reduction in the rate of photosynthesis; hormonal disbalance; and a low capacity for nutrient uptake [12]. Two novel approaches were made to break the link between soil drying and reduced water uptake. As the soil dried, Passioura [13] used a pressure vessel around the roots of wheat and increased the pressure to balance the increase in soil suction. Under these conditions, the water relations of pressurized plants was similar to those of normally watered plant but the leaf growth and stomatal conductance was reduced. In another experiment, Gowing et al. [14] split the roots of young apple trees into two containers. Leaf expansion and leaf initiation were reduced by soil drying in one container, which was restored by severing the root. Both these novel approaches indicated that the water status of the shoot was not related to the observed growth reduction under soil drying, and a signal from the root regulated the shoot growth and the gas exchange. The logical explanation for the restriction in shoot growth is the unbalanced supply of growth promotors and inhibitors originating from a root experiencing soil drying. Stress effects on leaf growth and stomates are obvious in even the short term, before solutes have built up to the high levels for osmotic adjustments, to the changes in the water balance of the tissues [15,16]. It has been demonstrated that without any noticeable change in leaf water status, the plant's growth and stomatal closure can apparently be affected by a message (hormonal) received by the leaves or sent from the roots experiencing osmotic stress [17]. Similarly, it has been shown that shoot turgor was not the limiting factor to plant growth under salinity stress [18,19]. Therefore, the earlier the response, the more likely

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that it had to do with the primary response of stress itself rather than a consequence of one or more of the early events. For such stimulus-response coupling, most of the models proposed consist of a sequential four-component system that includes perception of the stimulus, transduction of the signal, alteration of gene expression leading to a cascade and amplification of the message in the form of a network of biochemical/molecular events, and a physiological response in the form of a morphological adjustment/modification in the growth form. At present, we know nothing about the response of plant/crops growing in a stressful environment, and we have some understanding of the hormonal mode of transduction, but information about the perception mechansim is lacking. It has been suggested that, in eukaryotic cells, the major mechanism of signal transduction is via protein phosphorylation and dephosphorylation (via kinases and phosphorylases, respectively) [20,21].

Under a stressful environment, marked and often rapid changes in the hormonal balance of plants are commonly observed. Since a given stress induces resistance to unrelated stress(es) [12,22], and hormones also influence a stress response, changes in their relative levels may enable the plant to adjust its growth despite suboptimal conditions [23]. Our current understanding of plant growth processes from seed germination through vegetative growth, reproductive development, maturity, senescence, and seed production is influenced by hormones (Table 1). Since plants/crops lack a rapid communication system similar to the central nervous system of animals, they adjust their hormonal balance to regulate growth in response to environmental perturbations. Of the five commonly acknowledged hormones, auxins, gibberellins, cytokinins, ethylene, and abscisic acid, the

Table 1 Some Physiological and Developmental Processes Affected by Known Classes of Hormones

Devlopmental and Physiological Processes	Auxin	Gibberellin	Cytokinin	Abscisic acid	Ethylene
Abscission	+	+	+	+	+
Cell					
Differentiation	+	−	+	−	+
Division	+	+	+	−	+
Expansion	+	+	+	+	+
Permeability	+	−	+	+	+
Dormancy	−	+	+	+	−
Flower initiation	+	+	+	+	+
Fruit					
Growth	+	+	+	−	+
Ripening	+	+	+	−	+
Set	+	+	+	−	+
Gene expression	+	+	+	+	+
Germination	+	+	+	+	+
Juvenility	+	+	−	−	−
Metabolism					
Nucleic acid	+	+	+	+	−
Protein	+	+	+	+	−
Rooting	+	+	+	−	+
Senescence	+	+	+	+	+
Sex determination	+	+	+	−	+
Stomata	+	−	+	+	−
Transpiration	−	+	+	+	−
Tuberization	+	+	+	+	+

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levels of auxin and cytokinins are reduced, whereas that of abscisic acid is enhanced; there is not enough available information to generalize about the status of gibberellins and ethylene. It is known that ethylene causes a reduction in abscisic acid (ABA) which antagonizes the action of gibberellins; and gibberellins are the key hormone promoting internodal growth [24]. Therefore, a commonly observed enhancement in the ABA level would suggest that the levels of ethylene and gibberellins may not be as important as the other three hormones in playing a significant role in the overall stress physiology.

Plant hormones (also called *phytohormone*) are *organic compounds*, *synthesized in one part of a plant and translocated to another part that*, *in very low concentrations*, *cause a physiological response*. This definition sets forth three criteria which separate plant hormones from other nutrients and metabolites: they are endogenously produced, transported to a target area from the site of synthesis, and act in low concentrations. From the outset, plant hormones have been heavily involved with ''action at a distance'' and their ability to move within the plant body has been a paramount consideration. Their transport characteristics are unlike those of most other substances. Water moves up from the root hairs to the transpiring surface along a gradient of (negative) hydrostatic pressure; sugars move from the leaf chlorenchyma down to the cambium or roots along a gradient of both (positive) hydrostatic pressure and sucrose concentration. Ions can be accumulated against concentration gradients, but these are not considered to be growth substances. Auxin does not appear to move along an auxin gradient, but what really is a morphological gradient: polarly and predominantly from the apex to the base in leaves and shoots and from the apex to a short distance behind the apex in roots in most of plant species. As a rule, only slight polarity is shown by gibberellins, which in most concentrations travel freely in both directions in plants. Cytokinins may be carried in small amounts in the transpiration stream or in the bleeding sap, but they mostly appear to remain close to the site of their formation. ABA also seems to move with only slight directionality. In a large number of instances, it appears that the levels of hormones in a tissue/organ relative to one another are a more important consideration than are their absolute concentrations. They exist in plants at concentrations lower than 10^{-6} M [25], and an endogenous concentration above this is generally considered to be supraoptimal. It is also known that not only does each hormone affect the response of a number of plant parts, but that these responses also depend on the species, the plant organs, its developmental stage, cell and tissue sensitivity, concentration and interaction among the hormones, and the environmental factors.

The mechanism(s) by which hormones trigger a response is still far from clear. Specific receptors have been suggested, but no convincing evidence for their function in mediating hormone action has been given. There is, however, considerable evidence that these hormones induce gene expression, but how this is done biochemically is not well understood [1]. These hormones are detectable in all actively growing plant organs; younger leaves and apical buds are paticularly high in auxin, whereas root apices are high in cytokinins, gibberellins, and ABA. Fruits and seeds are generally rich in plant/crop hormones. Therefore, plant/crop hormones are ubiquitous and generally not species specific.

To demonstrate the hormonal control of a physiological process/response, either the balance of the test hormone must be experimentally manipulated (as by excising young organ[s], supplying the hormone exogenously, or using a hormone-deficient or overproducing mutant) to establish its control. In this respect, the Mitscharlich law of diminishing returns [26] can be modified as follows: the increase in plant response produced by a unit increment of a deficient (limiting) hormone is proportional to the decrement of that hormone from the maximum. In this chapter, we cover the hormonal relations of plant hormones elicited under moderate stresses and not at the levels that cause injury/toxicity.

HORMONES

Plant hormones are not only involved in cell division and/or cell differentiation, but there is also a wealth of information about many other processes, including induced gene expression and bio-

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chemical changes (see Table 1). These changes point to the nature of the control exercised by hormones at the subcellular or molecular level. Unlike most animal hormones, which usually have relatively specific types of physiological regulatory functions, plant hormones have considerable interplay between the various groups in the overall regulatory process. Owing to their inherent flexibility, plants/crops adapt to fluctuating environments to complete their life cycle. But when environmental conditions become stressful, plants/crops cope with this pressure by adapting a strategy of reducing leaf expansion and closing their stomates to limit water loss. These adaptive responses are elicited before any measurable change in the water or turgor potential is detected [15–18]. The reduction in leaf expansive growth to stressful environmental conditions has a profound effect on crop production independent of stomatal or biochemical effects. It has been suggested that the rate at which any cell enlarges is determined by the product of two cellular parameters: wall extensibility and effective turgor [27]. Hormonal modulation of the rate of cell enlargement is affected by altering one or both of these parameters. Cell differentiation and enlargement proceed concurrently with division during much of the period of leaf expansion. Stress-induced growth inhibition in the apical meristematic region and the expanding leaves may also be dependent on events external to these regions. Owing to the complexity of the control mechanisms of leaf growth by endogenous hormone(s), the issue remains unresolved. It has, however, been demonstrated that cell enlargement rather than cell division and auxin transport was reduced in coleoptiles (a leaf structure) subjected to salinity stress [28,29]. These observations along with that of Virk et al. [30] and the proposed model based on the auxin-cytokinin countercurrent [31], to explain the basic mechanism underlying plant axial growth, suggest that the reduction of plant/crop growth in response to a stressful environment may have been due to an impaired hormonal balance. The commonly accepted plant/crop hormones are identified as auxins, gibberellins, cytokinins, ethylene, and abscisic acid; jasmonic acid and salicylic acid have also been proposed to be included in this group of organic compounds.

Auxins

Auxin is a term derived from the Greek word *auxein*, meaning ''to increase.'' It is a generic name for chemicals which typically stimulate cell elongation, but auxins also influence a wide range of other growth and developmental processes (see Table 1). The existence of growth-regulating chemicals that control plant/crop growth, and the interrelations between their parts, was the outcome of experiments on the root and shoot responses to external stimuli. In some plant/crop species, a perceptible reduction in the root and shoot growth could be observed when the external water potential is reduced to -0.1 MPa [32,33]. Considering the importance of the auxin indole-3-acetic acid (IAA), it is surprising that very little attention has been paid in elucidating its role in the hormonal balance under a stressful environment. Since we completed this same chapter in the previous edition of this volume in 1993, it is surprising that no further advancement to our knowledge has become available since then [34].

To recollect, it is known that salinity stress reduces the recovery of the free or diffusible IAA from maize (*Zea mays*) coleoptile tips [35]. Similarly, water stress has also been shown to reduce the recovery of this hormone in *Helianthus annuus* (sunflower) and *Anastatica hierochuntica* [36] and from the abscission zone of *Gossypium hirsutum* (cotton) fruits [37,38]. The explanation for the reduction in the recovery of the free IAA was considered to be due to enhanced conjugation. Kannangara et al. [39] observed fluctuations of auxin concentration in the field-grown sorghum (Sorghum bicolar) and concluded that the change in the concentration could not be correlated with the diurnal changes in the ABA or in the leaf water status. Contrary to these observations, Sakurai et al. [40] reported an increase in the auxin level in the squash (*Cucurbita maxima*) hypocotyls subjected to a decreasing leaf water potential. Compacted soil [41,42] or shaking [43] as a source of mechanical stress enhanced or reduced, respectively, the auxin level in maize. Similarly, reduced recovery of auxin was observed from the leaves of tobacco (*Nicotiana tabacum*) subjected to wounding stress [44].

The above findings indicate that the observed reduction in diffusible auxin under a stressful environment may have either been due to a reduction in the biosynthesis, an enhancement in its metabolism, or an affect on the transport kinetics. There is reasonable amount of work on the biosynthetic pathway of auxin available, but we are not aware of any work conducted with plants raised under a stressful environment. Therefore, the only option left to have an understanding about this parameter is to rely on the studies reported on the other two parameters: metabolism and transport kinetics.

In critically evaluating the published work, we see that decreasing the water potential increased the in vitro IAA-oxidase activity in pea (*Pisum sativum*) which correlated with the endogenous auxin level [45,46]. On the other hand, the IAA-oxidase activity decreased with increasing the water deficit in wheat (*Triticum aestivum*) [47]. Similar results were obtained when oat (*Avena sativa*) seeds were imbibed for 48 h in various Na^+-salt solutions; that is, a decrease in the in vitro enzyme activity at all the concentrations tested. However, exposure for longer time periods caused a sharp enhancement in the IAA-oxidase activity proportional to an increase in the Na^+-salt concentrations [48]. Contrary to these results, Naqvi et al. [31] did not find any change in the in vitro endogenous enzyme activity in the coleoptiles (leaf structures) of salinity-stressed maize seedlings. These observations support the hypothesis that the organization of the cytosol is such that enzymes in vivo do not respond to osmotic/ionic environment as they do in vitro [49].

Naqvi [50] studied the absorption and transport properties of ^{14}C-labeled indole-3-acetic acid (^{14}C-IAA) in coleoptile segments excised from salinity-stressed maize seedlings. Using the classic donor:tissue:receiver system, it was demonstrated that the above two parameters were not influenced by the stress. Further studies on the kinetics of ^{14}C-IAA in the same system showed that 0.5 or 1.0% salinity did not materially affect either the velocity or the intensity (capacity) even though the seedling growth was adversely affected.

Kaldewey et al. [51] using internode segments of water-stressed pea seedlings observed that stress did not affect the velocity but did reduce the capacity of ^{14}C-IAA transport. Studies using cotton cotyledonary petioles also showed a reduction in auxin transport capacity by 50% when water stress was enhanced from -8 to -12 bar [52]. Testing the effect of aging, Davenport et al. [53] excised petiole segments from the upper, middle, and lower canopy of mature water-stressed cotton plants and obtained similar results regardless of the age of the petioles. Critical examination of their data, however, indicates that, contrary to their conclusion, no material effect of stress up to -20 bar was observed. Sheldrake [54] raised oat seedlings under nonstressed conditions for 4–6 days and isolated mesocotyl segments which were osmotically stressed (0.5 M sorbitol) for 2 h. These stressed segments when used for auxin transport studies indicated an enhancement in this parameter. However, in the absence of an effect on either the absorption or the transport velocity, the results could not be logically explained.

Nullifying the gravitational force by using Clinostat, it was demonstrated that gravity compensation did not affect either the auxin biosynthesis or the absorption and transport of exogenously supplied ^{14}C-IAA [55].

From a practical point of view, treatments with auxin have been reported to alleviate some of the adverse effects of stress on germination, seedling growth, and the yield of a number of crop species as well as fresh- and seawater algae [56–65].

Gibberellins

In 1926, Kurosawa discovered that gibberellins increase the growth of plants by greatly elongating the cells [66]. Studying the symptoms of the rice disease *bakanaebyo* (''foolish seedling disease''), Takahashi [67] observed that the causal pathogen was a soilborne fungus, *Gibberella fujikorai*, the sexual or perfect stage of *Fusarium moniliforme*, which caused infected seedlings to grow abnormally taller and to fall over owing to a spindly stem structure. He further observed that when a pure culture filterate was sprayed onto rice seedlings, it produced the same abnormal growth. This suggested that the filterate contained some soluble substance which caused the growth abnormality.

Other Japanese workers demonstrated that the effect was not confined to rice but could be reproduced in many other species. However, in 1938, Yabuta and Sumiki isolated two crystalline active substances from the culture filterates of the fungus and named them gibberellin A and B. Currently, 84 gibberellins (i.e., GA_1, GA_2, GA_3–GA_{84}) have been characterized from fungi, plants, and crops and all of them are active on plants/crops [1].

Besides other effects, the primary action of gibberellins is on stem elongation, which is a consequence of both increased cell division and cell elongation. Depending on the intensity and duration of water stress, excised lettuce (*Lactuca sativa*) leaves exhibited a rapid decline in gibberellin-like activity [68]. The reduction was also closely related to the increase in the leaf water saturation deficit and a concomitant elevation in the ABA level. The gibberellin-like activity was barely detectable after 6 h of stress, but after 4 h of relief from the stress, it returned to the normal level. It was further shown that a 10% decrease in the relative water content (RWC) of the detached leaves did indeed accelerate the decline in the gibberellin level [69]. Water-stress studies on gladiolus (*Gladiolus psittacinus*) flower bud growth also indicated a reduction in the level of gibberellin [70]. Similarly, when bean (*Phaseolus vulgaris*) seedlings were subjected to mechanical stress, a decline in the gibberellin content as well as in the stem growth was observed [71]. But studies using aeroponically grown sunflower plants did not show any change in the total gibberellin level or in their distribution when subjected to high-pressure liquid chromatography (HPLC) analysis or to dwarf rice (*Oryza sativa*) bioassay [72]. Gibberellic acid (GA_3) transport through cotton petiole segments has not been found to be influenced by either water or anaerobic stress [73], and the velocity remained at 1 mm/h.

Improvement in germination and seedling growth of onion (*Allium cepa*), sesame (*Sesamum indicum*), flax (*Linum usitatissimum*) [74], lettuce [75], and the polymorphic seeds and seedlings of atriplex (*Atriplex triangularis*) [76] under stressful environments has been observed. Similarly, treatment with GA_3 effectively enhanced the α-amylase activity and the coleoptile lengths in wheat [77,78] and seed germination as well as the seedling growth in barley (*Hordeum vulgare*) [79]. Rao and Ram [70] have demonstrated that bud opening in gladiolus was sensitive to stress which was alleviated by GA_3 indicating stress affected the hormone supply adversely. In addition to these beneficial effects, GA_3 treatment increased the nutrient uptake and enhanced the yield of field-grown wheat [80]. Starck and Kozinska [81] have concluded that gibberellin-treated bean absorbed more P and Ca^{2+} and less Na^+ and partially reestablished the monovalent/divalent ion ratio, which increased in the apical organs of salinity-stressed plants.

Ethylene

Ethylene is the simplest organic compound and is biologically active in trace amounts. Since ethylene is biosynthesized and emitted as gas by plant tissues directly into the atmosphere, it is easily detected by gas chromatography in nanoliter amount in less than 1 min without going through extraction and purification protocols. Besides, the classic *triple response*, characterized by growth retardation, an increase in diameter, and horizontal growth of shoots, is still used as a bioassay to identify and measure ethylene. There is increasing evidence that ethylene influences many plant growth and developmental processes and interacts with all the other plant hormones (see Table 1).

Plant tissues subjected to injury by a variety of stresses, for example, wounding; chemical; mechanical, and temperature extremes; and pathogens, are the site of enhanced ethylene biosynthesis. However, it is not the injured or dead cell but the cell adjacent to it which produces ethylene. Since it is a gaseous hormone, its emission has been detected to increase from 2 to 50 times or more depending on the intensity of the stress and the sensitivity of the tissue. However, this surge is short lived, peaking rapidly and returning to normal level within 24 h or less [82]. No work is available which suggests any deviation in the normal biosynthetic pathway of the stress ethylene surge. However, Kacperska and Kubacka-Zebalska [83] suggested two prerequisites for such stress-induced endogenous ethylene biosynthesis; (a) the promotion of 1-aminocyclopropane-1-carboxylic

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acid (ACC) synthesis and (b) activation of a free radical–generating system. The latter system was needed for the nonenzymatic conversion of ACC to ethylene and depended on the activation of the membrane-associated lipoxygenase caused by stress-induced alterations in cell membrane properties.

When intact plants of sunflower, bean, cotton, and miniature rose (*Rosa hybrida*) were moderately water stressed, no enhancement in ethylene emission was observed [72,84]. But whenever detached plant organs were stressed, ethylene production was enhanced [85,86]. When detached wheat leaves were subjected to dehydration to loose 9% of water of the initial fresh weight, a 30-fold enhancement in ethylene emission was detected within 4 h; thereafter it rapidly declined at or even higher water-stress [85].

The detection of ACC as a water-soluble precursor of ethylene helps to answer some unresolved questions referring to the movement of ethylene in plants. ACC was found to be present in the xylem sap of tomato plants. Its concentration was higher in the sap from waterlogged than from aerated control plants. The appearance of ACC in the sap preceded the onset of epinastic curvature of the petioles, and its concentration correlated with the production of ethylene in the shoot. Therefore, it seems that instead of ethylene, its precursor, ACC, moves within the plants/crops [87].

Ethylene promotes cell extensibility as well as elongation and has been shown to play an important role in many water plants with aerial parts to adjust to the water level. When plants are submerged, elongation is greatly accelerated until the aerial parts regain the water surface. The organs which elongate may be the stem, petioles, or flower stalks [87,88]. But generally the hormone is known to inhibit elongation growth [89,90] and to accelerate senescence [91]. By manipulating the endogenous levels of ethylene and auxin, cells of different shapes and sizes can be produced [92]. This hormone has also been suggested as a marker for screening stress-tolerant lines, because it correlated well with its seedling growth [93]. However, ethylene is well known to play an important role against pathogenic stress [20].

Cytokinins

The discovery of cytokinins was an outgrowth of in vitro technology of plant regeneration developed by Skoog and associates [10]. The isolation and identification of kinetin (6-furfurylaminopurine) from aged or autoclaved herring sperm DNA and its promotion of cytokinesis (cell division) at concentrations as low as 1 μM greatly stimulated research on plant growth and development. Although kinetin does not occur naturally, its discovery greatly supported the concept of the existence of a cell division factor postulated by Wiesener in 1892 [94] and experimentally demonstrated by Haberlandt [95]. However, it was later isolated from immature maize kernel and was named zeatin (Z) [96]. Since then 25 free cytokinins have been isolated and identified from plants/crops among which some are active in inducing maximum tobacco callus growth at concentrations as low as 4 nM [96]. The active cytokinins have not been clearly identified, mainly because mutants or inhibitors that block particular metabolic steps are not availabe. Therefore, it may have been a problem as to which of the 25 cytokinin(s) should be measured under stressful conditions. Cytokinins are a group of compounds that stimulate water uptake, increase cell division, promote organ development, and lead to regeneration and proliferation of shoots [97].

Cytokinins have been classified by a substituted base into three groups: zeatin (Z), dihydrozeatin ([diHZ]), and N^6-(Δ-isopentenyl) adenine (2ip). In many species, the zeatin-type cytokinins are the most active and most prevalent forms of cytokinin [97]. McGaw [98] has concluded that of the hormonally active cytokinins, the nucleotides are probably associated with uptake, with the ''active'' form comprising the ribosides and the bases. Letham and Palni [97] observed that the ribosides were the major forms transported through the xylem and phloem. But O-glucosides and nucleotides were observed to be the major constituents in bean xylem exudate [99]. Thus, there is little doubt that vascular exudate contains cytokinins [100,101]. Benzyladenine (BA), a synthetic cytokinin, is readily taken up by roots, and within 60 min after its addition, the internal cytokinin level was 30%

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of the external concentration [102]. It has also been demonstrated that if the internal BA concentrations were raised by external supply, the adverse effects on various growth parameters were compensated [103].

In an elegant experiment, Carmi and Van Staden [104] demonstrated that the growth of the primary leaves of decapitated and partially defoliated bean plants was strongly influenced by the roots. Partial excision of the roots reduced the leaf area and weight as well as the mesophyll thickening. Metabolic activities such as chlorophyll and protein biosynthesis were dramatically reduced by partial root excision. The balance between the root and shoot systems and the ratio between the dry weights of the root to the leaves also were affected. These effects could be correlated with the lower levels of cytokinin in the leaves, stems, and roots of the partially excised root plants. Actively dividing regions of plants are the sites of cytokinin synthesis [98,99,105], and because of the root morphology, there are more actively dividing tips in the root system; therefore, the major portion of the cytokinins is biosynthesized there and transported to the shoot [105,106]. These root-originated cytokinins along with shoot-synthesized ones influence the control of both the development and senescence of the whole plant [107]. Generally, the growth-enhancing effects of BA are considered to occur via an increase in the protein content [97] by stimulating the RNA polymerase activity [108] and reinforcing the binding of RNA to ribosomes by a higher rate of incorporation of BA nucleotides [97]. This increase in the protein content is reflected in a higher photosynthetic activity [109] and in an enhanced nitrate reductase activity [110]. Besides BA retards the protein degradation by inhibiting ribonucleases [111] and senescence [112].

Vascular exudates and/or leaves of stressed plants exhibit a reduced cytokinin activity, and the response is known to be rapid [16,34,72,98,106,113]. After the exposure to nutrient stress, transfer from 100 to 2%, the cytokinin concentrations of the shoot and root of *Plantago major ssp. pleiosperma* dropped to 50% in a 2-day period, whereas the mineral contents decreased much later [113]. Similarly, a rapid drop in the cytokinin concentration was correlated with a reduction in barley growth paraments [114]. Contrary to the general consideration that a reduction in growth under stressful environment signifies sensitivity, Kuiper and associates interpret it as an strategy for tolerance [103,113,114]. Treatments with exogenous BA or relieving of stress enhanced the internal cytokinin concentration and alleviated growth reduction in both the species. Similarly, tomato plants recorded reduced cytokinin activity 8 days after they were subjected to salinity stress, which was correlated with growth reduction. When the stress was relieved, the cytokinin activity reached the normal level in the next 4 days (Fig. 2) [10]. Hubick et al. [72] water stressed aeroponically grown sunflower plants and observed a rapid decrease in the cytokinin activity. Neuman et al. [115] reported that root hypoxia reduced cytokinin in the xylem sap, but zeatin riboside (ZR), dihydrozeatin riboside (diHZR), and their equivalents were not reduced in the leaves of bean and poplar (*Populus tricocarpa* x *P. deltoides*). However, Bano et al. [15] have observed that rice seedling roots experiencing 30 h of drying exhibited a reduced level of cytokinins, isopentenyladenine (2ip) + isopentenyladenosine (2iPA), and zeatin (t-Z)+zeatin riboside (t-ZR) in the xylem sap which increased after the relief of stress by rewatering.

Despite their established role in plant/crop development, information as to how endogenous cytokinins are reduced under stressful conditions is meager. The reduction in the cytokinin level raises the question whether the hormones' biological activity was minimized by enhancing temporary storage or whether it was metabolized. Studies show that N-glycosides are very stable and have a low biological activity in vitro and may withdraw cytokinin from the potential pool [97]. On the other hand, the O-glycosides are deglycosylated readily and may be involved in homeostatic control of the hormone levels [97,98]. A reduction in the cytokinin activity under stressful conditions may have been either due to reduced biosynthesis or enhanced metabolism. Binns [116] has defined cytokinin metabolism as the conversion of (9R-5′P)iP to any other N^6-substituted adenine derivative cytokinin. Accordingly, the major conversions are the dephosphorylation and deribosylation yielding the riboside and free base, respectively. The hydroxylation of the side chain produces the trans-zeatin (t-Z) derivatives (9R-5′P)t-Z, (9R)t-Z, (9R-5′P)-diHZ, (9R)diHZ, and diHZ. These derivatives can be further metabolized; often by O-glycosylation of Z or diHZ and N-glycosylation at the 7 and

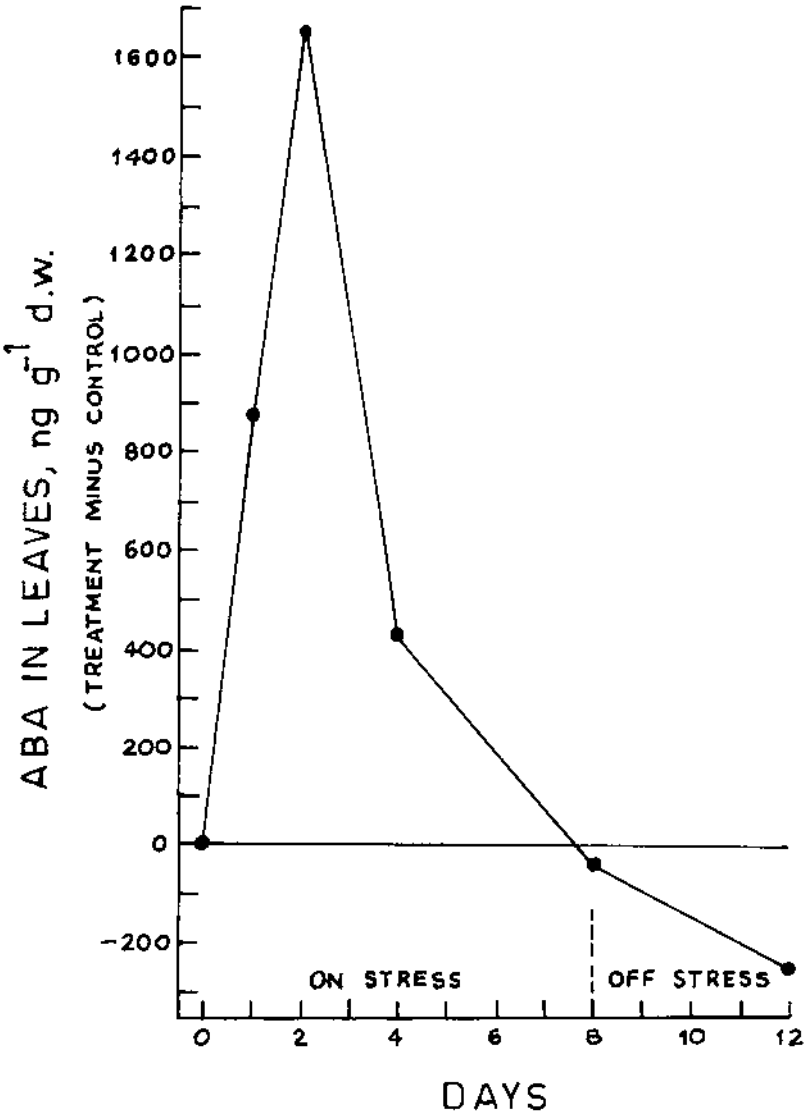

FIGURE 2 Effect of salt stress on the free ABA content of tomato leaves. (Reproduced from Ref. 10.)

9 positions of the adenine base. However, such studies under stressful conditions are not available to answer similar questions. Thus, basic questions regarding the biosynthesis, metabolism, and activities of cytokinins remain unanswered. Kuiper [103] has, however, observed that *Plantago* plants exposed to low salts had reduced cytokinins and had a limited number of active root tips.

Kinetin treatment enhanced seed germination and seedling growth in tomato and pea under salinity stress [117,118]. Under salinity stress, wheat seedling growth retardation was partially alleviated by kinetin treatment [119]. That alleviation of salinity induced a reduction in the germination and seedling growth by kinetin treatment seems to be well documented [120]. Kuiper and associates [11,103,113,114,121] have reported that mineral and salinity stress induced a reduction in the endog-

enous cytokinin concentration and the growth parameters of *Plantago major* ssp. *pleiosperma*, species of wheat and barley, were effectively enhanced by cytokinin. Treatments with cytokinin have been reported to enhance transpiration by causing maximum stomatal opening in a number of plant/crop species [122,123]. Saunders [124] has shown that cytokinin exerts at least part of its effect by stimulating Ca^{2+} uptake by responsive cells.

Certain cytokinins (6-benzylaminopurine, zeatin, 2-isopentyladenine) have also been shown to be efficient elicitors in inducing phosphoenolpyruvate carboxylase (PEPCase), the key enzyme in crasssulacean acid metabolism (CAM); proline and pinitol accumulation, and an osmotin-like protein in *Mesembryanthemum crystallimum* [125–127], a *halophyte*, and PEPCase and carbonic dehydrogenase in maize, a *glycophyte* [128]. Similarly, another cytokinin (BA) enhanced the accumulation of nitrate reductase (NR) mRNA in etiolated barley leaves [129]. This cytokinin (BA) also has been shown to regulate the accumulation of jasmonic and salicylic acids caused by mechanical wounding and pathogenic signals, respectively. As a consequence of these observations, in wild-type and transgenic tobacco plants, Sano et al. [130] concluded that cytokinins were indispensable for the control of jasmonic and salicylic acids, elicitors of wounding and pathogenic stresses.

Abscisic Acid

Hemberg is credited with being the pioneer who advanced the idea that plant growth and development are regulated by endogenous levels of both a promotor (auxin) and an inhibitor. Employing an *Avena* bioassay, he observed that potato peels contained a high level of growth inhibitors. He further demonstrated the presence of a similar inhibitor which was correlated with the degree and levels of bud dormancy in *Fraxinus excelsior* (ash) [131].

Bennet-Clark and Kefford [132], using paper chromatography to analyze plant extracts for growth substances, observed the inhibitory activity at R_f 0.6 and 0.7. This was later shown to be present in a number of plant species and the levels responded to environmental changes. After diverse approaches, that is, dormancy (dormin) and abscission (abscisin I and II), it converged that ABA was the hormone responsible for both the physiological states [133]. Like other plant/crop hormones, ABA also is ubiquitous among vascular plants/crops besides being present in some algae, fungi, and mosses, and it is known to influence many physiological functions (see Table 1).

The naturally occurring enantiomorph of ABA is (S)-ABA, which is sesquiterpenoid (a 15-carbon compound), and by its biogenesis is related to monoterpenes, diterpenes (gibberellins), carotenoids, and triterpenes. Endogenous (S)-ABA is optically active, having one center of asymmetry at C-I′, whereas synthetic ABA is recemic and composed of equal amounts of (S)-and (R)-enantiomers. The synthetic (R)-ABA accounts for 50% of the recemic mixtures of ABA and has a biological activity equal to that of the natural (S)-ABA in most cases; except in stomatal closure where it is inactive. Since the observation that the endogenous ABA level increases under environmental stress and that it causes stomatal closure reducing transpirational water loss [134], later workers named it the ''stress hormone''. However, the total content of ''free'' ABA per unit leaf area does not increase before stomata close under stress [135]. ABA influences many physiological processes (see Table 1), and its enhancement under stressful environments convinced Quarrie [136] to remark that, '' a plant that cannot make abscisic acid (ABA) is in trouble.'' Wright [137] surveyed over 70 plant/crop species and observed an increase in the ABA levels when their excised leaves were subjected to a period of wilting. Besides, it also accumulates in the absence of water deficit [15]. ABA also has been reported to increase under pathogenic stress [138,139] and enhances resistance to pathogens [140]. It is a typical sesquiterpene, consisting of three isoprene units, and is known to be synthesized through carotenoid pathways with xanthophylls being immediate precursors [141].

During the past 20 years or so the endogenous level of ABA has been reported to rapidly increase 5- to 100-fold or more under stressful conditions. Therefore, ABA is considered to be involved in stress signal transduction and to serve to coordinate the physiology and development of plants/crops experiencing a stressful environment [10,141]. However, gene analysis has revealed that both ABA-independent as well as ABA-dependent signal transduction cascades operate between

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the initial signal and the gene expression under drought stress [142]. It has further been suggested that factors other than ABA may be involved in the osmotic signal transduction pathway [143].

ABA is synthesized in the shoots and roots and moves bidirectionally and not polarly in plants/crops and is also transported rapidly from cell to cell. Under water stress, Hoad [14] observed that ABA was actively translocated out of the mature leaves in the phloem and transported to the stem apices, fruits, and seeds in white lupin (*Lupinus albus*). It has, however, been reported that, under a stressful environment, translocation of ABA form the shoot to the root is inhibited causing the hormone to accumulate in the aerial parts [145].

ABA reduces seedling growth, but Sharp and associates [146–149] conclude that it affected the shoot and root growth differentially; that is, maintaining primary root elongation and inhibiting shoot elongation in maize seedlings. The inhibition of shoot growth is unequivocal, but the maintenance of root elongation is contradictory and needs a satisfactory resolution. In short-term experiments (100 h), using variously moist vermiculite, they concluded that the accumulation of ABA under water-stress conditions functioned both to maintain the primary root elongation and to inhibit the shoot elongation of maize seedlings. Analysis of their data [148] reveals that seedlings raised under −0.03 MPa (control) had an ABA content of 15 ng^{-1} H_2O and a root length of 210 mm. Reducing the water potential to an order of magnitude (−0.30 MPa) enhanced the endogenous level to 37 ng g^{-1} H_2O (2.5×) but reduced the root length to 179 mm (−15%), and an exogenous ABA treatment of 2×10^{-6}M (−0.03 MPa) raised the endogenous level to 44 ng g^{-1} H_2O (3×) and also reduced the root length to 175 mm (−17%). Similarly, the data presented by Sharp et al. [149] (Fig. 1A, B) clearly indicate that in -FLU (fluridone, inhibitor of ABA biosynthesis) treatment, the increase in the root lengths between 50 and 100 h was independent of the ABA content. On the other hand workers using hydroponic [150–156], aeroponic [157], and soil [154,158–160] culture system to raise seedlings or mature plants have reported inhibition of both the shoot and root growth. It is therefore possible that maintenance of root elongation in the absence of calcium in such short-term experiments may not sustain it in long-duration studies. It is also known that roots are strongly hydrotropic in nature and grow in the direction of increasing soil moisture. This was elegantly demonstrated by raising cotton seedlings in soils of differing water content [161,162] and the hydrotropic response studies with mutant pea *Ageotropum* [163]. The threshold of osmotic perception was observed to be less than 2 min, and the transduction and transmission of the stimulus to the basal growing zone required 90–120 min to elicit the response [164]. Besides, parallel variation in the maintenance of the root growth to the varying ABA concentration has yet to be demonstrated.

During the last 30 years, the role of ABA in the control of stomatal closing in the context of the plant water relation has been established. Therefore, enhanced levels of ABA were very conveniently correlated with the closing of stomata as an initial response to the water-limiting environment. But recent reports by Davies and associates [17,165] suggest that stomata may start closing before changes in the xylem ABA level are detected unless it is modified by the influence of the leaf. ABA has been shown to act indirectly by increasing the cytosolic Ca^{2+} [166]. This observation is consistent with the long-known fact that external Ca^{2+} depresses the stomatal aperture [167].

Proline is probably the most widely distributed compatible osmolyte which accumulates in living organisms, including plants, under stressful environments [168,169]. The enhanced accumulation of proline was shown to be induced by ABA [170,171], but other workers using ABA-deficient plants [172,173] or maize cultured cells [174] have demonstrated that stress-enhanced proline was independent of ABA.

ABA has been reported to suppress the accumulation of nitrate reductase (NR) mRNA by inhibiting the level of transcription in etiolated barley leaves [129]. Similarly, it has been observed that ABA treatment did not lead to an increase in the osmotin protein in ice plants (*Mesembryanthemum cystallinum*) [127].

HORMONE INTERACTIONS

The growth-regulatory activities of plant hormones depend on their interactions, but the mechanisms by which interactions control the physiological processes and development is unknown. Some might

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consider this to be a pessimistic description of our state of knowledge which comes from the difficulty of carrying out genetic studies in this area [116].

In plants, cytokinin interacts with auxin to produce the callus and regenerate the shoot and/or root; it opposes auxin in lateral bud development (apical dominance or compensatory growth); it resembles auxin in inhibiting root elongation; it does strongly what auxin does weakly in promoting protein synthesis; and it acts in the same way as auxin to cause cell division. Similarly, gibberellin acts like auxin in promoting the elongation of etiolated stems and the formation of parthenocarpic fruit (although it generally delays fruit set); it reacts with auxin in producing the elongation of isolated green stems; it acts far more powerfully than auxin in the elongation of intact stems; and it does what auxin cannot do in causing flowering of long-day plants on noninductive photoperiods and the elongation of monocotyledonous leave sheaths. Yet gibberellin acts in the opposite direction of auxin regarding root formation by leaves and stem cuttings. It was suggested that auxin exerted its influence on lateral bud growth via enhanced ethylene synthesis, but recent evidence using transgenic overproducing auxin, cytokinin, or ethylene tobacco and *Arabidopsis thaliana* plants has shown that it was the auxin/cytokinin ratio and not ethylene which controlled the lateral bud growth [175,176]. The auxin-cytokinin countercurrent seems to be the basic regulatory mechanism controlling the plant axial growth [31]. Ethylene causes a reduction in the level of ABA, which is an antagonist of gibberellin's action, and gibberellin is the hormone that promotes internodal growth.

Gibberellic acid reverses the ABA induction of aldolase reductase, *rab/lea* genes, and α-amylase mRNA inhibition, and cytokinin enhances the accumulation of NR mRNA, which is suppressed by ABA, and the hormonal influence on NR gene expression was concentration dependent in the range of 10^{-7}–10^{-4} M [131]. ABA inhibits and cytokinin promotes the opening of leaf stomata and cotyledon expansion [129]. Plant senescence involves the interaction among auxins, cytokinins, ethylene, and ABA, and the accumulation of chlorophyll caused by cytokinin is an opposite effect of the senescence resulting from ABA treatment.

CONCLUSIONS

Early work on the environmental effects on plant/crop growth and production concentrated on the aspects of shoot growth and functioning. But some elegant experiments demonstrated that hormonal signals from stressed roots controlled leaf growth and stomatal behavior. The models suggested have a sequential four-component system that includes the perception of the stimulus, the transduction of the signal, the alteration of gene expression, and the physiological/morphological response. We now have some understanding of the hormonal mode of transduction, but information about the perception mechanism is lacking. Under a stressful environment, particularly in the root zone, hormonal imbalance in the form of enhanced ABA (inhibitor) and reduced cytokinins (promotors) seems to be coupled. These two hormones have an opposite influence on the leaf growth, stomatal behavior, senescence, and some important enzymes of the photosynthetic pathway. Recent molecular studies have shown that ABA-independent and ABA-dependent signal transduction systems operate under a stressful environment. Therefore, an important goal for future research needs will be to understand the influence of cytokinin and ABA on the early events of perception and transduction, by uncoupling their response. These studies may not be possible until suitable mutants or a chemical(s) are available to interfere with the biosynthetic pathways of the hormones. Attention also is needed to further explore the role of indole acetic acid in order to achieve the hormonal balance and ameliorate plant growth to enhance crop productivity under a stressful environment.

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Abscisic Acid—A Hormonal Long-Distance Stress Signal in Plants Under Drought and Salt Stress

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ABSCISIC ACID AS A STRESS HORMONE IN PLANTS

When abscisic acid (ABA) is applied externally to plants, their water relations are improved. ABA reduces water loss by promoting stomatal closure and can increase water uptake into roots. ABA application also promotes characteristic developmental changes that can help the plant cope with a range of environmental stresses. Examples of such changes are the restricted growth of shoots, the reduction in leaf surface area, a stimulation of root extension, lateral root growth, and root hair development. All these effects of ABA application, together with the observation that environmental stress stimulates ABA biosynthesis and ABA release from sites of synthesis to the sites of action, suggest a role for ABA as a stress hormone in plants.

MECHANISMS TO INCREASE THE ABA CONCENTRATION AT THE PRIMARY SITE OF ACTION AT THE STOMATA

It was shown previously [1] that the biosynthesis and metabolism of ABA are stimulated by the plant water deficit when the bulk leaf turgor is reduced close to zero. Stomatal reactions, however,

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can be observed at much smaller water deficits. It was also observed [2] that increases in ABA concentrations in leaves and in epidermal tissues are seen only after a lag phase of 30–60 min after the onset of stress, a time period within which stomatal reactions to the initiation of the stress have often been completed.

We must therefore ask how ABA concentrations can be increased at the primary site of action under conditions in which ABA biosynthesis is not affected? The primary site of action for ABA on the stomata was shown to be the outer surface of the plasmalemma of the guard cells [3–5]. Even if we assume that ABA may be recognized by cytosolic structures of the guard cells [6], the apoplast surrounding the guard cells is regarded as the relevant compartment so far as ABA action is concerned, because this is the only compartment from which ABA can be taken up into the guard cells.

Several possible mechanisms explain the ABA-dependent closure of guard cells under these special conditions:

1. ABA may be released rapidly from the mesophyll cells to the apoplast.
2. ABA may be transported from the roots in the transpiration stream to the leaves and to the stomata. In this respect, it may be important that under certain specialized conditions, only a few roots in the shallow soil layers, which dry out more rapidly than other parts of the root system, may be sufficient to supply the leaves with sufficient ABA to promote stomatal closure. This may occur even when the water relations of the shoot are unaffected by the soil-drying treatment.
3. Guard cell sensitivity and responsiveness to ABA may be changed by environmental stress, which could result in rapid and sensitive ABA responses.

Redistribution of ABA in Leaves Under Environmental Stress

ABA is the only one of the known acid plant hormones that distributes across the mesophyll cell compartments according to the anion trap concept [7]. The undissociated abscisic acid ABAH is the only permanent ABA species that passes the membranes by diffusion and is trapped in alkaline compartments (cytosol and chloroplast) as the almost completely nonpermeant anion ABA. Alterations of intracellular pH gradients therefore redistribute ABA between the leaf cell compartments. From the stress physiological perspective, apoplastic alkalization accompanied by a slight cytoplasmic acidification, as observed by Hartung et al. [8], is of particular interest.

This flattening of pH gradients, which occurs before turgor is reduced to zero, results in a substantial and rapid increase in the ABA concentration in the apoplast. Hartung and Slovik [7,9] performed a computer simulation of ABA redistribution and confirmed that this can be a rapid and sensitive process.

ABA AS A MESSENGER FROM THE ROOT SYSTEM TO THE STOMATA

It is now clear that, under many circumstances, stomatal behavior can be closely related to changes in the soil water conditions even when leaf water relations are not affected by these changes. Blackman and Davies [10] showed with split root experiments with maize (in which one part of the root is well watered but the other suffers from drought stress) that stomata close despite the maintenance of a high water potential in the leaves. These results, together with many other findings (for references see the review of Davies and Zhang [11]) strongly indicate that roots can sense some aspect of the soil water status. Stomatal behavior can be regulated as a function of the strength of these signals.

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ABA Biosynthesis in Roots

It was shown by several research groups that dehydration of isolated root systems increased ABA biosynthesis. Zhang and Davies [12] showed this for isolated maize root segments, Cornish and Zeevaart [13] and Cornish and Radin [14] for tomato and cotton roots, and Hartung and Abou-Mandour [15] for bean roots. Roots contain all the necessary precursors for ABA biosynthesis via the indirect violaxanthin pathway [16]. Burbridge et al. [17] have studied the gene expression of zeaxathin epoxidase (ZE), an enzyme that is believed to catalyze two early steps of the indirect ABA biosynthetic pathway. Drought treatment showed no effect on the abundance of ZE mRNA in tomato leaves. More recently, Burbidge et al. [18] have isolated a cDNA encoding a protein capable of catalyzing the oxidative cleavage of a 9-cis xanthophyll. They note that this is probably a key regulatory step in ABA biosynthesis. Northern analysis showed that there was a substantial drought stress–induced rise in the transcript level. The upregulation of gene expression is consistent with the proposed role of the cleavage enzyme as a key regulatory step in the ABA biosynthesis pathway. Nevertheless, the exact target of stress in the ABA biosynthesis pathway is still not known.

Carandang and Hartung (unpublished data) reinvestigated the increase in the ABA concentration in dehydrated maize and bean roots and observed the strongest capacity of a biosynthesis in root tips indicating that ABA synthesis occurs mainly in the cytosol of nonvacuolated cells (Fig. 1). Cortical and stelar tissues exhibit a comparable capacity for ABA biosynthesis (Fig. 2). As reported by other investigators [13,14], however, a quite drastic loss of water is required to stimulate ABA biosynthesis. As discussed, sensitive mechanisms are necessary to meet our postulations. It is therefore necessary to consider whether stress-dependent ABA redistribution in roots can play a role in stress signaling.

ABA Redistribution in Roots Under Stress

The question arises of whether mechanisms of ABA compartmentation and redistribution, which were found to be valid in leaves, can also be applied to root systems. Although, in leaves, stress

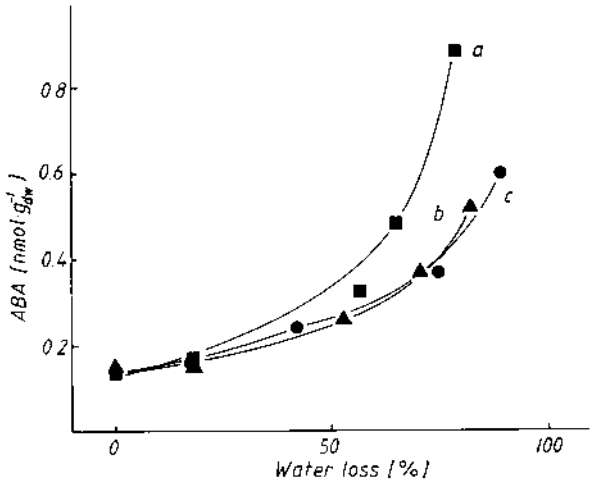

FIGURE 1 Accumulation of ABA in isolated root tip segments of *Zea mays* dependent on the percentage of water loss. Root segments (a, 0–3 mm; b, 3–6 mm; c, 6–9 mm behind the tip) were allowed to lose water to a certain percentage. The segments were then kept for 2 h under these conditions and then extracted and analyzed for ABA. (From J. Carandang and W. Hartung, unpublished data.)

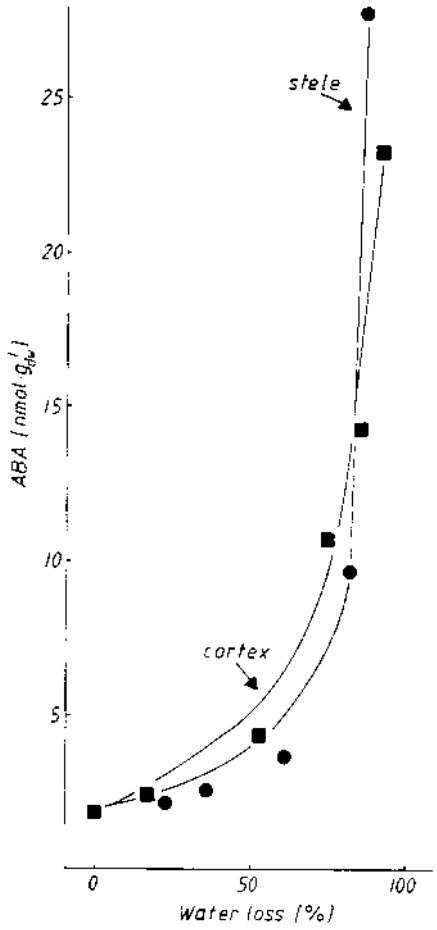

FIGURE 2 Accumulation of ABA in isolated 15-mm long cortical and stelar sections of *Phaseolus coccineus* dependent on the percentage of water loss. For further details see Figure 1.

must increase the apoplastic ABA concentration by an enhanced release of ABA from the mesophyll cells, the situation in roots is more complex.

Assuming that radial ABA transport in roots is symplastic predominantly, the ABA concentrations should be increased in the cytoplasm of cortical cells. This allows transport symplastically via the passage cells of the endodermis to the stele. Having arrived in the stele, ABA should be released easily from the xylem parenchymal cells to the xylem vessels.

To explain this process by the anion trap concept, which works perfectly in leaf tissues, we must postulate an increase in the pH gradients in the cortical tissues rather than a flattening, as occurs in the mesophyll. Thus, stress should acidify the apoplast of the cortex or alkalize the cortical cytosol, or both.

In fact, ^{31}P nuclear magnetic resonance (^{31}P-NMR) investigations [19] have shown that osmotic stress (caused by polyethylene glycol) and salt stress alkalize both the root tip cytosol and the vacuoles of maize. Daeter et al. [20] also determined the permeability the coefficients of the cortex root plasma membranes of maize for ABA and found them to be lower by one to two orders

of magnitude than those of plasma membranes from other tissue types. This would diminish loss of cytoplasmic ABA during symplastic transport to the stele. Incorporation of these findings into a computer model of Slovik et al. [21] predicts only small changes in the ABA concentration in the leaf apoplast. However, when the pH_{cyt} of the xylem parenchymal cells in the model is reduced by only 0.1 unit ABA_{xyl} is significantly increased by drought stress. Armstrong et al. [22] have shown that stelar tissues form an anoxic core even in well-aerated roots. Anoxic conditions can reduce pH_{cyt} by 0.2 unit for at least 16 h [23].

FACTORS AFFECTING ABA CONCENTRATION IN THE XYLEM SAP

Soil Water Content

Zhang and Davies [24,25] have convincingly shown that soil drying increases the ABA concentrations in both the root tissue and the xylem sap ascending from the roots. Presumably, a reduction in the root turgor volume will increase ABA synthesis in the roots. There are, however, characteristics of a drying soil that may also be responsible for increased ABA biosynthesis. Soils from extreme habitats like deserts exhibit, besides a reduced water content, an increased salt concentration, alkaline pH values, reduced nutrient supply, and high soil strength [26].

Salt Factor

High concentrations of NaCl in the soil solution can significantly increase the ABA concentrations in plant organs and transport fluids. Wolf et al. [27] studied the long-distance transport of ABA in salt-stressed lupins (*Lupinus albus*) and showed that the salt stress increased the phloem sap ABA by a factor of 5 and the xylem sap ABA by a factor of 10. Mathematical analysis of their data led to the conclusion that a significant portion of the xylem sap ABA was derived from the leaves and was transported from there to the roots and then retranslocated in the xylem back to the leaves. This recirculation of ABA also was stimulated significantly under conditions of salt stress.

Peuke et al. [28] and Jeschke et al. [29] have studied ABA flows in mildly stressed *Ricinus communis*. The strongest effect could again be observed on ABA accumulation in the roots and the ABA transport in the xylem. The stimulation of ABA accumulation in the tissues and the ABA export in the phloem were observed predominantly in fully mature but nonsenescent leaves.

Effects of Salts Different from NaCl

The effect of different cations and anions on ABA accumulation in wheat leaves has been studied by Varma [30]. Among cations, excessive sodium and magnesium had the strongest effect and calcium and potassium the weakest effect on ABA accumulation. Chloride salts, on the other hand, proved to be more effective than sulfate, nitrate, and carbonate salts.

Alkaline pH

Soils with high salt concentrations usually have a very alkaline pH. When we apply the anion trap concept to roots growing under these conditions, we should expect a release of ABA into the rhizosphere. Such an increased ABA release to the alkaline soil solution that was predicted by computer simulations of Slovik et al. [21] would result in severely ABA-deficient plants, assuming that the soil solution was free of ABA. According to Slovik's mathematical model, the dramatic loss could be prevented if ABA would be present in the soil solution in the low nanomolar concentration range. Indeed, ABA has been detected in soil solutions of different soil types under different crops in the range of 0.5–5.0 nM [31]. On the other hand, however, many roots acidify their rhizosphere, especially under conditions of nutrient deficiency. This was shown for phosphate-starved members of

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the *Brassicaceae* [32]. Such a proton release would increase pH gradients between root cytosol and apoplast (which is in direct contact with the rhizosphere) and prevent ABA starvation of roots. From a preliminary series of experiments with *Lupinus angustifolius* (alkali susceptible) and *Cicer arietinum* (alkali tolerant) (W. Hartung and N.C. Turner, unpublished results), it seems that ABA biosynthesis in the roots of the alkali-tolerant chickpea is increased causing a high ABA concentration in both the xylem sap and the soil solution.

Reduced Nutrient Supply

Application of Nitrogen to the Roots

It has been known since the 1930s [33] that nitrogen (N) deficiency mimics drought stress. Nitrogen deficiency also increases ABA concentrations in leaves and roots [34]. In sunflower, concentrations of leaf and root ABA increased following a 60% reduction in nitrate availability [35]. Interestingly, a similar reduction in the nitrate supply reduced the ABA concentration in the xylem of *Ricinus* plants [28].

The source of N may also be important for the stress physiology of the plants. NH_4^+ supplied as the only N source has strong inhibitory effects on leaf and root development. Peuke et al. [28] (Fig. 3) observed a threefold increase in the xylem ABA flow and a twofold increase in the phloem ABA flow of NH_4^+-stressed *Ricinus* plants compared with nitrate-nourished plants. In *Phaseolus vulgaris*, the NH_4^+-dependent increase in the xylem sap (ABA) was five- to sixfold, which is a particularly dramatic increase for this plant.

Foliar Application of Nitrogen

When nitrate or ammonium is sprayed on the leaves of *Ricinus* and the root nutrient medium is kept free of nitrogen, the ABA biosynthesis in the leaves of the nitrate-sprayed plants is stimulated. A high portion of this ABA is loaded to the phloem and translocated to the roots where most of the ABA is metabolized. In ammonium-sprayed plants, a significant ABA net biosynthesis can be observed in the roots resulting in a root to shoot ABA transport in the xylem which is four to five times higher compared with the nitrate-treated plants. Again, a large portion of phloem-transported ABA is recirculated to the shoot. Surprisingly, this resembles strongly the situation of the plants in Figure 3, where roots were supplied with nitrogen. We have to postulate a shoot to root signal of the ammonium-sprayed plants that causes stimulation of ABA biosynthesis in roots and ABA xylem transport. The nature of this signal is unknown.

Phosphate Deficiency

As pointed out above, saline soils can be very alkaline. A high pH of the soil solution causes a reduced availability of phosphate for the roots as long as the roots are not able to acidify their rhizosphere. Radin [36] studied the influence of phosphorus (P) deficiency on the accumulation of ABA in cotton leaves. The ABA levels were only slightly increased at water potentials below -1.5 MPa. The sensitivity of guard cells, however, was greatly enhanced.

In castor bean, P deficiency causes similar effects as mild salt stress as far as C and N flows are concerned. Different from salt-treated castor bean, however, the ABA accumulation in the leaves was very low despite a massively increased import of ABA in the xylem to the leaves [29].

Potassium Deficiency

Only very few data have been published on effects of potassium (K^+) deficiency on ABA in plants. In K^+-deficient wheat plants, the ABA levels in the leaves and the grains are much higher than in the controls, which may be responsible for the premature ripening of grains [37].

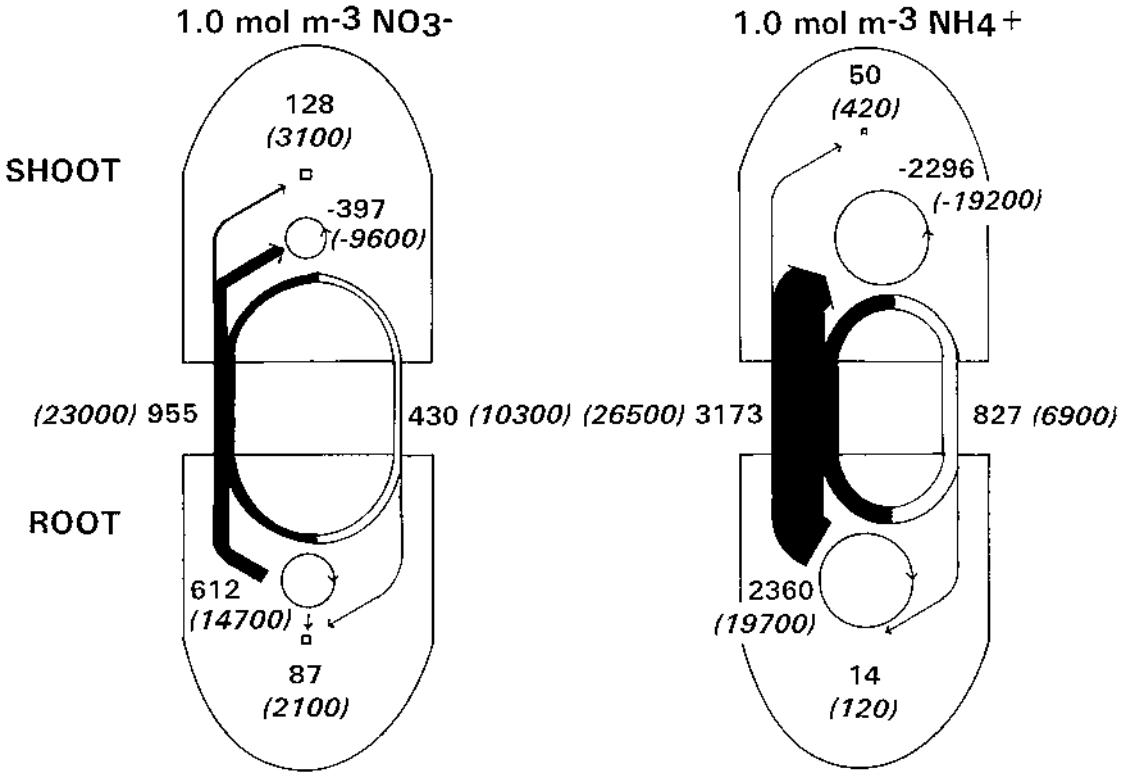

FIGURE 3 Flow profiles for transport, utilization, and net-synthesis or metabolism of ABA in castor bean plants that have been supplied with 1 mM nitrate or ammonium. Open arrows indicate flows in the phloem, black arrows the flows in the xylem. The widths of the arrows (flow in phloem and xylem), the area of squares (deposition), and circles (net synthesis/degradation) are drawn in the relation of the rates of flows. The numbers beneath the arrows, squares, and circles indicate net flows in pmol g^{-1}_{frwt} $(10\ days)^{-1}$. Upward arrows of the circles denote net synthesis, downward arrows net degradation. Numbers in italics show nmol $plant^{-1}$ $(10\ days)^{-1}$. (Redrawn after Ref. 28.)

Micronutrients

It has been shown recently that mutants which are deficient in the molybdenum cofactor (MoCo) also are ABA deficient. The MoCo is not only an important component of the nitrate reductase but also of aldehyde oxidase, the latter of which catalyzes the last step of ABA biosynthesis, the oxidation of ABA aldehyde [38,39]. Indeed, foliar sprays of molybdate to wheat leaves increase the ABA content, responsiveness to applied ABA, and dormancy of embryos significantly [40]. Peuke et al. [28] (see Fig. 4) have shown that an optimal nitrate supply stimulates ABA synthesis in the roots and leaves (foliar spray) of *Ricinus*. Nitrate treatment induces nitrate reductase which includes the MoCo and consequently the oxidation of ABA aldehyde.

High Soil Strength

It has long been known that a high soil strength (or mechanical impedance) inhibits plant growth and development. Masle and Passioura [41] observed that growth-inhibiting effects on shoots can

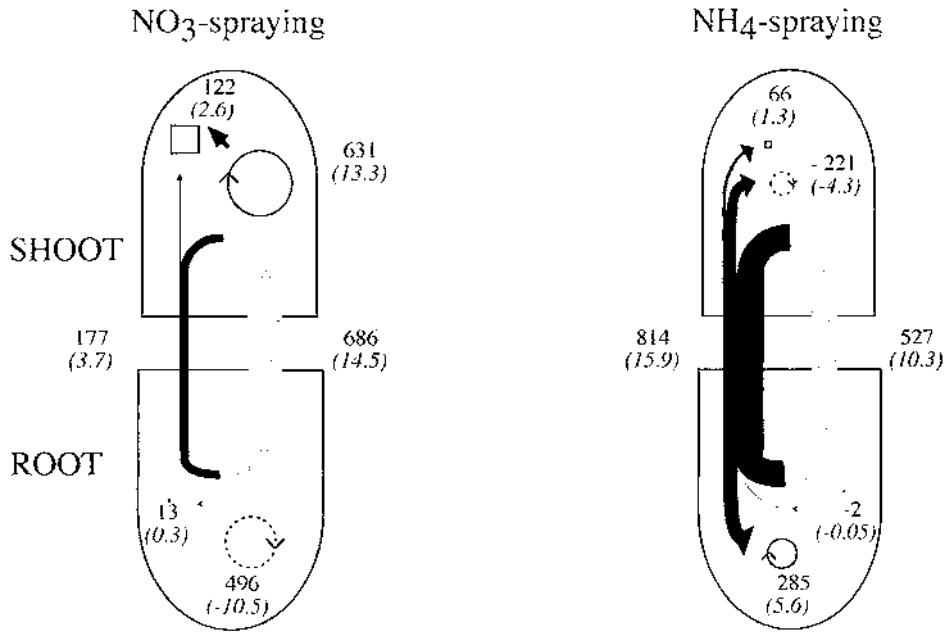

FIGURE 4 Flow profiles for transport, utilization, and net-synthesis or metabolism of ABA in castor bean plants that have been sprayed with nitrate or ammonium. The root nutrient medium was free of nitrogen. For further details see legend of Figure 3. (From A.D. Peuke, W.D. Jeschke, and W. Hartung, unpublished data.)

be greater than those on roots. These responses occur even under conditions in which the water relations of the shoots were not affected by mechanical impedance. These investigators [41] conclude that roots may signal to the shoots the effects of the interaction between the root and the compacted soil. Such a signal may modify the growth and development of the plant. Tardieu and coworkers [42–44] detected increased ABA concentrations in the xylem sap of maize plants growing in compacted soil. This extra ABA apparently reduced stomatal conductance. Enhanced ABA concentrations seemed to be a result of poor water supply and root dehydration in compacted soil rather than a direct effect of compacted soil [45,46]. When roots are forced to penetrate compacted soil, they increase radial growth, the number of root hairs, and the size of the root cap. Root hairs can act as an anchor to facilitate the penetration of the compacted soil. Very similar morphological changes can be observed when seedlings are cultivated in a nutrient medium containing 0.1–1.0 μM ABA. Maize seedlings growing in compacted soil show a 10-fold increase in the xylem sap ABA, especially during the first days after germination. It therefore seems that enhanced ABA can aid root penetration with secondary effects on shoot physiology [45,46].

Recently, Mulholland et al. [47,48] studied the influence of soil compaction on ABA relations in barley plants. They also found a transient increase of ABA_{xyl} as a result of soil compaction. Different from maize [46], however, the investigators concluded that ABA acts as a leaf growth promoter to maintain leaf growth under conditions of compaction stress.

SENSITIVITY OF STOMATA TO ABA

In most plants, leaf water deficit causes ABA concentrations to increase by a factor of 10–30, but the response of bean plants (*P. vulgaris*) to stress is very weak (except ammonium-stressed beans).

Trejo and Davies [49] found that ABA in the xylem sap increased by only a factor of 2, even in particularly stress-resistant varieties that were very severely droughted. Similar observations were made with *Phaseolus coccineus* [50]. Smith and Dale [50] also observed very weak ABA increases in the leaves of bean plants that were stressed by root cooling.

Two conclusions can be drawn from these findings:

1. Guard cells of bean leaves are particularly sensitive to ABA.
2. ABA is not the only chemical regulator generated by environmental stress.

Field experiments with maize [51] and with desert-grown almond trees [52] showed a biphasic relationship between the xylem sap ABA concentration and the leaf conductance (Fig. 5) under circumstances in which other environmental and plant factors (including leaf water status) vary greatly. Within a relatively narrow range of xylem sap ABA concentration, small increases in ABA have a dramatic consequence for leaf conductance, and a further increase is largely ineffective. These results suggest that, in many plants, a highly sensitive stomatal response to ABA can be observed.

In field experiments, Tardieu and Davies [51] observed diurnal variations in the sensitivity of maize stomata to ABA. They provide strong evidence that the leaf water potential modifies sensitivity, with an increased stomatal sensitivity to ABA during the afternoon hours when the leaf water potentials are low. These data are consistent with the findings of Burschka et al. [53], who observed that *Arbutus unedo* guard cells exhibited a diurnal variation in sensitivity to small doses of ABA injected into the transpiration stream. Burschka et al. [53] found that, in their system, sensitivity was also increased during the later afternoon hours. In cotton leaves, nitrate and phosphate deficiency also enhance stomatal sensitivity to ABA [36,54].

Model calculations of Hartung and Slovik [7] may also be important in this context. These investigators concluded that a fivefold increase of ABA in the xylem sap is sufficient to increase

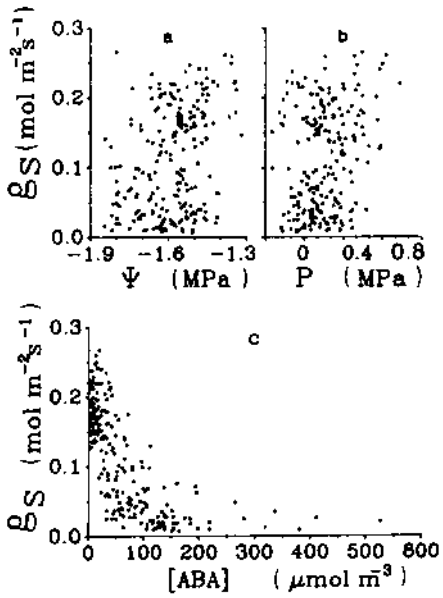

FIGURE 5 Stomatal conductance of maize plants growing in the field as a function of leaf water potential (a), leaf turgor (b), or xylem ABA concentration (c). (From Ref. 44.)

ABA at the primary site of action and that a twofold increase keeps the ABA at a constant high level over several days.

All these data show that small increases in ABA, as observed in beans, can be sufficient to explain the postulated stomatal responses, especially when changes in the ABA sensitivity of guard cells dependent on water and nutritional status are taken into consideration.

Detailed field observations [55] have suggested that the concentration of ABA in the xylem is determined not only by the degree of root dehydration but also by the rate of water movement through the soil-plant-air continuum. A high evaporative demand increases water flux and dilutes the ABA added to the transpiration stream from the drying roots. It is clear that without an increase in stomatal sensitivity to ABA, as the leaves dehydrate at higher fluxes, a chemical signaling system would have a little effect on hot and dry days, precisely the conditions under which it would be of most use.

Recent investigations of Daeter and Hartung [56] and Hartung et al. [57] demonstrated that ABA carriers at the epidermal plasma membranes and the metabolism of ABA in the cytosol of the epidermis and mesophyll cells are important for keeping the apoplastic ABA concentration in unstressed leaves low and for speeding up ABA redistribution under stress.

Recent experiments by Wilkinson (personal communication) suggest that even the very low concentration of ABA found in well-watered plants is important in the regulation of stomatal behavior. This is apparent, because the stomata of ABA-deficient mutants open in response to an alkaline pH treatment. The same treatment closes the stomata of wild-type plants. This means that low ABA concentrations may prevent excessive stomatal opening when conditions are such that an alkalinization of sap is promoted.

ABA Conjugates as Stress Signals

It has been postulated recently [58,59] that ABA-conjugates can act as root/shoot stress signals in a range of plants. Indeed, Bano et al. [60,61] have detected glucose esters of abscisic acid (ABA-GE), phaseic acid, and dihydrophaseic acid in the xylem sap of drought-stressed rice and sunflower plants, and Jeschke et al. [29,62] found ABA conjugates in the xylem sap of castor bean and maize plants.

As an example of ABA-GE as a potential long-distance hormonal signal in castor bean, the data of Figure 6 are shown which originate from the experiment shown in Figure 4, where leaves were sprayed with nitrate and ammonium. Under these conditions, ABA is conjugated in the leaves and deposited there, presumably in the vacuoles. Additionally, a significant part is loaded to the phloem and transported to the roots. There part of the ABA-GE is recirculated to the shoot. Similar to the case of free ABA (see Fig. 4), flows of conjugated ABA are stronger in ammonium-treated than in nitrate-treated plants.

ABA conjugates are believed to be extremely stable. In most cases, investigated conjugated ABA was not hydrolyzed to free ABA, especially when ABA-GE has been deposited in the vacuoles. The question arises of how ABA conjugates are taken up by the mesophyll cells after arrival in the leaf apoplast, because the permeability coefficient of the mesophyll plasma membranes for ABA-GE is extremely small (10^{-11} ms^{-1}) [63]. Additionally, we have to keep in mind that ABA-GE is physiologically inactive [64] and needs to be hydrolyzed before acting on the guard cells. Therefore, we postulate hydrolytic enzymes in the apoplast which may release ABA from the conjugate and allow free ABA to be taken up by the mesophyll cells and to act on the guard cells.

ABA as a Shoot to Root Stress Signal

The computer simulations by Slovik et al. [21] predicted that large quantities of ABA that has been synthesized in the mesophyll tissues should be loaded to the phloem and transported to sinks, mainly

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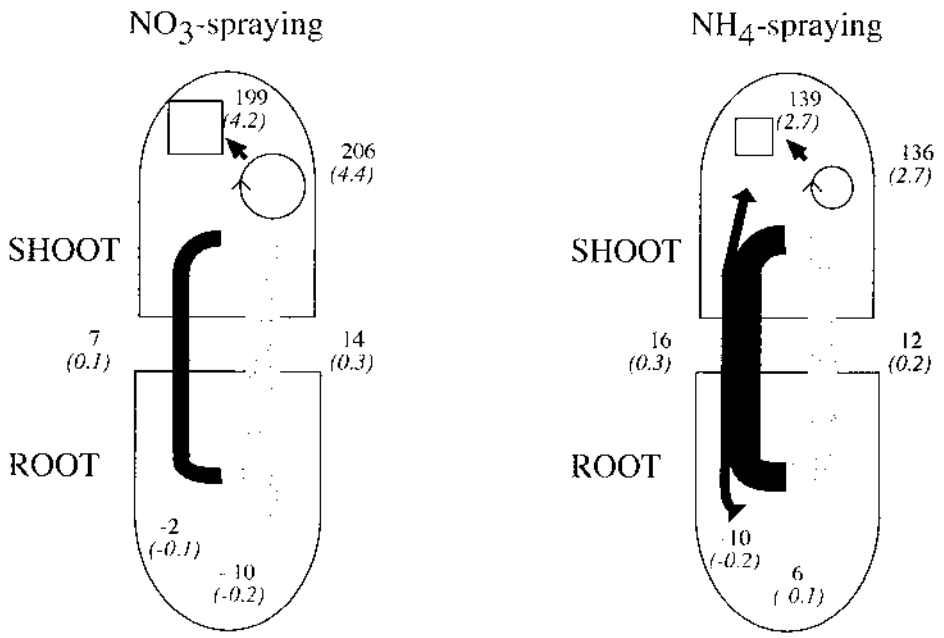

FIGURE 6 Flow profiles for transport, utilization, and net formation/degradation of conjugated ABA in castor bean plants that have been sprayed with nitrate or ammonium. For further details, see legend of Figure 3. (From A.D. Peuke and W. Hartung, unpublished data.)

the roots. As discussed earlier, a significant fraction of this ABA can be recirculated to the shoot again. ABA also can be taken up by the stelar and cortical tissues where developmental processes, such as lateral root and root hair formation, could be initiated.

A role as a shoot to root signal has been postulated for castor bean seedlings by Zhong et al. [65]. They observed that mild reduction of water potential in cotyledonal cells in the direct neighborhood of the phloem elements of *Ricinus* caused an increase of the ABA concentration in the phloem sap up to 50-fold. Our investigations of ABA long-distance transport of salt-stressed lupins and castor beans showed that fully expanded leaves acted as a net ABA donor for other sinks. This was not the case under nutrient deficiency. Neither nitrate- nor phosphorus-deficient *Ricinus* leaves exported significantly increased amounts of ABA to sinks [28,29].

Leaves of maize plants that were cultivated with their seminal roots only experienced drought stress because water flow was restricted by the constant number of xylem vessels in the mesocotyl, although the roots were sufficiently supplied with water and nutrients. The ABA content of the leaves of single-root maize plants were elevated up to 10-fold and leaf conductance was reduced significantly. Surprisingly, however, the ABA concentrations in the roots of single-rooted plants were clearly increased compared with the controls; most likely due to the increased ABA import via the phloem. In this system, ABA as a shoot to root stress signal may stimulate compensatory root growth of the seminal root system and may increase the hydraulic conductivity of the root system [62].

Figure 7 shows the relation between the leaf ABA concentration of castor bean plants and the phloem sap ABA concentration and the ABA phloem flow. These data support a role of the leaf ABA that is loaded predominantly to the phloem vessels and may act as a shoot to root stress signal.

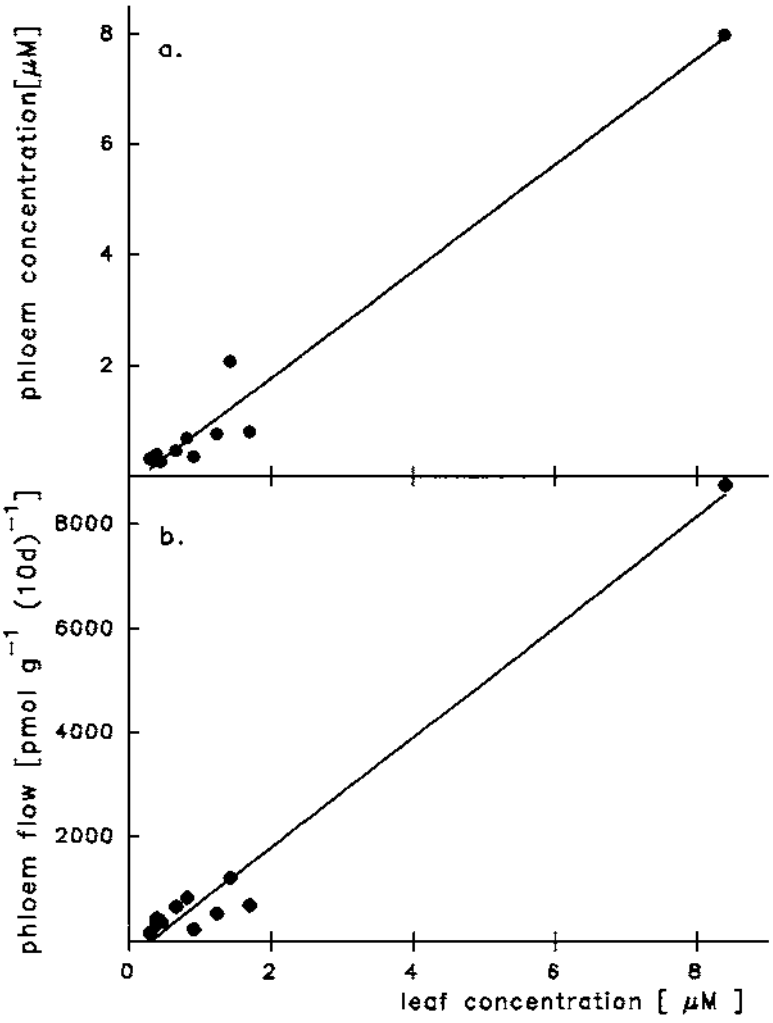

FIGURE 7 Relation between ABA concentration in castor bean leaves and ABA concentration and flows in the phloem. (Data taken from Refs. 28 and 29 and Fig. 4.)

ABA AND NONSTOMATAL REACTIONS

There has recently been much discussion of whether ABA affects nonstomatal processes in leaves, such as photosynthesis. Many conclusions have been drawn from gas-exchange experiments in which internal CO_2 concentrations are calculated before and after the application of ABA to the transpiration stream [66,67]. The results of many of these types of experiments suggest that ABA directly affects the photosynthetic processes in the leaf, but these results must be open to the question, since ABA may cause nonuniform stomatal closure [68], which can cause difficulties in the calculation of intercellular CO_2 concentrations.

ABA effects on the photosynthetic metabolism also have been investigated using the oxygen electrode. These results seem to be far more reliable, and with these techniques, the direct effects of ABA are negligible [69]. Experiments in which measurements of intercellular CO_2 were made

confirm this conclusion but suggest that water deficits may have a rather different effect on photosynthetic processes.

An additional nonstomatal stress physiological role has been suggested recently by Hollenbach et al. [70]. They observed that expression of the lipid transfer protein (LTP) gene (*ltp*) in barley leaves is stimulated when ABA is accumulated. LTPs play an important role in transfer of cutin and wax monomers from the site of synthesis to the cuticle. Thus, again, ABA influences the water relations of plants by increasing the wax layers on the cuticular surface.

ABA AND DEVELOPMENTAL PROCESSES

It has long been known that reduced soil moisture status can change the ratio of shoot to root in favor of the root system [71] and that similar responses and the formation of root-hairs and lateral roots can be stimulated by ABA applications [72,73]. Root growth at a reduced water potential was studied in detail by Sharp and coworkers [74]. This group showed that the tips of primary maize roots continue to grow under a reduced soil water potential when the growth and development of shoots are already inhibited. Maintenance of root growth at a low water potential is associated with a very substantial deposition of proline in the cells in the growing zone [75] and an increased activity of xyloglucan endotransglycosylase (XET) and the expansins. These enzymes may loosen cell walls and thereby sustain cell elongation in roots even when turgors are slightly reduced.

Treatment of roots with the carotenoid synthesis inhibitor fluridone reduces the ABA concentration in the root tips and substantially reduces growth at a low water potential [76] but not at a high water potential. This result suggests that roots have a requirement for ABA to help sustain cell expansion at a low water potential. Interestingly, fluridone treatment reduces proline deposition rates into the growing zone, but deposition rates are increased by ABA application to fluridone-treated roots.

A large number of developmental processes that depend on ABA accumulation under stress are listed by Trewavas and Jones [77].

CONCLUSIONS

There is an interesting parallel between the interacting effects of ABA and low water potential on both the root growth and the stomatal behavior. In both cases, the effects of ABA are increased as water potential falls. These results support the suggestion that there is a central role for ABA in the control of gas exchange and the growth and development of drought plants, but they also show that this chemical regulator cannot be considered to act entirely independently of changes in plant water relationships.

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Changes in Gene Expression in Response to Ultraviolet B–Induced Stress

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INTRODUCTION

Stratospheric Ozone Depletion and Ultraviolet B Fluxes

Ozone (O_3) is a minor constituent of the earth's upper atmosphere (stratosphere: 20–50 km above the earth's surface) and is produced by the combination of oxygen molecules formed by the breakdown of O_2 by the sun (Fig. 1). It is now well established that ozone concentrations in the stratosphere have declined during the last 25 years as a direct result of anthropogenic pollution of the atmosphere with a variety of chlorine- and bromine-containing compounds [1,2].

The importance of ozone in the stratosphere, with respect to life on earth, is that this is the only gas in the upper atmosphere that appreciably absorbs radiation below 300 nm. The absorption coefficient increases by many orders of magnitude with decreasing wavelength. Only the levels of ultraviolet B (UVB: 280–320 nm) radiation reaching the earth's surface will be affected by ozone loss (Fig. 2).

The detection of slight long-term increases in UVB levels due to ozone loss are difficult because the flux of UVB reaching the earth's surface is influenced by numerous atmospheric conditions apart from ozone content [3]. Nevertheless, increases in the levels of UVB attributed to ozone depletion have been reported over the Swiss Alps (0.7–1.0% increase per annum from 1979 to 1990) [4] and over New Zealand [5]. Although the precise nature of changes in the UV environment are not certain, it is apparent that there is likely to be increased amounts of UVB radiation reaching the earth's surface; an estimated 1.5- to 2.0-fold increase for every 1% loss of ozone. The regions in the Southern Hemisphere, where ozone depletion is most severe and the atmosphere relatively unpolluted, will likely be more significantly affected.

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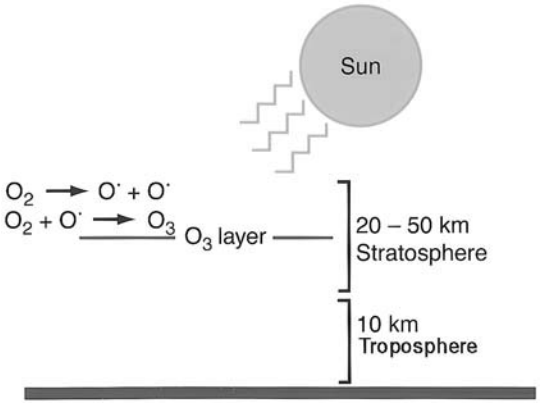

FIGURE 1 A schematic representation of the earth's upper atmosphere. The position of the ozone layer is indicated.

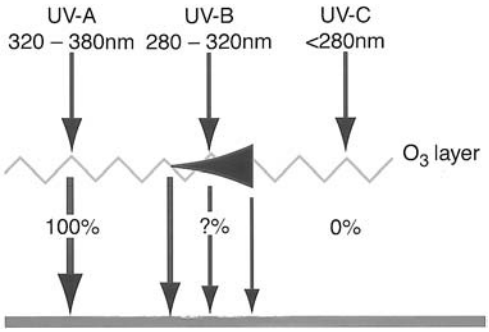

FIGURE 2 Diagrammatic representation of changes in UV penetration to the earth's surface in relation to changes in stratospheric ozone. At shorter wavelengths, <280 nm (UVC), absorption by ozone is so great that a small fraction of the present ozone layer is sufficient to block this radiation completely. At wavelengths >320 nm (UVA: 320–380 nm) absorption by ozone is so weak that changes in ozone are of no consequence. Only the levels of ultraviolet B (UVB: 280–320 nm) radiation reaching the earth's surface will hence be affected by ozone loss.

UVB radiation is potentially damaging to all living organisms, but it is especially so for plants, as they require sunlight for survival and as such are unable to avoid exposure to changes in the UVB environment. There have been many studies on the effects of UVB on plants, and this work has been extensively reviewed [6–8] and the most common effects are summarized in Figure 3. In addition, the exposure of plants to UVB radiation has been more recently shown also to have profound effects on cellular metabolism. These effects include the suppression of primary metabolic functions such as the expression and synthesis of the photosynthetic proteins Rubisco large (*rbcL*) and small (*RbcS*) subunits and chlorophyll *a*/*b*–binding proteins (*Lhcb*) (reviewed in Refs. 9, 10); the induction of elevated antioxidant responses, such as the expression and activity of superoxide dismutase (SOD), glutathione reductase (GR), and ascorbate peroxidase (APX) [11–13]; and the accumulation of phenolic pigments [14,15]. The effects of UVB at this level are likely mediated

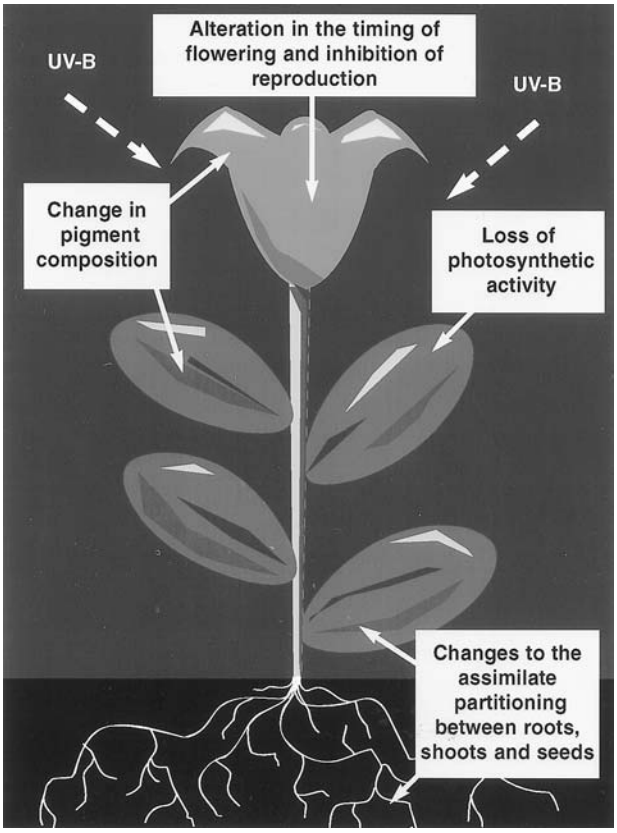

FIGURE 3 Diagrammatic representation of the most common effects of UVB on plant physiology and biochemistry.

through specific photoreceptors and subsequent signal transduction pathways and/or through absorption of UVB by DNA leading to subsequent changes in gene expression.

This chapter reviews the effects of UVB radiation on cellular metabolism, and more specifically gene expression, and the mechanisms and pathways by which UVB mediates these effects.

EFFECTS ON GENE EXPRESSION

In contrast to the physiological and biochemical impacts of UVB on plants, little is known about the effects of UVB on gene expression. The best-characterized genes are those encoding key proteins involved in photosynthesis, which are downregulated in response to UVB, and several genes encoding ''defense'' proteins, which are upregulated. This section will consider these two sets of genes separately.

Chloroplast Proteins

The exposure of plants to supplementary UVB leads to a reduction in photosynthesis, and biochemical studies have shown that chloroplasts are a major site of UVB damage. UVB exposure leads to a reduction in the efficiency of electron transport, photophosphorylation, and carbon fixation, all of

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which contribute to the decreases observed in the rate of photosynthesis [16,17]. More recently, it has become clear that UVB radiation also effects the expression of genes encoding key photosynthetic proteins and that UVB effects at this level can account, to some extent, for the overall inhibition of photosynthesis by UVB radiation [17–19]. In these studies, transcripts for key chloroplast proteins decline in response to UVB exposure within hours leading to a subsequent loss of enzyme activity and protein content over a period of days [17,19]. These studies have also highlighted that UVB-induced changes in gene expression are complex and dependent on several parameters, including the developmental stage of tissue studied and the level of background light provided throughout the UVB treatment [17,20]. These points will be discussed in detail in the section on Factors Affecting Gene Expression below.

Chloroplast proteins are encoded on the nuclear and chloroplast genomes. Both nuclear- (*Lhcb, RbcS* and the γ-CF_1-ATPase) and chloroplast-encoded (*rbcL, psbA,* CF_1-ATPaseB-E, cytochrome *b*, and subunit IV of the cytochrome *b*/*f* complex) transcripts are reduced in response to UVB exposure [9]. The actual extent of the downregulation is dependent on the severity of the UVB exposure; however, in general, chloroplast-encoded transcripts are maintained for longer periods than nuclear-encoded transcripts (Fig. 4). Hence, the reduction in the nuclear-encoded *Lhcb* transcript occurs rapidly, with as much as about an 80% reduction after 4 days (Fig. 4a[i]) in contrast to the chloroplast-encoded *psbA* transcripts which remain at about 90% of the control level even after 4 days of UVB exposure (Fig. 4a[ii]) [19,20]. These differences, however, do not necessarily provide a true reflection of the decline in the protein levels or, in fact, the extent of the loss of a particular photosynthetic function.

Nuclear genes are primarily regulated at the transcriptional level, whereas plastid-encoded genes also are subjected to posttranslational regulation [21]. The differences in the response of nuclear- and chloroplast-encoded photosynthetic genes may simply be a reflection of these different regulatory processes. Although there is only limited data available to define at what level the inhibition of UVB on gene expression takes place, studies carried out so far are consistent with this idea. The decline in the steady-state level of the nuclear-encoded *Lhcb* gene corresponded to the loss in the protein concentration of the chlorophyll *a*/*b*-binding protein (see Fig. 4a[i] and b[i]). In addition, the functionality of the mRNA, as determined by in vitro translation assays, was consistent with the mRNA and protein profiles. These results indicate that nuclear-encoded genes appear to be largely regulated at the transcriptional level in response to UVB [19]. The regulation of these genes may, however, be more complex. For instance, in ^{35}S-methionine labeling of pea leaf disks, the

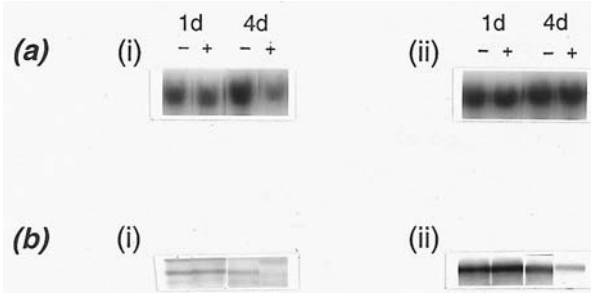

FIGURE 4 Changes in expression and protein levels of photosynthetic genes in response to UVB radiation. (a) Autoradiographs of Northern blot analysis of total RNA from fully expanded leaves of 17-day-old pea seedlings treated with (+) or without (−) supplementary UVB exposure. The RNA was then hybridized to ^{32}P-labeled (i) *Lhcb* and (ii) *psbA* CDNA. (b) Western blot analysis of total protein isolated from fully expanded pea leaves treated with (+) or without (−) supplementary UVB exposure. Antibodies were to (i) chlorophyll *a*/*b*–binding proteins (ii) D1 polypeptide of PSII.

results indicated that the methionine may have been incorporated into the initiation complex (80 S.Met-tRNA.mRNA) without further elongation of the peptide chain [18]. This suggests that there may also be some posttranslational inhibition. In contrast, the picture is very different for chloroplast-encoded proteins. The decline in the level of the chloroplast-encoded D1 polypeptide of photosystem II (PSII) was not in parallel with *psbA* mRNA profiles, with the polypeptide levels declining prior to any loss of mRNA (see Fig. 4a[ii] and b[ii]). ^{35}S-methionine–feeding experiments followed by chase experiments illustrated that UVB exposure enhances both the rate of synthesis and the degradation of these proteins [10,19], as has been demonstrated under conditions of high visible light [22] indicating that the levels of this protein are primarily regulated posttranslationally [19]. Interestingly, the photosensitizers of D1 degradation in the different regions of the spectrum are distinct: the bulk photosynthetic pigments in the visible and red region and plastoquinone in the UV region [23,24].

Defense Genes

Plants have evolved a number of protective mechanisms against UVB-induced damage with the three most important being attenuation of UVB radiation by protective pigments, the production of antioxidants, and the repair of UVB-induced lesions in nucleic acids. The latter will be discussed in detail in the section on DNA Damage and Repair, whereas this section will concentrate on the production of pigments and on the various antioxidant mechanisms.

The most extensively studied defense mechanism in response to UVB stress is the production of protective pigments, flavonoids, a group of secondary products which include the flavones, flavonols, isoflavonoids, and anthocynins [14,15]. These compounds are considered to be major attenuators of UVB radiation. Direct evidence for the protective function of flavonoids has come from the use of *Arabidopsis* transparent testa (*tt*) mutants defective in flavonoid biosynthesis. These studies have illustrated that a few specific flavonoid compounds, including sinapate esters and hydroxycinnamate esters, may be particularly important in UVB tolerance [25,26] and in the protection of photosynthetic transcripts against UVB irradiation (see below) (B.R. Jordan, P.E. James, S. A.-H.-Mackerness, unpublished data). However, it is important to note that flavonoids are effective antioxidants and may play an important role in this respect [27].

The increase in the level of these protective pigments in response to UVB irradiation is due to a dramatic and coordinated increase in the expression and activity of the enzymes of the phenylpropanoid pathway (e.g., chalcone synthase [*Chs*], phenylalanine ammonia lyase [*PAL*], chalcone isomerase [*CHI*], and dihydroflavonol reductase [*DHFR*]) [28]. Studies in pea and *Arabidospsis* have shown that, concomitant with the UVB-induced downregulation of gene expression for photosynthetic proteins (section on Chloroplast Proteins above), there is an increase in *Chs* (Fig. 5a) [18,19] and *PAL* expression [12,29] and a subsequent increase in the composition of protective pigments (Fig. 5b) [18,19,30]. These increases have been shown by run-off transcription assays on isolated nuclei to be caused by changes in transcription [31].

The role of flavonoids in protecting gene expression has only recently been confirmed. Using *tt*-5 mutants, it was demonstrated that flavonoids do play a part in the protection of photosynthetic transcripts against UVB (B.R. Jordan, P.E. James, and S. A.-H.-Mackerness, unpublished data). Transcript levels declined faster in the *tt*-5 mutants than in the wild-type plants on exposure to supplementary UVB radiation (Fig. 6). One of the proposed roles of flavonoids in response to UVB exposure has been shielding of DNA from UV-induced damage [32,33]. Thus, the greater reduction in transcripts in *tt*-5 mutants as compared with wild-type plants in response to UVB was likely due to UV-induced damage to the DNA in the mutants. However, the absence of these pigments did not affect the relative sensitivity of photosynthetic transcripts at different developmental stages; photosynthetic transcripts from older leaves were reduced faster than transcripts in younger leaves in these mutants, as in wild-type plants (Fig. 6; see the section on Factors Affecting Gene Expression below); thus indicating that it is unlikely that DNA damage could be the primary mechanism by which transcripts are regulated in response to UVB in wild-type plants.

The regulation of the flavonoid biosynthetic pathway may be controlled by UVB, UV/blue

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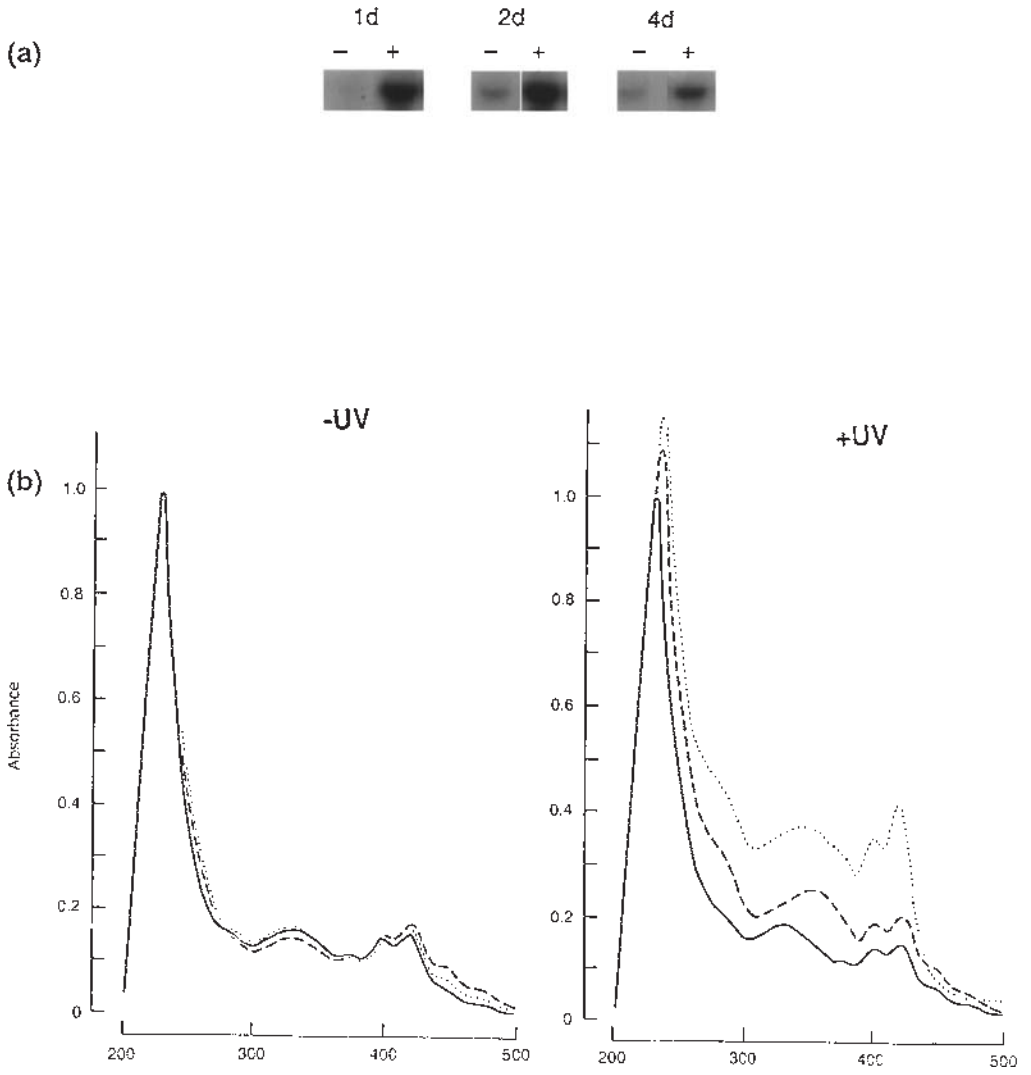

FIGURE 5 Changes in expression of *Chs* gene and pigment composition in *Arabidopsis thaliana* in response to UVB radiation. (a) Autoradiographs of Northern blot analysis of total RNA from the outer leaves of 6-week-old *Arabidopsis* plants treated with (+) or without (−) supplementary UVB exposure. The RNA was then hybridized to ^{32}P-labeled *Chs* CDNA. (b) Flavonoid profile obtained from the outer leaves of 6-week-old *Arabidopsis* plants treated with (+UV) and without (−UV) supplementary UVB for (———) 8 h, (----) 3 days, and (·····) 7 days.

photoreceptors, and phytochrome depending on the species and the stage of development [34]. In mature leaves of several species, including *Arabidopsis, Chs* expression is primarily regulated by UVB and UV/blue light, whereas in young or dark-grown seedlings, phytochrome is involved [35–38]. The light-dependent regulatory transcription elements within the *Chs* genes in several species have been characterized. The signal transduction pathways acting via different photoreceptors merge at a small light regulated unit (LRU1) of 52 bp on the *Chs* promoter. LRU1 has been shown to be

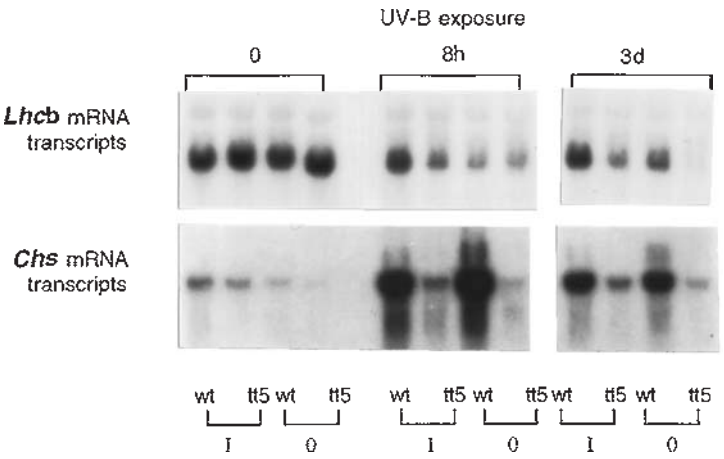

FIGURE 6 Changes in expression of *Lhcb* and *Chs* genes in wild-type and *tt-5* mutants in response to UVB exposure. Autoradiographs of Northern blot analysis of total RNA from inner (I) and outer (O) leaves of 6-week-old *Arabidopsis* wild-type (wt) and *tt-5* mutants (tt5) treated with supplementary UVB for a period of 3 days.

sufficient to confer a signal to gene expression by activation of transcription factors binding to this element [39,40].

In addition to light, the enzymes of the phenylpropanoid pathway are also induced in response to other abiotic and also biotic stresses, including treatment with fungal elicitors. However, treatment with elicitors, unlike UVB exposure, results in the transcriptional activation of *PAL* but not the *Chs* genes [31]. In fact, the treatment of parsley culture cells with fungal elicitors strongly inhibits transcription of the *Chs* gene and blocks its UVB induction. Interestingly, it has been shown that the same elements within the *Chs* promoter are involved in the downregulation of *Chs* gene expression in response to elicitors and upregulation in response to UVB [39,40].

A common feature in plant responses to different environmental stresses is the rapid generation of reactive oxygen species (ROS) and the rise in intracellular oxidative stress suggesting that different environmental challenges may be linked to induced cellular responses by common signal mechanisms (further details are in the section on Signal Transduction below). Consistent with the induction of oxidative stress, UVB exposure leads to an increase in the concentration of lipid peroxides [41–43] and changes in mRNA transcripts [12,44] and the activities of antioxidant enzymes [11,13,45]. The actual defense mechanism(s) involved remain to be elucidated and do vary between species. In pea, RNA transcripts for cytosolic Cu/Zn-SOD and mitochondrial Mn-SOD increase substantially in response to UVB exposure. Similarly, the mRNA transcripts for all three enzymes of the ascorbate/glutathione cycle increase. In contrast, the transcripts for the chloroplastic Cu/Zn-SOD are decreased [12,46]. It is possible that the reduction of the chloroplastic SOD is either a reflection of the need for the protection of the rest of the cell at the expense of the chloroplasts or an indication of the site of action of UVB, which may be at the cytoplasm or cell exterior and not within the chloroplast. Studies using *gun* mutants have added weight to the later theory. Oxidative damage within the chloroplast, mediated by the chloroplast signal transduction pathway, is frequently cited to explain the downregulation of nuclear-encoded genes for chloroplast proteins [47,48]. The *gun* mutants are defective in this pathway [48]; however, gene expression was still inhibited by UVB exposure in these mutants suggesting that the chloroplast signal is not involved in the downregulation of the nuclear-encoded photosynthetic proteins [9]. However, recent studies have indicated that the ROS generated in response to UVB do play a role in the signal transduction pathway leading to the downregulation of photosynthetic genes [49] (S. A.-H.-Mackerness, L. Surplus, B.R. Jordan,

and B. Thomas, unpublished data). This will be disscussed in more detail in the section on Signal Transduction below.

Stress-mediated changes in the abundance of antioxidant enzyme transcripts do not always correlate with a corresponding change in the antioxidant enzyme protein level/or activity [50,51]. A few studies have looked at the effect of UVB on the activity of antioxidant enzymes. However, the limited information available indicates species differences. For example, in cucumber, SOD and APX activity increases in response to UVB exposure [11], whereas in *Arabidopsis*, only APX [13] and in pea APX, GR, and SOD activity increases [46] (S. A.-H.-Mackerness, L. Surplus, B.R. Jordan, and B. Thomas, unpublished data). The most decisive way to determine the importance of a particular defense response in relation to a stress is to examine its role in over- or underexpressing transgenic plant system. Unfortunatly, studies using these transgenic plants modified in the content of various antioxidant enzymes have highlighted the complexity and perhaps species variablity present in response to various stresses and have not provided definitive answers. In addition, few have been studied with respect to UVB damage [9].

In addition to antioxidant enzymes systems, plants contain an array of nonenzymatic antioxidants, including ascorbic acid (vitamin C), glutathione, α-tocopherol (vitamin E), and carotenoids [52]. An increase in the level of these compounds has been reported in response to UVB exposure [12,43], and the importance of ascorbic acid has been confirmed in UVB tolerance by the use of an ascorbic acid–deficient *Arabidopsis* mutant [53].

From this short review of the mechanisms involved in UVB defense, it is clear that our knowledge remains limited and that further investigation will bring substantial benefits in our understanding of cellular defense mechanisms.

FACTORS AFFECTING GENE EXPRESSION

Little is known about the interaction of UVB with other stresses and the factors that affect the sensitivity or response of plants to UVB radiation. Plants respond to an array of stresses through common defense mechanisms, and therefore exposure to one stress may lead to protection or limited tolerance to another (reviewed in Ref. 9). For example, limited water availablity (water stress) increases the tolerance of some plant species to UVB stress [54]. However, the information available on the effect of various stresses on the sensitivity to UVB is limited and even less is known about the effects on gene expression. This section will therefore concentrate on the influence of the developmental stage of the tissue and high photosynthetically active radiation (PAR) on the UVB-induced effects on gene expression, which have been recently investigated further.

The majority of studies conducted on the molecular impact of UVB have been carried out in pea and using fully expanded leaves. However, recent studies have highlighted the differential sensitivity of plants to UVB irradiation at different developmental stages. In *Arabidopsis*, the older leaves become damaged by UVB faster and to a greater extent than the younger leaves [55]. A similar pattern of sensitivity is noted with respect to mRNA transcripts which are not as severely downregulated in younger leaves as they are in older tissue (see Fig. 6) (B.R. Jordan, P.E. James, and S. A.-H.-Mackerness, unpublished data). In pea, where these differences have been best characterized, the mRNA transcripts for chloroplast genes are maintained in young buds and not downregulated to the same extent as they are in older fully expanded leaves (Fig. 7a, b). In addition, during greening of etiolated buds, gene expression continues apparently unaffected by irradiance with UVB (Fig. 7c) [18]. Similar to *Arabidopsis*, visual assessment of UVB-induced damage correlates with the effects on RNA transcripts. Therefore, the differential sensitivity to UVB at different developmental stages is evident at the physiological, biochemical, and molecular levels.

The mechanism of the differential sensitivity of photosynthetic transcripts to UVB has been recently studied further in pea. Using a highly sensitive gene-specific polymerase chain reaction (PCR) technique [56], the role of individual members of the *Lhcb* genes family in pea was tested for their role in this response (S. A.-H.-Mackerness, L. Liu, B.R. Jordan, B. Thomas, and W.F.

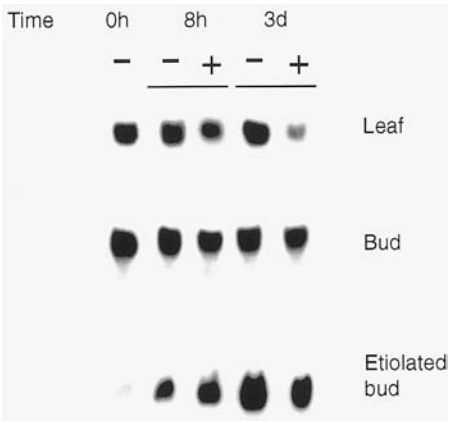

FIGURE 7 Changes in expression of the *Lhcb* gene at different developmental stages. Autoradiographs of Northern blot analysis of total RNA from (a) fully expanded leaves, (b) green buds of 17-day-old pea seedlings, and (c) 6-day-old etiolated buds treated with (+) or without (−) supplementary UVB radiation.

Thompson, M.J. White, unpublished data). The results indicated that the differential sensitivity to UVB at the different developmental stages is not due to the expression of different members of the *Lhcb* multigene family. The results, did, however, show that the genes could be divided into two groups with respect to their response to UVB at different developmental stages. These two groups had been previously identified in pea based on their responses to red and blue light and the differences in their relative levels of expression in buds and leaves [56,57]. These observations therefore indicate that the response of the two *Lhcb* gene groups to different light qualities may involve a common regulatory or signal transduction pathway.

In addition to the developmental stage of the tissue studied, the level of background light provided throughout the UVB treatment has a profound influence on the sensitivity of plants to UVB radiation. A high PAR (400–700 nm) can ameliorate the damaging effects of UVB [58–60] and the reduction in RNA transcripts to some extent [17,20]. Two mechanisms for this protection have been examined: (a) an increase in the repair of DNA damage by an increase in the activity of DNA repair enzymes, photolyases, and (b) an increase in the rate of photosynthesis. Photolyases are light-activated DNA repair enzymes involved in the repair of lesions formed in DNA on UVB exposure [61,62]; these are discussed further in the section on DNA Damage and Repair below. Several studies have indicated that the protective effect of high PAR is primarily due to increases in the activity of these enzymes under these higher light conditions [58,63,64]. However, recent studies in wheat have illustrated that the activities of these enzymes are saturated at very low light levels [65]. Therefore, the effects of PAR noted at much higher light levels are unlikely to be due to increases in the activity of these enzymes. Adamse and Britz [60] were the first to provide an alternative mechanism. They illustrated that increases in photosynthesis alone were sufficient to account for the protection against UVB-induced physiological damage in cucumber leaves under high light conditions. Using a similar approach, A.-H.-Mackerness et al. [20] illustrated that the high PAR protection of gene expression was also related to photosynthetic activity and not photorepair mechanisms (Fig. 8).

The carbohydrate products of photosynthesis are important regulators of chloroplast function, and it is well established that increases in the amount of sugars lead to the feedback inhibition resulting in a reduction in photosynthetic efficiency [66–68]. More specifically, these studies have shown that the decline in RNA transcripts encoding photosynthetic proteins, resulting in decreases in the content and activity of these proteins, leads to the reduction in the rate of photosynthesis

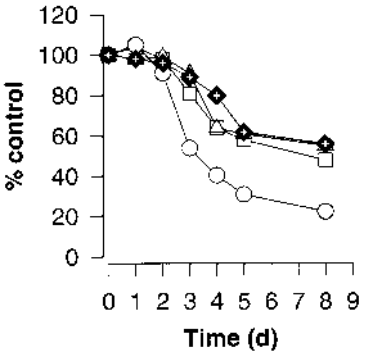

FIGURE 8 Changes in expression of *rbcL* gene under four light treatments in response to UVB radiation. Pea seedlings were grown under low-light conditions (150 μmol $m^{-2}s^{-1}$ PAR) and then exposed to supplementary UVB under low light (LL: ○), high light (HL_w, 350 μmol $m^{-2}s^{-1}$ PAR: □), high CO_2 (H_{CO_2}, 1500 ppm CO_2: △) or HL levels provided by high-pressure sodium lights (HL_{Na}: ◆). H_{CO_2} and HL_{Na} were used to increase photosynthetic rates to HL levels without altering the activity of photolyase. Northern blot probed with ^{32}P-labeled *rbcL* CDNA were quantified by determining the amount of bound radioactivity by liquid scintillation counting. (Modified from Ref. 20.)

[68,69]. In fully expanded leaves of pea seedlings, UVB treatment was reported to increase the levels of soluble sugars and starch [70] suggesting that the rapid decrease in mRNA levels for photosynthetic proteins, in response to UVB, could be regulated through a form of metabolic feedback. The relationship between photosynthesis and the carbohydrate status will vary substantially during chloroplast and organ development (e.g., switch from sink to source.) and at different light intensities. As such, therefore, this was an attractive proposal, as it could account for the differential effect of UVB at various developmental stages and also provide a mechanism for high light protection. However, the levels of UVB used in this study were high, and more detailed studies using more realistic UVB levels have revealed that UVB exposure leads to a decrease in the level of glucose rather than an increase in the response to UVB irradiation [71] (Fig. 9). Furthermore, comparison of the UVB effects on carbohydrates in source and sink organs indicated that changes in carbohydrates in response to UVB are clearly indirect and arise from the effects of UVB on photosynthesis in source organs [71].

Recent studies have highlighted similarities between plant responses to UVB and pathogen stress with generation of ROS being a common feature of both responses (see the section on Signal Transduction below) and have thus provided a starting point for dissecting the UVB-induced signal pathways. Initially by using cell cultures systems [49] and more recently using whole plants (S. A.-H.-Mackerness, L. Surplus, B.R. Jordan, and B. Thomas, unpublished data), it has become clear that UVB regulates photosynthetic genes through an ROS-dependent pathway (further details in the section on Signal Transduction below). As a result of these findings, a common mechanism was postulated to explain developmental sensitivity and high light protection against UVB damage, taking into account the role of photosynthesis and ROS in the sensitivity to UVB radiation.

The level of ROS within a cell is a balance between their generation and removal [72], and recent studies have shown that, consistent with this, the sensitivity of photosynthetic transcripts and the whole plant to UVB radiation is dependant on the antioxidant capacity of the tissue studied (S. A.-H.-Mackerness, L. Surplus, B.R. Jordan, and B. Thomas, unpublished data). The antioxidant capacity of a cell is primarily dependent on the rate of photosynthesis which in turn is dependent on, among other parameters, the developmental stage of the tissue [73,74] and the irradiance levels [75,76]. Therefore, younger tissues and plants under high light have higher antioxidant capacity,

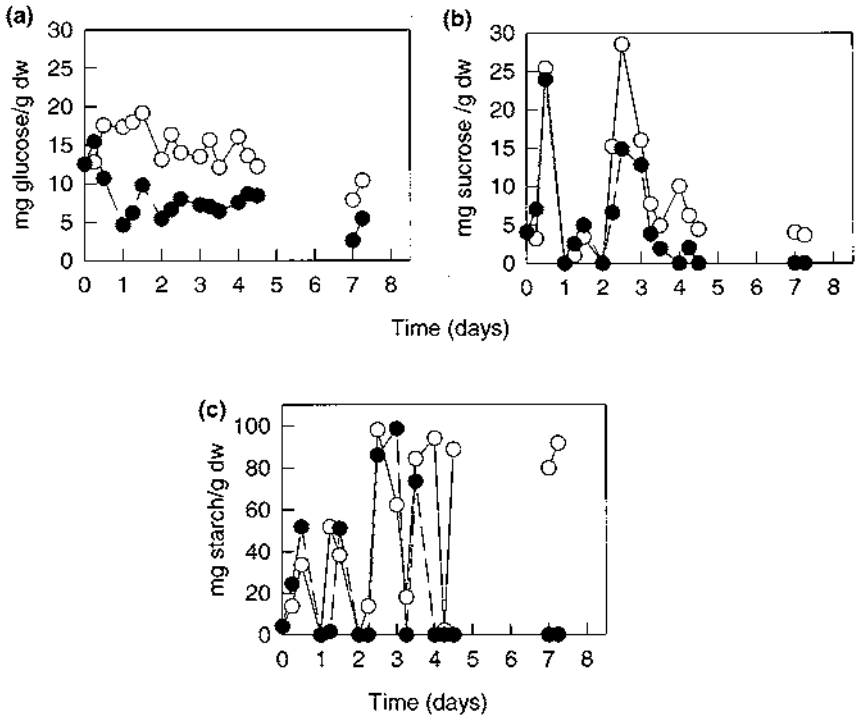

FIGURE 9 Effect of UVB on the carbohydrate status of pea leaves. Carbohydrate levels in the third leaf pair of 17-day-old pea seedlings were determined from plants treated with (●) or without (○) supplementary UVB radiation. (Modified from Ref. 71.)

as their photosynthetic rates are higher, and are thus able to remove ROS more effectively and hence are less sensitive to UVB-induced stress (S. A.-H.-Mackerness, L. Surplus, B.R. Jordan, and B. Thomas, unpublished data).

Further work is required to clarify the role of ROS in UVB-mediated stress and to determine the importance of different antioxidant enzymes in UV resistance. This information could provide potential targets for genetic engineering of plants for UV tolerance.

DNA DAMAGE AND REPAIR

DNA strongly absorbs UVB radiation and is therefore likely to be damaged by this highly energetic radiation. Most studies of UVB-induced damage and repair of DNA have been carried out in animals or microorganisms and the results extrapolated to plants. More recently, however, detailed research on DNA damage and repair in plants has been carried out (for further details see Refs. 9, 61, and 62).

The major DNA damage caused by UVB radiation is the formation of dimeric photoproducts, cyclobutane pyrimidine dimers (CPDs), and pyrimidine (6-4) pyrimidone photoproducts. The (6-4) photoproducts are also readily isomerized to Dewar photoproducts by UVA radiation, and this should be accounted for in the determination of UVB-induced damage. The formation of CPDs is the most common lesion and takes place throughout the DNA molecule. Formation of (6-4) photoproducts, however, takes place in actively transcribed regions of the DNA [77,78]. Consequently, the presence of (6-4) photoproducts may have more deleterious consequences, especially as they do not appear to be repaired as rapidly as CPDs. Although dimeric DNA lesions form the predominant DNA

damage, other lesions can occur, including the formation of monomeric photoproducts, DNA strand breaks and cross linking of DNA to protein [79]. These lesions may still have a significant impact on the biological function of the cell despite being a minor proportion of the DNA damage if the appropriate repair mechanisms are not available.

DNA damage, if not repaired, will have a profound influence on the function of plant cells, pyrimidine dimers are known to inhibit DNA and RNA polymerase in mammalian and microbial systems [78,80]. Consequently, CPDs or (6-4) photoproducts will prevent transcription of the DNA. To overcome this, cells contain a range of repair mechanisms that recognize lesions in the DNA. These repair mechanisms can be grouped as photoreactivation, excision repair, and recombinational repair [61,81].

The photoreactivation of DNA dimers (CPDs and 6-4 photoproducts) is carried out by photolyases that use a range of wavelengths in the UVA and blue region (300–500 nm) depending on the source and the presence of different chromophores within the photolyase [61,82]. Photoreactivation is both economical, efficient, and error free and has now been demonstrated in a range of plant species [79], including *Aradidopsis thaliana* [83,84] and maize pollen [85]. In wheat leaves, photorepair activity was found to be effective even at very low irradiance (2 μmol $m^{-2}s^{-1}$ PAR), with the removal of thymine dimers being much more efficient in the light than in the dark [65]. Photolyase activity, as well as being dependent on light, is also temperature dependant [83], which could be of substantial significance given the predicted scenario of changes in the global temperature. Recently, the first plant CPD photolyase gene was isolated from *Arabidopsis*. This sequence was found to be similar to the mammalian type II photolyases and not the type I photolyases found in bacteria [86]. Expression studies indicated that photolyase was not expressed in etiolated tissues and was inducible by blue and UVB light, which is similar to the photoreactivation activity in plants. Expression levels were highest in flowers, perhaps reflecting a need to protect exposed gametes from DNA damage.

Few plant systems have been studied in great detail with respect to DNA repair, and to date only a handful of studies have been carried out under field conditions. A substantial variation in the levels of DNA damage between different tissues was noted in field-grown maize exposed to ambient levels of UVB radiation [87]. No difference in DNA damage levels was, however, found between green tissue (leaf sheath and leaf blade), suggesting that architecture has no influence, with photoreactivation activity occurring in both the epidermis and inner cell layers of leaves. Although there was a slight increase in DNA damage throughout the day, no accumulation of damage was noted over the growing season.

In *Arabidopsis* seedlings, little or no photolyase activity was observed in the organellar genomes and high levels of DNA damage accumulated on UVB exposure. However, as only marginal effects on growth were noted, it was suggested that *Arabidopsis* must possess very efficient mechanisms for the tolerance of UV-induced DNA damage [84]. Surprisingly, in contrast, studies in maize indicated that all organelles that contain DNA (nucleus, plastid, and mitochondria) also contain the capacity to photorepair DNA [87]. Monocots tend to be more UV resistant than dicots. It is possible that if a more diverse distribution of photorepair enzymes is a general feature of monocots, then this could perhaps explain the difference in UV tolerance between these different plant types.

The mechanisms of excision repair of DNA in plants are not well characterized and are frequently referred to as ''dark repair'' to distinguish them from photorepair. In some species, no excision repair has been detected and in others the levels are significant but are much slower than in the presence of photoreactive light [62]. Interestingly, it has been suggested that the relative contribution of DNA repair mechanisms may differ depending on the levels of UVB damage [88]. Thus, at higher levels of damage to alfalfa seedlings, both photorepair and excision repair made significant contributions to CPD removal. At lower levels, however, only photorepair could be detected. The third type of repair, recombinational repair, has not yet been demonstrated in plants.

The information on DNA repair mechanisms in plants lags greatly behind that of mammalian and microbial systems. However, owing to the concern over increases in UVB radiation, there recently has been considerable effort made to better characterize the repair systems in plants. The

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information available indicates very efficient repair systems both in the light and dark with genes being rapidly and specifically repaired. Owing to this efficiency, it is unlikely that the effects of UVB on gene expression (see sections on Factors Affecting Gene Expression above) can be due to nonspecific DNA damage.

SIGNAL TRANSDUCTION

Our understanding of the signal transduction pathways leading to various UVB responses is very limited. In recent years, the application of knowledge from mammalian systems has provided some insight into the details of these pathways. In addition, the realization that plant responses to UVB stress have commonalties with plant responses to a variety of other stresses, including pathogen [89,90] and wounding [91] responses, has provided a starting point to allow the elucidation of these complex cascades. For example, in both responses to pathogens and UVB, photosynthetic genes are downregulated and the acidic and basic pathogenesis-related (PR) genes are upregulated (Figs. 10 and 11a).

Salicylic acid (SA) has been recognized as an endogenous regulator in plants and it is now accepted as a component of the signal pathway leading to plant responses to pathogens [92,93]. In addition, ROS generated after pathogen attack are considered to play a role in the regulation of disease resistance [94,95]. Regulation of genes in response to UVB radiation has been shown to involve components of this pathway. UVB exposure can result in the production of ROS [43,96,97] and can lead to increases in SA levels [98].

Numerous studies have demonstrated that SA levels rise after pathogen attack and that SA is the endogenous signal for the induction of several plant defense responses, including the expression of the acid-type PR genes [92,93,99]. The role of this compound in upregulation of the PR genes was confirmed in experiments using transgenic plants (NahG plants). These plants overexpress the salicylic hydrolase gene from *Pseudomonas putida* which catalyzes the conversion of SA to catacol. Therefore, these plants are unable to accumulate significant levels of SA [100]. In these plants, pathogenic infection did not lead to the accumulation of PR transcripts. In similar experiments, using these transgenic plants, it was demonstrated that SA was a component of the UVB signal pathway leading to the upregulation of three acid-type PR transcripts (PR-1, PR-2 and PR-5) (see Fig. 11b). However, photosynthetic genes were still downregulated in response to UVB in these transgenic plants indicating that SA is not involved in this pathway (see Fig. 11b). Feeding/spraying

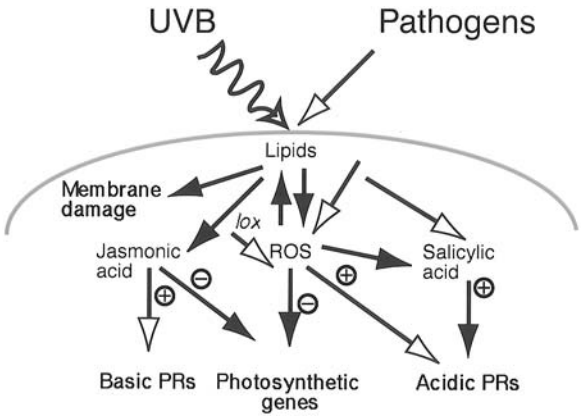

FIGURE 10 Schematic representation of pathogen-induced signal transduction pathways. Possible commonalties with responses to UVB stress are indicated with solid arrows.

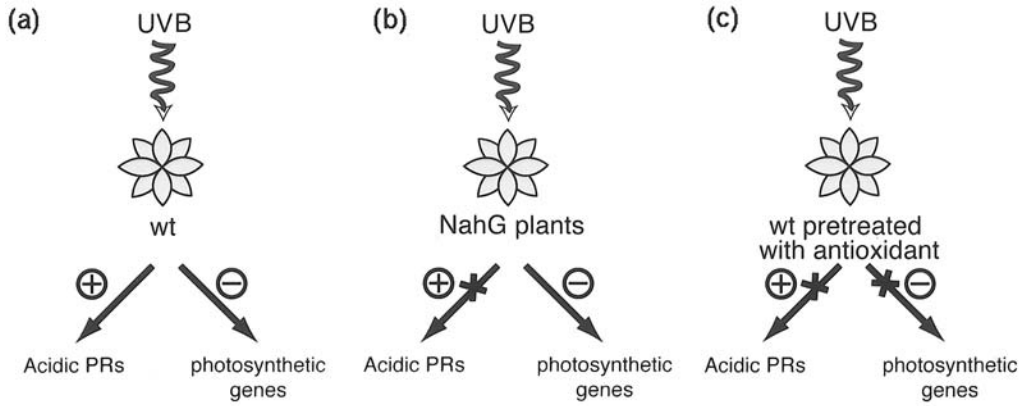

FIGURE 11 Schematic representation of the role of salicylic acid and reactive oxygen species (ROS) in the signal pathway leading to the downregulation of photosynthetic genes and the upregulation of the acid-type PR genes in response to UVB. (a) UV treatment of wild-type (wt) *Arabidopsis* leads to an increase (+) in PR transcripts and a decrease (−) in photosynthetic transcripts. (b) UVB treatment of transgenic NahG plants, which cannot accumulate SA, does not lead to an increase in PR transcripts but the decrease in photosynthetic transcripts still occurs. (c) Treatment of wild-type (wt) plants with antioxidants [e.g., ascorbic acid or pyrrolidine dithiocarbamate (PDTC)], prior to UVB treatment prevents both the increase in PR transcripts and the decrease in photosynthetic transcripts in response to UVB exposure.

experiments using antioxidants and oxidants in wild-type plants have shown that ROS are also involved in the upregulation of PR genes [89] (L. Surplus, B.R. Jordan, B. Thomas, and S. A.-H.-Mackerness, unpublished data) as well as the downregulation of photosynthetic genes in response to UVB radiation (see Fig. 11c) [49]. (L. Surplus, B.R. Jordan, B. Thomas, and S. A.-H.-Mackerness, unpublished data). These studies therefore have illustrated that, as in pathogenic attack, the increase in PR transcripts in response to UVB is via a SA- and ROS-dependant signal transduction pathway. In contrast, a separate UVB signal pathway, which is ROS dependant but SA independent, leads to the decrease in photosynthetic transcripts.

In contrast to SA, which is involved in the signal pathway in response to pathogens, jasmonic acid (JA) is involved in the response to wounding and pathogen attack as well as other important plant physiological responses such as senescence [101]. UVB irradiation also has been shown to lead to an accumulation of JA [91]. UVB leads to the upregulation of a set of genes normally associated with responses to wounding, and the role of JA in the regulation of these genes in response to UVB exposure was confirmed by using a similar approach as that described above by the use of mutants blocked in the octadecanoid pathway [91]. UVB exposure also leads to an increase in ethylene production [102]. Ethylene, like JA, is produced in response to wounding and senescence [103]. The role of ethylene in plant responses to UVB stress has not been studied.

In addition to molecular genetics approaches described above, various studies have taken advantage of the expanse of knowledge on UVB effects on mammalian systems and have used biochemical approaches to dissect these pathways. Initial studies have been carried out using cell cultures and a variety of pharmaceutical compounds with known targets. These studies have concentrated on the mechanism leading to the upregulation of the *Chs* gene in response to UVB. However, although considerable progress has been made in elucidating the steps of the phytochrome-mediated signal transduction pathway, little is at present known about the UVB signal pathways.

Intracellular calcium, calmodulin, and serine/threonine kinases, but not tyrosine kinases, and

phosphatase activity have been shown to be involved in UVB light-dependant *Chs* expression [104,105]. This pathway is distinct from the previously characterized cGMP-dependant pathway utilized by phytochrome to upregulate *Chs* expression [106] and the blue/UVA pathway which does not appear to involve calmodulin. In addition, it is also separate from the ROS-dependant pathway leading to the upregulation of PR genes in response to UVB [105].

Little is known about the components of the UVB-induced pathway at present; nevertheless, it is apparent that plant cells utilize different signal pathways to regulate the same gene (e.g., *Chs*) depending on whether activation is caused by a phytochrome, blue/UVA, or UVB light receptor. In addition, there are distinct signal pathways leading to the regulation of different genes in response to UVB involving ROS, SA, and JA. Although it is clear that these studies are in their infancy, it is quickly becoming evident that UVB-induced signaling is complex and, similar to other stresses, crosstalk between various pathways must occur. A combination of the types of approaches described above should, however, provide us with a wealth of information that was to a great extent out of our reach a few years ago.

CONCLUSIONS

The effects of UVB radiation on plant growth and development have been extensively investigated in the last two decades and a wide range of responses have been reported. However, the mechanisms underlying these UVB responses are poorly understood. It has become more obvious in recent years that, although UVB can directly damage DNA, responses to UVB radiation are not a result of this nonspecific damage. The involvement of specific signal transduction pathways in response to UVB are adding further weight to this argument. Further work will need to be directed toward the identification and further characterization of these and other pathways, which provide the most promising avenue for the identification of potential targets for future engineering of UVB tolerance in important agricultural and horticultural crops.

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Molecular Mechanisms of Plant Responses to Elevated Levels of Tropospheric Ozone

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OZONE STRESS IN PLANTS: ORIGINS AND SYMPTOMS

Atmospheric Ozone and the Biosphere

Ozone, the three-atomic molecular combination of oxygen (O_3), plays the dual role of a friend and a foe to the biosphere, being either protective or damaging to living beings according to the atmospheric height where it accumulates. Stratospheric ozone, produced mainly by photochemical reactions of sunlight with oxygen, attains its highest concentration in a layer, extending from 25 up to 30 km over the earth's surface, that protects the biosphere from the sun's ultraviolet radiation [1]. The progressive thinning of this layer due to chemical pollution is currently giving rise to an ozone ''hole'' with the subsequent damages to life produced by exposure to intense low wavelength (potentially mutagenic) radiation. However, this chapter focuses on ozone at the troposphere, the lowest region of the atmosphere in contact with earth's surface, where it is an important component of the photochemical air pollution associated with human activities, and causes direct damage to terrestrial animals and plants (among them agricultural crops) owing to its high reactivity and oxidizing nature. Both ozone stores, the bigger stratospheric one (holding about 90% of total ozone), and the smaller tropospheric one (with the remaining 10%) are fairly well delimited and isolated by the colder tropopause barrier, which is thought to prevent convection between them. However, upward transport of tropospheric air is known to occur through equatorial deep convective systems, whereas evidence for transient downward inputs of stratospheric ozone-rich air into the troposphere also has been recently found in tropical zones of the Atlantic Ocean [2]. Even if locally significant, the global contribution of these exchanges to tropospheric ozone levels is uncertain.

Origin of Tropospheric Ozone

Most of the current tropospheric ozone is thought to be a result of human pollution, and it has accordingly increased during the last century with the development of the industrial civilization.

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Ozone is spontaneously synthesized from volatile air pollutants, mainly nitrogen oxides (NO and NO_2), which are primary components of ''smog'' [3,4]. Nitrogen oxides are generated by burning organic matter (mostly fossil fuels), being present in most industrial and motor vehicle exhausts. NO_2 is readily photolyzed to give atomic oxygen:

$$NO_2 + h\nu(\lambda < 400 \text{ nm}) \rightarrow NO + O$$

whereas atomic oxygen combines with molecular dioxygen to produce ozone:

$$O + O_2 \rightarrow O_3$$

Furthermore, NO is usually oxidized back to NO_2 through photochemically generated oxidants, thereby closing an ozone-producing cycle. These oxidants arise from organic air pollutants (such as volatile hydrocarbons) in a cyclic chain reaction involving the hydroxyl free radical ($OH^{\bullet}$), which is itself a photochemical product of ozone [1].

In addition, ozone can also be generated in the presence of molecular oxygen by many ''high-energy'' events, such as intense low-wavelength radiation or high-voltage electric discharges. The sources for these processes may be artificial (among those well known for scientists are spectrofluorimeter lamps, photocopying machines, and laser printers) or natural (such as thunderstorms).

The irregular geographical distribution of the ozone sources (highly correlated with human population density and industrial activity) together with climatic trends (such as wind cycles) account for the observed spatial variability of ozone levels [4]. However, temporal patterns of variation also have been observed, with the highest ozone peaks being achieved during warm and sunny days. These changes have been attributed to the higher overall pollution induced by intense solar irradiance and high temperatures due to easier volatilization and photochemical transformation of many organic compounds that contribute, either directly or indirectly (by oxidizing NO to NO_2), to ozone production. As a result, ozone levels show a seasonal variation, being higher during the summer, when short peak episodes of high ozone levels have been described in industrialized countries [4,5].

Evidence of Plant Damage by Ozone

Although the nature of the initial chemical damage of ozone to plant tissues has proven elusive and controversial (see section on Ozone Injury: First Events below), there is an overwhelming amount of data supporting a correlation between exposure to high levels of ambient ozone and plant growth decay. The physiological and anatomical effects of ozone exposure were early described in the cigar wrapper varieties of tobacco, in which elevated levels of ozone cause the necrosis of leaves known as ''weather flecks.'' A careful work carried out during the late 1950s led to the selection of ozone-tolerant varieties of tobacco, and also to the isolation of the hypersensitive Bel W3 line. This mutant responds to ambient (subcritical) concentrations of ozone-producing necrotic flecks in presenescent mature tobacco leaves, and it has been used as an ozone biomonitor for more than 30 years [6].

Even at doses similar to those of moderately polluted air (such as occurs over much of the United States during the summer), ozone has been shown to reduce the photosynthetic activity of several tree and crop species, thereby limiting plant growth and crop yield [7]. Other studies have correlated plant exposure to ozone with other changes at the physiological and metabolic level, such as leaf yellowing (i.e., photosynthetic pigments loss) and abscission, stimulation of dark respiration, phloem unloading, and alterations in the patterns of assimilate distribution (reviewed in Refs. 8 and 9). Since most of these symptoms are also characteristic of the natural decay processes of plants, ozone is often reported to accelerate senescence.

In general, a threshold ozone concentration for the adverse effects on plant growth can be only approximately defined owing to the significant dependence of the induced damage on the plant species, the presence of other pollutants, soil moisture, and other environmental conditions. However, the figure of 40 nL/L (or 40 ppb) has been acknowledged as a convenient (if rough) consensus value in order to express ozone treatments as a cumulative dose over a critical threshold. Thus, an

exposure index has been defined, being referred to as AOT40 (accumulated ozone exposure over a threshold of 40 ppb) [10]. AOT40 is computed as the product of the ozone concentration (ppb) by the time (hours) that it exceeded the threshold value. Nevertheless, the generalized use of this threshold in the prospective evaluation or economic assessment of crop or forest damage by ozone is strongly discouraged in favor of detailed approaches that take into account the characteristics of the particular species and environmental modifying factors [10].

Most studies devoted to ozone effects on plants have been carried out in controlled ambients either in the laboratory or in open-top chambers in the field. In the latter case, experiments monitored the ambient ozone levels while appropriate controls were run in parallel under the lower ozone concentration achieved through charcoal filtering of the air or by the addition of the protective antioxidant ethylene-diurea, N-(2-(2-oxo-1-imidazolidinyl)ethyl)-N′-phenylurea [5]. Under more controlled conditions in the laboratory, experiments are usually performed either with acute treatments (i.e., short moderate to high [>200 nL/L] doses of ozone [similar to transient peak episodes which occur naturally during the summer]) or chronic treatments (i.e., long-term exposure to low [40–60 nL/L] doses [closer to seasonal means]). Plants subjected to chronic treatments are usually affected at the physiological and metabolic level, undergoing restricted growth and premature senescence but without visible anatomical injuries. Symptoms of ozone damage under such moderate conditions may be difficult to recognize in the field because of their similarity to natural senescence or to the effects of other stress factors [5]. In contrast, acute treatments habitually produce symptoms which are readily perceptible such as yellowing, necrotic wounds, and leaf abscission even if the integrated dose (ozone concentration multiplied by exposure time) in these experiments is habitually much lower than the natural one corresponding to ambient levels sustained throughout a growing season [11].

In addition to the evidence obtained in laboratory (or tightly controlled) experiments, the incidence of ozone exposure on plant growth also has been evaluated under natural field conditions, in forest pine trees subjected to ambient ozone levels, by simultaneously monitoring the stem circumference of 28 trees and the local ozone level over a period of 5 years. Analysis of the collected data indicated that a high ozone concentration (above 40 nL/L) interacted with low soil moisture and high temperatures as one of the critical factors that reduced the short-term rate of stem expansion [12].

Summing up, exposure of plants to ozone causes dose-dependent metabolic, physiological, and anatomical alterations that result in diminished growth and crop yield, enhanced senescence, and generalized decay (which may eventually lead to abscission and death). The whole of this behavior, which includes cell and tissue deterioration as well as plant defense processes in a complex cross-talk (showing even species-specific features), is known as the ozone stress syndrome. In the following section, we will further analyze the characteristics of this complex response aiming to find explanations for the observed changes at the molecular level.

OZONE INJURY: FIRST EVENTS

Ozone Penetration

The nature of the primary interaction of ozone with plants that leads to its damaging effects is still a disputed matter, but the way by which ozone penetrates into plants is fairly well established. A comparative study between several tree and crop plants indicated that those species with a higher stomatal conductance exhibited more intense deleterious response (as measured by photosynthesis reduction) [7]. Accordingly, a study of ozone penetration in sunflower leaves [13] showed that stomatal diffusion was indeed the main path of entrance. In fact, the ozone uptake rate (Q) may be anticipated from the following formula [13]:

$$Q = C \cdot \frac{g_w}{1.68}$$

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where C is the ambient concentration of ozone, g_w is the gas phase (stomatal + boundary layer) conductance of the plant for water vapor, and 1.68 is the estimated ratio of the diffusion rates for water vapor and O_3.

Direct measurement at intercellular spaces indicated that the ozone concentration is extremely low there irrespectively of the ambient (external) concentration (up to 1500 nL/L) [13]. This was interpreted as that ozone is absorbed and rapidly decomposed in the cell wall and plasmalemma of the cells surrounding the substomatal chambers and it does not penetrate into the deeper layers of cells [13]. If this is so, the direct effect of ozone would be highly restricted to the cell walls and plasma membranes of a few cells.

Free Radicals

It has been long suspected that the direct damaging effects of ozone may be due to the generation of free radicals. Oxygen-derived radicals belong to a group of reactive species, known as ROIs (reactive oxygen intermediates), which are naturally produced in plants as a result of the partial reduction of molecular dioxygen. In order of increasing reduction of oxygen, ROIs include the superoxide anion ($O_2^{-\bullet}$) and its protonated form, the hydroperoxyl radical ($O_2H^\bullet$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($OH^\bullet$) (Fig. 1A). The latter is the most active and potentially damaging to biological structures, whereas hydrogen peroxide, and even superoxide, are comparatively much less reactive in aqueous solution [14]. Nevertheless, hydroxyl radicals can be generated

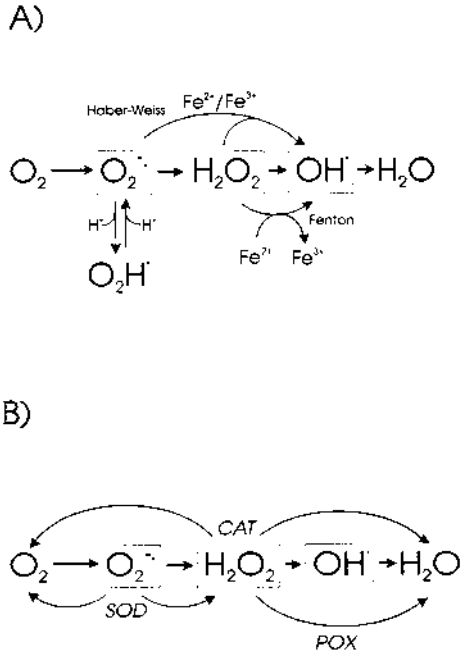


FIGURE 1 Reactive oxygen intermediates (ROIs). (A) Main ROIs and exchange reactions. ROIs produced by progressive oxygen reduction: superoxide anion ($O_2^{-\bullet}$) (the protonated [peroxyl] radical form is also shown [$O_2H^\bullet$]), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^\bullet$). The transition metal–induced Haber-Weiss and Fenton reactions also are indicated. (B) ROIs scavenging reactions and enzymes implicated in them: superoxide dismutase (SOD), peroxidase (POX), and catalase (CAT).

from hydrogen peroxide in the presence of transition metal ions in the reduced state (specially Fe^{2+} and Cu^{+}) by means of the Fenton reaction:

$$H_2O_2 + Fe^{2+} \rightarrow OH^- + OH^{\bullet} + Fe^{3+}$$

The oxidized metal ion can be reduced back in the presence of superoxide:

$$O_2^{-\bullet} + Fe^{3+} \rightarrow O_2 + Fe^{2+}$$

The net results of these two reactions

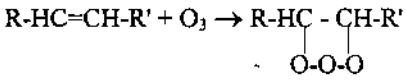
$$H_2O_2 + O_2^{-\bullet} \rightarrow O_2 + OH^- + OH^{\bullet}$$

is known as the Haber-Weiss reaction and by which hydrogen peroxide and superoxide anions are converted to the highly reactive hydroxyl radical through the catalytic effect of metal traces. Free metal ions are scarce inside cells, but they can be released from metal-protein complexes found in vivo through the oxidative attack of hydroxyl radicals. Thus, the production of hydroxyl radicals may become autocatalytic in vivo provided that other ROIs are present [14]. ROIs, which may arise naturally in plants as a by-product of mitochondrial respiration or photosynthetic activity in the chloroplast, are usually damaging to the cell functions. Therefore, they are habitually disposed off by an array of enzyme-catalyzed protective reactions [15]. However, under certain conditions, the cellular production of ROIs may be topically allowed, or even stimulated, as a part of a defensive strategy against pathogens [16]. In addition, ROIs also are known to act as cellular messengers triggering manifold protective and defensive responses [17]. Just because of their signaling role, oxygen radicals are good candidates to mediate the elicitation of the diversified effects that constitute the plant's response to ozone. Actually, induction of free radicals inside intact leaves of legumes by ozone treatment has been demonstrated in situ by means of electron spin resonance spectroscopy [18]. Unfortunately, the chemical nature of the radicals could not be unambiguously identified because of the need of a spin trap to enhance sensitivity. Ozone is known to decompose spontaneously in aqueous solution in a chain reaction that involves the ozonide radical ion ($O_3^{-\bullet}$) and produces hydroxyl radicals and superoxide anions. The reaction is initiated by hydroxide ions through the following sequence [19]:

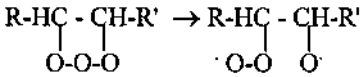
$$O_3 + OH^- \rightarrow O_2 + HO_2^-$$
$$HO_2^- + O_3 \rightarrow O_3^{-\bullet} + HO_2^{\bullet} \leftrightarrow O_3^{-\bullet} + H^+ + O_2^{-\bullet}$$
$$O_3^{-\bullet} \rightarrow O_2 + O^{-\bullet}$$
$$O^{-\bullet} + H_2O \rightarrow OH^- + OH^{\bullet}$$

However, owing to its dependence on hydroxide ions, the decay of ozone in aqueous medium takes place at a significant rate only at relatively high pHs. Thus, ozone decomposition has been deemed to be too slow at physiological pH, at least under laboratory conditions (half-life of about 5 min), compared with other competing reactions of ozone with biological molecules, such as singlet oxygen production [20].

Alternatively, ozone may also produce other free radicals as a result of its reaction with a number of compounds, such as phenolics and other organic molecules containing carbon-carbon double bonds. The reaction appears to proceed through ozone addition to the double bond:



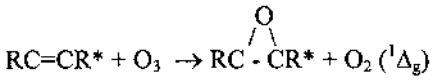
yielding an unstable ozonide, which may break down to radicals (among other possible outcomes):



Other free radicals (including ROIs) may secondarily arise from these as a result of electron propagation reactions. This mechanism may explain the observed effect of phenolics, enhancing the production of hydroxyl radicals in aqueous solutions exposed to ozone [21]. In order to explain the synergistic effect of ozone with the gaseous plant hormone ethylene (see section on Ethylene and Polyamine Bioynthesis below), Melhorn et al. [18] proposed that ozone toxicity may result from the production of free radicals on reaction with stress ethylene (presumably by adding on across the carbon-carbon double bond, as above), which is produced by the plant and is present in intercellular spaces. This would explain why inhibitors of endogenous ethylene production supress or reduce the deleterious effects in plants exposed to ozone [22]. Even if ethylene cannot account quantitatively for the rapid disappearance of ozone once it penetrates into the plant [23], it is plausible that most damaging effects could arise from ethylene-induced free radical production, whereas the excess ozone could be almost immediately consumed by reaction with the abundant phenolic acids bound to cell walls [18]. Alternatively, other investigators propose a direct ozone damage to membranes or even to intracellular molecules.

Singlet Oxygen Production

Ozone may combine with several organic functional groups in a reaction in which one oxygen atom is incorporated to an oxidized product, whereas the remaining two atoms are released as singlet oxygen ($O_2[^1\Delta_g]$), a highly reactive overenergized state of molecular dioxygen, which may easily oxidize a variety of biological compounds [14]. Kanofsky and Sima [20] have carried out a study on a number of biological molecules as potential targets of ozone reactivity and found that several amino acids (especially methionine and cysteine), redox protective compounds (ascorbic acid, uric acid, reduced glutathione), and coenzymes (NADH and NADPH) as well as proteins (human serum albumin) do indeed react in vitro with ozone producing singlet oxygen in high yield. Reaction with molecules containing carbon-carbon double bonds (such as NADH, NADPH, ascorbic, and uric acids) apparently proceeds via the formation of an unstable epoxide:



which may further hydrolize spontaneously to a diol or break down to different subproducts. On the other hand, the reaction with sulfur-containing molecules (methionine, cysteine, glutathione) leads to a sulfoxide:

$$RSR^* + O_3 \rightarrow RSOR^* + O_2(^1\Delta_g)$$

which, in the case of thiols (R* = H; such as cysteine or reduced glutathione), may turn to a disulfide by condensation with another thiol molecule:

$$RSOH + R'SH \rightarrow RSSR' + H_2O$$

Thereafter, the original thiol group may be restored by disulfide exchange:

$$RSSR' + R''SH \rightarrow RSH + R'SSR''$$

This could explain why the activity of some cysteine-containing enzymes, which is lost through ozone exposure, can be regained by treatment with an excess of low molecular weight thiols [24,25].

Singlet oxygen released in these reactions may in turn react with lipids to give peroxide derivatives or oxidize other amino acid residues (such as tryptophans or histidines) [14]. These reactions (together with the generation of free radicals) are likely to be involved in the oxidative modification of certain amino acid residues observed on treatment of purified proteins from plants [26], bacteria, and animals [27] with ozone. In these experiments, histidines were converted to aspartic acid residues, tyrosines to 3,4-dihydroxyphenylalanines, and other side chains to carbonyl derivatives (apparently generated by a direct attack of ozone to ethylenic double bonds) in a protein-dependent manner, indicating that susceptibility of the residues to oxidative modification is subjected to sequence and/or structural constraints [27]. By means of these (or similar) reactions, ozone could plausibly inactivate membrane proteins through modification of functional residues. Indeed, Dominy and Heath [25] have reported that ozone inhibits the K^+-stimulated ATPase from bean plasma membranes by oxidizing a critical sulfhydryl group. Thus, damaging of membrane pumps (thereby altering the ionic balance inside the cell) is a possible mechanism by which ozone could elicit a diversified cellular response [9]. The case of the abundant photosynthetic enzyme ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), which is a preferred target for proteolysis during natural and stress-induced senescence [28], and whose inactivation and degradation are thought to be regulated by oxidation of critical residues [29,30] also is relevant owing to the habitual use of its turnover to monitor plant senescence in photosynthetic organs. Rubisco levels have been shown to decline in several plants on exposure to ozone [31–33]. Indeed, treatment of the purified potato Rubisco with ozone results in modification of residues to carbonyl derivatives, with subsequent inactivation and aggregation of the enzyme [26]. Moreover, the potato enzyme also inactivates and aggregates inside isolated intact chloroplasts (kept at low temperature to prevent proteolysis of the aggregates) as a result of exposure to ozone [33]. Even if Rubisco modifications are not detected in vivo when the whole plant foliage is treated with ozone, it is plausible that they are nevertheless taking place provided that oxidation targets the protein for degradation and the modified Rubisco is efficiently removed in vivo [26,34]. However, since the chloroplastic localization of the enzyme makes unlikely its direct interaction by ozone, it appears that Rubisco modifications may result from the production of secondary oxidants (such as singlet oxygen or ROIs) [26] or other specific signals, elicited by ozone damage, which may trigger redox imbalance inside the chloroplast [34].

Lipid Peroxidation

The deterioration of membrane structure through lipid peroxidation is a common process typically associated with senescence and decay processes in plants. It usually results from the oxidative modification of polyunsaturated lipids in a radical chain reaction that involves molecular dioxygen and propagates from an initial event triggered by a free radical [14]. The process is initiated by the removal of a hydrogen atom from a methylene group (usually one weakened by adjacent double bonds) by a reactive free radical such as hydroxyl:

$$—CH{=}CH—CH_2—CH{=}CH— + OH^{\bullet} \rightarrow —CH{=}CH—{}^{\bullet}CH—CH{=}CH— + H_2O$$

The resulting carbon radical rearranges to a more stable conjugated diene:

$$—CH{=}CH—{}^{\bullet}CH—CH{=}CH— \rightarrow —{}^{\bullet}CH—CH{=}CH—CH{=}CH—$$

which then reacts with molecular oxygen to give a peroxyl radical:

$$—{}^{\bullet}CH—CH{=}CH—CH{=}CH— + O_2 \rightarrow —{}^{\bullet}OOCH—CH{=}CH—CH{=}CH—$$

In turn, the resulting peroxyl radical can abstract another hydrogen atom (thereby becoming a peroxide) from a neighboring lipid molecule. Thus, the process is reinitiated and propagated as a chain

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reaction. Ozone treatments are known to induce lipid peroxidation in membranes [35]. From the chemical point of view, the reaction with ozone could proceed following three different paths. First, ozone can initiate lipid peroxidation as a secondary effect of generating free radicals (specially hydroxyl ones). Second, peroxidation of lipids can also result from the production of singlet oxygen, since this species can directly react with unsaturated lipids to give hydroperoxides [14]. Nevertheless, the latter is not a chain reaction and hence does not propagate (a molecule of singlet oxygen is needed for each oxidized double bond). Third, ozone could directly add on across double bonds of lipids, yielding an unstable ozonide, which could decompose to give free radicals, thereby initiating peroxidation [14]. Whatever the mechanism, lipid peroxidation (together with oxidative modification of membrane proteins) is expected to alter membrane permeability and function, thereby initiating a diversity of cellular responses. On the other hand, peroxidized lipids can be further metabolized through different pathways to compounds (such as volatile aldehydes, hydroxy acids, or jasmonates) that may act as diffusible signals [36]. For these reasons, and given the easy accesibility of membranes from the intercellular space, lipid peroxidation is also a likely candidate to be the critical primary effect of ozone that mediates the subsequent complex response.

In summary, ozone damages cellular structures by oxidative modification of key molecules, most likely at the membrane and cell wall level. This oxidative injury may result either from direct combination with ozone or as a secondary result from free radical or singlet oxygen production. It is plausible that all the above paths of oxidation occur simultaneously, the qualitative and quantitative relevance of each of them being dictated by the physical, chemical, and anatomical constraints of the particular case. At any rate, there is little doubt that this primary effect of ozone is the origin of chemical signals that are able to trigger secondary responses at the genetic, metabolic, and hormonal level.

PLANT RESPONSES TO OZONE

Ozone exposure modulates plant gene expression both inducing and repressing specific messages [36,37]. All ozone-repressed genes described to date code for chloroplastic proteins, such as photosynthetic components and antioxidative enzymes [38,39]. This fact may reflect the special sensibility of the chloroplast to cellular redox changes. However, not all nuclear genes encoding chloroplast proteins are repressed by ozone indicating that downregulation of chloroplast gene expression is not a generalized trend [38]. Table 1 lists some of the genes regulated by ozone exposure and its abbreviations (intended for quick reference here). Most of these genes have been shown also to be regulated by other stresses, such as pathogen attack, drought, and exposure to ultraviolet (UV) irradiation as well as other air pollutants and heavy metals. Moreover, oxidative stress caused by treatments with copper or aluminum results in a gene-induction pattern which is very similar to the ozone response [34,40].

Reactive Oxygen Intermediates Scavenging

Plants produce ROIs (see Fig. 1A) under normal developmental conditions mainly through the mitochondrial respiratory and photosynthetic electron transport chains. Although plants make use of ROIs in several metabolic processes, such as lignin formation in the cell walls, ROIs are very reactive, instantaneously damaging proteins, lipids, and DNA. In order to deal with ROIs, plants have a battery of constitutive antioxidative defense mechanisms that include metabolites and enzymatic systems.

Ascorbate (vitamin C), glutathione, α-tocopherol, and β-carotenes are among the metabolites implicated in scavenging ROIs [11]. Besides, and since transition metals ions promote the production of ROIs through the Haber-Weiss and Fenton reactions (see Fig. 1A), metal chelators (fitochelatins and metalothioneins) have been shown also to have antioxidant properties [41]. Similarly, proteins

TABLE 1 Ozone-Regulated Gene Expression in Plants

Function	Protein	Abbreviation	References
Downregulated			
Photosynthetic proteins	Chlorophyll *a*/*b*–binding protein		38,39
	Ribulose-1,5-bisphosphate carboxylase oxygenase small subunit	Rubisco	38,39
Chloroplast antioxidant enzymes	Iron-superoxide dismutase	Fe-SOD	38
	Glutathione reductase	GR	39
Upregulated			
Antioxidative enzymes	Copper/zinc-superoxide dismutase	Cu/Zn SOD	56
	Catalase	CAT	58
	Ascorbate peroxidase	APX	59–61
	Glutathion peroxidase	GPX	58
	Glutathion-S-transferase	GST	38
	Glutathion reductase	GR	63
Ethylene and polyamines metabolism	S-adenosyl-L-methionine synthetase	SAM	79
	Aminocyclopropane synthase	ACS	39,79,80
	Aminocyclopropane oxidase	ACO	79
	Arginine decarboxylase	ADC	77
PRs proteins	β-1,3-Glucanase		37,92,94,95
	Chitinase		37,92,94,95
Phenylpropanoid metabolism	Phenylalanine ammonia lyase	PAL	47
	Chalcone synthase	CHS	101,102
	Stilbene synthase	STS	102
Cell wall biosynthesis and modification	Cinnamyl alcohol dehydrogenase	CAD	99
	Lypoxygenase	LOX	133
	Extensins		47
	Glicine rich		109
Others	Heat-shock proteins	HSP	37
	HMG-CoA synthase		108
	Thiol protease		109
	Protease inhibitors		109

that participate in the homeostasis of metals in yeast also have been implicated in protection against oxidative stress [42].

The antioxidative enzymatic system (Figs. 1B and 2) is composed of superoxide dismutases (SODs) that convert the superoxide anion into H_2O_2 and O_2, catalases (CATs) that eliminate H_2O_2, and peroxidases (POXs) that reduce H_2O_2 into water using electron donors, such as ascorbic acid in the case of ascorbate peroxidases (APXs), or reduced glutathione in glutathione peroxidases (GPXs). Ascorbate is recycled back at the expense of NADPH in the ascorbate-glutathione or Halliwell-Asada cycle (Fig. 2A). The enzymes that participate in this pathway are APX, dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), and glutathione reductase (GR).

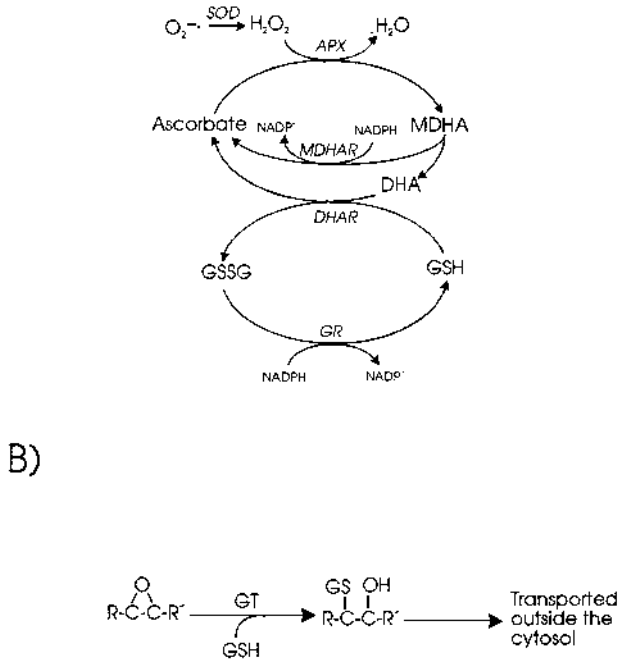

FIGURE 2 Some antioxidant plant enzymes. (A) The ascorbate-glutathione, or Halliwell-Asada, cycle connects the photosynthetic reductive power of NADPH to the scavenging of ROIs using glutathione and ascorbate as intermediates. Enzymes participating in the pathway are superoxide dismutase (SOD), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), and glutathione reductase (GR). Other abbreviations are MDHA, monodehydroascorbate; DHA, dehydroascorbate; GSH, reduced glutathione; and GSSG, oxidized glutathione. (B) Glutathione transferase (GT) conjugates glutathione to oxidative damaged molecules (an epoxide is shown as an example) targeting it for transport outside the cytosol.

In addition, glutathione S-transferase (GST) plays an important role in detoxifying oxidative products by catalyzing its conjugation with reduced glutathion (Fig. 2B). After further chemical modification, these conjugates are transported out of the cytosol by specialized pumps [43]. Several isoforms with different cellular locations have been described for most of the antioxidative enzymes suggesting that isoenzymes could reduce ROIs to a different permisive level in each compartment [44].

In general, both reductant metabolites and antioxidant enzymatic activities decline in plant tissues with age, and this may be one of the main factors that promote the onset of oxidative processes associated with senescence [45].

Exposure to different stresses may cause a localized and pronounced rise in ROIs, which in some cases is due to the activation of a plasma membrane NADPH-oxidase enzyme [46]. This sudden local increase in ROIs, known as ''oxidative burst,'' is part of a defensive strategy against pathogens and elicits an array of plant responses, including an increase in the biosynthesis of the antioxidative enzymes in the surrounding cells.

Since ozone may generate ROIs by several pathways (see section on Free Radicals above),

it is likely that the overlapping responses of ozone with other stresses at the gene-induction level (the so-called cross induction [47]) are due to the initial production of ROIs which can mimic the oxidative burst. In turn, this may trigger signaling events leading to a variety of plant responses that are common to these stresses [11].

Antioxidant responses include the synthesis of metabolites such as ascorbic acid that aids cells to detoxify lipid peroxides caused by ROIs. In this regard, apoplastic ascorbate levels have been found to increase in response to ozone exposure in different plants [48–51]. An ascorbic acid–deficient mutant, which accumulates only 30% of the normal ascorbate concentration as a result of a biosynthetic deffect [52], has been shown to be ozone sensitive demonstrating the important role that ascorbate plays in resistance to oxidative stress [53]. Glutathione, which is connected to ascorbate through the Halliwell-Asada cycle (see Fig. 2), also increase both in the reduced and oxidized forms during ozone treatment [54–56]. Perhaps the presence of elevated levels of antioxidant compounds in ripe fruits is the cause of their extraordinary resistance to the high doses of ozone used as fungicidal in post-harvest technology [57].

Contradictory results have been obtained with regard to the effect of ozone on the activities and expression levels of antioxidant enzymes; maybe as a result of differences between plant systems and ozone treatments utilized by several investigators. An extensive study on mRNA accumulation of antioxidant genes after ozone, sulfur dioxide, and UVB light exposure has been performed in *Nicotiana* by Willekens et al. [58]. Their results suggest that CAT and GPX activities, but not SODs and cytosolic APX, contribute to the antioxidant response in tobacco. However, other studies have shown that both the APX transcript and the derived enzymatic activity increase after ozone exposure [38,59–61] presumably enhancing plant tolerance to ROIs. Accordingly, the reduction of endogenous cytosolic APX mRNA levels in transgenic tobacco plants by antisense technology made these plants significantly more susceptible to ozone injury than control plants [62]. These results indeed demonstrate the importance of cytosolic APX in relieving the oxidative stress generated by ozone.

Ozone exposure correlates with a decline in chloroplastic GR transcripts [38]. The role of GR activity in oxidative stress responses is also rather controversial. Experiences with tobacco plants transformed with a pea GR expressed either in the cytosol, chloroplasts, or chloroplast plus mitochondria indicated that only certain lines of the latter combination were more resistant to ozone [63]. In addition, tobacco plants expressing the GR coding sequence from *Escherichia coli*, both in cytosol and chloroplasts, improve their tolerance to paraquat but show no differences in the response to ozone [64,65]. This may reflect the different location of ROIs induced by paraquat (chloroplast) and ozone (apoplast).

In contrast, transcription of GST is dramatically affected during ozone stress. Its mRNA increases rapidly and transiently about 20-fold within 3–6 h of ozone treatment suggesting that GST could play an important role in detoxifying cells [38,56]. Owing to this prominent change, GST is considered to be a sensitive molecular marker of ozone.

Ozone treatment has been shown to increase both SOD transcript [56] and SOD activity [54]. This appears to be due to enhanced transcription of the cytosolic Cu/Zn SOD, since mitochondrial Mn SOD remained unchanged and the chloroplastic Fe SOD transcript declined in ozone-treated plants [38]. The general increase of reduced glutathione in plants exposed to oxidative stress [66] has been proposed to influence the expression of cytosolic SOD in tobacco, which is thought to be under redox control [67,68].

Results of overexpressing SODs in transgenic plants are controversial with regard to ozone resistance. Engineered tobacco plants producing elevated levels of chloroplastic Cu/Zn SOD activity display the same sensitivity than control plants to superoxide toxicity caused by paraquat [69] or ozone [70]. However, tobacco plants overexpressing chloroplast-directed MnSOD show enhanced protection against oxygen radical and ozone damage [71,72]. In addition, transgenic tobacco plants that overproduce chloroplast-located pea Cu/Zn SOD have a higher resistance to oxidative stress and also exhibit an increase in APX activity [73]. These discrepancies could be explained by differences in the levels of SOD overproduction, in the extent of inhibition of Cu/Zn SOD by hydrogen peroxide, or in the ozone dosage among different experiments [72].

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Ethylene and Polyamine Biosynthesis

Ethylene and polyamines play antagonistic prosenescent and antisenescent roles, respectively [74]. Their synthetic pathways (Fig. 3) share the same precursor metabolite, S-adenosyl methionine (SAM); a fact that could explain in part their competing interactions. SAM also acts as a methyl group donor in numerous methyl transferase reactions, including the biosynthesis of lignin. The key regulatory step in the ethylene biosynthetic pathway is the conversion of SAM to 1-aminocyclopropane-1-carboxylic acid (ACC) catalyzed by the ACC synthase (ACS) [75]. The final reaction in the pathway is the conversion of ACC to ethylene, which is catalyzed by the ACC oxidase (ACO) (Fig. 3).

Ethylene production is involved in many physiological processes throughout the plant life cycle [75]. Besides, most plants synthesize ethylene when subjected to different stress conditions, including ozone exposure [76,77]. Tobacco and tomato plants subjected to ozone treatment show increased levels of the ethylene precursor ACC [78,79]. Accordingly, two genes encoding ACS from tobacco have been found to be transcriptionally activated after ozone exposure, thereby increasing ethylene production [80,81]. In a recent study, Tuomainen et al. [79] have compared the expression of different genes involved in ethylene biosynthesis on exposure of tomato plants to ozone. Their conclusions are that specific genes from the families of the SAM synthase, ACS and ACO are indeed induced by ozone, but postranslational modification (phosphorylation) of ACS also contribute to the activity rise, especially during the early stages of response.

A remarkable correlation between stress ethylene synthesized in response to ozone and plant sensitivity to ozone has been detected by analyzing the levels of ethylene in ozone-sensitive and ozone-insensitive plant varieties [77,82]. When ozone-sensitive tobacco plants were exposed to ozone, ethylene synthesis increased considerably in parallel with leaf injury, whereas levels of ethylene remained low in the insensitive variety Bel B. Moreover, younger tobacco plants are more

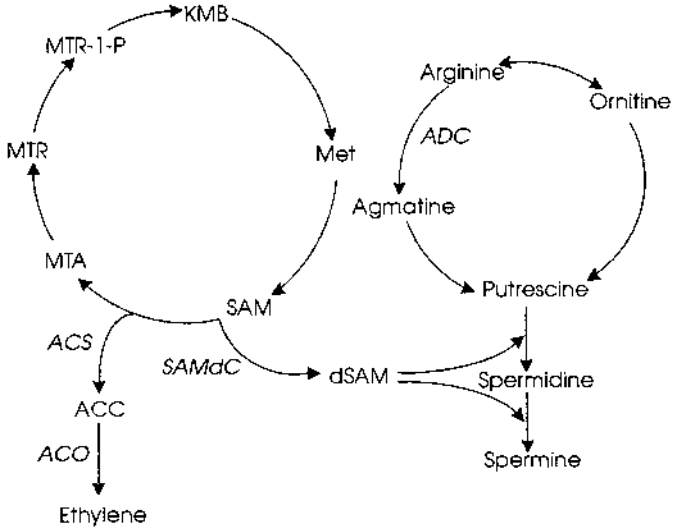

FIGURE 3 Biosynthetic pathways of ethylene and polyamines in plants. Only main intermediary metabolites and key enzymes are indicated. Metabolites: ACC, 1-amino-cyclopropane-1-carboxylic acid; KMB, 2-keto-4-methylthiobutyric acid; MTA, 5′-methylthioadenosine; MTR, 5-methylthioribose; SAM, S-adenosyl-methionine; dSAM, decarboxylated SAM. Enzymes: ACS, ACC synthase; ACO, ACC oxidase; ADC, arginine decarboxylase; SAMdC, SAM decarboxylase.

resistant than older plants to ozone injury correlating with their ability of producing stress ethylene. Furthermore, ozone toxicity is reduced in mung bean plants when pretreated with different inhibitors of ethylene biosynthesis acting at different metabolic steps. It also has been demonstrated that these inhibitors prevent ozone injury without affecting stomatal opening [22]. These results illustrate a synergic effect between stress ethylene and ozone, which has been attributed to the nonenzymatic reaction between them in the gas phase of the substomatal chambers to produce free radicals and reactive aldehydes that might be responsible for plant damage [76].

Contrarily, ethylene treatment, previous to ozone exposure, has been shown to minimize the subsequent damage to plants [18,83] probably through the induction of defense systems which protect plants from the toxic effects of ROIs [59].

ADC is the key enzyme regulating polyamine synthesis during plant stress [74]. The activity of this enzyme has been studied in different plant species subjected to ozone treatment. These studies support a strong positive correlation between ozone exposure, ADC activity, and polyamine contents of the plants. High ADC activity and putrescine levels are characteristic responses of the Bel B tolerant variety of tobacco to ozone exposure [77]. In barley leaves, the application of difluoromethyl arginine, a specific inhibitor of ADC, extended the visible injuries resulting from a subsequent ozone fumigation [84]. Moreover, root-applied polyamines increased the foliar levels of polyamines in tobacco Bel W3, thereby protecting the leaves of this sensitive line from ozone damage [85].

Hydroxycinnamic acids conjugates of polyamines also have been shown to increase after ozone exposure [77]. In general, elevated levels of polyamines correlate with oxidant stress resistance [86] probably because they can act as free radicals scavengers [87]. A model for the inhibition of senescence by polyamines has been proposed in which polyamines bind to membranes preventing lipid peroxidation by quenching free radicals that catalyze this process. Free radicals also are needed for the ACO activity, and this could be the reason why polyamines inhibit ethylene biosynthesis [74]. The same model could also apply for protection against ozone-induced damage by polyamines. The scavenging effects of polyamines has been shown not to be due to the amine group itself but to the conjugated phenolic hydroxy group, since polyamine conjugates, but not the free polyamines, are effective scavengers for ROIs [85].

Pathogen-Related Proteins

Close similarities between ozone- and pathogen-induced reactions in plants have been stressed in several reviews [36,88,89]. The relationship between these two processes became evident when mutants screened for their susceptibility to fungal pathogens turned out also to be ozone sensitive [90]. Moreover, ozone treatments habitually lead to changes in plant susceptibility to pathogenic attack [11].

Plant responses to biotic stress caused by a wide range of pathogens is currently becoming one of the leading research fields in plant molecular biology [46]. Plant-pathogen interactions may be divided into compatible and incompatible reactions depending on if pathogen invasion takes place or not, respectively. Plant defense mechanisms against pathogens are mediated by ligand-receptor interactions (also named gene-for-gene) between *Avr* (the pathogen avirulence gene) and *R* (the plant resistance gene) resulting in the locally induced hypersensitive response that leads to localized plant cell death and thereby to the isolation of the pathogen at the necrotic site. This interaction stimulates further responses, including the signal that triggers the systemic acquired response (SAR), a whole-plant and wide-range resistance developed by the plant after being attacked by pathogens.

The local and controlled production of ROIs by the cell is one of the main components in the plant response against pathogens. Fungal and bacterial elicitors trigger a biphasic ROI production. Phase I is a very rapid response (within minutes) and not always correlated with plant disease resistance. Phase II is produced many hours later and correlates with the degree of resistance of the plant to the pathogen [16,17].

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Pathogen-related proteins (PRs) are a group of polypeptides induced in plant hosts by pathogens which share some biochemical characteristics, such as low molecular weight, stability at low pH, and resistance to degradation by proteases. PRs are classified in five functional groups containing both acidic extracellular and basic vacuolar members. For example, some are β-1,3-glucanases and chitinases, enzymes that degrade the cell wall of fungal pathogens, and others are proteinase inhibitors or proteases degrading the pathogen proteins [91]. β-1,3-Glucanases and other hydrolases could be also involved in the recycling of monomers from polysaccharides of necrotic areas, thereby salvaging useful products [92]. In any case, as a result of the coordinate expression of PRs, the plant becomes more resistant to pathogenic attacks [93].

PRs induction both at the postranscriptional and postranslational levels have been demonstrated in several instances after ozone treatment [92,94,95]. Furthermore, PRs genes have been isolated in a differential screening in ozone-treated against control parsley plants [37].

Phenolic Compounds and Cell Wall Constituents

Many potentially protective phenylpropanoid compounds, including flavonoids, furanocoumarins, and lignin, play a role in plant defence responses. These compounds are synthetized under different environmental stress conditions such as wounding, pathogenic attack, UV light, and ozone exposure [96].

The first regulatory enzyme in this pathway is the phenylalanine-ammonium lyase (PAL) that converts phenylalanine in cinnamic acid initiating the phenylpropanoid secondary metabolism. Several branched pathways, leading to different families of compounds, emerge after the PAL-catalyzed first step (Fig. 4).

Rapid and transient increases in PAL activity due to transcriptional regulation of the PAL genes have been described in plants exposed to ozone [47]. There are several isoforms of PAL and only some of them are induced by stress. The PAL induction patterns by ozone, plant pathogens, or wounding are extremely similar, which is evidence of a remarkable parallelism among these responses [88].

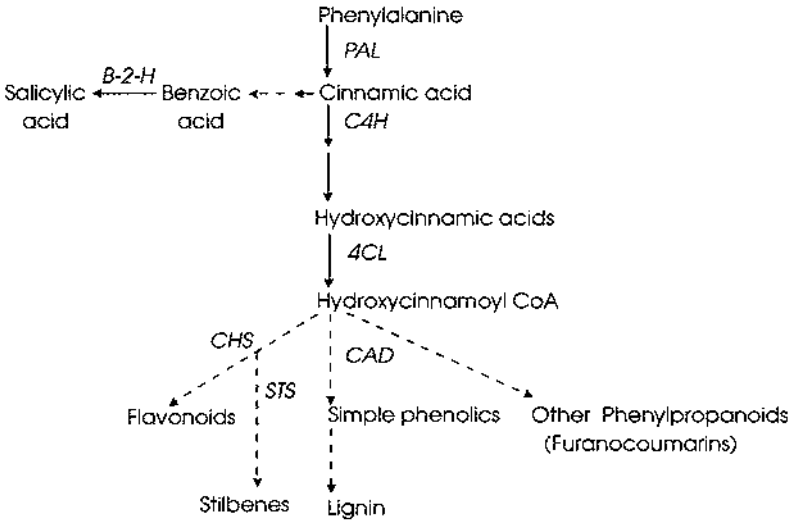

FIGURE 4 Schematic phenylpropanoid metabolism in plants. Indicated regulatory enzymes are phenylalanine ammonium lyase (PAL), benzoate-2-hydrolase (B-2-H), p-coumarate: CoA ligase (4CL), chalcone synthase (CHS), stilbene synthase (STS), and cinnamyl alcohol dehydrogenase (CAD).

Salicylic acid is a cinnamic acid–derived signaling compound that accumulates in response to several stresses, including ozone treatment [97]. Salicylic acid is plausibly implicated in the ozone-signaling pathway (see section on Salicylic Acid below).

Lignin and suberin are complex polymers formed by the oxidative combination of simple phenolics, which are usually conjugated with sugars and organic acids [98]. These reactions are particularly important in woody tissues. However, in response to stress conditions, lignin-like products can be formed in other tissues as part of the general defense strategy of plants. Cinnamyl alcohol dehydrogenase (CAD) is the key regulatory enzyme in lignin biosynthesis and its activity also has been shown to increase through transcriptional activation following ozone treatment [99,100].

Flavonoids are synthesized via chalcone synthase (CHS), a branching enzyme in the pathway. Some of these compounds absorb UV light–protecting cells against UVB damage by avoiding DNA dimerization and breakage [96]. Accumulations of flavonoids seem to be required for normal pollen development, and their induction by stress may also serve to prevent pathogenic infection. Increases in CHS activity after ozone exposure also have been reported [101,102].

Stilbenes are phytoalexins related to flavonoids. They branch out of the common pathway through the regulatory enzyme stilbene synthase (STS), which is strongly induced by pathogens [103]. Primary needles of 6-week-old pines exposed to ozone increase several hundred- to thousandfold the activity of the STS in a dose-dependent manner; therefore being useful as an ozone marker [102].

Flavonoids and furanocoumarins, which usually accumulate in response to UV light and pathogens respectively, are both induced by ozone treatment in parsley. This result suggests that ozone could produce a cross induction of the UV- and pathogen-defense responses [37,47].

Other Ozone-Induced Gene Products

New molecular approaches are leading to identification of genes induced by ozone. Sharma and Davis [104] have isolated a novel ozone-induced cDNA (AtOZ1) of unknown function in *Arabidopsis* by the technique of mRNA differential display [105]. Eckey-Kaltenbach et al. [37] have isolated a cDNA corresponding to a heat-shock protein (HSP) through a differential screening. Although the involvement of HSP in oxidative stress defense is well known in yeasts [106,107], this is the first time that these proteins have been described to participate in oxidative responses in plants. Transition metal metabolism also has been implicated in defense reactions against oxidative stress in other organisms [107]. In this regard, a transcript for a cytosolic copper transport protein, which may be implicated in delivering copper to the secretory compartiments, has been found to increase following ozone treatment in *Arabidopsis* (H. Mira and L. Peñarrubia, personal communication). Further examples of transcripts induced by ozone exposure are an enzyme for the biosynthesis of terpenes [108], a thiol protease and protease inhibitors [109], and polyubiquitin, which suggest the involvement of the ubiquitin system in ozone-damaged protein degradation [108].

MOLECULAR BIOLOGY OF OZONE PERCEPTION AND SIGNAL TRANSDUCTION MECHANISMS

Contrary to other initiation events in plant transduction processes, the ozone response apparently does not begin with the recognition of the effector molecule by a receptor. No ozone receptor has been reported to date, and it is not unreasonable to postulate that this receptor simply does not exist. After all, plant ozone stress is a very recent phenomenon, and it is likely that the components of the ozone response have been sorted out from preexisting responses to other stresses activated through coincident steps of chemical damage. This conjecture is supported by the fact that every reported ozone-induced gene (see Table 1) has been shown also to be affected by other stresses or natural senescence. For example, the expression of a heat shock (sHSP) and two PR proteins is induced by ozone, but only sHSP is activated by heat shock [37]. This fact suggests that ozone can activate

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different signal transduction pathways, with one of them merging with paths of the heat-shock response perhaps through interaction with some heat-shock transcription factors, as has been shown to happen in other eukaryotic organisms during oxidative stress [107,110].

Signaling Molecules

Also with regard to the signaling cascade, no specific molecules have been described in the case of the response to ozone which probably means that common cellular intermediate signals could mediate this response. This fact would explain the rather inespecific nature of the ozone response and the cross induction with other stresses. A wide variety of chemical signals that are known to participate in other processes also have been implicated in the ozone transduction pathway. However, since some molecules could act as a chemical signal only within an extremely narrow range of concentration and under particular circumstances, quantitative, spatial, developmental, and species-specific aspects must be taken into account before assuming their role in a process. Among these potential signal molecules are the following.

Reactive Oxygen Intermediates

During pathogenic attacks, ROIs are produced at primary infection sites, and they act as early diffusible signals leading to the activation of detoxification systems in distant cells [16,111].

$O_2^{-\bullet}$ has been shown to be the signaling molecule that induces plant defense genes in the mutant *lsd1* which mimic disease lesions [112]. However, owing to its longer life and higher permeability across membranes, H_2O_2 has been proposed as the best candidate for signaling among ROIs. Indeed, H_2O_2 is involved in the signal transduction pathway of the hypersensitive response [113]. Moreover, H_2O_2 has been implicated in limiting the spread of cell death by induction of cell-protectant genes in surrounding cells [114,115]. GST transcripts increase in response to a narrow range of H_2O_2 [114]. In addition, H_2O_2 inhibits the growth of diverse microbial pathogens [116] and also contributes to plant cell wall strengthening through peroxidase-mediated cross linking of Pro-rich structural proteins [117,118].

Overproduction of H_2O_2 in tobacco transgenic plants by expression in the apoplast of an H_2O_2-generating glucose oxidase has been shown to increase the resistance to pathogenic attacks by activation of host defense mechanisms [116,119]. Besides, H_2O_2 increases benzoic acid-2 hydroxylase enzyme activity [120], which is required for the salicylic acid biosynthesis (see Fig. 4) associated with the systemic acquired response. However, exogenous application of H_2O_2 did not induced PAL or CHS gene expression [114].

Baker et al. [46] have suggested a parallelism between the mammalian immune and the plant pathogenic responses with regard to the function of ROIs. In the mammalian immune response, ROIs induce the expresion of genes through the activation of the redox-regulated transcription factors NF-κB and AP-1 [121]. Different ROIs are able to activate NF-κB, whereas $O_2^{-\bullet}$-producing agents are not [122]. Antioxidants also have different effects on the activation of these transcription factors [123]. AP-1 activation is involved in the UV response pathway, which is highly conserved from yeast to mammals [124]. Curiously, proteinase inhibitors I and II from potato tubers have been shown to block UV-induced AP-1 activation in mammals probably through inhibition of the signaling mechanism that mediates UV-induced carcinogenic cell transformation [125]. The DNA-binding domain of AP-1 have cysteine residues that only bind to its target *cis* DNA element when they are reduced by the nuclear redox factor Ref-1 [126,127]. An *Arabidopsis* homologue of Ref-1 has been described in plants [128].

ROIs, produced in the apoplast of ozone exposed plants, may be either detoxified by the extracellular radical scavengers or they can react with plasma membrane components initiating the production of intracellular signals. Similar to pathogenic attack responses, a biphasic production of ROIs have been detected in the apoplast of tobacco plants exposed to ozone [36]. Endogenously produced ROIs, most likely generated through secondary alterations of energy transduction components in the chloroplasts and mitochondria, could further contribute to the diversification of responses, especially to the numerous changes described in the chloroplasts.

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Jasmonates

Jasmonic acid and its methyl ester, methyl jasmonate, are oxidation products of the linolenic acid from the plasma membrane via the octadecanoic pathway [129]. The role of jasmonates as signal transduction intermediates have been well characterized during induction of proteinase inhibitors in tobacco and tomato after UV irradiation and wounding [129a]. These compounds have been demonstrated to play a critical role in promoting transcription of several wound- and senescence-related genes [130], although both jasmonate-dependent and jasmonate-independent responses have been described during wound-induced signal transduction [131].

Jasmonates could also be involved in plant senescence, since their exogenous application to leaves decreases the expression of some photosynthetic genes causing chlorosis [132]. In contrast, high levels of jasmonates have been described in young and reproductive tissues. In order to conciliate both findings, Creeman and Mullet [129] have suggested that the role of jasmonates could be related to inhibition of the photosynthetic apparatus, thereby avoiding premature accumulation in young organs and helping chloroplast dissassembly during senescence.

Jasmonates could also play a role in the ozone response. It has been suggested that ozone-induced ROIs could mediate lipid peroxidation and induction of the lipoxygenase activity that releases jasmonic acid from linolenic acid [133]. Moreover, methyl jasmonate and its precursors participate in the gene induction of flavonoid glycosides by ozone [47] and mediate the induction of PAL mRNA accumulation by H_2O_2 [134].

Ethylene

Ethylene has been widely associated with both natural and stress-induced senescence. Recent studies with transgenic plants, where ethylene biosynthesis have been blocked, suggested that senescence is initiated by an age-dependent developmental program which is ethylene independent. However, ethylene influence senescence timing as a way to respond to environmental conditions [135].

Ethylene signal transduction has been deciphered in recent years, and it is becoming one of the best-known plant hormone transduction pathways. Genes involved in the ethylene response have been identified in *Arabidopsis*. The ethylene receptors [136,137] show homology with two-component sensor-regulator bacterial proteins and resemble a hybrid protein sensor-regulator kinase responsible for signaling hyperosmotic stress in yeast. Dowstream in the ethylene signaling pathway is CTR1 [138], a negative regulator which encodes a Raf-1–like protein kinase homologous to those involved in multiple signaling cascades of eukaryotic cells. The participation of other genes in ethylene signal transduction has been recently reviewed [139–141].

Ethylene induction is one of the first events detected in plants after ozone treatments, correlating with ulterior visible injury and decline of the photosynthetic apparatus [34]. Owing to the similarities shown between ozone damage and the plant/pathogen–mediated hypersensitive response, a role as an abiotic elicitor has been proposed for ozone [79,89]. Together with other damage-related factors, ethylene is thought to play a role in the development of symptoms controlling or promoting the spread of cell death.

Since both ethylene and jasmonates are probably involved in ozone transduction, they might perhaps interfere with each other. For example, methyl jasmonate is known to stimulate ethylene production in fruits mainly through enhancement of the activity of the ACC oxidase [142]. Ethylene and jasmonates have been shown also to cooperate in the signaling pathway following wounding in tomato, rendering complicated patterns of gene expression which may differ between species [131,143].

Salicylic Acid

Salicylic acid is required to induce systemic acquired resistance (SAR) and PR gene expression [144,145]. However, it remains unclear if salicylate is or is not the long-distance systemic signal for SAR induction. The role of salicylic acid in the plant defense is complex and may differ from species to species. Elevated levels of salicylic acid can also inhibit jasmonic acid and ethylene

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biosynthesis interfering with the signaling pathways of these molecules during wound-induced responses [143,146,147].

Salicylic acid stimulates ROIs production owing in part to the direct inhibition of the catalase activity of a salicylic acid–binding protein [148,149], and ROIs in turn activate salicylic acid biosynthesis, acting as a positive effector of the benzoate-2-hydroxylase. Recent data have suggested a model for the role of the oxidative burst and/or the salicylic acid in the hypersensitive and SAR responses [150]. In this model, pathogen-induced oxidative burst (phase I) leads to rapid activation of the phenylpropanoid pathway, thereby promoting salicylic acid synthesis. Pathogen-induced phase II of the oxidative burst means a persistant rise in H_2O_2 by the recognition of an incompatible pathogen. The sustained activation of salicylic acid synthesis, together with other factors, would potentiate the ROIs production in phase II leading to cell death (in the hypersensitive response) and to systemic acquired resistance [150].

Exposure to ozone or UV light trigger salicylic acid biosynthesis in tobacco probably through chemical release of the precursor, benzoic acid, from its conjugated forms [97]. Both glycosylated and free forms of salicylic acid accumulate rapidly after ozone treatment. Free salicylic acid induction is transient, reaching a maximum after 6 h of ozone treatment in correlation with the activation of plant defense mechanisms, as measured by PAL and GST-1 mRNA accumulation [151]. This fact suggests that ozone activates the hypersensitive response and the SAR through signaling pathways that involve salicylic acid.

Plants engineered to be deficient in salicylic acid, through the expression of a bacterial salicylate hydrolase (nahG), did not produce a normal systemic acquired resistance response [152]. Moreover, transformed plants also are affected in their response to ozone, being more susceptible than control plants, developing necrotic lesions and becoming slightly chlorotic. This response was more pronounced in aged plants; probably due to the fact that the activation of the antioxidant response depends partly on salicylic acid accumulation. The study of these plants by Sharma et al. [151] allows us to dissect the different signaling routes in the ozone response and has led to the description of three different gene expression behaviors:

- Genes whose mRNA induction by ozone was abolished in transgenic plants (e.g., PR1) indicating that their induction is salicylic acid dependent.
- Ozone-induced genes which also accumulate in these plants (e.g., PAL). This indicates that there is also a salicylate-independent pathway in the ozone response.
- Genes that accumulate habitually under ozone treatment but their expression is attenuated in these plants (e.g., GST1), indicating that they are under control of at least two independent regulatory pathways.

Genetic Studies

Genetic approaches are providing insights for understanding the role of salicylic acid in defense responses (for a recent review, see Ref. 153).

Nonexpresser of PR genes (*npr*1) mutants are defective in the SAR response expression at a step downstream of salicylic acid. When these mutants are treated with ozone, accumulation of PAL mRNA is unaffected, whereas PR1 transcripts are detected at very low levels [151]. This result shows that NPR1 is necessary in the signal transduction for PR1 expression. However, GST1 transcripts remained at almost the same level than in wild-type plants, suggesting that the salicylic acid–dependent component in the regulation of GST1 does not require NPR1. Ozone-induced disease resistance also is disrupted by the *npr*1 mutation. This mutation appears not to interfere with the hypersensitive response [154] indicating that ozone-induced resistance is mediated basically by the SAR pathway [151]. Moreover, *npr*1 plants were not more sensitive to ozone damage suggesting that the antioxidant response does not require NPR1, a protein containing ankyrin repeats [155], which probably mediate protein-protein interactions. NPR1 shares similarity at the gene sequence level with the mammalian IκB, an inhibitor of NF-κB, that regulates the cytosolic localization of

this transcription factor. This similarity suggests that NPR1 could act as a transcriptional regulator of PR gene expression [46].

Mutants with spontaneous hypersensitive response lesions in the absence of pathogenic infection have been described in several plant species indicating that the hypersensitive response is under genetic control [156]. The *Arabidopsis* mutants *lsd1* (lesions simulating disease) constitutively express PR proteins and produce salicylic acid [157]. LSD1 encodes a zinc finger protein that participates as a negative regulator in the transcription of death-response genes [158].

Cis Elements

Conserved regulatory protein-binding nucleotide sequences in the promoters of several stress genes [159,160] are probably responsible for the coordinate induction by converging signaling pathways. The study of *cis*-acting regions in the promoters of ozone-induced genes is providing some information about these interactions. Recently, the stilbene synthase promoter from grapevine fused to the glucuronidase reporter gene has been analyzed in transgenic tobacco plants [161]. Glucuronidase activity after ozone treatment was distributed all over the leaf in small spots, mainly in palisade and parenchymal cells. Analysis of the promoter revealed an elicitor-responsive element (ERE). Interestingly, deletion analysis demonstrated that regions responsible for its inducibility by pathogens and ozone are located in different, nonoverlapping zones of the promoter. Moreover, the fact that the ERE element is included inside the ozone-responsible area of the promoter appears to indicate that the ozone effects on gene regulation are related to elicitation [161].

CONCLUSIONS

Although a relatively recent environmental problem, ozone is already causing important agricultural losses, while short-term predictions regarding a desirable attenuation of the pollution levels in industrialized countries are pessimistic [162]. In this context, sustainable agriculture would require to address seriously the problem of ozone damage to plants, the molecular details of which are recently beginning to emerge thanks to the wealth of data supplied by the molecular biology approach. Some of the main conclusions of these studies are summarized in the following scenario (Fig. 5).

Ozone enters the leaf through the stomata and, once in the apoplast, it may react with the cell wall and membrane components. As a result of some of these reactions, ROIs, singlet oxygen, and other free radicals are produced. These species have three possible destinies. First, they can be detoxified by the antioxidant compounds present in the apoplast. Second, they can diffuse (especially H_2O_2) inside the cell. Third, they may further react with membrane components generating a variety of products that can act as intra- or extracellular signals. These putative signals could be detected by nuclear sensors leading to the biosynthesis of ethylene and salicylate which would mediate further responses.

Intracellular ozone-induced signals, other than those acting on the nucleus, could be responsible for changes in the chloroplast membranes that have been described in ozone-exposed cells. Alterations in energy transducing membranes or electron carriers of chloroplasts and mitochondria may potentially lead to enhanced ROI production that in turn results in further damage to these molecules. This positive-feedback chain, or ''vicious circle,'' of ROIs in these organelles has to be quenched by antioxidative systems, or else it has been proposed as the mechanism responsible for accelerated senescence. The enhanced production of ROIs may contribute to the global increase in the oxidative conditions inside the cell and trigger a senescence program responsible for downregulation of chloroplastic gene expression. Ethylene could also participate in the timing of stress-induced senescence. Probably all three plant cell genomes (nuclear, mitochondrial, and chloroplastic) possess redox sensor mechanisms to detect the rise of oxidants. Under these circumstances, cross-talk between the nucleus and the organelles appears to be critical to integrate the global antioxidative response. Transcriptional and posttranscriptional mechanisms of nuclear control over the chloroplast

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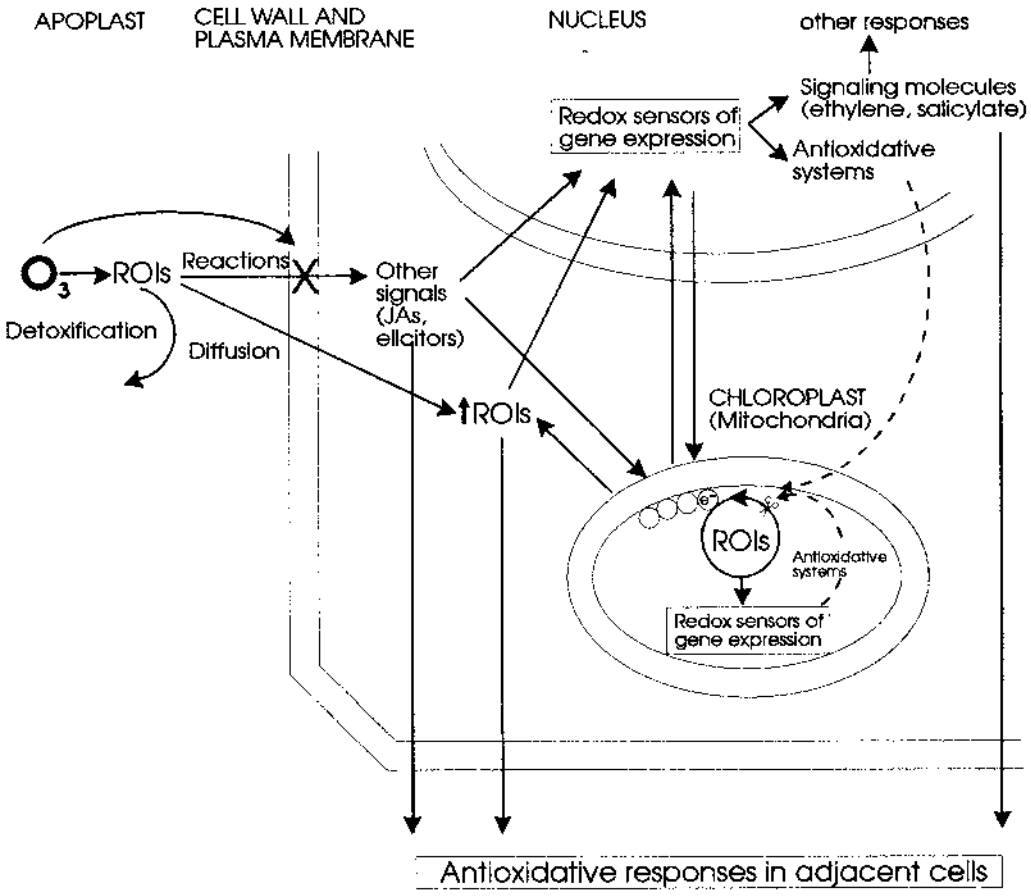

FIGURE 5 A model of the signaling mechanisms whereby ozone exposure induces cellular responses and modulates gene expression. Perception of intracellular conditions and cross-talk among the different plant cell genetic systems is central to this model. Postulated signaling molecules are ROIs, ethylene, salicylate, jasmonates (JAs), and chloroplast-targeted nuclear effectors.

protein expression have been shown to act under different environmental conditions [163–165]. Jasmonates could additionally (or alternatively) mediate nuclear-directed chloroplastic alterations in the gene expression during ozone exposure, since these compounds have been proposed to inhibit the chloroplastic functions inducing senescence.

Depending on the intensity of the stress caused by ozone, adaptative (under low-level long-term exposures) or necrotic (under acute treatments) responses can take place. These qualitatively different behaviors could result from a threshold phenomenon. Antioxidative defenses, both nuclear and chloroplastic, would act controlling the levels of ROIs up to the point where they become overwhelmed. Below this permissible level, intracellular conditions would be compatible with cell survival and the response would be adaptative. However, if the critical threshold is exceeded, uncontrolled ROI production will probably lead to irreversible oxidative injury, and the damaged cells may initiate a death program that would produce a necrotic lesion. Meanwhile, the surrounding cells would receive diffusing signals that activate their defenses against oxidative stress.

The diversity in the responses to ozone could depend on multiple interacting factors, for example, the ozone dose and time of exposure, plant species characteristics, and developmental stage, and simultaneous presence of other stressing factors. Taken together, these factors could modulate the response through the variety of signals, interactions, and cross-talks mediating the ozone response. Currently, a clear picture of these interactions at the molecular level is lacking, probably because several important pieces of the complex signal transduction pathway are still missing. New approaches, such as the study of the ozone response in specific mutants or transgenic plants, are helping to solve questions about the role of single signal molecules in the whole transduction pathway. Besides, further work is needed to investigate the plausible involvement in the ozone response of other habitual signaling components, such as G proteins, calcium, protein kinases (MAPKs), and phosphatases, since these components have been shown to participate in closely related plant signal transduction processes, such as plant-pathogen interactions [166–170].

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Genetic Factors Affecting Abiotic Stress Tolerance in Crop Plants

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INTRODUCTION

Plant growth and development is a result of the interplay between the genetically governed potential of the plant and the plant environment in which it grows [1,2]. Therefore, a plant's peaceful way of living is often disturbed by a number of environmental factors among which abiotic factors are of crucial importance. The earth's surface, which is 70% saltwater and 30% land [3], possesses only half of its land area free from extremes of water and temperature, and to soil erosions and difficult geography. The other half, which is used for agricultural production, also faces various abiotic stress problems that are considered to be the main source of yield reductions all over the world [4]. These abiotic stresses are either stable or fluctuating in nature. Stable stresses are those due to abnormal pH or metal toxicity. Unstable or fluctuating stresses include abnormal levels of water (drought/flooding), temperature (cold/hot), and other factors such as pollutants.

Abiotic stresses are characterized by the occurrence of more than one stress at the same time or throughout the growing cycle even though one stress may dominate [5]. Since the whole biotic world, directly or indirectly, is dependent on plants for survival, any disturbance to plants gets reflected in their own disturbance. This is of greater reality for humans and their agriculture, and it is even greater in the present situation where all our natural resources are shrinking except the number of mouths to feed. Therefore, providing relief to crop plants from abiotic stresses is providing relief to ourselves. Relief from abiotic stresses is possible either by changing/avoiding the environment or changing the genotype of the plant itself. Genetic manipulation of crop plants, to make them ready for abiotic stress areas, now seems possible with our increased knowledge about the genetic factors affecting abiotic stress tolerance. However, in some cases, it is difficult to differentiate between the genetics of the traits associated with stress and the tolerance of the stress itself. Breeding for abiotic tolerance may be direct (selection pressure under stress) or indirect (selection pressure under a stress-free environment) [3]. The recent, advent of molecular tools, especially molecular markers, has revolutionized the genetic analysis of crop plants and provided not only

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geneticists but also physiologists, agronomists, and breeders with valuable new tools to identify traits of importance in improving resistance to abiotic stresses [6].

DROUGHT TOLERANCE

Crops all over the world are exposed to chronic or sporadic periods of drought [4], a multidimensional stress affecting plants at various levels of organization [7]. Drought stress affects yield by reducing both sink and source. It can be a result of stress affecting either one directly or their interaction with one another [7]. Before seeing the face of the sun or facing the external world, the living plant (the dormant embryo) within the seed is highly tolerant to desiccation but loses its tolerance on germination and emergence. Plants survive under drought through avoidance or postponement of dehydration or tolerance [8–12]. The ability of a crop to grow satisfactorily in areas subjected to water deficits has been termed drought resistance [8]. Plants tolerate drought stress through various morphological, biochemical, and molecular adjustments at the whole-plant level.

Morphophysiological Traits: Genetic Basis

Although there are no traits that confer global drought tolerance [13], numerous constitutive traits carry a large impact on crop performance under drought stress [7]. According to Wilson [14], traits associated with water-use efficiency act through their effect on (a) timing of crop development, (b) efficiency of root to harvest water, (c) effective transpiration control by the shoots and the relationship of transpiration and photosynthesis, and (d) the ability of plants to endure stress. Any trait that reduces transpiration or increases photosynthesis will increase water-use efficiency. Ludlow and Muchow [15] have reviewed important traits in crops for success in water-limited environments. Although several traits join to fight drought, the genetics of some of the traits useful under drought conditions is given below:

Earliness

Rapid plant development and early maturation requires less water thus works through avoidance [8–12,15]. There is genetic variation for earliness both across and within species [12,16,17]. For example, in wheat, there is much genetic variation in flowering time and maturity [18]. The *Rht* genes in wheat possibly possesses the pleiotropic effect on earliness [18]. In maize, a strong quantitative trait locus (QTL) for flowering date and anthesis to silking interval was identified on the same chromosome as the genes for yield under drought [19].

Roots

An extensive root system is desirable for efficient water extraction in different crops [12,20–22]. Genetic variation in root characteristics do exists in crop plants [20,23–28]. Many root characteristics have been shown to be under genetic control and are quantitatively inherited. But in rice, the difference in the depth of rooting is controlled by only a few genes [29,30]. There is genetic variation for root hydrolic conductance in wheat, and this is heritable [31]. Genetic variability for root size was found in sorghum [32], wheat [33], rice [34], soybean [25], and oat [35]. The density of root-hairs also shows considerable genetic variability (200 cm^2 in trees to 2500 cm^2 in cereals), but little is known about the intraspecific variability and genetics of this trait [36]. Root traits (root length, root number, root-tip thickness, and root/shoot weight ratio) in rice have been found to posseses moderate heritability and are under the control of both additive and dominance gene effects [29,30,34]. The predominance of additive gene action for maximum root length and number has been reported in rice [37]. Some of these traits also showed heterosis in several crop plants [7,38,39].

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Stomatal Conductance

Reduced stomal conductance through various characters such as stomatal frequency, length, and behavior [15] increases, water-use efficiency. Genetic variation has been reported for various stomatal characters [40–42], and they seem to be highly heritable [40,43].

Epicuticular Wax

The waxy layer covering the plant parts, for example, the glaucous characteristic in wheat and the bloomed trait in sorghum, improve water-use efficiency. Genetic variation has been reported for the bloomed trait in sorghum [44,45] and glaucousness in wheat [46]. Variation for cuticular wax also has been reported in other crops; for example, oat [47], rice [48], and barley [49]. The genetics of epicuticular wax has been investigated in several crop species; the presence of waxy bloom was found to be controlled by a single dominant gene [36]. Heritability for bloomed trait of sorghum was low [45].

Osmotic Adjustment

Osmotic adjustment (OA) reduces the rate of leaf senescence (stay green trait), because it increases both avoidance and tolerance of dehydration. Genetic variation for this trait has been found in wheat (*ms* gene) [50–55], sorghum [56–59], millet [60], cotton [61], rice [62], and pigeon pea [63]. OA is simply inherited and only one or few genes are involved in wheat [50,55,64] and soybean [65]. In rice, the *indica* cultivars tend to be more dehydration tolerant than *japonica* cultivars [62]. A gene for OA was located in chromosome 7A of wheat [64]. On the basis of homeology between a small segment of chromosome 7 of wheat and chromosome 8 of rice [66,67], it has been suggested that there might be association between the OA gene of wheat and rice.

Transpiration Efficiency

The transpirational efficiency of C_4 plants is greater than of C_3 plants [68,69]. Genetic variation for transpiration efficiency has been reported in wheat, barley, cotton, peanut, and sunflower [31,70]. Its inheritance is complex [15] but heritability is high [70,71]. High heritability has been noted in crested wheat grass [72], peanut [73], and wheat [74–76] and moderate in cowpea [77]. It has been reported that transpiration efficiency may be under the control of few genes in tomato [75].

Several other traits such as, for example, the mobilization of preanthesis assimilates, leaf movements, epidermal conductance, developmental plasticity, and leaf area maintenance, also are important for drought tolerance [15]. Genetic variation for some of these traits also has been reported, for example, for mobilization of preanthesis assimilates [78,79] and leaf area maintenance [80,81]. Richards and Passioura [82] found an intraspecific variability in bread wheat for xylem vessel diameter which possess high heritability. Leaf rolling showed genetic variation in sorghum [83,84] and rice [10,84]. Genetic variation for epidermal conductance has been observed in rice [85,86], sorghum [87,88], and soybean [89]. Low lethal water status also exhibits genetic variation in sorghum [87,89], wheat [90], pigeon pea [91], and cotton [92].

Genetics

The genetics of drought resistance can be partly understood through the inheritance of traits responsible for drought avoidance or postponement or tolerance as described above. However, the situation is complex, as single genes that substantially change water-use efficiency are difficult to find and generally it involves many genes and many interactions [12]. Tolerance to drought is rare in the vegetative parts of plants, whereas angiospermous seeds and pollen are able to survive extreme dehydration [93]. Genes responsive to drought, desiccation, high osmoticum, or wilting have been identified in tomato [94], *Craterostigma plantagineum* [95], maize [96], barley [96], rice [97], *Arabidopsis* [98], tobacco [99], soybean [100], and cotton [101].

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To understand the genetic mechanism of osmotic adjustment and dehydration tolerance in rice, a recombinant inbred (F_7) population was mapped with 127 restriction fragment length polymorphism (RFLP) markers; one major locus was found to be associated with osmotic adjustment in rice [62]. This locus may be homeologous with a single recessive gene previously identified for the same trait in wheat; the putative osmotic adjustment locus and two of five quantitative trait loci (QTLs) associated with dehydration tolerance were close to chromosome regions associated with root morphology [62].

The location of genes having a major effect on drought-induced abscisic acid (ABA) accumulation in wheat was determined by using molecular markers, a set of single chromosome substitution lines, and populations derived from a cross between high-ABA–producing and low-ABA–producing genotypes [102]. A similar drought test with detached and partially dehydrated leaves confirmed the location of gene(s) regulating ABA accumulation in the long arm of chromosome 5A [102]. MAPMAKER QTL showed the most likely position for the ABA quantitative trait locus to be is between the loci *Xpsr575* and *Xpsr426*, about 8 cm from *Xpsr* 426; other QTLs for ABA accumulation may be present on chromosome 3BS and 6AL [102]. It was reported that xylem ABA (not root ABA) was associated with stomatal conductance and root characteristics; genes for yield under drought and ABA were located on different chromosomes in maize and, therefore, were unlikely to be related [19].

A QTLs for response to drought has been reported in different crops such as maize [19,103–107], sorghum [108,109], rice [110,111], wheat [102,112], and barley [113]. There is clear evidence now that all the major cereal species have extensive linkage blocks where gene order is conserved [114], which probably represents fragments of a hypothetical ancestral cereal ''chromosome'' [6].

Gene Expression

Water deficit is one of the most common abiotic stresses that effects the growth and development of plants through alterations in metabolism and gene expression [115]. Genetic information to withstand drought is present in plants in stress genes, but these genes are expressed only in particular developmental stages [116]. The molecular studies of dehydration stress are mainly based on (a) desiccation tolerance of the maturing embryo [117] and resurrection plants (angiosperms that are able to survive dehydration and revive upon hydration) [95,118], (b) *Arabidopsis thaliana* [119–121], and (c) other crops such as, for example, tomato, pea, wheat, and barley [122]. Genes regulated by drought stress can be divided in to three groups [122]: genes encoding polypeptides of unknown function, genes encoding *Lea* proteins and related polypeptides, and genes encoding polypeptides of known function.

DNA sequence analysis of osmotic stress–inducible cDNAs indicate that genes responsible for drought [6,19,62,102–113] encode a variety of proteins [123]. Many of the proteins encoded by these cDNAs have been classified into various groups; namely, LEA (late-embryogenesis abundant) [101], RAB (responsive to ABA) [124], or dehydrin [96] proteins. LEA proteins and dehydrins are classified on the basis of their characteristic amino acid motifs, whereas RAB proteins are classified based on expression in response to ABA [123]. The LEA proteins, first identified during seed maturation and desiccation, express in water-stressed vegetative tissues in almost all plants [123]. They are supposed to protect the dehydrating cells by a variety of mechanisms, including renaturation of unfolded proteins, sequestration of ions, and stabilization of native protein structure [123]. Recently, it has been demonstrated that the accumulation of barley HVA1 protein, a group of three LEA proteins, in transgenic rice confers increased tolerance to water deficit as well as to salt stress [125].

Genes have also been isolated from the resurrection plant, *Craterostigma plantagineum*, which can recover completely from complete dryness with in 24 hs of contact with water [126,127]. Many of the desiccation-induced genes share sequence homology with LEA genes [126,128]. The early responsive to dehydration stress (ERDS) genes in comparison with ABA-responsive genes are preferentially responsive to the dehydration stress [129].

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The dynamics of water transport in plants is influenced by water channel proteins in plants [130,131] which are related to the superfamily of membrane intrinsic proteins (MIPs) first characterized in *Escherichia coli*. The function of different MIP channels vary [132,133]. Several MIP-related proteins have been identified in plants. A member of this family, *Trg* 31 (in pea), was initially identified when gene expression was induced in partially dehydrated leaves [134]. Another dehydration-inducible gene was isolated from *A. thaliana* [135]. NOD 26, the first plant MIP protein to be characterized [136], is abundant in the prebacteroid membrane root nodules. MIP-like tonoplast intrinsic proteins (TIPs) and their corresponding genes have been identified [137]. Water deficit results in diminished growth of young leaves. In soybean, water-stressed plants show increased expression of genes encoding the vegetative storage proteins *(Vsp)* [138]. But the same does not occur in the mature leaves even though they possess the potential to express *Vsp* when leaves are wounded [139].

Breeding

Despite many decades of research, drought continues to be a major challenge to agricultural scientists probably because of the difficult nature of the target environment [140,141] and the interaction of drought with other abiotic as well as biotic stresses [142]. This is supported by the observation that, in water-limited environments, the yield of the biomass of current cultivars is about the same as cultivars from over a century ago [143]. However, the wonderous display of plant adaptations to dry habitats points to the fact that a substantial genetic variation for drought tolerance exists and this may be used for plant breeding [144].

The problem of breeding crops for drought environments is not due to the want of enough genetic variation but probably lies in the elusive design of the ideal plant, the ideotype [145], which has been in attention [146,147] for both normal and stress conditions [7]. The demands of genes depend on the type of ideotype required for the water-stress area. Since a large number of traits take part in the plant-stress response, the task is not easy in practical terms.

The water status of a plant is a function of uptake (by roots) and loss (via stomata and cuticle) of water; therefore breeding strategies may broadly focus on either of these two parameters [148]. So far, the breeding strategies for drought areas have been suggested to depend on (a) selecting genotypes with improved yield in water-stress environments [139]; (b) identifying and selecting traits that contribute to drought avoidance, drought tolerance, or water-use efficiency [15]; and (c) even selecting under nonstress environments and then trying in stress areas [144,149]. Without plant selection under water-deficient conditions, traits beneficial under water stress may be missed [12]. However, in favorable environments, there is less error and thus high yield potential expressed in favorable environments can also have a spinoff in less favorable environments [144]. In Australia, where wheat is grown in a water-limited environment, 95% of the current cultivars can be traced to the CIMMYT germplasm where breeding is done under highly favorable environments [144].

There are few reports of the transfer of alien DNA into crop species specifically to improve drought responses [6], but an extensive introgression program is in progress to transfer useful traits from *Festuca* into *Lolium* [150–152]. In some of the introgression lines with *F. arundinacea* in *L. multiflorum* × *F. arundinacea* crosses, drought resistance was equivalent to the *Festuca* donor [151]. This single chromosome addition lines of *F. arundinace* onto *L. multiflorum* also showed improved drought resistance [6]. Hence, this work looks promising for the future to improve the drought responses of other graminaceous crops [6]. The introgression of the drought-tolerant machanism present in wild species in cultivated plants is in progress in several laboratories [153,154]. Another strategy for the genetic improvement of plants under drought has been to identify gene(s) of desiccation tolerance, for example, in desert plants or wild species [155,156], and transfer them to agronomic crops [157]. Transgenic rice plants having tolerance to water deficit and osmotic stress have been reported [158]. However, this effort is not expected to be of much significance, and the mechanisms of desiccation tolerance at the plant level are believed to be quite efficient [107].

The QTL mapping techniques are a hope for the future with regard to improved abiotic stress

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tolerance and drought stress in particular [6]. The locations of QTL are now easy to compare across species, as demonstrated through the comparison of the QTLs of root characters in maize and rice [103,110]. Marker-assisted selection, using Random Amplified Polymorphic DNA (RAPD), improved the yield performance in common bean in stress conditions [159]. Although molecular genetics might prove to be important, the identification of stress-responsive genes or even their cloning and insertions seems to be beyond practical application unless their function and value within the ideotype can be demonstrated [7].

SUBMERGENCE TOLERANCE

Tolerance to waterlogging or submergence tolerance is associated with crops grown in high-rainfall areas of the world. Most of the knowledge regarding submergence tolerance is on account of the studies on rice crops, 16% of whose total world area is affected by this problem [160]. Limited gas diffusion in water is considered to be the principal cause for the adverse effects of submergence [161]. Therefore, tolerance to flooding is associated with the ability to cope with the problems associated with submergence, such as, for example, anaerobiosis, lower carbon assimilation due to less CO_2 and radiation [162,163], and high ethylene levels. This is partly achieved by avoidance—through maintenance of growth processes leading to elongation of plants to maintain their foliage above water [164].

Morphophysiological Traits: Genetic Basis

Several morphophysiological traits have been reported to be associated with submergence tolerance [165]. Setter et al. [165] listed 17 morphophysiological traits as part of the mechanism explaining submergence tolerance in rice. These traits were classified into presubmergence, submergence, and postsubmergence traits. Among these, the three important traits are (a) carbohydrate concentration, (b) alcoholic fermentation, and (c) elongation of the stem. The favorable effects of high carbohydrate concentration [166–170] and high alcoholic fermentation [171–175] are well documented. Stem elongation does favor avoidance but, on the other hand, there is a strong negative correlation between elongation growth and percentage of survival of seedlings during submergence [176,177], because elongation growth competes for energy and carbohydrates required for maintenance processes for survival [165]. The elongation mechanism is effective only when the water level remains high for a considerable period, as in deep-water rice cultures. It is not desirable under flash-flood conditions, because when the water recedes, the plants tend to lodge [178].

Genetics

The existence of varietal differences for submergence tolerance has been reported by several workers in rice [179–182]. Mohanty et al. [183] reported that submergence tolerance in rice was dominant over suceptibility and that both major and minor genes were involved in the inheritance. A 10×10 half-diallel analysis [184] showed a significant additive and nonadditive gene action for submergence tolerance, but additive effects were more important. Tolerance was dominant over nontolerance, and the average dominance was within the range of incomplete dominance; Wr/Vr graphic analysis also suggested the involvement of both major and minor genes [184].

In an earlier study [185], it was reported that at least three submergence-tolerant genes are present in the four most tolerant of rice varieties; namely, FR13A, Thavalu, Kurkardppan, and Goda Heenati. Analysis of F_2 and backcross generations of the above four rice varieties and two susceptible (IR 42 and Nona Bokra) lines indicated the presence of a single dominant gene for submergence tolerance [165]. It was also found that the first three tolerant lines possessed the same gene for submergence but was different in the cultivar Goda Heenati. This finding was supported by the bimodal distribution of rice lines for submergence in Thailand [186], but it was contradicted by the normal distribution noted in the Phillipines [186]. The finding of the three most tolerant rice lines

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having the same gene for submergence tolerance suggested that a common factor related to tolerance of limited gas diffusion (e.g., one of the enzymes of alcoholic fermentation) may be responsible for genotypic differences in the submergence tolerance of rice [165]. It also suggests that a gene for a transcription factor is involved in the expression of a multiple gene cascade that confers submergence tolerance [165].

Gene Expression

Gene expression for anaerobic (submergence) stress is better known than other abiotic stresses [165,187,188]. The knowledge of gene expression during anaerobisis is due to the finding in maize that alcohol dehydrogenase (ADH) activity increases owing to flooding [189–191]. ADH activity which allows maize to survive in flooding reflects a simultaneous expression of two unlinked genes, *Adh1* and *Adh2* [192]. The *Adh1* and *Adh2* cloned cDNA families have been identified and analyzed extensively [193,194]. The regions of *Adh1* and *Adh2* genes upstream from the site of transcription initiation show homology with respect to an 11-bp homologus region that includes a TATA box and three other segments 8 bp in size [195]. The other important enzyme is pyruvate decarboxylase (PDC) [196], which is governed by three *Pdc* genes in rice [197].

During anaerobiosis, a shift in protein synthesis was reported [195] where it was found that there is repression of preexisting protein synthesis followed by de novo synthesis of a new set of proteins. This has been reported in several crops; for instance, soybean [198], maize [195,199], rice [200], sorghum, barley, pea, and carrot [165]. The shift in protein synthesis is quite fast with a transition period of few hours. The polypeptides (33 kd) formed during this transition period are referred as transition polypeptides (TPs). Another group of 20 polypeptides (anaerobic polypeptides; ANPs) also is induced after a 90-min gap which includes isozymes of alcohol dehydrogenase [201], glucose-6-phosphate isomerase [202], fructose-1, 6-diphosphate adolase [203], and sucrose synthetase [165]. In rice, the two *Adh* genes [204] and three *Pdc* genes have been cloned and characterized [197,205,206]. Genes of these two enzymes are now being used for over- and underexpression studies in rice [165] by using the coding regions of *Adh* and *Pdc* genes through two types of promoters: constitute (CaMV35S and Act1) [207] and inducible (6 XARE) [208].

Submergence tolerance being a complex character, it is likely that the putative single gene for submergence tolerance either (a) encodes a transcription factor (*trans*-acting factor) (i.e., a protein or RNA which binds to specific regulatory DNA sequences [209]) or (b) affects the signal transduction pathway (i.e., the number of steps after the submergence treatment by which the plant activates the set of genes required for survival [210]). Around a dozen major genes known to be induced under anaerobic condition in maize [211,212] and rice [213,214] possess a similar sequence in their promoters [215] and therefore suggests the involvement of a common transcription factor [165].

Breeding

Breeding for submergence tolerance has been an important objective for rice breeders. Systemic screening at International Rice Research Institute (IRRI), the Philippines, has resulted in the identification of flood-resistant rice cultivars such as FR13A, FR43B from India and Kurkaruppan and Goda Heenati from Sri Lanka [184]. Another submergence-tolerant elite line of IRRI, IR 49830 (-7-1-2-2) whose ancestry includes FR13A, IR42, and IR48 [216], has been reported to have a four to five times higher yield than FR13A [165]. Shuttle breeding programs between IRRI and the national agricultural research institutes of several countries now provide promising advance lines as well as segregating populations for use in their target environments [165]. However, the success achieved in submergence tolerance all over the world is far below our need and expectations. A probable reason for the absence of the significant introduction of improved submergence-tolerant rice cultivars during the last two decades has been the confusion between genuine submergence tolerance and shoot elongation [217]. Another factor for the above might be intolerance to other common stresses like phosphorus and zinc deficiency and presubmergence drought [218]. Despite

a limited knowledge in the physiological and molecular basis of submergence stress, the association of submergence tolerance in traditional tolerant genotypes with poor agronomic traits direct us to harness the tools of molecular biology to rebuild rice plants for better fitness [165].

HIGH-TEMPERATURE TOLERANCE

Heat stress, especially during reproductive development, causes severe yield reductions in different crops. It is an important problem in tropical and subtropical environments and is believed to increase in the future because of global warming [219]. In dry land areas, heat stress may occur in association with radiation and drought stress [220]. Plant responses to heat stress are diverse, but photosynthesis is considered to be the most heat-prone plant process [221–224]. Photosynthesis and respiration are more sensitive to heat stress in cool-season species such as wheat than in warm-season plants [223]. The thermal stability of warm-season species is associated with the properties of the photosynthetic system, including key enzymes and the thylakoid membrane, with the thylakoid membrane being more heat sensitive than the cell membrane [223]. Other reports [225–227] suggest that high temperature retards the conversion of sucrose to starch in developing grains (e.g., in wheat). Thus, any of a number of important metabolic functions may be disrupted because of heat stress, but a cell membrane system that remains functional during heat stress appears to be central to the adaptations of plants to high temperature [228]. Understanding the molecular and physiological bases of heat tolerance in higher plants has proved difficult owing to its complexity [229,230].

Genetic Basis

Knowledge regarding traits conferring high temperature tolerance and their genetics is essential for the creation of genotypes capable of giving high yields under high-temperature environments worldwide [231]. However, no single trait can be said to be directly responsible for heat-stress tolerance, although several traits have been found to be associated with this mechanism. Therefore, high-temperature tolerance is characterized by measuring whole-plant productivity traits (e.g., yield traits) or by utilizing bioassays in different crops [231]. Different traits utilized in such studies are, for example, flower bud abortion and a reduction in pod fill in common bean [232]; electrolyte leakage [233,234], membrane thermostability [235], a reduction of 2,3,5 triphenyl tetrazolium chloride [231,236–239] and chlorophyll fluorescence in wheat [240]; electrolyte leakage in soybean [241–243] and common bean [243]; and heat-shock proteins in sorghum [244,245], cotton [246], wheat [237,247], and maize [248,249]. Most of these traits are measures of the effect of the heat stress rather than the cause of heat-stress tolerance. Therefore, the genetics of traits causing heat-stress tolerance and the heat tolerance itself is difficult to be seperate. Hence, all traits are discussed below to understand the genetics of heat-stress tolerance.

Substantial genotypic variation for heat tolerance was found in groundnut, soybean, pigeon pea, and chickpea and they were ranked from heat tolerant to heat sensitive in the same order [250]. Quantitative inheritance with a large environmental effect was reported for heat tolerance at pod and seed set in snap bean [251]. In another study, a single dominant gene in one snap bean accession and two genes with epistatic effect in another were reported [252]. In a study involving two resistant and two susceptible genotypes of common bean (*Phaseolus vulgaris*) crossed in all possible combinations, including reciprocals, quantitative inheritance was noted for heat tolerance [232]. Additive effects were significant for two heat-tolerance traits (flower bud formation and pod filling) in common bean. Cytoplasmic effects, including the interaction of cytoplasmic and nuclear genes, were also recorded [232]. In cowpea, tolerance to the inhibition of flower bud development under high temperature and a long day was due to a recessive gene [253], whereas tolerance during pollen formation was under the control of a single dominant gene [254]. In tomato (*Lycopersicon esculentum*), it was reported that heat tolerance during fruit set was conferred by a few partially dominant genes, but narrow sense heritability was very low (8%) owing to large environmental effects [255].

Genetic effects on membrane thermostability in wheat in 90 F_2-derived lines of heat-tolerant

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and heat-susceptible lines showed that heat tolerance is not simply inherited [235]. Genetic differences in membrane thermostability were noted in soybean (*Glysine max* L. merr.) [241,256]. Hybrids of heat-tolerant and heat-susceptible soybean genotypes were found to be intermediate but closer to the tolerant parent in tolerance; the number of genes involved could not be estimated [241].

Marsh et al. [243] examined the inheritance of membrane stability in common bean and found that heat tolerance was under the control of few genes. They also found low additive effects along with epistasis. In a diallel including the reciprocal of six wheat genotypes, significant general combining ability effects and maternal effects were noted [240]. On the basis of the relation of the heat-stress effect and the reduction of 2,3,5 triphenyl tetrazolium chloride, which produces a red formazen, significant differences in acquired high-temperature tolerance were reported in wheat [239]. Using 20 F_1 progenies produced through a complete 5×5 diallel mating design of tolerant and susceptible genotypes showed that only the general combining ability effect was highly significant, accounting for 67% of total genetic variation [231].

There is extensive evidence of both qualitative and quantitative intraspecific genetic variability for low molecular weight (LMW) heat-shock proteins (HSPs) in crops; for example, sorghum [244,245], cotton [246], wheat [237,247], and maize [248,249]. Very few reports are available regarding HSP gene transmission in plants. Additive inheritance for some HSPs has been reported in barley where the presence of hybrid-specific HSPs indicated the activation of genes that were suppressed in one parent [257]. Both additive and nonadditive inheritance were demonstrated in the F_1 hybrids of maize [248]. Intraspecific qualitative polymorphism in LMW synthesis is extremely rare [258], and quantitative variation in HSP synthesis may determine relative thermal tolerance levels [259]. A genetic analysis of HSPs in maize, HSP synthesis revealed both qualitative and quantitative polymorphism implicative of the differences in HSP structural genes and regulatory factors [260]. The F_1 hybrid HSP profile indicated that the synthesis of all parental HSPs conformed to dominant inheritance patterns, including complete dominance, overdominance, and codominance; there was evidence for unlinked loci of four different HSP gene pairs, but data for three other HSP gene pairs were inconclusive [260].

Gene Expression

Heat stress is known to induce HSPs, which [261] are known to be associated with acquired thermal tolerance in many species, including bacteria [262], mamalian fibroplasts [263], and higher plants such as, for example, soybean [264,265], wheat [237], cotton [266], and maize [249]. In plants, a heat shock of 8–10° C above normal growing temperature induces the synthesis of both high (65–110 kDa) and low (15–27 kDa) molecular HSPs, with the LMW proteins being the most prevalent [267,268]. A subset of the LMW group, 15–18 kDa, is unique to higher plants [195].

The LMW HSPs are a complex group, with as many as 30 members being present in soybean [269]. The induction of LMW HSPs has been well documented [270], and their number is known in some monocot species [268]. Detection of low and high molecular weight HSPs synthesized in seedlings and flag leaves in flowering plants suggest that HSPs are synthesized before leaf temperatures reach levels that are considered injurious to growth and development [271].

HSPs are among the fastest known gene expressions in plants [272]. The heat shock response may be the accumulation of damaged proteins [273]. This is supported by the fact that small-protein ubiquitin, which has a role in the ATP-dependent breakdown of abnormal proteins [274], is itself an HSP [275].

Limited information is available about the structural relationships among the HSP genes and the molecular regulation of their transcription and translation to protein [248,260,276]. On the basis of nucleotide and amino acid similarities and protein localization, there are four families of structural LMW HSP genes known in plants [277]; one each encoding plastid localization and endomembrane proteins and two that encode cytoplasmic proteins (classes I and II). The HSPs of 17–18 kDa comprise classes I and II. There are several class II gene sequences [278,279] as well as class I cDNA HSPs [280].

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Breeding

Increasing productivity under heat-stress conditions requires the development of heat-tolerant genotypes in all crops. Improvement of heat-stress tolerance can contribute to sustainability and provides a way to extend the area under cultivation [250]. Limited progress has been made with regard to breeding heat-tolerant genotypes probably because yield losses due to heat are more subtle than biotic stresses [281]. The two most important hurdles are the absence of substantial information on the genetic diversity for heat-tolerance traits and effective screening techniques [282]. Both in vivo and in vitro methods are used for screening but in vitro methods are advantageous in that they are plant conserving, and this feature is important in plant breeding for heat tolerance [250].

The level of tolerance to high temperature varies among genotypes [240,283,284] suggesting that the trait can be improved [240]. The indication of larger additive genetic variation with regard to some traits believed to be associated with heat stress also indicates that gains from selection for improved heat tolerance should be possible. Breeding for heat tolerance in cowpea has involved a pedigree breeding approach with selection beginning in the F_2 generation [285] to incorporate major recessive genes conferring heat tolerance during early floral bud and seed coat development [286]. It has been suggested that to overcome difficulties caused by environmentally induced variation, incorporating a heat-tolerance pod set into other genetic backgrounds will require family selection in advanced generations to ensure that the trait is fixed [254].

Recurrent selection has been suggested to accumulate genes favoring high-temperature tolerance based on chlorophyll fluorescence measurements in wheat [240]. One of the most popular wheat varieties of India, HUW234, which currently occupies more than 3 million hectares of area, possesses the unique feature of both avoidance and tolerance of heat stress of the north eastern plains zone of the country. Its early maturity, profuse tillering, high grain number per spike, and fast ripening provides it with a clear superiority over other varieties under late to very late sown conditions when the hot winds of early summer cause serious yield losses.

COLD TOLERANCE

Low temperature, especially in the northern region of the temperate climatic zone, presents substantial obstacles to the survival of plants throughout the winter [287] and is one of the most severe stresses that limits crop growth and productivity [288]. Reductions in grain yield are incurred not only as a direct result of winter damage but also as a result of limiting the areas where such crops can be sown [289]. Although low-temperature stresses are usually of two types, chilling at above 0° temperature and freezing at subzero temperature [290], the winter may expose young seedlings to many kinds of stresses (as in wheat) such as, for example, a direct frost effect, cold winds, snow cover, intense freezing and glaciation of the soil, and frost lifting in the spring. In grasses and wheat, there are two different mechanisms of tolerance to ice encasement: rapid (wheat) and slower (grasses) glycolysis [287]. Following cold acclimation, a number of forage species show a high tolerance to the extremely cold condition of ice encasement [291] even greater than that of winter wheat [287]. In grasses, Berings hairgrass has recently been shown to have an extremely high tolerance to anoxia [292], a property that is common to many arctic plants [287]. The physiological and biological processes which lead to cold tolerance or the adaptation of plants to low temperature are extremely complex [293].

Morphophysiological Traits: Genetic Basis

Like other abiotic stresses, tolerance to low temperature also is due to the joint action of several traits of plants. Significant correlations were established between cold hardiness and days to head [294] and growth habit [295–297] in wheat. In general, spring wheat lines are less hardy than winter

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lines and the spring growth habit is dominant over the winter growth habit. The winter growth habit of wheat is possibly inherited by a *Vrn* (vernalization requirement) gene [297]. Chahalan and Law [298] found no evidence of a genetic linkage between cold hardiness and the vernalization requirement in wheat even though chromosomes in homeologus group 5 were implicated in the control of both of these characters. Studies in grass species (e.g., clover) also do not show correlation between a single trait and cold tolerance [299–301]. Photoperiodism, an important trait for the adaptability in cold climate, is governed by genes present in the group 2 chromosome of wheat [302]. At least three genes, *Ppd1, Ppd2*, and *Ppd3*, governing photoperiodism are known in chromosomes 2D, 2B, and 2A respectively [303]. Low temperature has been found to enhance anthocynanin synthesis in plants, such as sorghum, cabbage, maize, Arabidopsis, apple, roses, and petunia [304].

Genetics

Winter hardiness is a genetically programmed integrated process [305,306]. The genetics of winter hardiness was studied as early as 1912 when Nilsson-Ehle [307] of Sweden investigated winter hardiness in wheat, and on the basis of the appearance of transgressive segregants in a cross of two cultivars intermediate in winter hardiness, he reported that it is a quantitative trait under the control of a polygenic system [307]. Since then no general opinion has arisen on the issue. The trait has been reported to be recessive [308], intermediate [309,310], dominant [308], or overdominant [308,311]. It has been reported that winter hardiness is under the control of dominant genes in mild cold, whereas under severe cold, it is governed by recessive genes [296,312–315]. Winter hardiness behaved as a recessive factor as early as 1923 in a cross made by Schafer [316].

A majority of studies indicate a polygenic control of cold tolerance [295,312,317–321]. The quantitative nature of winter hardiness also is supported by the absence of a drastic improvement in the winter hardiness of different crops [322–324], the appearance of transgressive segregants [299], and a complex of factors influencing winter hardiness [325]. However, all the genes do not work together and different genes affect tolerance at different levels of stress [313]. There is evidence from studies showing that winter hardiness genes may act as dominant or recessive depending on the type of environment [296,305,312,326,327]. In wheat, 11 chromosomes carry genes for cold tolerance with chromosome number 5 being the most important [328]. Some studies have implicated 15 of 21 chromosomes pairs of wheat to be associated with cold tolerance [315] with chromosomes 5A [298,329] and 7A, 4D, and 5D [315,330] being most commonly mentioned. In barley, a major QTL was found associated with chromosome 7 [331,332]. In *Solanum* spp., the nonacclimated freezing tolerance and acclimation capacity were found to be separate heritable traits controlled by few genes [333]. In rye (*Secale cereale*), cold hardiness is controlled by genes with mainly additive effects [334].

The genetics of frost tolerance, studied in winter wheat by using complete diallel [313,335, 336], showed that frost tolerance is controlled by an additive-dominance system [314,315,337]. Several studies [305,336,338,339] have shown that frost tolerance is a complex character controlled by at least 10 of the 21 pairs of chromosomes [305]; chromosomes 5A and 5D have been implicated most frequently and they appear to carry major genes [336]. The gene for frost resistance (*Fr1*) was located on the long arm of chromosome 5A [336,340], and there might be close genetic linkage between *Vrn1* and *Fr1* [341]. Studies done so far indicate the presence of four major genes for vernalization requirement: *Vrn1, Vrn2, Vrn3* [342,343], and *Vrn4* [344]. Another gene, *Vrn5*, also was reported [345].

Cold tolerance is under the control of both additive and nonadditive gene effects in chickpea [346] and pea [347,348]. Genetic interactions also play an important role in cold tolerance [346]. Three additive genes or linkage groups are reported to control winter hardiness in pea [349]. The expression of low-temperature tolerance has been found to be under the control of the same genetic factors in the sporophyte and gametophyte of potato [350–352]. High heritability estimates for cold hardiness have been reported in wheat [297,315,337], barley [353,354], and oats [355].

Gene Expression

The molecular mechanism which regulates cold tolerance is not sufficiently well known [292]. In 1970 [306], Weisner suggested that cold acclimation might involve changes in gene expression. Since then, however, more and more information has been reported in this field [293,356–359]. The realization that cold acclimation requires altered expression of tolerance-related genes not seen under nonacclimating conditions has the basis for isolation and characterization of cold-induced genes [293]. Several studies have demonstrated that plants synthesize a new set of proteins when exposed to a cooler environment [324]. The existence of partially different mRNA populations in nonacclimated and acclimated plants has allowed the isolation of cDNAs corresponding to acclimation-specific mRNAs by differential screening, as in alfalfa [358,360], *Arabidopsis* [361], and barley [290,362–363]. The temporal pattern of low-temperature (LT)–induced gene activation varies between different plant species ranging from few hours (*A. thaliana*) [119,364–366] to several days [358,367,368].

The LT responsive genes are transcriptionally regulated through sequence-specific transcription factors that bind to their target sequence on the corresponding promoters [290]. LT-induced genes contain certain sequence elements that resemble ABA-responsive [369] and drought-responsive elements [370]. Homologous regions are also present in the promoters of LT-induced genes: *cor15a* [371], *rab18, kin1, kin2* [369], *lti29*, and *cor47* [290].

Identification of an mRNA group which only functions during the cold effect and codes proteins found only in frost-resistant wheat varieties [359] suggests a positive correlation between the quantity of proteins synthesized during the cold effect and the frost resistance of the varieties [293]. Some mRNAs decline during exposure to low temperature, as in *Brassica* [372], rice [373], and spinach [324].

Cold-induced rRNA synthesis in wheat takes place in seedlings as the result of low temperature during the first few days of cold treatment [374,375], and the cold-induced rRNA synthesis is closely correlated to the rRNA cistron number [375]. Quantitative and qualitative changes have been noted in the rRNA maturation processes owing to low temperature in a weak frost-resistant line of wheat as a consequence of which there is an increase in the last precursors (1.4 and 0.9 MDa) of the two stable cytoplasmic rRNAs [293].

Freezing tolerance includes tolerance to freeze-induced dehydration [324,376]. This is further substantiated by the fact that several of the LT-induced proteins are similar to proteins induced in response to water stress (dehydrins) [101,377]. Certain proteins having a putatative protein-stabilizing function (*Bip, Hsp70, Hsp90*) have been identified among LT-induced proteins [378,379]. Cryoprotective polypeptides capable of protecting the plant thylakoid membrane in vitro against the freeze-thaw cycle also has been reported in cabbage and spinach [380,381]. A structural similarity of the gene product of *kin1* from *A. thaliana* with an antifreeze protein of winter flounder [361] led to the speculation of the presence of an antifreeze protein, which was later contradicted [382]. However, there are reports of the presence of an antifreeze protein in winter rye [383,384].

In *A. thaliana* leaves, pigment accumulation in response to low temperature results from the activation of phenylalanine ammonia-lyase (*pal*) and chalcone synthase (*chs*) gene transcription in a light-dependent manner [385]. It was suggested that light dependency is a general feature of cold-induced gene expression. However, in *Petunis corolas*, cold activation of *chs* expression was not light dependent [304]. The effect on *chs* expression was not always correlated with that on anthocyanin content suggesting a posttranslational effect [304]. Earlier, Christie et al. [386] suggested that the effect of temperature is associated with transcription, transcript stability, translation, and enzyme activity. Low temperatures do not simply create conditions that facilitate the developmental activation of *chs* expression; they act as a separate inducing signal [304,386]. The transduction of a low-temperature signal (2–5° C) for the activation of a cold-acclimation–specific (*cas*) gene also has been studied that probably does not belong to the *chs* group [304].

The transcripts of enzymes of the fermentation pathway, alcohol dehydrogenase (ADH) and

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pyruvate decarboxylase (PDC), have been found to increase as a result of hypoxic acclimation in wheat [174] and maize [387]. However, more tolerant forage grasses, timothy (*Phleum pratense*) and Berings hairgrass (*Deschampsia berengensis*), show lesser activity of ADH and PDC [287].

Desication often accompanies cold acclimation and freezing stress [324]; therefore, at the molecular level, genes induced by water stress and ABA also are induced by cold stress in barley, rice, and spinach [373]. In contrast, genes induced by cold temperature can respond to water stress and ABA [315,361,363]. Homology between HSPs and cold-induced proteins has been reported in potato [363].

Breeding

Conventional breeding has been utilized by breeders worldwide to enhance cold tolerance in different crops. It has been suggested that, in chickpea, selection would be more effective if dominance and epistatic effects were reduced after a few generations of selfing [346]. Sutka [336] suggested three ways to improve genetic variation for frost tolerance in wheat: interspecific crossing, chromosome manipulation, and the induction of somaclonal variation. Wild species related to cultivated wheat, for instance, *Aegilops cylindrica, Agropyron glaucum* (intermedium), and *Agropyron elongatum*, are extremely promising sources of increased genetic variation [336] for cold tolerance. Disomic additions of *Agropyron glaucum* were able to survive freezing to a temperature as low as $-18°$ C. A somaclone of wheat was significantly better than control for cold tolerance, and thus it is of practical importance [388].

In vitro selection through anther culture has been suggested as a useful tool for breeding low-temperature tolerance in crops [350–352] on the ground that there is genetic overlap between the sporophyte and the gametophyte [389]. Gametophytic selection for low-temperature tolerance has been succesfully demonstrated in tomato [390], maize [391–393], and potato [394].

Despite great progress in understanding the molecular basis for plant cold acclimation, the complexity of the system hampers the genetic engineering of plants having freezing tolerance [290]. Among the possible approaches to enhance cold tolerance in plants, the ways that hold promise are [290] (a) increasing the freezing resistance of the plant plasma membrane by increasing the amount of phospolipid [394,395], as shown successfully in tobacco [396,397]; (b) metabolic alterations alleviating the detrimental effects of desiccation stress (e.g., osmotic adjustment through osmoprotectants) [290]; (c) exploiting cryoprotective and antifreeze proteins (e.g., fish antifreeze proteins [398]); and (d) manipulation of signal pathways leading to the expression of tolerance genes.

SALINITY RESISTANCE

Soil salinity is a major agricultural problem, particularly in irrigated agriculture. Around 10% of the world's arable land is affected by salinity [399,400]. One third of the land in Australia is salt affected [401], whereas in India and Pakistan, such areas constitute around 5% of their total cultivable land. In irrigated areas, the percentage of salt-affected land is much higher. In the United States, 23%, of the irrigated land is under salt effect [400]. With as much as half of the world's existing irrigation systems under the influence of secondary salinization, alkalization, and waterlogging [402], the coexistence of irrigation and salinization threatens the current agricultural productivity [403]. Despite the importance of salt-affected areas in world agriculture, too little progress has been made in improving the salt tolerance of crops [400,403]. The problem is expected to increase in the future and an intregrated approach seems to be the only answer.

Morphophysiological Traits: Genetic Basis

Traits associated with salt tolerance have been investigated by several workers [399,400,404–408], but exact traits are still to be identified [399]. The differential response of plants to salt stress at

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different growth stages has added further problems in this area. In wheat and sorghum, salt tolerance is associated with seed size, with larger seeds having greater tolerance [409,410]. Seedling survival in saline solution also is an indicator of the salt tolerance of crops and has been studied in *Medicago* [411,412], forage crops [413], and potato [414]. The results showed that characters underlying short-term tolerance may contribute to long-term tolerance but did not themselves confer long-term tolerance [415].

Two broad physiological mechanisms by which plants respond to salt stress [408] are (a) inclusion and the use of inorganic ions as osmotica to maintain a favorable water balance (halophytic response) and (b) partial exclusion of ions and the synthesis of organic solutes for osmotic adjustment (glycolytic response) [416,417]. Salt tolerance is associated with an increased capacity of ion regulation through compartmentation and the transport of toxic ions, osmotic adjustment, and the maintenance of membrane integrity [418]. Most of the crops respond to salt stress by excluding ions from the shoot, and genetic variation exists for the threshold level at which the exclusion mechanism fails [408]. Physiological and genetic factors that contribute to the growth of glycophytes at a very high salt concentration are related to survival more than yield potential and hence are of little interest to growers except those engaged in subsistence agriculture [419]. Low sodium transport has been suggested as an important heritable trait for salt tolerance in rice [420–421]. Chlorine ion exclusion was responsible for the genetic difference for salt tolerance in soybean [422] and was found to be a heritable character in white clover (*Trifolium repens*) and lucern (*Medicago sativa*) [406]. Under salt stress, a tolerant tomato genotype accumulated significantly less Na^+ and Cl^- and more Ca^{2+} than the leaves of a sensitive genotype [408]. Generation mean analysis indicated that, under salt stress, both absolute and relative growth and the Na^+ and Ca^{2+} accumulation in the leaf were genetically controlled with additivity being the major genetic component [408]; a moderate estimate of narrow sense heritability (0.49 ± 0.09) was obtained for shoot dry weight under salt-stress treatment [423]. Potassium ion selectivity was identified to be a principal adaptive mechanism to salt stress in legumes and cereals [424,425].

Genetics

The degree to which different plants can tolerate high concentrations of salt in their rooting medium is under genetic control [411,426–430]. The genetics of salt tolerance has been investigated in several crops and results so far indicate monogenic to polygenic control. Salt tolerance was recorded as a heritable trait in *Agropyron intermedium* [431] and barley [432]. In sorghum, the genetic variation for osmotic adjustment was studied in 10 inbred lines and variation was noted to be due to more than a single gene and both general and specific combining ability effects were found to be significant [433]. Greater tolerance of wild sunflower, *H. paradoxus*, is due to a single dominant gene, Sa_1, but a modifier may also be present [434]. Salt tolerance in wheat grass showed that tolerance behaves in additive fashion [435]. In tomato, stage-specific polygenic control was suggested to control salt tolerance [436–438]. In another study, generation mean analysis showed that additive gene action was the predominant component for salt tolerance in tomato; narrow sense heritability was estimated to be moderately high [439]. Six marker loci in tomato have shown an association with QTLs involved in yield under salinity [440].

The *GPert* (''Golden Promise'' erectoides) mutation, produced by gamma-ray irradiation in barley variety Maythorpe in the late 1950s, which is allelic to *ari-e* mutants (short awned, *brevi-aristatum*), has a significant effect on salt tolerance [441]. *GPert* performs similar to other *ari-e* mutants (*ari-e*. 1, *ari-e*. 119, *ari-e*. 156, and *ari-e*. 228) and possesses a relatively low shoot Na^+ content and a higher salt-tolerance index [442] in comparison with nonmutants. These mutants show greater tolerance than *denso* (*sdw*) or *ert-k*^{32} dwarfing mutants [441,442]. The *GPert* mutation is modified by the genetic background [443,444] and also is associated with drought-tolerance characters [442].

In saline environments, bread wheat, *Triticum aestivum* (genomes AABBDD), accumulates less Na^+ and more K^+ on expanding and young leaves than durum wheat, *T. turgidum* (genomes

AABB) [444]. Chromosome 4D accounts for 50–60% of the difference between bread wheat and durum wheat for this trait [425,445]. Dvorak and Gorham [425] recombined chromosome 4D with durum wheat chromosome 4B by using the *phlc* mutant of durum wheat and found that K^+/Na^+ discrimination is controlled by a single locus on the long arm of chromosome 4D, which was designated *Kna1*. The *Kna1* locus was mapped on a short region in the 4DL arm and was completely linked to *Xwg199, Xabc305, Xbcd402, Xpsr567*, and *Xpsr375* [444]. The 5J chromosome of *Agropyron junceum* carries a major dominant gene(s) conferring tolerance to salt [446]. In barley, genes with positive effects for salt tolerance were located on chromosomes 4H and 5H of *H. vulgare* and $1H^{ch}$, $4H^{ch}$, and $5H^{ch}$ of *H. chilense* [447].

Gene Expression

Adaptation of plants to a saline environment must be due to some salt-related changes in the pattern of gene(s) expression [408]. More than 100 genes were estimated to be expressed when subjected to salt stress [449]. There are several reports of alterations in protein accumulation due to salinity [405,448,449]. One of the most characterized genes associated with salt tolerance is the gene encoding a 26-kDa protein called osmotin, which is responsive to several environmental and hormonal signals, including osmotic and pathogenic stress [450–454]. Osmotin gene expression and protein accumulation were elicited in the vegetative tissues of tomato in response to short- or long-term exposure to NaCl as well as after severe water loss [455]. This gene also is stimulated by ABA [124,454]. Although NaCl can induce the osmotin gene through changes in the ABA levels, this signal also can regulate osmotin mRNA accumulation by ABA-independent signal transduction pathways, as suggested for the *Em* gene in rice [456] and other ABA-inducible mRNAs from wheat [457] and rapeseed [458]. *Cis*-deletion analysis of the osmotin promoter indicated that the induction by NaCl, ABA, and ethylene is associated with the same region of the promoter [459]. Many molecular responses to salt stress in the common ice plant (*Mesembryanthemum crystallinum*) are elicited primarily by the transcriptional induction of specific genes [460,461].

Breeding

Like all other stresses, breeding of tolerant cultivars is crucial to fight the ill effects caused by high-salt concentration. Breeding for salt tolerance has been proposed to the extent of possible crop production in seawater [462,463]. Of various ways to tackle the salinity problem, exploitation of the genetic mechanism is the most important strategy. Deliberate exploitation of the genetic mechanism is mainly possible through (a) direct use of halophytes [464–466] or choosing salt-tolerant crops according to the problem; (b) introgression of tolerant genes from salt-tolerant genotypes (related or distant) [403]; and (c) use of nonconventional approaches such as tissue culture and molecular biology.

Despite having knowledge about a long list of halophytes and their economic potential as fodder, fuel [403,464,465], and oilseed [466], their direct use as an economic crop is still in its dormancy. Among these, jojoba (though not too salt tolerant) is a suitable crop for such areas [400]. On the basis of our knowledge about the sensitivity of crop plants to high-salt concentration, appropriate crops can be grown according to the intensity of the salt concentration in the soil. Cultivated crop plants can be classified into tolerant, intermediate, and sensitive types. Shanon [400] has presented an elaborate review on the genetic variability of domesticated crop plants to salt stress. Barly is one of the most salt-tolerant crops; other tolerant crops are, for example, sugar beet, cotton, canola (*Brassica* spp.), asparagus, red beet, zucchini squash, date palm, pomegranate, grape, wheat grass, and bermuda grass. Crop plants having intermediate tolerance are, for example, sorghum, sunflower, saffflower, sugarcane, potato, alfalfa, faba bean, almond, plum, orange, grapefruit, peanut, chrysanthemum, carnation; the salt-sensitive group includes, for example, rice, corn, wheat, legumes, linseed, cowpea, lentil, chickpea, citrus, avocado, stone fruits, apricot, peach, blackberry, strawberry, aster, poinsettia, gladiolus, azalea, gardenia, gerbera, amarylis, and African violet. Introgression of

salinity tolerance has been attempted from related genera and species in some crops, such as wheat and tomato, but without success because of the absence of correct information about the exact kind of traits, their genetics [467,468], and the difficulties in recovering the traits of agronomic value.

However, intervarietal crossing has yielded successful salt-tolerant genotypes in some crops. For example, few salt-tolerant wheat varieties (KRL 1–4, Raj 3077, WH 157, JOB 66) have been developed in India during the past few years and are being successfully grown in salt-affected areas. Based on the problems associated with the breeding of salt-tolerant genotypes in crops, it has been suggested that it is better to select for yield rather than salt tolerance [448,469]. Rosielle and Hamblin [470] also suggested that selection for productivity will increase yields in both stress and nonstress environments. However, this strategy may not work in all agroeconomic environments, for instance, in a waterlogged condition where salt-tolerant rice is the only alternative [403]. Therefore, the use of physiological parameters might prove to be a useful component of breeding through pyrimiding component physiological traits, at least in sensitive species [403]. According to Flowers and Yeo [403], salt-tolerant genotypes can be developed through a crossing program which maximizes recombination followed by single-seed descent and selection for resistance along with agronomic characters. Among novel ways of enhancing the salt tolerance of crops, the important ones proposed are the use of undifferentiated cells in tissue culture and gene manipulation through molecular biology. Although difficulties are still present in both methods, some success has been obtained in crops such as alfalfa [471], bent grass [472], potato [473], and citrus [474,475].

ACID SOIL TOLERANCE

Similar to other abnormal environments, a low pH of soil also retards plant growth and development, thereby causing yield reductions. Soil acidity is a function of H^+ activity in the soil solution [476] and shows both chronological and spatial (horizontal and vertical) variation [477]. Acid soils are phytotoxic owing to a complex of nutritional disorders which includes both deficiency (Ca, Mg, Mo) and excess (Al, Mn, H) availability of different nutrients [478,479]. In this complex situation, the most damaging is the Al toxicity [480], which causes a number of disorders and may also influence the water-stress tolerance of crops [477,481]. Manganese toxicity, which is not as important as Al toxicity, also has received attention during recent years [482,483].

Acid soils are scattered worldwide in patches with the greater proportion being in tropical regions. Hence, this is of concern to a vast population of growers. The increased awareness about the soil acidity problem and the consequent yield reductions have attracted researchers to unravel the mechanism of resistance against the acid pH of soils [477,482,484–486]. Aluminium toxicity, being most crucial, has received the most attention in our attempt to understand the tolerance to soil acidity.

The mechanisms involved in Al tolerance are complex and could differ among species [487]. Plants tolerate Al toxicity in two ways: (a) Al exclusion from plant tissues, especially the symplastic portion of root meristems (e.g., by chelation of Al by organic acids), and (b) internal Al detoxification by converting Al into a harmless form [477,487]. In contrast to Al, Mn tolerance seems to be largely based on an internal mechanism only. The probable reasons for this are the role of Mn as an essential element and the biological and chemical similarities between Mn and Mg [477].

Morphophysiological Traits: Genetic Basis

There is a strong correlation between the soil acidity and the root growth of plants [487–489]. Inheritance of root length under acid soils in wheat showed polygenic control with a wide range of the degree of dominance [488]. In rice, the relative root length under acidic pH showed both additive and dominance effects with a preponderance of the additive effect; the trait was partially dominant with high heritability, and one group of genes was detected [489]. In soybean, along with

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thicker roots, increased seed weight also was found as an associated response with selection for seedling tolerance to acidity [487,490]. For Mn, a common gene system in both the root and shoot of wheat has been suggested [491].

Among physiological mechanisms countering a low pH effect, exudation of organic acids by plant roots is the most acceptable mechanism of external tolerance to Al toxicity [492]. Al tolerance also is reported to be associated with a greater efficiency of phosphate uptake [480,493] and cation exchange capacity [494]. Although there are indications of variation for these traits [492–494], the genetics of these traits has not been elucidated.

Genetics

Substantial genetic variation for tolerance to acid soil has been reported in different crops, such as, for example, wheat [477,495–498], rice [499], maize [500–502], and soybean [487]. Genetic studies on acid tolerance in crops indicate both qualitative and quantitative inheritance. Monogenic control was reported in wheat [495,496,503–505] and maize [500,501]. Two dominant genes have been shown to be responsible for Al tolerance in wheat variety Atlas 66 [506], whereas several genes were found in relatively the less tolerant line Chinese Spring [477]. Resistance to Al toxicity may be different at the seedling and adult plant stages [477].

Quantitative inheritance for tolerance in acid soils is reported in wheat [488,507,508], rice [489], maize [509–513], and soybean [487]. Al tolerance was dominant over sensitivity, but, at the same time, it showed a greater role of the additive gene effect [488,507]. Allelic variation for genes controlling Al tolerance has been noted in wheat [514], barley [515], and maize [500]. A change in the direction of dominance with a change in Al toxicity has been reported in wheat [488,506,516] and barley [515], which might be due to the differential expression of tolerant genes at varying levels of Al concentrations. In rice, both GCA and SCA were important for Al toxicity [489], but GCA was more prevalent. Reciprocal effects were also noticed [489]. Similarly, in maize, both additive and doimant genetic variations were reported for yield under acid soils [509–513].

In wheat, hexaploid (AABBDD) wheat is more tolerant than the tetraploid or diploid variety. The tolerance of the D genome is maximum followed by A and B genomes, respectively. The R genome of rye (*Secale cereale*) possesses even greater tolerance than the D genome of wheat. The Un genome of wheat (*T. ventricosum*) also possesses acid soil tolerance. Although the genes associated with Al tolerance are present in all the three genomes of wheat, the most important locations are 2DL and 4DL [517–519]. In rye, Al-tolerance genes are located on chromosomes 3R, 4R, and 6R [518], but when transferred to wheat, they show reduced tolerance probably due to their suppression by the unknown genes of wheat [518].

A wide genetic variation for Mn tolerance also has been recorded in wheat [482,520] with the suggestion that only few genes are involved in the Mn tolerance [482]. It has been suggested that the inheritance of tolerance to Al and Mn is independent and different genes may be involved [491,521–523], but there might be genes for the coregulation of their inheritance [477].

Gene Expression

The ability of plants to tolerance soil acidity is associated with a syndrome of cellular and molecular activities. A large number of genes take part in the whole operation by synthesizing different proteins. The idea that plants develop Al tolerance through the synthesis of proteins capable of inactivating Al [524] has now grown to a near reality with the identification of several proteins showing increased synthesis in response to Al [525–528]. A protein called RMP51 (51 kDa) has been reported to occur in the roots of an Al-tolerant cultivar of wheat; this protein has shown insensitivity to Mn, Cu, and heat stress, but is induced by Cd also [529]. However, none of the known proteins can be said to be the product of a gene conferring Al tolerance [477].

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Breeding

In view of the increasing food demand and the shrinking of land resources, the need for the genetic improvement of crops for their tolerance to acid soils is beyond question. This might be a case of necessity rather than economics, although it might prove useful in the long run.

It is true that genetic tolerance does not correct the problem of soil acidity and only postpones the need to take corrective action [477], but a judicious crop cultivation may have a beneficial effect on the soil in a variety of ways. Tolerance to acid soils varies from crop to crop and genotype to genotype. For example, rye is more tolerant than common wheat and common wheat is more tolerant than barley. In wheat, a number of Al-tolerant genotypes have been identified; for example, Atlas 66. Genotypes of Brazilian origin show a high tolerance to Al toxicity [477].

Breeding for acid soil tolerance has gained momentum with the development of reliable screening techniques. Both laboratory and field screening methods are used. The most common screening medium for Al and Mn tolerance is solution culture, which is a nondestructive measurement of tolerance. During screening, the tolerance is generally measured based on the damage to the root/shoot [530] and the degree of severity following exposure to Al [507].

The presence of dominance for acid soil tolerance has enabled breeders to use the backcross method of breeding for improving acid soil tolerance. A successful example is the transfer of a major gene for Al tolerance from the Carazinho variety of wheat to the Egret variety [531] in Australia. It has been suggested that early generation selection may be beneficial in breeding for Al tolerance in rice, and a pedigree method may also be used [489]. Recurrent selection also has been suggested as an alternative method to exploit the additive gene action related to Al tolerance in wheat [515,532] and maize [510,511,533]. In the CIMMYT breeding program, which utilizes the shuttle breeding program, selection for acid tolerance is generally done following the evaluation of the genotypes for yield and quality [534].

Interspecific hybridization also is a possible way of improving acid soil tolerance in crops. For example, tetraploid hybrids of *T. aestivum* × *T. ventricosum* [535] were developed for meeting the same objective. Although under investigation [528,529,536], molecular biology has still to play a role in breeding for tolerance to acid soils.

CONCLUSIONS

Increasing pressure on our natural resources, including land, is being realized by everyone. As our land area cannot be increased, the only hope of extending cultivable area is through greater utilization of the so-called ''unfit areas'' which suffer from various stress problems. A large portion of this area suffers from severe abiotic stresses. However, this does not mean that abiotic stresses do not occur in other areas. In fact, abiotic stresses are prevalent in almost all cultivable lands, although in variable intensities and durations. It is common to see our crops suffering from or fighting with abiotic stresses such as abnormal levels of water, temperature, and soil pH. When our crop plants suffer, we also suffer directly or indirectly.

Abiotic stresses despite so vivid and important for crop production, tolerance mechanisms are not fully understood and efforts to enhance tolerance of crop varieties are still far from satisfactory. Of the various reasons for this slow progress, the absence of clearcut traits conferring tolerance and the complexity caused by the simultaneous occurrence of more than one stress in variable intensities and durations are the most important ones. However, the efforts of various researchers have succeeded in correlating some morphophysiological traits with concerned stresses along with the genetic mechanism involved. Molecular genetics also is contributing in a slow but sure manner to expand our knowledge in this direction. Several varieties have been released in different countries for meeting the challenge posed by abiotic stresses. Even manmade crops like ''Triticale'' have been created to occupy marginal lands with high-stress pressure. Although abiotic stresses can be tackled better by an intregated approach, genetic manipulation should remain as the main strategy.

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When we are thinking of settling planets other than earth, our hopes are alive to design plants sturdier than ever before.

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Response of Green Beans (*Phaseolus vulgaris* L.) to Salt Stress

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INTRODUCTION

The gradual progress of desertification due to the detrimental effects of natural stress factors such as low precipitation, long-term drought, heat, and erosion coupled with improper human activities as a result of overgrazing, the overutilization of land, and the application of insufficient management decisions and improper agricultural practices, urbanization, and industrial activities has left extensive arable lands at the potential risk of conversion to unusable soils. These problems are more severe in arid and semiarid regions where the soils already encounter salinity and sodicity problems and are more vulnerable to stress conditions.

The accumulation of high soluble salts in a soil can significantly decrease the value and productivity of agricultural lands. Salt and water stress have been recognized as major agricultural problems, especially in arid and semiarid regions. Retardation of crop yield by salinization also has been known for a long time. Since the early 1900s, various investigations of the effects of salts on plant growth have been undertaken covering a range of aspects from the plant response to the salinity to salt behavior in soils [1–26]. Physiological studies have revealed that the major effects of salinity on plant growth retardation are osmotic and specific ion effects [10,16,18–21,24,26–40]. Furthermore, the reduced nutrient uptake by plants grown in saline environments has been observed in several species of plants [5,14,16,18,20,24,33,35,41–50]. Differences in salt tolerance among plant species also have been long recognized [21,28,35,39,51–82]. Although the agricultural scientists started studying the salinity tolerance of plants almost 50 years ago in the early 1950s (see Refs. 51–53 and the references cited therein), there is still a great deal of interest in working on this subject (for the recent work on this subject, see Refs. 39 and 80–85). According to Qadir et al. [83], cultivation of salt-tolerant grasses in a saline or saline-sodic soil may mobilize the native lime ($CaCO_3$) in these soils through root action. This may substitute the chemical approach for reclamation of such soils. Recently, Apte and Thomas [84] reported that the simultaneous application of a

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halotolerant nitrogen-fixing cyanobacteria during crop growth seems to be an attractive possibility for the reclamation and improvement of saline soils, especially since it also can supplement the nitrogen requirement of the corp. Also, De Villiers et al. [85] in recently assessing salinity tolerance of different plant species found that the perennials seemed to be better suited for rehabilitation purposes under saline soil conditions. However, the role that salt tolerance plays in causing differences in the growth and development, nutrient uptake, and metabolism between various plants, among plant species, and at different stages of growth is still a major concern among investigators, and it is not yet fully understood. The discovery of the physiological basis of salt tolerance in crops and the use of this knowledge to obtain more tolerant cultivars by modern plant breeding procedures should result in substantial increases in world food production.

The effect of salt stress on nutrient element utilization and nutrition as well as metabolism in plants has been studied for various plants using different methods. The results are still inconclusive. However, the change in nutrient metabolism induced by excessive salt is commonly accepted among scientists as one of the most important factors responsible for abnormal plant metabolism and reduced growth. Bernstein et al. [86] found that despite the decrease in total N uptake the leaf N concentration of some grain and vegetable crops increased with increasing salinity at all N fertilization levels. Increased in the N concentration of corn (*Zea mays L.*) and cotton (*Gossypium hirsutum* L.), plants under salinity stress was reported by Khalil et al. [41]. The uptake and metabolism of $^{15}NH_4^+$ and $^{15}NO_3^-$ in red kidney beans (*Phaseolus vulgaris* L.) was adversely affected by both salt and water stress at −0.4 MPa (−4 bar) osmotic potential [42,43,87]. Reduced ^{15}N uptake and metabolism as well as impaired protein synthesis under stress conditions by various crops also have been reported by several other investigators; Helal and Mengel [88] (barley, *Hordeum vulgare* L.); Pessarakli and Tucker [45] and Al-Rawahy et al. [49,50] (tomato, *Lycopersicon esculentum* Mill.); Pessarakli and Tucker [46] (eggplant, *Solanum melongena* L.); Pessarakli et al. [47] (corn); Pessarakli [48] and Pessarakli et al. [89,90] (green beans, *Phaseolus vulgaris* L.). However, Pessarakli and Tucker [44,91] found that ^{15}N uptake and protein synthesis by cotton plants increased under low levels (−0.4 Mpa, −4 bar, osmotic potential) of NaCl salinity. Increased total N concentration of plants grown in saline substrate was also reported by Bernstein and Pearson [4].

To explain these different results, a dilution or concentration effect (depending on the relative severity of salt stress on growth or nutrient, i.e., N uptake) was reported as a cause of the fluctuations in N content or concentration in plants [42,44–46,48].

Among the various environmental stress factors, salinity appears to have been given more attention than any other factors both in the past and at present. This is clearly seen from the continuous investigations and the voluminous reports that are continuously being generated on this subject. Hundreds of publications dealing with plant and crop stress caused by salinity are annually added to the literature on this subject [1–35,38–129]. For some recent reports during the 1990s, see elsewhere [22–26,39,40,48–50,80–85,92,93,95,96,102–129].

Despite the voluminous publications dealing with the effects of salt stress on plant growth and nutrient (i.e., N) nutrition, the literature concerning this issue on green beans is scarce. The reports of Gauch and Wadleigh [3], Balasubramanian and Sinha [13], Bhivare and Nimbalkar [14], Csizinsky [16], Coons and Pratt [19], Wignarajah [23], Frota and Tucker [42], Saad [43], Hoffman et al. [64], Maliwal and Paliwal [65], Harbir-Singh et al. [70], Salim and Pitman [72], Ashraf and Rasul [73], Alislail and Bartels [92], and Velagaleti et al. [93] deal primarily with the effects of salinity on the growth and/or chemical composition of other types of beans and are not concerned with green beans. Among the cited references in this chapter, in addition to the author's own research work, only that of Bernstein and Pearson [4] reported the influence of exchangeable sodium ions on the yield and chemical composition of green beans. However, their work was not concerned with N (labeled or nonlabeled) uptake and metabolism by the reported plant species. Thus, green beans were selected to be covered in this chapter primarily because they are classified as salt-sensitive plants [94]. Also, the effects of salinity on the growth and nutrient uptake and utilization by these plants have not been studied and documented sufficiently. In addition, these salt-sensitive plant species were selected for discussion in this chapter to compile information regarding the re-

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sponses of different plant species which are discussed by different authors in this book. This information is being compiled in a volume to assist the readers in comparing all these various salt-tolerant plant types under the stressful environmental conditions.

Thus, this chapter is concerned with responses in terms of growth, nitrogen (total and ^{15}N) uptake, protein synthesis, and water absorption by three cultivars of green beans (Tender Improved, Slim Green, and Kentucky Wonder) at the vegetative stage of growth under normal and NaCl stress conditions with the following objectives.

1. To compare the growth of these cultivars by evaluating their dry matter yield under normal and NaCl-stress conditions
2. To compare total N and ^{15}N uptake and distribution in plant roots and shoots by these cultivars as affected by salinity
3. To evaluate protein synthesis by these plant species under normal and salt-stress conditions
4. To study the water absorption by these cultivars as influenced by NaCl stress

FACTORS EVALUATED REGARDING THE RESPONSES OF GREEN BEANS TO SALT STRESS

Dry Matter Production

The effects of NaCl salinity on dry matter production of the three cultivars of green beans have been examined in several studies [48,89,90,95]. All these studies reported that the NaCl stress significantly reduced total dry matter yield for all three cultivars, but Tender Improved was the least severely affected at all salinity levels (Table 1; data from Ref. 89). The degree of reduction in dry matter yield increased with the increasing salt-stress level and over time. Other investigators also have

TABLE 1 Dry Matter Yield of Three Green Bean Cultivars Under Various NaCl-Stress Levels at Different Harvest Times

		Dry weight of plant parts[a] (g)					
		Harvest[b]					
		Shoots			Roots		
Cultivar	Salt stress (osmotic potential) (MPa)	1	2	3	1	2	3
Tender Improved	Control (−0.03)	3.12	5.18	7.25	0.84	0.98	1.48
	−0.25	2.73	4.45	6.53	0.68	0.93	1.45
	−0.50	1.92	3.64	4.56	0.46	0.81	1.14
Slim Green	Control (−0.03)	1.76	4.24	7.22	0.36	0.78	1.51
	−0.25	1.32	2.16	3.51	0.34	0.49	0.88
	−0.50	0.76	0.92	1.34	0.21	0.32	0.41
Kentucky Wonder	Control (−0.03)	3.12	4.33	7.54	0.67	0.85	1.53
	−0.25	1.67	2.45	3.18	0.55	0.77	1.28
	−0.50	0.95	1.24	2.41	0.43	0.51	0.72
	LSD (0.05)[c]	0.42	0.76	0.96	0.18	0.24	0.35

[a] Represents the means for pots containing two plants with three replications.
[b] Harvests 1, 2, and 3 are for 5-, 10-, and 15-day ^{15}N-uptake periods, respectively.
[c] Represents the least significant difference between the treatment means at the 0.05 level of confidence.
Source: From Ref. 89.

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reported reductions in dry matter production and decreased in yields of other bean cultivars [4,16,19,42,43,48] and a number of other crops [6,12,15,17,25,44,47,49,50,58,86,87,91,97,107,108, 113,117,129].

Under NaCl stress, shoot and root growth were substantially lower for the Slim Green and Kentucky Wonder cultivars as compared with the Tender Improved cultivar [48,89,90,95]. This phenomenon indicates the presence of significant interaction effects between salinity and cultivars. Roots appear to be affected less than shoots by salt stress for all cultivars.

The dry matter production and growth period were linearly correlated (r^2 values ranged from 0.89 to 0.99 for different treatments) [48]. For all cultivars, the dry-matter yield increased as the growth period progressed [48,89,90,95].

Total N Uptake by Plants

According to Pessarakli [48] and Pessarakli et al. [89,90], the total N uptake by green bean plants was significantly decreased with increasing salinity of the nutrient solutions for all cultivars at all three harvests. The results of Pessarakli's study [48] is presented here (Table 2). The Slim Green cultivar contained substantially lower total N than the other two cultivars at each harvest for all corresponding treatments except for the control shoots at the third harvest. The uptake values were markedly lower at the first harvest for this cultivar indicating a slower initial N uptake and a slower early growth rate for the Slim Green cultivar. For all cultivars, shoots contained substantially more

TABLE 2 Total N Uptake of Plant Parts of Three Green Bean Cultivars as Affected by Three Levels of NaCl Stress at Three Harvest Times

		Total N uptake of plant parts (mg N pot^{-1})[a]					
		Harvest[b]					
		Shoots			Roots		
Cultivar	Salt stress (osmotic potential) (MPa)	1	2	3	1	2	3
Tender Improved	Control (−0.03)	104.8	146.2	210.4	24.4	27.6	40.6
	−0.25	68.6	97.4	184.2	18.8	26.8	38.9
	−0.50	42.8	82.8	113.4	13.1	24.1	28.8
Slim Green	Control (−0.03)	46.6	118.8	215.3	10.4	23.5	44.5
	−0.25	33.2	58.7	89.2	9.3	14.4	22.6
	−0.50	18.2	24.5	35.7	6.0	8.7	10.2
Kentucky Wonder	Control (−0.03)	99.2	126.2	204.5	21.6	27.1	48.3
	−0.25	48.6	71.1	86.4	16.8	21.1	38.1
	−0.50	26.6	32.6	65.6	12.5	14.7	20.4
LSD (0.05) salinity × cultivar		3.4	5.3	13.1	1.6	2.3	3.1
Summary of the significance of variance sources							
Cultivar (C)		**	*	*	**	*	*
Salinity (S)		**	**	**	**	**	**
C × S		**	**	**	**	**	**

[a] Represents the means for pots containing two plants with three replicates.
[b] Harvests 1, 2, and 3 are for 5-, 10-, and 15-day ^{15}N-uptake periods, respectively.
*, ** Significant at $P = .05$ and .01, respectively.
Source: From Ref. 48.

total N than roots [48,89,90], probably due to the larger dry weights of shoots than roots (larger sink size).

The reduction in total N uptake was similar to the reduction pattern for total dry matter yield by plants under NaCl stress. The similar reduction pattern for total N uptake and dry matter yield indicates that the major portion of the absorbed N was incorporated into protein and contributed to plant growth and development. As N uptake decreased, dry matter yield also decreased under the NaCl-stress condition. This is supported by reports of several investigators [3,11,43,47,87,88,91,96], which indicated that the changes in N metabolism caused by salinity stress is one of the most important factors responsible for abnormal plant metabolism, reduced growth, and decreased crop yield.

Total N Concentration in Plant Tissues

All three studies conducted by Pessarakli [48] and Pessarakli et al. [89,90] reported that total N concentrations in all three cultivars generally were lower in plants subjected to salinity, especially at the highest NaCl stress levels as compared with controls. Table 3 indicates this finding. However, for a salt-tolerant cotton plant, the N concentration was significantly higher in NaCl-stressed plants even at a higher level of salinity (−0.8 Mpa, −8 bar, osmotic potential as observed by Pessarakli and Tucker [44]. An increase in N concentrations of corn and cotton plants under salt-stress conditions was also reported by Khalil et al. [41]. Therefore, differences in N concentrations of these

TABLE 3 Nitrogen Concentration of Plant Parts of Three Green Bean Cultivars as Affected by Three Levels of NaCl Stress at Three Harvest Times

		N concentration of plant parts (mg N g^{-1}DW)[a]					
		Harvest[b]					
		Shoots			Roots		
Cultivar	Salt stress (osmotic potential) (MPa)	1	2	3	1	2	3
Tender Improved	Control (−0.03)	33.6	28.2	29.0	29.0	28.2	27.4
	−0.25	25.1	21.9	28.2	27.6	28.8	26.8
	−0.50	22.3	22.7	24.9	28.4	29.7	25.3
Slim Green	Control (−0.03)	26.5	28.0	29.8	28.9	30.1	29.5
	−0.25	25.2	27.2	25.4	27.4	29.4	25.7
	−0.50	23.9	26.6	26.6	28.6	27.2	24.9
Kentucky Wonder	Control (−0.03)	31.8	29.1	27.1	32.2	31.9	31.6
	−0.25	29.1	29.0	27.2	30.5	27.4	29.8
	−0.50	28.0	26.3	27.2	29.1	28.8	28.3
LSD (0.05) salinity × cultivar		1.4	1.5	1.3	1.3	1.3	1.3
Summary of the significance of variance sources							
Cultivar (C)		**	NS	*	*	*	**
Salinity (S)		*	*	*	*	*	**
C × S		*	*	*	*	*	**

[a] Represents the means for pots containing two plants with three replicates.
[b] Harvests 1, 2, and 3 are for 5-, 10-, and 15-day ^{15}N-uptake periods, respectively.
*, ** Significant at $P = .05$ and .01, respectively.
Source: From Ref. 48.

different crops (cotton as compared with green beans) under salt stress are probably due to differences in their salt tolerance. At each stress level, the total N concentration of the Tender Improved cultivar generally tended to be lower than those of the other cultivars [48,89,90]. This is probably due to a dilution effect, since the Tender Improved cultivar produced significantly higher dry matter than the other cultivars at each stress level for each harvest. The total N concentration of the roots was generally higher than that of the shoots at each harvest for each cultivar for any corresponding treatment except for the control Tender Improved plants [48].

Nitrogen-15 Uptake by Plants and Distribution of ^{15}N in Plant Shoots and Roots

The results of several studies [48,89,90] on different cultivars of green beans showed that the total ^{15}N uptake by plants was decreased with increasing salinity of nutrient solutions at all three harvests for all three cultivars. The ^{15}N results of an experiment completed by Pessarakli [48] is presented here in Table 4. The reduction in the ^{15}N uptake followed the same reduction patterns as total N and dry-matter yield under stress conditions. This is an indication that the absorbed ^{15}N was incorporated into protein and contributed to plant growth and development as reflected in dry matter production. The Slim Green cultivar absorbed the least amount of ^{15}N under NaCl stress conditions. The absorbed ^{15}N values were higher for the Kentucky Wonder cultivar and generally highest for the Tender Improved cultivar under stress conditions. However, the Tender Improved cultivar contained significantly lower ^{15}N in both shoots and roots at the third harvest under normal (nonsaline) condi-

TABLE 4 Nitrogen (^{15}N) Content of Plant Parts and Shoot to Root ^{15}N Ratios of Three Green Bean Cultivars as Affected by Three Levels of NaCl Stress at Three Harvest Times

		^{15}N content of plant parts (mg ^{15}N pot^{-1})[a]								
		Harvest[b]								
	Salt stress (osmotic potential)	Shoots			Roots			Shoot to Root ^{15}N Ratio		
Cultivar	(MPa)	1	2	3	1	2	3	1	2	3
Tender Improved	Control (−0.03)	3.23	5.58	8.57	0.98	1.19	1.91	3.30	4.69	4.49
	−0.25	1.56	3.37	7.22	0.69	1.24	1.88	2.12	2.72	3.84
	−0.50	1.08	2.94	4.15	0.38	1.06	1.30	2.84	2.77	3.19
Slim Green	Control (−0.03)	1.29	4.19	9.46	0.40	1.06	2.13	3.23	3.95	4.44
	−0.25	0.74	2.04	3.51	0.34	0.63	1.09	2.18	3.24	3.22
	−0.50	0.31	0.71	1.06	0.19	0.36	0.39	1.63	1.97	2.72
Kentucky Wonder	Control (−0.03)	3.06	4.94	9.44	0.87	1.23	2.40	3.52	4.02	3.93
	−0.25	1.22	2.34	3.33	0.66	0.89	1.76	1.85	2.63	1.89
	−0.50	0.55	0.98	2.37	0.44	0.63	0.82	1.25	1.56	2.89
LSD (0.05) salinity × cultivar		0.14	0.22	0.32	0.03	0.06	0.10	0.17	0.21	0.20
Summary of the significance of variance sources										
Cultivar (C)		**	**	*	**	*	**	*	**	*
Salinity (S)		**	**	**	**	**	**	**	**	**
C × S		**	**	**	**	**	**	**	**	**

[a] Represents the means for pots containing two plants with three replicates.
[b] Harvests 1, 2, and 3 are for 5-, 10-, and 15-day ^{15}N-uptake periods, respectively.
*, ** Significant at $P = .05$ and .01, respectively.
Source: From Ref. 48.

tions as compared with the other two cultivars [48]. Substantial differences between the ^{15}N uptake by cultivars at each salinity level (Table 4) implies a significant interaction effect between salinity and cultivars at each harvest for each plant part. Significant decreases in the ^{15}N uptake by these plants under high salinity level is in agreement with experimental data obtained with red kidney beans [42,43], cotton [44], barley [88], tomato [45,49,50], and eggplant [46]. However, the low level of NaCl salinity (−0.4 MPa, −4 bar, osmotic potential) in cotton slightly enhanced the ^{15}N uptake [44]. This phenomenon is probably due to the difference in the salt tolerance of these different plant types (cotton as compared with green beans).

The nitrogen-15 contents of green bean shoots were reported [48] as being of higher magnitudes than those in roots for all three cultivars at all salinity levels (see Table 4). These differences are considered to be due to the larger dry weights of shoots than those of roots for all cultivars (larger sink size). The shoot/root ratios of the ^{15}N content tended to increase with time and decrease with increasing salinity for all cultivars except for the Kentucky Wonder cultivar at the third harvest with −0.25 MPa (−2.5 bar) stress. This may have been due to the retarded translocation of ^{15}N from roots to shoots caused by salt stress and the accumulation of ^{15}N in shoots as the growth period progressed. This effect also can be clearly seen by comparing the ^{15}N concentration values between shoots and roots (Table 5; data from Ref. 48). The concentration of ^{15}N in both shoots and roots increased as the growth period progressed and decreased as the salinity level increased for all cultivars. This pattern was similar to the shoot/root ratios of the ^{15}N content of plants. The ^{15}N concentration in roots was far greater than that in shoots for all cultivars. This higher concentration of ^{15}N in roots can be explained, in part, as the absorption of NH_4^+ onto the root surface or infusion of

TABLE 5 Nitrogen (^{15}N) Concentration of Plant Parts of Three Green Bean Cultivars as Affected by Three Levels of NaCl Stress at Three Harvest Times

		^{15}N concentration of plant parts (mg ^{15}N kg^{-1} DW)[a]					
		Harvest[b]					
		Shoots			Roots		
Cultivar	Salt stress (osmotic potential) (MPa)	1	2	3	1	2	3
Tender Improved	Control (−0.03)	1035	1077	1182	1167	1214	1291
	−0.25	571	757	1106	1015	1333	1297
	−0.50	563	808	910	826	1309	1140
Slim Green	Control (−0.03)	733	988	1310	1111	1359	1411
	−0.25	561	944	1000	1000	1286	1239
	−0.50	408	772	791	905	1125	951
Kentucky Wonder	Control (−0.03)	981	1141	1252	1299	1447	1569
	−0.25	731	955	1047	1200	1156	1375
	−0.50	579	790	983	1023	1235	1139
LSD (0.05) salinity × cultivar		21	25	27	16	18	20
Summary of the significance of variance sources							
Cultivar (C)		**	**	*	*	**	**
Salinity (S)		**	**	**	**	**	**
C × S		**	**	**	**	**	**

[a] Represents the means for pots containing two plants with three replicates.
[b] Harvests 1, 2, and 3 are for 5-, 10-, and 15-day ^{15}N-uptake periods, respectively.
*, ** Significant at $P = .05$ and .01, respectively.
Source: From Ref. 48.

TABLE 6 Nitrogen (^{15}N)–Uptake Rate of Plant Parts of Three Green Bean Cultivars as Affected by Three Levels of NaCl Stress at Three Harvest Times

Cultivar	Salt stress (osmotic potential) (MPa)	^{15}N–uptake rate of plant parts (mg ^{15}N kg^{-1} DW d^{-1})[a]					
		Harvest[b]					
		Shoots			Roots		
		1	2	3	1	2	3
Tender Improved	Control (−0.03)	207	108	79	233	121	86
	−0.25	114	76	74	203	133	87
	−0.50	113	81	61	165	131	76
Slim Green	Control (−0.03)	147	99	87	222	136	94
	−0.25	112	94	67	200	129	83
	−0.50	82	77	53	181	113	63
Kentucky Wonder	Control (−0.03)	196	114	84	260	145	105
	−0.25	146	96	70	240	116	92
	−0.50	116	79	66	205	124	76
LSD (0.05) salinity × cultivar		11	10	7	8	6	5
Summary of the significance of variance sources							
Cultivar (C)		**	*	NS	*	**	*
Salinity (S)		**	**	**	**	**	**
C × S		**	**	*	**	**	**

[a] Represents the means for pots containing two plants with three replicates.
[b] Harvests 1, 2, and 3 are for 5-, 10-, and 15-day ^{15}N-uptake periods, respectively.
*, ** Significant at $P = .05$ and .01, respectively.
Source: From Ref. 48.

ammonium and nitrate ions into the root apparent free space, as suggested by Pessarakli and Tucker [44–46] and Pessarakli [48].

Nitrogen-15 Uptake Rates

The ^{15}N-uptake rate, expressed as milligrams of ^{15}N absorbed per kilogram of dry matter produced by plants per day, is presented in Table 6 (data from Ref. 48). At each salinity level, ^{15}N uptake rates peaked at the earliest harvest and decreased as the growth period progressed for both the shoots and roots for each cultivar. This finding indicates that the younger plants absorbed ^{15}N at a faster rate than the older ones regardless of the stress level. Nevertheless, ^{15}N-uptake rates significantly decreased under NaCl stress as compared with the controls at each harvest for all cultivars in both plant parts except for the roots of the Tender Improved cultivar at the second and third harvest. At the earliest harvest, Slim Green shoots, had a substantially lower ^{15}N-uptake rate than the other two cultivars under normal conditions.

Protein Synthesis by Plants

The crude protein contents of both shoots and roots of the three green bean cultivars were markedly lower under stress conditions as compared with the controls (Table 7; data from Ref. 89). Under stress conditions, the Tender Improved cultivar produced significantly more protein than the other two varieties. Protein synthesis in shoots was substantially higher than that in roots for all the three

TABLE 7 Crude Protein Content of Three Green Bean Cultivars Under Various NaCl-Stress Levels at Different Harvest Times

Cultivar	Salt stress (osmotic potential) (MPa)	Crude Protein content of plant parts (mg)[a]					
		Harvest[b]					
		Shoots			Roots		
		1	2	3	1	2	3
Tender Improved	Control (−0.03)	361	521	766	92	108	149
	−0.25	239	453	653	56	113	165
	−0.50	181	314	416	47	101	108
Slim Green	Control (−0.03)	172	379	866	50	71	180
	−0.25	156	260	465	43	51	93
	−0.50	63	91	138	27	32	41
Kentucky Wonder	Control (−0.03)	259	350	683	79	89	256
	−0.25	142	187	332	61	68	157
	−0.50	103	161	295	46	51	66
	LSD (0.05)[c]	43.2	61.5	101.4	16.8	18.3	35.1

[a] Represents the means for pots containing two plants with three replications.
[b] Harvests 1, 2, and 3 are for 5-, 10-, and 15-day ^{15}N-uptake periods, respectively.
[c] Represents the least significant difference between the treatment means at the 0.05 level of confidence.
Source: From Ref. 89.

cultivars. This significant difference appears to be due to the higher dry matter production of shoots than roots for any treatment for any of the three cultivars. Pessarakli et al. [90] used two sources of N (ammonium and nitrate) for evaluating protein synthesis in green beans and found that, under normal (nonsaline) conditions, the nitrate-treated plants synthesized appreciably more protein than the ammonium-treated ones at each harvest for all three cultivars. This phenomenon was more noticeable in roots than in shoots for each cultivar. However, except for the Tender Improved cultivar at the first harvest, the crude protein content of plants was substantially lower under stress as compared with the controls for either source of N. Among the three cultivars, salt stress had the most severe effect on protein synthesis in the Slim Green for both NH_4-N and NO_3-N sources of N.

The impaired protein synthesis under stress conditions by other bean cultivars such as red kidney beans [43,87] and other types of plants such as barley [88], cotton [91], alfalfa (*Medicago sativa* L. [96], peas [98], wheat (*Triticum aestivum*) L. [99], tobacco (*Nicotiana tobaccum* L.) [100], and corn [130] have been reported previously by many investigators. In these studies, either the decreased amino acid incorporation into protein or the reduction in polyribosome levels due to the salt stress was reported as the reason for the depressed protein synthesis by plants. This may be a reason for the reduction in protein synthesis in green beans.

Water Uptake by Plants

For all three cultivars of green beans, the total water uptake decreased with increasing salinity (Table 8; data from Ref. 48), and the decrease patterns were similar to those of dry matter production [48]. The Tender Improved cultivar absorbed more water than the Kentucky Wonder and Slim Green cultivars under NaCl-stress conditions. However, under normal condition, the Kentucky Wonder cultivar absorbed significantly more water than the other two cultivars at the second and the third harvests [48]. The absorbed water values for the Slim Green cultivar were the lowest among the three cultivars at each harvest for any corresponding treatment. The reduction in water uptake by

TABLE 8 Total Water Absorption and Water-Use Efficiency by Three Green Bean Cultivars as Affected by Three Levels of NaCl Stress at Three Harvest Times

Cultivar	Salt stress (osmotic potential) (MPa)	Harvest[b] Water uptake (mL H_2O pot^{-1})[a] 1	2	3	Water-use efficiency (ML H_2O g^{-1} DW)[a] 1	2	3
Tender Improved	Control (−0.03)	2035	4160	6010	514	676	689
	−0.25	1310	3275	4800	384	610	602
	−0.50	800	2125	3325	336	478	584
Slim Green	Control (−0.03)	1085	2985	5685	513	1057	1150
	−0.25	840	2005	3555	506	757	815
	−0.50	485	1310	2010	500	596	652
Kentucky Wonder	Control (−0.03)	1800	4315	7840	500	1019	905
	−0.25	1085	2225	3525	490	834	865
	−0.50	690	1780	2830	475	692	792
LSD (0.05) salinity × cultivar		86	118	143	42	47	61
Summary of the significance of variance sources							
Cultivar (C)		**	**	**	NS	*	**
Salinity (S)		**	**	**	*	**	**
C × S		**	**	**	*	**	**

[a] Represents the means for pots containing two plants with three replicates.
[b] Harvests 1, 2, and 3 are for 5-, 10-, and 15-day ^{15}N-uptake periods, respectively.
*, ** Significant at $P = .05$ and .01, respectively.
Source: From Ref. 48.

other plants or other bean cultivars due to salt stress has been reported by many investigators [9,15,25,42–47,101,102], who generally agreed that plant root permeability (expressed as the hydraulic conductivity of the root system) decreased significantly under salt-stress conditions. This may explain the reduction in the water-uptake rate and may contribute to a similar reduction in nutrient absorption resulting in retarded plant growth and decreased dry matter production under salt-stress conditions.

Water-Use Efficiency of Plants

Water-use efficiency, expressed as milliters of water absorbed per gram of dry matter produced by plants is presented in Table 8 (data from Ref. 48). These data indicate that all three cultivars tended to use water more efficiently at the earliest harvest than at later harvests either under normal or stress conditions. This appears to be due to the faster rate of growth and higher dry matter production rate (gram of dry matter produced per day, which can be calculated from the dry matter data; see Table 1) at the earliest harvest than at the later harvests (a dilution effect). Nevertheless, all cultivars at each harvest (except for the Slim Green and Kentucky Wonder cultivars at the first harvest) used substantially less water for each unit of dry matter produced under stress conditions as compared with the controls. At each harvest for any corresponding treatment (except control plants at the first harvest), the Tender Improved cultivar used substantially less water for each unit of dry matter produced (used water more efficiently) than the other two cultivars.

SUMMARY AND CONCLUSIONS

The effects of NaCl stress on dry matter production, total N, ^{15}N, crude protein, and water uptake by three green bean cultivars have been discussed in this chapter.

The total dry matter production was greater for the Tender Improved cultivar than for the Kentucky Wonder and Slim Green cultivars for any corresponding treatment at each harvest. For all three cultivars, the total dry weight decreased significantly with the increasing salinity level. The reduction in dry weight due to NaCl stress was less for the Tender Improved cultivar than for the other two cultivars. Total N and ^{15}N uptake by all three cultivars substantially decreased under NaCl-stress conditions. The nitrogen-15 concentration and shoot/root ratios of ^{15}N decreased with increasing salinity. The nitrogen-15 concentrations of shoots were less than those of roots for all plants. Sodium chloride stress severely reduced the crude protein content of plant parts for all three cultivars at all three harvests. However, the Tender Improved cultivar appears to be less affected by salinity than the other two varieties. The shoots of all plants contained substantially higher total crude protein than the roots for all treatments. This appears to be due to the higher biomass of the shoots than of the roots for any corresponding treatment. Nevertheless, the shoots were more severely affected than the roots by salinity when salinized plants were compared with the controls for each plant part. Sodium chloride stress severely decreased the crude protein content of all three cultivars at each harvest for both sources of ^{15}N. However, the Tender Improved cultivar appeared to be the least and the Slim Green the most severely affected by salinity among the three varieties.

Under normal (nonsaline) conditions, green beans appear to absorb and utilize more NO_3-N than NH_4-N into protein synthesis. In contrast, under salt stress, NO_3-N seems more severely affected than NH_4-N for being incorporated into protein. Furthermore, any level of salt stress will likely cause a drastic reduction in the protein content and N metabolism in the salt-sensitive bean plants.

For all cultivars, water uptake also was substantially decreased under stress conditions, particularly at the highest level of stress. Among the three cultivars, the Tender Improved variety was the least and the Slim Green variety the most severely affected by salinity in all aspects of stress. This is an indication of the difference in the salt tolerance of these cultivars. Therefore, among the three cultivars discussed here, the Tender Improved cultivar of green beans appears to be the most suitable for growing under field conditions. Furthermore, since there are numerous cultivars of green beans, additional testing of their response under saline conditions could detect a wider range of tolerance and susceptibility to soil salinity. This will enable researchers to select the most salt-tolerant cultivars to be recommended to the growers.

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Salt and Drought Stress Effects on Metabolic Regulation in Maize

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INTRODUCTION

Salinity may occur naturally or can be induced by agriculture. In any case, it is an environmental stress that limits the growth and development of salt-sensitive plants [1]. Worldwide about 33% of the irrigated land is affected by salinity, and more land is not being irrigated because of salinity than there is new land made available by means of advanced irrigation procedures [2,3]. Even when the proper technology of saltwater irrigation is applied, salt is accumulated in the soils, which often impairs the growth of crop plants of low-salt tolerance. Salt tolerance is relatively low in most crop species. Therefore, not only selection and breeding for salt tolerance are important issues for traditional agricultural production in arid and semiarid regions, they may also offer the potential for utilizing the unlimited resource of seawater for irrigation [3]. Epstein et al. [4] showed that grain yields of up to 1 t/ha can be reached when undiluted seawater, supplemented with nitrate and phosphate, is used for irrigation. The interests in the economic feasibility of halophyte utilization also increased during the last decade, and in several projects halophytes were cultivated for greenification, fodder or vegetables [5–8]. Breeding of salt-tolerant glycophytes and evaluating the economic feasibility of halophytes are both promising attempts to reduce the destruction of ancient agarian societies.

Salinity Effects on Plant Physiology

The breeding of salt-tolerant crops depends on the knowledge of the physiological responses to salt stress. There are three major constraints for plant growth on saline substrates: water deficit, ion excess, and nutrient imbalance. Salt exclusion minimizes ion toxicity but accelerates water deficit, whereas absorption facilitates osmotic adjustment but also can lead to toxicity and nutritional imbal-

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ance [1,9,10]. Moreover, as will be pointed out below, salinity can affect the regulation of metabolic pathways, because metabolites active as regulatory ligands may become sequestered to the vacuoles. It is often not possible to distinguish the relative contribution of these constraints to growth inhibition at the whole-plant level. There also may be differences between plant tissues and stages of plant development. The long-term exposure of a plant to salinity may result mainly in ion toxicity in the older leaves or leaf parts and in water deficit and shortage of carbohydrates in the younger leaves or in the meristematic zone.

Most studies on the effcts of salt stress on plants focused on the growth and development of leaf tissues [11,12], but some data on the salt effects on the root system are available as well [13]. The root is the fist plant organ to become exposed to soil salinity, and in many instances it plays a crucial role in the exclusion of salt from the leaves [14]. Moreover, the response of the root apical zone to salt stress is critical to furhter growth and development of the root system.

A high selectivity during the ion uptake and transport are prerequisites for the adaptation of plants to saline habitats [15]. It helps to diminish a nutritional imbalance. However, there is no significant difference between the content of the major nutrients in halophytes and in glycophytes [16]. The specificities for the uptake of K^+, Mg^{2+}, and Ca^{2+} are neither in halophytes nor in glycophytes sufficient to hinder a dilution of these ions at the tissue level. A second prerequisite is the homeostasis of the ions in the metabolically active compartments. The salt-induced changes of ion relations can be tolerated because Na^+ and Cl^- concentrations are relatively low in the cytoplasm [17]. Simultaneously, the K^+, Mg^{2+}, and Ca^{2+} concentrations decrease mainly in the vacuoles and stabilize the homeostasis of these ions in the cytoplasm. A compartmentation of Na^+ and Cl^- into the vacuoles is essential to avoid flooding of the cytoplasm. The accumulation of high concentrations of essential ions in the leaf tissues during periods of low salinity can be one mechanism to enhance NaCl tolerance.

It was shown that the halophytes *Beta vulgaris spp. maritima* and *Laguncularia racemosa* have a special inter- and intracellular K^+, Ca^{2+}, and Mg^{2+} buffer in plants grown without NaCl. It helps the salt-treated plants to grow under salinity and to exclude Na^+ from the plants without significant ion deficiency. The sea beet, for example, has crystal cells with high Ca^{2+} concentrations in the spongy parenchyma and high Mg^{2+} concentrations in the photosynthetically active cells. K^+ is uniformly distributed in all tissues. *Laguncularia* also has crystal cells with high Ca^{2+} concentrations, but the Ca^{2+} concentrations too are high in all other spongy parenchymal cells [10,18]. The highest Mg^{2+} concentrations can be measured in the vacuoles of the epidermal cells, and the K^+ concentrations are high in all leaf tissues but in the spongy parenchyma. The distribution of these elements among different cell layers enables the plant to store them and reduces the deficiency if uptake is hindered because of salinity. Further investigations will have to show if similar buffers exist in maize and if they are effective only for a short period of time or if they enhance the NaCl tolerance in general.

It may be generally assumed that plants have an encoded capability for stress perception, signaling, and response. Biochemical studies have revealed similarities in processes induced by salt stress that lead to the accumulation of metabolites, such as (a) nitrogen-containing compounds like proline, other amino acids, quarternary amino compounds, and polyamines, and (b) hydroxyl compounds like sucrose, polyols, and oligosaccharides. More recent results indicate that the gene pattern activated in response to salt stress is common to most plant families. Learning about the biochemical and molecular mechanisms by which plants tolerate environmental stress is necessary for genetic engineering approaches to improve crop performance under stress. We will discuss these phenomena taking salt stress on maize as an example.

Metabolites accumulated on salt stress seem to function in three ways: (a) They act as osmolytes, faciliating the retention of water in the cytoplasm and allowing sodium sequestration to the vacuole or apoplast. (b) Protection of cellular structures might be accomplished through interactions with membranes, protein complexes, or enzymes. Their function may involve scavenging active oxygen. Compatible solutes are strong water structure formers [19]. Thus, according to the preferential exclusion model [19–21], such molecules will be preferentially solubilized in the bulk water

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of the cell rather than in the hydration shell of proteins or other labile macromolecules. They may then contract with small, highly charged molecules, which preferentially solubilize in the water of the hydration sphere where they may interact electrostatically with the macromolecule causing damaging effects at high concentration. (c) There is a growing body of evidence that plants are capable of sensing photosynthesate concentrations. Sensing sugar concentrations appears to control gene expression and by this cell differentiation is regulated [22–26].

Whereas the accumulation of compatible solutes contributes to the maintainance of cell growth under conditions of increased ion concentration, many organisms also have developed efficient methods to keep the ion concentrations in the cytoplasm at low levels. In bacteria, Na^+/H^+ antiporters provide both a mechanism for pH homeostasis and the primary mechanism for Na^+ extrusion [27]. In plants, antiporters have been observed on both the plasma membrane and the tonoplast, which in principle are capable of evacuating the cytoplasm. Increased antiporter activity on the tonoplast has been observed by treatment or adaptation to salt [28,29]. The plasma membrane and tonoplast antiporters appear to be organized differently at the molecular level and may both be composed of multiple subunits. These characteristics may complicate the ability to modify such activities.

In the past, many studies of abiotic stress tolerance have monitored the physiological status of stressed plant compared with unstresed controls. Mechanisms have been deduced from such descriptions. But, until recently, these studies have not included molecular and genetic analyses of stress-tolerance principles. Knowledge from physiological measurements has led to a few studies on the biochemical mechanisms underlying tolerance to salt stress. Our growing understanding of the biochemical mechanisms involved in stress tolerance makes it possible to search for specific genes.

Adaptation of Plants to Saline Habitats

The salt resistance of vascular plant tissues depends on the mechanisms of salt tolerance [30,31]. Salt regulation comprises avoidance of intake, elimination, and dilution. On the other hand, salt tolerance is defined as the property of the protoplasm to cope with high salt concentrations via compartmentation [17]. Adaptation by salt exclusion requires mechanisms for the avoidance of an internal water deficit [3]. In terrestrial halophytes, salt tolerance is based mainly on the inclusion of salts in the leaves and their utilization for turgor maintainance and for the replacement of K^+ by Na^+ in various metabolic functions [10,18]. Exclusion also can be an important factor to high salt tolerance, but adverse effects on water balance and reduced growth rates are the consequences [32,33]. Most crop species such as *Zea mays* are glycophytes and show an inverse relationship between salt uptake and salt tolerance [1]. However, there are large differences in sodium exclusion ability between different cultivars of maize [34]. Even when Na^+ and Cl^- concentrations remain relatively low in the shoot, growth is inhibited [3]. The application of excessive NaCl or Na_2SO_4 concentrations showed that Na and not Cl is the most toxic ion in maize under salinity stress [35]. There was a stronger negative correlation between the Na^+ concentration in the third leaf with the survival of 26 maize cultivars [36,37]. Cultivars with lower Na^+ concentrations in the shoot were more salt tolerant. Additionally, growth depression seems to be not a result of ion deficiency (Mg^{2+}, K^+, Ca^{2+}) but of an impaired osmoregulation [3] (H.-W. Koyro et al., in preparation). The calcium status in salt-treated root tissues has been found to be an adequate measure, being a prerequisite for the maintenance of a high K^+/Na^+ selectivity [38–40]. The K^+/Na^+–uptake ratio increases in the presence of Ca^{2+} in maize. There too was an increase of the Mg^{2+} concentrations in the leaves after salt treatment (H.-W. Koyro et al., in preparation).

Mechanisms inhibiting excessive Na^+ transport to the shoots of plants grown in saline substrate operate mainly in the cortex cells, in the endodermis, in the innermost layer of the cells of the cortex, and in the xylem parenchymal cells of the root. The Casparian band constitutes an effective barrier against passive solute movement into the stele. In most plant species, suberin lamellae are found in the exodermis, hypodermis, and endodermis [41]. The free space accessible for passive flux of saline solutions represents only 5% in maize [42]. However, it has been shown that the

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rhizodermal cells play a central role in ion uptake and are the predominant location of P-ATPases [43,44]. NaCl salinity enhances the V- and P-ATPase activities in roots of the salt-sensitive and Na^+-excluding *Sorghum* much more than in roots of the Na^+ includer *Spartina* [45]. In the same study, a correlation was shown between increases of Intra Membraneous Particle (IMP) frequencies on rhizodermal membranes (tonoplast and plasmalemma) and ATPase activities.

In maize cultivars, the differences of the sodium exclusion seem to be related to differences of passive Na^+ permeability of the root cell membranes [34]. The ability to maintain K^+ homeostasis and low Na^+ concentrations in the cytoplasm of these cells seems to be the crucial factor of the degree of salt tolerance [46]. The partitioning of the remaining Na^+ and Cl^- between old and young leaves and vegetative and reproductive organs and among distinct cell types within the leaf blade are of crucial importance and are characteristic for salt-tolerant species [11,19] (H.-W. Koyro et al., in preparation). A steep gradient between young and old leaves by restricting the import into young leaves, inflorescences, and seeds has been shown for a more salt-tolerant maize cultivar [36]. High K^+ and low Na^+ concentrations are achieved by low xylem transport rates of both ions and a high supply of K^+ through the phloem of mature leaves [47]. This system depends on the K^+ storage capacity of the mature leaves and the restricted Na^+ import via the shoot. The collapse of this way of regulation leads to a nearly spontaneous and distinct decrease of the K^+/Na^+ ratio in leaf tissues (H.-W. Koyro et al., in preparation).

In salt-excluding species like maize, growth in a saline substrate with a relatively low water potential requires an increase in the synthesis of organic solutes such as sugars and amino acids or a high selectivity at the uptake of K^+, Mg^{2+}, and Ca^{2+}. The osmotic adjustment of salt-excluding plants has a distinct influence on the energy balance. It is much more economical in terms of energy for a plant to accumulate NaCl than to synthesize sugars for osmoregulation [48,49]. Growth depression is a logical consequence, because, in terms of energy demand, exclusion of NaCl is most energetically costly. It has been shown that salt stress can lead to an increase of the water-use efficiency in maize and the change from a C_3 plant type to a C_4 plant type [50]. As will be discussed below, both factors can extend the degree of salt tolerance.

SPECIAL FEATURES OF MAIZE PHYSIOLOGY

Leaf Development

The characteristic strap shape of monocot leaves is generated through polarized patterns of cell division and expansion that maintain cells in files [51]. It was originally thought that the majority of divisions in monocot leaves take place at a basal intercalary meristem [52]. More recent data have shown that cell divisions in maize occur in a different manner during two phases of leaf development: During the first phase, divisions occur throughout the leaf and become restricted to the base of the leaf only after initiation of the ligule at the leaf blade/leaf sheath boundary during the second phase of leaf development [53]. Despite the apparent age mosaicism, cellular differentiation tends to occur in a basipetal direction [54,55].

A clonal analysis of the bundle sheath and mesophyllic cells in maize leaves has shown that the mesophyllic cells in the middle layer of the leaf (these are cells having no epidermal contact) are more closely related to the bundle sheath than to other mesophyllic cells [56]. Since all mesophyllic cells are functionally equivalent, this information suggests that photosynthetic cell type differentiation is position dependent and not lineage dependent. In C_4 monocots and dicots, the vascular system obviously provides the framework around which photosynthetic bundle sheath and mesophyllic cells are arranged in a radial pattern referred to as ''Kranz anatomy'' [57–59].

Bundle sheath and mesophyllic cells are distinguished by a number of morphological features [57,59]. The most obvious ones are: Bundle sheath cells have thick cell walls and numerous large starch-containing plastids. Mesophyllic cells have thin cell walls and smaller, randomly distributed chloroplasts that do not accumulate starch during the daytime. Unlike bundle sheath cells, mesophyllic cells are never in contact with the vascular tissue. In maize, the chloroplasts of bundle sheath

and mesophyllic cells are morphologically similar early in development (both have been found to contain grana stacks) [60,61]. Subsequent maturation of bundle sheath cell choroplasts results in the granaless bundle sheath chloroplasts found in the mature leaf and described in most textbooks. We will refer to leaf maturation in the following sections and discuss the salt-stress effects on both morphology and enzyme pattern. The bundle sheath cells of NADP-ME (malic enzyme specific for $NADP^+$)–type grasses, such as maize, most often contain granaless chloroplasts in a centrifugal location and include a suberized lamella.

Division of Labor Among Mesophyll and Bundle Sheath Cells

Photosynthesis by C_3 plants suffers from the low affinity of ribulose-1,5-bisphosphate caboxylase oxigenase (Rubisco) toward atmospheric CO_2 and limitation by photorespiration. The major function of the C_4 pathway is to overcome the limitation by low CO_2 concentration, which results in significant increases in photrespiration. The C_4 photosynthesis pathway serves as a CO_2 pump to concentrate CO_2 at the site of Rubisco and thus supresses its oxygenase activity and the associated photorespiration [62].

As shown in Figure 1, the C_4 photosynthesis pathway eliminates photorespiration by splitting photosynthetic reactions between two morphologically distinct cell types: the bundle sheath and the mesophyll. Rubisco is physically separated from atmospheric oxygen by its compartmentalization in the internally localized bundle sheath cells. This scheme is advantageous in hot, dry conditions but can be energetically wasteful. Some studies have suggested that certain C_4 plants function as C_3 plants under conditions when the use of the C_3 pathway is energetically favorable [63,64].

C_4 function depends on the morphological and functional differentiation of bundle sheath and mesophyllic cells and on the spatial regulation of photosynthetic gene expression. It is generally accepted that the mesophyllic cells contain all of the phosphoenolpyruvate carboxylase (PEPC) in the leaf and that the bundle sheath cells contain all of the Rubisco and most of the enzymes of the Calvin cycle. The carboxylation of PEP thus occurs in the mesophyllic cells, with the formation of oxaloacetate (oxac) and malate (mal) or aspartate (asp). In a subsequent reaction sequence, this C_4 compound is transferred to the bundle sheath cells where it is decarboxylated to form pyruvate (pyr). Pyruvate is transferred back to the mesophyll and becomes phosphorylated inside the mesophyllic choloplasts to form PEP again. As shown in Figure 1, the effect of this C_4 cycle is the transfer of CO_2 from the mesophyll to the bundle sheath at the expense of two ATPs per molecule of CO_2 transferred. In the final reaction sequence, CO_2 is fixed inside the bundle sheath chloroplasts in the reaction sequence of the Calvin cycle.

C_4 plants have been classified in three subgroups based on the different enzymes which decarboxylate C_4 acids in the bundle sheath: (a) the NAD^+-malic enzyme, (b) the PEP carboxykinase, and (c) the $NADP^+$-malic enzyme. Plants of the NAD^+-malic enzyme and PEP carboxykinase types tend to form aspartate, whereas plants of the $NADP^+$-malic enzyme type tend to form malate. The extent of this different behavior depends on external factors such as the nitrogen nutrition of the plants. Malate can carry reducing equivalents from the mesophyll to the bundle sheath. If aspartate is transferred, the plants need to have photosystem (PS) II active in the bundle sheath to generate the reductant required for CO_2 assimilation. The disadvantage of this situation is that O_2 from the water-splitting system will compete with CO_2 sites on the Rubisco.

$NADP^+$-malic enzyme–type plants possess chloroplasts which lack grana stacks inside the bundle sheath. The bundle sheath and mesophyllic cells of maize leaves have different metabolic functions. The initial steps of CO_2 assimilation in the C_4 cycle are spatially separated from the Benson-Calvin cycle [65–67]. The maize mesophyllic cell contains chloroplasts with both PSI and PSII. In contrast, the bundle sheath chloroplasts are deficient in PSII and exhibit very little net O_2 evolution [65]. Hence, the bundle sheath chloroplasts are restricted in their capacity for noncyclic electron flow and NADPH formation. Therefore, there is a net NADPH deficit in the bundle sheath cells, and a large proportion of the assimilated carbon, in the form of 3-phosphoglycerate, has to

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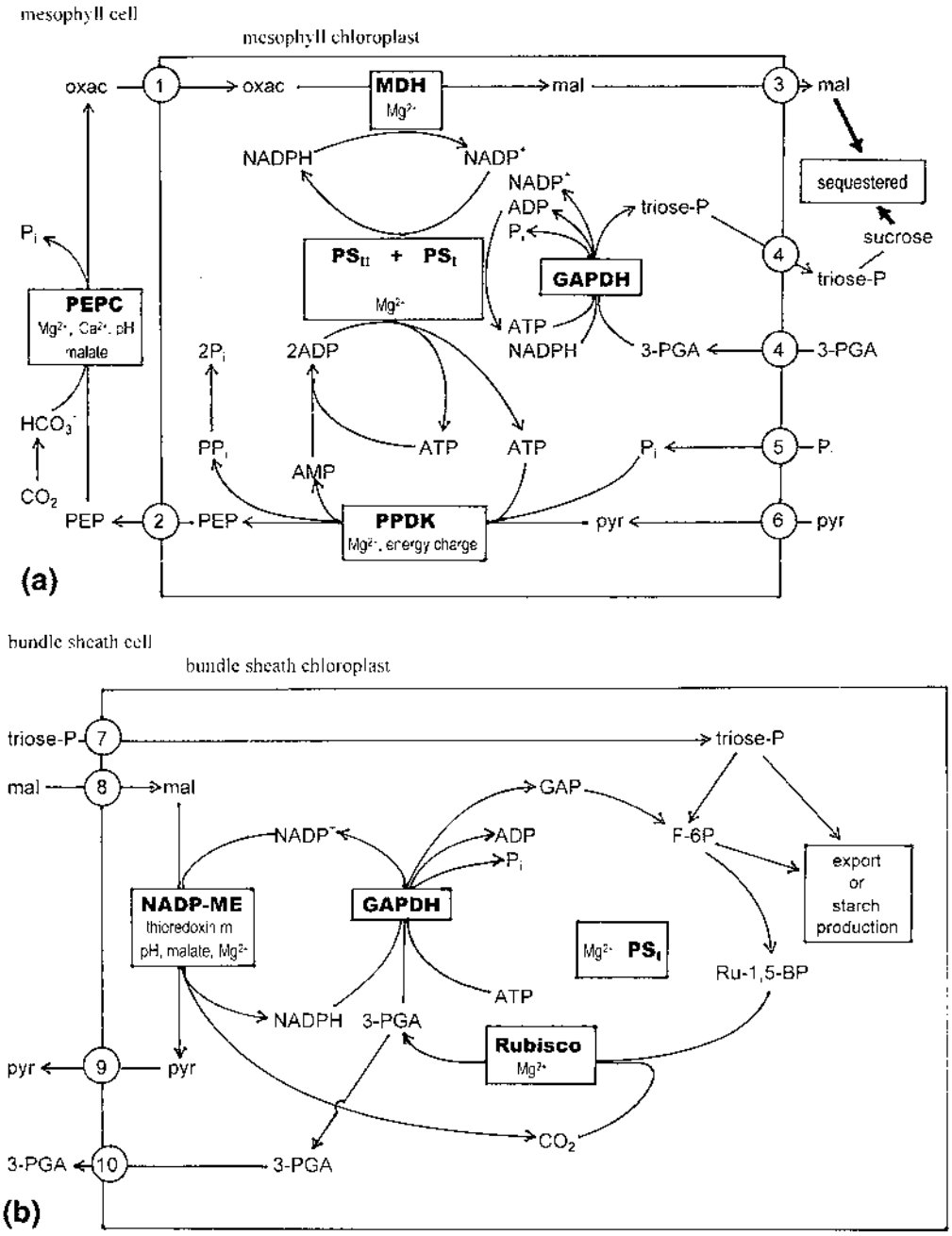

FIGURE 1 Pathways of CO_2 assimilation in maize leaves. Photosynthetic reactions are split between mesophyll (1a) and bundle sheath cells (1b). Effectors are called regulating enzyme activities essential for the operation of the total system. A prerequisit for the functioning of the C_4 cycle is an exchange of intermediates between both cell types. Membrane transport is brought about by translocators at a rate equal to the rate of CO_2 assimilation: (1) The oxalo-

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travel to the mesophyllic cells for reduction to triose phosphate, which is then returned to the bundle sheath [65]. The operation of C_4 photosynthesis requires that the intermediates malate (aspartate), glycerate-3-phosphate, and triosephosphates) move between bundle sheath and mesophyllic cells at rates equal to those of CO_2 assimilation. Owing to this, C_4 plants have different transport properties compared with C_3 plants. It may be mentioned here that malate may become sequestered inside the vacuole as a primary response to salt stress (see Fig. 1). This reaction interferes with the redox status of cells active in photosynthesis, and will reduce the rate of CO_2 fixation. Moreover, other essential reactions, nitrate reduction, for instance, too may be affected. As a consequence of this distribution of assimilate, sucrose synthesis from triose phosphate is restricted to the mesophyllic cells, whereas starch synthesis is predominant in the bundle sheath [68,69]. Similarly, the alternate respiratory pathway and the enzymes of nitrogen assimilation are differentially partitioned between the two cell types [68,70,71]. Since NADPH in the bundle sheath cells is in short supply, the question arises as to whether other NADPH-requiring reactions also are limited to the mesophyllic cells and are depleted in the bundle sheath tissues.

PHYSIOLOGICAL DESCRIPTION OF SALT STRESS EFFECTS ON MAIZE

General Observations

Much of the physiological research into salinity has concentrated on three topics: (a) water relations, (b) photosynthesis, and (c) the accumulation of a particular metabolite, assuming that one or more of these processes would limit growth in saline conditions. Most of these studies have been descriptive and have established patterns of responses in many crop species. One approach toward understanding the mechanisms of salt tolerance at the whole plant level is to follow the series of events that exposure to salinity initiates. Such time studies do not prove causal relations, but they can eliminate some possibilities. For example, if leaf expansion slows before photosynthesis does, then the decrease in photosynthesis cannot be causing the decrease in leaf expansion.

Salinity stress is composed of the osmotic and ionic components, both of which could potentially affect plant performance. Shoot growth is usually more affected than root growth [1]. Even though Na^+ and Cl^- are not the dominant ions in all saline soils, almost all research on salinity is done using NaCl as the stress agent [72–74]. The water-stress effect observed after NaCl treatment also can be induced by the addition of the nonpenetrating osmolyte polyethylene glycol (PEG) to the root medium [75]. These investigators conclude that hardening of leaf cell walls is a primary event in the chain of growth-regulatory responses to PEG-induced water deficits in maize.

The discovery of carrier proteins specific for water is an important step toward understanding the mechanism by which plants adopt to water stress [76]. Water channels facilitate the flux of water along an existing osmolarity gradient. Expression of tonoplast and plasma membrane aquaporin transcripts, some of which are water-stress inducible, is correlated with cell elongation [77].

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acetate translocator shows an extremely low affinity to malate and thus allows oxaloacetate uptake in the presence of malate. (2) The PEP translocator catalyses the export of PEP in exchange for P_i. (3)(8) The dicarboxylate translocator predominantly is active in transporting malate. (4)(10) The triosephosphate-phosphate translocator is transporting in exchange dihydroxyaceton phosphate, glycerinaldehyde-3-phosphate, and phosphate. (5) This means that phosphate may enter the chloroplasts via two different translocators: [2,4]. (6) The pyruvate translocator is active in the light-dependent uptake of pyruvate into the mesophyll chloroplasts. It is under discussion whether it operates in the bundle sheath chloroplasts in a different way [9]. As indicated, photosynthetate may become sequestered on salinization.

Ion uptake and compartmentation are crucial not only for normal growth but also for growth under dry and saline conditions, because both stresses lead to a disturbance of ion homeostasis. Key components for homeostasis under salinity stress are Na^+/H^+ antiporters. Physiological studies have observed Na^+ excretion in the root system and vacuolar deposition of Na^+ in leaves. The results argue for the existence of a Na^+/H^+ antiporter activity that removes Na^+ from the cytosol, but until now no plant Na^+/H^+ antiporter protein has been purified. Despite the lack of progress in isolating a plant Na^+/H^+ antiporter, a putative antiporter has been isolated from yeast [78]. The finding that overexpression of a Na^+/H^+ antiporter in yeast increases Na^+ tolerance indicates that pH regulation is not disturbed. This implies that endogenous H^+ATPases and H^+PPases can sufficiently supply protons to drive Na^+ efflux. Overexpression of Na^+/H^+ antiporters solely in the root epidermis might make transgenic plants better Na^+ excluders.

Increases in the concentrations of solutes that contribute substantially to the osmotic pressure are often measured on the assumption that their accumulation improves growth. As pointed out by Munns [79], there is no clear evidence to support this. In a more recent paper, Munns [80] states that salts taken up by the plant do not directly control plant growth by affecting turgor, photosynthesis, or the activity of any one enzyme. Rather the build-up of salt in old leaves hastens their death, and the loss of these leaves affects the supply of assimilates and hormones to the growing regions and thereby affects growth. This statement may be kept in mind when analyzing the information summarized below.

Does Turgor Affect Growth Rate?

It appears to be widely accepted that turgor regulates stomatal conductance and cell expansion and hence plant growth in soils of low water potential. But, at a higher time resolution, some data prove that there are transient effects of a change in the leaf turgor on leaf expansion. On a time scale of days and weeks, the turgor of salt-affected plants is not always reduced. Turgor is sometimes lower in expanded leaves of salt-affected plants than controls [81], but often turgor is similar to or higher than controls [82–85].

A reduction of turgor was thought to be a factor limiting leaf elongation when plants were osmotically stressed (see Refs. 86 and 87 and references therein). This concept has been challenged by several other groups [81,86,88–93]. In an excellent study on the elongating regions of soybean stem [92], turgor was estimated by four different techniques using psychometers, a pressure chamber, and a pressure probe. All methods were comparable and indicated that water stress did not inhibit the turgor pressure in the growing zone of soybean stems.

In grasses, where the growing zone of the leaf is tightly enclosed by other leaves and not exposed to the atmosphere, the turgor pressure has been estimated from measurements with a pressure probe [81], psychrometers [89–91], and the Chardakov technique [86,90]. In each of these cases, the turgor was not reduced by osmotic stress once leaves had recovered to steady-state elongation rates even though these rates were below that of the control.

The turgor of leaves of salt-sensitive plant varieties is usually higher than their salt-tolerant relatives [80]. This is presumably because salt sensitivity is usually associated with poor exclusion of salt by roots, and salt concentrations are higher in leaves of sensitive genotypes than in tolerant ones. With two genotypes of wheat differing in salt tolerance, the more sensitive one, with the higher turgor, had a greater reduction in stomatal conductance than the tolerant one [89]. Such observations suggest that something other than turgor is controlling stomatal conductance.

Thiel et al. [81] and Yeo et al. [94], using a pressure probe, found no detectable change in the turgor of elongating leaf cells of plants growing in saline solution. Using a different experimental approach, data have been collected for plants in soil or nonionic solution of low water potential with the same outcome: Turgor in elongating tissues is either not affected by water deficit [93,95,96], or when it is, there is no correlation between the local elongation rate and the turgor of the cells [97,98]. The same lack of effect of turgor was shown for plants growing in a drying soil [99].

These results imply that turgor does not control growth, and that measurements of leaf water relations and osmotic adjustment have little value in predicting or explaining the growth rates of

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salt-affected plants. Of course, turgor is essential for growth. Without turgor, there would be no extending force acting on the cell wall, and it could not expand. But the rate of cell wall expansion is controlled by the rheological properties of the cell wall; described by Green et al. [100] as ''wall-softening'' and ''strain-hardening'' processes, and not directly by turgor. A molecular explanation for these processes is given by Passioura and Fry [101].

There is consensus in the literature indicating that turgor does not limit the growth of osmotically stressed maize regardless of the method used to estimate turgor [11,12]. Measurements of apparent turgor indicated that, initially, there was a reduction of the apparent turgor, but after 4 h, when leaf elongation reached new, but reduced steady-state rates, the apparent turgor returned to control values.

Salt Effects on Ca^{2+} Functioning

Calcium has been recognized as a transducer of hormonal and environmental signals to the responsive elements of cell metabolism [102,103]. Changes in cytosolic Ca^{2+} activity trigger the chain of events that result in tuning a phosphorylation process on and off, thus ultimately affecting a large number of biochemical reactions [103,104]. The hypothesis of Ca^{2+} being the primary physiological transducer of environmental stress effects has been advanced for chilling injury [105], Al^{3+} toxicity [106,107], and salt stress [108–110]. Therefore, it appears that disturbance of the cell Ca^{2+} homeostasis may be the primary response to a variety of environmental stresses. As such, it may precede the hormonally mediated decline in both the growth rate and the rate of acquisition of all resources as common features of plants growing in suboptimal environment [111].

Cell wall loosening (breaking of calcium load-bearing bonds) as a consequence of acidification is a prerequisite for increased wall extensibility, and therefore cell elongation [112]. It has been shown that salinity does not reduce the activity of H^+-ATPases in the plasma membrane [113,114], and that the zone of acidification close to the root tip is similar in both salt-stressed and control roots [114].

Salinity severely affects Ca^{2+} uptake and transport, so that shoots frequently show symptoms of Ca^{2+} deficiency [73,115–119], especially in salt-sensitive genotypes [120,121]. Increased salinity reduces the amount of Ca^{2+} bound to endomembranes in *Zea mays* protoplasts [109].

Supplemental Ca^{2+} alleviates deleterious salt effects probably through mitigating the toxic effect of Na^+ ions rather than the osmotic effects associated with salt stress [122]. Therefore, the ratio Ca^{2+}/Na^+ in the rooting medium (or the Ca^{2+}/Na^+ activities) appears as the more reliable indicator for salt stress than the Na^+ concentration alone [123,124].

No apparent change in permeability of the plasma membrane after exposing *Zea mays* root protoplasts to 100 mM NaCl for up to 1 h was observed [109]. Such a result places more importance on the direct effect of Na on both the plasma membrane and endomembrane Ca^{2+} transport systems.

In intact root-hairs as well as root protoplasts of *Zea mays*, other monovalent cations may also inhibit Ca^{2+} binding to the plasma membrane [125]. The order of inhibition, $Li > Cs \gg Rb > Na > K$, precludes any inference about possible specific ionic effects in connection to the ion radius (with and without hydration shell) and/or specific ion charge. Therefore, the observed effect on Ca^{2+} displacement from the plasma membrane by monovalent cations [108,125,126] may be due to the nonspecific effects of the increased ionic strength of the external solution on both Ca^{2+} activity in the apoplasmic space and electrical properties of the plasma membrane.

Direct Toxic Effects of Salts on Meristematic Tissues

Much of the scientific attention has focused on the limitations caused by ion toxicity [1,9,87,127]. This factor is important for sure, but removal of the long-term limitation to plant growth by the development of plants capable of ion exclusion or compartmentation will not alleviate the water-stress component. Thus, long-term growth will still be limited by this primary response. Therefore, it also is important to study the mechanisms by which salinity inhibits growth in the short term.

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Na^+ Exclusion and Na^+ Sequestering in Leaf Tissues

Bernstein and colleagues have shown, in early experiments, that cultivars with low rates of salt accumulation in leaves yielded best in saline soils. Grafting experiments showed that salt accumulation in leaves was controlled by roots [128]. No difference in Na^+ efflux but higher Na^+ influx was noted in salt-sensitive genotypes of *Zea mays* compared with the salt-tolerant ones; sodium uptake was greater in seedlings than in plants at later stages of growth [34]. Sodium ions that entered the cytoplasm may be sequestered into the vacuole. The Na^+/H^+ antiport activity in tonoplast vesicles that is being fueled by the pH difference across the tonoplast (lower pH in the vacuole) appears to be responsible for Na^+ accumulation in the vacuole. Stellar parenchyma cells of the roots and mesocotyl accumulate Na^+, thus reducing its load to the *Zea mays* leaf cells via the xylem transport [129]. Hajibagheri et al. [38] showed that, in 26 salt-stressed maize cultivars, shoot Na^+ concentration and plant survival were negatively correlated. Other experiments did not support the hypothesis that Na^+ is toxic to maize. A simple positive correlation between Na^+ exclusion and salt tolerance was not observed by Lessani and Marschner [130].

The data of two greenhouse experiments with 100 mM NaCl and 50 mM Na-sulfate revealed significantly greater Na^+ exclusion from the shoots of cultivar Pioneer 3906 than from Across 8023. These findings show that the Pioneer cultivar is a strong Na^+ excluder, which has also been demonstrated by Maas et al. [131] and Schubert and Läuchli [132]. Besides effective Na^+ exclusion at the root surface of cultivar Pioneer as a result of the relatively low passive sodium permeability of the epidermal and cortex plasmalemma [34], the lower Na^+ and higher K^+ concentrations observed by Fortmeier and Schubert [37] in the shoot tissue of the Pioneer cultivar compared with the Across cultivar indicate that there is efficient Na^+ exclusion from the xylem by parenchymal cells, where a K^+/Na^+ exchange may be responsible for the control of Na translocation to the shoot [14,133].

The results of an investigation presented by Fortmeier and Schubert [37] suggests that the leaf sheath is important for salt tolerance as a result of sequestering of Na^+. The Na^+ concentration of the leaf sheaths were almost two times higher than those of the leaf blades. This was true for both cultivars, but the level of Na^+ was much higher in the Across cultivar than in Pioneer one, which indicates the storage capacity of Na^+ in the roots of cultivar. Across is lower than that of cultivar Pioneer.

Turgor Maintainance and Photosynthesate Sequestering

On salt stress, the reduction of growth is greater than the decrease in photosynthesis, and the reduction of shoot growth is much greater than the reduction of root growth [134]. It is broadly acceptable that the total carbon usage can be partitioned to growth, maintenance, transport, and storage. Analysis of this partitioning is by no means trivial. The best-resolved effects of salinity are those on the maintenance costs of the shoots. As would be expected, the maintenance respiration of rapidly growing plants is generally much higher than that of more slowly growing, environmentally less-responsive species. Salinity-induced changes also are greater. This probably reflects, in part, the additional costs of transport associated with salt exclusion. Increased maintenance costs, however, cannot explain all loss of growth. Increases in carbohydrate accumulation with salinity are already known. Although this use does not result in loss of carbon from the plant, it may well remove it from the pool available for immediate metabolism, growth, or regulation.

Growing tissues of plants respond to external salinity by rapid adjustment of internal osmotic potentials and thus maintain the water potential gradient required for turgor maintenance [90]. Moreover, cell micropressure probe measurements and indirect bulk estimates of turgor in elongating stem or leaf tissues of maize [11,12] consistently revealed the absence of any long-term (hours to days) salinity effects on turgor. Thus, longer term effects on growth might be regulated by induced reductions in the mechanical extensibility characteristics of the growing tissues (''wall hardening'') [100,101]. Estimates of turgor pressure, yield threshold, and hydraulic conductivity of growing

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maize leaf tissues indicated that they were not greatly affected by long-term salinization, and that, by default, changes in wall extensibility were responsible for growth inhibition [135]. Two other reports indicated that wall extensibility in pea stems and maize leaves were altered by salinity [136,137]. They found that growth-inhibitory effects of salinization for 5 h were primarily related to reductions in the wall extensibility coefficient. However, Cramer and Bowman [11,12,137] detected significant changes in the wall extensibility coefficient only after 24 h of salinization, and they related the earlier inhibition of growth to changes in yield threshold pressure and effective turgor.

Osmotic adjustment, the lowering of osmotic potential by the net increase in intracellular solutes, is recognized as an adaptive mechanism to water stress in many crops and is considered to be a major component of drought-tolerance mechanisms. Osmotic adjustment is one aspect of a highly complex and integrated system of adaptation to water deficits. The degree of osmotic adjustment depends on the rate of the decrease in the leaf water potential [138]. The compounds involved in osmotic adjustment are principally soluble sugars, K^+, organic acids, Cl^-, and free amino acids [139].

It is clear from numerous similar studies of water and salt relations, however, that turgor maintainance alone does not assure continued leaf expansion [87,93]. It may be that photosynthetic capacity is insufficient to provide the carbon both for wall synthesis and for ''turgor-driven cell expansion.'' Or it may be that some higher level controls operate to limit expansion in spite of the available potential. Munns and Termaat [87], for example, argued that shoot growth was not limited by the lack of substrate. Instead the existing carbohydrates were metabolically unavailable for wall synthesis, and they supported the hypothesis that the controlling message originated in the roots. This interpretation is supported by Cramer and Schmidt [140], who reported that maize leaf elongation is limited by the reduction of effective turgor, and that cell wall extensibility is unaffected by salinity.

The difference in the long-term growth response of the two cultivars under salt conditions indicates that Na^+ exclusion is positively correlated with salt tolerance in maize. A comparison of the growth responses and ionic concentrations of the plants between the two experiments with Na-chloride and Na-sulfate indicates that Na^+ is the most toxic ion in maize under salinity. In the 100-mM NaCl treatment, Cl^- concentrations in the shoot of the Across cultivar were not significantly higher than those of the Pioneer variety [35]. Furthermore, in the 50-mM Na-sulfate treatment, the Cl^- concentrations in the shoots and roots did not differ between the two cultivars. Cultivar Across 8023 showed the same toxic symptoms in the NaCl and the Na-sulfate treatments, with higher Na^+ concentrations in the shoot. The root and shoot growth of cultivar Across with 50 mM Na-sulfate was decreased to a greater extent than that of the Pioneer cultivar in the NaCl experiment.

The response of the leaf elongation rate to water deficit has most often been analyzed by considering the behavior of plants subjected to both the soil water potential and the low evaporation demand. In this case, at least a large part of the control of the leaf elongation rate is linked to a message originating from the roots and traveling to the shoots. This was demonstrated using experimental designs where the leaf water potential was maintained high and constant by pressurizing [141] or splitting the root system [142] while soil water was progressively depleted. Abscisic acid (ABA) could play a major role in this message [143,144], and it has recently been shown to induce mRNA populations in intact plants [143], with some of them being associated with inhibition of the elongation of the maize mesocotyl at low soil water potential [144].

On the basis of these data Ben Haj Salah and Tardieu [145] conclude that ABA signaling alone cannot account for the changes in the leaf elongation rate of droughted plants. The time course of the leaf elongation rate markedly diverged from that of changes in the ABA concentration in the xylem in droughted plants subjected to varying evaporation demands and diverged from that of the ABA fluxes in ABA-fed plants. The daytime and nighttime effects of soil water deficits also clearly differed in time and space in spite of nearly constant ABA concentrations inside the xylem. It is suggested [145] that the effect of water deficit on the leaf elongation rate should be analyzed as the superposition of two effects—one linked to the soil water status and probably involving ABA signaling and the other linked to the transpiration rate or the leaf water status and involving local events in the leaf. A hydraulic signal is the most likely possibility to account for this second effect.

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Instant Effects of Changes in Salinization

Some reports indicate that the salinity-related inhibition of leaf elongation may be extremely fast (observed after only 1 min following addition of salt to the root medium) [94] and independent of the roots [11,12]. This apparent controversy places a great significance to the research that would identify a primary response to salinity. Yeo et al. [94] argued that the water supply to the roots is the only factor that can be preceived, transmitted, and translated into a stoppage of leaf growth that was detected after only 1 min.

The short-term response (up to a few days) of plants to salinity appears to be different from the long-term response. According to Munns and Termaat [87], short-term responses of shoot growth to salinity may not be limited by water deficit, ion toxicity, or carbohydrate supply. Nevertheless, growth is still inhibited. However, the short-term response was not correlated with Na^+ exclusion, a result which was also obtained by Schubert and Läuchli [132] and Cramer et al. [146]. In this first phase, water stress rather than ion toxicity affects plant growth [80]. When salinity is applied to the root medium of maize plants, leaf elongation is inhibited immediately [147], but it recovers to a new steady-state rate which is below that of the control. In contrast to the observations of Fortmeier and Schubert [37] and results obtained by Schubert and Läuchli [132], Cramer [147] found that the short-term effects of 75 mM NaCl, with or without supplemental Ca^{2+} (10 mM), on the kinetics of leaf elongation of maize cultivars Pioneer abd DeKalb (a Na^+ includer) were different. After 24 h salinization, cultivar DeKalb appeared to be more salt sensitive and responsive to supplement Ca than the Pioneer cultivar.

Neumann [135] has found a rapid and reversible modification of the extension capacity of the cell walls in elongating maize leaf tissues responding to root addition and removal of NaCl. The long-term inhibition of the cell wall extension capacity was reversed within 20 min of salt withdrawal from the root medium. The rapid reversal of the inhibition of the wall extensibility and leaf growth after salt removal from the root medium of long-term salinized plants suggested that neither the deficiencies in growth essential mineral nutrients nor the toxic effects of NaCl on the plasma membrane viability were directly involved in the inhibition of leaf growth. Rapid metabolically regulated changes in the physical properties of growing cell walls, caused by osmotic and other effects, appear to be a factor regulating maize leaf growth responses to root salinization.

Calculations

Leaf elongation is a function of the rate of cell production and expansion. Cell expansion is dependent on water uptake and the rheological properties of the cell wall. According to Boyer [148], expansion can be described by equation (1):

$$v^{-1} * dv * dv^{-1} = \mathrm{mL} * (m + L)^{-1} * (y_0 - y_s - Y) \quad (1)$$

where v, t, m, L, y_o, y_s, and Y are the volume of the cell, time, cell wall extensibility, hydraulic conductivity, xylem water potential, cell osmotic potential, and yield threshold, respectively. Although cell expansion is three dimensional, it can be adequately described in one dimension as well [149].

When the rates of cell expansion are plotted as a function of $(y_o - y_s)$, the plot is theoretically linear with the x intercept equal to Y and the slope equal to the yielding coefficient $\mathrm{mL}/(m + L)$ [66]. If hydraulic conductivity is not limiting, then equation (1) can be reduced to:

$$v^{-1} * dv * dv^{-1} = m * (y_p - Y) \quad (2)$$

where y_p is the cell turgor. The slope of equation (1) simplifies to m in the equation (2) [148].

Experimentally, the cell expansion rates may be modified by lowering the osmotic potential around the cells or roots, which changes cell turgor [86,100]. Okamoto et al. [150] took a different approach and modified the shoot elongation rates by excising the roots and hydraulically increasing

the xylem pressure to increase elongation. Their technique has the advantage of increasing the force for cell expansion three dimensionally, but it has the disadvantage that the plant must be excised, which may eliminate regulatory factors from the root or add regulatory factors due to wounding. Estimates of Y have been presented for water-stressed plants [86,100,149,151,152]. In maize leaves, Y increased with water stress from approximately 0.37 to 0.44 MPa [86], and it was the single factor regulating the short-term steady-state LER of water-stressed maize.

The yielding coefficient, mL/(m + L), was unaffected by salinity in experiments with maize, and it therefore was not a limitation to the RER [11,12]. Moreover, these investigators found that *m* is not affected by salinity. This result is in good agreement with earlier work using the Instron technique on the leaves of barley [153] and bean [154]. Likewise, the data presented by Cramer and Bowman [11,12] suggest that, in maize, *L* was not affected by salinity, because both the yielding coefficient and *m* were unaffected by salinity. This conclusion is supported by data indicating that *L* of the roots does not appear to limit the LER of salt-stressed maize [12]. Furthermore, *L* did not limit the leaf growth of salt-stressed barley [93,155].

Summary of Physiological Description of Salt Stress Effects on Maize

Maize (*Zea mays* L.), a C_4 plant of tropical origin, is one of the most important crops for animal and human food and agroindustrial purposes worldwide [156,157]. An understanding of the factors that determine the sensitivity of maize are particularly important, since stress tolerance remains a major selection criterion in current maize-breeding programs. The complexity of stress syndromes, however, requires that many different techniques be utilized. Faster progress could certainly be made if groups of investigators with complementary expertise work together.

Maize is classified as a salt-sensitive crop plant [158]. The response of maize to salinity varies depending on the stage of development [131,159,160]. Vegetative growth appears to be more sensitive to salinity, whereas plants are much less affected at later stages [161]. The vegetative growth of maize is severely impaired even at rather low salt concentrations [131]. Maize excludes sodium, in contrast to the more salt-tolerant barley, which has Na^+-including abilities [162], but there are large differences in the sodium exclusion ability between different cultivars of maize [34].

Munns [80] has proposed a biphasic model of the growth response to salinity. According to this model, growth is first reduced by a decrease in the soil water potential. This phase of growth reduction is a water-stress effect and may be regulated by inhibitory signals from the roots. One such signal may be ABA as it increases in the xylem sap of plants in saline soil [163,164]. The water-stress effect observed after NaCl treatment can also be induced by the addition of the nonpenetrating osmolyte PEG to the root medium [75].

Schubert and Läuchli [132] showed that there was no positive correlation between sodium exclusion and salt tolerance in maize in a short-term experiment of 17 days. The more recent data of Cramer et al. [146] are in aggreement with this result. The investigators found that the salt tolerance of maize was not associated with sodium exclusion. In this first phase, water stress rather than ion toxicity affects plant growth [80], and genotypes differing in salt tolerance respond identically in this first phase [80]. Therefore, it can be concluded that the growth response of maize within the first 2 weeks of salinity is primarily affected by osmotic factors. When salinity is applied to the root medium of maize plants, leaf elongation is inhibited immediately [147], but it recovers to a new steady-state rate which is below that of the control. In contrast to the observations of Fortmeier and Schubert [37] and results obtained by Schubert and Läuchli [132], Cramer [147] found that the short-term effects of 75 mM NaCl, with or without supplemental 10 mM Ca^{2+}, on the kinetics of the leaf elongation of maize cultivars Pioneer and DeKalb (a Na^+ includer) were different. After 24 h of salinization, cultivar DeKalb appeared to be more salt-sensitive and responsive to supplemental Ca^{2+} than cultivar Pioneer.

In the second phase, the concentration of toxic ions increases rapidly, especially in old leaves, which die as a result of the fast increase of the salt concentration in the cell wall or the cytoplasm

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when vacuoles can no longer sequester incoming salts. In this second phase, genotypes which vary in salt tolerance may respond differently as a result of their different abilities to exclude toxic ions or to sequester them in the vacuoles [80].

Fortmeier and Schubert [37] argued that the difference in the long-term growth response of two maize cultivars under salt conditions indicates that Na^+ exclusion is positively correlated with salt tolerance. A comparison of the growth responses and ionic concentrations of the plants between experiments with NaCl and Na_2SO_4, respectively, indicates that Na^+ is the most toxic ion in maize under salinity.

BIOCHEMICAL ASPECTS OF SALT-STRESS EFFECTS ON MAIZE

Primary Effects of Salinity on Photosynthesis and Respiration

It has been pointed out by many investigators that growth is limited by the rate of photosynthesis. This view is based on the frequent observation that photosynthesis in salt-affected plants is reduced. However, the growth is affected before photosynthesis, as has been clearly demonstrated by Yeo et al. [94]. Further, long-term studies have found that growth declines more than photosynthesis [82,165,166]. So, salinity affects carbon assimilation per plant via a smaller leaf area rather than a reduced rate of photosynthesis [23]. A special situation occurs after prolonged periods of exposure to salinity. Then, levels of reserve carbohydrates can become low. The leaf area of such plants is probably quite low because of salt injury. So it is not surprising that high CO_2 improves the growth of plants for long periods under salinity [167].

When measuring the relative growth rates and the net assimilation rates of maize cultivars in water-stress treatment and controls at 20 days after treatment [138], it was found that the relative growth rate and the net assimilation rate decreased under stress. The results indicate substantial adaptation to water stress via osmotic adjustment and turgor maintenance. Sugar and K^+ were the major osmotic contributors in maize. Sugar concentration during drought treatment was almost twice that of controls at both 10 and 20 days. K^+ concentration increased at 10 days after withholding water, but a considerable difference was not seen at 20 days. Ca^{2+}, Mg^{2+}, and Na^+ concentrations increased slightly and phosphate decreased during stress treatment at both 10 and 20 days. K^+ was the major osmotic contributor in well-watered controls, whereas sugar became the major contributor with increasing water deficits [138]. These results suggest that sugar plays a major role in decreasing the osmotic potential under water-deficit conditions in maize. Under low leaf water potential conditions, stomata respond by closing, with a consequent reduction in transpiration as well as assimilation [168], leaf rolling occurs in minimizing evapotranspirational water loss [169] during water deficits. Cultivars with the ability to maintain turgor are capable of maintaining physiological processes at low leaf water status and are tolerant to drought.

Another aspect of discussions has been that decreases in growth with salinity may be due to increased respiration rates resulting from higher energy requirement [170,171]. We will refer to this point when discussing the salinity effects on ATPase activity, and we explain that this idea is not in accordance with in vivo observations with salt tolerant plants.

Inhibiting the Metabolism of Unidentified but Essential Metabolites by Salinity

Several investigators have discussed the fact that the uptake of salt might directly affect the production of a metabolite essential for cell growth. But it is unlikely that salt would be toxic in meristems: The salt concentrations in shoot apices [172–175] is not high enough to affect metabolism. It is

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unlikely that salt in rapidly expanding tissues would be toxic to enzymes. The expanding vacuoles would readily accommodate the salt and would prevent their build-up in the cytosol or the cell wall. It is more likely that salt reaches toxic levels first in mature leaves. As soon as roots encounter salinity, salts start to build-up in leaves. These salts are preferentially sequestered in vacuoles, and there is much evidence to suggest that the compartmentation of salts in the vacuoles is an important feature of salt tolerance [176]. The sodium concentration in the cytosol and chloroplasts is kept at a level of 100–150 mM by sodium uptake into the vacuoles until its concentration becomes so high that the net uptake by the vacuole becomes zero [134]. If the leaf is still transpiring when the vacuole is ''full,'' incoming salts must then build-up in the cytoplasm or the cell wall. Thus, the cell dies of salt poisoning or dehydration depending on whether salts build-up in the cytoplasm or cell wall. In either case, death of the cell would occur within a few days of the vacuole ceasing to take up incoming salt. The rise of the salt concentration would occur so rapidly that it is likely to affect all enzymes almost simultaneously.

There are some enzymes that are more sensitive to salt than others [177], but the salt concentration in the cytoplasm could increase by 10 mmol per hour, so there would be at most a day's difference between the most sensitive and the most tolerant enzyme being poisoned by salt [80]. But, within the context of this chapter, we should be interested in tolerable salt concentrations rather than poisonous ones. Therefore, we will focus on salinity affecting or regulating metabolic pathways, taking as an example experimental data on some key enzymes.

H^+-ATPases

In the context of active transport, V-type and P-type H^+-ATPases have to be distinguished, being predominantly active in the tonoplast and plasmalemma membranes, respectively. This can be done easily by applying the phosphomolybdate method and by the sequential inhibition of the different ATPase types [178–180]. Whereas V-type ATPases exclusively catalyze the ATP-driven export of protons, the different P-type ATPases can transport other cations as well. It has been found that ATPases vary their apparent K_m for ATP with the size of the membrane potential and the ion transport rate. At low transport rates, K_m may be as low as 1–2 μM ATP, whereas K_m values of 50–100 μM have been measured at high transport rates brought about in the presence of the K^+-ionophore valinomycin (U. Homeyer and B. Huchzermeyer, unpublished results). Anyway, these affinities towards ATP are very high. ATPases efficiently can reduce the phosphorylation potential, when their activities become enhanced in response to salt stress.

The plasmalemma H^+-ATPase links ATP hydrolysis to the extrusion of protons from the cytoplasm to the apoplast [181]. This provides the driving force for solute transport at this membrane. A characteristic property of the H^+-ATPase is the stimulation of its activity by K^+ [182]. Owing to the complex kinetic profile observed for maize plasmalemma preparations, it was proposed that the H^+-ATPase might conduct a H^+/K^+ exchange that would contribute to K^+ uptake into the maize cells [182]. This transport mechanism was thought to be of special importance when external K^+ concentrations are low compared with other ions. But, in a more recent paper, Briskin and Gawienowski [183] showed that the H^+-ATPase provides the driving force for cellular K^+ uptake by secondary mechanisms such as K^+ channels or H^+/K^+ exchange systems. The plasma membrane H^+-ATPase does not directly mediate ATP-mediated K^+ transport.

As the plasma membrane H^+-ATPase provides the potential for the transport of Na^+ and Cl^- between the apoplast and symplast, the activity of this pump must be critical to the energy-dependent transport required for ionic homeostasis in saline environments. In the fully expanded leaves of *Atriplex nummularia*, Niu et al. [184] found that H^+-ATPase mRNA levels were induced specifically by NaCl. mRNA accumulated in the bundle sheath cells, but not in the gland cells, implicating xylem unloading into the bundle sheath cells as a crucial control process for ionic homeostasis when the shoot is responding to NaCl stress. Salt-induced plasma membrane H^+-ATPase gene expression, which is assumed to be a basis for at least part of higher H^+ transport activity, occurs during stress

adaptation but not after the new adaptive state has been achieved. When ionic gradients have been established, altered membrane permeability restricting the Na^+ influx may be the primary basis for ionic homeostasis [185].

The capacity to alter the membrane permeability may be another criterion to differentiate halophytes and plants do not tolerate salts. Plants lacking this ability permanently depend on the energy-consuming active salt transport processes. In contrast to these experiments with a halophyte, we did not find a distinct NaCl-induced H^+-ATPase activity in maize leaves at a first glance. We rather recognized that photosynthetic active leaves died as soon as the cytosolic NaCl concentration started to increase. But, meanwhile, some NaCl stimulation of the H^+-ATPase activities in fully developed leaves could be detected: After 1 day of salinization, there was a transient increase of P-ATPase in the leaves in salinized plants compared with the leaves of the control plants. After a salinization period of about 30 days, V-ATPase increased until it suddenly collapsed at a time when all other enzyme activities became reduced. This latter point probably indicates the moment when salt concentrations build-up in the cytosol and the leaf cells start to die. Owing to variations in the growth rate of different plant cultures, it is difficult exactly to reproduce, in terms of days, these results and define the periods of enhanced ATPase activities (H. Klenke, unpublished results).

In maize roots, most of the V-ATPase activity is located in the stelar parenchyma, with only very low activity in the cortex. Growth of the roots in 100 mM NaCl brought about a marked increase in V-ATPase activity of the cortex, with only a small effect on the stele suggesting that expression in the cortex is under environmental control. The promoter region of a V-ATPase gene for the catalytic subunit of carrot has been sequenced and shown to be active in enhancing gene expression. Interestingly, an ABA box identified as sufficient to confer responsiveness to this hormone is present. In experiments with tobacco cells, it has been shown that ABA increases V-ATPase gene expression [186].

These experimental results match our data on NaCl sequestering in maize on salinization. Isolated enzymes appeared to be more sensitive to NaCl than photosynthetic $^{14}CO_2$ incorporation into sugar compounds. But, owing to our experimental technique, we measured the total NaCl concentration in the cell sap rather than inside the cytosol or chloroplasts. The easiest explanation of the discrepancies observed in our experiments is to assume that most of the NaCl was sequestered to the vacuole and toxic effects on enzymes involved in photosynthesis became manifest no earlier than the enhanced NaCl concentrations appeared in other cell compartments (H. Klenke, unpublished results). Vacuolar compartmentation of Na^+ and Cl^- is an essential mechanism for salt tolerance, since it results in lower cytosolic ion levels and faciliates osmotic adjustment, as already discussed above [134].

Development of C_4-Type Metabolism in Maize

The control of photosynthetic gene expression in plants is often complex, with regulation occurring at many levels and in response to numerous developmental, metabolic, or environmental signals. For Rubisco, the principal photosynthetic enzyme present in the chloroplasts of all higher plants, alterations in the expression of chloroplastic genes encoding the large subunit and nuclear genes encoding the small subunit can be mediated by (a) light [187,188,189], (b) developmental processes [190], or (c) photosynthetic metabolism [191–194]. Therefore, varied Rubisco concentrations under salinity are difficult to allocate to direct or indirect effects (via salt interfering with enzymes active in metabolism). In many cases, the control of Rubisco gene expression has been shown to be due to the control at the level of transcription [190,195], although examples of regulation occurring at posttranscriptional levels, such as RNA turnover, transport, processing, or regulation of translation, have been documented [190,196,197].

In C_4 plant species, genes encoding Rubisco and other photosynthetic enzymes have acquired additional or modified forms of regulation [67]. C_4 development appears to require many independent modifications to already existing C_3-type expression patterns for numerous photosynthetic genes, and these added levels of regulation may involve both transcriptional and posttranscriptional

control mechanisms [67,198–200]. Steady-state levels of C_4 enzymes and their mRNAs increase severalfold if dark-grown plants are illuminated [201–204]. This is an increase from the low levels that are developmentally induced even in darkness.

Early in leaf development, maize shows a ground state with Rubisco in all photosynthetic cells. This ground state is modified to give the more specialized C_4-type gene-expression pattern. The first step in this modification appears to be the repression of the Rubisco expression in the mesophyllic cells. This repression requires light and is only effective in the mesophyllic cells in proximity to a vein [205–208]. The low levels of Rubisco found in dark-grown maize seedlings represent accumulation in both the bundle sheath and mesophyllic cells [202,207]. If dark-grown plants are illuminated, the Rubisco accumulation is restricted to the bundle sheath cells and mesophyll-localized Rubisco is turned over more efficiently in the leaf blade than in the leaf sheath. This suggests that illumination is an essential component of the positional information that represses Rubisco in the mesophyllic cells of C_4 plants. Run-off experiments with nuclei from separated cell types suggest that this repression occurs posttranscriptionally, because Rubisco small subunit transcripts appear to be initiated in both cell types in maize [198]. The possibility of cross contamination of cell types in such experiments makes it impossible to rule out a role for transcriptional regulation.

The light dependence of the bundle sheath–specific and mesophyllic cell–specific gene expression has been further examined in situ [207]: Maize husk leaves with widely spaced veins were allowed to develop under various levels of illumination. In low light, Rubisco accumulates in the mesophyllic and bundle sheath cells and C_4 enzymes are absent. In high light, cell-specific C_4 enzymes accumulate principally in cells close to veins. This observation is consistent with measurements of photosynthetic enzyme levels in maize plants grown under high and low levels of illumination [209–211]. These results suggested that development in low-light levels favored the accumulation of the C_3 enzyme Rubisco, whereas higher light levels resulted in greater levels of both Rubisco and the C_4 fixation enzyme PEPC. This C_3/C_4 shift is accompanied by a new spatial pattern of gene expression. Compared with those in leaves grown in low light, mesophyllic cells of high-light–grown leaves have higher levels of pyruvate, phosphate dikinase (PPDK) and PEPC but lower levels of Rubisco and NADP-ME than the bundle sheath cells [212]. With respect to the above-mentioned observation that the Rubisco gene expression is controlled by photosynthetic metabolism, it may be argued that the C_3/C_4 shift is under the control of metabolite levels conditioned by the neighboring vein. The shift from the C_3-like to C_4-type Rubisco gene expression occurs in the basipetal direction, so that bundle sheath cell–specific expression of Rubisco was observed initially at the apex of young leaves and progressed rapidly downward to the leaf base. We will refer to these observations when discussing the salt-stress effects on enzyme patterns in developing maize leaves.

Key Enzymes of C_4 Metabolism in Maize

The enzymes addressed here may be easily localized in Figure 1, which shows the pathways of carbon metabolism within mature mesophyllic and bundle sheath cells, respectively. Moreover, in parts a and b of Figure 1, as well as in Table 1, possible salinity effects are indicated. Applying low salt concentrations, none of these enzymes will become inactivated or even denaturated, but salt stress will interfere with the regulation of the catalytic activities of these enzymes.

Phosphoenolpyruvate Carboxylase

Catalysis: Phosphoenolpyruvate carboxylase (PEPC, EC 4.1.1.31) in higher plants is a cytosolic, oligomeric, highly regulated enzyme [213,214]. It catalyzes the β-carboxylation of phosphoenol pyruvate in the presence of hydrogencarbonate and divalent metal cations to yield oxaloacetate and orthophosphate.

$$\text{Oxaloacetate} + \text{ATP} <=> \text{Phosohoenol Pyruvate} + \text{ADP} + CO_2$$

TABLE 1 Key Enzymes of C_4 Metabolism in Maize

Enzyme	Essential elements for catalysis and regulatory lignads	Other effectors	Salinity effects
PEPC	Mg^{2+} essential for catalysis Strictly alkaline pH optimum, stimulated by Ca^{2+} Allosteric effects: Positive: glucose-6-P, triose-P Negative: L-malate, aspartate	Phosphorylation potential affects catalytic activity via its effect on protein kinase activating the enzyme.	Direct effects via salt interfering with Mg^{2+} and Ca^{2+} uptake Indirect effects by reducing phosphorylation potential due to enhanced ATPase activity
NADP-MDH	Light activation via thioredoxin m	High $NADP^+$/NADPH ratios result in reduced catalytic activity via $NADP^+$ competition for electrons with thioredoxin m	Sequestering of reducible substrates oxaloacetate, 3-PGA, malate will affect the $NADP^+$/NADPH ratio
NADP-ME	Mg^{2+}, pH, and malate concentration directly affect substrate binding to the catalytic site Allosteric effects: Positive: CoA, fructose-1,6-bis-P Negative: Cl^-, NO_3^-, HCO_3^-		Direct effects via impaired ion uptake Indirect effects via sequestering of effectors as well as effects brought about by ATPase and translocator-mediated pH shift
PPDK	Mg^{2+} promotes PET/pyr exchange in the catalytic site K^+, NH_4^+ stimulate catalytic activity ADP inhibits enzyme activity; oxalate prevents from ADP-inhibition. Inhibited by proteinphosphorylation; activated by light dependent dephosphorylation	Mg^{2+} essential for forming the catalytic active tetramer Sugar, polyols, and pyruvate protect the tetramer form dissociation	Direct effects via salt interfering with ion uptake Rate of enzyme inactivation depends on ATP/AMP and pyr/PEP ratios: minimal variations in phosphorylation potential bring about massive effects on catalytic activity

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Therefore, it is obvious that this enzyme is involved intimately in the dicarboxylic acid metabolism in plants. In addition to its cardinal role in the initial fixation of carbonate during C_4-type photosynthesis, PEPC functions in several anaplerotical metabolic pathways, such as C-N partitioning in C_3 leaves [213]. Moreover, PEPC isoforms have been identified in various plant tissues playing specialized roles; PEPC of guard cells is only one such example [215].

In the presence of hydrogencarbonate, phosphoenolpyruvate can undergo hydrolysis to form pyruvate. Under in vivo conditions, that is, in the presence of Mg^{2+}, this hydrolysis contributes less than 5% to the total reaction flux, but it becomes more important in the presence of other cations, with a yield of 50% being found in the presence of Ni^{2+} [216]. Thus, salt cations competing for uptake with Mg^{2+} may interfere with the apparent carboxylation yield. Recent studies on the catalytic mechanism indicated that there is a strict synergism in substrate binding to the active site of the enzyme: Mg^{2+} binds first, and this binding is at equilibrium, phosphoenolpyruvate binds second, and hydrogencarbonate binds third. Moreover, all three substrate components have to be present inside the catalytic site before the reaction starts [217].

From stereochemical studies, it may be concluded that during catalysis phosphate is transferred first to form a carboxyphosphate. In a subsequent intermediate state, an enzyme base depronates the carboxyl group of the carboxyphosphate, which decomposes to form enzyme-bound CO_2 and P_i. In this state CO_2 is located above the plane of the enolate within bonding distance of the carbon-3. In this state, the divalent metal cation plays a crucial role. Obviously, metals other than Mg^{2+} lower the reactivity of the enolate. The result is a loss of CO_2 in the presence of other divalent metal cations [216].

Regulation: PEPC and sucrosephosphate synthase are well-known examples of plant enzymes in vivo regulated by enzyme phosphorylation [218,219]. Moreover, it is well documented that various isoforms of plant PEPC are subjected to allosteric regulation by both positive (glucose-6-phosphate and triose phosphates) and negative (L-malate, aspartate) effectors, and that the affinity of these effectors is strictly dependent on the pH value. (See Table 1 for a summary.) For instance, the K_i for L-malate of the C_4 PEPC from *Sorghum* is decreased 25-fold when measured at pH 7.3 compared with the one determined at pH 8.0 [220]. Indirect effects on PEPC activity of salinization may be expected when these effectors become sequestered to the vacuoles during the first phase of the salt-stress response. These modulators of PEPC activity may be imported from the bundle sheath. This feature provides a means of communication between the Calvin cycle and the C_4 cycle.

It has been shown that the C_4-PEPC is reversibly light activated in vivo by a phosphorylation of a serine residue at the 110-kDa subunit's N-terminus that directly or indirectly depends on photosynthesis. This activation mechanism is modulated by the incident photosynthetic photon flux density above a minimum threshold of about 300 μmol m^{-2} s^{-1}. In maize, Ser-8 and Ser-15 are the two candidates that might become phosphorylated [221–223]. Phosphorylation of PEPC not only results in the activation of its catalytic activity but also fine tuning by allosteric regulation becomes changed: A reduced sensitivity toward L-malate inhibition (K_i is sevenfold increased) and a higher affinity of the allosteric sites toward glucose-6-phosphate (K_a becomes fivefold decreased) have been observed [220,224].

It has been concluded from available experimental data that PEPC phosphorylation is brought about by a Ca^{2+}-independent 30- to 39-kDa kinase that is light-dark regulated in C_4 plants in vivo [225]. It is generally accepted that the light-induced C_4 transduction cascade is initiated in the illuminated chloroplasts by photosynthesis. From in situ experiments, it may be concluded that increases in the pH and Ca^{2+} concentration within the cytosol of the mesophyllic cells function as regulatory elements. On the other hand, it is still a matter of discussion whether 3-phosphoglycerate, generated in the Calvin cycle of bundle sheath chloroplasts, functions as a messenger as well. In addition to the above-mentioned allosteric effects, there is strong evidence for a regulation of cytosolic protein synthesis under the control of another protein kinase [226,227]. This reaction cascade tuning PEPC catalytic activity is summarized in Table 1.

The maize PEPC enzyme family posseses at least five nuclear genes; the C_4-PEPC is unique and is located near the centromer of chromosome 9 [228,229]. The C_4-PEPC gene is expressed in

photosynthetic tissues during greening via a phytochrome-mediated response [230]. Gene expression is not correlated to the Kranz leaf anatomy, because, in maize, it also occurs in inner leaf tissues and tassels [231]. In maize, it has been found that cytokinins upregulate the transcriptional activity of the C_4-PEPC gene [232]. In the facultative Crassulacean acid metabolism (CAM) plant *Mesembryanthemum crystallinum* (ice plant), the transcription of the CAM-PEPC gene is induced by ABA, which is produced under salt stress [233,234]. It has been shown that salt stress in *M. crystallinum* causes three protein factors (PCAT-1, -2, and -3) to bind to AT-rich regions of the Ppcl promotor [235]. Similar promoter sequences have been found in *Sorghum* as well [236]. But, up to now, no clear results concerning salt-stress–induced regulatory mechanisms in maize are available. Nevertheless, the above-sumarized direct effects of divalent cations on the catalysis and regulation as well as the regulatory effects of metabolites seem to suggest salinity effects on PEPC.

Malate Dehydrogenase Specific for NADP$^+$

The malate dehydrogenase specific for $NADP^+$ (NADP-MDH, EC 1.1.1.82) was originally found by Hatch and Slack [237] in plant leaves. It catalyzes the reversible reduction of oxaloacetate in the presence of NADPH to form malate and $NADP^+$.

$$\text{Malate} + NADP^+ <=> \text{Oxaloacetate} + NADPH$$

The enzyme is predominantly located in mesophyllic chloroplasts. The C_4 acid is transported to the bundle sheath cells. It was shown that the active enzyme, but not the enzyme in its inactive disulfide form, is irreversibly inactivated by the thiol-reacting reagent N-ethylmaleimide. In accordance with its physiological function, the production of C_4 acid, the affinity toward malate is low as compared with the other substrates. Ashton and Hatch [238] published the following data: $K_{m/NADP} = 45\ \mu M$, $K_{m/NADP} = 50\ \mu M$, $K_{m/malate} = 24$ mM, and $K_{m/oxaloacetate} = 18\ \mu M$.

The catalytic activity of NADP-MDH (malate dehydrogenase specific for $NADP^+$) is rapidly and reversibly inactivated when leaves are darkened. The same observation can be made with isolated mesophyllic chloroplasts from maize [239]. Light activation can be inhibited by 3-(3,4-Dichlorophenyl-1,1-dimethylurea (DCMU), and thiol reagents like DTT are essential to keep the catalytic activity in leaf extracts [239,240]. Thioredoxin *m* has been shown to be involved in the light-dependent activation of NADP-MDH [241–245]. The degree of activation of NADP-MDH varies with light intensity, and it has been concluded that high catalytic activity depends on the conditions of the rapid ferredoxin-mediated reduction of thioredoxin *m* [246]. This interpretation is supported by the finding of Leegood and Walker [247] that adding oxaloacetate or 3-Phosphoglycerate (3-PGA) reduced the level of activation of NADP-MDH possibly via $NADP^+$ production by the reduction of these substrates. The enhanced $NADP^+$ concentration inside the mesophyllic chloroplasts results in a decrease of the steady-state level of reduced ferredoxin and hence reduced thioredoxin.

Such correlations are important for the understanding of salt-stress–induced variations of enzyme activity as well. Experiments with maize leaves [248–250] indicate that the level of NADP-MDH activity under physiological conditions depends on the source to sink ratio of photosynthesates: Reducible substrates (oxaloacetate and 3-PGA) produced by CO_2 fixation result in an increased $NADP^+$ level and a decrease of the ferredoxin (thioredoxin *m*) redox state and the degree of NADP-MDH activation. Finally, it has to be kept in mind that low O_2 concentrations inside the mesophyllic chloroplast will decrease the rate of direct oxidation of thioredoxin *m*, and this would favor an increase in the activation state of NADP-MDH [246].

NADP$^+$-Malic Enzyme

The $NADP^+$-malic enzyme (NADP-ME, EC 1.1.1.40) catalyzes the oxidative decarboxylation of malate to form pyruvate and CO_2 inside the bundle sheath chloroplasts. Pyruvate subsequently is transported back to the mesophyll. The decarboxylation is paralleled by a reduction of $NADP^+$, thus providing NADPH for the Calvin cycle.

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$$\text{Malate} + \text{NADP}^+ <=> \text{Pyruvate} + \text{NADPH} + \text{CO}_2$$

NADP-ME is sensitive to malate, pH, and Mg^{2+} in a manner indicating that it probably is largely inactive in the dark. It is activated by coenzyme A and fructose-1,6-bisphoshate, which increases its activity 4- and 16-fold, respectively. Chloride and nitrate are inhibitory ligands; high levels of bicarbonate give some inhibition [251]. In summary, it may be speculated that there are both direct and indirect effects of salinity on the catalytic activity of this enzyme.

Pyruvate, Phosphate Dikinase

Pyruvate, phosphate dikinase (PPDK, EC 2.7.9.1) catalyzes the reversible phosphorylation of pyruvate to form phosphoenol pyruvate (PEP) [252–254]:

$$\text{Pyruvate} + \text{ATP} + \text{P}_\text{i} <=> \text{PEP} + \text{AMP} + \text{PP}_\text{i} + 2\ \text{H}^+$$

In C_4 plants, the operation to form PEP inside the mesophyllic chloroplasts is favored by the presence of very high levels of adenylate kinase and pyrophosphate activities [255]. The fact that the PPDK activity is lowest among the C_4 cycle enzymes and close to the observed rates of photosynthesis in C_4 leaf tissues suggests that this enzyme might catalyze the rate-limiting step of C_4 photosynthesis [246,255–257]. This view is supported by the fact that the activity is subject to many regulatory controls. PPDK from maize has been purified by Sugiyama [258]. The enzyme has a molecular weight of 387 kDa and represents a tetramer of its 94-kDa subunit. The enzyme is inactivated in the absence of Mg^{2+} and dithiol compounds like DTT. Inactivation in the absence of Mg^{2+} results in the dissociation of the active tetramer to an inactive dimer.

The isolated enzyme from maize can be protected from dissociation not only by the addition of Mg^{2+} but also by high concentrations of sugars, polyols, and pyruvate [246]. This Mg^{2+} dependency suggests that the PPDK activity may be reduced if external salts compete with Mg^{2+} for uptake. Andrews and Hatch [252] proposed a two-step mechanism for catalysis:

$$\text{Step 1: PPDK} + \text{ATP} + \text{P}_\text{i} <=> \text{PPDK-P} + \text{AMP} + \text{PP}_\text{i}$$
$$\text{Step 2: PPDK-P} + \text{Pyruvate} \Leftrightarrow \text{PPDK} + \text{PEP}$$

They observed that the PEP/pyruvate exchange required Mg^{2+} but not other substrates, and the AMP-ATP exchange was very strongly affected by varying both P_i or PP_i concentrations. The requirement for Mg^{2+} in excess of ATP under in vitro conditions could not be overcome by the addition of other divalent cations like Mn^{2+} or Ca^{2+}. Moreover, the activity of the maize enzyme can be stimulated by both ammonium ions and K^+ but not by Na^+ [246].

In vivo PPDK activity declines in the dark and is rapidly reactivated on illumination of maize leaves. For activation of phosphate, Mg^{2+} and a reducing agent (DTT under in vitro conditions) are required. But it was clearly shown by several investigators that the thioredoxin system is not involved in PPDK activation [246]. A protein factor, PDRP (a dimer consisting of two identical 45-kDa subunits) exclusively located in the mesophyllic chloroplasts is involved in ATP as well as in ADP-dependent inactivation and light-dependent activation of the PPDK catalytic activity [259].

ADP-dependent inactivation is inhibited by oxalate. On the other hand, oxalate as well as pyruvate are inhibitors of catalysis [260,261]. The transfer of the β-phosphate from ADP phosphorylating a PPDK threonine residue is one possibility to inhibit the catalytic activity. In the presence of ATP, a histidine residue of the catalytic site becomes phosphorylated, and this phosphorylation also results in an inhibition of the enzyme. During light-dependent reactivation, the phosphate is transferred on orthophosphate to form pyrophosphate. This reaction can be inhibited in vitro by the addition of AMP, ADP, or PP_i.

It has been clearly shown that the rate of ADP-mediated PPDK inactivation depends on the phosphorylation status of the enzyme. This status is controlled by the ATP/AMP and pyruvate/PEP ratios inside the mesophyllic chloroplasts. Activation of catalytic activity is brought about by a high-energy charge resulting in a high ATP/ADP ratio in the chloroplast. Relatively small changes in

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the level of the energy charge result in dramatic changes in the relative activity of the PPDK population. These observations imply that salt stress might affect the PPDK activity not only via competition among cations but also by variations of the energy status and the metabolite concentrations inside the mesophyllic cells. Such variations will be brought about by increased ATPase activities under salinity.

Photorespiration and Nitrogen Metabolism

Labeling studies with $^{14}CO_2$ and $^{18}O_2$ have indicated that glycine and serine are synthesized in C_4 plants particularly under conditions of high O_2 or low CO_2 [262–265]. Using isolated bundle sheath strands, Farineau et al. [264] demonstrated the incorporation of $^{14}CO_2$ into glycine and serine in maize at low bicarbonate concentrations. The addition of α-hydroxypyridinemethanesulphonic acid, an inhibitor of glycollate oxidase, resulted in both a reduced ^{14}C-labeling of glycine and serine and an increased labeling of glycollate. In a similar experiment, isoniazid, an inhibitor of glycine decarboxylase, increased the labeling of glycine and decreased that of serine. These data suggest that, even in C_4 plants, there can be an appreciable metabolism of carbon through the photorespiratory pathway under some conditions. It must be assumed that the majority of the CO_2 evolved during photorespiration in C_4 plants is immediately reassimilated by Rubisco and is not lost from the bundle sheath cells [266].

A major source of cytosolic NADH for the reduction of nitrate is a malate/oxaloacetate shuttle that operates in green leaves between the chloroplasts and the cytosol. In the leaves of C_4 plants, photorespiration is restricted to bundle sheath cells, whereas nitrate assimilation is found in the mesophyll [267]. If the pyruvate dehydrogenase complex (PDC), located in the mesophyllic cells, were sensitive to ammonia produced by photorespiration, the spacial separation in the mesophyll and bundle sheath, respectively, would result in a protection of the PDC. The advantage of the C_4 morphology would thus be twofold: Photorespiration is reduced relative to its contribution in C_3 photosynthesis and the ammonia production by photorespiration and nitrate reduction are spacially separated in C_4 plant leaves [268].

The actual separation of reductive steps in the bundle sheath and mesophyllic cells of C_4 plants would contribute to reduce photorespiration in the bundle sheath cells and enhance the potential NADH supply derived from the malate shuttles in the mesophyllic cells. In mesophyllic cells, the NADPH produced in the chloroplasts can be used to reduce oxaloacetate or the 3-phosphoglycerate imported from the bundle sheath. The reduction products from either reaction would then be available to generate NADH in the cytosol, which in turn is available for nitrate reduction. Thus, the compartmentation of metabolism in C_4 plants is designed for the maximal supply of NADH to the cytosol.

Nitrate reductase has been shown to be light activated via ATP and Mg^{2+}-dependent protein phosphorylation [269]. Any treatment that affects the levels of cytosolic ATP or Mg^{2+} concentrations can inhibit the light-mediated activation of the enzyme. Moreover, salts competing with Mg^{2+} for uptake into the cytosol or affecting the malate/oxaloacetate status of the cell will interfere with the nitrate reduction and affect growth.

Biosynthesis of Compatible Solutes

Glycinebetaine is synthesized by many halophytes and is thought to play an important role in the salt tolerance of these species as a compatible osmotic solute [270]. In higher plants, glycinebetaine is synthesized from choline in a two-step, chloroplast-localized pathway [270]. Whereas homozygous glycinebetaine-accumulating maize plants exhibit less severe growth inhibition than near isogenic glycinebetaine-deficient plants under salinity stress, the high glycinebetaine accumulation in maize appears to be associated with a 5% grain yield penalty under well-irrigated field conditions [271]. Glycinebetaine is a known growth stimulant of certain pathogenic fungi; thus, it is plausible that glycinebetaine accumulation in maize confers susceptibility to stalk pathogens.

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In leaves of maize, starch is usually exclusively located in the bundle sheath chloroplasts. However, on continuous illumination with high-light intensity, enzymes necessary for starch synthesis appear in the mesophyllic chloroplasts and starch synthesis takes place there as well [272]. The major portions of sucrosephosphate synthase, fructose-6-phosphate kinase, and fructose-2,6-bisphosphate are present in the mesophyll. In line with this observation, sucrose synthesis occurs predominantly in the mesophyll and sucrose has to be transported through the bundle sheath cells to the phloem [272].

The accumulation of carbohydrates in source leaves leads to the inhibition of photosynthesis and the concomitant decrease in Rubisco protein, some Calvin cycle enzymes, and chlorophyll. Jang and Sheen [24] used a maize protoplast transient expression system to monitor the effects of a variety of sugars and metabolic intermediates on the promoter activity of genes encoding photosynthetic enzymes. Glucose and other substrates of hexokinase, but not the phosphorylated products, acted as repression signals. Therefore, hexokinase was proposed to mediate the sugar-sensing control of metabolic pathways. Potential intermediary steps in signaling have been implied for protein phosphorylation/dephosphorylation and Ca^{2+}/calmodulin [25,26]. These data suggest that building high concentrations of photosynthesate to compensate for the external osmotic potential of salts not only sequesters sugars from metabolism in maize but also the decreases the expression of C_3 and C_4 cycle enzymes.

Summary of Salinity Effects on Maize Photosynthesate Metabolism

From the above discussion, it becomes obvious that salinity affects metabolism mainly at two levels: gene expression and enzyme catalysis. Moreover, it appears to us that there are only a few key reactions responsible for bringing about a cascade of secondary responses. In this context, it is interesting to note that enzyme patterns and chloroplastic morphology are not strictly coupled, and that metabolic pathways may become varied. Preiss et al. [50] could show, for example, that malate may become an additional substrate to acetate to form fatty acids, when in the chloroplasts of young maize plants, the C_4 pathway becomes dominant over the C_3 pathway.

Recent discussions of the salinity effects on maize metabolism are based on the results published by Aoyagi and Bassham [273] and Crespo et al. [274]: Pulse-chase–labeling and enzymic studies indicated that maize leaves begin to express C_4 enzymes during several stages of their differentiation. Full C_4 photosynthesis is probably missing in the base section of young maize seedlings and appears later in the middle and top section of the leaves [273]. In a study of 1- to 5-week old *Zea mays* plants, different activities of PEPC and Rubisco have been found in the lower and upper leaves. The data have been interpreted as indicating the existence of C_3 and C_4 pathways in the same plant [274].

In leaves of graminaceous plants, cell divisions occur primarily in the basal meristem, with older cells being displaced by younger cells below them. This process results in a positional gradient of cell ages with younger, less-differentiated cells at the base and older, more-differentiated cells toward the tip of each leaf. This developmental gradient has been used by several investigators to examine the aspects of leaf development in maize [23,275–277]. Although small quantities of PEPC mRNA are detectable in the basal region of the leaf, a significant mRNA accumulation is coincident with that of the polypeptide at 4–6 cm from the leaf base [278]. The strict correlation between PEPC mRNA and its protein concentrations suggests that regulation of the genes encoding this enzyme may be primarily transcriptional [278].

A recent study [23] has investigated whether salinity interferes with the induction of the C_4 type enzyme patterns in maize. Twenty-one days after germination, one half of a well-watered maize population was stressed with an additional 50 mM NaCl in the nutrient, and development and enzyme patterns were watched during the following 3 weeks. In order to monitor the development of C_4-type metabolism in the fourth leaf, it was portioned into five sections (tip to base) and PEPC, NADP-MDH, and GAP-DH activities isolated from these portions were measured day by day. The central

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results of the experiment can be summarized as follows: (a) Salinity reduces leaf growth (increase of fresh weight) and leads to an increase of extractable enzyme activity. (b) The enzyme pattern in the leaf tip section at the beginning of salinization (day 21 after germination) already represented the C_4 type and did not vary during the rest of the experiment. (c) All five leaf sections of the control plants showed the same C_4-type enzyme pattern 4 weeks after germination indicating that the fourth leaf was differentiated at this time. The salt-stressed plants, on the other hand, did not finish differentiation during the course of the experiment. (d) Most interesting is the result that the activities of the C_4 pathway enzymes PEPC and NADP-MDH remain strictly coupled, although these enzymes are localized in the mesophyllic and bundle sheath cells, respectively.

In analogy to the above-discussed variation of Rubisco activities in the mesophyllic and bundle sheath cells during the initiation of the C_4 metabolism in young leaves, a ground state of the enzyme pattern with glycerinealdehydephosphate dehydrogenase (GAPDH) present in the mesophyll and bundle sheath may be assumed. As enzyme activities have been measured after extraction from the leaf sections, increasing enzyme activities in the extracts have to be attributed to a newly synthesized enzyme protein. It is a generally accepted observation that the induction of the C_4 enzyme pattern needs high light intensity. Taken together with the observation that photosynthesates are capable of regulating the expression of genes and hexokinase may function as a sensory element [22–26], it may be concluded that the primary metabolites of CO_2 fixation are active in C_4 gene activation as soon as their concentrations in the cytosol reach a threshold value. Consequently, it may be concluded that C_3-C_4 pathway maturation will be affected under salt stress, because photosynthesate becomes sequestered inside the vacuoles in response to salinity. Such an interpretation would suggest that salt affects the differentiation of the pathway of CO_2 fixation by reducing the concentration of photosynthesate. The most probable way to bring about such a reduction is by sequestering photosynthesates inside the vacuole as a response to a salt-stress signal transmitted from the maize root system.

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Water Stress and Alfalfa Production

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ALFALFA ADAPTATION

Alfalfa (*Medicago sativa* L.), or lucerne as it is sometimes called, is a perennial herbaceous plant with superior forage quality. It is the most important forage crop in the world and was the first forage crop to be domesticated [1]. Alfalfa is a legume, and it is able to fix nitrogen from the air through a symbiotic relationship with *Rhizobium* bacteria. It has a tap root that can reach 11 m or more that allows it to escape drought [2]. Alfalfa becomes dormant during periods of drought and will resume growth once water becomes available. Alfalfa can be feed as hay, green chop, silage, cubes, pellets, or grazed.

Alfalfa originated in areas with hot, dry summers in the Near East (Iran) and Central Asia [1]. Alfalfa spread long before recorded history and is found on every continent [1]. Alfalfa may have been disseminated as early as 7000 BC from Mesopotamia through trade with other regions [3]. It has become adapted to many areas throughout the world since its original spread from its center of origin thousands of years ago. Alfalfa is best adapted to the temperate zones throughout the world and prefers slightly alkaline, well-drained soil. Most of the world's alfalfa production is centered in the United States, Argentina, southern Europe, and parts of the former Soviet Union [4]. It can survive in extremely cold areas, but other forage crops are better adapted in these regions. Alfalfa is not usually grown in the tropics because of problems with disease, weeds, and stand life and of poor curing conditions. Stands do not last more than 2–3 years in many regions of the southern United States owing to factors other than acid soils or drought stress [5]. The highest alfalfa yields are obtained in warm, arid areas where production is possible nearly year round.

Alfalfa growing regions have been classified according to temperature, latitude, and precipitation [6–8]. Water stress may occur in both humid and arid regions. Water stress in humid regions may occur as a result of inadequate soil moisture at the start of the season or untimely rainfall or drought during the season. These episodes of water stress in humid regions may occur frequently, but they are not common or severe enough to warrant the expense of an irrigation system. Water stress rather than direct temperature effects is responsible for decreased mid season growth that occurs in many alfalfa production regions of North America [9]. Water stress can occur in arid regions even with irrigation. The irrigation system, for example, may not be engineered to meet

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peak water demands by the crop during the hottest part of the year. Sometimes irrigation systems fail because of mechanical breakdowns, problems with the uniformity of water distribution, or the unreliability of the water-delivery system to the farms. In addition, during peak water-use periods, other crops on a farm may also require water and have priority over the water originally designated for alfalfa. Whether in humid or arid regions, water stress commonly limits alfalfa production.

ALFALFA WATER REQUIREMENTS

Water use is driven by solar radiation, temperature, wind speed, and crop canopy development [10]. Seasonal water use also is affected by the number of cuttings or the length of the growing season and cultural factors that enable the crop to reach its yield potential. Seasonal evapotranspiration in alfalfa can range from 407 mm in Geneva, NY [10], to 1887 mm near Phoenix, AZ [11]. Maximum daily water use can be near 0 on rainy days to 9–15 mm d^{-1} in Saudi Arabia [12]. The highest consumptive use is obtained when the days are longgst in June and July in most areas. The consumptive use during other months is less, being almost in proportion to day length. The monthly consumptive use curve for alfalfa is bell shaped with a peak in June and July and ''tails'' at the beginning and end of the growing season [11]. An example of seasonal water use for alfalfa compared with other crops near Phoenix, AZ, is 1887 mm for alfalfa, 1046 mm for cotton, 993 mm for oranges, 902 mm for wheat, and 216 mm for lettuce [11]. Alfalfa has high seasonal water use compared with other crops, since it is a perennial crop with a long growing season.

Forage yield and root mass are related to evapotranspiration. Evapotranspiration and forage yield are linearly related in alfalfa [13–15] and other crops. The relationship between evapotranspiration and grain yield, however, is often curvilinear owing to a higher proportion of vegetative growth compared with grain at higher evapotranspiration levels [16,17]. After water stress is relieved, growth greater than normal has been observed [18]. However, compensatory growth is sometimes due to an increased leaf growth and not stem length or dry matter, which may actually be reduced [19]. The relationship between evapotranspiration and root mass is curvilinear [20]. In the case of water stress, however, root growth can be affected positively or negatively. Salter et al. [21] measured a decrease in root weight but an increase in root fibrousness as water stress increased. Root growth may be increased by limited water stress under low evapotranspirational demand but the opposite may occur under high evaporative demand [22].

Water-use efficiency (WUE), or the ratio of crop production to evapotranspiration, has been reported in alfalfa to decrease with water stress [23,24]; increase with water stress [25], especially if evaporative demand is low [24]; or not be affected by water stress [26]. The relationship between water-use efficiency and water stress is inconsistent possibly due to the type of water stress or factors other than water stress affecting yield. Water-use efficiency for alfalfa varies considerably and examples of reported values are 1.0 kg m^{-3} in the Imperial Valley of California [26]; 1.0–3.0 kg m^{-3} in Minnesota [27], 1.2 kg m^{-3} composited from Las Cruces, NM, Reno, NV, North Dakota, and Nebraska [28]; 1.2–2.3 kg m^{-3} in North Dakota [29], and 2.3 kg m^{-3} in the San Joaquin Valley of California [15]. In Israel, a seasonal decline in WUE was reported from 2.8 kg m^{-3} between April and early June to 1.0 kg m^{-3} in August [30]. The seasonal decline in WUE reported in hot areas has been attributed to high nighttime temperatures that reduce forage yield [31]. Reported values of WUE for alfalfa compared with other crops are 1.2 kg m^{-3} (alfalfa), 1.8 kg m^{-3} (wheat and cotton), 2.9 kg m^{-3} (corn), and 3.3 kg m^{-3} (grain sorghum) [32].

If we assume that alfalfa has a water-use efficiency of 1–3 kg m^{-3}, then 100–33 mm of water, respectively, will be consumed in evapotranspiration to produce 1 t ha^{-1} of alfalfa. In low rainfall areas, more irrigation water needs to be applied than is actually consumed by the crop owing to the nonuniformity of application and losses from evaporation, runoff, and deep percolation. Nevertheless, water-application efficiency, or ratio of evapotranspiration to applied water, is usually high for alfalfa, since it is a deep-rooted crop [33,34]. Water-application efficiencies reported for Arizona crops are 75% for alfalfa, 76% for cotton, 62% for wheat, 31% for lettuce, and 22% for oranges

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[34]. Water-application efficiencies vary depending on the type of irrigation system and how well it is managed. The amount of irrigation water applied and yield are linearly related in alfalfa [28,29,35] similar to consumptive use and yield, but the relationship becomes curvilinear at high levels of water application. If more irrigation water is applied than the plant can use, additional increases in yield will not be obtained. Alfalfa can extract water from depths of 11 m [2], but the effective rooting zone for water extraction has been reported to be 2.4 m for irrigated alfalfa [11,36]. The actual effective rooting zone can vary, of course, depending on soil characteristics, irrigation practices, amount and distribution of rainfall, and depth of the water table. A water-uptake pattern of 46–26–18–10% over depth increments of 0.6 m has been reported [37]. The water-uptake pattern can differ depending on the amount of water available in various soil layers and other factors.

ALFALFA RESPONSE TO WATER STRESS

The water status of plants is usually described by the water potential. The water potential is the sum of the osmotic, pressure, matric, and gravitational potentials [38]. The osmotic potential is related to the solute concentration, the pressure potential is the pressure or turgor exerted against the cell wall, the matric potential is capillary or adsorptive forces, and the gravitational potential is the component attributed to gravity. The matric and gravitational potentials are usually small and can be ignored. The plant maintains turgor as the water potential decreases by increasing the solute concentration. Turgor pressure maintenance allows stomates to remain open, photosynthesis to continue, and growth to be uninterrupted. Alfalfa yields usually decline once the plant water potential falls below -1.0 to -1.5 MPa [15,39–41]. The plant will close its stomates under water stress in an attempt to maintain turgor. Stomatal closure prevents CO_2 from entering the plant and affects carbon fixation, photosynthesis, and growth.

Stomatal closure causes the canopy temperature to increase. Stomatal closure due to water stress elevated the leaf temperature by 8.5°C in the study of Carter and Sheaffer [41]. The crop water stress index (CWSI) uses this change in the leaf or canopy temperature to assess the water status in alfalfa and other crops. The CWSI normalizes the differences between the air and canopy temperature using the vapor pressure deficit (VPD). Water stress decreases the difference between the canopy and air temperature [24]. Yield is linearly related to the CWSI in alfalfa but quality is not predicted adequately [42]. The canopy temperature and the related CWSI values can differ based on dormancy class [43]. Temperature stress days (TSD) [44] and the difference between canopy and air temperature [45] have been used as indicators of water stress, but the CWSI is superior, since it accounts for environmental differences. The canopy temperature and associated parameters such as evapotranspiration, leaf conductance, and leaf water potential usually cycle diurnally under high moisture conditions but the leaf conductance and the water potential may remain low throughout the day if the water stress is severe [41,46].

The first outward signs of water stress in alfalfa is the blue-green or gray-green appearance of the plant and wilting and cupping of leaves. Cupping is related to a decline in the plant water potential [47] and to an ambient vapor pressure deficit [48]. The stems become shorter and thicken and fewer are produced. Water stress causes leaf cells to become smaller, which reduces water loss under arid conditions [49]. In cool climates, plants selected for larger leaves are expected to have a greater water-use efficiency under water stress than plants with smaller leaves owing to a higher growth rate and reduced transpiration rate [23]. However, when stomates are closed, small leaves reduce the increase in leaf temperature above air temperature [50]. Plants subjected to water stress have lower cell wall, neutral sugar, and glucose concentrations [51]. Water stress decreases the leaf chlorophyll concentration [52]. Water stress increases the epicuticular wax production but the wax production was not found to decrease cuticular transpiration [53]. Alfalfa exhibits heliotropism, adjusting its leaf surface perpendicular to the sun (diaheliotropism) early and late in the day and adjusting its leaf surface to avoid the sun (paraheliotropism) at midday [48]. Paraheliotropism is assumed to be a stress-avoidance mechanism, but no clear relationship has been shown between

paraheliotropism and the xylem water potential [48]. However, Moran et al. [54] showed that water stress diminished diurnal tracking of the sun by alfalfa leaves and the canopy assumed a more vertical profile compared with well-watered plots.

Certain biochemical changes occur because of water stress. Water stress results in an increase in the total amino acid concentration in the phloem of alfalfa, especially of proline [55]. Asparagine accounts for up to 70% of the amino acid content in the phloem sap of alfalfa but does not vary with water stress [55]. The ability to metabolize oxidants may be an important adaptation to water stress [56]. The maintenance of leaf cytokinins also is important during drought, and mycorrhizal symbiosis may play a role in this process [57]. Abscisic acid appears to mediate adaptive responses in plants to a variety of stresses including water deficit. Yeast and animals use kinase to mediate stress signals, and the kinase pathway mediates drought in alfalfa independent of abscisic acid [58]. A family of genes exist in alfalfa that encode a group of proteins that are inducible by abscisic acid and environmental stresses [59].

Photosynthesis, respiration, and nitrogen fixation are decreased by water stress. The net photosynthesis usually declines with water stress owing to closing of stomates or nonstomatal factors. Nonstomatal factors have been shown to be of equal or major importance compared with stomatal factors [60,61]. Nonstomatal factors responsible for a decrease in the net photosynthesis from water stress include decreased light-saturated photosynthetic activity, apparent quantum yield, electron transport rate, intercellular response to CO_2, and RuBP carboxylase activity [61]. Alfalfa plants suffering from water stress increase the partitioning of photoassimilates to the roots [19]. Respiration decreases under severe water stress in alfalfa [62] or, in other forage species, if water stress causes stomatal closure [63,64]. Water stress decreases symbiotic nitrogen fixation in alfalfa [14,62,65]. Nitrogenase activity and nodule number and size is reduced by water stress [66,67]. Nodules may resume activity when water stress is relieved or may be shed under severe water stress [68]. Symbiotic nitrogen fixation requires 1–2 days to recover once water stress is relieved [69]. Mychorrhizal symbiosis may play a role during drought by maintaining the leaf cytokinin levels [57].

Alfalfa quality is usually increased by water stress, since stem growth is slowed [40] resulting in an increased leaf to stem ratio [14,70]. In some studies, however, quality components were not consistently affected by water stress [14,27]. The effect of water stress on alfalfa quality depends on the timing and severity of the stress. If water stress is severe enough to cause leaves to shed, then quality can decrease. Stress at the bud or flower stage after the stem has extended also can decrease forage quality owing to a reduction in the leaf to stem ratio from leaf loss and a deterioration of leaf quality [71]. A forage quality increase due to water stress is accounted for mainly by the slowing of growth and a delay in maturity [70]. Water stress also can increase the quality of forage by decreasing the cell wall concentration [51,70] but not necessarily the cell wall degradability [51]. Water deficit can increase in vitro digestible dry matter and crude protein [70,72] and increase concentrations of Ca, Mg, Zn, K, and P in the entire plant [72,73].

Alfalfa may adapt to water stress through avoidance or tolerance mechanisms. Increased root mass and depth of rooting are examples of dehydration avoidance [21,30,49]. A small root system may restrict water loss and delay the onset of drought. Mild dehydration tolerance is related to the accumulation of osmotically active substances such as proline and sucrose [50,74,75]. Osmotic adjustment maintains turgor and is related to tolerance of low water potential [76], may enhance water uptake by maintaining root extension [50], and delay the time to reach the lethal leaf water content [77]. Sucrose and proline not only serve as osmoticants but also as protectants of membranes and proteins during dehydration [78–80]. In more extreme cases of water stress, the tolerance of protoplasmic dehydration is necessary for the plant to survive [81].

PRODUCTION FACTORS

Many factors related to alfalfa production affect water stress directly or indirectly. Deep ripping increases root development and the water infiltration rate [82]. The addition of manure before plant-

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ing can increase soil porosity and water penetration [83]. Firming of the seedbed may be necessary if soil moisture is limiting for better seed/soil contact and stand establishment [84]. If soil moisture conditions are not favorable, deeper seeding is recommended than if moisture conditions are optimum [84]. Companion crops seeded with alfalfa help control wind and water erosion and suppress weeds but compete with alfalfa for water as well as light and nutrients. Placing a band of phosphorus fertilizer near the seed at planting time is advantageous on low-fertility soils when dry periods follow seeding [85,86]. Increased water availability increases top growth and uptake of all major and secondary nutrients [87]. Water stress decreases the yield benefit of P and K fertilization [88,89], and P fertilization increases water-use efficiency [88]. The addition of lime to acid subsoils has been observed to increase root penetration [90,91] and performance under water stress should improve. Soil ammendments such as gypsum or sulfur increase water infiltration in sodic soils and thereby decrease susceptibility to water stress. Harvest schedules are usually dictated by soil moisture conditions, especially in irrigated areas. Cutting too early when the soil is wet can compact the soil, which could result in water infiltration and water-stress problems later. Cutting too late may result in yield and quality losses from water stress, and inadequate soil moisture for regrowth while the hay is drying in the field before irrigation water can be applied.

Water stress usually lessens the expression of alfalfa diseases that are associated with excessive soil moisture. For example, fewer symptoms of verticillium wilt (*Verticillium albo-atrum* Reinke et Berth) were observed under drought stress than nonstressed conditions, and verticillium wilt altered the leaf potential [92] and reduced the stomatal conductance under drought [93]. Low soil moisture reduced the effects of the stem nematode (*Ditylenchus dipsaci* [Kühn] Filipjev) and fusarium wilt (*Fusarium oxysporum* f. sp. *medicaginis*) [94]. Phytophthora root rot (*Phytophthora megasperma* [Drechs] f. sp. *medicaginis* Kuan & Erwin [Pmm]) is associated with excess soil moisture, and relieving saturated soil conditions can help control the disease. Scald is a physiological disease caused by flooding during periods of high temperatures that can be managed by avoiding standing water.

Water stress adversely affects insects such as potato leafhopper (*Empoasca fabae* Harris) and silverleaf whitefly (*Bemecia argentifolia*). Potato leafhoppers tend to probe fewer cells and cause less injury if the alfalfa plant is water stressed [95]. Under water stress, potato leafhopper population declined by half, the development period of the nymphs increased, and adult survivorship, fecundity, and oviposition rates decreased with water stress [96,97]. Silverleaf sweetpotato whitefly damage was less on water-stressed compared with well-watered alfalfa, presumably because the insect preferred the more succulent growth of the well-watered plants [98].

Excess soil moisture and loss of the alfalfa stand can lead to weed infestation. Certain weeds can survive saturated soil conditions in contrast to alfalfa. Alfalfa can usually survive when soil moisture is low, whereas many weeds desiccate. In fact, summer irrigation termination was commonly practiced as a weed-control measure in the southwestern United States until the early 1960s when effective herbicides were introduced.

SUBOPTIMAL IRRIGATION STRATEGIES

Suboptimal irrigation strategies for alfalfa include deficit irrigation and irrigation termination. Deficit irrigation involves applying less irrigation water than the crop actually needs by altering the amount or frequency of the irrigation. The irrigation amount can be altered with sprinkler irrigation systems by applying a certain percentage of the crop evapotranspiration requirement. Yield decreases in direct proportion to the difference between the amount of water applied and the amount of water required for maximum yield in a particular environment [15]. Irrigation frequency can be altered by applying fewer irrigations. Irrigating once per cutting resulted in 87% of the yield of irrigating twice per cutting in the study of Frate et al. [99]. Water-application efficiency, or the ratio of evapotranspiration to applied water, often increases with deficit irrigation, since irrigations are applied to relatively dry soils and less water is potentially lost to deep percolation and surface runoff.

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Irrigation termination usually involves not irrigating alfalfa for one or several cuttings. Irrigation termination has the advantage of not incurring production costs but the disadvantage of potential residual effects on yield even after irrigations are resumed. Some of the earliest research on alfalfa irrigation termination was conducted at Mesa, AZ, by Schonhorst et al. [100]. In this study, stands were actually enhanced if alfalfa irrigation was terminated during the summer for two cuttings and yields recovered when irrigations resumed. Schneiter [101] conducted a similar study in Tucson, AZ, and found that alfalfa stands and subsequent yields were not damaged by withholding irrigation water during the summer. Alfalfa yields recovered in the second cutting after irrigations were resumed in an irrigation termination study in the San Joaquin Valley of California that included a July/August irrigation skip and an irrigation termination from July to the following season [99]. Irrigation termination lasting two cutting cycles during the summer had no long-term effect on the alfalfa yield in Cyprus, but the yields were reduced by 20% the first harvest after irrigations were resumed [102]. Drought stress that lasted 2 and 8 months did not affect alfalfa growth the following winter and early spring in Israel [30]. In a study conducted in the Imperial Valley of California, alfalfa stands were not affected by irrigation termination the first and second years presumably owing to the reliance on subsoil moisture during the termination period but were severely reduced the third year [98]. Hay yields generally rebounded by the second cutting after irrigations were resumed except during the third year where modest yield reductions were noted. Irrigation termination treatments resulted in soil salt build-up and increased weed pressure. Ottman et al. [103] conducted irrigation cutoff studies at Maricopa and Yuma, AZ. At Maricopa, alfalfa yields following the summer termination treatment were not affected the first year, but, in the second year, were 67% of the control the first cutting after irrigations were resumed and 85% of the control the second and similar thereafter. The summer, fall, and winter termination strategy resulted in reduced alfalfa yields for most cuttings even after irrigations were resumed. On an alfalfa stand at the Yuma location, summer irrigation termination (July through October) resulted in severe stand loss (33% of the control) and permanent productivity damage. Winter irrigation termination (November through February) had no effect on the alfalfa stand and reduced yield 41% during the termination period. Stand loss occurs when the crown moisture content drops below 40%, according to Wissuwa et al. [104], and can be used as a guideline of when to reirrigate alfalfa to avoid permanent damage. Whether crown desiccation is a cause or effect of stand loss that can occur during irrigation termination is not known. A decrease in root nonstructural carbohydrates has been correlated with stand loss during irrigation termination in one study [105] but no correlation was found in another study [103]. Irrigation cutoff strategies have resulted in greater applied water-use efficiency or more forage produced per unit of water applied [99,100,103]. However, applied water-use efficiencies also have been decreased by irrigation cutoff strategies if yields after irrigations are resumed are permanently damaged [98,103].

Alfalfa is a deep-rooted crop that can deplete subsoil moisture. The classic work of Kiesselbach et al. [2] demonstrated the importance of subsoil moisture to depths up to 10 m for alfalfa production. If shallow water tables are present, surface irrigation can be reduced [106]. The success of suboptimal irrigation strategies may depend on the availability of subsoil moisture to maintain the crop during periods of water stress.

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Salinity Tolerance in Turfgrasses

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INTRODUCTION

The importance of turfgrass has increased rapidly in the last several decades. In 1993, the annual expenditure for maintaining turfgrass in the United States, including labor but excluding capital expenses, was approximately $45 billion [1]. This was double the amount reported in 1985. And turfgrass maintenance expenditures in the United States are projected to be $90 billion by the year 2000 [2].

The need for salt-tolerant turfgrasses is ever increasing. Growth of the turfgrass industry, accompanied by rapid urban population growth, has put enormous pressures on limited freshwater supplies. Many state and local governments have reacted by placing restrictions on the use of potable water for irrigating turfgrasses. This is particularly evident in a number of western states, where current laws now *require* the use of saline secondary water sources (such as effluent) for irrigation of golf courses and other large turf facilities [3,4]. In coastal states, where urbanization also has been particularly rapid, overpumping, and resultant salt water intrusion of coastal wells used for irrigating turf facilities has widely occurred [5,6]. Finally, in northern areas, the use of salt for deicing roads has resulted in soil salinization along roadsides planted to turfgrass [7].

DEFINING SALINITY TOLERANCE IN TURFGRASSES

Although substantial differences in salinity tolerance exist among turfgrasses [8–10], many environmental, edaphic, and plant factors also interact with salinity level to influence plant salinity tolerance [11–14]. Salinity tolerance often differs with the stage of plant development (e.g., seedling, juvenile, mature) [15]. Climatically, temperature, relative humidity, and air pollution can influence the plant response to salinity [11]. For example, most plants are more sensitive to salinity under hot, dry conditions than under cool, humid ones, probably due to increased evapotranspirational demand, favoring increased salt uptake [16]. Air pollution increases the apparent salt tolerance in oxidant-sensitive crops [11]. Edaphic factors also influence the plant response to salinity [12,13]. Soil water content changes have a direct effect on rootzone salinity. Indeed, soil salinity varies with time and depth, increasing as the soil dries between irrigations, and also as depth increases, with salt

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concentrations approximately that of the irrigation water near the soil surface, to many times higher at the bottom of the rootzone [11,17]. In most saline situations, sodicity problems can occur, as the primary ion in most saline soils is sodium. In finer textured soils, this can result in anaerobic conditions in the rootzone, which can have a more profound effect on plant growth than the salinity itself [13,17]. To minimize the effects of variable edaphic and climatic conditions on plant responses to salinity, some researchers have utilized solution or hydroponic culture under controlled environmental conditions (growth chambers, greenhouses) in plant salt-tolerance research.

Owing to interacting factors discussed above, the ''absolute'' salinity tolerance level of a particular genotype or cultivar cannot be determined [11,12]. For example, the salt tolerance of Bermudagrass cv. Tifway, indicated as the level of salinity required to reduce the shoot dry weight by 50% was reported as 33 dS m^{-1} [18], 27 dS m^{-1} [19], 18.6 dS m^{-1} [20], and 12 dS m^{-1} [21]. The use of different criteria to measure salinity tolerance further complicates comparisons. For example, shoot weight [15], shoot weight reduction relative to nonsalinized plants [22], root weight or length [23,24], shoot/leaf length [25,26], shoot visual injury [27], plant survival [28], and seed germination [29] have all been used as measures of salinity tolerance in turfgrasses. Finally, the units used in measuring salinity often vary from study to study. Units of measurement frequently used include salts on a weight basis (parts per million total dissolved solids [TDS ppm], total dissolved solids in milligrams per liter [TDS mg L^{-1}], total dissolved solids in milliequivalents per liter [TDS meq L^{-1}]), or on a conductivity basis (millimhos per centimeter [mmhos cm^{-1}], decisiemens per meter [dS m^{-1}]). Wherever possible, I have standardized units to dS m^{-1} to facilitate comparison of studies.

Even with these limitations, the relative (to one another) salinity tolerance of turfgrasses can be estimated within studies and between studies having at least one entry in common. This chapter presents the results of existing turfgrass salt tolerance studies and attempts comparisons, where possible, to derive relative salinity tolerances of currently used turfgrasses and also of alternative, proposed turfgrasses. Cultivar studies also are reviewed, and salt-tolerant cultivars within species are presented. The C_3 (cool-season) turfgrasses are presented first, followed by C_4 (warm-season) turfgrasses. See Table 1 at the end of this chapter for a summary of the estimated relative salinity tolerance of turfgrass species.

RELATIVE SALINITY TOLERANCE OF C_3 (COOL-SEASON) TURFGRASSES

Alkaligrasses (*Puccinellia* spp.)

Alkaligrasses are found inhabiting saline and alkaline sites throughout the cooler portions of North America [30]. These low-growing, perennial bunchgrasses were first considered for use as turfgrass in Illinois [31] and Colorado [32] when alkaligrasses were found along roadsides where deicing salts had eliminated other grasses. Fults [33] listed three *Puccinellia* spp. as being the most valuable for turf: weeping alkaligrass (*Puccinellia distans* [L.] Parl.), Nuttall alkaligrass (*P. airoides* [Nutt.] Wats and Coult.), and Lemmon alkali grass (*P. lemmoni* [Vasey] Scribn.). Alkaligrasses are mainly suited for low-maintenance turf, and they have been used successfully along roadsides and on some residential and athletic grounds [9].

Alkaligrasses are by far the most salt-tolerant cool-season grasses having turflike growth characteristics. Weeping alkaligrass has been reported surviving in soils with EC_e (salinity, in electrical conductance, of soil-saturated paste extract) over 46 dS m^{-1} [32]. The U.S. Salinity Laboratory [17] listed Nuttall alkaligrass as having high salt tolerance (EC_e 12–18 dS m^{-1}), being more salt tolerant than bermudagrass (*Cynodon dactylon* L.) but less salt tolerant than saltgrass (*Distichlis spicata* var. *stricta* (Torr.) Beetle; *D. spicata* var. *spicata* (L.) Greene). Harivandi et al. [34] found a weeping alkaligrass accession to be more salt tolerant than an accession of Lemmon alkaligrass in terms of leaf yellowing and survival. Lunt et al. [35] reported weeping alkaligrass survived relatively well

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in sand culture when irrigated with EC_{iw} (salinity, in electrical conductance, of irrigation water) 32 dS m^{-1} over a 4-month period, suffering less injury than creeping bentgrass (*Agrostis palustris* Huds.), tall fescue (*Festuca arundinacea* Schreb.), colonial bentgrass (*Agrostis tenuis* Sibth.), and Kentucky bluegrass (*Poa pratensis* L.). Hughes et al. [15] reported forage yields of a weeping alkaligrass accession were reduced 33% over a 5-month period when irrigated with EC_{iw} 32 dS m^{-1} (NaCl) in a greenhouse pot trial. In contrast, perennial ryegrass (*Lolium perenne* L.) was reduced 44% and Kentucky bluegrass 47%. Ahti et al. [28] reported weeping alkaligrass cv. Fults to be more salt tolerant than other fine fescues (*Festuca* spp.) or Kentucky bluegrass cultivars tested, continuing to exhibit healthy vigorous growth with essentially no leaf injury following 80 days of exposure to EC_e 32 dS m^{-1}. Root elongation of Fults weeping alkaligrass seedlings was inhibited to a lesser extent than seedlings of perennial ryegrass, creeping bentgrass, or red fescue (*Festuca rubra* L.) when exposed to 25 dS m^{-1} NaCl [36]. In a greenhouse pot trial irrigated with salinized (final levels not given) water for 5 weeks, Nuttall alkaligrass suffered no leaf chlorosis, whereas weeping and Lemmon alkaligrasses suffered slight leaf chlorosis. Other turfgrasses, including perennial ryegrass, creeping bentgrass, tall fescue, red fescue, and Kentucky bluegrass suffered higher degrees of leaf firing [27].

Percentage of germination after a 15-day expousre to 75% seawater (EC_{iw} 28.5 dS m^{-1}) was greater for Lemmon and weeping alkaligrasses than for Dawson creeping red fescues, perennial ryegrass cv. Pennfine, creeping bentgrass cv. Seaside, and Kentucky bluegrass cv. Merion [37].

Bentgrasses (*Agrostis* spp.)

Creeping Bentgrass (*Agrostis palustris* Huds.; *A. stolonifera* L.)

Creeping bentgrass is considered to be relatively salt tolerant by most investigators, being classified as tolerant of soil EC_e from 8 to 16 dS m^{-1} [10,13,38,39] or 6 to 10 dS m^{-1} [8]. Hannon and Bradshaw [40] reported natural ecotypes of creeping bentgrass to be slightly more salt tolerant than red fescue ecotypes. In a pot experiment irrigated with equimolar concentrations of NaCl and $CaCl_2$ over a 5-month period [35], creeping bentgrass cv. Seaside was more tolerant than tall fescue, colonial bentgrass, and Kentucky bluegrass. The Seaside cultivar survived EC_{iw} 31 dS m^{-1}, with a 50% reduced growth rate occurring at EC_{iw} 18 dS m^{-1}. Greub et al. [27] found salinity tolerance of Seaside to be greater than the perennial ryegrasses, rough bluegrass, and Kentucky bluegrasses tested. Grasses were irrigated with 2.6 M NaCl for 5 weeks, and salinity tolerance determined as the percentage leaf firing.

There appears to be a good deal of variability in the salinity tolerance among genotypes of creeping bentgrass. Substantial variation in salinity tolerance has been reported among natural populations (ecotypes) of creeping bentgrass, with seaside selections being more salt tolerant than inland selections [24,40–42]. Younger et al. [43] reported differences in the salinity tolerance among seven creeping bentgrass cultivars grown in solution cultures up to EC_{iw} 26 dS m^{-1}. Salinities resulting in 50% relative shoot growth reduction ranged from EC_{iw} 9 to 26 dS m^{-1}. The salinity tolerance decreased in the order Seaside, Arlington, Pennlu, Old Orchard, Congressional, Cohansey, and Penncross cultivars. No other creeping bentgrass turfgrass cultivar salinity trial has been published to date.

Colonial Bentgrass (*Agrostis tenuis Sibth.*), Velvet Bentgrass (*A. canina* L.), and Redtop (*A. alba* L.)

Very little salinity work has been done on these bentgrass species. Colonial bentgrass has been rated as having poor salinity tolerance, tolerating EC_e less than 4 dS m^{-1} [10,13,39] or EC_e less than 3 dS m^{-1} [8]. In a greenhouse experiment in which pots were irrigated with saline water until death, a tall fescue accession survived longer than Astoria colonial bentgrass [44].

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Salinity tolerance of velvet bentgrass has not been ranked, but, in a greenhouse pot study, colonial bentgrass cv. Bardot and velvet bentgrass cv. Novobent were compared with three creeping red fescue and two perennial ryegrass cultivars [45]. The bentgrasses were the least salt tolerant.

In a germination experiment using salinized agar [5], salt levels required to reduce germination by 50% decreased in the order redtop cv. Streaker (16,000 ppm $\approx$ 25 dS m^{-1}), creeping bentgrass cv. Seaside (23 dS m^{-1}), velvet bentgrass cv. Kingston, colonial bentgrass cv. Exeter (23 dS m^{-1}), colonial bentgrass cv. Highland (22 dS m^{-1}), creeping bentgrass cv. Penncross (21 dS m^{-1}), creeping bentgrass cv. Pennlinks (20 dS m^{-1}), and creeping bentgrass cv. Penneagle (18 dS m^{-1}).

Bluegrasses (*Poa* spp.)

Kentucky Bluegrass (*Poa pratensis* L.)

A number of salinity studies have been done on Kentucky bluegrass in the United States, reflecting its wide use among cool-season turfgrasses. Kentucky bluegrass has been ranked as having poor salinity tolerance, tolerating EC_e less than 4 dS m^{-1} [10,13,38,39,46] or EC_e less than 3 dS m^{-1} [8]. Lunt et al. [35] reported a Kentucky bluegrass accession to survive an EC_{iw} of only 8 dS m^{-1}, whereas creeping bentgrass cv. Seaside and tall fescue cv. Alta survived an EC_{iw} of 19 and 13 dS m^{-1}, respectively. Butler et al. [9] reported that Kentucky bluegrass will not grow well in soils with an EC_e greater than 4 dS m^{-1}. Horst and Taylor [26] reported the growth of Kentucky bluegrass cultivars, on average, was reduced 50% at EC_{iw} of 11 dS m^{-1}.

There does not appear to be an extensive range of salinity tolerance differences among the Kentucky bluegrass cultivars studied. Six Kentucky bluegrass cultivars had greater shoot weight reductions and more shoot tissue injury than two cultivars of perennial ryegrass and a creeping bentgrass exposed to 4.5 Mg NaCl ha^{-1} $week^{-1}$ over a 3-week period [27] (actual concentrations of irrigation water were not given). Although there were no differences in the shoot dry matter yield, the shoot salt injury was significantly less for Nugget than other Kentucky bluegrass cultivars (Fylking, Park, Pennstar, Newport, and Merion). Nugget, Ram I, and Baron Kentucky bluegrasses suffered less shoot visual injury than Adelphi when irrigated with EC_{iw} of 15 dS m^{-1} over a 2-month period [47]. In a greenhouse pot experiment, 23 Kentucky bluegrass cultivars were subirrigated with EC_{iw} of 14 dS m^{-1} for a period of 97 days [28]. Appearance (percentage live leaf tissue) decreased in the order Nugget, K1-148, Bristol, and Parade. Subsequent statistical groups were Sydsport, Windsor, Cheri, Victa, Touchdown, A-34, Fylking, Newport, and Rugby followed by Park, South Dakota Certified, Aquila, Majestic, Baron, and Bonnieblue followed by Pennstar, Adelphi, Merion, and Vantage. Ahti et al. [28] reported that there was not a very wide range of salinity tolerance among Kentucky bluegrass cultivars compared with other turfgrasses studied. Torello and Spokas [48] tested 37 Kentucky bluegrass cultivars by spraying NaCl at increasing weekly concentrations onto field plots. At the end of 9 weeks, cultivar differences in turf quality (composite of color and shoot density) were small. Majestic, Princeton, and Galaxy had the highest turf quality, whereas Haga, Plush, and Victa had the lowest ratings. These two cultivar groupings were significantly different from each other, but both groupings were statistically similar to the remaining 31 tested cultivars. In a subsequent experiment, Torello and Symington [49] measured the leaf and root length of seedlings of five Kentucky bluegrass cultivars grown in nutrient agar medium containing up to 1% NaCl. Variables consistently showed Adelphi and Ram 1 to be more salt tolerant than Nassau, Bensun, and Baron. Fylking performed better than Merion Kentucky bluegrass in field plots having an average EC_e of 11.4 dS m^{-1} [50]. The seedling leaf blade growth of 44 Kentucky bluegrass cultivars was tested under a range of salinity from 7500 to 15,000 ppm (EC_e 25 dS m^{-1}) of a NaCl/$CaCl_2$ mix [26]. Cultivars having the greatest leaf blade growth included Arista, Nugget, Delta, Prato, Baron, Park, S-21, Pennstar, Fylking, Windsor, Victa, Birka, Banff, Cheri, and Oregon Common.

In comparing cultivar salt tolerance ratings in the above studies, there are conflicting trends, perhaps due to a narrow range of salt tolerance within this species. Only Nugget was consistently in the top group in the four studies in which it appeared. In contrast, Fylking was in the top statistical

group in two studies, but, in two others, it had only intermediate salt tolerance. In two studies, Park was ranked fairly low in salt tolerance, but it was in the top group in the Horst and Taylor [26] study. Also, in some studies, Adelphi and Baron ranked as being salt tolerant, whereas they ranked as being salt sensitive in others. Merion consistently ranked as being salt sensitive in the three studies in which it was included.

Reiten et al. [51] reported germination of Kentucky bluegrass cv. SD Common to be completely inhibited by 0.6% NaCl (EC_{iw} 11 dS m^{-1}). Forty-four Kentucky bluegrass cultivars were tested for percentage of germination under a range of salinities from 7500 to 15,000 ppm (EC_e 25 dS m^{-1}) [26]. There was a continuous decrease in the percentage of germination from 100 to 4%, with Delta, Park, Prato, Warrens 113, and Nugget having the highest percentage of germination.

Rough Bluegrass (*Poa trivialis* L.)

In a greenhouse pot experiment in which grasses were exposed to 4.5 Mg NaCl ha^{-1} $week^{-1}$ over a 3-week period, a rough bluegrass accession suffered more shoot injury than creeping bentgrass cv. Seaside, was equal in the salt tolerance to perennial ryegrass cv. Common and three Kentucky bluegrass cultivars (Pennstar, Nugget, and Park), and was more salt tolerant than Kentucky bluegrass cultivars Fylking, Newport, and Merion [27].

Fescues (*Festuca* spp.)

Creeping Red Fescues (*F. rubra* L.)

Probably more salinity studies have been done on creeping red fescues than any other turfgrass, particularly in Europe and Canada, owing to their widespread use in cooler climates. There are two types of creeping red fescues: a strong creeping type with 56 chromosomes (*F. rubra* L. ssp. *rubra*) and a slender creeping type with 42 chromosomes (*F. rubra* L. ssp. *trichophylla* Gaud. or ssp. *litoralis* [Meyer] Auguir) [52]. However, most salinity research does not distinguish these two types (accessions are listed simply as ''red fescue'' or *Festuca rubra* L.).

There appears to be a broad range of salinity tolerance within creeping red fescues. Creeping red fescue is generally rated as having poor salt tolerance, tolerating an EC_e less than 4 dS m^{-1} [10,13,38,39] or an EC_e 3–6 dS m^{-1} [8]. However, Butler et al. [46] rated red fescue as having medium salt tolerance, tolerating EC_e of 8–12 dS m^{-1}. Salt-tolerant naturally occurring coastal populations of creeping red fescue have been described [53,54]. Michelmann [55] reported accessions of strong and slender creeping red fescue to have greater salinity tolerance than perennial ryegrass and velvet and colonial bentgrasses. Greub et al. [27] reported Ruby red fescue to be intermediate among cool-season grasses in salt tolerance: equivalent to perennial ryegrass cultivars (Common, NK-200), less tolerant than tall fescues (Alta, K-31) and creeping bentgrass cv. Seaside, but more tolerant than a rough bluegrass and redtop accession. However, Gibeault et al. [50] reported red fescue to have poor salt tolerance (equivalent to colonial bentgrass but less than Kentucky bluegrass and perennial ryegrass) when grown in field plots having EC_e averaging 11 dS m^{-1}. Shildrick [56] stated that slender creeping fescue cultivars (such as Dawson and Oasis) are generally more salt tolerant then strong creeping red fescues (such as Ruby and Bargena). Humphreys [57] found a salt-marsh ecotype of creeping red fescue to be much more salt tolerant than the tolerant slender creeping red fescue cultivars Dawson and Oasis. Potted grasses were sprayed with a 16% w/v NaCl spray over a period of 4 weeks. Humphreys [57] stated that differences among creeping red fescue cultivars are in fact more closely related to the point of origin than to the subspecies. Leaf number, leaf length, root number, and root length were measured in seedlings exposed to up to 170 mM NaCl for 30 days [49]. All measured parameters indicated creeping red fescue cv. Dawson above even Fults weeping alkaligrass, followed by creeping red fescue cv. Checker, and then 5 Kentucky bluegrass cultivars. In contrast, Fults weeping alkaligrass was reported as being the most salt tolerant in a study comparing a number of cool-season grasses [28]. Potted grasses were irrigated with water containing 1.25% NaCl (EC_{iw} 21 dS m^{-1}) for 90 days, with salt tolerance

measured as the percentage of leaf firing. Salt-tolerance ratings were as follows: Fults weeping alkaligrass > creeping red fescues > Kentucky bluegrasses > Chewings fescue > hard fescue = sheep fescue. Among creeping red fescues, Dawson and Golfrood were more tolerant than other cultivars. The salt tolerance of seven slender creeping red fescue cultivars was determined by measuring the root length of seedlings growing hydroponically in up to 250 mM NaCl (EC_{iw} 25 dS m^{-1}) [58]. Oasis, Hawk, Polar, Merlin, and Dawson were more tolerant than S59 and Jupiter. Saltol, a creeping red fescue collected from a saline tidal marsh on the St. Lawrence River, was more salt tolerant than Biljart, Carlawn, Highlight, Ottawa 1 creeping red fescues, Baron, Bristol, Merion, Nugget, Touchdown Kentucky bluegrasses, and Manhattan, Norlea, NK-200, Pennfine, Yorktown perennial rye grasses [59]. Potted grasses were sprayed five times with 4% road salt (85% NaCl) solution and visually rated for salt injury. Comparing all studies, it appears that Dawson, Oasis, and Saltol have superior salinity tolerance to other creeping red fescue cultivars. It appears that the red fescues merit better than the rating of poor salt tolerance given by some investigators.

Germination of slender creeping red fescue cv. Dawson was superior to Kentucky bluegrass cv. Merion and creeping bentgrass cv. Seaside and equivalent to perennial ryegrass cv. Pennfine and alkaligrass [37]. Grasses were germinated in Petri dishes containing 75% seawater (EC_{iw} 28 dS m^{-1}). Relative percentage of germination was compared among 16 creeping red fescue cultivars following 7 weeks' exposure to 260 mM NaCl (25 dS m^{-1}) [60]. Polar had the highest % germination, followed by Dawson, and then Koket and Novorubra, Erika, Famosa, Jamestown, and Reptans.

Chewings Fescue (*F. rubra* L. ssp. *commutata* Gaud.)

Chewings fescue has been ranked as being moderately salt sensitive, tolerating only EC_e 3 to 6 dS m^{-1} [8]. Torello and Symington [49] ranked Chewings fescue as being less salt tolerant than creeping red fescues. In a greenhouse pot experiment, Greub and Drolsom [44] reported a Chewings fescue accession to have poor salt tolerance, being equivalent to Kentucky bluegrass cv. Merion and colonial bentgrass cv. Astoria but less tolerant than Nuttall alkaligrass, tall fescues cv. Alta and K-31, and creeping red fescue cv. Ruby. An inland population of Chewings fescue was less salt tolerant than a maritime one [61]. Yield reduction occurred at 100 mM NaCl (EC_{iw} 11 dS m^{-1}) for the inland population but not until 200 mM (EC_{iw} 21 dS m^{-1}) for the maritime population. Chewings fescue cv. Highlight was found to be less salt tolerant than seven slender creeping red fescue cultivars in a seedling root growth trial [58].

Hard Fescue (*F. longifolia* Thuill.)

Hard fescue has been ranked as being moderately sensitive to salinity, tolerating only EC_e 3–6 dS m^{-1} [8]. In a study in which pots were subirrigated with 1.25% NaCl (EC_{iw} 32 dS m^{-1}) for 80 days, hard fescues cv. Scaldis, Centurion, and Durar had poorer salt tolerance than creeping red fescue cultivars; measured as visual leaf firing [28].

Meadow Fescue (*F. elatior* L.)

Meadow fescue has been ranked as having poor salt tolerance, tolerating EC_e less than 4 dS m^{-1} [10,13,39,46]. In a greenhouse study, a meadow fescue accession was less salt tolerant than four tall fescue cultivars (K-31, Falcon, Rebel, Houndog). Pots were subirrigated with 0.8% NaCl (EC_{iw} 14 dS m^{-1}) and visually rated for salt injury over a 2-month period [47].

Sheep Fescue (*F. ovina* L.)

Sheep fescue has not been ranked for salinity tolerance. Sheep fescue cultivars Firmaula and Barok were equivalent in salt tolerance to hard fescues, being less salt tolerant than creeping red fescues [28].

Tall Fescue (*F. arundinaceae* Schreb.)

Tall fescue has been rated as medium in salinity tolerance, tolerating EC_e 4–8 dS m^{-1} [10,13,39] or EC_e 6–10 dS m^{-1} [8,38,46]. Lunt et al. [35] reported salinity tolerance to decrease in the order

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alkaligrass, creeping bentgrass cv. Seaside, tall fescue cv. Alta, Kentucky bluegrass, and colonial bentgrass cv. Highland. Alta shoot growth was reduced 50% at 160 meq L^{-1} (EC_{iw} 14 dS m^{-1}). Pots were irrigated with a 50/50 mix of NaCl and $CaCl_2$ over a period of 5 months. In a greenhouse experiment in which pots were irrigated with EC_{iw} 16 dS m^{-1}, salt tolerance decreased in the order tall fescue cv. K-31, Kentucky bluegrass cv Nugget, buffalograss, blue grama, and Kentucky bluegrass cv. Alelphi [47]. Greub and Drolsom [44] reported tall fescue cv. Alta and K-31 to be even more salt tolerant than Nuttalls alkaligrass as well as creeping red fescue cv. Ruby, Kentucky bluegrass cv. Merion, and colonial bentgrass cv. Astoria. In contrast, Greub et al. [27] reported Nuttall alkaligrass to be more salt tolerant than tall fescue cv. Alta and K-31, whereas Lemmon alkaligrass was equivalent to the tall fescues. More sensitive grasses were creeping bentgrass cv. Seaside, followed by creeping red fescue cv. Ruby and perennial ryegrasses cv. Common and NK-200, followed by redtop, and finally rough bluegrass. Horst and Beadle [25] determined germination rates and seedling growth of 16 tall fescue cultivars exposed to up to 15,000 ppm of 50/50 NaCl and $CaCl_2$ (EC_{iw} 25 dS m^{-1}). After 3 weeks' exposure to 25 dS m^{-1}, cultivars with the highest seedling fresh weight included four Belt series, Houndog, Alta, Gallway, and Kenwell. The germination rate was somewhat associated with growth with the top group including the four Belt series, Houndog, Kenmont, Gallway, T-5, and K-31. Comparing the limited information available, Alta would be ranked as being salt tolerant among tall fescue cultivars.

Ryegrasses (*Lolium* spp.)

Perennial Ryegrass (*Lolium perenne* L.)

Perennial ryegrass is typically ranked as having medium salinity tolerance, tolerating EC_e of 4–8 dS m^{-1} [10,13,38] or 6–10 dS m^{-1} [8]. In a field trial with soil EC_e averaging 11.4 dS m^{-1}, perennial ryegrass cultivars maintained better quality than red fescue, Kentucky bluegrass, and colonial bentgrass cultivars [50]. Although statistical comparisons were not given, the shoot dry matter yield of perennial ryegrass cv. Common and NK-200 was reduced less than in six Kentucky bluegrass cultivars, a rough bluegrass, a creeping bentgrass, and two tall fescue cultivars [27]. In contrast, Michelmann [55] reported perennial ryegrass to be less salt tolerant than red fescue but more salt tolerant than velvet or colonial bentgrasses.

The shoot dry weight, as a percentage of control plants, was not different among the perennial ryegrass cultivars tested (Vic. Cert., Tasdale, Barlata, and Linn) [29]. Grasses were exposed to 300 mM NaCl for 2 weeks (EC_{iw} 30 dS m^{-1}).

Dudeck and Peacock [62] germinated six perennial ryegrass cultivars in Petri dishes containing salinized (10,000 ppm seawater) agar. Total germination was greater for Pennant, Citation II, and Palmer than for Horizon, Derby, and Fiesta, whereas the germination rate was the highest for Pennant.

Annual Ryegrass (*Lolium multiflorum* Lam.)

Marcar [29] reported an accession of annual ryegrass to be less salt tolerant than perennial ryegrass Vic. Cert., with 50% shoot growth reductions occurring at 100 and 150 mM NaCl, respectively (EC_{iw} 10.5 and 15.5 dS m^{-1}). However, the effect on germination was reversed, with 50% germination occurring at 330 mM NaCl for annual ryegrass and at 250 mM NaCl for perennial ryegrass (32 and 29 dS m^{-1}).

RELATIVE SALINITY TOLERANCE OF C_4 (WARM-SEASON) TURFGRASSES

Bahiagrass (*Paspalum notatum* L. Flugg.)

Harivandi et al. [8] ranked bahiagrass as being moderately sensitive to salinity, tolerating EC_{iw} of 3-6 dS m^{-1}. However, In a pot study, shoot growth of bahiagrass cv. Pensacola was reduced by an EC_{iw} of only 0.4 dS m^{-1} and did not survive an EC_{iw} higher than 0.8 dS m^{-1} (EC_e 2.5 dS m^{-1}) over

the 255-day trial [63]. Dudeck and Peacock [22] reported bahiagrass cv. Argentine to be less salt sensitive than other warm-season turfgrasses, with 50% shoot yield occurring at EC_{iw} of 9.3 dS m^{-1}. Grasses were grown hydroponically and exposed to salinity increments of up to 42.6 dS m^{-1} for 6 months. The same investigators [64] reported germination of bahiagrasses cv. Argentine and Pensacola were more affected by synthetic sea salt ranging from 0 to 5800 mg L^{-1} (0–≈11 dS m^{-1}) than were other warm-season turfgrasses.

Bermudagrasses (*Cynodon* spp. Rich.)

Bermudagrasses widely used for turfgrass consist of two species. Common Bermudagrass (*Cynodon dactylon* L.), being cosmopolitan in distribution, is one of the world's worst weeds [65]. It also is the most widely used warm-season turfgrass. Interspecific hybrids of *Cynodon dactylon* and *C. transvaalensis* Burt-Davy (African Bermudagrass), often called ''hybrid'' Bermudagrasses, are typically sterile triploid, fine-textured grasses used for golf courses and other high-value areas. These have been produced in turfgrass-breeding programs. There also are some occurrences of natural crosses of *C. dactylon* and *C. transvaalensis*, commonly known as *C.* x *magennisii* [66]. As these types are typically referred to, and grouped together as Bermudagrass in the literature, I have avoided subsectioning this genus.

Bermudagrasses are invariably ranked as having excellent salinity tolerance, tolerating EC_e 8–16 dS m^{-1} [10,13,38,39], >10 dS m^{-1} [8], 12–18 dS m^{-1} [17], or 16–18 dS m^{-1} [46]. The shoot growth of bermudagrass cv. Santa Ana was reduced 50% relative to control plants when exposed to 160 meq L^{-1} of a 50/50 mix of NaCl and $CaCl_2$ (EC_{iw} 16 dS m^{-1}) for 6 weeks [67]. Pasternak et al. [68] reported bermudagrass cv. Suwannee to be more salt tolerant than seashore paspalum in a field experiment irrigated with EC_{iw} of up to 14 dS m^{-1}. There seems to be a good deal of variability in salt tolerance in the bermudagrasses. An ecotype collected from an alkaline soil in India had much greater salinity tolerance than one from a normal soil [69]. The shoot dry matter yield of the alkaline soil ecotype was not reduced over an 8-week period by EC_{iw} of 12 dS m^{-1}. Two common bermudagrass selections collected from the windward coast of Oahu, Hawaii, were more salt tolerant than Tifgreen [70]. Other bermudagrasses (Sunturf, Tifdwarf, and FB-137) were intermediate in salinity tolerance. Dudeck and Peacock [18,22] compared bermudagrasses cv. Tifway and Tifway II with other warm-season turfgrasses. Grasses were exposed to saline hydroponic solutions for 6 months and shoot dry weights compared with control plants. Tifway was more salt tolerant than Tifway II, with a 50% shoot growth reduction occurring at 33 and 24 dS m^{-1}, respectively. Smith et al. [21] compared Tifway with Tifway II in solution culture. Tifway was slightly more salt tolerant than Tifway II, with 50% shoot growth reductions occurring at EC_{iw} 12 and 11 dS m^{-1}, respectively. Eight bermudagrasses were grown in hydroponics for 10 weeks at EC_{iw} up to 32.5 dS m^{-1} [20]. The range of salinity tolerance among cultivars was relatively narrow, with 50% reductions in the shoot growth ranging from EC_{iw} 17.4 to 22.5 dS m^{-1}. Salinity tolerance decreased in the order Tifdwarf, Tifgreen, FB-137, Tifway, Tiflawn, Everglades, Common, and Ormond. Francois [71] compared three bermudagrass cultivars in sand culture and exposed to EC_e up to 35 dS m^{-1} for 10 months. Tolerance decreased in the order Tifton 86, Tifway II, and Tifton 10, with a 50% shoot growth reduction ranging from EC_{iw} 31 to 24 dS m^{-1}.

Using the existing studies to rank bermudagrass cultivars is problematic owing to the variability among studies. A common bermudagrass selection was ranked as being relatively tolerant in one study [70] but as sensitive in another [20]. This is probably a result of the highly heterozygous character of common bermudagrass, having a large range of ecotypes [21]. Tifway has been ranked as being more tolerant than Tifway II in the two studies in which they were compared [21,22]. However, Tifdwarf was ranked as being tolerant in one study [20] but intermediate in another [70]. Similarly, Tifgreen was ranked as being tolerant [20] and sensitive [70] among cultivars studied.

Germination of Common bermudagrass was not affected by salinity from 0 to 5800 mg L^{-1} ($EC_{iw} \approx 10$ dS m^{-1}) synthetic sea salt [64].

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Buffalograss (*Buchloë dactyloides* (Nutt.) Englem.)

Buffalograss has been classified as being moderately tolerant to salinity (EC_e 6–10 dS m^{-1}) [8]. A salinity level resulting in 50% shoot growth reduction of four cultivars (Sharp Improved, Texoka, Topgun, and Plains) was reported to be EC_{iw} 15 dS m^{-1} [72]. Grasses were evaluated after 40 days of exposure. buffalograss cv. Prairie was less salt tolerant than zoysiagrass cv. Meyer and bermudagrass cv. Arizona Common [73] and also less tolerant than tall fescue cv. K-31 but more tolerant than Nugget and Kentucky bluegrass cv. Adelphi [47]. Wu and Lin [74] found substantial differences in germination and seedling survival among diploid and polyploid buffalograss clones, but overall they ranked buffalograss as being moderately sensitive to salinity, with 50% shoot dry weight reductions occurring at 8–10 dS m^{-1}. In a subsequent study, the Wu and Lin [75] found no significant differences in salt tolerance at the seedling stage among nine buffalograsses of different ploidy levels, although the germination rate was different among genotypes, being substantially inhibited at EC_{iw} 5 dS m^{-1}). Germination of two buffalograss clones was invariably better than Kentucky bluegrass cv. SD Common, with the upper limit for germination being at 2.8% NaCl ($EC_{iw} > 40$ dS m^{-1}) [51].

Carpetgrass (*Axonopus* Beauv.)

Two species of carpetgrasses have been used to a limited extent for turfgrass: common carpetgrass (*A. affinis* Chase) and tropical carpetgrass (*A. Compressus* [Swartz.] Beauv.) [39]. Carpetgrass has been classified as having poor salt tolerance [39]. A germination test revealed a common carpetgrass accession to be more sensitive to salinity than bermudagrass cv. Common but less sensitive than bahiagrasses cv. Argentine and Pensacola [64].

Centipedegrass (*Eremochloa ophiuroides* [Munro] Hack.)

Centipedegrass is invariably classified as having poor salt tolerance, tolerating $EC_e < 4$ dS m^{-1} [10,13,38,39], or <3 dS m^{-1} [8]. Centipedegrass has been the least salt-tolerant warm-season turfgrass in several studies. Centipedegrass cv. Common and bahiagrass cv. Argentine were equivalent in salinity tolerance, with a 50% reduction of shoot growth occurring at 9 dS m^{-1} [18,22]. St. Augustinegrass, bermudagrass, seashore paspalum, and zoysiagrass were more salt tolerant. In another study, centipedegrass cv. Common was least tolerant among warm-season turfgrasses, with a 50% shoot yield reduction occurring at EC_{iw} 6 dS m^{-1} [19].

St. Augustinegrass (*Stenotaphrum secundatum* [Walt.] Kuntze)

St. Augustinegrass has been ranked as being tolerant to salinity, tolerating EC_e 8–16 dS m^{-1} [10,13,38,39] or >10 dS m^{-1} [8]. A Hawaiian selection [19] and St. Augustinegrasses cv. Floratine [70] were reported to be equally salt tolerant to seashore paspalum and more tolerant than several bermudagrasses, Japanese lawngrass cv. Korean Common, and centipedegrass in solution culture. A 50% shoot dry weight reduction occurred at 40 dS m^{-1} for the Hawaiian selection [19]. St. Augustinegrasses cv. Seville and Floratam were more tolerant than Tifway and Tifway II in solution culture, with a 50% shoot dry weight occurring at 30, 19, 12, and 11 dS m^{-1}, respectively [21]. In contrast, Dudeck and Peacock [22] reported Floratam to be less salt tolerant than hybrid zoysiagrasses cv. Emerald, seashore paspalum cv. FSP-1, and bermudagrass cv. Tifway but more tolerant than centipedegrass and bahiagrass. A 50% shoot reduction occurred at 22 dS m^{-1} for Floratam. Meyer et al. [76] reported the shoot and root growth of St. Augustinegrass cv. Seville to be less affected by salinity than Floratam in solution culture containing up to 34 dS m^{-1}. Dudeck et al. [77] compared four St. Augustinegrass cultivars in solution culture. Seville was more salt tolerant than Floratine, Floratam, and Floralawn, with a 50% shoot dry weight reduction occurring at 28

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and 23 dS m^{-1}, respectively. In comparing studies for cultivar salinity tolerance, Seville was invariably found to be more tolerant than other cultivars in three studies.

Seashore Paspalum (*Paspalum vaginatum* Swartz)

Seashore paspalum has been ranked as the most salt-tolerant warm-season turfgrass, tolerating $EC_e > 16$ dS m^{-1} [13,38]. Seashore paspalum cv. Futurf has been reported to exist in soils with EC_e 40–45 dS m^{-1} [78], although it is more generally accepted that seashore paspalum can withstand up to EC_e 22 dS m^{-1} [79]. Major [80] reported both Fults weeping alkaligrass and Futurf seashore paspalum survived $EC_e > 50$ dS m^{-1} in greenhouse pot culture. Marcum and Murdoch [19] reported a Hawaiian selection of seashore paspalum to have a 50% shoot dry weight reduction at 40 dS m^{-1} in greenhouse solution culture, being equal in tolerance to St. Augustinegrass and Manilagrass but more tolerant than bermudagrass, Japanese lawngrass, and centipedegrass. In contrast, a seashore paspalum accession was reported to be less salt tolerant than bermudagrass cv. Suwannee in field plots [68]. Differences in salinity tolerance among seashore paspalum genotypes has been noted. Dudeck and Peacock [22] compared two Florida seashore paspalum genotypes (FSP-1 and FSP-3) to other warm-season turfgrasses in solution culture. The Emerald zoysiagrass hybrid was found to be the most tolerant, followed by FSP-3, then bermudagrass cv. Tifway and FSP-1, then bermudagrass cv. Tifway II and St. Augustinegrass cv. Floralawn, and finally centipedegrass cv. Common and bahiagrass cv. Argentine. Dudeck and Peacock [81] compared four seashore paspalum cultivars in solution culture. FSP-1 was the most tolerant, with a 50% shoot growth reduction at EC_e 28.6 dS m^{-1}, followed by Futurf and FSP-2, and finally Adalayd (EC_e 18.4 dS m^{-1}). Not enough studies have been done to make salt-tolerance comparisons among seashore paspalum cultivars possible.

Zoysiagrasses (*Zoysia* spp.)

Zoysiagrass used as turf grass consists of several species: Japanese lawngrass (*Zoysia japonica* Steud.), Manilagrass (*Zoysia matrella* [L.] Merr.), and Mascarenegrass (*Zoysia tenuifolia* Willd. ex Trin.), as well as the interspecific hybrid cultivar Emerald (*Z. japonica x Z. tenuifolia*). Other zoysiagrass species (*Z. sinica* Hamce, *Z. macrostachya* Franch. and Sav., *Z. koreana*) are either being considered for use as turfgrass or are being used as turfgrass in Asia [82]. General salt-tolerance rankings which compare turfgrass genera have traditionally not made this species distinction, although recent studies have revealed large differences among species. Zoysiagrass in general has been ranked as being salt tolerant compared with other turfgrasses, tolerating EC_e 8–16 dS m^{-1} [10,13,38,39] or 6–10 dS m^{-1} [8]. Japanese lawngrass cv. Meyer was reported to be equivalent in salt tolerance to bermudagrass cv. Arizona Common but more tolerant than buffalograss cv. Prairie and grama grasses [73]. Dudeck and Peacock [22] reported Emerald hybrid zoysiagrass to be more salt tolerant than any other warm-season turfgrass in the study, having a 50% shoot dry weight reduction at 37 dS m^{-1}. In decreasing order of salt tolerance: Emerald > FSP-3 seashore paspalum = Tifway bermudagrass > FSP-1 seashore paspalum > bermudagrass cv. Tifway II = Floralawn St. Augustinegrass > Common centipedegrass = Argentine bahiagrass. FC13521 Manilagrass was more salt tolerant than Korean Common Japanese lawngrass in solution culture, with 50% shoot dry weight reductions at 40 and 12 dS m^{-1}, respectively [19]. An accession of *Z. koreana* was reported to be the most salt tolerant, followed by an accession of *Z. sinica* and *Z. matrella*, and finally an accession of *Z. japonica* [83]. Fifty-nine zoysiagrass genotypes were compared for salt tolerance in solution culture [84]. Zoysiagrass species decreased in salt tolerance in the order *tenuifolia, matrella, japonica x tenuifolia, sinica, macrostachya*, and *japonica*. Diamond Manilagrass was the most salt tolerant among 17 cultivars tested, being superior to EI Toro, Belair, Meyer, Korean Common Japanese lawngrass, and Emerald zoysiagrass hybrid.

Alternative C_4 Grasses

Several warm-season grasses having turf-type characteristics are known for their exceptional tolerance to salinity or drought stress. These are saltgrass (*Distichlis spicata* var. *stricta* [Torr.] Beetle;

D. spicata var. *spicata* [L.] Greene), *Sporobolus virginicus* (L.) Kunth, curly mesquite (*Hilaria belangeri* [Steud.] Nash), and gramagrasses (Bouteloua spp. Lag.) [9,15,32,85]. Although generally thought to be too coarse in texture for use as turfgrass, these grasses can be mowed and are currently used as ground covers in marginal areas.

The USDA Salinity Laboratory [17] ranked salt-tolerant (tolerating EC_e 12–18 dS m^{-1}) grasses in order of decreasing salt tolerance: saltgrass, Nuttall alkaligrass, and bermudagrass. In a solution culture experiment [73], saltgrass was more salt tolerant than Common bermudagrass,

TABLE 1 Estimated Relative Salinity Tolerance of Turfgrasses

C_3 (cool season) turfgrasses	Salinity tolerance[a] (dS m^{-1})	C_4 (warm season) turfgrasses
	40+	Saltgrass
		Sporobolus virginicus
Nuttall alkaligrass	30	Seashore paspalum
Weeping alkaligrass		Mascarenegrass
Fults		Manilagrass
Lemmon alkaligrass		Diamond
	22	St. Augustinegrass
		Seville
	18	Hybrid zoysiagrass
		Emerald
		Bermudagrass
		Tifway
	15	Japanese lawngrass
		El Toro, Palisades
Creeping bentgrass	12	
Seaside, Mariner		
Tall fescue		
Alta, K-31		
Creeping red fescue	10	
Dawson, 'Oasis' (slender),		
Ruby (strong)		
Perennial ryegrass	8	Buffalograss
Manhattan		Gramagrasses
Redtop		
Rough bluegrass	4	Centipedegrass
Kentucky bluegrass		Carpetgrass
Nugget		
Chewings fescue		
Hard fescue		
Sheep fescue		
Meadow fescue		
Annual ryegrass		
Annual bluegrass	3	Bahiagrass
Colonial bentgrass		
Velvet bentgrass		

[a] Relative salinity tolerance is an estimate (based on literature) of the salinity (EC_e in dS m^{-1}) at which the grass can acceptably grow or the point at which shoot growth is reduced by approximately 50%.

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Meyer zoysiagrass, sideoats and black gramagrasses, and Prairie buffalograss. Saltgrass continued to grow in solutions containing 50 dS m^{-1}, equivalent to full-strength seawater. Saltgrass was found to be more salt tolerant than seashore paspalum or bermudagrass in field plot experiments [68]. The shoot growth of *Sporobolus virginicus* was stimulated under moderate salinity, peaking at 15 dS m^{-1}. The shoot growth continued at a lower rate even at 45 dS m^{-1}, whereas root growth increased, relative to control, to the highest salinity level (45 dS m^{-1}) [86]. *Sporobolus virginicus* continued to grow without injury after 4 months' exposure to full-strength seawater (45 dS m^{-1}) [87]. Kinbacher et al. [47] reported blue gramagrass to be salt sensitive, being equivalent in tolerance to Kentucky bluegrass. Research has shown that saltgrass, alkali sacaton, and *Sporobolus virginicus* continue to grow well in full-strength seawater (>45 dS m^{-1}), and, thus they can be considered to be true halophytes.

OVERALL SALT-TOLERANCE RANKING OF TURFGRASSES

There is considerable difficulty in precisely ranking the salinity tolerance of turfgrasses because of factors discussed at the beginning of this Chapter. These include the different methods of quantifying the relative salt tolerance used in the reviewed studies (i.e., relative shoot dry weight reduction, percentage of leaf burning, root growth changes, germination) and the difference in environmental conditions under which studies were done, such as temperature, light, and soil differences (or lack of soil, as in solution culture experiments), all of which are known to interact with relative salinity tolerance measurements. Also the units used to quantify salinity levels differed among studies. Hence, there was a good deal of variability in the results among studies, often resulting in contradictory cultivar rankings within species, but sometimes even resulting in contradictory species rankings. Even more affected by these factors are estimates of actual salinity tolerance levels in terms of salinity (dS m^{-1}). I have attempted to summarize the current literature concerning turfgrass salt tolerance into a table (Table 1) ranking turfgrass species relative to one another. If information regarding cultivar differences is available, salt-tolerant cultivars are listed immediately below each turfgrass.

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Stress in Wildland Plants: Implications for Ecosystem Structure and Function

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INTRODUCTION

Humans have been interested in factors that constrain the distribution of plants at least since Aristotle considered this concept over 23 centuries ago. This early interest has been sustained to the present, and it is reflected in contemporary definitions of ecology (e.g., see Ref. 1): the analysis of interactions that determine distribution and abundance of organisms. Further, physiological ecology and response to stress have been the central foci of ecological investigations since the inception of ecology as a formal discipline (e.g., see Refs. 2 and 3 and citations therein). Nonetheless, a significant gap divides physiological ecology from population ecology. Although physiological ecology provides a solid foundation for the post hoc interpretation of ecological patterns, a mechanistic link between physiological ecology and population ecology has yet to be fully explored. The inability to integrate physiological ecology and population ecology belies extensive literature in both disciplines.

Grime [4] defined stress as, ''the external constraints which limit the rate of dry-matter production of all or part of the vegetation.'' This definition was refined by Welden and Slauson [5] to exclude biotic interactions: ''abiotic stress is an external condition, apart from the activities of other organisms, that induces strain in an organism.'' We adopt this more precise definition in order to exclude interference. Inter- and intraspecific competition are important processes that constrain the structure and function of vegetation systems [6]; however, they are beyond the scope of this chapter.

Whereas the term *stress* refers to an external condition or process acting on a plant, *strain* refers to the internal physiochemical changes within that plant in response to a stress. Thus, strains mediate the response of plants to stresses. Strain may be assessed in several ways (e.g., by measuring

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growth rate, photosynthetic rate, or tissue water potential). Although strains refer to suboptimal physiological states (relative to a sometimes only theoretical optimal state), they are not always expressed at the whole plant level, and they may not reduce fitness or affect population dynamics [5].

Stresses on wildland plants considered in this chapter include water, temperature, nutrient stress, defoliation, and climatic variation. Although defoliation and climatic variation are often considered as disturbances, or ''mechanisms which limit the plant biomass by causing its partial or total destruction'' [4], we discuss them herein as stresses important to the structure and function of wildland plant systems.

Stress indirectly affects the structure and function of all naturally occurring communities and ecosystems. Adaptation to various kinds and intensities of stress (e.g., drought, frost, defoliation) is requisite to the survival of wildland plants. Understanding how wildland plants respond to stress provides important insights into patterns and processes in communities and ecosystems. Nonetheless, stress per se does not exist at levels of organization higher than the individual plant. Communities and ecosystems are not organismal entities but rather comprise variously interacting species that often cease to coexist when the environment changes (e.g., see Refs. 7–9). The responses of communities and ecosystems to the environment are most appropriately viewed as a product of the responses of individual organisms to their environment. As such, the terms *health, integrity*, and *degradation* are inappropriate descriptors of ecosystems [10–12] and will not be used herein.

This chapter provides an overview of the importance of stress in wildland ecosystems. We review the major sources of stress in these systems and discuss the relevance of stress to ecosystem structure and function. Finally, we review recent attempts to link physiological response to ecosystem processes.

INTEGRATION OF STRESS RESPONSES: THE PLANT AS A BALANCED SYSTEM

The mechanistic, or bottom-up, approach in ecology represents one important strategy for understanding current vegetation patterns and predicting vegetation responses to changing conditions. Within this context, responses of populations, communities, and ecosystems to environmental stress are best understood at the level of the whole plant. By impairing the function of individual plants, stress has important implications for groups of plants (e.g., populations, communities). Different species, and even individuals within a species, display different levels of tolerance to various stresses. These differences form the basis for competitive exclusion or coexistence of individuals and species in environments where conditions are sometimes limiting to plant growth. Growth and survival of an individual plant may be constrained by any of several stress factors, and the existence or importance of these factors may depend on the abundance of neighboring plants. These factors operate independently or in combination, and they frequently interact with one another, so that stress ultimately constrains the distribution and abundance of plant individuals. As such, these constraints on distribution and abundance of plants exert primary control over ecosystem structure and, sometimes, ecosystem function. Stress responses of individual plants, therefore, play a central role in our understanding of the dynamics of natural ecosystems.

A plant functions as a balanced system with respect to resource acquisition and use. If natural selection has molded plant function to maximize growth or fitness under stressful conditions, then plants should allocate internal resources (C, N, P) in such a way that source and sink activities are kept in equilibrium. Allocation to different resource-acquiring structures in response to stress, therefore, should occur so that growth is equally limited by all resources [13] and internal resource pool sizes remain constant [14]. In economic terms, investment in a particular function (e.g., soil nutrient uptake, photosynthetic capacity, defense) should cease if the return in terms of growth or fitness is lower than the investment or lower than returns from alternative investments [13,15]. For example, under light limitation, plants will invest proportionately greater amounts of nitrogen to light-harvesting chlorophyll and less to the enzyme Rubisco such that photosynthesis per unit nitrogen allocated to leaves is maximized. Magnitudes of stress response, therefore, may be interpreted as the degree to which the balance of plant functions and internal resource limitations deviate from the optimum

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achievable by a particular genotype or species. This balancing act should be directly related to the impact of stress on growth or fitness. Consequently, across habitats differing in resource availability or stress conditions, populations frequently evolve genetically fixed allocation and growth responses that match the overall limitations of the environment [16].

The concept of optimal allocation or functional equilibrium has fostered numerous theories about the mechanistic basis for community and ecosystem dynamics [17–19] and provides a framework for managing ecosystem functions [19] and for elucidating scaleable ecosystem processes [20]. It is not clear, however, at what temporal or spatial scale one should interpret allocation shifts or physiological acclimation to stressful conditions. Plants can respond almost instantaneously to fluctuations in resource supply or to conditions which reduce the efficiency of particular organs. Stomatal conductance and leaf photosynthetic capacity respond rapidly to changes in light levels [21] or humidity [22], but allocation shifts at the level of the whole plant occur over longer periods. Plants that differ in life span or growth rate employ different strategies for balancing resource demands of different functions and have different patterns of allocation in response to stress or changes in resource supply. Short-lived herbaceous plants or plants from resource-rich environments tend to shed and redeploy new resource-acquiring organs (leaves, roots) in response to changes in the resource supply. Long-lived perennial plants or plants adapted to resource-poor environments often rely on physiological acclimation of existing organs rather than redeployment [16]. In both instances, it is assumed that the return is maximized from allocation investments (redeployment or acclimation) and that physiological and fitness trade-offs or costs accompany the different strategies.

Current patterns of allocation in response to stress, however, may have evolved under selective pressures no longer present within a plant's environment. Consequently, not all responses may be the optimum achievable for keeping the plant as a balanced system. The evolution of plastic responses to stress (as opposed to genetically fixed responses among populations or species) may depend on the spatial and temporal dynamics of the stress as well as other life history or genetic constraints [23–25]. Constraints on the adaptive response to stress, in fact, may determine the rate or pattern of vegetation responses to global change [26,27].

The appropriate resource for evaluating the cost and return from allocation in response to stress is assumed to be carbon or mass, but other plant nutrients (N and P) can be allocated in a manner independent of mass [28]. Furthermore, the cost of acquiring and processing these nutrients, in terms of grams of glucose [29], may be difficult to assess [30–32]. Even the level at which one assesses the cost of growth or reproduction may have a bearing on how we interpret optimal allocation responses. Physiologically based measures of cost are often preferred over measures based on fitness because of the difficulty in estimating the contribution of reproduction to future generations in long-lived plants.

Because allocation to different functions involves trade-offs between costs and returns, the response of plants to stress and the role of these responses at population, community, and ecosystem levels depend on how plants have evolved to manage functional trade-offs within a given environment. The role of plant life forms, adaptive strategies, and functional types in community and ecosystem processes is determined by these allocation trade-offs at the whole plant level. Here we review examples of plant functional trade-offs associated with temperature, water, nutrient, and defoliation stress in wildlands and describe how these trade-offs influence processes and dynamics at the ecosystem level.

Temperature Stress

Temperature affects many aspects of plant growth and development and has a primary influence on the distribution of plant species [33–35]. Plants in natural environments frequently experience temperatures or conditions that cause tissue temperature to be suboptimum for growth or photosynthesis [36]. All tissues and life stages of the plant can be affected by temperature. Temperature stress affects processes at different organizational levels, including gene expression [37], enzyme function [38], membrane integrity and function [39], cell division [40,41], photosynthesis [42,43], respiration [44–46], phloem translocation [47], root and shoot allocation [48], and reproductive

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development [43,48,49]. It is beyond the scope of this chapter to address in any detail the physiological responses of wild plants to temperature or any other single environmental condition at all of these organizational levels. Comprehensive reviews of temperature effects on wild plants are provided by Berry and Bjorkman [50], Berry and Raison [36], and Long and Woodward [51].

In general, temperature stress can affect the efficiency of resource capture and use by plants and may be an important selective pressure shaping patterns of allocation to different plant parts or functions. Adaptation to temperature stress can involve modifications in the energy balance characteristics of leaves and canopies to maintain favorable tissue temperatures or physiological stability across the range or extremes in temperature conditions. C_4 photosynthesis represents a major biochemical and anatomical adaptation that enhances photosynthetic efficiency at high temperature [52] and has ramifications for species and vegetation distribution along altitudinal and latitudinal gradients [53,54]. Many studies have observed photosynthetic acclimation or stability to changing temperature conditions [43,55], as well as ecotypic variation for photosynthetic function across habitats that differ in growing season temperature [56]. Unfortunately, few studies have addressed the physiological or fitness costs associated with photosynthetic acclimation or tolerance to broad ranges of temperature.

Modifications in leaf orientation or morphology (e.g., wilting under high heat loads or vertically oriented leaves) that decouple tissue and ambient temperatures can have positive physiological and/or fitness consequences [57–59]. The production of reflective hairs or waxes on leaf surfaces may serve a similar function [60,61], but like fixed patterns of leaf orientation, the development of a reflective epidermis can have physiological and demographical costs. Allocation to reflective leaf hairs in the desert shrub *Encelia farinosa* increases through the growing season and differs among populations along an aridity gradient in the arid southwestern United States. Reflective leaf hairs are costly in terms of the energy required for their production and they reduce the amount of radiation absorbed for photosynthesis [60]. It is not surprising that when water is available to *Encelia* plants, either in spring or as an intrinsic part of the habitat, transpirational cooling rather than radiation reflectance becomes the dominant mechanism by which plants decouple leaf and air temperature [60,62]. Although it is widely known that sensible versus latent heat loss varies across aridity gradients, the implications of plant level trade-offs between reflectance and transpiration for ecosystem level evapotranspiration, energy exchange, and productivity seem apparent but have not been fully explored.

The ability of plants to meet their nutrient and water demands for photosynthesis, growth, and reproduction is linked to the root responses to soil temperature [63,64]. Water and nutrient acquisition and transport from the roots to shoots can be impacted by temperature in several ways. First, the roots themselves are sensitive to temperature. Across the natural range of most plant species or over the course of the season, soil temperature varies greatly. Unfortunately, few studies have addressed the impact of these temperature variations on root function in wild plants. The roots of plants from warm climates, however, express a greater tolerance to high temperatures than do the roots of plants from cooler environments [36]. Root growth, demography, and respiration are apparently strongly affected by the soil temperature under natural conditions [63,65] and may determine patterns of belowground resource uptake in many situations. High surface soil temperature may be one factor contributing to the inability of certain shrub and tree species to utilize summer rains in southern Utah [66,67] potentially limiting the productivity and distribution of temperature-sensitive species.

Low soil temperature in the spring may limit root growth and contribute to water stress and low water transport efficiency in forest trees even when the soil is relatively moist [68]. Additionally, the hydraulic conductivity of the xylem is influenced by frost and by temperature-dependent variations in the viscosity of liquid water [69,70]. Susceptibility to frost cavitation increases with the vessel size [71,72] and constrains seasonal leaf phenology in a number of species [73]. Because vessel size is inversely related to conducting efficiency, a trade-off between hydraulic conducting capacity of stems and susceptibility to damage by frost is unavoidable. Susceptibility to frost cavitation is an important yet relatively unexplored mechanism that may limit species function and distribution near the margin of their range [72,74,75].

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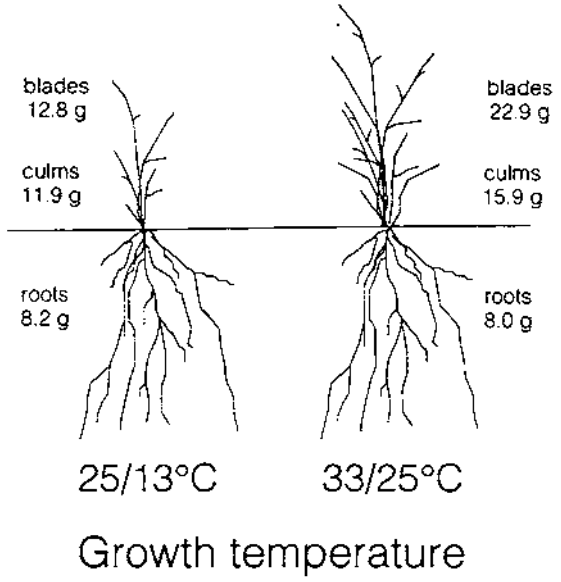

FIGURE 1 Biomass of root, culm, and leaf blades of the invasive C_4 grass *Pennisetum setaceum* (Fountain grass) grown for 56 days at high and low temperatures in a growth chamber environment. (Redrawn from Ref. 43.)

Biomass accumulation in above- and belowground organs is strongly affected by temperature. In general, ecotypes of plant species from colder climates allocate proportionally more mass to roots than to shoots compared with ecotypes from warmer regions. This allocation response may have evolved to offset the demand for limiting soil nutrients in colder climates [76–79]. Plasticity also is observed when plants are grown experimentally at different temperatures. Williams and Black [43] found that the C_4 grass *Pennisetum setaceum* maintained proportionally greater mass in roots than in shoots when grown at 25/13°C (day/night) temperature conditions than when grown at 33/25°C (Fig. 1). Apparently, shoot growth was inhibited exclusively by different temperature conditions for this warm-season grass. Greater dry mass accumulation in roots for plants grown in colder temperature conditions may be due to slower fine root turnover and death [80] coupled with a greater impact of the low temperature on shoot expansion and growth [81]. However, patterns observed for plant biomass allocation responses to experimental temperature stress at the population level are not seen in broad comparisons at the community level across natural gradients [81]. High belowground dry matter accumulation, therefore, may not be an essential trait for survival in cold climates.

Water Stress

Water stress is a dominant feature of most wildland ecosystems, and the importance of water balance on community structure is widely recognized (e.g., see Refs. 82–86). Nearly all wildland areas are characterized by periodic drought, and plants possess an impressive array of adaptations for surviving these periods.

Water loss from leaves to the atmosphere is an unavoidable consequence of CO_2 exchange in terrestrial plants. Consequently, water is often the single most limiting factor to plant growth and ecosystem productivity in arid and semiarid regions [87] and has a primary influence on the distribution of species and vegetation. Plants have evolved a variety of morphological and physiological features that allow them to complete their life cycles and persist in water-limited environments.

Reviews on plant physiological responses to drought stress from different perspectives are provided by Turner [88], Schulze [22], Tyree and Sperry [69], and Passioura [89].

Water stress has been cited as the ultimate constraint on plant distribution. For example, Merriam [90] invoked water stress as the primary factor influencing the distribution of dominant plants in the mountains of the southwestern United States. Merriam's descriptions of life-zones along altitudinal gradients have been supported by contemporary research bolstered by sophisticated analytical tools unavailable to Merriam (e.g., see Refs. 91 and 92). This extensive body of work on plant water relations indicates that species respond differently to water stress, and this differential response explains why plants are ''sorted'' along elevational gradients.

At spatial scales of a few meters or smaller, water stress may be mediated by the presence of neighboring plants. These neighbors may exacerbate water stress by interfering with water uptake or they may alleviate water stress by ameliorating the microclimate. Negative effects on plant survival have been attributed to interference in most wildland ecosystems (e.g., see Ref. 6 and references therein). The presumed mechanism for this interaction is that one individual preempts the use of the resource (water) more quickly or more efficiently than other individuals. Positive effects (facilitation) have been attributed to amelioration of the microclimate in arid and semiarid sites (e.g., see Refs. 93–95) and hydraulic lift (nocturnal transport of soil water from deep to shallow layers) [96]. Alternatively (and possibly concomitantly), shade provided by the canopy of one individual reduces the evaporative demand and, hence, water stress on individuals beneath the canopy.

Much work has been conducted on the cellular and molecular components of drought response (e.g., osmotic adjustment, compatible solutes, membrane structure and function), but physiological ecologists have tended to focus on the patterns and processes of water stress at the leaf, root, and whole-plant levels, particularly focusing on the impacts on plant gas exchange and growth. Adaptations to water stress at the whole-plant level involve trade-offs among different organs and functions of the plant. At the leaf level, minimizing transpirational water loss by stomatal closure under drought conditions reduces net CO_2 uptake but increases instantaneous water-use efficiency [97] while allowing the plant to avoid low shoot water potentials. Debate continues, however, on the exact control mechanism for stomatal closure—root abscisic acid (ABA) signals or feedforward or feedback responses at the leaf [98–101]—and what measure of humidity is sensed by plants in drying air [102–104]. Because both soil and atmospheric drought influence transpiration rates and stomata in similar ways, it is difficult to distinguish their effects at the whole-plant level, especially along complex climatic gradients typical of natural vegetation. Despite these unresolved issues, it is clear that plants have evolved numerous responses to water stress that maximize fitness in arid and semiarid environments.

Rooting profiles of plants illustrate obvious trade-offs between different solutions to coping with limited water in dry environments. Deeply rooted perennial species such as mesquite (*Prosopis* spp.) overcome periods of little or no rainfall during the growing season by using groundwater or water stored deep in the soil from prior rains. Construction and maintenance of an extensive root system, however, is energetically costly. Alternatively, shallow-rooted perennial plants are very effective at capturing moisture from growing season precipitation (e.g., summer monsoonal rains), but they can potentially experience wide fluctuations in soil moisture availability that can limit gas exchange to periods when rainfall is high. Consequently, seasonal patterns of water use, drought stress, and productivity vary widely between deep- and shallow-rooted plants even within the same habitat [105]. Diverse rooting profiles may limit competitive interactions among perennial plants in arid and semiarid ecosystems and may be one explanation for species coexistence [82,106,107].

Generalizations about soil resource partitioning and coexistence of different plant life forms should incorporate explicit consideration of plant life history. For example, mature *Quercus emoryi* Torr. (Emory oak) trees and perennial bunchgrasses within temperate savannas of the southwestern United States obtain water from relatively deep and shallow depths in the soil profile, respectively (Fig. 2) [108]. Such soil moisture resource partitioning may facilitate the coexistence of these life forms. However, grasses and 1- and 2-year-old *Q. emoryi* seedlings obtained water from similar depths in the soil profile, which suggests that soil moisture partitioning between *Q. emoryi* and

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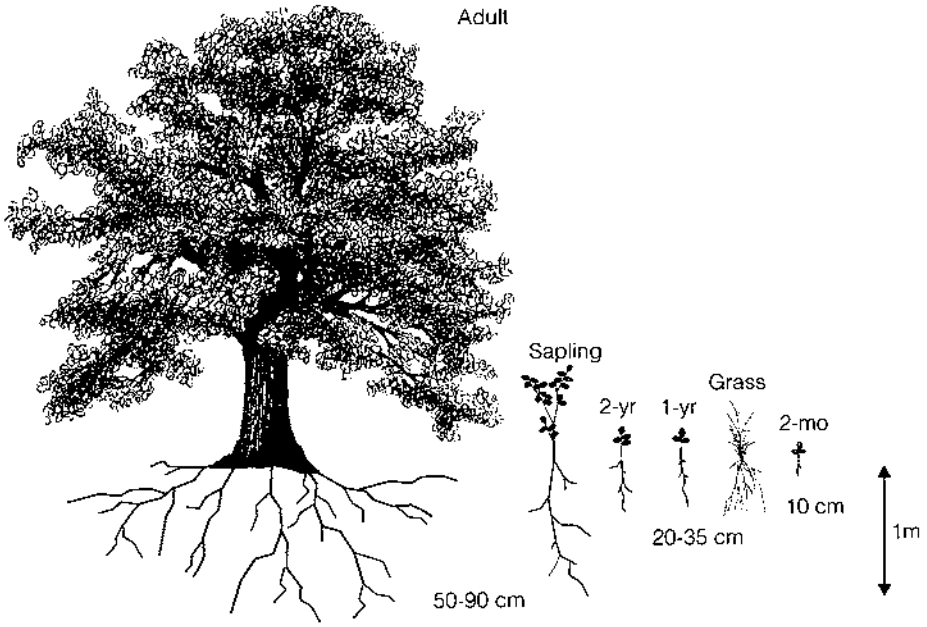

FIGURE 2 Acquisition of soil moisture from different depths in the soil profile for *Q. emoryi* (Emory oak) at different phenological stages, and coexisting grasses, in a temperate savanna of the southwestern United States [108]. Both life forms, and all phenological stages of *Q. emoryi*, utilize soil moisture derived from summer precipitation in September. Adult and sapling *Q. emoryi*, trees obtain soil moisture from 50–90 cm, 2-year-old and 1-year-old *Q. emoryi* and coexisting bunchgrasses access soil depths of 20–35 cm and 2-month-old *Q. emoryi* utilize water from the top 10 cm of the soil profile.

coexisting grasses does not occur for at least 2 years after seedling germination. Further, very young tree seedlings (about 2 months old) use water from shallower depths in the soil profile than grasses, which may facilitate germination and early establishment of *Q. emoryi*. Thus, soil resource partitioning occurs at some, but not all, developmental stages of woody plant development, with potential implications for woody plant population dynamics.

The hydraulic architecture of plants plays a critical role in determining species response to drought conditions regardless of where in the soil profile plants may be taking up water. Water flow from roots to leaves conforms to Darcy's law where the volume flow rate is a function of the plant hydraulic conductance (inverse of resistance) and the pressure drop from the root to the leaf resulting from evaporation at leaf surfaces. Conductance to flow along the soil-plant-atmosphere continuum has been the subject of much investigation, particularly in light of how plants may have evolved to optimize water usage for maximum production or fitness in arid regions. Hydraulic conductivity of the soil and root-soil contact are potentially important in limiting water flux to roots in drying soil [109,110]. Hydraulic conductivity of the root-to-leaf pathway represents an additional constraint on transpiration and is impacted by water stress in several ways. The xylem can be impaired by air embolisms that cause cavitation during periods of drought or high transpiration. The xylem water potential necessary to induce this cavitation varies widely among plants [111] and has been shown to correlate with the lowest xylem water potentials normally experienced under natural conditions [71]. Plants tend to control stomata such that the xylem water potential does not fall below cavitation-inducing pressures [112,113]. As soil moisture or humidity declines, either transpiration is reduced

or leaf-specific hydraulic conductivity is increased. In this way, plants balance the demand for transpirational water loss and carbon uptake by leaves with allocation to root absorption or stem-conducting tissue [68,114]. There is only a modest negative relationship or trade-off between the hydraulic conductivity and the susceptibility to drought cavitation for the wildland species that have been examined to date [115]. This may be because susceptibility to cavitation is more a function of vessel and tracheid pit anatomy than conduit size [116].

Preferential allocation to roots is a common response to water-stress conditions in wild plants [32,117]. Theoretically, plants should allocate energy to the growth and maintenance of the roots in a patch of moist soil if the physiological cost of this allocation is lower than the energy gained via photosynthesis from uptake of that moisture [13]. Unfortunately, there are very few data to validate this hypothesis for wildland species. Nobel et al. [63,118] found that the roots of succulent species in the Sonoran Desert have very low combined construction and maintenance costs. These succulents employ the crassulacean acid metabolism (CAM) photosynthetic pathway and are known to have relatively high water-use efficiency. The combination of high water-use efficiency and low root growth and maintenance costs likely contributes to the success of CAM succulents in very arid environments (root efficiency is maximized). Rapid, but low-efficiency, exploitation of water by roots may be favored under some circumstances such as intensely competitive environments or when belowground water resources are not limiting [64].

In addition to the impacts at the cellular, leaf, or whole-plant level, water stress may have important direct effects on ecosystem processes. For example, water stress at the leaf level may influence the stand level or regional transpiration fluxes. Of importance is the degree to which stomatal behavior controls transpiration from whole trees and stands [119] and the influence of stand structure and composition on canopy level transpiration [120]. Stomatal behavior and the development of a boundary layer may differ among patches of vegetation dominated by different species [121], thereby influencing the rates or patterns of transpiration and energy exchange on larger scales [99,119].

Nutrient Stress

Soil nutrient stress is a common feature in most wildland ecosystems [122]. Competitive interactions among plants, plant distribution at local and regional scales, and ecosystem productivity are strongly influenced by soil nutrient availability. Even in arid and semiarid regions where water imposes the primary constraint on plant growth and productivity, nutrient limitation can be a dominant feature of the environment experienced by plants. Numerous studies have shown greatest growth enhancement when soil mineral nutrients (particularly nitrogen) and water are experimentally added together in arid and semiarid ecosystems [123–125].

Soil-derived nutrients are allocated internally in a manner consistent with resource optimization theory. The plant internal nitrogen and carbon pools regulate efficient allocation under changing environmental conditions and resource demands by the plant [32]. Experimental additions of soil nutrients tend to shift allocation of biomass away from roots towards the shoots bringing internal carbon and nutrient reserves to a balance that is most favorable for maximum growth [13]. Plants adapted to nutrient-poor conditions tend to have inherently low relative growth and leaf turnover rates, use the limiting nutrient very efficiently by reabsorption from senescing tissues, and respond only modestly to experimental nutrient amendments [17,122,126]. Plants from nutrient-rich sites, in contrast, often have high relative growth rates and leaf turnover rates, and they are not effective at remobilizing nutrients to new growth or recovering nutrients from senescing tissues, but respond greatly in terms of growth to nutrient amendment. In general, evergreen perennials and sclerophyllous shrubs tend to dominate nutrient-poor sites, and deciduous or herbaceous plants tend to dominate nutrient-rich sites [17,122].

The degree to which preferential allocation occurs to above- or belowground plant components may depend on plant growth form. For example, evergreen Emory oak seedlings (*Quercus emoryi*)

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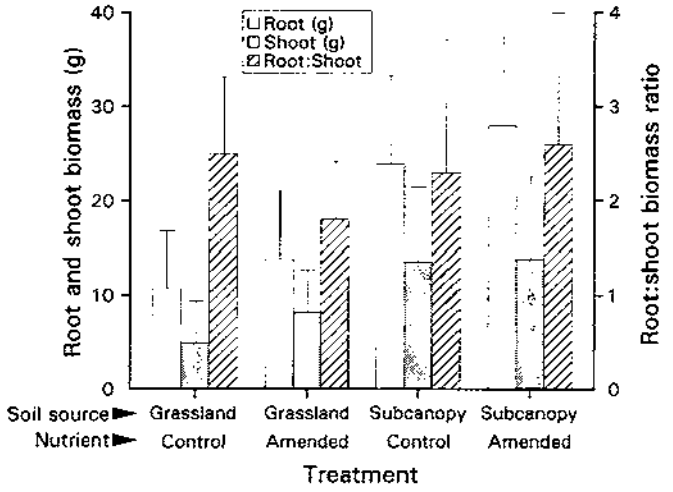

FIGURE 3 Root and shoot biomass (g) and root:shoot biomass ratios for 10-month-old *Q. emoryi* (Emory oak) seedlings grown in a greenhouse in Tucson, Arizona (J. F. Weltzin et al. unpublished data). Seedlings were grown within 1-m columns filled with soil collected from the subcanopy of *Q. emoryi* and in adjacent treeless grassland; soils received either a full nutrient amendment (equivalent to 12.0, 10.6, and 10.0 g m^{-2} yr^{-1} N, P, and K, respectively) or no nutrient amendment (control) ($n = 5$). Nutrient and nutrient $\times$ soil source interactions were not significant ($P > .23$) for any parameter. Vertical lines represent 1 standard deviation.

characteristic of southwestern savannas were grown in soils collected from beneath mature conspecifics and from adjacent treeless grassland [127]. Seedlings in each soil received either a full nutrient amendment or no nutrient amendment. Contrary to expectations, seedlings in the amended grassland soils (that had relatively low nutrient contents) did not exhibit preferential allocation to shoots (Fig. 3). Similarly, *Q. emoryi* grown from acorns in the field along a gradient of annual water inputs from 359 mm yr^{-1} to 846 mm yr^{-1} exhibited no reallocation response to watering treatments [128]. These results contrast with the observations of the growth response of deciduous *Q. douglasii* seedlings to resource manipulations in California (e.g., see Refs. 129 and 130). However, intrinsically low potential growth rates of evergreen plants such as *Q. emoryi* may constrain their ability to respond to resource additions [17,126]. In addition, resources other than those experimentally added may limit plant growth [131]. Further, biomass allocation in drought-adapted species such as *Q. emoryi* may be relatively insensitive to variations in soil resource availability, particularly soil moisture [132,133], or may be ontogenetically constrained [134].

The dynamics of vegetation are strongly influenced by soil nutrient conditions. Early ideas on primary succession implied a linkage to soil organic matter and nutrient accumulation fostered by colonizing plants [135]. The more subtle spatial and temporal dynamics of vegetation also are clearly linked to the effect that plants have on soil nutrient dynamics. Species adapted to conditions of low soil nutrient supply produce leaf litter that is low in nitrogen concentration and chemically protected from herbivores. These traits tend to reduce rates of decomposition and mineralization in the soil and reinforce low levels of nutrient supply to plants [126]. Dominant plants need only affect the mineralization dynamics of a small fraction of the total soil nutrient pool (labile nutrients found in surface soil) to have large impacts on overall ecosystem nutrient and vegetation dynamics. For example, Wedin and Tilman [136] found that Eurasian C_3 grasses produced

higher quality litter that released nitrogen at higher rates in soil than did C_4 perennial grasses in Minnesota old fields. These differences in litter quality were enough to alter soil nitrogen mineralization rates.

Although plants possessing the C_4 photosynthetic pathway require less nitrogen for photosynthetic metabolism on a per unit leaf basis than do C_3 plants, the ecological advantage in nutrient-poor soils is not always realized by C_4 plants. Sage and Pearcy [137] found that *Chenopodium album*, a C_3 herb, was more productive at low nitrogen supply rates than was the C_4 herb *Amaranthus retrofexus*. Fertilization experiments in Minnesota prairie ecosystems, however, caused alien C_3 grasses to become community dominants [138] in place of native C_4 perennial grasses. The outcome of competition and the community composition changes following disruption or changes in the nutrient supply, furthermore, may depend on other plant resources and their temporal or spatial distribution. For instance, Ehleringer et al. [139] predict that C_4 invasive species like *Salsola* will become dominant in native desert shrub ecosystems on the Colorado Plateau region in North America following disturbance of the nitrogen-fixing cryptobiotic soil crusts and increases in the intensity of the summer monsoon system predicted by global circulation models [140]. The soil crusts are responsible for up to 80% of the nitrogen inputs to these systems and are very sensitive to surface disturbance [141]. Summer season precipitation increases are important for the predicted outcomes, since C_4 species in this ecosystem maintain greater growth and photosynthetic rates in the hot summer season and utilize greater amounts of monsoon precipitation than do the native C_3 shrub species.

Defoliation Stress

All wildland plants experience defoliation (i.e., loss of aboveground tissue), and perennial plants usually experience repeated bouts of defoliation during their lives. Herbivores and fire are primary sources of defoliation of wildland plants. Similar to other sources of stress in plants, defoliation influences physiology, growth, and survival and ultimately may influence distribution of individual plants.

Considerable research has documented the responses of wildland plants to various seasons and intensities of defoliation, and this research has been summarized by several investigators (e.g., see Refs. 142–145). However, the physiological bases for predicting the plant response to defoliation are not known for most wildland species. Rather, most knowledge about defoliation has been derived from case studies of individual plants or plant assemblages. The vast literature on defoliation has been used to invoke many physiologically based hypotheses for plant responses; tests of these hypotheses are becoming more common in concert with technological advances.

Plant responses to defoliation are strongly dependent on the morphological and physiological features of plants. Relationships between these factors enable us cautiously to provide a few generalizations about the plant response to defoliation, as described below.

In general, the morphological trait that confers maximum resistance to defoliation involves the location of the primary growing points (e.g., meristems). Protection of these tissues by soil or plant tissue such as bark or dense leaves minimizes the risk of defoliation-induced mortality. Thus, rhizomatous grasses and woody angiosperms which are capable of resprouting from belowground tissues usually tolerate fires and high intensities of herbivory. In contrast, stoloniferous grasses (e.g., *Bouteloua eriopoda* [Torr.] Torr.) and woody plants with unprotected aboveground buds (e.g., most conifers) are susceptible to mortality induced by herbivory or fire. However, species with unprotected buds may possess characteristics that enable rapid recolonization of burned or grazed areas (e.g., serotinous fruits, recalcitrant seeds), which indicates that no single trait can reliably predict the plant response to defoliation.

The response to defoliation is closely related to the availability of water. In fact, plants may be particularly susceptible to defoliation-induced mortality during periods of limited water availability regardless of the plant phenology or the intensity of defoliation [146]. Both above- and belowground growth are reduced by defoliation [147–151] and reductions are especially pronounced when soil

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moisture is limited [146], presumably because reductions in the root growth constrain water uptake from the soil. Reductions in the root growth also may affect nutrient uptake.

Plant phenology influences the response to defoliation. For example, herbaceous plants are particularly susceptible to defoliation during the period of late vegetative and early floral growth [148,152,153]. Seedlings of the arborescent legume *Prosopis glandulosa* Torr. (honey mesquite) become increasingly intolerant of repetitive top removal with increasing age at initial defoliation (Fig. 4). Retention of functional cotyledons that otherwise abscise within about 20–40 days of shoot emergence may enable seedlings to tolerate defoliation early in their life cycle [154] (cf. Refs. 155 and 156). Similarly, delayed senescence and stimulated photosynthesis of leaves remaining on partially defoliated plants have been reported for various plant growth forms [157]. Plant functions are relatively unaffected by defoliation during the period after seed formation and before initiation of spring growth [146,158,159], presumably because plant physiology is largely restricted to maintenance activities rather than growth or reproduction.

Characteristics that influence the plant response to defoliation, depending on species and sites, include canopy architecture, rooting habit, carbohydrate status, growth form, and inherent growth rate among others [160,161]. For example, large bunchgrass plants with fine leaves tend to be more susceptible to fire than small, coarse-leaved plants, because abundant litter which accumulates in the crowns of the former plants burns longer and more completely than the litter in the latter plants; the associated heat load on meristematic tissue apparently causes high mortality [162].

Fires and herbivores may produce dissimilar effects on plants. Fires usually remove more biomass than herbivores during a specific temporal period, which produces several distinct local differences between these two types of defoliation. Unlike herbivores, fires increase solar insolation at the soil surface, increase soil temperature, and volatilize more nutrients. These differences in the physical environment are responsible for many of the differences in the biological response between fire and herbivory.

There also is considerable variability in the plant response within these broad categories of defoliation. For example, defoliation by invertebrates may be quite dissimilar to defoliation by vertebrates; similar caveats pertain to generalist herbivores versus specialist herbivores and different intensities of fire among other factors. In addition to the variability associated with differences

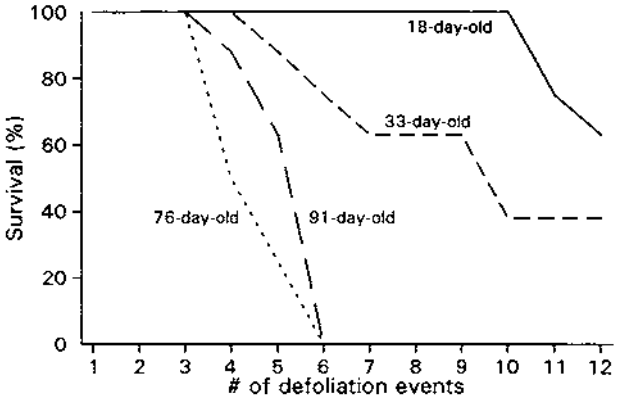

FIGURE 4 Survival of *Prosopis glandulosa* (honey mesquite) initially defoliated above the cotyledonary node at 18, 33, 76, and 91 days after shoot emergence and defoliated thereafter at 2-week intervals (n = 8). Seedlings were grown from seed in commercial potting soil in a controlled environment chamber. Soil water content was maintained near field capacity, and water soluble fertilizer was applied twice monthly. (Adapted from Ref. 154 and J. F. Weltzin, unpublished data.)

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between and within these sources of defoliation, additional variability arises from comparisons across taxa and environments. The responses of individual plants are highly variable and are dependent on environmental conditions before, during, and after defoliation events [163]. Therefore, the response of individual plants may deviate from the generalizations described in this section [146,154,161].

Several investigators (reviewed in Refs. 164–166) have suggested that herbivory increases total productivity, reproductive output, or fitness of some plant species. Empirical evidence for ''overcompensation'' by grazed plants is generally weak, and responses have been poorly linked to physiological mechanisms. Nonetheless, the phenomenon has been at least partially documented for a few systems [167].

STRESS IN THE FACE OF GLOBAL ENVIRONMENTAL CHANGE

The concepts and examples described in the previous sections indicate that much is known about the response of individual plants to various agents of stress. In some cases, the responses of plant assemblages can be explained on the basis of individual plant responses. It also is evident that stress factors interact with one another. Given the anticipated rapid and substantial changes in atmospheric composition, considerable knowledge about the response of individual plants to stress must be re-evaluated in light of these changes. Current knowledge may help predict the responses to some future changes but may be deficient for other predictions. However, the functional equilibrium or optimal allocation concept of the plant response to stress provides the theoretical framework for predicting responses to changing environmental conditions and resource limitations of the future [32]. Since shifts in the allocation at the whole-plant level determine the rates of resource use or processing by the plant, it may be feasible to at least generate crude predictions of the vegetation responses to future conditions. A mechanistic approach is preferable here, because we have few good analogues or environmental gradients that may provide the information needed to predict the responses to future conditions.

Atmospheric concentrations of carbon dioxide have been rising since the beginning of the 19th century and are predicted to reach about 700 ppm by the middle to end of the 21st century [168]. Increases in CO_2 levels are predicted to have both direct (e.g., reduction of stomatal conductance) and indirect (e.g., alteration of surface temperatures or precipitation regimens) effects on vegetation [169]. The response of plants to elevated CO_2 has been documented for a great number of species and growth and life forms [170]. Increases in CO_2 typically benefit plants with C_3 photosynthetic metabolism by decreasing photorespiration and by increasing net CO_2 assimilation, quantum yield, temperature optima for photosynthesis, and water- and nutrient-use efficiency (see reviews in Refs. 171–178). Furthermore, tolerance of C_3 plants to heat, drought, salinity, and other stresses is improved by elevated CO_2. As such, C_3 plants exposed to elevated carbon dioxide levels generally exhibit increases in net photosynthesis and above-and belowground biomass production. For example, a recent comprehensive literature survey of 250 C_3 species grown individually under conditions of elevated CO_2 reported a 47% increase in dry matter accumulation [170].

In contrast, plants with C_4 photosynthetic metabolism have been regarded as being relatively unresponsive to elevated CO_2, or responses have been inconsistent [170,174,175,179]. However, recent research with C_4 species suggests that they may also benefit from rising CO_2 levels [180–183]. In their review of literature, Poorter et al. [170] found a statistically significant 10% increase in the net biomass accumulation for C_4 plants grown singly under elevated CO_2 concentration. Positive responses of C_4 species to elevated CO_2 levels have been attributed to small but consistent increases in photosynthesis [184], increases in water-use efficiency (WUE) caused by reductions in stomatal conductance [181,185], or interactive effects of leaf water potential and photosynthesis [180].

However, the actual response of plants to CO_2 enrichment varies among species and photosyn-

thetic pathway types and depends on other biotic and abiotic environmental conditions such as soil fertility and moisture, species characteristics, or competitive environments [179,186–188]. For example, based on the response of C_3 and C_4 plants grown separately, a priori one might predict that elevated CO_2 would favor C_3 plants over coexisting C_4 plants (see Refs. 175 and 189, but see Ref. 190). However, studies of the effects of CO_2 enrichment on plants grown together have produced a variety of outcomes. In a recent review of the effects of elevated CO_2 levels on C_3 and C_4 plants grown together, Reynolds [179] concluded that when environmental factors are not limiting, C_3 species typically increase performance relative to C_4 species. Conversely, when competitive interactions are strong or soil fertility is high, CO_2 increases may favor species that are superior competitors for light regardless of their photosynthetic pathway (e.g., see Refs. 185, 188, 191, and 192).

Regardless, it is often hypothesized that shifts in the competitive abilities of C_3 and C_4 plants that experience increased atmospheric CO_2 concentration or other indirect effects of climate change may result in changes in their relative abundance or spatial distribution [173,193–196]. For example, it has been hypothesized that increases in CO_2 may enhance the growth and establishment of C_3 shrubs in C_4-dominated grasslands of the southwestern United States [175,186,189,197]. Increases in the WUE and fine root biomass of C_3 woody plants under elevated CO_2 (e.g., see Refs. 186, 198, and 199) suggests that these plants may be able to expand their distribution into ecosystems where water is otherwise a limiting factor [194]. A simple WUE model developed by Idso and Quinn [200] suggested that a doubling of atmospheric CO_2 levels would cause oak woodlands dominated by *Q. emoryi* Torr. in the southwestern United States to shift downslope and displace extensive regions of semidesert grassland. The indirect effects of CO_2 enrichment on soil water availability include increased WUE for most species, with ramifications for species currently limited by dry conditions [83].

LINKING PHYSIOLOGICAL AND ECOSYSTEM PROCESSES

Linking stress responses of individual plants with ecosystem processes (i.e., scaling up) represents a major challenge, and it is a primary focus of contemporary research. The goal of this exercise is to develop methods for extending very limited ground measurements of ecosystem function (CO_2 uptake, evapotranspiration, energy exchange) to large areas (region, globe) and predict ecosystem functional responses to future climatic and human land-use conditions [201].

Individual responses to any number of future environmental changes (elevated CO_2, precipitation redistribution, climate warming) can be interpreted within the theoretical framework of optimal allocation. Furthermore, interactions at the community level, either positive or negative, that impact local resource availability or that affect essential plant functions, likewise can be viewed within this context [32]. These interactions, however, are complicated and may limit our ability to use simple bottom-up approaches to predict the responses of vegetation to global changes.

Field [20] argues that if plants have evolved a limited set of optimal responses to resource limitations and stress, then nitrogen distribution and allocation within plants will be good predictors of plant carbon gain capacity and can be scaled to the ecosystem level. The whole canopy photosynthetic capacity, therefore, could be sensed remotely for the purposes of global assessment of carbon fluxes. Essential to this approach is a consistent scaleable relationship between maximum photosynthetic rate and nitrogen concentration of photosynthetic tissues and the spectral qualities of the leaf canopy. In this sense, one could view the plant canopy as a ''superleaf'' that has predictable characteristics under different resource supply rates.

The variety of solutions that plants have evolved to meet the demands of environmental stress and resource limitation in natural environments is exemplified by the diversity of plant life forms, phenologies, and physiological systems. Limitations to scaling the plant ecophysiological processes to the ecosystem are due to the complexity of resource-use patterns displayed by plant species and life forms and the patterns of micrometeorological fluxes and feedbacks in natural ecosystems

[202,203]. A complicated but widely applicable approach to scaling plant responses to the ecosystem level involves classifying plant species into functional groups and assessing the abundance of these groups across the landscape. The plant functional type (PFT) concept has emerged as a useful way to organize plant species with similar responses to and impacts on ecosystem processes into manageable and meaningful categories. Useful traits for a PFT classification would include the characteristics of the plant form and function that influence the rates and processes of transpiration, energy exchange, nutrient cycling, and migration into new habitat [201]. Depending on the spatial scale of interest, the traits could be very specific or very general such that modeling and prediction are practical and explanatory. PFT description of dominant plant species should recognize species-environment responses and potentially account for habitat conditions. This is similar to the ''norm of reaction'' concept that is useful for interpreting the ecological and evolutionary responses of genotypes, populations, and species [204] to environmental heterogeneity but are applied here to changes in species' functional roles at the ecosystem level.

The PFT concept explicitly recognizes that the relationship between species diversity and ecosystem function is tenuous. Identification of functionally different groups for specific ecosystems is necessary to generate meaningful predictions about the effects of species removals on ecosystem properties [205]. To make PFT classifications robust, experiments must be carefully executed to minimize confounding factors between diversity and other factors that may influence ecosystem function. Thus, results from the few experimental investigations to date, which indicate that increased diversity of plant functional types increases ecosystem productivity and stability [135,206–210], may not apply to other systems. Research being conducted on the Jornada Experimental Range in southern New Mexico, wherein species diversity and PFT diversity are being experimentally manipulated by selective removals, should shed additional light on the interactive effects on this issue (L. Huenekke and W. Schlesinger, unpublished data).

CONCLUSIONS

Stress is a phenomenon that occurs at the scale of individual plants but that has important manifestations at the ecosystem level via the effects on organismic interactions and fluxes of energy and materials. Because plants function as a balanced system, stress will have somewhat predictable effects on allocation patterns and resource use that potentially can be scaled to higher levels. Consequently, predictions of the stress effects at the ecosystem level will necessarily be improved with the development of robust physiological models. Although optimal physiological responses to stress are difficult to model and measure, and are not expected in every situation, the concept provides a useful starting point for predicting vegetation responses to altered resource distributions brought about by anthropogenic or natural perturbations. Recent research has focused on empirical descriptions of the plant response to elevated CO_2 levels and illustrates the complex nature of responses of plant assemblages. A clearer understanding of processes that link physiological stress responses with population, community, and ecosystem processes will greatly enhance our ability to predict the structure and function of wildland ecosystems into the next century.

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Response of Woody Species to Salinity

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INTRODUCTION

Saline soils are of worldwide occurrence, but their incidence is more severe in arid and semiarid regions. Higher evapotranspiration than precipitation in these areas aggravates the situation further. The capillary rise, often of bad-quality water, brings salts to the surface from the deeper horizons, which are not leached down and out of the root zone. In the presence of a shallow water table, the process of salinization of soil surface is further accelerated.

In spite of our efforts to counter the menace of salinity, it remains a major threat to agriculture. It is affecting yields on large areas and is creeping into fertile lands at an alarming pace. In most of the developing countries, the ever-increasing population and soil degradation at a rate higher than reclamation make the problem of saline soils even worse. These lands, which are abundant, are not generally suited for most of the field crops. The solution lies in leaching the excess salts out of the rootzone and subsequent drainage. Sinking of tube wells and the installation of tile drains may help, but this is a costly proposition.

The persisting demand for conventional agricultural production will continuously recharge the groundwater and subsequent discharge will make the reversal of the situation unlikely at least in the near future. Being a long term problem, soil salinity requires suitable land utilization choices to be made. One option may be the planting of trees and shrubs, many of which can withstand much higher salinities than most of the conventional agricultural crops [1].

The following sections highlight some of the candidate species for salinity and relevant soil

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problems, like sodicity and waterlogging. The species mentioned here are only meant for illustrative purposes, and the list is not exhaustive. Many more and possibly even better ones for a particular condition may exist. There also is a need to conduct more experiments to match the suitability of species for the conditions of a site. Efforts to introduce *Leucaena leucocephala* in the Sahel, for instance, did not bring desirable results, because the pH there was much lower (6.0 or below) than required (above 6.5) for most of the varieties. A proper approach would be to assess the detailed physicochemical properties of the site and make recommendations accordingly. An alkaline soil may, for instance, allow better growth of *Casuarina equisetifolia*, *Acacia auriculiformis*, *Phoenix dactylifera*, and *Tamarix* spp. *Pinus* and *Bambusa* spp. do better under acidic pH, *A. raddiana* and *Acacia senegal* grow well in loose, light, and sandy soil, and *A. nilotica* and *Bauhinia reticulata* prefer heavy, clayey soils that may become waterlogged during the rainy season [2].

TREES ON FARMS

The importance of trees needs no emphasis, and their products and by-products are too many to cite here. There is no denying the fact that there is no farm, big or small, without trees. Trees are known to conserve soil and water supplies, check erosion and runoff, enrich the soil, and reduce the hardships of rural life. They not only provide shelter for workers during the hot sunny days, but in many cases supply the much needed fuel, complement fodder requirements, and provide cash return through sale of their wood.

In areas of high wind velocity, trees may be useful for windbreaks and shelter belts. The effectiveness of shelter belts in reducing wind erosion has been demonstrated in many parts of the world. When used as windbreak, one often hears of root competition and other undesirable effects of trees (e.g., shade, allelopathy), but the yield gains of the protected crop often outweigh the losses if any. Root competition is of major concern, because it shares the nutrients and water with the crops. This can be avoided by digging a trench between the tree row and the field to check the access of tree roots.

It is, however, also worth mentioning here that the resources of a small farmer are limited. The farmer has to meet the requirements of his or her family and livestock from the farm. It necessitates the maximum use of the land at the farmer's disposal. Consequently, he or she is a mixed system farmer, who is interested in immediate gains and cannot afford to invest in ventures with a sizeable time gap between investment and return. Growing trees is a proposition which requires a waiting period. The introduction of fast-growing multipurpose tree species has reduced this time gap to some extent [3]. These species may meet several needs, and the overall returns may be comparable with other farming systems, but growing annual crops will still be more attractive. However, through education and by providing proper incentives, a farmer can be convinced to accept other profitable proposals.

The practice of the block plantation of *Acacia nilotica* (known locally as ''hurries''), which has been in vogue in the Sindh Province of Pakistan since the 1850s, is a case of the adoption of tree planting for improving marginal lands, including salt-affected ones. In this system of planting, seed of *A. nilotica* is scattered over the marginal plot which, after the initial soaking dose, receives only the run-off from adjoining fields. The seedlings grow among a crop for the first season, after which very close trees are removed for wider spacing, but the stand is still so thick that the canopies intermingle. The stems are thin, straight poles and after 5–6 years can be sold at a profit. The root biomass and the leaf fall enriches the soil for normal cultivation. Thus, the farmer not only gets a monetary benefit with a minimum input but also ends up with a much improved piece of land.

CHARACTERIZATION OF SALT-AFFECTED SOILS

Saline soils are characterized by an EC_e (electrical conductivity of the soil saturation extract) greater than 4 mS/cm, pH below 8.5, and an ESP (exchangeable sodium percentage) less than 15. These

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soils generally contain neutral soluble salts comprising chlorides and sulfates of sodium, calcium, and magnesium and they possess good physical condition and permeability owing to the flocculating effect of neutral salts. Sodic/saline-sodic soils, on the other hand, have a high pH and exchangeable sodium. They have greatly impaired physical and chemical conditions owing to the deflocculating effect of sodium on soil resulting in, for example, surface crusting, compaction of subsoil, reduced infiltration rate, and poor hydraulic conductivity. A more precise classification of salt-affected soils is given:

Salinity		Sodicity	
ECe (mS/cm)	Class	ESP	Class
<4	Nonsaline	<15	Nonsodic
4–8	Slightly saline	15–25	Slightly sodic
8–15	Moderately slaine	25–35	Moderately sodic
>15	Highly saline	>35	Highly sodic

EXTENT OF SALT-AFFECTED LANDS

Of the approximately 13 billion ha total land areas on earth, about 1 billion ha are affected by salinity/sodicity [4,5] (Table 1). This does not include the former Soviet Union (about 2.22 billion ha), for which data are not available. According to another report [6], saline/sodic soils cover about 26% of the world's cultivated land. Incidentally, most of the developing and underdeveloped countries of south and Southeast Asia, Africa, and South America lying in arid/semiarid regions, are the worst affected by this threat.

India, for instance, has about 7 million ha of saline/sodic lands [7], whereas the neighboring Pakistan, with a much smaller land area, has about 4.2 million ha of such lands in the Indus Basin only [8]. The whole country may have as much as 6–7 million ha [9], resulting in a national economic loss of about 32 million US$ annually [10]. In these countries, waterlogging is the main cause of the upward flux of salts, which is due to the intensive and continuous use of surface irrigation and has altered the hydrological balance of the affected areas.

TABLE 1 Worldwide Occurrence of Saline/Sodic Soils in Million Hectares

	Total area	Affected
North America	2137.80	17.72
South America	1753.47	129.16
Africa	2963.63	80.54
Australia	788.66	357.33
Europe	472.96	50.80
South Asia	678.02	84.83
North and Central Asia	1103.01	211.69
Southeast Asia	897.62	19.98
Former USSR	2227.00	N.A.
Total	13022.17	952.05

Source: Refs. 4 and 5.

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Adding to the problem is a more serious and perpetual loss of water through seepage from canals and watercourses. Waterlogging, even for short periods and with nonsaline water, may have adverse effects on plant growth. Stagnant water, even if nonsaline, reduces the growth of most flood-tolerant species (e.g., *Taxodium distichum*) [11].

TREES ON WATERLOGGED SOILS

Tolerance to waterlogging has not been studied systematically in the field because it is very difficult to maintain uniform waterlogging during extended periods. The information available has been derived from observations recorded from natural habitats (i.e., swamps, seasonally waterlogged areas). Modifications like the development of aerenchyma in roots and the proliferation of roots which run laterally near the ground surface help in flooding tolerance. The former helps in relieving anoxia, and the surface roots start functioning normally as soon as the water recedes from the surface, but the deeper roots are still inundated [12]. van der Moezel et al.[13] reported that *Casuarina obesa* and *C. glauca* developed aerenchyma in the roots and, consequently, they grow better than *C. cristata*, *C. cunnighamiana* and *C. equisetifolia*. Data derived from other reports suggest that *Eucalyptus camaldulensis* may tolerate long-term flooding [14]. Other waterlogging-tolerant species include *Melaleuca* spp., *Eucalyptus tereticornis*, *E. robusta, Salix* spp., *Syzygium cuminii*, *Terminalia arjuna*, and *Albizia procera* [15].

Unfortunately, vast areas of saline lands have a shallow water table or they get inundated during the rainy parts of the year. In saline waterlogged soils, the effects are compounded, and it has been observed that plants which can withstand fairly high salinities do not grow well or even fail to survive if salinity is combined with waterlogging. This makes the choice of species difficult for such conditions.

Many *Eucalyptus*, *Casuarina*, and *Acacia* species exhibit high salt tolerance, but they will not tolerate waterlogged conditions associated with salinity, whereas some *Acacia* spp. (e.g., *nilotica*, *stenophylla*) are fairly tolerant of the combination of waterlogging with low to moderate salinity [16–18].

TREES IN SODIC SOILS

Sodic soils are characterized by poor physical conditions, nutritional imbalance, and ion toxicity. Sodicity tolerance has generally been characterized by ESP: sensitive species are affected at ESP of about 10, moderately tolerant at 20–25 and highly tolerant at >25 [15]. Some tree species having fairly high sodicity tolerance are *E. tereticornis*, *E. camaldulensis*, *Prosopis juliflora*, *A. nilotica*, *A. auriculiformis*, *Zizyphus* spp. [19], *A. modesta*, *A. stenophylla*, *P. chilensis*, *P. siliquestrum*, *P. alba*, and *C. obesa* [20].

SALINITY AND PLANT GROWTH

Plants exposed to saline condition face various stresses, which may be (a) reduced availability of water due to the low osmotic potential of the external medium compared with that of the cell sap (also sometimes termed *physiological drought*); (b) specific ionic effects, especially those of Na and Cl; and (c) nutritional, enzymatic, hormonal, and other disturbances due to combined effects of (a) and (b).

Plants which are capable of withstanding the harmful effects of these stresses do so, for example, through controlled transpiration (i.e., reduction in uptake of harmful ions), the production of organic osmoregulants (e.g., proline, glycinebetaine), the selective uptake of K in preference to Na (avoidance), succulence (i.e., dilution of harmful ions), the excretion through salt glands, salt hair,

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and the shedding of salt-loaded leaves (reducing salt load) [21]. Such plants consequently complete their life cycle better than those which succumb to the above pressures.

Glycophytes, the class of plants to which all of field crops belong, have only limited tolerance to salinity. Halophytes, the salt-loving plants, on the other hand, can grow in fairly high salinities, and they may even require certain salt concentrations for optimum growth [22]. The potential use of most of the halophytes has, however, yet to be explored, although these plants have a wealth of very useful species having food, industrial, medicinal, land conservation, and esthetic values [23–25].

SALINITY AND TREES

Gainful utilization of the salt-affected lands would be possible through the use of salt-tolerant glycophytes or suitable halophytes; the former have a low threshold level of salt tolerance [26] and the later have low utility. Trees may prove an attractive alternative, as the threshold of many species for stress tolerance is higher than most field crops. If it is a multipurpose tree, then the owner gets a good return of desirable products from an area where nothing worthwhile grew before. The microenvironment, root action, and leaf fall, for example, may gradually improve the soil condition and allow growth of less stress-tolerant species after the land has been under trees over a period of time.

A number of fruit trees are known to be appreciably salt tolerant. These include, among others, date palm (*Phoenix dactylifera*), fig (*Ficus carica*), jojoba (*Simmondsia chinesis*), jujube (*Zizyphus jujuba*), olive (*Olea europaea*), papaya (*Carcia papaya*), pineapple (*Ananas comosus*), pomegranate (*Punica granatum*), guava (*Psidium guajava*), sapodila plum (*Acharas zapota*), black plum (*Syzygium cuminii*), and grewia (*Grewia asiatica*).

While going through the literature, we often come across plant species which are common to different stress conditions. One striking example is a general similarity between drought and salinity tolerances. Although not universal, many woody species growing in arid areas cope fairly well with at least moderate salinity levels. This may be due to the fact that the two stresses of salinity and aridity have one thing in common. Whereas a shortage of water per se is an impediment in arid drought–affected soils, salinity causes ''physiological drought'' due to a lowered osmotic potential, which is of course accompanied by specific ionic effects.

Nearly one third of the earth's surface, excluding polar regions, has been classified as arid or semiarid. About 15% of world population lives in these regions where conventional crops may not be successful. Some woody species relevant to such conditions are *A. albida, A. nilotica, A. auriculiformis*, *A. tortilis* (sand dune stabilization), *A. catachu* (tannin), *A. senegal* (gum exudate), *Albizia lebbek*, *A. procera*, *Azadirachta indica* (nonedible oil, pesticides, fertilizers), *Cassia siamea* (pepper), *Leucaena leucocephala*, *P. juliflora*, *P. cineraria*, *Sesbania grandiflora* (paper, pulp), *Phyllanthus emblica*, and *Zizyphus mauritiana* (fruit) [27].

A number of other species may tolerate exclusively either salinity or drought stress, but many of the above-mentioned drought-tolerant species perform satisfactorily under moderate to high salinity levels. It is thus evident that a knowledge of drought-tolerant species narrows our search for identifying salt-tolerant species.

One point worth noting here is that a wide variation may often be found between provenances (stands in a particular area developed through natural selection), because species evolved under certain environmental conditions are expected to have acquired these characteristics [28]. For instance, the Petford provenance of *E. camaldulensis* had 200% greater wood volume than the Walpole provenance at 5 years of age [29]. In fact, in *E. camaldulensis* [30] and *E. occidentalis* [31,32] intraspecific variation is reported to be so wide that to assign a tolerance classification at the species level is not reliable.

Efforts have been made in the past to screen plants for salt tolerance. A number of floras and directories have lately been published which provide information about species suited for cultivation under particular conditions. These also provide a basis for exploiting the potential capacity of these

plants for the benefit of humankind. Aeronson et al. [33], for instance, have evaluated the response of 120 halophytes to irrigation with seawater and subsequently published a data-based record of salt-tolerant plants [34]. Other bibliographies of a similar nature also are available (e.g. see Refs. 35–37).

It is, however, evident that screening of woody species for salt tolerance has received comparatively less attention than field crops. The dynamic nature of salinity and its changing influence on plants during phases of growth makes it easier and probably more precise to study annuals than perennials. The beneficial influence of trees on the earth's atmosphere and their land conservation values are increasingly being appreciated, resulting in diverting the attention of researchers toward this important but relatively less recognized reality of the present day. For instance, this recognition has led to the widespread introduction of *E. camaldulensis* in the saline lands of Pakistan.

Today, attention is being focused worldwide to identify suitable woody species for use as fodder or fuel. Timber is a high-value product of trees, but it often receives a lower priority than fodder/fuel, especially in less-developed countries where energy and food shortage attain immense significance. Table 2 summarizes some such species found to be tolerant to irrigation water salinities of 10–20 mS/cm in sandy areas [38–43].

Woody species from Australia, especially *Acacia*, *Eucalyptus*, *Atriplex*, *Melaleuca*, *Casuarina* species, and mesquites from the Americas, figure prominently in many studies on salt tolerance. *Atriplex* species dominate saline plains over vast areas in Australia and provide useful supplemental fodder for cattle during periods of shortage. These are, however, shrubs and not trees, but in addition to utility as cattle feed, they may be used as fuel. Some other shrubs may be similarly relevant under particular conditions, but they are not fully exploited, and only *Atriplex* species have been studied in any detail for utilizing saline lands.

High salinity tolerance in *Atriplex* is expected owing to it being a halophyte. It attains this tolerance through accumulation of a high salt content (18–21% of dry matter, mostly as NaCl) in the forage. The in vitro dry matter digestibility may be about 75%. Sheep feeding on such diets require an increased water uptake. Limited evidence of diarrhea in the grazing animals was found, which persisted in the lambs born to these ewes, but cleared up within a few days after feeding from the salt bush was stopped [44].

A wide range of *Atriplex* and *Maireana* species were subjected to test under arid and/or saline conditions in a program on the use of forage shrubs for saline areas of Pakistan during 1991–1993. Results from these studies have provided useful information on salt tolerance and other related aspects of these species, which confirm the results from similar studies from other parts of the world. From these studies, it is evident that *A. amnicola*, *A. lentiformis*, *A. undulata*, *and A. halimus* have the potential to tolerate high–salinity/sodicity stress [1].

Felker and his colleagues have worked extensively in the United States on *Prosopis* species and reported them to be generally highly tolerant to stresses like salinity and drought (e.g., see Refs. 45 and 46). Similar reports also have come from other parts of the world [20,47–50]. *Prosopis* species are, however, such aggressive colonizers that they may become troublesome weeds and hence a nuisance.

Some other studies from India and Pakistan have reported *Prosopis* spp. (*juliflora*, *chilensis*, *alba*) to be more tolerant than *A. tortilis*, *E. camaldulensis*, *C. equisetifolia*, *Azadirachta indica*, *E. tereticornis*, *E. microtheca*, and *A. nilotica* [51–54].

In another study, Singh et al. [55] reported that *Dalbergia sissoo* did not perform well in saline/sodic soils of Utter Pradesh, India. This was later confirmed from a pot culture experiment [56], where *C. equisetifolia* was observed to be most salt tolerant (EC_e, 32.5 mS/cm), followed by *A. nilotica*, *Eucalyptus* hybrid, *Pongamia pinnata* (EC_e, 16.3 mS/cm), and *D. sissoo* and *Arucaria cunnanghamii* (EC_e, 8.1 mS/cm). Rankings for salinity tolerance have also been published for a number of species of *Acacia* [57,58], *Casuarina* [13,59], *Eucalyptus* [60–64], and *Melaleuca* [62,64].

Eucalypts dominate a forestation program for cultivating moderately saline lands in many countries, and *E. camaldulensis* is probably the species that has the widest adaptability [65–68].

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TABLE 2 Some Forage and Fuelwood Species Grown in Sandy Areas of Various Countries Using Irrigation Water of EC 10-20 mS/cm

Countries	Plant species for forage (grasses and others)	Plant species for fuel
Argentina	*Atriplex undulata*	*Prosopis juliflora*
Australia	*Maireana brevifolia*	*Acacia ampliceps*
	Atriplex amnicola	*Eucalyptus camaldulensis*
	Atriplex bunburyana	*Eucalyptus occidentalis*
	Atriplex paludosa	*Casuarina equisetifolia*
	Atriplex cinerea	
Chad/Senegal	—	*Tamarix senegalensis*
		Parkinsonia aculeata
		Prosopis juliflora
		Acacia linearoides
Chile	—	*Prosopis tamarugo*
		Prosopis chilensis
		Prosopis pallida
China	*Atriplex cana*	*Salsola passerina*
		Haloxylon aphyllum
Egypt	*Atriplex nummularia*	*Tamarix aphylla*
	Atriplex halimus	*Acacia tortilis*
	Kochia indica	*Ziziphus spina-christi*
	Salsola tetrandra	*Prosopis juliflora*
		Casuarina glauca
Ethiopia	—	*Acacia senegal*
		Acacia tortilis
		Commiphora africana
India	*Atriplex halimus*	*Prosopis juliflora*
	Atriplex amnicola	*Acacia nilotica*
		Acacia tortilis
		Tamarix articulata
		Ziziphus nummularia
		Casuarina equisetifolia
		Azadirachta indica
Iran	*Atriplex halimus*	*Tamarix articulata*
		Tamarix aphylla
		Casuarina equisetifolia
Iraq	*Atriplex nummularia*	*Tamarix articulata*
	Atriplex lentiformis	*Salvadora persica*
	Atriplex halimus	*Acacia nilotica*
	Atriplex amnicola	
	Salsola rigida	
	Maireana brevifolia	
Kenya	*Indigofera califordiana*	*Acacia africana*
	Euphorbia shimperi	*Acacia tortilis*
		Commiphora riparis
		Salvadora persica
Kuwait	*Salsola kali*	*Prosopis juliflora*
	Atriplex nummularia	*Tamarix aphylla*
	Atriplex amnicola	
	Atriplex halimus	

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Table 2 Continued

Countries	Plant species for forage (grasses and others)	Plant species for fuel
Libya	*Atriplex nummularia*	*Acacia tortilis*
	Atriplex halimus	*Eucalyptus camaldulensis*
		Tamarix aphylla
Oman	*Atriplex farinosum*	*Acacia tortilis*
	Atriplex coriacia	*Ziziphus spina-christi*
Pakistan	*Sesbania sesban*	*Prosopis juliflora*
	Indigofera oblongifolia	*Tamarix indica*
	Leucaena leucocephala	*Eucalyptus camaldulensis*
	Atriplex nummularia	*Calotropis procera*
	Atriplex amnicola	*Azadirachta indica*
	Atriplex cinerea	*Parkinsonia aculeata*
Syria	*Salsola vermiculata*	*Prosopis stephanian*
	Atriplex halimus	*Tamarix indica*
	Atriplex canescens	
	Atriplex nummularia	
Thailand	*Sesbania grandiflora*	*Prosopis juliflora*
	Leucaena leucocephala	*Casuarina equisetifolia*
	Cassia siamea	*Acacia auriculiformis*
		Cassia siamea
Tunisia	*Atriplex halimus*	*Acacia saligna*
	Atriplex nummularia	*Acacia tigulata*
	Salsola tetranda	*Tamarix aphylla*
	Halocnemum strobilaceum	
Saudi Arabia	*Atriplex halimus*	*Acacia tortilis*
	Chenopodium album	*Prosopis juliflora*
	Salsola kali	*Calotropis procera*
	Salsola baryosma	*Cloris gayana*
Sudan	*Atriplex amnicola*	*Acacia tortilis*
		Acacia saligna
		Tamarix aphylla
		Prosopis juliflora
UAE	*Atriplex nummularia*	*Acacia tortilis*
	Atriplex amnicola	*Tamarix aphylla*
	Atriplex halimus	*Tamarix stricta*
USA/Canada	*Atriplex triangularis*	*Salsola passerina*
		Parkinsonia aculeata
		Prosopis alba

Source: Refs. 38–43.

Other species, like *Casuarina and Melaleuca*, may attain significance under particular conditions; for example, cultivation in sandy/costal areas [13]. A number of *Acacia* species are gaining attention in the context of the utilization of salt-affected lands because of their high utility and land-conservation properties. Aswathappa et al. [57], working on the response of a number of tropical and subtropical acacias of Australia to salinity, found that, based on the classification of Pedley [69], species belonging to the section Juliflorae were generally the least tolerant, whereas those of Phyllodineae generally performed the best and Plurinerves were intermediary. A 50% reduction in the growth of

tolerant species occurred at an average salinity level of about 1500 mM, at 1100 mM in intermediary, and at 800 mM in least tolerant species. Tolerance to salinity in *Acacia* species is generally through exclusion of sodium, as Craig et al. [58], working on the salt tolerance of 10 taxa of *Acacia*, found that the slowest rates of growth were associated with the accumulation of the highest concentration of sodium in the uppermost phyllodes.

In order to capture greater genetic gain, obtain a uniform population rapidly, matching closely with specific environmental conditions, it may often be more desirable to reproduce individuals through clonal vegetative propagation [70]. Shoot/rooted cuttings and micropropagation or tissue culture may be employed for this purpose. Recent studies on the comparison between clones of *E. camaldulensis* and unselected seedlings on saline sites have shown the superiority of such clones [71,72]. However, a wider variation between provenances still remains a comparatively easier option for identifying suitable candidates for particular soil/environmental conditions.

Taking the lead from an Australian Center for International Agricultural Research (ACIAR) publication [73], one of the first screening trials outside Australia, on Australian *Acacia* and *Casuarina* species for salt tolerance was conducted by Ansari et al. [74], where the *Acacia* species were generally more tolerant than *Casuarina* species. Field trials were conducted later by Ansari et al. [16] using more diverse species. Observations recorded at 2 years after outplanting showed that *Acacia* species and *Atriplex lentiformis* were generally more tolerant than other species under test. Among the species of *Acacia*, *A. auriculiformis* and *A. salicina* did not survive, whereas *A. ampliceps* proved to be the best, followed by *A. stenophylla*, *A. machonochieana, A. nilotica*, and *A. victoriae. Casuarina glauca* performed fairly well, but its growth was much slower than that of acacias. Among eucalypts, *E. microtheca* showed better tolerance with *E. occidentalis* and *E. camaldulensis* barely surviving.

Among the indigenous species under test, *Cassia sturtii*, *Azadirachta indica*, and *Prosopis cineraria* did not survive, whereas *Conocarpus lancifolius* and *Parkinsonia aculeata* had better survival but poor growth compared with *A. nilotica*.

Interprovenance differences also were noted. *A. ampliceps* (15741), for instance, had better survival and growth than its counterparts. Similarly, *A. stenophylla* (15736) and *Casuarina glauca* (15929) were superior to nos. 14670 and 15941, respectively, and total mortality occurred in *E. camaldulensis* (15319), but a few plants survived in 15441. This illustrates the need of conducting provenance/progeny trials for more tangible results.

This experiment was badly damaged by the monsoon rains and, consequently, most of the species suffered high mortality. However, the surviving plants of *A. stenophylla*, *A. nilotica*, *E. microtheca*, and *Atriplex lentiformis* grew fairly well even after the waterlogging. Some plants of *Casuarina glauca*, *C. obesa*, *Conocarpus lancifolius*, and *Parkinsonia aculeata* also survived, but the recovery from shock due to waterlogging was not complete, as these plants had very poor growth.

The observations of the growth performance of individual trees of *A. ampliceps* (15741), categorized on the basis of rootzone salinity, irrespective of replicates, showed a typical halophytic response (i.e., stimulation in growth at EC_e 5–10 mS/cm compared with a lower [EC_e <5] salinity level, and a gradual depression at higher EC_e). *A. ampliceps* is not a halophyte, and this response may not be real but more of an illusion due to scatter of the observation points. This, however, illustrates the potential of *A. ampliceps* to withstand the adverse conditions of salinity, but without waterlogging, as the two stresses proved fatal for this species. Table 3 presents a spectrum of broad categorization of some important woody species for their performance under moderate to extreme salinity/sodicity conditions [75].

STRATEGIES TO IMPROVE INITIAL GROWTH

A high seeding mortality and difficulty in establishing at the initial stages is often encountered after outplanting in salt-affected areas due to osmotic and/or specific ionic effects. If this situation could some how be improved, subsequent growth might not be severely hampered. In spite of their high

Table 3 Categories of Woody Species Based on Reasonably Good Performance Under Conditions of Moderate to High Salinity/Sodicity

Severity	Salinity	Sodicity
Moderate	*Acacia auriculiformis*	*Acacia auriculiformis*
	Acacia nilotica	*Acacia saligna*
	Acacia saligna	*Casuarina glauca*
	Casuarina cunnighamiana	*Eucalyptus occidentalis*
	Casuarina equisetifolia	*Melaleuca bracteata*
	Eucalyptus camaldulensis	*Melaleuca halmaturorum*
	Eucalyptus coolabah	*Tamarix aphylla*
	Eucalyptus robusta	
	Eucalyptus tereticornis	
	Melaleuca arcana	
	Melaleuca bracteata	
	Sesbania formosa	
	Leucaena leucocephala	
	Populus euphratica	
High	*Acacia salicina*	*Acacia nilotica*
	Casuarina glauca	*Casuarina equisetifolia*
	Casuarina obesa	*Casuarina obesa*
	Conocarpus lancifolius	*Eucalyptus camaldulensis*
	Eucalyptus occidentalis	*Eucalyptus coolabah*
	Eucalyptus rudis	*Eucalyptus tereticornis*
	Melaleuca leucadendra	*Prosopis juliflora*
Extremely high	*Acacia ampliceps*	*Acacia ampliceps*
	Acacia machonochieana	*Acacia machonochieana*
	Acacia stenophylla	*Acacia stenophylla*
	Melaleuca halmaturorum	*Tamarix articulata*
	Prosopis juliflora	
	Tamarix aphylla	
	Tamarix articulata	

For severity classes: EC_e 4–8 mS/cm: moderate, 8–16: high, >16: severe salinity, and pH 8–9: moderate, 9–10: high, >10: severe sodicity [75].

salt tolerance, even the tree species need appropriate strategies to reduce the environmental stress during the early establishment phase. This helps to improve growth, as our sole objective is to have flourishing plants on a saline site, without which one cannot expect any tangible effect on the soil as a result of, for example, due to litter fall and root action.

Efforts are, hence, needed to improve the survival and growth of trees through cultural and other methods. Land leveling and subsurface loosening [76], planting on raised beds at nearly one third of the way below the peak [77], and mulching with sand or straw [78,79] have been reported to alleviate the adverse salt effects on trees. Mounding may improve survival under conditions of waterlogging resulting from a shallow water table or poor soil permeability [80]. A combination of treatments may often have greater impact than a single treatment. For instance, in a trial in Thailand, *A. ampliceps* performed best under a combined application of rice hull mulch, gypsum, and NP fertilizers [75].

Preconditioning of tree seedlings before transplanting may affect their subsequent performance in the field. In an experiment, some seedlings, when sown in a field of fairly high salinity (EC_e 20–30 mS/cm) tolerated salts better than those transplanted directly from the nursery [81].

Ahmad and Ismail [82], in their experiments at Karachi University, Pakistan, have observed that a prehardening treatment with saline water of 10 mS/cm resulted in better surival and establishment of *A. ampliceps* and *P. juliflora*.

Another trial conducted at Tandojam, Pakistan, under the same program using *A. ampliceps*, *A. nilotica*, and *C. lancifolius* showed that mulching with wheat straw had a beneficial effect on initial survival, and these effects persisted until the later stages of growth [16]. Here, irrespective of the species, control generally had the lowest survival, whereas mulching alone or in combination with other treatments (extra watering or addition of NP fertilizers) had the best survival. This advantage due to mulching, however, was not reflected in height and DBH (diameter at breast height), where no significant differences between treatments were observed. The effects probably did not last for very long as the plants grew and spread their roots gaining access to deeper soil layers.

TREE WATER USE

The removal of deep-rooted plants and replacement with shallow-rooted ones may often lead to an increased groundwater recharge and a rising groundwater level. Seepage from unlined water channels, low rates of infiltration, and the permeability of soil and poor irrigation management are some other factors responsible for raising the water table.

A shallow groundwater table is not only a deterrant for plant growth, but it also hinders soil improvement owing to a constant upward flux of salts. The difficulty of ameliorating such soils has been demonstrated in one of our earlier studies [83]. A deep groundwater table is hence a prerequisite for improving saline soil conditions.

Suitable trees, owing to their ability to adapt to saline conditions, and because of their high transpiration requirement, may be used in conjunction with mechanical means (i.e., tube wells, tile drains to lower the underground water table). The positive impact of trees and shrubs on the groundwater table has been under study during the last few years, and it has been concluded that these plantations may serve as biological pumps for lowering the water table [84–86]. In fact, tree planting on the banks of canals has often been practiced to check the seepage of water to adjoining areas. A number of species have been identified their for relative tolerance to the combination of salinity and waterlogging stresses [58,63,64,87,88].

Trees have often been incorporated in agroforestry programs for controlling the groundwater table and for salinity control [89–91]. From such studies, it has been observed that pastures consume far less quantities of water than trees; the difference over 1 year may be in the region of 70–80 mm. Among trees, those producing fodder (i.e., *A. blakeyi* and *Chamaecystisus proliferus*, or tagasaste) had better water-use efficiency than the oil-producing eucalypts [90].

High rates of evaporation from tree species is sustained by their greater ability to exploit soil water, which often leads to a reduction in the groundwater level. This will, of course, depend on the species used, the age and density of plants, depth and salinity of the water table, soil type, and climatic factors. In one such study for instance [92], detailed measurements of plantation water use with the help of ''heat-pulse'' or ''sap-flow velocity'' equipment, water table depth and soil conditions were recorded over 2 years in two small plantations with contrasting soil and groundwater salinity at Tandojam, Sindh Province, Pakistan. Species monitored included *A. nilotica*, *A. ampliceps*, and *P. pallida*. Annual water use by 3- to 5-years-old *A. nilotica* was 1248 mm on the severely saline site and 2225 mm on the mildly saline site. Water use by *A. nilotica* was considerably greater than the annual rainfall, implying uptake of groundwater, which was confirmed both by piezometric observations and chloride balance modeling to predict vertical water movement through the rootzone. The plantation water tables fell from 1.7 m below the surface after winter to over 2.9 m after the next summer and then rose again during irrigation of the surrounding farmland. The rootzone salt concentrations remained high at the more saline site throughout the monitoring period, but at the less saline site, there was evidence of increasing rootzone salinity as salt accumulated in the areas of the profile subject to the root water uptake. The salt concentration in the upper profile

decreased as the soil dried and water was absorbed from a greater depth. It was concluded that plantations using saline groundwater may be sustainable if occasional leaching and other salt-removing processes are sufficient to maintain rootzone salinity at a level which does not excessively reduce tree growth.

Tree species in uniform stands at a single location have, however, been observed generally to show little variation in the volume of water moving through the stem per square meter of sapwood per day (sap flux density). This was confirmed from observations on water-use data collected for 2 years at seven different sites in Pakistan, Thailand, and Australia in plantations of several species (i.e., *A. nilotica*, *A. ampliceps*, *P. pallida*, *E. camaldulensis*, *E. microtheca*, *C. cunninghamiana*), where differences in water use between species at any site were largely due to the variation in their growth rates [75]. These results highlight the importance of selection and breeding programs aimed at improving tree growth, as this will determine the sapwood area and, consequently, the total water used by a plantation.

CONCLUSIONS

Adverse soil conditions, especially salinity/sodicity, waterlogging, and drought, in most arid and semiarid regions necessitate the search for suitable plants for cultivation in these areas. The genetic make-up of a species is a predominant factor for survival under hostile conditions. This is evident not only in the performance of plants of different classes (i.e., glycophytes, halophytes, xerophytes) but may be observed in the species of each class and cultivars of a species, and some characters may show variations even within individuals of the same cultivar.

Successful agriculture in such problem areas requires intelligent use of the available information. In saline, soils, with the threshold level of all of our present-day field crops being low, lands not suitable to support these species may be put to some other productive use like growing salt-tolerant trees and shurbs.

It is evident that trees can play an important role on degraded lands, as they are known to be generally more tolerant to adverse soil conditions. There is, however, a need to exploit their genetic potential and to identify species/provenances acclimatized to particular conditions. If this is successful, then it could bring financial benefits from a land where nothing else can grow. There is a chronic shortage of fuel wood in most developing countries. These multipurpose trees not only alleviate this shortage, but their foliage may also cater to the needs of fodder for cattle. Good-quality wood could be sold at as high a price as timber.

Providing a cover on these degraded lands creates a microenvironment which reduces evaporation from the soil surface: the main cause of the capillary rise of the salts. The roots open up the soil, and, if it is a nitrogen-fixing species, add to the fertility. The falling debris enriches the surface soil through the addition of organic matter, and hence there is a good chance that a tree cover will result in an improvement of the soil fertility.

The immediate task for areas with a shallow water table is to lower the level of underground water. This could be achieved through engineering or biological methods. The potential of using trees as biological pumps has been demonstrated. This can provide a basis for calculating the impact of extensive farm planting of suitable tree species in an area with shallow groundwater and eventually develop management plans to reclaim saline lands, enhance farm production, and provide much needed fuel wood and timber. Contrary to our apprehensions [93], one of our studies in an area of low to moderate salinity showed that the rootzone under a block plantation did not deteriorate to any significant extent over a period of 1 year [92]. There is naturally a need for long-term monitoring to gather more data from diverse sites to reinforce the estimates and to explore the effect on the site and other environmental variables.

Soil salinization is, however, a very complex and dynamic phenomenon requiring constant efforts for effective management of the salt-affected lands. There is a need to continue this research

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and to look in more detail into the problems encountered in such ventures and to find ways and means to deal with the problems as and when they arise.

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Photosynthetic Responses of Citrus to Environmental Changes

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INTRODUCTION

Citrus is one of the most important fruit crops in world trade today. Its commercial cultivation has been extremely successful in tropical and subtropical areas, as well as in semiarid and arid regions with adequate irrigation. Citrus belongs to the subfamily Aurantioideae of the family Rutaceae. Among the six genera constituting the true citrus fruit trees, only three (*Fortunella, Poncirus*, and *Citrus*) are of economic importance [1]. These are native to a large Asiatic area extending from India to China and the Philippines in the east and Burma, Thailand, Indonesia, and New Caledonia in the southeast. An exception is the grapefruit (*Citrus paradisi* Macfadyen), which appeared in the West Indies (Barbados) before 1790 as a mutant or possibly a hybrid of a species introduced from the Far East [1].

Growth and development of citrus is the net result of many interacting processes, including photosynthesis and its direct relationship to crop yield. The photosynthetic rate is dependent on a multitude of reactions, each with a potentially unique response to environmental factors [2]. As with other crop species, the ability of citrus to adapt, acclimate, and/or compensate to environmental stress is critical to survival and performance. Understanding the physiological and biochemical responses, as well as the control mechanisms involved with acclimation and adaptation, of photosynthesis to stress conditions is necessary for devising methods to enhance growth and productivity.

Effort has been expended to improve citrus stress tolerance through breeding and selection. However, limited understanding of the physiological, biochemical, molecular, and genetic bases of the stress response hinders the application of genetic engineering to achieve stable and enhanced production under environmental variation. Increased research efforts should exploit the vast physiological, biochemical, and genetic variability inherent in different germplasms, with the ultimate goal of developing cultivars with more efficient production through tolerance of environmental stress. There is indeed a critical need to explore more deeply the effects of environmental stress on citrus metabolism, to understand the mechanisms of stress responses, and to identify targets for genetic

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manipulation [3–5]. This knowledge is basic to any genetic/breeding approach designed to enhance stress resistance/tolerance.

This chapter focuses on the current understanding of the physiological and biochemical responses of citrus photosynthesis to environmental changes. Citrus leaf photosynthetic capacity in relation to solar irradiance, ambient temperature and humidity, soil water availability, and elevated atmospheric CO_2 is discussed. Other more general responses of citrus to various environmental stresses have been reviewed elsewhere [6–8].

SOLAR IRRADIANCE

Photosynthesis consists of both light-dependent and light-independent reactions. In the former, solar radiant energy in the form of quanta (photons) (400- to 700-nm wavelength range) is captured by chlorophyll and converted to chemical energy-rich compounds (ATP) and reducing agents (NADPH) through an electron transport system. The stored energy and reducing power are used in the biochemical light-independent reactions to form carbon skeletons from the ''fixation'' of atmospheric CO_2. Thus, solar energy is stored as chemical energy in the form of carbohydrates and other organic compounds which are ultimately used for plant growth and productivity. A number of enzymes, including ribulose bisphosphate carboxylase-oxygenase (Rubisco), catalyzing the reactions of photosynthesis are regulated by light [2,9–11]. Rubisco ''fixes'' atmospheric CO_2 and thus plays a vital role in plant growth and productivity. This enzyme is a multimeric protein complex consisting of eight large subunits (LSU) and eight small subunits (SSU). It is the most abundant protein in the world, ''fixing'' about 10^{11} tons of CO_2 annually [12]. The LSU, with a molecular weight of 50–55 kDa, is encoded by the *rbcL* gene of the chloroplast genome; and the SSU, with a molecular weight of 12–15 kDa, is nuclearly encoded by the *rbcS* gene. Catalytic activity of the enzyme resides in the LSU, whereas the SSU seems to have a regulatory role [13].

The net CO_2 exchange rate (CER) of citrus is generally low compared with other woody perennials [14–19]. The CER of individual attached, fully expanded top leaves of field-grown citrus trees has a light compensation point (CER = 0) at a solar photosynthetic photon flux density (PPFD) of about 50 μmol m^{-2} s^{-1} and a maximum value (7–8 μmol CO_2 m^{-2} leaf area s^{-1}) at 600–800 μmol m^{-2} s^{-1} PPFD (Fig. 1). This saturation of the leaf CER at relatively low light has been found

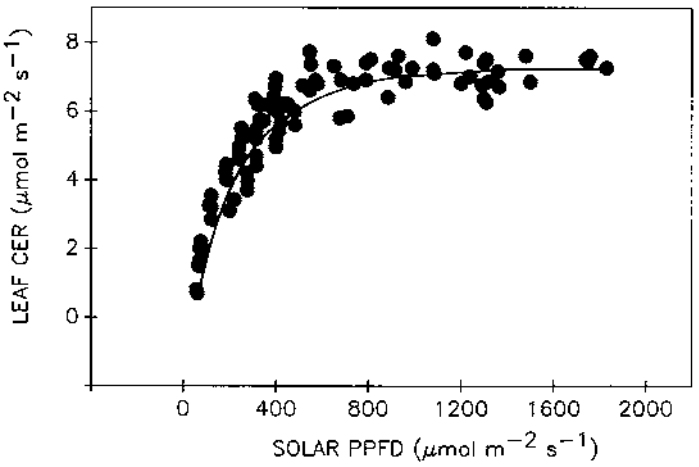

FIGURE 1 Net CO_2 exchange rates of single, attached, fully expanded top leaves of field-grown Valencia sweet orange trees measured during morning hours (0700–1100 EST). Data are plotted against the solar irradiance (PPFD).

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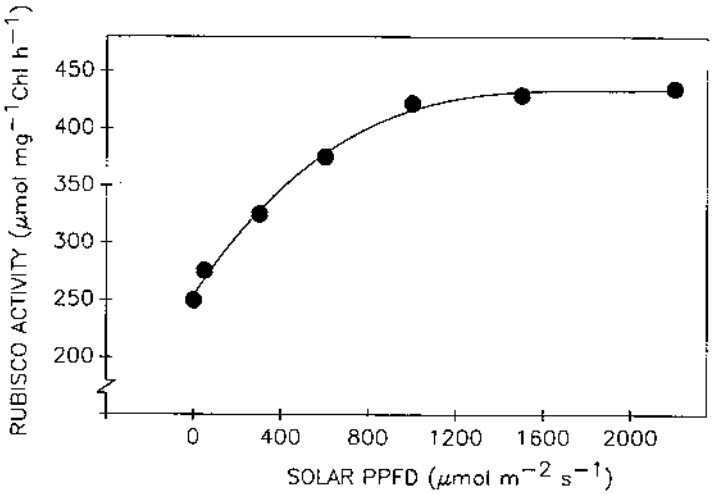

FIGURE 2 Total (CO_2/Mg^{2+}-saturated) Rubisco activity as a function of the solar PPFD from fully expanded top leaves of field-grown Valencia sweet orange trees.

for citrus grown both in glasshouses and under field conditions [14,16,18,19]. A solar PPFD of 800–1000 μmol m^{-2} s^{-1} also is the saturating level for activation of citrus Rubisco (Fig. 2). In addition, there is a diel change in Rubisco activity, which is 45–75% greater during the day than during the night [20] (Fig. 2). The percentage of activation of Rubisco, expressed as the ratio of the initial activity (the in vivo activity) to the total activity (the maximum activatable activity) at midday, reaches a light saturation at about 1000 μmol m^{-2} s^{-1} solar PPFD (Fig. 3).

In citrus leaves, the concentration of the Rubisco protein is 19–25% of the total soluble protein fraction [18,20]. The $K_m(CO_2)$ and K_{cat} values of Rubisco average 19 μM and 25 mol CO_2 mol^{-1} enzyme s^{-1}, respectively, which are typical for C_3 species [18]. The total (CO_2/Mg^{2+}-saturated)

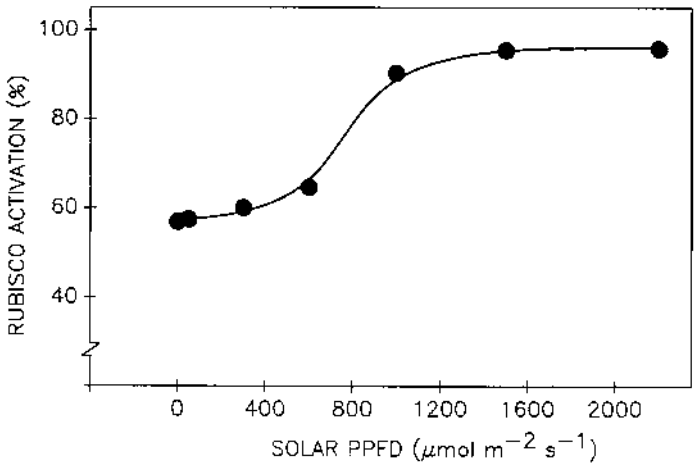

FIGURE 3 Percentage of activation of Rubisco as a function of the solar PPFD from fully expanded top leaves of field-grown Valencia sweet orange trees.

Rubisco activity, about 430 μmol CO_2 mg^{-1} Chl h^{-1} or 73 μmol CO_2 m^{-2} leaf area s^{-1} (see Fig. 2) [18], is more than adequate to support the CER values of 8–11 μmol CO_2 m^{-2} leaf area s^{-1} (see Fig. 1) [14–16,18,20,21].

COLD TEMPERATURE

Various aspects of plant responses to chilling or low temperatures (LT) have been extensively reviewed [22–30]. Temperature affects the rates of biochemical processes differently, thus inducing imbalances among metabolic processes [27]. Adjustments to alleviate LT effects are observed in most plant processes such as growth, photosynthesis, dark respiration, and reproduction [31]. Plants of tropical and subtropical origin, including citrus, have a limited ability to adjust to LT, as well as to develop resistance for survival under freezing conditions. In contrast, plants of temperate origin have significant freezing tolerance, a characteristic thought to involve a genetically programmed process enabling plant survival under severe winter conditions [26]. Citrus is typically an evergreen, and continual replacement of 1- to 2-year-old leaves occurs as trees grow; this process and ultimately growth and productivity are affected by temperature [32]. The continued existence of the U.S. citrus industry, with a 1996 production area of 347,300 ha for Florida, 13,300 ha for Texas, 15,200 ha for Arizona, and 109,200 ha for California [33], would greatly benefit by a common potential to survive devastating freezes. In Florida, three severe freezes in the 5-year period of 1981 through 1985, which destroyed 130,000 ha of the 340,000 ha total in 1980 [34], had a serious impact on the agriculture of the state, resulting in a shift of citrus production southward.

Of all the physiological processes, photosynthesis appears to be the most extensively investigated. It is one of the first processes to be affected when chilling-sensitive plants are exposed to LT [35]. LT effects on CER reduction involve changes in stomatal and nonstomatal characteristics [22]. Regarding the former, the primary change consists of reducing the physical diffusion of gases into and out of the leaf. This is likely due to lower stomatal conductance as a result of partial closure of the stomata. Nonstomatal characteristics, however, appear to change over a wide temperature range and require sufficient time following exposure of plants to the contrasting growth temperature regimen [22,36]. LT alters gene expression and protein metabolism, and it can cause impairment of photosynthesis through effects on catalytic proteins and carbon metabolism [26,28,37–42].

Few studies have been done on citrus carbon assimilation and the effects of temperature, and less is known about chilling effects on the component reactions of photosynthesis. LT reduces leaf stomatal conductance, transpiration, chlorophyll content, and CER of Valencia sweet orange [43]. Activities of Rubisco and phosphoenolpyruvate carboxylase (PEPCase) in fully developed leaves of Valencia orange trees maintained for 30 days at LT (15.6°C day/4.4°C night) are higher than their counterparts maintained at warm temperature (WT, 32.2°C day/21.1°C night) [43]. Of particular interest is a twofold increase in the activity of PEPCase in leaves of trees grown at the LT regimen, causing a change of the Rubisco/PEPCase ratio from 6.6 for the WT treatment to 3.5 for the LT treatment. Transfer of the 30-day WT-treated trees to a LT regimen for 4 days increases PEPCase activity by 45% and decreases the Rubisco/PEPCase ratio to that of the 30-day LT-treated trees [43].

Exposure of citrus trees to LT also alters the expression of several leaf proteins, among which the expression of Rubisco and PEPCase is differentially regulated by LT in the various genotypes [44]. Cold acclimation results in an increased amount of the Rubisco LSU but a decreased amount of the SSU in both cold-hardy and moderately cold-hardy citrus genotypes. In addition, the amount of PEPCase is enhanced by cold acclimation in the cold-hardy genotypes [44]. For C_3 plants, such as citrus, the role of PEPCase is anapleurotic, functioning in gluconeogenesis and nonautotrophic CO_2 fixation. Increased PEPCase activity would be an important part of the metabolic adjustment to the constraints imposed by LT. Thus, the capacity to enhance PEPCase expression during cold acclimation may be important in the acquisition of citrus freezing tolerance.

Accumulation of low molecular weight metabolites, such as soluble sugars and proline, with demonstrated cryoprotectant characteristics, has been reported for many plant species [26]. Sucrose accumulation is most commonly found in chilling-tolerant plants. Its concentration increases significantly during chilling exposure and is accompanied by a decrease in starch [45]. Although starch-sucrose conversion is well documented in cold-hardened plants, little is known about the enzymology of carbohydrate metabolism during cold acclimation [26]. Carbon translocation and regulatory mechanisms as related to carbon partitioning at source/sink under chilling temperatures are not understood [46]. In citrus, the association of sugars with cold hardiness is based primarily on the fact that there is a rapid accumulation of sucrose in the leaf and woody tissues during the cold-acclimation period [47–49]. Valencia sweet orange leaves accumulate more ^{14}C in the sugar fraction at 10°C than at 25°C [50]. In addition, sweet orange trees having high sugar/starch ratios in the leaves and wood withstand the −6.7°C freeze test without injury, whereas trees with low sugar/starch ratios are killed [51]. In plants, many sugar-modulating genes have both direct and indirect roles in sugar metabolism, suggesting that their altered expression may represent a valuable mechanism for adjusting to environmental change [52].

The accumulation of proline also is well known in plants subjected to cold temperatures and other environmental stresses [53]. The mechanism leading to proline accumulation, however, is still obscure. Free proline levels, which are associated with citrus frost hardiness, increase up to 10-fold in citrus leaves during chilling growth temperature regimens [43,49,54]. Proline is the most abundant amino acid found in the tracheal sap of orange trees throughout the entire year, and it is especially high in concentration during the winter season [49]. Whether the proline content is high enough to help protect citrus against frost injury is an enigma at the present time.

The association of proteins with cold hardiness is not as well documented in citrus as in other plant species. However, recent reports of three cold-induced polypeptides (glycoprotein-24, COR11, and COR19), which accumulate in *Poncirus trifoliata*, the most cold-hardy citrus germplasm, exposed to LT pose new and interesting questions as to the role of these proteins in citrus tolerance and survival to cold temperature [55,56].

For many plant species, light is required during exposure to LT in order to attain maximum tolerance [57,58]. In citrus, light has been reported to increase cold hardening for a number of cultivars exhibiting a wide range of cold tolerances [59]. Citrus trees conditioned in controlled-environment growth chambers at low temperatures in the light are injured less than those conditioned at similar temperatures in the dark during subsequent freeze tests. This increased cold hardening in the light has been postulated to result from the accumulation of excess fixed carbon (sucrose) from photosynthesis during the cold-acclimation period [58,59].

All commercial citrus trees are essentially combinations of scions and rootstocks [60,61]. These scion-rootstock interactions, along with different cultural environments and practices, complicate comparative ratings of citrus cold hardiness at various freeze intensities and durations [49]. Among the scion cultivars, mandarin (*Citrus reticulata* Blanco) is the most cold hardy, followed by sweet orange (*C. sinensis* L. Osbeck) and grapefruit (*C. paradisi* Macf.), whereas lime (*C. aurantifolia* Christm. Swingle) and lemon (*C. limon* L. Burm.) are the least cold hardy [49]. In addition, the rootstock definitely has a pronounced effect on the cold hardiness or tolerance of a citrus tree [61–64]. Trifoliate orange (*Poncirus trifoliata* L. Raf.), a deciduous relative of the genus *Citrus*, is a superior cold-hardy rootstock, whereas sour orange (*C. aurantium* L.) is an intermediate, and rough lemon (*C. jambhiri* Lush.) is one among the most cold-sensitive rootstocks. For citrus, it is not known whether protein alterations during cold acclimation are different in seedlings than in budded trees, but there is an indication that rootstocks influence protein alterations of budded trees [49,65].

HIGH TEMPERATURE

Numerous reviews of the effects of high temperatures or heat stress on different processes in plants, including acclimation, carbon fixation, carbohydrate metabolism, and protein synthesis, have been

compiled [2,22,66–72]. Virtually nothing is known of the biochemistry of citrus photosynthesis at high temperature (see section on Elevated Atmospheric CO_2). Heat stress in citriculture is of some concern, because major producing areas are between 35° north and south latitudes. Estimates of lethal temperatures for sweet orange fruits and leaves suggest that damaging heat stress probably does not occur on a large scale in most citrus plantings [73,74]. However, high-temperature effects on citrus growth and yield [75] do cause concern in light of global warming scenarios [76].

Citrus trees grown in subtropical climates generally bloom heavily in the spring, but many flowers and flower buds drop before fruit setting [77]. Furthermore, although the young fruits that remain after the first period of dropping are presumably capable of developing into mature fruits, there is generally a period of accelerated fruit drop as the weather becomes hot in the early summer months; this is referred to as the ''June drop'' [77,78]. As the result, only a small percentage of citrus flowers, about 0.2–7.0%, produce mature fruits [77,79]. However, if severe heat or hot, dry, and windy weather continues for several days, heavier fruit drops result in more serious crop losses. Although the actual mechanism controlling the dropping of flowers and young fruits is still not well understood, there is an indication that growth regulators, as well as nutrients and carbohydrates, may play a role in these abscission processes in response to environmental and/or internal changes [77–79].

The response of CER in citrus under conditions of elevated temperature, high evaporative demand, and low soil water availability is not well understood. Leaf photosynthetic rates of citrus, as previously discussed, are relatively lower than those of other tree crops. In addition, midday depression of leaf photosynthetic rates occurs frequently with outdoor citrus trees on days when atmospheric temperatures and vapor pressure deficits are high [16,18,19,80]. Diurnal measurements made on field-grown citrus trees on cloudless warm spring days in central and south Florida show that leaf CER increases in the morning, reaches the saturation level of 8–11 μmol m^{-2} s^{-1} when solar PPFD approaches 600–800 μmol m^{-2} s^{-1} at about 0900 Eastern Standard Time (EST), and remains relatively stable until late morning as solar PPFD reaches 2000 μmol m^{-2} s^{-1} [16,18]. However, there is a midday depression in CER which starts at about 1230 EST, and by 1330 EST, leaf CER is only one fourth of the saturation level. By 1530–1600 EST, a partial recovery of CER is observed [16,18]. Under hot and dry summer conditions in Phoenix, Arizona, leaf CER of sour orange trees is highest (about 7 μmol m^{-2} s^{-1}) at the morning's first measurement at 0700 Mountain Standard Time (MST), but it steadily decreases to about 1.5 μmol m^{-2} s^{-1} at 1300 MST, and then remains unchanged until 1700 MST [81]. Data collected over a 4-year period on sour orange trees also show that leaf CER declines from about 6.1 to 0.1 μmol m^{-2} s^{-1} as temperature rises from 31 to 47°C [82].

The response of leaf CER to high temperatures also is dependent on the atmospheric humidity [22]. As leaf temperature increases, the water vapor pressure difference (VPD) between the leaf and the surrounding air (i.e., leaf-to-air VPD) increases unless supplemental humidity is provided [2,22]. The increase in VPD promotes closure of the stomata, thus further depressing CER at high temperatures. Stomata of citrus close under these circumstances, presumably as an evolutionary adaptation to conserve water [8,16,83]. Studies conducted on glasshouse-grown sweet orange and grapefruit show that leaf CER is highest when measured at 22–26°C and 8 mbar VPD, but large reductions occur at leaf temperatures above 30°C or when VPD is increased to 24 mbar [15]. The reductions in leaf CER due to high temperatures are associated with reductions in mesophyll conductance to CO_2, whereas those due to high VPD are attributed to decreased total leaf conductance to water vapor [15]. When leaf photosynthesis of sweet orange is measured under controlled conditions at constant temperature (26°C) but varying VPDs, the CER and total leaf conductance determined at 24 mbar VPD are only one half and one third, respectively, of those measured at 8 mbar VPD [15]. For citrus grown under field conditions, although part of the midday depressions in leaf CER may be due to the direct temperature effects on the nonstomatal component reactions, there is indeed an implication of stomatal control mediated by VPD [16,18]. The midday depressions in CER are observed mostly on warm days with high VPD at midday, and stomatal closure occurs in response to such adverse climatic conditions [16]. Continuous monitoring of the leaf gas exchange of field-

grown citrus during several weeks in the spring in South Florida shows the majority of leaves exhibiting midday CER depression on days when the midday VPD is more than 28 mbar and maximum temperature is higher than 31°C [16]. Stable, maximum rates of leaf transpiration existing even with increasing VPD imply a changing stomatal conductance to restrict water vapor loss [16].

In a study with irrigated citrus grown in sunlit, controlled environmental chambers under three dry-bulb temperature (DBT)/VPD regimens, the photosynthetic rate and water-use efficiency are greatest at 24°C/17 mbar treatment and remain high even when the soil water content becomes low [83]. At DBT/VPD levels of 29°C/24 mbar and 37°C/36 mbar, CER and water-use efficiency are reduced, and midday CER depressions occur when the soil water content is low [83]. These findings suggest that midday depression of photosynthesis in citrus, which results in part from stomatal closure at high VPD but perhaps more significantly from increased mesophyll resistance induced by low soil water availability at high DBT/VPD levels, would not occur as long as soil water is easily available [83]. Thus, the rate of water supply to the leaves may be an important factor in mediating the control of stomatal conductance and the resultant midday depressions in CER [16].

SOIL WATER DEFICIT

Soil water deficit, or drought, affects plant growth and metabolism in numerous ways and is the single most important factor limiting crop yield [84,85]. Production of agricultural crops in semiarid and arid areas of the world is heavily dependent on irrigation. Even in normally humid areas, irrigation systems are installed to prevent yield reduction resulting from short dry periods. In Florida, 60% of agriculturally available water is used to irrigate citrus crops [86]. Problems created by drought are critical worldwide and present challenges to plant breeders for long-term solutions to improve yield in dry crop-producing regions. Breeding approaches need to identify drought-tolerant characters that can be genetically transferred to new crop cultivars to enhance drought tolerance and adaptation. A more comprehensive understanding of the metabolic processes affected by water deficit is essential, as are recovery mechanisms once drought stress is relieved.

Among the most prominent effects of drought on plants are the reductions of growth, carbon fixation, photosynthate translocation, protein synthesis, and enzyme activities [57,68,84,87–90]. Of prime importance is the reduction of the leaf photosynthetic rate of plants exposed to drought conditions. Although the pathways and enzymes involved in normal photosynthesis are well defined, the effects of drought on individual processes are not well understood. Exactly how much water deficit affects photosynthesis and its component reactions and the correlation between stomatal function and photosynthetic rates during stress exposure are still presently unclear [91]. In addition, plant responses to the environment also are related to the type of photosynthetic carbon metabolism, as C_3 plants are generally less tolerant to hot, dry conditions as compared with C_4 plants [92].

For many crops, including citrus, an increase in drought stress is followed ultimately by decreasing CER, but the mechanisms contributing to the reduction are incompletely understood. Reduction in CER has been partially attributed to stomatal closure which occurs during drought stress [93,94]. Nonstomatal components, such as decreased enzyme catalysis, also are involved in CER reduction [18,94–100]. In sweet orange, water deficit reduces the catalysis and protein concentration of Rubisco and shifts carbohydrate distribution in the leaves [18,101]. During short periods of drought, reduction in stomatal conductance and leaf transpiration and increases in proline concentration also are commonly observed for citrus [102,103]. Soil water deficit affects both citrus vegetative growth and tree size, as well as fruit yield and quality [104,105].

FLOODING

Temporary or continuous flooding is common in many land areas throughout the world, and soil waterlogging is a major problem in the growth and productivity of many crop plants, including citrus. In Florida, many new planting sites, especially in the south, are vulnerable to soil waterlogging

owing to the low elevation and shallow water table. The prospect of more citrus plantings in southern counties, because of winter freezes and severe losses in north central Florida during the 1980s, significantly increases concerns of anaerobiosis resulting from flooding and its impact on crop growth and productivity. Newer methods of land development that emphasize good drainage do not totally guard against excessively wet summers, and the annual threat of excessive rainfall during hurricanes intensifies the flooding problem in low, flat areas.

Soil flooding inhibits root and shoot growth, depresses root respiration, and reduces leaf photosynthesis in many plant species [106–115]. For citrus, root system deterioration in flooded soils is a major problem [116,117]. Leaves of root-flooded citrus trees show reduced Rubisco activity and dark respiration [117,118], but little is known about photosynthetic carbon metabolism or photosynthate partitioning due to soil waterlogging. There are survival differences among citrus rootstocks subjected to soil flooding [61,117,119]. For example, flooding for 10 days inhibits leaf photosynthetic rates up to 98% in various citrus rootstock seedlings [116]. Hamlin sweet orange trees flooded for 24 days show senescence, wilting, and abscission of leaves; these symptoms are more evident with Hamlin trees grafted on sour orange rootstocks than those grafted on rough lemon rootstocks [117]. Similarly, leaf CER, stomatal conductance, chlorophyll content, and Rubisco activity are significantly reduced in Hamlin trees grafted on sour orange. Dark respiration rates are greatly decreased in the fibrous roots of flooded trees but not in leaf tissues, whereas total nonstructural carbohydrates are higher in leaves (50% for Hamlin on sour orange and 80% for Hamlin on rough lemon) but lower in roots (60% for Hamlin on sour orange and 45% for Hamlin on rough lemon) [117]. This indicates that the selection of rootstocks is critical in reducing the impact of waterlogging.

ELEVATED ATMOSPHERIC CO_2

The global atmospheric CO_2 concentration ($[CO_2]$), presently at about 365 parts per million (ppm), is increasing and is expected to double by the end of the next century [120,121]. The increase in $[CO_2]$ and other ''greenhouse'' gases (e.g., methane, chlorofluorocarbons, nitrous oxide, ozone) may cause global air temperatures to rise, possibly by as much as 3–6°C, and shifts in regional scale rainfall patterns [122–124].

Approximately 95% of terrestrial plants are C_3 species, about 1% are C_4 species and 4% undergo crassulacean acid metabolism (CAM) [125]. The present atmospheric $[CO_2]$ limits the photosynthesis, growth, and productivity of many crop plants, especially C_3 species which could benefit from elevated CO_2 [125,126]. Numerous studies have shown that atmospheric CO_2 enrichment has beneficial impacts on crop growth and yield [127–129].

Elevated $[CO_2]$ enhances the photosynthesis, growth, and yield of citrus crops [81,130–133]. Citrus trees grown at about twice ambient $[CO_2]$ have a greater number of and larger leaves, a higher trunk and branch volume, and a greater fine-root biomass than their ambient-treated counterparts [130,132–135]. Under long-term growth (5 years), fruit production of sour orange doubles in CO_2-enriched trees as compared with ambient grown [133]. Trees of Valencia sweet orange grown under enriched CO_2 also produce more fruit, although similar in size and weight to that of control trees [131].

Rubisco activity is higher in leaves of Swingle citrumelo plants grown at twice ambient $[CO_2]$, a finding not seen in leaf samples from Carrizo citrange [130]. The net CER of citrus, measured at the $[CO_2]$ used for growth, is substantially enhanced by elevated $[CO_2]$ [19,81,131,136]. At elevated $[CO_2]$, the inhibitory effects of high VPD and decreased available soil water on citrus CER are lessened, and the CO_2 assimilation rate does not exhibit the midday depression commonly observed in trees grown under ambient $[CO_2]$ [19]. Elevated $[CO_2]$, in addition, can compensate for the adverse effects of high growth temperature relative to the net photosynthetic rate [81,82], as seen in other crops [137]. In sour orange, the mean daylight photosynthetic rate of the leaves under summer conditions in Phoenix, Arizona, is about 2.2-fold greater for the elevated (700 ppm) CO_2 treatment in comparison with the ambient (400 ppm) CO_2 treatment [81]. Also, there is a negative

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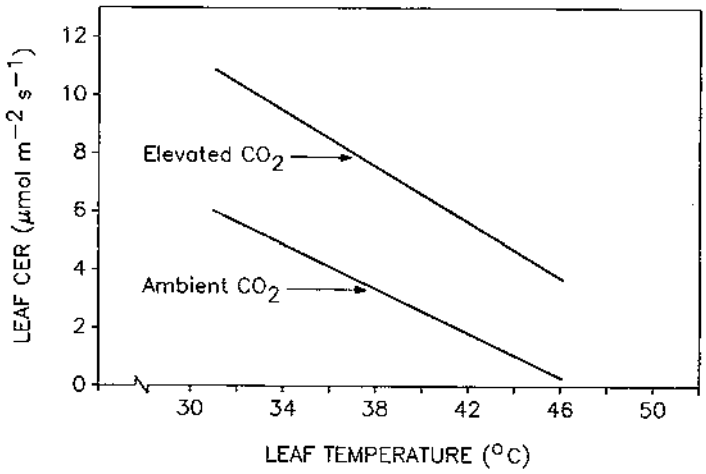

FIGURE 4 Linear relationship between net CO_2 exchange rate (CER) and leaf temperature (T) of foliage of sour orange trees maintained outdoors in Phoenix, AZ, at ambient (400 ppm) (CER = 17.68–0.375 T; $r^2 = 0.737$) and elevated (700 ppm) CO_2 (CER = 25.53–0.472 T; $r^2 = 0.475$). (From Ref. 82.).

linear relationship between the net photosynthetic rate and the leaf temperature from 31 to 47°C for both ambient and elevated CO_2-grown trees (Fig. 4), indicating that this range of temperature is above the optimum for net photosynthesis of this citrus species [82]. However, the degree of enhancement of net photosynthesis by CO_2 enrichment is 75% at a leaf temperature of 31°C, 100% at 35°C, and 200% at 42°C [82]. These enhancements fall in the range of the predictions for an idealized C_3 plant, showing that a rise in temperature from 28 to 40°C increases the degree of enhancement from 66 to 190% when the atmospheric [CO_2] is raised from 350 to 650 ppm [138]. At 47°C, the net photosynthetic rates of sour orange trees grown at ambient CO_2 drop to zero and become negative thereafter, whereas the CO_2-enriched trees still maintain their photosynthetic rates at a significantly high level [82]. Theoretically, a 300-ppm increase in atmospheric [CO_2] could raise the temperature optimum of light-saturated CER of C_3 plants by 5°C [138].

Citrus trees grown under long-term CO_2 enrichment and natural field conditions do not experience the downregulation of the photosynthetic capacity or growth rate [139] that occurs in some other plant species [125]. In a crop canopy, photosynthesis is light limited for all leaves for part of the day and for all of the day for the leaves of the lower canopy [129]. For a citrus canopy, although the absolute benefits of CO_2 enrichment on CER are greatest at high-light intensities, the relative benefits are, however, more significant at low-light levels [140]. The positive direct effect of enriched CO_2 on citrus photosynthesis more than compensates for the negative self-shading effect due to the increased leaf area under elevated CO_2 growth conditions [140].

CONCLUSIONS

Freezing and drought are excellent examples of stress-related agricultural catastrophes that threaten the ability of the world to feed itself. Producing crops under stress conditions is a growing problem in world agriculture, and new strategies are required to improve and maintain world food supplies and nutrition. Citrus, with its high value for nutritional and palatable qualities, will enter the 21st century as a major world crop. A better understanding of the regulatory mechanisms of citrus metab-

olism in response to environmental changes will aid in finding and/or developing cultivars with stress tolerance.

Physiological and biochemical studies have shown a reduction in the photosynthetic capacity owing to environmental stress; however, the mechanisms involved have yet to be identified [141,142]. In recent years, there has been a rapid advance in our understanding of the molecular biology of the metabolic pathways in the photosynthetic carbon reduction cycle [143], and effort has focused on genes encoding key photosynthetic enzymes [144]. Current technical advances offer the opportunity to understand the molecular mechanisms involved in plant stress responses, which will be useful in designing experiments for genetic engineering and breeding to produce stress-tolerant crop species [145].

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Ecophysiology of *Ajuga reptans* L. at the Northern Boundary of Its Distribution

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INTRODUCTION

This chapter provides information on the changes in some morphological and physiological traits of a perennial herbaceous plant *Ajuga reptans* L. (bugle), growing in the taiga zone of the northeastern region of Europe as compared with that growing in the broad-leaved forests of Europe. *A. reptans* L. has recently been shown to contain biologically active substances, known as ecdysteroids [1]. It also is widely used in Europe and North America as a decorative plant for landscape gardening. There are different cultivated varieties of bugle, which are distinguished by the leaf color. The plant is important as a good source of honey in apiaries. Wild *Ajuga* plants grow only in Europe.

Growth conditions in the northern part of bugle's growing area differ significantly by climatic and edaphic factors from those in central Europe. The adaptation of plants to habitat differences occurs via coordinated changes at the morphophysiological, anatomical, and biochemical levels. The acclimatory responses ensure maximal fitness by balancing resource uptake under varying environmental conditions. CO_2 exchange is the major external manifestation of plant metabolism. Hence, investigation of the dependence of photosynthesis and respiration on light, temperature, nutrition, and other factors is important to characterize the plant metabolic activity and adaptability in different environmental conditions [2,3]. Experiments were carried out with the plants growing in the birch-aspen forest floor in the vicinity of Syktyvkar, Komi Republic, Russia. To estimate *Ajuga* plants' interactions with the environment, we studied the growth and CO_2 exchange responses to temperature and light conditions. We also transplanted about 100 rosettes into an open site where they grew for 2 years.

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AJUGA REPTANS L., A RARE AND RELICT SPECIES IN EUROPEAN NORTHEAST FLORA

The present global distribution of plants reflects both their evolutionary history and effects of past and recent climatic conditions. *Ajuga reptans* L. is a nemoral herbaceous plant widely spread almost all over Europe [4]. The northern boundary of distribution of *Ajuga* extends to the middle taiga subzone of the European northeast of Russia. It is a rare and relict species in this region. Nemoral and nemoral-boreal species appeared here during the second part of Atlantic period of the Holocene era (6–7 million years ago) after considerable warming. At that time, broad-leaved forests with heat-loving herbaceous accompanying species moved to the north farther than they currently exist. On the following climate recooling, many warm-adapted species disappeared and boreal forests (dark coniferous taiga) again became dominant in the northern region. Nemoral species, which were adapted to unfavorable environmental conditions, remained here as relicts of the Holocene climatic optimum. The current boundary of many nemoral species extends to the middle taiga subzone and is limited by the Vychegda River [5].

The growing season in this region does not exceed 90–110 days. Spring begins commonly in late April. Frosts can occur at the end of June and at the beginning of August. The mean annual air temperature only slightly exceeds 0°C. The mean January temperature is −15.2°C and the mean July temperature is +16.8°C. Annual precipitation is typically about 650 mm and exceeds evaporation by 30%. Most of the precipitation occurs during the frost-free season. Approximately 40% of the precipitation falls in summer.

Day-length variations of the vegetative period are determined by the location of the northern region up to the latitude of 60° N. At the latitude of Syktyvkar (62°52′ N), the sun is less than 6°C below the horizon in early summer.

Podzolic soils of the middle taiga according to their temperature regimen are classified as moderately cold soils [6]. In winter, the temperature at the soil surface drops to −2°C. Soil temperatures optimal for plant growth (near 15°C) set in the middle of June and persist for about 1 month. Heat deficiency and poor natural fertility of the soil are unfavorable for plant growth.

BIOLOGICAL AND ECOLOGICAL TRAITS OF *AJUGA* PLANTS

Ajuga reptans L. (Lamiaceae) is a herbaceous perennial semirosulate summer-winter green plant with clonal type of growth (Fig. 1). The plant forms runners (stolons) and two generations of leaves [7]. Spring-summer leaves grow on the stolon nodes. Rosulate leaves are formed in summer and most of them overwinter. In the broad-leaved forests of the Temperate Zone of Russia (at the Moscow latitude), *Ajuga reptans* is characterized as a species with a reactive life strategy [8]. The spacing of stolons allows large areas to be rapidly but loosely occupied, and this allows rapid formation of the new vegetative units which separate rapidly from the old ones. *Ajuga* plant shoots are developed dicyclically and flower in the second year of life. Flowering of plants lasts from the middle of May until July and sometimes until the end of August. Plants from the optimum part of the area typically propagate by seeds [8].

In the middle taiga subzone of the European northeast of Russia, *Ajuga* plants are developed polycyclically. A recent study [9] also indicates that the sensitive features of the species decrease and the tolerant features appear. The growth and development of plants are delayed and they remain for longer time in the same area. The average leaf length and the number of runners are 2.0 and 3.5 times less, respectively, as compared with broad-leaved forests (Table 1).

Expression of vegetative reproduction is one of the most remarkable adaptations of *Ajuga* plants to the environmental stress conditions at the northern boundary of its distribution. The individual shoots (ramets) of a clonal plant are produced from the apical buds of runners. The connections

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FIGURE 1 Plant of *Ajuga reptans*. (A) Floriferous shoot. (B) Rooting rosette at the end of runner.

that initially exist between older (mother) and new (daughter) ramets of the plants remain for longer periods than those in the central part of the area.

In the middle taiga subzone, *Ajuga* plants grow in a floor of mixed and parvifoliate forests. It is suggested that nemoral plants cannot compete successfully with spruce roots for nutrients, especially nitrogen [10]. Therefore, plant growth is impeded in the floor of pure coniferous stands. Small-leaved trees, such as *Populus tremula* L., *Betula pubescens* Ebrh., and *Betula pendula* Roth, predetermine (through the leaf fall) more favorable conditions. The soil nitrogen content is higher, and soil acidity is low in such sites.

It should be noted that an elevated ambient CO_2 concentration usually occurs near the soil surface in a forest floor. Carbon dioxide is produced by aboveground plant respiration and by microbial and root respiration from soil, and it is consumed in plant photosynthesis. Depending on the amount of organic matter in the soil and its temperature and moisture, the soil can be the main source of CO_2 for the forest floor vegetation. Elevated CO_2 is expected to increase leaf photosynthesis of the forest floor herbs under both cases: when they are shaded from direct sunlight, and when sunflecks penetrate under the forest canopy [11]. Bugle has a prostrate growth form. This allows it to photosynthesize at 30–50% higher CO_2 concentration in the forest floor, especially in the early morning [12]. *Ajuga* plants apparently use a substantial amount of CO_2 that has been respired by other vegetation and soil organisms as a substrate for photosynthesis.

The available data characterizing the physiological traits of *Ajuga* plants growing in the central

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Table 1 Characteristics of *Ajuga reptans* Plants in Different Parts of the Area of Distribution (Midsummer)

Part of the area	Index	Reference
	Total plant mass, g DW	
North	0.9–1.2	Our data
Center	3.0–3.5	8
	Length of runner, m	
North	0.20–0.45	9
North	0.30–0.38	Our data
Center	0.30–0.50	8
	Runners per plant, n	
North	1–2	9
North	2–3	Our data
Center	3–7	8
	Net photosynthesis, $\mu molCO_2\ m^{-2}\ s^{-1}$	
North	1.5–3.0	Our data
Center	4.0–6.0	13
	Chlorophyll, $g\ m^{-2}$	
North	0.215 ± 0.005	Our data
Center	0.440 ± 0.010	14
	Leaf carbohydrates, $mg\ g^{-1}$ FW	
North	35	Our data
Center	75–85	13
	Leaf 20-hydroxyecdisone, $\mu g\ g^{-1}$ DW	
North	24 ± 1	15
Center	28 ± 5	1

part of the area of distribution are very scarce. Bachmann et al. [13] studied photosynthesis and seasonal carbohydrate changes in different parts of *A. reptans* Atropurpurea in the Zurich area. In *Ajuga*, significant seasonal variations in soluble nonstructural carbohydrate levels in aboveground-grown plant parts and the predominance of RFO (raffinose family of oligosaccharides) were found throughout the whole year. RFO was lowest in summer (75 mg g^{-1} fresh weight) and highest in fall/winter (200 mg g^{-1} fresh weight). The maximal net photosynthetic rate attained 6 μmol CO_2 m^{-2} s^{-1}. The temperature optimum of net photosynthesis was decreased (from 16 to 8°C) during cold treatment. According to Masarovicova [14], the chlorophyll content was about 0.44 g m^{-2} for *Ajuga* plants growing in a temperate hardwood deciduous forest in southeast Slovakia. Our studies show that at the northern boundary of distribution, *Ajuga* plants have twofold less photosynthetic rates, chlorophyll content, and total carbohydrates than those in the central part of the area. The phytoecdysteroid content, products of specialized metabolism, is equal to 24 μg g^{-1} [15] and close to that cited for plant growing in Spain [1].

TEMPERATURE RESPONSE OF NET PHOTOSYNTHESIS

Photosynthesis is one of the most temperature-sensitive aspects of growth. The study of the temperature dependence of net photosynthesis (P_N) indicated that, in midsummer, the leaves of *Ajuga* plants were able to uptake CO_2 intensively at a temperature lower than 10°C (Figs. 2 and 3). We failed

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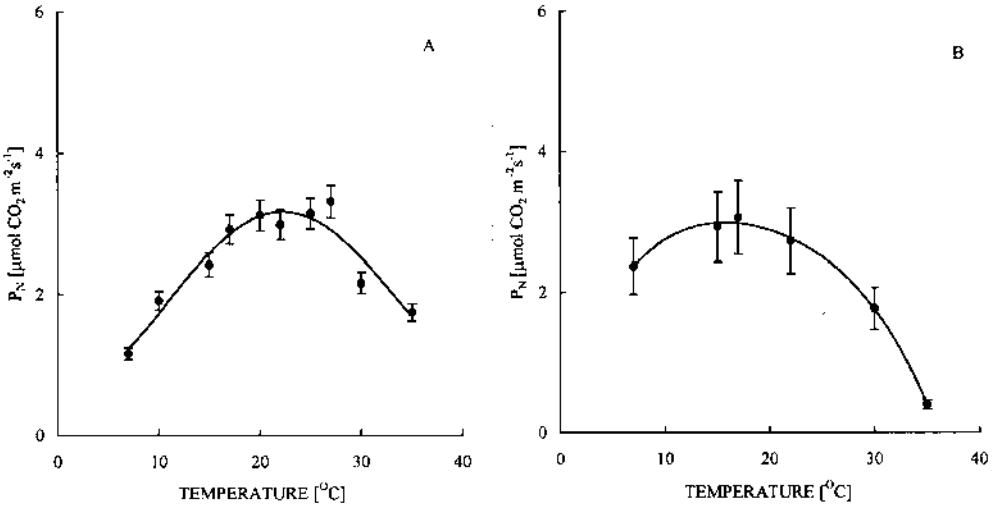

FIGURE 2 Temperature response curves for CO_2 uptake in the leaves of *Ajuga* plants. (A) warm and dry (July 1995) and (B) cool and wet (July 1996) seasons.

to determine the lower temperature limit of photosynthesis, because the temperature in the leaf chamber could only be lowered to 5°C. At 5–7°C, the rate of net photosynthesis was equal to about 60% of the maximum values of P_N. Maximum values of P_N were measured in the temperature range of 15–25°C. In a cool and wet season (1996), the zone of optimum temperature of photosynthesis was 12–22°C (Fig. 2B). In a warm and dry season (1995), the optimum temperature for net photosynthesis shifted to higher temperatures; that is, to 18–26°C (Fig. 2A). The rate of leaf photosynthesis was considerably depressed at temperatures higher than 30°C, especially in a cooler summer.

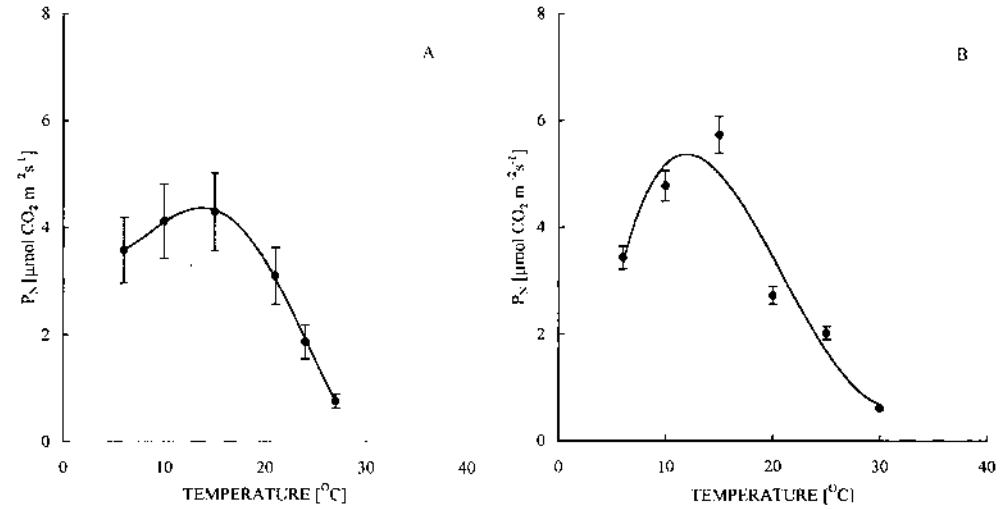

FIGURE 3 Temperature response curves for CO_2 uptake in overwintered (A) and new-grown leaves (B), June 1997.

At the beginning of summer, new-formed rosulate leaves showed maximum values of P_N at 10–15°C (see Fig. 3B). Only small differences in the optimum temperature and rate of CO_2 uptake at optimal temperatures between new-grown and overwintered rosulate leaves were obtained at this time (see Fig. 3A). Overwintered leaves commonly died by the end of June. New rosulate leaves were able to uptake CO_2 after the first frost in autumn. Leaves collected from *Ajuga* plants after the average daily temperatures near −2°C at the end of October were able to photosynthesize with a rate of about 0.5 μmol CO_2 m^{-2} s^{-1} at 5–7°C.

The optimum temperature for photosynthesis is a genotype-dependent characteristic, and it is modified by environmental conditions. One characteristic of the stress-tolerant species is a strongly developed ability to acclimate photosynthesis, particularly in fluctuating temperatures [16,17]. Our data have shown that *Ajuga* plants had an ability to acclimate thermally their photosynthesis. Plants demonstrated a relatively flexible photosynthetic apparatus which could respond to changing temperatures during the growing season. The photosynthetic apparatus of this species is quite well adapted to the moderate temperatures of the growing season. The optimum temperature of leaf net photosynthesis was comparatively low in *Ajuga* plants.

LIGHT RESPONSE OF NET PHOTOSYNTHESIS

Light is one of the most important factors for plants. Light influences plants as an energy source and as a medium to transfer information from the environment to the plant. Photosynthesis is a major physiological process depending on the light conditions of plant growth. Natural selection to the light environment may favor plants whose physiological and morphological characteristics tend to maximize the effeciency and productivity of their photosynthesis [18].

At the northern boundary of its area of distribution, wild *Ajuga* plants grow commonly in the floor of the birch-aspen forest, where light environment is characterized by a low level of photosynthetically active radiation (PAR) and reduced red/far-red ratio. Approximately 5% or less of light penetrates under the forest canopy in summer. Our measurements of the leaf P_N were conducted in a wide range of irradiance from dark to 1800 μmol m^{-2} s^{-1} PAR. In addition, the content of pigments and nitrogen in the leaves were measured. The light-harvesting complex (LHC) chlorophyll (Chl) contents were calculated as described by Lightenthaler [19]. Plant growth was monitored by measuring biomass accumulation.

Shade plants were distinguished by low biomass and slow growth (Table 2). They had 25–35 leaves per plant. A high total Chl content low ratio chlorophyll a/chlorophyll b (Chl a:b) and high contents of the LHC per leaf area were found in these leaves. The leaves had low values of light compensation point and adaptation irradiance. The photosynthetic rate at saturating irradiance (P_{Nsat}) and dark respiration (R_D) were equal to 1.8 and 0.24 μmol m^{-2} s^{-1}, respectively. The rate of net photosynthesis at adaptation irradiance was 2.5 times lower than P_{Nsat}. The low Chl*a*:*b* ratio, high percentage of Chl belonging to the LHC, and values of the key parameters of the light-response curve of photosynthesis showed the high-shade tolerance of *Ajuga* plants.

Various acclimatory traits were determined in the plants transplanted from the forest understory (shady environment) into an open site (sunny environment) (see Table 2). A month after transfer, all leaves were dropped and new leaves were grown. A year after transplantation, each plant had 70–100 leaves. Their mass and area were 20 and 10 times, respectively, greater than those in the shady environment. Sunlight had a significant impact on the leaf area ratio and specific leaf area. But the leaf weight ratio and root/shoot ratio did not change significantly, and showed no acclimation on the basis of biomass allocation.

The light curves of photosynthesis were modified by a high-irradiance environment. The values of the light compensation point and the adaptation irradiance increased twofold. The growth of plants in a sunny environment led to an increase in photosynthetic and respiratory rate in leaves. But we found no significant impact of light conditions on the respiration/photosynthesis ratio. This ratio was near 0.15 or near 0.37, if the P_N value at saturating irradiance or at adaptation irradiance

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TABLE 2 Characteristics of *Ajuga* Plants Acclimating to Shady and Sunny Environments

Characteristics	Shade	Sun
Physiology		
1. Net photosynthetic rate at saturating irradiance, P_{Nsat}, $\mu molCo_2\ m^{-2}\ s^{-1}$	1.8 ± 0.1	2.8 ± 0.3[a]
2. Net photosynthetic rate at adaptation irradiance, P_{Nai}, $\mu molCO_2\ m^{-2}\ s^{-1}$	0.7 ± 0.1	1.2 ± 0.1[a]
3. Dark respiration rate, R_d, $\mu molCO_2\ m^{-2}s^{-1}$	0.24 ± 0.04	0.48 ± 0.02[a]
4. Photosynthetic CO_2-fixation capacity, PFC, $\mu molCO_2\ \mu mol^{-1}$ (chl a+b) $s^{-1} \times 10^{-4}$	75 ± 5	153 ± 17[a]
5. Saturating irradiance, I_S, $\mu molCO_2\ m^{-2}\ s^{-1}$	Close to 200	Close to 200
6. Adaptation irradiance, I_A, $\mu molCO_2\ m^{-2}\ s^{-1}$	20.1 ± 1.8	42.4 ± 2.2[a]
7. Compensating irradiance, I_C, $\mu molCO_2\ m^{-2}\ s^{-1}$	6.0 ± 0.4	11.9 ± 1.2[a]
Biochemistry		
8. Chlorophyll a content, Chla, $\mu mol\ m^{-2}$	167 ± 5	136 ± 3[a]
9. Chlorophyll b content, Chlb, $\mu mol\ m^{-2}$	72 ± 4	47 ± 4[a]
10. Chlorophyll a + b content, $\mu mol\ m^{-2}$	239 ± 6	183 ± 5[2]
11. Chlorophyll *a*:*b* ratio, Chl*a*:*b*	2.3 ± 0.2	2.9 ± 0.2
12. Carotenoids content, Car, $\mu mol\ m^{-2}$	51 ± 4	58 ± 2
13. Chlorophyll:carotenoids ratio, Chl:Car	4.7 ± 0.2	3.2 ± 0.2[a]
14. Chlorophyll of light-harvesting complex, LHC-Chl, % total Chl	66	56
15. Content of N, % leaf DW	2.8	1.3
Assimilation and allocation		
16. Total plant mass, g DW	0.9 ± 0.1	25.0 ± 3.5[a]
17. Leaf mass per plant, g DW	0.48 ± 0.8	11.4 ± 2.4[a]
18. Leaf weight ratio, LWR, $g\ g^{-1}$	0.51 ± 0.11	0.46 ± 0.12
19. Leaf area per plant, m^{-2}	0.024 ± 0.004	0.240 ± 0.010[a]
20. Specific leaf area, SLA, $m^2\ g^{-1}$	0.040 ± 0.007	0.020 ± 0.004[a]
21. Leaf area ratio, LAR, $m^2\ g^{-1}$	0.025 ± 0.005	0.010 ± 0.002[a]
22. Leaf number per plant	29.1 ± 3.3	93.2 ± 6.8[a]
23. Shoot number per plant	2.5 ± 0.8	17.1 ± 1.5[a]
24. Root:shoot ratio, $g\ g^{-1}$	0.12 ± 0.03	0.12 ± 0.03
25. Daily productivity of photosynthesis, PP_N, g/(plant day)	0.0240 ± 0.0001	0.44 ± 0.01[a]
26. Respiratory losses, R, g/(plant day)	0.0150 ± 0.0002	0.45 ± 0.01[a]
27. Gross photosynthesis, P_g, g/(plant day)	0.0310 ± 0.0002	0.67 ± 0.01[a]
28. Net assimilation, NAR, $g/(m^2\ day)$	0.46 ± 0.10	0.65 ± 0.20
29. Relative growth rate, RGR, g/(g day)	0.012 ± 0.001	0.006 ± 0.002
30. R/P_g ratio	0.480 ± 0.001	0.670 ± 0.005[a]

[a] Significant differences at $P \leq .05$.

Note: Leaf net photosynthetic rate (P_N) was measured by an open system with an infrared analyzer as described in detail recently [17]. Leaf chlorophyll (Chl) and carotenoids (Car) contents were determined at 662.0, 644.0, and 440.5 nm spectrophotometrically after extraction by 100% boiling acetone. Total nitrogen (N) was measured with an automatic analyzer ANA-1500. Total nonstructural carbohydrate (TNC) content was analyzed according to Pochynok [20].

Means and standard errors are presented.

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were used for calculation, respectively. The chlorophyll content, as well as Chl*a*:*b* ratio and chlorophyll/carotenoids ratio were modified by sunlight conditions. Both Chl *a* and *b* contents per leaf area decreased and their ratio increased. Although there were no differences in the content of carotenoids between leaves of plants grown in the shade and those grown in sun, the chlorophyll/carotenoids ratio was lower in sunny plants. It is possible that the function of carotenoids in the leaves of plants grown in the sun was related more to the protection of their photosynthetic apparatus against exceeding light energy, whereas in the leaves of shade plants it was related more to the absorption and conversion of light energy.

The data show a twofold difference in the nutrient-use efficiency (NUE), as an amount of CO_2 assimilated per unit of nitrogen content, in the leaves of sun-grown and shade-grown plants. The low NUE of shade-grown plant leaves was related to their high nitrogen content rather than to the low photosynthetic rate. There are reasons why plants growing in the forest floor can accumulate more nitrogen: (a) the ground cover is rich in humus, (b) plants grow slowly, and (c) leaves contain more chlorophyll. The leaf chlorophyll content is a stable informative parameter for the evaluation of soil nitrogen uptake at different growth conditions [21]. In turn, a high nitrogen content can reflect the accumulation of pigments in the leaves of shade-grown plants.

Our data show that *A. reptans* is a shade-enduring plant also capable of growing at a high irradiance. Since at significant differences in the light environment the morphophysiological parameters of the photosynthetic apparatus were changing within a narrow range, it can be concluded that the shade tolerance is a genetically determined trait of *Ajuga* plants, and a weak trade-off exists between the ability to achieve high photosynthetic activity in the sun and the ability to survive in the shade.

VEGETATIVE REPRODUCTION AND CARBON BALANCE

Northern environmental pressures led to the dominance of vegetative reproduction. It is one of the most remarkable adaptations of *Ajuga* plants growing at the northern boundary of its distribution. There is a great interest in clonal growth owing to its importance in ecological and evolutionary consequences. *Ajuga* plants produced more ramets and they were distinguished by intensive growth in a more favorable light environment. The number of runners and their biomass were 7 and 25 times higher in the sun-grown plants than in those grown in the shade (see Table 2). Since the root/shoot ratio was 0.12 in both shade- and sun-grown plants, we can assume that distribution of the plant biomass strongly depends on genotype.

The accumulation of the plant biomass results from an interaction of the photosynthetic assimilation of CO_2, respiratory losses, and growth. A quantitative investigation of the carbon balance parameters based on the CO_2 exchange measuring showed that the daily photosynthetic productivity (PP_N) correlated with the leaf area and was by a factor of 10 higher in the sun-grown plants (see Table 2). Absolute respiratory losses (R) and gross photosynthesis (P_g) accompanying the greater net photosynthetic productivity were higher in sun-grown plants. Conversely, the relative growth rate (RGR) decreased with the increasing leaf area and was twofold lower in the sun-grown plants than in the shade-grown ones. The growth rate is controlled by the leaf area rather than the net assimilation rate (NAR). The NAR was fairly constant under different environmental conditions.

The increase in respiratory losses (R) in sun-grown plants had a direct effect on their R/P_g ratio (0.67), which was 30% higher than that is shade-grown plants. The decrease in RGR in the sun-grown plants resulted from the reliable biomass accumulation and the rise of respiratory losses for maintenance.

A positive carbon balance is regarded as a criterion of adaptation [22]. At adaptation to high-light conditions, *Ajuga* plants maximize the net photosynthetic carbon gain, which promotes the formation of new ramets and successful vegetative reproduction.

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SUMMARY AND CONCLUSIONS

We found that the variance of *Ajuga* characteristics depends on different habitat conditions. At the northern boundary of its distribution, this species is represented by the ecotype which is characterized by slow growth and development, as well as by the predominance of vegetative reproduction over seed multiplication. Comparatively, a low leaf photosynthetic rate of the shade-grown plant is the main factor limiting plant growth in the forest floor. As *Ajuga* plants have been transferred from the forest understory to an open site, higher CO_2 uptake promoted an increase in the leaf area and number of stolons. Vegetative reproduction predominating in northern conditions provides for considerable success through a lower reproductive cost, because stolons carry out both photosynthetic and multiplication functions simultaneously. The ability of the *Ajuga* northern ecotype in a temperature range of 10–15°C to photosynthesize closely to the maximal possible intensity is the most important component of the plant adaptation in a cold climate. Light appeared to be more critical for growth than temperature in determining the plant biomass in northern conditions.

Ajuga reptans L. has a prostrate life form and a creeping type of growth, which allows it to benefit from leaf fall and snow cover, thus alleviating the effect of low temperatures in winter. Also, accumulation of osmotically active compounds (sugars, free amino acids) contributes to frost resistance by decreasing the freezing point of the plant tissues. This is especially important for overwintering leaves.

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Adaptation of Plants to Anthropogenic and Environmental Stresses: The Effects of Air Constituents and Plant-Protective Chemicals

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INTRODUCTION

Although precise calculations and detailed evidence are still lacking with respect to many parameters, it is generally ''expected'' that the earth's climate, in general, and the average temperature of the atmosphere, in particular, will increase within the decades and centuries to come. During the last 100 years, an increase in the mean values which have been registered worldwide has been observed already and amounts to about 0.7%. According to the actual state of science, the forms of air pollution which affect the environmental climatic conditions include carbon dioxide, nitrogen oxides, photooxidants, especially ozone, dusts, which often contain heavy metals, acid precipitation, and organic compounds [1,2]. Recently developed models deal with the participation of sulfur compounds and aerosols which might counteract the increasing greenhouse effect. It is discussed in this context that sulfur particles which derive from the oxidation of fossile fuels function like a type of mirror and reflect large amounts of the irradiation from the sun. As a second effect, it has been proposed that, in combination with clouds, the sulfur particles might alter the size of the aqueous drops thereby influencing the washing out of air pollutants, micro particles, and aerosols from the atmosphere. In any case, it might be necessary to use sulfur-containing aerosols as a correction factor for the calculation of the expected increase in the average temperature. Under the assumption

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that the amount of emitted components of any type is not changed, an increase in the average temperature of 3°C in the next 100 years was calculated taking into account the absence of sulfur-containing aerosols, whereas a prediction of 2°C was the result when these additional parameters were included in the calculations. In order to illustrate the complexity of the problem, it should be mentioned that, in many cases, modern computer processors need several months to calculate the respective values, and that not even a change in the solar radiation intensity can be reliably excluded. (From paleoclimatic studies, it is known that about 6000 years ago this radiation was in fact slightly more intense than it is today, but it is not clear whether such alterations and ''periodicities'' might really suffice for the problem in question.)

Without any doubt, CO_2 is the most prominent gas in connection with the temperature on earth, and this also can be derived from the conditions on our neighboring planet, Venus, with its extremely dense atmosphere, a carbon dioxide partial pressure of 95%, and a homogeneous temperature of 470°C. (On Mars, the dilute atmosphere results in a temperature range between −10 and −150°C whereas on Mercury—with virtually no atmosphere at all—temperature changes between +350°C during the day and −180°C during the night have been calculated!) For this planet, it has been calculated that CO_2 contributes 75%, CH_4 about 24.5%, and N_2O about 0.2% of the warming expected in the 100-year period beginning in 1993 [3], but many more parameters have to be included. For example, methane and N_2O are several times more radiatively active than CO_2 [4]. The estimated emissions from vegetation burning in the subcontinental African countries are 0.5 Tg CH_4, 14.9 Tg CO, 1.05 Tg NOx, and 1.08 Tg of particles smaller than 2.5 μm. The 324 Tg CO_2 emitted is expected to be reabsorbed in the following years [5]. Senegal's total emissions are estimated at 17.6 Tg ECO_2. The major gases emitted are CO_2 (61%) followed by CH_4 (35%) and N_2O (4%) [6]. Nigeria is one of the 13 low-latitude countries with significant biomass-burning activities; trace gas emissions were estimated to be 300 Gg CH_4, 2.4 Gg N_2O, and 24 Gg NO_x. CO_2 emissions from burning, decay of the biomass, and long-term emissions from soil amounted to a total of 125,561 Gg [7]. The emissions from motor vehicles in the northwest of England make up 52% of the NO_x emissions, whereas those from fossil fuel–fired power stations make up 20% and 58% of SO_2 emissions [8]. The largest contribution to NH_3 emissions is from cattle, but humans may contribute some NH_3 [8]. With the emission of HCl from the oxidation of polyvinylchloride (—CH_2—CHCl—) in waste-burning industries as an example, one might conclude that deposition of pollutants is heaviest in the immediate vicinity of the source; however, new forms of forest damage appear to show up even in so-called clean-air zones far from any polluting source [1]. The transport of air pollutants leads to a widespread distribution and can entail both chemical and physical transformations at different sites.

In general terms, it can be concluded that the effects of pollutants on the global climate does not necessarily show up as a dramatic and immediate negative impact. It is rather discussed that long-term influences with initial time lags—even intermediate positive effects—and only subsequent negative effects have to be assumed [9]. Observations like premature aging is a typical nonspecific stress symptom and reflects something like an alarm phase of the stressed plant; in the young parts of the plant, this is coupled with more or less efficient resistance processes which might work until the continued stress situation leads to definitive damages and to premature senescence of the whole plant [10]. However, the influence of air pollution on flora and fauna varies in intensity according to the ecosystem involved [1]. Analyses of the significant forest damages realized since 1983 in European countries shows that, besides conifer species, broad-leaved forest trees also appear to suffer from the stress conditions—in particular, alterations in the crown morphology, damages of foliage, and changes in the pigment content are among the relatively unspecific symptoms. On the other hand, many of the conclusions with respect to the details of the reaction mechanisms in this context turned out to be somewhat hasty—at least for such complex phenomena. As an example, the amount to which Al^{3+} ions contribute as a phytotoxic agent to the forest decline is far from clear.

It is obvious that the responses of plants (e.g., conifers) to air pollutants (ozone, sulfur dioxide,

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nitrogen oxides) are strongly influenced by additional factors like temperature, light intensity, water availability, and the overall nutrient content of the soil [11]. The microbial activity and the humus content influence both the carbon and the nitrogen cycles in forest soils [12]. When air pollutants decrease the photosynthetic activity, they appear to alter the structure of the chloroplasts as the most prominent cellular target [13]. Therefore, in young Norway spruce trees fumigated with nitrogen oxides, no damage was found and the trees' ability to fix carbon dioxide was even increased. Following the application of SO_2, there was a substantial decrease in photosynthetic activity, but as soon as the fumigation was stopped, the effect was reversed. The effect of ozone, however, was not reversible [14].

The plum poxvirus strongly influenced the level of economic feasibility and was in turn affected in its toxicity by various exhalations. The production of trees has no economic value to exceed in regions attacked by strong acid emission with the predominant component SO_2, NO_x, etc., acid emissions with ash and compounds of fluorine and chlorine, acid emissions with metallurgical ash, acid emissions with a significant proportion of organic matter, as well as by alkaline magnesium emissions [15]. However, a low or zero negative effect was observed when no industrial fertilizers were applied on evaluated trees and where calcium is predominant in the exhalates, i.e. in the ''alcalic-calcium'' emission types. Accordingly, Vanek, et al. [15], presumed that the deharmonization of ecology causes a restriction, almost a loss of the induced plant resistence to pathogens which renders these plants inadequate for production purposes. Foliar application of kinetin and ascorbic acid has minimized the foliar injury in *Oryza sativa* L. cv. GR 3 grown near a fertilizer plant which emits SO_2, NH_3, NO_2, and F as major air pollutants, thereby improving the photosynthetic leaf area, and increasing the number of panicles with high total dry standing crop. However, unfavorable climatic factors acted as an additional stress to hamper the productivity [16]. The reduced growth of mycorrhizal and nonmycorrhizal fine roots at the pollution (SO_2, NO_x, alkaline fly ashes)–impacted sites is seen as an adaptation mechanism of the root system to high nutrient inputs [17]. Buecker and Ballach [18], however, stated that the energy normally needed for growth and the development of frost hardiness is used for maintenance purposes and to repair damages (an air pollution–dependent demand for energy and carbon), which may be reflected in an increase in the susceptibility to freezing damage and a decrease in growth. Another point to be described in this context is the sensitivity of lichens to gaseous pollutants, which has been the subject of a number of experimental approaches in recent decades [19]. Although fumigation experiments have so far been concentrated mostly on the effects of sulfur dioxide, Loppi, et al. [20], however, pointed out that changes in lichen frequencies, as bioindicators of air quality, are largely determined by nitrogen oxides.

The effects of modern plant-protective chemicals on the physiology and on the metabolism of plants are often neglected even in modern experimental approaches. Generally, it is assumed that pesticides—other than herbicides—only have an effect on the respective target organisms without influencing the host plants. At this point, it should be emphasized that plants normally come into very close contact with, for example, insecticides, fungicides, nematicides, and that this might well represent serious problems. Following the application of a liquid-formulated plant-protective chemical, the solution or suspension dries and remains for a relatively long time on or near the plant, with all the implicated problems for the soil, the water, and the environment as a whole. Another very important aspect, however, is the question whether and how plants are affected or injured by the enormous amounts of substances which are used in modern agriculture. To date, there is little information about this point; few investigations dealt, for example, with the effects of the insecticides Thiodan (Hoechst), Carbaryl (Union Carbide), and other pesticides on the glycolipid and phospholipid contents of maize seedlings [21], but extended work on this subject is almost completely lacking. Therefore, we refer in this context to investigated details of the effects of synthetic pyrethroid insecticides with different molecular structures on higher plants—not least under the applied aspect that such analyses might help in the selection of efficient but less phytotoxic chemical products with less environmental problems [22].

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Carbon Oxides

Carbon Dioxide

Since the beginning of modern industrialization, the carbon dioxide partial pressure of the atmosphere has constantly increased, and this process appears to continue. Projection of the observed development for the next 50 years suggests that the carbon dioxide content of normal air will increase from the actual 340 ppm to about 700 ppm CO_2 or more. This increase will cause an additional ''greenhouse effect'' which will have enormous abiotic consequences on the general climate. The resulting gradual increase in the average temperature will entail further liberation of carbon dioxide from lakes and oceans, as the solubility of gases in liquids is strictly temperature dependent. Initial biological effects will surely impact plants (and only secondarily animals and human beings), because carbon dioxide, in contrast to the so-called air pollutants, is unproblematic for animals and humans, whereas plants, on the other hand, virtually always live under a strict limitation of this gas, because it is the necessary carbon source for carbohydrate formation. In terms of agricultural efficiency, the questions of how crop plants react to an elevated carbon dioxide content of the atmosphere and also how weeds perform under such altered conditions are of enormous both scientific and economic interest.

As C_4 and crassulacean acid metabolism (CAM) plants possess an internal CO_2 concentration mechanism, an impact of an increase in CO_2 concentration on plants is first of all expected for C_3 plants. Also, however, C_4 plants appear to react to alterations in the carbon dioxide partial pressure [23,24]. In recent years, an increasing number of publications is dealing with this problem which might have on a long-term level in particular both positive and negative implications. Many papers have been published thus far describing the details of the behavior of plants in reaction to an elevated carbon dioxide partial pressure. In conclusion, from these reports, we have learned that an increase in the CO_2 concentration is (in the first instance) largely beneficial for C_3 plants. For rice, an optimal carbon dioxide content of even 1500–2000 ppm has been calculated with respect to growth and yield of the plants [25]. In relation to the number of investigations on the effects of carbon dioxide (or other components of the air) alone, only scattered reports are available at present on the possible (and expected) joint effects of carbon dioxide together with other air constituents or pollutants. This is certainly due to the fact that such complex investigations pose enormous specific problems in terms of definite and unequivocal interpretations because of the complexity of the experimental approaches. Comprehensive and informative reviews on the effects of ozone on plants have been published (e.g., see Refs. 26–28).

When normally grown C_3 plants are analyzed under a 700-ppm CO_2 gas atmosphere, it is generally observed that the plants perform better as far as the overall photosynthesis, growth and a variety of parameters like, for example, biomass production, plant height, fresh and dry masses, are concerned. The experiments have been carried out with different crop plants such as tobacco, cowpea, sweet potatoe, tung-oil tree, and others [29–32]. Even the increase in the production of lateral branches has been investigated in the cases of white pine and crab apple seedlings [33,34]. The number of stomata increased [29], whereas the stomatal aperture was clearly reduced in the case of maize in a short-term experiment [35]. For stomata, however, long-term adaptation processes also have to be taken into account [36]. Similar adaptation phenomena have been described for the increased P_N values which are observed under conditions of high CO_2 concentrations but return to normal with time [37]. Details on the mechanisms of this ''backreaction'' have been proposed but are still unclear [38]. Respiratory processes also appear to be affected by high CO_2 values; in the case of *Eucalyptus*, a decrease of the dark respiration rate to about one half together with the concomitant increase of P_N has been described [39].

It should be noted, however, that in a variety of cases no or only minimal effects were described. Thus, in the case of soybean, the plant height appeared to be more or less unaffected by the elevated CO_2 concentration [40]. However, not only the impact of an elevated carbon dioxide partial pressure on crop plants has to be considered; the predicted alteration in the environmental

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conditions will also affect growth and photosynthesis of weeds, thereby indirectly influencing growth rates and efficiencies of agricultural crop plants. Beside the suggested stimulation of photosynthesis of C_3 plants and the reduced opening of stomata and the increased water-use ability of both C_3 and C_4 plants, the control of perennial weeds might become more difficult, as an increased photosynthetic rate also enhances the production of rhizomes as storage organs. In this context, not only the rate of individual survival but also the distribution of certain weeds and weed families in different regions might be altered [41]. Also, the interrelationship between a stimulated accumulation of mineral substances by a high CO_2 partial pressure and the need for and the doses of fertilizer applications are and will be of agricultural interest [42]. This aspect might be of particular interest not least because the nitrogen supply in particular will be influenced. If one assumes a substantial increase in photosynthetic power, it is easy to predict that the C/N ratio of plants will be one of the problematic points. The necessity of a higher nitrogen supply either by fertilizers or by symbiotic nitrogen-fixing organisms might be a consequence of agricultural reality [43]. Figure 1 summarizes the most relevant interrelationships between carbon dioxide and plant activities showing that via direct and indirect effects, a multitude of physiological and metabolic processes in plants are linked to the partial pressure of carbon dioxide.

Until now, it could not be predicted that an increased CO_2 partial pressure of the atmosphere would entail an elevated carbon dioxide content of the soil, thus affecting parameters like, for example, root respiration. Recent investigations, however, deal with the fraction of carbon required for root respiration and other processes [44]. In any case, it is obvious that an increased photosynthetic activity based on a higher carbon dioxide partial pressure in the atmosphere will also (directly or indirectly) lead to an elevated carbon content of the roots which in turn might influence mycorrizal symbioses. It has been reported that under such conditions, for example, the uptake of phosphorus is increased, whereas the nitrogen uptake remained unaffected. Apparently, changes in the activity of uptake processes have to be regarded here, as the overall mycorrhizal colonization inside the roots did not increase [45].

One of the striking observations in this context was the result that apparently photorespiration,

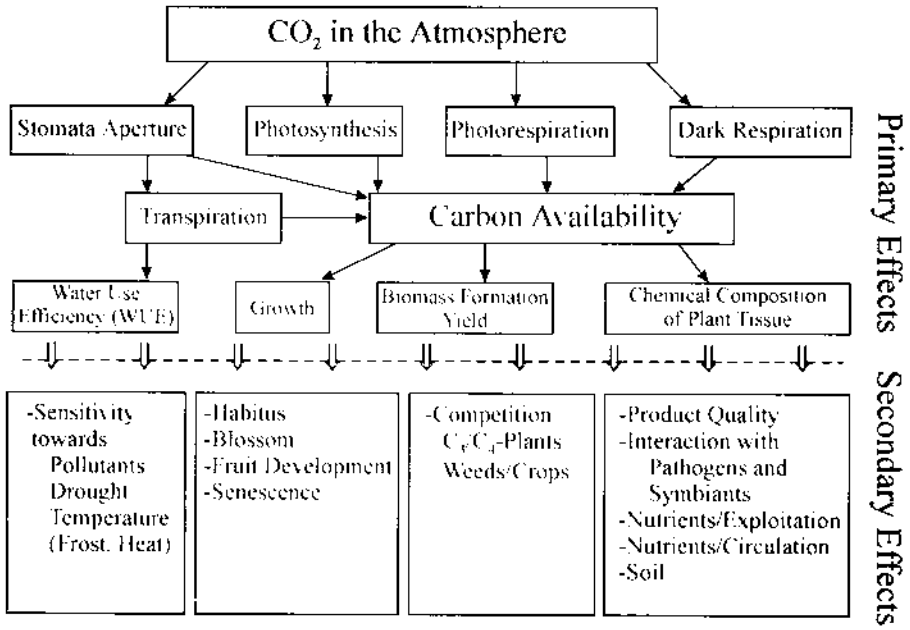

FIGURE 1 Summary of primary and secondary effects of an elevated carbon dioxide partial pressure in the atmosphere on plants. (Modified from Ref. 26.)

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which is still a somewhat unclear phenomenon, is not necessarily reduced under the conditions of an elevated carbon dioxide partial pressure. Via the bifunctionality of the ribulose bisphosphate carboxylase oxygenase (Rubisco), the enzyme also reacts with oxygen and not exclusively with carbon dioxide, thereby having an oxygenase activity besides a carboxylase function and ''counteracting'' the photosynthetic yield (to about 50%) by the formation of phosphoglycolate and phosphoglycerate instead of two molecules of phosphoglycerate. Consequently, it had been expected earlier that the amount of photorespiration of crop plants would decrease or even disappear when breeding experiments would have selected versus high yields exclusively. Today, we know that this is not the case, and that mutants with artificially lowered photorespiration showed even substantially impaired activity as soon as photorespiratory conditions prevailed (e.g., dislocation from low to normal oxygen partial pressure values of the test plants). In many cases, photorespiratory conditions even turned out to be lethal for such mutants. One of the principal technical problems in context with this and similar gas exchange reactions being composed of simultaneous but counteracting partial reactions like, for example, the oxygen uptake from respiratory processes and oxygen evolution derived from photosynthetic water oxidation is that many conventional analyzing techniques cannot discriminate between these two phenomena. This leads to the ''principal falsification'' of the quantitation, as only the net difference between the two rates is determined (Fig. 2).

This problem can, however, be overcome by mass spectrometric analyses with the application of appropriate gas isotopes [31]. Under the assumption that a reaction assay is composed of normal

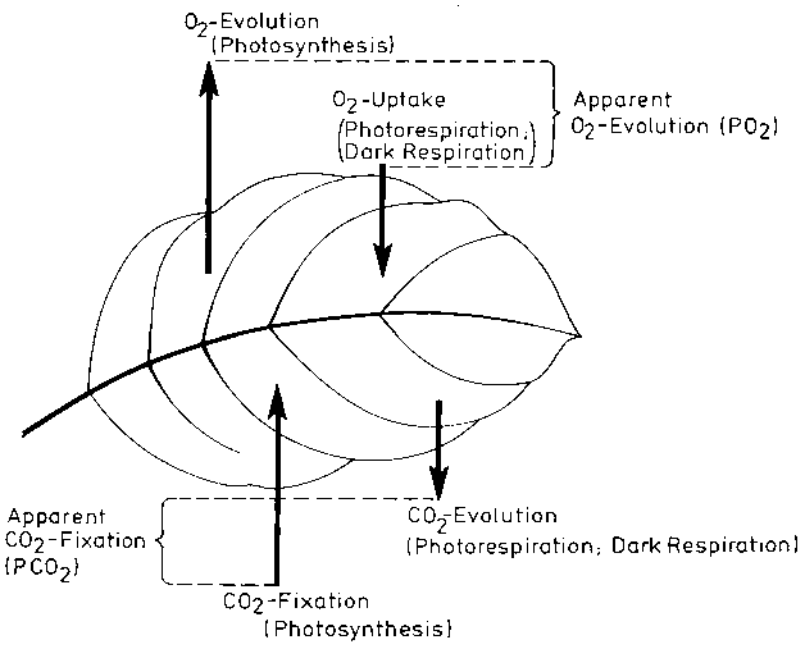

FIGURE 2 Schematic representation of the specificities of carbon dioxide and oxygen gas exchange reactions measuring intact leaves from higher plants, cell cultures from higher plants, algae, or cyanobacteria. The overall gas exchange is in any case composed of two counteracting parts; namely, uptake and evolution reactions. Thus, conventional electrodes can only measure the respective differences between the partial reactions. Mass spectrometry with the application of appropriate isotopes can, however, discriminate between the two partial reactions so that independent but simultaneous measurements (e.g., of photorespiration and photosynthesis) are possible (cf. Fig. 3).

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water (i.e., $H_2{}^{16}O$) and the (artificial) gas atmosphere is supplied with the stable oxygen isotope $^{18}O_2$, any oxygen evolution will show up as mass $^{16}O_2$, whereas the *concomitant* oxygen uptake can be *independently* detected at mass 36. (In many cases, analysis of the mixed oxygen isotope $^{16}O^{18}O$ as mass 34 allows the most decisive and specific conclusions with respect to details of a given reaction mechanism.) Principally, the same holds true for other gas exchange reactions and isotopes; for example, carbon dioxide (CO_2 fixation/photorespiration) or nitrogen fixation. Figure 3 depicts, as an example, the oxygen gas exchange in whole leaves from tobacco analyzed by means of mass spectrometry.

Figure 3 also shows that normally grown tobacco plants are carbon dioxide limited with respect to the photosynthetic capacities and that the phenomenon of photorespiration quantified as $^{18}O_2$ uptake is decreased on increasing the carbon dioxide partial pressure. Under these conditions, the competition between carbon dioxide and oxygen molecules for the binding site of Rubisco favors the carboxylase and diminishes the oxygenase function of the enzyme. Surprisingly, this interpretation turned out to be valid only for normally (350 ppm CO_2) grown plants; the effect disappeared as soon as plants were adapted to an elevated carbon dioxide concetration. Provided the tobacco plants had been cultivated (adapted) for several weeks under the conditions of 700 ppm CO_2, absolutely no reduction in photorespiratory activity was observed (Table 1).

In this case, photosynthetic activity was mass spectrometrically measured both as carbon dioxide fixation and as oxygen evolution ($^{16}O_2$). Both rates were enhanced when 350 ppm–grown plants were compared with 700 ppm CO_2–grown tobacco. When the gas atmosphere over the respective assays, however, was supplemented with the stable oxygen isotope $^{18}O_2$, it was clearly shown that high CO_2-adapted plants did not reduce their photorespiratory activity. Plants which had been cultivated under 350 ppm CO_2 or under 700 ppm CO_2 revealed exactly the same photorespiratory activity when they were analyzed in 700 ppm carbon dioxide. Thus, increased photosynthetic activity under an elevated carbon dioxide partial pressure is principally independent of the (completely

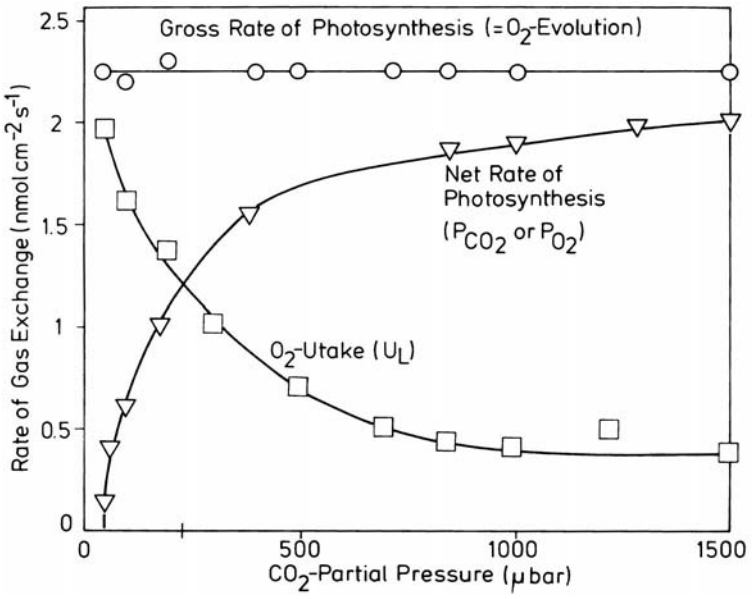

FIGURE 3 Oxygen gas exchange in leaves of *Nicotiana tabacum* var. John William's Broadleaf. Dependence of $^{16}O_2$ evolution in the light (photosynthesis) and of $^{18}O_2$ uptake in the light (photorespiration) on the CO_2 concentration in air. Measurements have been carried out simultaneously by mass spectrometry at a light intensity of 250 μE. (From Ref. 31.)

TABLE 1 CO_2 and O_2 Gas Exchange Rates in *Nicotiana tabacum* var. John William's Broadleaf in Normal Air (350 ppm CO_2) or in Air Enriched in CO_2 (700 ppm)

	Rates of photosynthesis (μmol CO_2 or O_2)					$^{18}O_2$-uptake (μmol O_2) in light (U_1)		
	P_{CO_2}		P_{O_2}		Ratio			Ratio
	mg Chl^{-1} h^{-1}	dm^{-2} h^{-1}	mg Chl^{-1} h^{-1}	dm^{-2} h^{-1}	P_{O_2}/P_{CO2}	mg Chl^{-1} h^{-1}	dm^{-2} h^{-1}	U_1/P_{O_2}
Control plants measured under 350 ppm CO_2	15.3 ± 1.3	79.6 ± 4	21.3 ± 2.8	109.9 ± 15	1.4 ± 0.12	17.7 ± 2.2	64.2 ± 8.6	.83 ± 0.22
Control plants measured under 700 ppm CO_2	22.9 ± 1.3	124.9 ± 8.7	26.0 ± 5.6	142.6 ± 23.6	1.1 ± 0.18	11.7 ± 1.9	42.4 ± 8	.45 ± 0.23
"700-ppm plants" measured at 350 ppm	26.4 ± 7.5	126.5 ± 22.8	34.6 ± 10.5	164.9 ± 32	1.3 ± 0.03	13.7 ± 2.4	49.0 ± 10.2	.400 ± 0.07
"700-ppm plants" measured at 700 ppm CO_2	43.9 ± 4	215.1 ± 5	45.0 ± 12.2	216.0 ± 36	1.03 ± 0.17	10.9 ± 1.9	39.2 ± 8.2	.243 ± 0.03

Age of the tobacco plants: 3 weeks after transplantation (i.e., 6 weeks after sowing). Control plants are grown in normal air containing 350 ppm CO_2. "700-ppm plants" are grown all the time under air having a CO_2 content of 700 ppm. Measured at 350 or 700 ppm CO_2 means that the leaves have been conditioned before the gas exchange measurement in the measuring cell in the respective atmosphere. The values are averages of three independent measurements on different leaves. The variations given represent absolute variations inherent to the performance of different leaves tested. The mass spectrometric technique itself works with an internal precision of less than 0.5%.
Source: From Ref. 31.

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unchanged) photorespiration rate, and this result might match with the breeding experiments concerning yields of crop plants (see above).

Consequently, many investigations deal with the question of whether an elevated carbon dioxide level will generally result in higher yields of crops. At present, a variety of analyses suggest this to be the case, although precise calculations are difficult to obtain—not least because of the complexity of the approaches. Taking results from the German Agricultural Research Institute as an example, one might predict that the cereal production has already increased owing to the higher amount of carbon dioxide which is available for the plants. Recent estimations proceed on the assumption that the annual average hectare yields might increase by about 0.6% with 2 ppm increase in the CO_2 content of the atmosphere [45a]. Table 2 summarizes the results from experiments with various growth and yield parameters of both C_3 and C_4 crop plants in relation to CO_2 concentrations of 372 ppm, 459 ppm, and 539 ppm, respectively. In this case, maximum stimulations of about 80% for the grain yield of barley and 25% for the grain yield of wheat were observed; these increases, however, also were brought in line with an increase in the number of ears per plant and also with a greater number of grains per ear. Based on the newly developed free air carbon enrichment (FACE) technique, one might hope that investigations on the effects of elevated carbon dioxide

TABLE 2 Effect of a CO_2 Enrichment on the Yields of Crop Plants

Crop	CO_2 concentration Cultivar	CO_2 response 372 ppm	459 ppm	539 ppm	% Yield ppm^{-1}
Beans					
Pods	Pfälzer	2.88a	2.50a	2.56a	0
	Juni	±0.20	±0.21	±0.18	
Maize					
Cobe yield	Bonny	5.85a	5.3a	4.46a	—[a]
		±0.67	±0.75	±0.95	
	Boss	14.4a	9.1b	10.4b	—[a]
Biomass yield	Bonny	89.1a	89.5a	93.5a	0.03
		±2.6	±3.5	±3.5	
	Boss	80.4a	79.0a	90.2b	0.07
		±2.9	±4.0	±2.9	
Spring barley					
Grain yield	Alexis	18.0a	21.0b	24.8c	0.22
		±0.90	±0.97	±0.69	
	Arena	11.2a	15.6b	20.2c	0.47
		±0.53	±0.70	±1.1	
Spring wheat					
Grain yield	Star	22.8a	24.7a	25.6a	0.10
		±0.88	±0.85	±1.2	
	Turbo	24.3a	27.6b	30.0b	0.16
		±0.55	±0.90	±1.1	

Yield values (g pot^{-1}) are given as means (± standard error, n = 14 for cereals and maize, and n = 20 beans, respectively). The value of the "CO_2 response" was calculated as the ratio of the increase in % yield per increase in the atmospheric CO_2 concentration (% ppm^{-1}). Values were calculated as the slope of the CO_2-yield curve (maize, barley) or from the 372-ppm and 459-ppm treatment level (wheat). Values within a row followed by the same letter are not significantly different at the 5% level.

[a] Cobes were harvested before ripening.

Source: From Ref. 45a.

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concentrations will supply more reliable and consistent results and allow more unequivocal conclusions. (Hitherto, one of the basic problems in this context is the fact that modified stable and homogeneous carbon dioxide concentrations for field experiments are not easy to establish.) Experiments of the American Ministery of Agriculture resulted in increased yields of about 10% for wheat and about 50% for cotton and citrus. Moreover, adaptation processes to drought stress appeared to be substantially improved. However, long-term adaptation processes of plants to the elevated carbon dioxide partial pressures might mitigate increased yields of the relevant crop plants that might be correlated with the different nutrient supply in natural and agricultural ecosystems. Therefore, recent and promising experiments are investigating a large area in North Carolina equipped with the FACE technique with respect to the concerted analysis of all possible parameters from plant behavior to the microbial activity within the soil. Recent analyses using the FACE technique dealt with the effects of higher CO_2 values on leaf and canopy specificities in a pine tree forest. Minor changes were observed with respect to the water use, the sap flux density, and direct stomatal responses. The net photosynthetic rate of the leaves, however, was substantially increased with trees of similar foliage but grown at 550 μM/M CO_2 [46]. The FACE technique also proved to be useful for investigations on the effects of an elevated carbon dioxide partial pressure on root morphological characteristics under free-air conditions. In this case, an increase in both the number of lateral roots and the dry weight of the roots was observed [47].

Carbon Monoxide

Carbon monoxide (CO) is a colorless gas with little water solubility, no smell, and hardly any taste. It most probably represents the most important pollutant with respect to its negative impact on animal and human life; that is, warm blooded organisms. This effect is, in part, based on the property of carbon monoxide to react in a competitive manner and with a more than 300-fold affinity (in relation to oxygen) with the 6 coordinative position of the iron in the heme group of the hemoglobin molecule. Consequently, the oxygen transport system of the organisms substantially impaires what affects many essential metabolic functions inside the central nervous and the cardiac circulatory systems. For plants, this pollutant is much less problematic; but some effects have been described. To date, there is still debate about the extent to which carbon monoxide is emitted from natural or anthropogenic sources. Values for the latter differ to about one order of magnitude between 4 and 50%. In any case, industrial processes, traffic, and private housekeeping are among the most significant sources. In general, oxidation of carbohydrates (vegetation burning), but also degradative reactions of, for example, chlorophyll play a role. Bullock, et al. [48] reported that when cheese whey was applied to growing alfalfa (Fortress variety) on silt loam calcareous soil, large amounts of carbon monoxide were emitted from the soil, which indicates a concern for whey disposal on agricultural ground and the resultant production of CO. Unfortunately, the oxidation of carbon monoxide to carbon dioxide, which is catalyzed by ultraviolet light or high temperatures, does not reach significant reaction rates. (In some cases, this oxidation is enhanced and accelerated by microbial activities within the soil.)

Detailed analyses of the effects of carbon monoxide revealed that this pollutant essentially acts as an inhibitor of respiration in plants, cyanobacteria, and photosynthetic bacteria. The principal target is the cytochrome oxidase complex, so that any reduction of oxygen at the level of complex IV is obviated. Visible spectra of mitochondrial and enzyme preparations showed that CO bound to cytochrome (cyt) oxidase at heme a_3, whereas N_2O and D_2O did not directly affect the ligand-binding site [49]. Specifically, carbon monoxide binds to a high-spin cyt *b* in the cytochrome *c* oxidase enzyme of the facultative phototrophic bacterium *Rhodobacter capsulatus* [50]. Mitochondrial CO effects were dose dependent and readily reversible, with maximal activity inhibition of 58 and 81% for mitochondria and oxidase, respectively, in the presence of 80% CO. However, a cytochrome P450 with low affinity (about $3 \cdot 10^{-3}$ M) for CO appears to be the major microsomal P450 in some plant tissues. The presence of such a low CO affinity cytochrome P450 correlates with its lack of NADPH reducibility and with the presence of high levels of 13(S)-hydroperoxy-

9(Z), 11(E)-octadecadienoate peroxidase activity [49]. Low CO affinity is characteristic of the allene oxide synthase P450s, and these P450s constitute a major portion of the microsomal P450 in a variety of plant tissues, particularly in monocot species. Carbon monoxide (CO), nitrous oxide (N_2O), and deuterium oxide (D_2O) as respiratory effector molecules revealed that cytochrome *c* oxidase (EC 1.9.3.1) activity is the first step in seed deterioration, resulting in the loss of seed viability or vigor. Seed germination was not changed in the presence of these molecules, but reductions were observed in seedling respiration and root length corresponding to reductions in cytochrome oxidase activity [51]. Unlike the other gas pollutants, little, if any, information is available concerning the morphology, growth, or productivity of CO-polluted plants. However, several pieces of evidence revealed a clear inhibition by CO of cytochrome oxidase–mediated pathways.

The conversion of (R)-reticuline to salutaridine, the key intermediate in morphine biosynthesis, is catalyzed by microsomal preparations from *Papaver somniferum* plants (roots, shoots, and capsules but not in latex) and inhibited by carbon monoxide (in darkness but not in light), suggesting that the enzyme is a cytochrome P450–dependent oxidase [52]. Carbon monoxide also significantly inhibited ethylene production and ACC (1-aminocyclopropane-1-carboxylic acid) conversion to ethylene in soybean (*Glycine max*) seedlings, indicating the existence of a relationship between cytochrome P450 activity and ethylene-forming enzyme activity [53]. These and many more investigations with different plant species suggest that the main site of action of carbon monoxide is the metabolic pathway of respiration and, in particular, the cytochrome oxidase (e.g., see Refs. 54–58)

However, positive effects of carbon monoxide also have been reported; for example, the (albeit slight) stimulation of cocklebur (*Xanthium pennsylvanicum* Wallr.) seed germination by CO [59]. In addition, Traunecker et al. [60] reported that a strictly anaerobic bacterium (tentatively called strain MC) could grow on carbon monoxide. Several C_3 plants (*Triticum aestivum, Gossypium hirsutum, Oryza sativa, Cicer arietinum, Arachis hypogaea, Cajanus cajan*) turned out to be relatively insensitive to CO and required a high CO to O_2 ratio of 40 to promote significant nitrate reductase activity [60]. On the other hand, the leaves of the C_4 plants (*Zea mays, Sorghum bicolor, Pennisetum americanum, Echinochloa crusgalli, Eleusine coracana, Panicum miliaceum, Chloris gayana, Panicum maximum*) were highly sensitive to CO even at CO to O_2 ratios of 5 or less. In these leaves, the uncoupler was without any effect, probably because the mitochondria, either from mesophyll or bundle sheath cells or both, lacked tight respiratory control [61]. Moreover, defined $CO:O_2$ ratios distinguish one C_3 plant from the other with respect to its CO sensitivity of cytochrome *c* oxidase [62]. In the leaves of C_3 plants (wheat, chickpea, and groundnut), the optimum ratios of $CO:O_2$ for the inhibition of cytochrome *c* oxidase were found to be 40, 30, and 10, respectively. Moreover, Cai et al. [63] found that the use of carbon monoxide provides a simple and specific test to differentiate between the multiple polyphenol oxidase activities laccase and catechol oxidase activity. ^{60}Co gamma-irradiated grains of allspice, cinnamon, cumin, polished rice, and wheat could be distinguished from nonirradiated ones by the level of retained CO gas even after 2 months of storage at room temperature [64].

Sulfur Dioxide

Sulfur dioxide appears to be the most prominent source of emission-dependent plant injuries. It originates from the burning of charcoal and oil products, metal roasting, and many other industrial processes. Owing to its specific weight, it accumulates near the soil—thus in the immediate vicinity of many plants. It is easily water soluble and reacts forming sulfuric acid. In contrast to other gaseous pollutants like ozone, SO_2 (and NO_2) can principally act as nutrients as sulfur-containing compounds are required by the plants. SO_2 affects the sulfate and the organic sulfur pools of the leaves and causes an enhanced export of sulfur. Thus, sulfur from atmospheric pollution can interact with the sulfur nutrition of plants [65]. As a consequence, plants fumigated with SO_2 also contained higher amounts of reduced sulfur compounds, mainly glutathione, in their roots. The interrelationship between sulfur dioxide (and other pollutants) and the whole-plant nutritional status has been expressively described (Fig. 4).

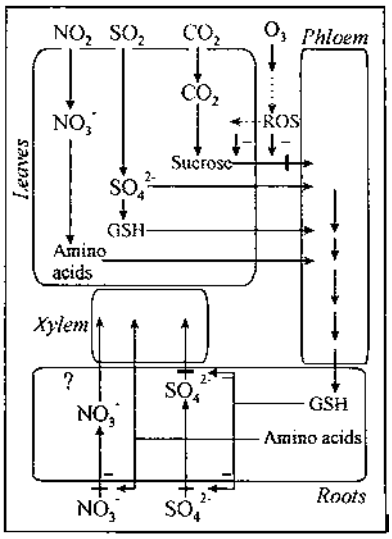

FIGURE 4 The influence of atmospheric pollution by O_3, SO_2, and NO_2 on shoot-root interactions in plants. Reactive oxygen species (ROS) released by ozone impact inhibit sucrose export from leaves. Absorbed NO_2 or SO_2 is used to synthesize amino acids or glutathione (GSH), respectively, which are translocated in the phloem to the roots. Amino acids may affect nitrate uptake; glutathione reduces sulfate uptake and the xylem-loading process in roots. (Modified from Ref. 65.)

However, any positive effect is strictly limited by the redox active capacities of the plants to detoxify sulfur dioxide into sulfate and then to organic compounds. Thus, some plants can very efficiently oxidize sulfur dioxide to sulfate, so that no macroscopically detectable damages are observed at moderate sulfur dioxide concentrations. Specifically sensitive plants are conifers, leguminoses, spinach, oats, maize, and citrus. The oxidation of sulfite to sulfate by intact chloroplasts isolated from spinach (*Spinacia oleracea* L. cv. Yates), which are capable of photoreducing carbon dioxide, was slower in the light than the reductive formation of sulfides [66]. Under these conditions, the electron transport inhibitor 3-(3,4-dichlorophenyl)-1, 1-dimethylurea (DCMU, diuron) decreased not only the reduction but also the oxidation of sulfite and the formation of additional compounds. In the dark, however, both the oxidative and the reductive detoxification of sulfite were very slow. Figure 5 summarizes the involved redox reactions as well as the relevant detoxification starting from stomatal sulfur dioxide uptake.

However, sulfur was found to behave conservatively within the canopy in the sense that the sulfur dioxide uptake balanced, within certain limits, the sulfate originating from the soil [67]. Comparison of the uptake rates of SO_2 by 11 lichen species which had been fumigated with increasing concentrations between 0.036 and 2.0 ppm sulfur dioxide revealed a linear correlation with the applied concentrations. No differences were observed when the fumigation was performed in the light or in the dark [68]. Moreover, thalli which had been inactivated by heat treatment or in which respiration was inhibited by azide treatment did not show SO_2 uptake significantly different from that of active thalli. After the first hour of fumigation, the uptake rate was almost constant during the following 5 h for concentrations up to 1.0 ppm SO_2, whereas at higher concentrations the uptake declined continuously. For *Arabidopsis thaliana* (L.) Heynh, it has been demonstrated that sulfur is accumulated preferentially in the shoots following the exposure of the plants to SO_2 [69]. However, the SO_2 flux to spruce (*Picea abies* (L.) Karst.) seedlings treated with 12.5 ppm Mn was about twice as high as to trees treated with 0.5 ppm Mn [70]. This is due to a synergism between manganese leaching and catalysis of the SO_2 oxidation by the leached Mn^{2+} ions.

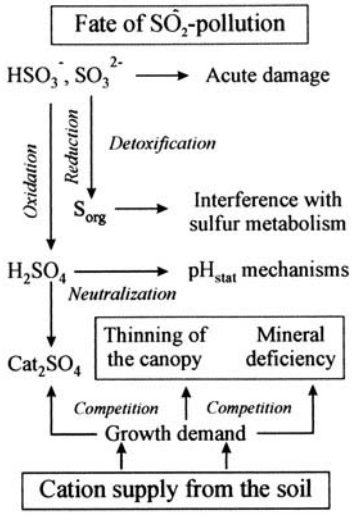

FIGURE 5 Overview of chronically activated detoxification ($SO_2 \rightarrow S_{org}$ or $SO_{2-} \rightarrow SO_4^{2-}$) and compensation pathways in spruce trees after long-lasting SO_2 pollution at ambient SO_2 concentrations in the field. Concerning NO_x (= NO_2 + NO), only reductive detoxification ($NO_x \rightarrow N_{org}$) is observed in the field at ambient NO_x concentrations. (Modified from Ref. 81.)

As mentioned before, sulfur dioxide, after being hydrated in the aqueous phase of the stomatal cell walls of spinach to form sulfite, can be oxidized to sulfate. This is based on the activity of an apoplastic peroxidase normally involved in phenol oxidation [71]. With no ascorbate present in the aqueous phase of the apoplast, a fast sulfite oxidation is catalyzed by a radical chain reaction, whereas in the presence of ascorbate, chain initiation and sulfite oxidation are inhibited with the participation of scavenging radicals. Even after exposure of leaves to high concentrations of SO_2, which inhibited photosynthesis, the redox state of ascorbate remained almost unaltered in the apoplastic space of the leaves. On the other side, any deleterious effect of sulfur dioxide on the leaf photosynthesis can be observed only with more or less intact plant systems. If, on the other hand, the flash-induced oxygen evolution in chloroplasts from higher plants (tobacco) is analyzed, absolutely no inhibitory influence of sulfur dioxide on the isolated organelles could be detected (K. P. Bader, unpublished results). Hence, the investigations on sulfur dioxide–induced damages require the analysis of whole plants or intact leaves on principle and cannot be studied with isolated organelle suspensions, as can be done in many other cases of pollution stress.

As mentioned above, the reaction rate of sulfur dioxide detoxification by oxidation is slow; its rate depends on the generation rate of apoplastic hydrogen peroxide and on the steady-state concentrations of phenolics and sulfite. The affinity of the peroxidase for phenolics is higher than that for sulfite. It has been reported that SO_2 was either oxidized to sulfate or converted into extra organic sulfur compounds in a sulfate/organic sulfur ratio 3:1, and this ratio was independent on the SO_2 concentration prevailing in the atmosphere [69].

The phytotoxic effect of sulfur dioxide consists mainly of the inhibition of the major enzyme of carbon dioxide fixation, Rubisco. The damage of leaves by sulfur dioxide due to the effect on Rubisco is mediated through the production of reactive oxygen species. Thus, the site of action of sulfur dioxide is mainly the chloroplast and both light, and the photosynthetic electron transport system affect the negative influence on the foliar tissue [72].

Partial pressures at around 0.3 ppm SO_2 resulted in dramatic effects on plants. In the case of *Aleurites montana* leaves which turned brown after 5 days and after 6–8 days, a strong leaf-shedding

occurred which affected 0.75% of the leaves [32]. Most interestingly, the two to three youngest leaves were always more resistant and survived with an apparently effective adaptation. Detailed analyses of cell components revealed the background of this drastic stress situation (Table 3).

Virtually all tested parameters were substantially decreased by the SO_2 stress; chlorophyll *a*/*b* decreased by 50%, soluble sugars by 52%, and Rubisco/area by 35%. In these experiments, it was clearly shown that any negative effect of a higher sulfur dioxide concentration on plants appear to be mitigated by the concomitant increase of the carbon dioxide concentration to 700 ppm (Table 4).

Similarly, chlorosis and browning were observed on the leaves of tomatoes cv. Pusa Ruba grown at polluted sites of a coal-fired thermal power in India [73]. The amount of sulfur in leaves was greatly enhanced and foliar injury was invariably greater on nematode-infected plants. Also, seriously deteriorated needle surfaces of Scots pine (*Pinus sylvestris* L.) was related to higher atmospheric SO_2 concentrations [74]. In full accordance with this, acute injury symptoms—leaf necroses in the vegetative period—appeared on *Picea abies* after average daily SO_2 concentrations of more than 200 $\mu g \cdot m^{-3}$. Higher concentrations of SO_2 (nearly 300–400 $\mu g \cdot m^{-3}$) often caused leaf necroses on *Picea omorica, P. pungens*, and *Pinus strobus*; the most tolerant conifers to acute pollution stress were *Pinus contorta* and *Abies alba* [75]. In two cultivars of *Cicer arietinum*, growth rate, stomatal index, amount of chlorophyll and carotenoids, total carbohydrate, and phosphorus content and, consequently, the yield were adversely affected by SO_2 exposure; the specific sensitivity against sulfur dioxide was dependent on the cultivar and of course on the concentration [76]. In other cases, SO_2-treated cultivars of wheat showed a significantly reduced leaf area; hence, the total plant biomass and the yield substantially decreased [77]. Even the root shoot ratio and also the leaf weight ratio were altered in plants grown at different nutrient levels and with different SO_2 treatments. Total chlorophyll, ascorbic acid, starch, and protein contents were as well reduced by SO_2 exposure, whereas total soluble sugar and reducing sugar levels were increased in quantity. A decreased activity of glutamine synthetase, lower concentrations of soluble protein, leaf pigments and P and K, and an increased activity of glutamate dehydrogenase were found in 3-year-old seedlings of Scots pine (*Pinus sylvestris*) exposed to SO_2 [78].

In a variety of cases, however, no substantial differences following exposure to SO_2 were observed with respect to the overall growth rate and the pigment contents of young Norway spruce (*Picea abies* [L.] Karst.) trees; neither the content of ascorbic acid nor its redox state was affected. [79]. Moreover, chlorophyll fluorescence measurements showed values of F_v/F_m ratios which are typical for plants with a healthy photosynthetic apparatus and a functional electron transport system. Other affected parameters, however, were the ''epoxidation state'' of the xanthophyll cycle, an increased foliar contents of sulfate, total glutathione (reduced and oxidized form), cyst(e)ine, and a slightly higher reduced redox state of glutathione; the latter appeared to act as a signal to control sulfate uptake from the soil and to inhibit the process of xylem loading [63]. Apart from an increase in water-soluble nonprotein sulfydryl content and a slight increase in the amount of glucosinolates (play a minor role as sinks for excess sulfur) no negative effects of exposure to SO_2 were observed for shoot biomass of *Arabidopsis thaliana* (L.) Heynh. [69]. Even the organic nitrogen to organic sulfur ratio did not change despite the increased sulfate content, which means that no changes in the composition of sulfur-containing compounds have to be assumed in such plants.

For tobacco (*Nicotiana tabacum* var. John William's Broadleaf), we observed that fumigation with 0.3 ppm SO_2 resulted in substantial damages of the older leaves of the plants (cf. Table 3). The youngest leaves, however, got adapted within the application period so that absolutely no injuries could be observed—not even under continued treatment (K. P. Bader, unpublished results). Similar effects have been described for the extent of visible injuries on soybean (*Glycine max*) seedlings fumigated with SO_2; no further damage of younger leaves were observed under long-term fumigation [80]. Accordingly, the increase of membrane permeability showed a significant recovery under prolonged fumigation. Even the SO_2-induced increase in free amino acid content was decreased down to the level of the control during the experimental fumigation. Moreover, pretreatment with low SO_2 concentration increased the resistance of soybean seedlings to high SO_2 concentration. In comparison, chronic SO_2 pollution was found to be 2.0–2.6 times more phytotoxic to Norway

TABLE 3 Protein, Chlorophyll, Sugar, Ribulose 1,5-Bisphosphate Carboxylase Oxygenase (Rubisco) and Content of Coupling Factor of Photophosphorylation (CF_1) as Well as Fresh Weight of Leaves of the Chinese Tung Oil Tree *Aleurites montana* Under the Influence of 14 days of 0.3 ppm SO_2 in Air

Aleurites montana	Fresh weight/area (mg/cm^2)	Protein/area (mg/cm^2)	Rubisco/area (mg/cm^2)	Chlorophyll/area (mg/cm^2)	Chlorophyll *a/b*	Sugar/area (mg/cm^2)	Sugar % of protein	Rubisco % of protein	CF_1 % of protein	Chlorophyll % of protein
Control plants	18.7	1.74	1.01	0.10	3.33	0.40	22.8	58.7	4.0	5.72
SO_2 plants	17.4	1.28	0.65	0.037	2.93	0.14	11.0	50.6	5.7	2.83

Control plants were 9 months old and then exposed for 14 days of 0.3 ppm SO_2 in air. Chlorophyll and protein determinations deviated by 2–3%.
Source: From Ref. 32.

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TABLE 4 Protein, Chlorophyll, Sugar, Ribulose 1,5-Bisphosphate Carboxylase Oxygenase (Rubisco) and Content of Coupling Factor of Photophosphorylation (CF_1) as Well as Fresh Weight of Leaves of the Chinese Tung Oil Tree *Aleurites montana* Cultivated for 14 days in Air Containing 0.3 ppm SO_2 and 700 ppm CO_2 and Comparison to the Values of Plants Grown Under Normal Air Conditions

Aleurites montana	Fresh weight/area (mg/cm^2)	Protein/area (mg/cm^2)	Rubisco/area (mg/cm^2)	Chlorophyll/area (mg/cm^2)	Chlorophyll a/b	Sugar/area (mg/cm^2)	Sugar % of protein	Rubisco % of protein	CF_1 % of protein	Chlorophyll % of protein
Control plants	15.0	2.31	0.71	0.043	3.22	0.49	24.3	34.5	5.1	2.11
SO_2/CO_2 plants	16.0	2.94	0.84	0.057	3.28	0.56	22.0	28.6	4.6	1.97

Control plants were 10 months old and then exposed to the combined mixture of 700 ppm CO_2 and 0.3 ppm SO_2 in air. Chlorophyll and protein values deviated 2–3%.
Source: From Ref. 32.

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spruce trees than equally high NO_2 concentrations in air [81]. Over a long period, fumigation with environmentally reasonable concentrations of SO_2 could, at least, affect leachate chemistry, and this might affect the decomposition rate of leaf litters of Scots pine (*Pinus sylvestris* L.) which was more affected than those of mixed angiosperms [82].

As with other pollutants, the response of plants to the gas varied among different plant species and within a given species with the age of the plant. When malonaldehyde and soluble protein were taken as parameters of cellular injuries, damages were more pronounced in *Dalbergia sissoo* than in *Cassia siamea*. The relatively smaller effects in *C. siamea* could be correlated with a generally faster sulfite turnover rate and also with substantially enhanced peroxidase and superoxide dismutase activities. Accordingly, older leaves of two *Populus* cultivars (*P. nigra* L. cv. Loenen and *P. maximowiczii* Henri) exhibited a severalfold increase in sugar (raffinose) after exposure to SO_2 [83]. In contrast, younger leaves were not affected [84]. With respect to the seasonal shift of the raffinose pool in poplar leaves, the alterations may be related to an acceleration of senescence. Unfavorable climatic factors were described as an additional stressor influencing the productivity of rice (*Oryza sativa* L. cv. GR 3) growing in the immediate vicinity of a fertilizer plant emitting SO_2, NH_3, and NO_2 [85]. It has been emphasized that sulfur deposition during the winter might have an impact on the total sulfur content in the needles of Scots pine (*Pinus sylvestris* L.) [86]. Investigations with *P. sylvestris* also revealed an inhibition of the photochemical efficiency of photosystem II (PSII) following fumigation with SO_2 and NO_2 in samples collected in December 1989 and January 1990 [87]. Moreover, low concentrations may have significant long-term effects and can possibly be triggered by unfavorable environmental conditions (particularly in fall and winter), and also here the relative or the absolute altitude of the fields is effective [88].

Up to a certain degree, there might be some protection against these deleterious effects by components of the antioxidant (photo) scavenging cycle. Relative resistance to sulfur dioxide and cross resistance to other oxidative stresses which originate in the chloroplast have been correlated in many cases with elevated levels of various antioxidant proteins and/or substrates. Recent studies utilizing differentially sensitive cultivars, antioxidant enzyme analyses, and genetically engineered plants have provided new insights into the mechanisms of resistance to sulfur dioxide and other stresses. It is suggested that complex regulatory mechanisms function at both the gene and the protein levels and coordinate antioxidant responses, and that a critical role is played by organelle localization and intercompartmental coordination [72]. When soybean (*Glycine max*) seedlings were exposed to a certain dosage of SO_2, an apparently freshly synthesized 15-kDa protein appeared in the leaves, increased during fumigation, and gradually declined again when the fumigation had ceased. The appearance of this polypeptide was accompanied by an increase of the resistance to SO_2 [69]. The effects of O_3, SO_2, and UVB on the ''antioxidant genes'' are very similar, although the response to SO_2 is generally less pronounced and delayed in relation to the other [89]. In wheat (*Triticum aestivum* L.), superoxide dismutase activity decreased with the increase of SO_2 fumigation dosage (but still higher than the controls) and produced a new isoenzyme pedigree [90]. However, peroxidase activity increased with SO_2 fumigation and showed an effect of relative gain with phosphate buffer; the isoenzyme pedigree increased markedly. The change of the protective enzyme system of scavenging free radicals was possibly because phosphate buffer eased the SO_2 insult of wheat seedlings. The level of two bands of superoxide dismutase isozymes (which did not appear in the controls) increased in the soybean (*Glycine max*) seedlings fumigated with SO_2 followed by increases in superoxide dismutase (SOD), an intense increase in the antioxidative ability which implied a higher resistance of the plant to SO_2 [80]. The availability and the effectiveness of defense systems, the size of internal storage pools, and the actual growth rate of the plant largely influences the significance of SO_2, NO_2, and O_3 in affecting root-shoot interactions [65]. Remarkably, SODs and cytosolic ascorbic peroxidase (cyt APx) were not affected. It is, therefore, proposed that alterations in mRNA levels of catalases and glutathione peroxidase, but not of SODs and cyt APx, form part of the initial antioxidant response to O_3, SO_2, and ultraviolet B (UVB) in *Nicotiana tabacum* L. cv PBD6.

Besides the above-mentioned cellular mechanisms, the nutritional status and some protectants

may be effective to overcome or detoxify the sulfur dioxide stress. In the case of underoptimal nutrient status, the total plant length of wheat (Malviya 206 and Malviya 234) was reduced significantly in SO_2-treated plants, whereas plants grown at twice the recommended fertilizer concentrations were not affected. Thus, the general mineral nutrient status of the soil has been found to modify the response of wheat plants [77]. In agreement, the positive effect of NH_3 (on the concentrations of N and Chl *a*) in Scots pine (*Pinus sylvestris*) counteracted the negative effects of SO_2 [78]. The nutrient balance was affected (NH_3 ''increased'' the concentration of N and SO_2 ''decreased'' the concentrations of P and K), whereas a mixture of both might cause serious nutrient imbalances provided the increased demands for nutrients in shoots cannot be adjusted and equilibrated by components of the soil. This led to the assumption that there might be a positive feedback between (moderate) acidification of soils and SO_2 and NH_3 inputs to terrestrial ecosystems [70]; foliar calcium seemed to be only a short-time buffer even under conditions of optimal calcium supply.

Positive effects were described following the application of kinetin and ascorbic acid to mitigate foliar injuries in *Oryza sativa* [85]. The accumulation of fresh and dry matter of the culms was not affected by air pollution on kinetin and ascorbic acid spray application, but instead plants produced an increased number of panicles. Uniconazole ((E)-(p-chlorophenyl)-4, 4-dimethyl-2-(1,2,4-triazol-1-yl)-penten-3-ol) provided similar protection against SO_2-induced injury [91]. However, high concentrations of uniconazole reduced leaf size and total chlorophyll concentration, decreased malondialdehyde accumulation, increased SOD activity, and delayed flowering slightly but had little or no effect on variable chlorophyll fluorescence nor on the number or dry weight of pods. Absolutely no correlation was found between stomatal resistance and uniconazole treatment.

Ozone

Ozone (O_3), as an important component of the atmosphere, has to be regarded under completely different aspects. First, it efficiently reduces the (short wavelength) UVB radiation which is (depending on the doses) deleterious for humans, animals, and plants. Consequently, the so-called ozone layer is an absolute requirement for higher life forms on earth, and the worldwide application of ozone-depleting substances (such as chlorofluorocarbons and other pollutants) is more than problematic. (This and other questions have been discussed at the recent World Climate Conference in Kyoto, and this turned out to be differently regarded depending on the different political and economical interests.) Apart from the primary effect for UV radiation, oxygen free radicals, peroxides, or superoxides will increase simultaneously as a result of higher UV radiation. Second, in the stratosphere, this gas is involved in many chemical and photochemical reactions (e.g., the conversion of SO_2 and NO_x). As a component of the troposphere, ozone belongs to the so-called greenhouse gases and absorbs those portions of the long-wavelength radiation which are (otherwise) reflected from the earth's surface, thereby trapping the energy and contributing to a global warming. However, increasingly high concentrations of ozone near the soil are themselves problematic for plants. (For information on the negative effects of ozone on animals and human beings, the reader is referred to medical publications; for example, the excellent review by Lippmann [92].) High ozone concentrations in the lower parts of the troposphere (i.e., in the vicinity of plants) are observed as the result of both photochemical reactions near the soil and vertical wind fluxes. Physicochemically, ozone is a molecule composed of three oxygen atoms; it is a colorless gas with a rather specifically pungent smell and with low water solubility. The overall toxicity of ozone can be explained by the fact that it is highly reactive and is one of the strongest known oxidants. At present, there is a strong debate about the qualitative and quantitative participation of anthropogenic activities in the production of high ozone concentrations during the summer months in particular. Industrial productions and automobile emissions appear to be among the principal sources of ozone. Moreover, intentional incendiarisms contribute without any doubt to the phenomenon and sometimes lead to a composition of the air over African and Asian regions which can hardly be distinguished from smog conditions in Los Angeles as far as the ozone concentration is concerned. Not too much is known about the detailed mode(s) of action of ozone in plants. It penetrates preponderently through the stomata and

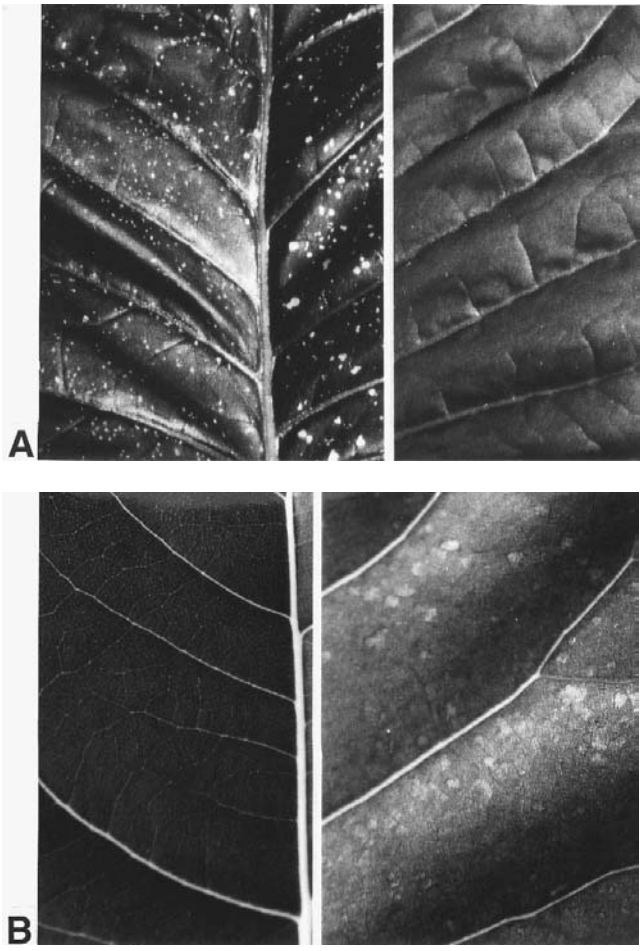

FIGURE 6 (A) Left tobacco leaf with early O_3 symptoms in a shaded place; right leaf exposed in the field in a sunny place. (B) Left poplar leaf grown under filtered air (control); right leaf with necrotic spots under the daytime O_3 treatment. (From Ref. 93.)

appears to affect the selective permeability of membrane structures; as already mentioned, the major deleterious and even destructive effect of ozone on plants and on plant metabolism can be traced back to the strong oxidative capacities of the molecule. In recent years, serious efforts have been made to elucidate the details of the consequences of elevated ozone partial pressures on plants in general and on crop plants, including plant defense mechanisms, in particular. Among the many investigations on the complex reactions of plants to increasing concentrations of ozone, the concerted chamber experiments of Günthardt-Goerg [93] describe results with tobacco (*Nicotiana tabacum* L. var. BelW3), poplar (*populus* X *euramericana* var. Dorskamp), birch (*Betula pendula* Roth), and alder (*Aldus glutinosa* (L.) Gaertn.). Figure 6 illustrates the obvious characteristics of ozone-dependent injuries on the above-mentioned plants. Tobacco exhibits characteristic white chlorotic spots in the shade (Fig. 6a); in the case of poplar leaves, small necrotic areas were detected following ozone treatment (Fig. 6b). In any case, damages were different depending not only on the concentration but also on the uptake into the plants via the stomata. This in turn is related to external factors like relative humidity of the air, light intensity, temperature, humidity of the soil and, again, the

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concentration of ozone itself. Internal factors are the age and the morphology of the leaves [93]. (Many herbaceous plants close their stomata during the night, whereas trees only diminish the diameter of the stomatal pores by narrowing in many cases.)

Pitcher and Zilinskas described the origin of ozone damage by the action of oxygen free radicals and ozone degradation products, and plants are supposed to develop and to apply cellular antioxidant systems as defense [94]. As with other pollutants, ozone-related damage of any type as well as the defense-related responses are strongly dependent on the plant species, age, and even on the time of in vitro treatment (day/night). Although present ambient concentrations of ozone decrease the yield of several important crops, plants substantially differ in sensitivity to ozone; for example, wheat (*Triticum aestivum* L.) is more sensitive than barley (*Hordeum vulgare* L.) [95]. Owing to its sensitivity, tobacco is even the so-called indicator plant for elevated ozone partial pressures. Fumigation of spring wheat (*Triticum aestivum* L. cv. Drabant) with elevated concentrations of ozone caused chlorosis of the flag leaves, a decrease in the amount of cytoplasm, and an increase in the vacuolization of the cells. In later phases, the chloroplasts became affected by decreasing in area and containing more plastoglobuli. The plasma membranes lost their intimate contact to the cell wall and convoluted. Mitochondria remained largely unaffected until the late phases of cell destruction. However, in most cases, the described changes were quantitatively, but not qualitatively, specific for ozone; they also occurred with charcoal-filtered air, although later in time. This indicates that ozone preferentially causes premature senescence in the investigated flag leaves [96]. A similar acceleration of (the visible symptoms of) senescence and a decrease in the activity and quantity of Rubisco has been described in various cases [97].

Ozone uptake through the stomata has been investigated and described by Luwe et al. [98], who found that fumigation with 0.3 $\mu l \cdot L^{-1}$ of ozone resulted in ozone uptake by the leaves close to 0.9 $pmol \cdot cm^{-2}$ of leaf surface area s^{-1}. As plants take up ozone (and other gases) preponderently through the stomata, the amount of ozone uptake is substantially dependent on and effectively controlled by factors influencing the stomatal aperture like illumination, humidity, and water status (see above). Water vapor pressure deficit was the climatic factor most closely correlated with ambient O_3 concentration [99]. Thus, when O_3 concentrations were highest, O_3 uptake tended to be restricted by stomatal narrowing. Consequently, the deleterious effect of ozone also can be correlated with the elevation of the cultivation area (cf. section on Sulfur Dioxide above), as high tillages are often less protected from soil water stress, so that stomata are in relation and per time unit longer and wider open, as might be the case at low-elevation locations.

It is obvious that the more the stratospheric ozone layer is injured, the more the biosphere will be exposed to higher doses of UVB (290–320 nm) radiation [100]. Damaging effects of such increased UVB radiation received by plants have been described and, at the same time, improved our understanding of the phenomenon of UVB tolerance [100,101]. Even in terms of the indirect effects on rice blast disease, enhanced UVB affected both the fungus itself (*Pyricularia grisea*) and the susceptibility of the rice plant to the fungus [102]. On the other hand, care must be taken in those cases when no direct or macroscopic visible symptoms could be observed when studying the effect of O_3 or UVB, because several plant species exhibited no visible symptoms, although the subcellular structure(s) had been severely affected and altered up to the level of chromosomal aberration [103]. Also, differences in the responses of plants to O_3 and UVB by invoking different antioxidant enzymes were reported [104]. UVB exposure preferentially induces peroxidase-related enzymes, whereas O_3 exposure invokes the enzymes of the superoxide dismutase/ascorbate-glutathione cycle, and in contrast to O_3, UVB exposure generated activated oxygen species by increasing NADPH-oxidase activity.

An important aspect when dealing with the effects of pollutants on plants is that very often laboratory investigations and conditions can by no means be transferred to real in vivo conditions. In this sense, one of the principal improvements of scientific investigations was the construction and application of the so-called open-top chambers. Barnse et al. [105] recorded different responses to ozone treatments between laboratory- and field-grown plants. Under laboratory conditions, seedlings of cucumber (*Cucumis sativus* L.) and tomato (*Lycopersicon esculentum* Mill.) exhibited appre-

ciable (approximately 50%) and rapid inhibition in hypocotyl elongation in response to UVB exposure [105]. For cucumber, it has been described that the UVB-induced inhibition was reversible, was not linked to concomitant changes in dry matter production, and was caused by UVB incident on the cotyledons and not the stem or growing tip. For mixed cultures of wheat (*Triticum aestivum* L.) and wild oat (*Avena fatua* L.), a common weedy competitor, supplemental UVB irradiation in the field differentially altered shoot morphology which resulted in changes in canopy structure, light interception, and calculated stand photosynthesis. It is argued that because of its asymmetrical nature, competition for light can potentially amplify the effects of UVB on the shoot morphology and may, therefore, be an important mechanism by which changes in the solar UVB spectrum associated with stratospheric ozone reduction could alter the composition and character of terrestrial vegetation. Similar expectations concerning modifications of whole ecosystems (plants and plant families) in the future are made with respect to the gradual increase in the carbon dioxide partial pressure of the atmosphere.

Nikolopoulos et al. [106] observed the first effect of UVB radiation on *Phlomis fruticosa* L.; namely, a growth response at late spring. The effect consisted of an inhibition of new leaf development and of a premature falling of (old) leaves leading to smaller leaf numbers and total leaf areas for the rest of the experimental period [106]. However, a single exposure of flowering racemes of *Brassica campestris* L. to 100 nL/L ozone for 6 h had no significant effect on the numbers of reproductive sites produced or aborted [107]. This result was in clear contrast to a related species, *B. napus* L., in which a single exposure to 100 nL/L ozone induced a significant loss of reproductive sites. However, multiple exposures of *B. campestris* to ozone had significant effects on seed abortion and on the number of mature seeds per pod at final harvest. However, the extent of the effect depended strongly on the developmental stage of the reproductive organ at the time of exposure. On the other hand, it was observed that various effects like seed number per plant, mean seed weight, and total seed weight per plant at maturity were not significantly altered, which implies a high degree of compensation during reproductive development.

In the southeastern United States, it was found that current ambient ozone concentrations might influence the carbon-fixation rates and also the growth of various forest tree species [108]. The loblolly pine trees exhibited a significant decrease in the photosynthetic rates of needles, with this effect being proportional to the cumulative ozone exposure (decrease of 50% after 350 ppm $\cdot$ h^{-1}; 12 h summation). The decrease went along with a substantial decline in Rubisco activity. Thus, current ambient ozone concentrations might lead progressively to a biochemical disequilibrium within leaf cells with a reduced production of assimilates and transient increased respiration. In many cases, there is clear evidence that present ambient concentrations of ozone decrease the yield of several important crops (e.g., see Ref. 95). *Arabidopsis thaliana* plants treated with either 150 or 300 parts per billion (ppb) ozone daily for 6 h revealed both reduced growth and extensive leaf curling [109]. Fresh and dry weights of ozone-treated plants were reduced by 30–50% compared with air controls. Nevertheless, the role of ozone in forest decline is still unclear, although several investigations on young conifers have shown that ozone can reduce net photosynthesis, disturb carbon allocation, and reduce growth. Furthermore, little information exists on the effects of ozone on adult trees.

In order to protect photosynthetic tissue from UVB radiation, plants synthesize different types and quantities of UVB-absorbing compounds (e.g., flavonoids). Apparently, plants with an elevated content of total flavonoids were significantly more UVB tolerant with respect to growth rate, pigments, and gas exchange reaction rates [101]. In accordance, Ziska and Teramura [110] analyzed the degree of sensitivity of photochemical reactions at different UVB radiation conditions and found that leaves from different rice cultivars produced completely different amounts of UVB-absorbing compounds. Fujiyama-5 had a significantly higher concentration of these compounds than IR-36 in any of the investigated environments, and the production rate in Fujiyama-5 was stimulated by UVB influence. Greenberg et al. [100] reported the biosynthesis of flavonoids and other UV-absorbing pigments also in *Brassica napus* exposed to such levels of UVB radiation causing cotelydon curling [100]. Approximately 20 distinct UV-absorbing pigments were produced in response to UVB radia-

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tion. Although synthesis of flavonoids is induced by UVB radiation, its protective role on photosynthetic pigments is still under debate in other cases [101]. Also, the formation of the stilbenes pinosylvin and pinosylvin 3-methyl ether, as well as the activity of the biosynthetic enzyme stilbene synthase, were reported to be induced several hundred- to thousandfold in primary needles of 6-week-old pine (*Pinus sylvestris* L.) seedlings on exposure to a single pulse of ozone of at least 0.15 μL/L [111].

The chlorophyll fluorescence kinetics of broad bean (*Vicia faba*) plants showed significant alterations following the treatment reflecting a perturbation in the photochemical functioning of the thylakoids and specific disturbances of the water-splitting enzyme system of the PSII of two broad bean cultivars; recovery took about 1 week [112]. Absorption, trapping, or electron transport increased considerably under the conditions of elevated O_3 or CO_2. This increased activity seems to be due to an increased antenna size in O_3-treated samples. Similar conclusions have been made in connection with our analyses on the effects of higher carbon dioxide partial pressures on *Nicotiana tabacum* and *Aleurites montana* where we described an increase in the light-harvesting complex and the extrinsic polypeptide of 33-kDa molecular mass [31,32]. An inhibition in the photosynthetic activity was associated primarily with a stomatal limitation rather than a real PSII damage [101]. Although a decrease in Rubisco was obvious, Dizengremel, et al. [108] observed the largest increase in the activity of glucose-6-phosphate dehydrogenase. However, this substantial increase and the slighter increases in phosphofructokinase and fumarase activities (about 25%) showed a tendency to a further decline when the loblolly pine spaldings were exposed to higher cumulative ozone doses. Rennenberg, et al. [65] concluded that ozone interacts with carbon allocation most likely by inhibiting sucrose export which causes an accumulation of carbohydrates and starch in leaves and results in a reduction of photosynthesis. Thus, O_3 exposure can diminish the availability of photosynthetate for growth and development and result in an increased shoot/root ratio and an overall reduction in the biomass. In agreement with this conclusion, Grantz and Yang found [113] that Pima cotton (*Gossypium barbadense* L. cv S-6) exhibited foliar injuries and yield reduction at ambient concentrations of O_3. Eight weeks after planting, stem basal diameter, leaf area, and total plant dry weight decreased by 61, 83, and 88%, whereas the root/shoot dry weight ratio declined from 0.16 to 0.09 g/g. Results from these investigators support the hypotheses that O_3 reduces the allocation of the biomass to the root system, and that the disrupted carbohydrate allocation impairs the root hydraulic capacity relative to the transpiring leaf area even though the leaf area development is itself reduced by O_3. Sensitive birch clones which had been exposed to a single 8-h ozone pulse of 150 ppb suffered from partial tissue chlorosis and necrosis, whereas insensitive clones were unaffected [114].

Guidi et al. [112] observed that in this case, subsymptomatic exposure to 150 ppb of ozone for a single 3-h period led to a rapid and significant reduction in photosynthetic activity coupled with a reduction in stomatal conductance and transpiration in two cultivars of broad bean (*Vicia faba* cvs. *Reina blanca* and Gigante d'Ingegnoli). The two cultivars behaved quite differently in the postfumigation stages. *R. blanca* recovered quickly; its photosynthetic rate returned to prefumigation values within 48 hours [112]. In Gigante d'Ingegnoli, the recovery process took much longer; 72 h after ozonization, the net photosynthesis was only 59% that of the unfumigated controls. The contributions of stomatal conductance and photochemical quantum conversion to the observed reductions in photosynthesis rates also differed between the two cultivars.

The mean of the red/far-red fluorescence ratio from spectra collected 24 h after exposure to ozone was significantly different from the prefumigation R/FR ratio mean ($P = .10$) in white pine (*Pinus strobus* L.). However, a potential recovery of photosynthetic processes from the effects of ozone exposure was also observed [115]. Wallin, et al. [116] studied the effect of ozone at different plant ages of one clone of Norway spruce, *Picea abies* (L.) Karst, and found that in 1- and 2-year-old shoots, the apparent quantum yield decreased with increasing shoot age and ozone concentration, whereas no effect was found in the current-year shoots. The decrease could probably partly be attributed to a lower efficiency of light capture due to a lower content of chlorophyll. In another work, these investigators [116] found no significant effects on photosynthesis or on leaf conductance to CO_2 in current-year shoots. In 1- to 3-year-old shoots, leaf conductance to CO_2 and rates of net

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photosynthesis at both 330 μM/M CO_2 and saturating concentrations of CO_2, decreased with increasing shoot age and ozone concentration [117]. The carboxylation efficiency significantly decreased in 2- and 3-year-old shoots from the nonfiltered air and nonfiltered air plus ozone treatments compared with shoots from the charcoal-filtered air treatment. The gas phase limitation of photosynthesis decreased with the increasing shoot age and ozone concentration. In the experiments performed by Carlsson, et al. [118], spinach was not at all sensitive, whereas both pea and wheat leaves of different ages reacted specifically to ozone. In pea, the sensitivity to ozone increased substantially with the age of the leaves. A decrease in the relation of chloroplast membrane lipids to nonchloroplast membrane lipids was observed for both pea and wheat, whereas again spinach was unaffected [118]. Similar to what had been described in the section on sulfur dioxide above, older leaves were shown to be more sensitive to the pollutant, whereas younger leaves were generally less sensitive.

Genetics

Although there were no visible symptoms on spruce trees directly after the fumigation had ceased, the treated plants showed a significantly increased number of chromosomal aberrations in comparison with the control plants [103]. Five further investigations of both variants of this experiment up to 2 years after the ozone fumigation had ended showed a long-term hangover in the genetic material of spruce trees. The observed chromosomal aberrations in all variants of the experiment consisted of chromosomal stickiness, chromosomal breakage, and fragmentation. The most important type of observed chromosomal abnormalities was a chromosomal stickiness leading to cell death. It has been suggested that an intensive site effect is significant rather than the soil or the provenance of the individual. This cytogenetic plant test system also was used to investigate 5-year-old spruce trees exposed in environmental chambers to elevated concentrations of carbon dioxide (750 cm^3/m^3) and ozone (0.08 cm^3/m^3). The pollutants were applied singly or in combination; thereafter the plants were transferred to the field for observation of a ''memory effect.'' The fumigated variants showed an increased number of chromosomal aberrations compared with the controls, which carried on as a memory effect in the root meristems far beyond the fumigation period.

When plants were exposed to 0.08 μL/L for 5 h per day, a decrease in the steady-state levels of rbcS mRNA was observed in expanding leaves after 3 days of ozone exposure; the ethylene levels had increased 6- to 10-fold [119]. The expression of OIP-1, a 1-aminocyclopropane-1-carboxylate synthase cDNA from potato, correlated with the increased production of ethylene and the decreased levels of rbcS mRNA. In plants exposed to 0.30 μL/L ozone for 4 h, rbcS transcript levels were reduced to 0.25%. At least in part, the loss of rbcS mRNA might be due to posttranscriptional regulation. The levels of transcripts for other chloroplast proteins, glyceraldehyde-3-phosphate dehydrogenase, and a PSII chlorophyll *a*/*b*–binding protein decreased in O_3-treated plants in parallel with the decrease in rbcS mRNA. The steady-state mRNA level of a cytosolic glyceraldehyde-3-phosphate dehydrogenase increased in O_3-treated plants. The induction of ethylene and changes in transcript levels was followed by visible leaf injuries and decreases in Rubisco protein levels. Apparently, an increase in the chloroplastic Cu/Zn superoxide dismutase is not sufficient to reduce ozone toxicity, as no consistent protection was provided to transgenic tobacco plants (*Nicotiana tabacum* cultivar W38) plants that overproduce petunia chloroplastic Cu/Zn superoxide dismutase under the conditions of exposure to ozone concentrations which harm control plants [120].

Nitrogen Oxides

Nitrogen oxides are unavoidable compounds playing a substantial role both in the projected global warming of the atmosphere and in the depletion of the protective ozone layer. Moreover, NO_2 emission from cultivated areas (soils and plants) leads to nitrogen loss from ecological system of agriculture [121]. Motor vehicles and fossil fuel–fired power stations are important sources of NOx emission. The nitrogen which is contained in wood fuels is mainly converted to molecular atmo-

spheric nitrogen, although under the conditions of high temperatures where the combustion is most efficient, there also is some conversion to nitrogen oxides [122]. On addition of vanadium pentoxide, however, the formation of nitrogen oxides can be decreased during the burning of oil, primarily because of the reduction of the attainable gas temperature [123]. For the practical use of heating greenhouses, low-NO_x propane burners seem to be an effective alternative to liquid CO_2 [124], although it cannot be excluded that, in this case, some nitrogen oxides might also be contained in the oxidation products.

In soils, however, processes which produce and consume both NO and N_2O are principally microbiological in nature and are linked directly and indirectly with the chemical and physical factors that control gaseous transport through the soil medium; such as temperature, water-content soil composition, nutrient availability, vegetation, disturbances (e.g., burning, agricultural practices), and others [125]. About 2–6% of the total annual NO_x emission and 16–64% of the total annual N_2O emission in Great Britain are supposed to stem from agriculture [8]. In this domain, nitrogen fertilizers are without any doubt among the most important sources of anthropogenic N_2O emissions [2]; about 5% of the applied N was lost as N_2O from NH_4NO_3 which had been applied in the spring. This value is significantly higher than the amount lost as N_2O in case of urea fertilizing or NH_4NO_3 which had been applied in fall. On the average, 12.4% of the N input were released as N_2O and N_2 during the vegetative growth and the stem fruit stage of cucumbers. This process corresponds to a mean emission rate of 0.62 kg nitrogen per hectare greenhouse area and day. Additional factors like the growth of green algae on the substrate surface further stimulating the production of N_2O have to be taken into account [126]. For other plants and depending on the cultivated crop, approximately 0.5–3.0% of the added inorganic N fertilizers were calculated to be lost as N_2O [127]. Nitrate addition increased the total N_2O emission rate substantially, but the percentage emitted through rice plants was lowered [128]. Without any tillage, emission of N_2O was generally higher as with conventional tillage, and in the same experiments, more N_2O was emitted from corn fields than from soybean or alfalfa cultivations. However, in a corn system using conventional tillage, legumes in rotation, and moderate fertilizer, N would reduce N_2O emission [129]. The N_2O and N_2 emissions showed clear diurnal variations [130], whereas frost in early winter did not lead to higher amounts of nitrogen loss as N_2O [131].

Precipitation in spring and fall is generally conducive for higher N_2O emissions from wheat fields, whereas in winter, this appears not to be the case; in the case of rice fields, no correlation was observed [132]. It must be emphasized that substantial amounts of (trapped) N_2O were detected within the soil down to a depth of 90 cm, and this observation shows that agricultural production systems might contain a considerable pool of N_2O and this N_2O will subsequently be reduced to N_2 [133]. It has been demonstrated that the presence of manure on and in cultivated fields modifies microbial activity in the soils, and this effect is based on the incorporation of additional quantities of C and N; as a whole, various physical and chemical properties of the soil are modified [134]. In the case of animal manure composts, 0.2–3.3% parts of nitrogen contained in it were lost as nitrous oxide [135], and under these conditions of nitrogen supply, highest annual emissions were recorded [136]. Therefore, in order to make an effective use of animal manure, the respective application time must be correlated with the time of rice planting. In the subsequent fallow periods following the harvest of rice, the soils contribute to significant N_2O emission which is maximal during the drainage period and decreases to about zero at the time of reflooding [4,137].

Denitrification represents an essential factor within the global nitrogen cycle (cf. Ref. 138, for example). Losses of nitrogen by denitrification and N_2O emission from irrigated corn amounts to about 1–5% of the N applied as fertilizer or in irrigation water. Depending on the soil level (upper parts), a significant correlation was found between denitrification activity on the one hand and the level of ground water, water-filled pore space, and nitrate content on the other hand [139]. It also was dependent on the variety of rice [140]. With lucerne, the denitrification rate was nearly four times higher than with rye grass (*Lolium perenne* L.) or from fallow soil [141]. The availability of organic carbon compounds which are easily decomposed was an important limiting factor for the denitrification activity in the subsoil of peat soils [139]. It was observed that when the carbon

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to nitrate ratio was lower, the amount of the released N_2O increased [133]. Enzymatic activity by the nitrous oxide reductase was another important factor regulating N_2O emission in the case of paddy soils. This enzyme contains the Cu-A center as a structurally novel metal site similar to cytochrome *c* oxidase. Therefore, it has been suggested that both N_2O reductase and NO reductase may be types of ancestors of members of the heme–copper oxidase enzyme family [138]. However, not all the nitrogen oxides released come from real denitrification processes. Additional amounts originate from nitrate reductases produced by plants, algae, fungi, cyanobacteria, or eubacteria acting anomalously on nitrite to liberate small amounts of NO and N_2O, which may further contribute to global atmospheric stocks of nitrogen oxides. Such enzymes are used by nitrifying bacteria, thereby reducing nitrite when oxygen is limiting [142]. Detailed analyses have shown that nitrous oxide was the major gas emitted from less aerated soils (conditions that allowed denitrification to occur), whereas nitric oxide played this role in the case of well-aerated soils (conditions that favor nitrification) [143]. Recent discussions deal with the contribution of nitrogen inputs caused by air pollution to the overall danger for forest ecosystems [144], but as with the general discussion on forest decline, many details of the complex mechanisms and implications remain to be elucidated and early conclusions should be avoided.

Pollution of the atmosphere by oxides of nitrogen can lead to various responses in plants among which are modifications of the amount of nitrate and/or nitrite in plant tissues, the stimulation or the inhibition of enzyme activities, changes in the reaction rates of photosynthesis and CO_2 fixation, and even the general growth of the plants may be affected [145]. Similar effects have been described for conditions of an increased fumigation frequency or enhanced concentrations of nitrogen oxide at a given single application in the case of four *Eucalyptus* species [146]. Saarinen reported that oxides of nitrogen (NO_x) and traffic emissions are possible factors affecting the light reactions of photosynthesis and the pigment content of pine needles in an urban environment [147]. When two spring wheat cultivars (*Triticum aestivum* L. cvs. Minaret and Eridano) were exposed to ozone with and without small amounts of nitrogen oxides, the Minaret plants reacted with less influenced leaf dry weight and inhibition of growth when O_3 contained the by-products N_2O_5 and N_2O, which could be explained by more nitrogen content per plant [148]. In some investigations, nitric oxide (NO) appears to have even a positive effect in the sense that plants better overcome other stress phenomena [149]. This situation of ''stress coping'' can be interpreted by the observation of a substantial deceleration of stress ethylene production. NO_2 fumigation caused no macroscopic damages to the cuttings of a poplar clone (*Populus times euramericana* Dorskamp). Fumigation enlarged the foliar area, elevated the net CO_2 assimilation rate, and enlarged the width of xylem and bark tissue in the main stem. Fumigation also had a stimulating effect on the total biomass production during the exposure period. Exposure of Scots pine seedlings inoculated with the mycorrhizal fungus *Pisolithus arhizus* to low concentrations of NO had no effect on growth, total nitrogen concentration, ergosterol (as a measure of mycorrhizal infection) concentration, or total protein concentration but resulted in a significant uptake of NO and distinct modifications in amino acid composition [150]. Fumigated plants, however, showed elevated activity of nitrate reductase and higher leaf nitrogen concentrations relative to the control, indicating nitrogen assimilation from NO_2 [151]. Despite the fact that germination of bean seeds was not changed in the presence of the respiratory effector molecules N_2O, D_2O, or CO, it was observed that the respiration of seedlings and the length of roots were reduced corresponding to reductions in cytochrome oxidase activity [152]. Pollutants including NO_x induced deesterification of lipids as evidenced by the accumulation of myoinositol, serine, and raffinose, which are components of the hydrophilic head groups of membrane-associated phospholipids and galactolipids [153].

Nitrogen derived from NO was found in the shoots and roots, indicating the transport of such nitrogen from shoot to roots. Nitrate reductase activity can be used as a biomarker for the foliar uptake of nitrogen oxides during periods of air pollution [154], although the nitrate assimilation in leaves can include nitrate taken up from the soil in the case of red spruce (*Picea rubens* Sarg.). In many cases, NO_2-induced nitrate reductase activity was observed within 24 h after the start of fumigation [155]. In contrast, exposure to NO caused a rapid decline in activity within 24 h. Addition

of the nitrification inhibitor dicyandiamide reduced N_2O fluxes form ammonium sulfate, whereas increasing calcium ammonium nitrate (CAN) increased the emitted N_2 on grassland. Accordingly, there is room for reducing N_2O emission from grasslands by choosing the N fertilizer type depending on the soil moisture status. Avoiding excessive N application rates may also minimize N_2O emission from intensively managed grasslands [156]. Dicyandiamide- and polyolefin-coated urea showed the potential to be used to decrease N_2O emissions from N fertilizer [154].

PLANT-PROTECTIVE CHEMICALS AND PATHOGENESIS

Fungicides

In order to maintain and even further increase the yields of crops and other relevant agricultural and horticultural plants, enormous amounts of plant-protective chemicals of any type and specificity are applied every year. Actual calculations and projections proceed on the assumption that, on the average, 35% of the possible yields are lost because of fungal infection albeit the worldwide use of enormous amounts of fungicides.

In recent years, scientists in many laboratories have investigated the mode(s) of action of herbicides under completely different aspects. Besides the application of such chemicals to control weeds generally (and also other plants at undesired locations), some herbicides like dichlorphenyldimethylurea (DCMU; Diuron) have entered all photosynthesis laboratories worldwide as a standard inhibitor for PSII reactions. Consequently, herbicides and their specific properties have been thoroughly analyzed and described, and many excellent reviews are available with respect to their impact on the photosynthetic electron transport (e.g., see Ref. 157) or the so-called bleaching herbicides acting on the phytoene desaturase [158]. Much less attention has been paid to the question whether plant-protective chemicals other than herbicides are in fact as unproblematic for the treated plants as is generally thought (in most cases, without really regarding the problem). Apart from the trivial idea that a fungicide is intended to be toxic for a fungus and an insecticide for an insect, and so forth, only scattered reports have been published dealing with the question whether different chemicals of this kind interact with the treated plants in the sense that physiological and metabolic activities of the plants are affected or even inhibited. We show here for the first time that fungicides, for example, of the Triforin (Saprol) type, exert a strong inhibitory effect on electron transport reactions of higher plant chloroplasts (Fig. 7), whereas on the intact leaf, no macroscopically detectable symptoms are observed. Even under these conditions, recording of the fluorescence emission from the intact leaf shows that, in fact, the photosynthetic electron transport is impaired.

Figure 7 shows the effect of the fungicide on the fluorescence emission of tobacco leaves 1 day after the leaf had been sprayed several times with $2 \cdot 10^{-3}$ M of the fungicide Triforin. The maximal fluorescence is enhanced (what is normally observed, e.g., after the application of a urea-type herbicide and the ratio $F_{max}-F_0/F_{max}$ (significant for the efficiency of the electron transport) is shifted from the normal value of 0.8 for controls to lower values tending to zero. This hints at a substantial interruption of the electron transport chain (most probably in the region of the acceptor side of PSII).

The observation of an impaired photosynthetic electron transport in the region of PS II can be substantiated by the measurement of the flash-induced oxygen-evolution amplitudes in chloroplast preparations from peas in the frame of the coherent Kok model. Figure 8 depicts the typical oxygen-evolution pattern of higher plant chloroplasts (upper curve) and the inhibited yields in the presence of 25 μM of the fungicide (lower curve). Any further increase of the concentration of the fungicide abolished any oxygen-evolution activity of the pea chloroplasts.

Thus, fungicides immediately have significant herbicidal properties which are even more pronounced as soon as accessibility of the product to the inner parts of the plants is allowed. This also means that under such aspects the integrity of the outer plant surfaces like the cell walls and cutin layers are of enormous importance. (Cultivation manipulations of any kind like cutting twigs, branches, and roots should be minimized not least with respect to this point.) In most of the described

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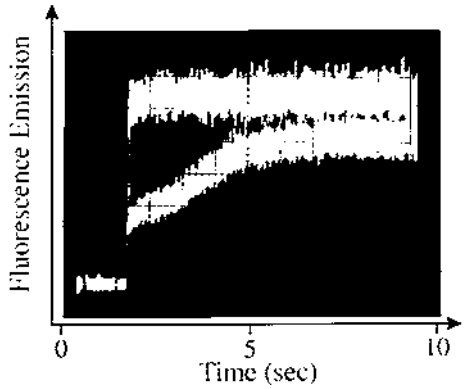

FIGURE 7 Effect of a Triforin-type fungicide on the fluorescence-induction curves in whole leaves from *Nicotiana tabacum*. The control leaf was dark adapted for 10 min before the fluorescence emission was recorded, whereas another leaf (identical in size) has been sprayed several times with a solution of the fungicide ($2\ 10^{-3}$ M). Note that the increase in the maximum fluorescence yield and the disappearance of the Kautsky kinetics are virtually identical to the effects observed with various herbicides.

cases, application of plant-protective chemicals other than herbicides did not in fact harm the plant provided only intact parts of the surface were sprayed. (In general, no inhibitory concentrations of the chemicals were attained via the physiological plant openings like stomata.)

Most interestingly, the type of effect on plants which is induced by some plant-protective chemicals can in fact be very similar or identical to the one observed in other cases; for example, pollutant stresses. It is known that paraquat (a standard herbicide)–induced injuries are attributed

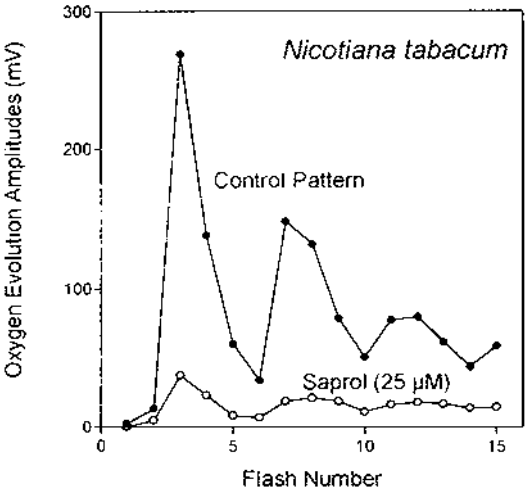

FIGURE 8 Effects of a Triforin-type fungicide on the oxygen-evolution capacity in chloroplasts from tobacco (*Nicotiana tabacum*). The photosynthetic oxygen evolution was detected as the consequence of short (5 μs) saturating light flashes spaced 300 ms apart. The fungicide was added to give a final concentration of 25 μM.

to the generation of free oxygen radicals. Recently, it was described in context with this herbicide that an in vitro translation product was formed at significantly increased levels during paraquat stress, and that this product was identical to the one that was observed in higher amounts during ozone stress [159].

Insecticides

Relatively little research has been done with respect to the effect of insecticides on the physiological and metabolic processes of cyanobacteria and higher plants. The analyses carried out by Bhunia et al. [160] described alterations in the gluthatione content and in the enzyme activity in a cyanobacterium (*Nostoc muscorum*) induced by Carbaryl, an often-used insecticide. This compound is a carbamate derivative which is used as contact and stomach poison and which has slight systemic properties. The chemical is often applied in fields of cotton, soft fruit, top fruit, vegetables, and various other crops. Higher plants have been analyzed with respect to lipids in maize chloroplasts under pesticide stress in the work by Mishra et al. [21]. Recently, we have investigated in a detailed study the effects of the synthetic pyrethroids cypermethrin, deltamethrin, fenvalerate, and permethrin on the photosynthetic electron transport reactions in cell cultures from tomato (*Lycopersicon peruvianum*) and in chloroplast suspensions from tobacco (*Nicotiana tabacum* var. John William's Broadleaf) [22]. These and similar insecticides might deserve specific scrutiny, under environmental aspects in particular, as they are derived from the naturally occurring pyrethrines which are physiological components of several *Chrysanthemum* species. Therefore, analyses of the modes of action of pyrethroids might be not only essential for basic plant physiological research but also could lead to new insights into structural specificities and necessities of modern and ecologically acceptable plant-protective chemicals.

Figure 9 shows that intact leaves from *Nicotiana tabacum* and cell cultures from *Lycopersicon peruvianum* suffer significant stress following the application of permethrin and cypermethrin. In this case, the leaves had been sprayed (wetted) with the chemical, whereas cell suspensions had been supplied with the pyrethroid in the respective concentration only 5 min before the measurements. The maximal fluorescence is substantially increased, and in the case of permethrin, the kinetics of the so-called Kautsky effect are virtually absent. The induction curves strongly resemble the ones which are observed in the presence of standard herbicides like, for example, Diuron. Thus, it might be concluded from the experiments that pyrethroid insecticides can interact with the photosynthetic electron transport chain between PSII and PSI. In this case, the primary acceptor of PSII (Q) is in the reduced state Q^-, which means that fluorescence is principally high, as the excitation energy cannot be dissipated versus PSI. When partial reactions of the photosynthetic electron transport chain were analyzed, it was shown that in fact the pyrethroid insecticide fenvalerat seems to interact directly with the herbicide-binding site Q_B of PSII.

When the photosynthetic electron transport running through both photosystems was analyzed by means of a water → methylviologen Mehler-type reaction, fenvalerat concentrations at about 40–60 μM inhibited the reaction almost completely. The same held true for a ferricyanide-mediated (i.e., a typical PSII reaction) Hill reaction. Both a silicomolybdate-driven Hill reaction and a dichlorphenolindophenol/ascorbate → methylviologen photosystem I reaction (which are normally herbicide insensitive) were not affected by increasing concentrations of fenvalerate (Fig. 10). *It can be concluded from the results that insecticides like the investigated pyrethroids do not only have substantial herbicidal activity, but they do interact with the photosynthetic electron transport chain at exactly the same site as herbicides of the urea type*. Furthermore, pyrethroids interact with the redox state system of the water-splitting system of photosynthesis. When the photosynthetic oxygen evolution is analyzed as the consequence of short saturating light flashes and when—based on the corresponding amplitudes—the dark distribution of the so-called S states is calculated, a strong effect of the pyrethroid deltamethrin was observed.

Figure 11 shows that increasing concentrations of deltamethrin modify the dark distribution of the S states in the sense that the overreduced state S_{-1} is preferentially formed at the expense

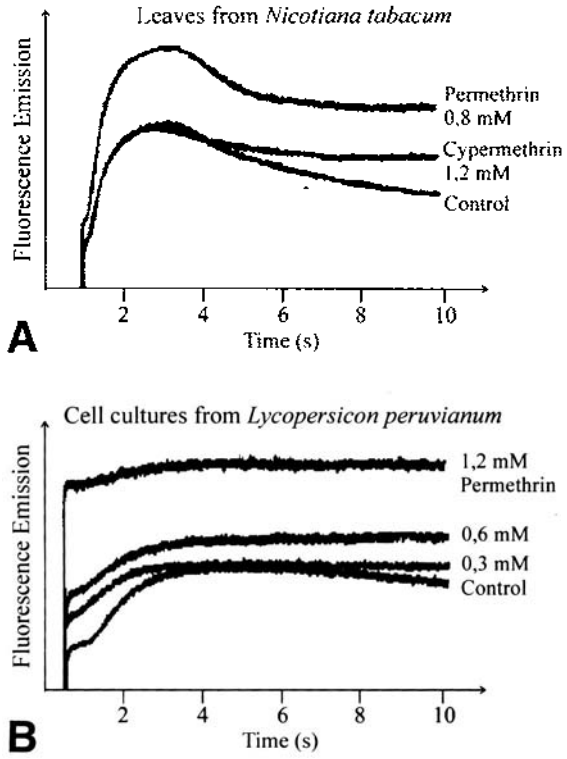

FIGURE 9 Effect of synthetic pyrethroid insecticides on the fluorescence induction curves from tobacco leaves (*Nicotiana tabacum*) (A) and cell cultures from *Lycopersicon peruvianum* (B). Plants were sprayed (completely wetted) with the pyrethroids in the respective concentration 1 day before the measurements. Leaves were cut from the plants and dark incubated 10 min before illumination (A). Cell suspensions were supplemented with the pyrethroid and dark adapted 5 min before the measurements (B). (From Ref. 22.)

of the ground state S_0. However, Figure 11 also shows that the described effects (and others) are highly specific for a given molecular structure. Apparently, the halogen side of the pyrethroid molecule plays a substantial role in this context, as fenvalerate, with its different structure in this region of the molecule, did not absolutely influence the redox conditions of the water oxidation complex (results not shown).

In conclusion, from these and similar results, we might say that more detailed analyses of pesticides (other than herbicides) are required with respect to their specific phytotoxicity and their interaction with the physiological and metabolic reactions of plants. The trivial statement that such compounds as insecticides and fungicides are well tolerated and are plant compatible does not allow us to conclude that plants are not drastically affected and inhibited by treatments with them. Moreover, it turned out that the deleterious effects on plants might well be influenced and up to a certain degree regulated by in some cases minor modifications of the molecular structures without affecting the specific toxicity toward the real target organisms. Thus, these and extended measurements together with the screening of appropriate chemicals and even the directed synthesis of compounds might help in the choice of effective but less phytotoxic and more ecologically acceptable plant-protective chemicals.

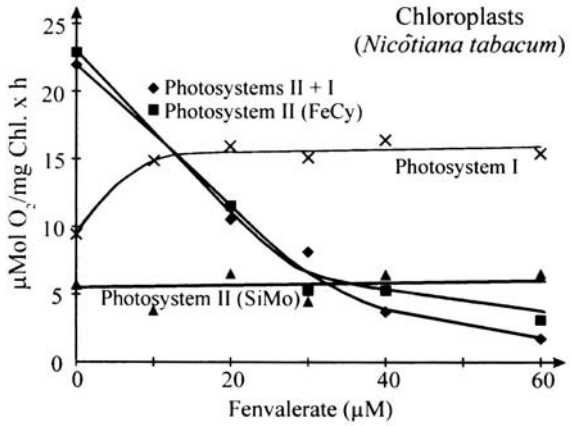

FIGURE 10 Effect of increasing concentrations of the insecticide fenvalerate on partial reactions of the photosynthetic electron transport in thylakoids from *Nicotiana tabacum*. $H_2O \rightarrow FeCy$; $H_2O \rightarrow MV$; PS I-DCPIP/asc $\rightarrow MV$; $H_2O \rightarrow SM$. For comparison, positive values of O_2 evolution and negative values of O_2 uptake are depicted in the same coordinate system. (From Ref. 22.)

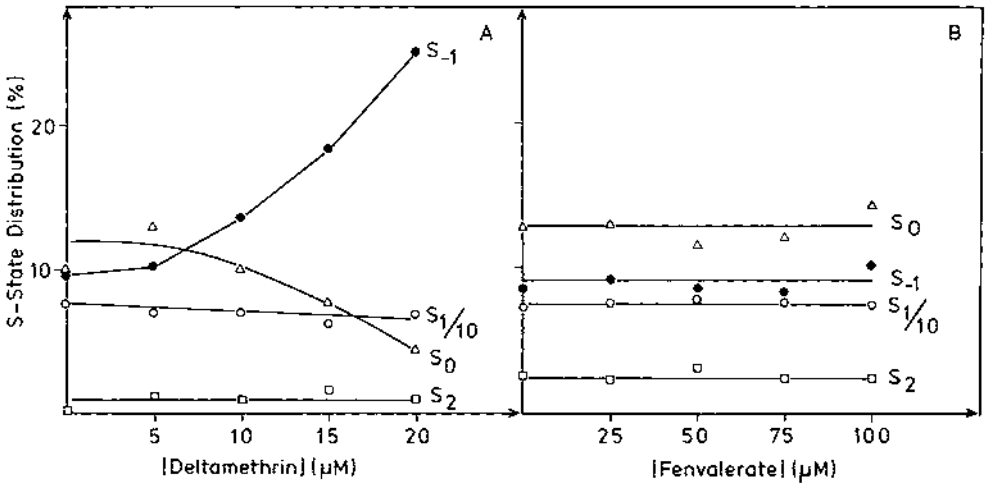

FIGURE 11 Effect of deltamethrin (A) and fenvalerate (B) on the S state distribution in tobacco chloroplasts calculated on the basis of a S state Kok model. (From Ref. 22.)

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Ecophysiological Adaptations and Genetic Variability in Mangroves

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INTRODUCTION

Mangroves are unique, taxonomically diverse plant communities inhabiting predominantly the estuarine and intertidal regions of tropical coasts. The exact total area of mangrove forests is not known, but it is estimated that mangroves cover nearly 15.2 million hectares worldwide (Table 1) [1,2]. Mangrove vegetation comprises approximately 70 species from 20 different angiosperm families [3]. The distribution of mangrove forests is limited to areas where the mean annual temperature of the coastal water is above 16°C. About 80% of all known mangrove plants are found in the Indo-Pacific region between South India, Oceania, and northern Australia, with 9% occurring in East Africa, 6% in West Africa, 5% in the Caribbean, and 5% in South Africa [2].

Mangrove vegetation in most cases is continuously exposed to harsh environmental conditions, being often dominated by salinity, flooding, high temperature, and high irradiance [4–9]. To withstand these environmental adversities, mangroves have evolved several ingenious solutions, which are well reflected in their distinct morphological, anatomical, and physiological adaptations [1,10]. In the past, much of the research on mangroves has been focused on the physiological ecology, species diversity, and systematics [1,10,11,12]. In this chapter, we discuss the physiological bases of various adaptative strategies, particularly those which are evolved to cope with high salinity and waterlogging, and emphasize the urgent need for more information on the genetic variability of mangroves for effective conservation of this overexploited ecosystem.

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TABLE 1 Common Plants of Mangrove Forests

Family	Genus	No. of species	Geographical distribution
Acanthaceae	*Acanthus*	3	India to Western Pacific, Australia, Philippines
Apocyanaceae	*Cerbera*	3	Southeast Asia, New Guinea
	Rhabdadenia	3	Subtropical and tropical South America
Avicenniaceae	*Avicennia*	8	Southeast Asia, Australia, South and West Africa
Bombacaceae	*Camptostemon*	2	Borneo, Moluccas, Australia, New Guinea, Philippines
Combretaceae	*Laguncularia*	1	South America, West Africa
	Lumnitzera	2	East Africa, Western Pacific, Australia, Indochina
	Conocarpus	2	East and West Africa, Tropical America
Euphorbiaceae	*Excoecaria*	2	Tropical Africa and Asia, Western Pacific
Lecythidaceae	*Barringtonia*	2	West Africa, Polynesia
Leguminosae	*Dalbergia*	2	South America, Caribbean, West Africa
Meliaceae	*Xylocarpus*	3	Indo-Malaya, East Africa
Myrsinaceae	*Aegiceras*	2	India, Australia
	Myrsine	1	Malaysia
Palmae	*Nypa*	1	Indo-Pacific, West Africa
Pellicieraceae	*Pelliciera*	1	South America
Plumbaginaceae	*Aegialitis*	2	Australia, Indo-Pacific
Pteridaceae	*Acrostichum*	3	Caribbean, Indo-Pacific
Rhizophoraceae	*Bruguiera*	6	Indo-Pacific, East and West Africa
	Ceriops	2	Indo-Pacific
	Kandelia	1	India, South East Asia
	Rhizophora	8	Pantropical
Sonneratiaceae	*Sonneratia*	6	East Africa, Indo-Malaya, Australia
Sterculiaceae	*Hertiera*	2	Tropical Africa, Indo-Pacific
Tiliaceae	*Brownlowia*	2	Southeast Asia

SALINITY

Salinity Tolerance, Plant Growth, and Distribution of Mangrove Species

Salinity refers to the occurrence of various soluble salts in soil or water in concentrations that may interfere with plant growth. Although NaCl is sometimes the most predominant salt present, the term *salinity* includes chlorides, sulfates, and bicarbonates of sodium, calcium, magnesium, and potassium [13,14]. The concentrations of these salts can be expressed in a multitude of ways, but the preferred expression by physiologists and soil scientists is electrical conductivity, stated as decisiemens per meter (dS/m) or millimhos per centimeter (mmhos/cm). According to U.S. Salinity Laboratory recommendations, a soil with an electrical conductivity of 4 dS/m, or if all the dissolved salt is NaCl with an ionic concentration of 44 mM or more, can be considered as saline. Salinity in the mangrove environment is largely due to NaCl and varies considerably in time and space [10,15]. It is noted that the salinity level in a mangrove habitat fluctuates considerably depending on the season, and occasionally it may even become hypersaline, with salinities ranging from two

to three times that of seawater. Thus, the relative salt tolerance of different mangrove species will largely determine the structure of mangrove forests along salinity gradients.

Mangroves, like many other glycophytes (plants of nonsaline habitat) [16,17] and halophytes (plants of saline habitat) [18], exhibit a variety of growth responses to salinity. For instance, *Sonneratia lanceolata* grows maximally in salinities ranging from freshwater to 5% seawater, but the growth declines drastically in 50% seawater [19]. In contrast, *Ceriops decandra* and *Sonneratia alba* showed extremely poor growth and a time-dependent decline in vigor under freshwater conditions [19]. In the same study, propagules of *Bruguiera parviflora* and *Ceriops tagal* var. *australis* failed to grow in freshwater, but vigorous growth was attained with 5% seawater, indicating that these species could be considered as being obligate halophytes. Of the 16 mangrove species examined by Ball and Pidsley [19], most of them could grow in freshwater, but growth was stimulated considerably in the presence of 5–50% seawater depending on the species. Several previous studies showed that halophytes, in general, grow faster with higher than the standard levels of salt in the growth medium [18,20–22]. The physiological or biochemical basis of this growth stimulation is not clear. However, it is suggested that the excessive accumulation of water and inorganic ions in the cells occurring under saline condition results in increased turgor pressure which in turn causes growth enhancement [23]. Although some earlier studies [22,24] lend support to the concept of a turgor-controlled growth response, there is evidence that increasing salinity in the bathing medium enhances growth by directly influencing various metabolic processes in mangroves. For example, Critchley [25] reported a positive requirement for high chloride levels for photosynthetic electron transport in *Avicennia marina*. In the same species, the stimulation of oxygen uptake and respiration of roots with an increasing salt concentration accompanied a significant stimulation of plant growth [24]. It is, therefore, very probable that, in *Avicennia marina*, a highly salt-tolerant mangrove species, the enhancement of growth by increased salinity levels of the growth medium may not be solely due to turgor-controlled extension growth.

Sensitivity to salinity varies with the developmental stage of plants [26], and this is evident even in highly salt-tolerant mangroves [1]. Like other salt-marsh species [27,28], seeds of mangroves germinate at relatively low levels of salinity. Clarke and Hannon [29], in their studies of mangrove and other salt-marsh species of Sydney swamps, reported a decreased germination rate with increased salinity levels for most species. In fact, seeds of all species examined germinated in tap water. Interestingly, mangrove species showed a requirement for seawater for further growth of seedlings, and maximum seedling growth for all mangrove species was attained in nutrient solution containing 20% seawater. Unfortunately, there are not many studies related to germination of mangroves under saline conditions, and thus little is known about the mechanism(s) by which NaCl inhibits germination in mangroves. In *Avicennia marina*, as in many glycophytes [30,31], salinity affected seed germination by inhibiting cotyledonary reserve mobilization; thus, the viviparous development of the propagules seems to be an adaptation to circumvent the adverse effects of salinity on germination in this species [32]. Although germination was adversely affected by salinity, further growth and establishment of *Avicennia marina* seedlings were maximal in 50% seawater [20,29,32]; again suggesting the requirement for NaCl for optimal vegetative growth in mangroves.

There have been several studies on the various aspects of vegetative growth of mangroves in relation to salinity [10,22,29,33–35], but detailed analysis of relationships between interspecific differences in salt tolerance and species distribution is rather limited [4,36–38]. In a recent investigation, Ball and Pidsley [5] observed distinct interspecific differences in salt tolerance between two mangrove species, *Sonneratia alba* and *S. lanceolata. S. alba* grew in salinities ranging from freshwater to seawater, with growth being maximal in 5–50% seawater, whereas *S. lanceolata* grew in 0–50% seawater, with maximal growth occurring in 0–5% seawater. A change in the net assimilation rate accounted for most of the differences in growth between these two species with an increase in salinity. It appears that there is a tradeoff between the growth rate and the acquisition of salinity tolerance as the less salt-tolerant *S. lanceolata* achieved twice the height, leaf area, and biomass of the more salt-tolerant *S. alba* under low-salinity conditions. Thus, *S. lanceolata* becomes an effective competitor under low-saline conditions and successfully excludes *S. alba* from establishing in such

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environments. From these results, Ball and Pidsley [5] concluded that the differential distribution of *S. alba* and *S. lanceolata* along tidal river systems may be a reflection of the difference in salt tolerance of these species as the riverine salinity regimens vary with distance upstream from the mouth of the river. A similar conclusion also was made by Smith [39] while comparing the growth and dispersal of the closely related species *Ceriops tagal* and *Ceriops australis* grown under a saline condition.

From the above discussion, it is interesting to note that a saline condition is a requirement for the optimal growth of mangrove species; salinity stimulated growth phenomenally in most of the species studied. Although the physiological basis of this attribute is not clearly understood, it may have some implications in developing salt tolerance and colonizing a saline habitat. The remarkable variation in salt tolerance within the mangrove species may, at least partly, account for their characteristic segregation in different mangrove environments.

Mechanisms of Salt Tolerance

Much of the physiological investigations on plant adaptations to salinity has concentrated on water relations, carbon acquisition and allocation, and metabolite production with the assumption that these processes would be the most severely affected ones under salt-stress conditions. Although physiological knowledge gained in the past helped characterize various patterns of responses, no clear understanding of salt-tolerance mechanisms, either in halophytes or glycophytes, has emerged from these studies. From the available evidence, the consensus is that all plants face qualitatively similar problems in a saline environment. Plants, including salt-tolerant mangroves, need to adapt osmotically and avoid ion toxicity, nutrient deficiency, and water stress to sustain growth under saline conditions [18,40–44]. To overcome such problems, mangroves have adopted several mechanisms, including salt exclusion, salt accumulation, and salt secretion [45–48]. Apparently, some of these mechanisms may interact with each other at the whole-plant level. For instance, salt exclusion is a common strategy to regulate the influx of salts into mangrove roots, but it appears to be less efficient in mangrove plants with salt-secretion glands [15,46,49]. Similarly, accumulation of ions for osmoregulation is a common strategy of mangrove plants, although they differ in the extent to which ions can be accumulated without any adverse effects on metabolism [50–52]. Irrespective of the strategies employed, the separation of the osmotic and metabolic roles of ions remain crucial for the survival of mangroves under saline conditions.

Osmoregulation and Ion Toxicity Avoidance

The ability to regulate the transport of ions and water in relation to growth is a distinguishing feature of mangrove species. Most of the glycophytes do not possess an efficient mechanism to adjust salt influx to maintain a favorable water balance [30,41], whereas halophytes accumulate large amounts of Na^+ and Cl^- to maintain osmotic adjustment and turgor to sustain growth [45]. But maintaining high intracellular ion concentrations without affecting growth can be achieved only if the plants possess high metabolic tolerance of the resulting ion build-up. If Na^+ is selectively excluded as in some species, then osmotic adjustment is normally achieved with an increased uptake of K^+ as the cation [45]. But high concentrations of K^+ are as inhibitory as Na^+, and again metabolic tolerance becomes necessary. Apparently mangroves accumulate large amounts of Na^+ and Cl^- for osmoregulation [50,53]. For instance, the salt concentration in the leaves of *Avicennia marina* reached around 600 mM NaCl when plants were grown in the presence of 500 mM NaCl [53]. A similar situation also was evident in *Agiceras corniculatum* grown under high-saline conditions [53]. In another independent study, Downton [22] observed a large accumulation of inorganic ions in the leaves of *Avicennia marina* exposed to salinity stress. Together these results indicate that the leaves of *Avicennia* species accumulate high levels of inorganic ions, which were in fact more than sufficient to maintain leaf osmotic pressures at higher levels than those experienced at the roots. But several studies have shown that enzymes and metabolic processes such as protein synthesis have a narrow

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range of ion concentration for optimal activity in both glycophytes and halophytes [26]. Enzymes extracted from salt-adapted halophytes were found to be salt sensitive. Conspicuously, the activity of these enzymes was considerably inhibited in vitro at salt concentrations similar to those that were found optimum in the medium for growth of the source plants [54,55]. Also, the salt sensitivity of amino acid incorporation into proteins by microsomes from salt-adapted halophytes indeed did not differ from that of microsomes obtained from glycophytes [56]. So it is apparent that high levels of inorganic ions cannot be maintained in the cytoplasm, and mangroves, like other halophytes, overcome this problem either by salt secretion [47,57] or probably by compartmentation of ions to less metabolically active sites [46]. Analysis of the chemical composition of Australian mangroves grown under saline conditions showed a substantial accumulation of low molecular weight organic compounds and compatible solutes that did not interfere with cell metabolism [50–52]. Although there is a lack of direct evidence, the above findings indicate that, in mangroves, the excess ions entering into the cells may be sequestered in vacuoles to minimize ion toxicity, as synthesis of metabolically compatible solutes to balance the osmotic potential of the cytoplasm is a common response of salt-stressed plants compartmentalizing excess ions in vacuoles [18,26]. In vitro studies have shown that low molecular weight organic solutes such as proline and glycinebetaine are compatible with enzyme activity even at 1000 mM [58].

At the cellular level, salt balance must be maintained either by the effective exclusion of Na^+ and Cl^- ions initially or by other strategies like salt secretion or ion compartmentation. In many salt-tolerant plants, ion compartmentation seems to be a highly effective mechanism to minimize ion toxicity [59], and the importance of this adaptive strategy to metabolic processes is well exemplified by the Na^+ compartmentation of cell organelles, chloroplasts, and mitochondria in the leaf cells of the halophyte *Suaeda maritima* [60]. In *Suaeda maritima* grown at 340 mM NaCl, Na^+ was about 150 mM in the cytoplasm and 600 mM in the vacuole of the leaf cells, thus showing an efficient compartmentation of Na^+ in the vacuole under a saline condition [60]. Unfortunately, the regulatory aspects of transmembrane ionic movements in relation to salinity stress have not been studied extensively in mangroves. Also, little is known about the significance of this strategy to physiological functioning in mangroves. Only a few investigations on the subcellular estimation of ions have been undertaken in mangroves [47,61]. Salt-tolerant plants usually accumulate large quantities of Na^+ and Cl^- ions in their leaves, but there is no evidence that chloroplasts and mitochondria are the sites of salt adaptation capable of tolerating large amounts of Na^+ and Cl^- [62]. For example, analysis of the ionic composition of chloroplasts isolated from salt-tolerant and salt-sensitive plants showed similar levels of Cl^- [63–67]. It has been observed that chloroplasts of both salt-tolerant and salt-sensitive plants maintained K^+ as the major cation, but in salt-tolerant species, Na^+ may be substituted for K^+ to a considerable extent, as Na^+/K^+ ratios in chloroplasts of salt tolerant species were much higher than those found in salt-sensitive ones [66]. This indicates that, in halophytes, the entry of Na^+ in the chloroplasts may be selectively regulated. However, the mechanism of ion compartmentation appears to fail under higher salinity levels even in highly salt-tolerant mangroves. For instance, in *Avicennia marina*, high concentrations of NaCl in the growth medium caused severe disorganization of photosystem II, thus making the plants more vulnerable to photoinhibition [68].

Control of Ion Uptake

Control of ion uptake is perhaps one of the most poorly understood salt-adaptive mechanisms of mangrove species. There have been very few investigations into the structure and function of the mangrove roots in relation to ion uptake [69,70], although the anatomical features of mangrove roots have been studied extensively with respect to certain functions such as aeration, water absorption, and mechanical support [1,71]. Also, there appears to be very limited information on the ionic status, particularly of Na^+, K^+, and Cl^-, in the xylem sap of mangroves [47,72]. It is estimated that the xylem sap of field-grown *Aegialitis annulata* contained 118 mM Na^+, 14 mM K^+, and 122 mM Cl^- [46]. In the case of *Avicennia marina* grown in seawater, Field [72] gave a value of 792 mM Na^+, 118 mM K^+, and 799 mM Cl^- for the xylem sap of the stem. A comparable level of Na^+ and

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Cl^- ions also was found to be present in the xylem sap of root, but the K^+ content was twice that recorded for the stem xylem sap. In another study with *Avicennia marina* grown under a natural habitat, it was found that the ion concentration fluctuates considerably during the day [47]. All the three ions measured, Na^+, K^+, and Cl^-, had higher concentrations in the mornings and evenings. The average concentration of Na^+, K^+, and Cl^- was, respectively, 130, 30, and 120 mM, with a Na^+/K^+ molar ratio of approximately 4/1. Clearly, more quantitative data of the ion content in the xylem sap of various mangroves are required to ascertain the true relationship between different ionic species as well as to gain more insight into the regulation of ion uptake.

A quantitative analysis of the contribution of various factors regulating the salt balance of *Avicennia marina* leaves indicated that salt filtration by the root is by far the most important salt-exclusion mechanism operating in this species [47]. With the salt filtration by the roots, *A. marina* prevents nearly 80% of the salt, which is carried toward the root surface by the transpiration stream, from entering the plant [47,48]. Such a system for salt exclusion may require energy for its operation. In the case of *A. marina*, Waisel et al. [47] noted that the extremely low water potential in the xylem would serve as the driving force for the operation of this ultrafiltration system. A similar explanation also was advanced by Scholander [15] to account for the operation of the salt-exclusion system existing in *Aegialitis*.

The mechanism by which mangroves regulate the uptake of ions at the root surface is not understood. In an earlier study, Lawton et al. [69] found that the inability of *A. marina* to prevent salt influx completely resulted from a structural gap existing between the fully developed endodermis and the proximal end of the root tip. Using the same species of *Avicennia*, Moon et al. [70] demonstrated that the access of external salt solution to the symplasm was restricted to the distal 17 mm of the third and fourth order roots, and a barrier to apoplastic transport in the periderm and endodermis isolated the bulk of the root system from the external salt solution. It is noted that the location of an apoplastic barrier at the root periphery helps prevent the cortex from accumulating deleterious levels of Na^+ and Cl^- from the salt solution. With fluorescent tracer dyes, Moon et al. [70] also showed that only minimal apoplastic uptake of water and ions took place in *A. marina* grown under a saline condition. Obviously, this functional attribute and the structural barrier at the periderm and endodermis to apoplastic ion transport in *A. marina* roots are well suited to cope with a highly saline environment. However, the occurrence of similar characteristics in other mangrove species remains unknown.

Control of Salt Balance in the Shoot System

Continuous absorption of salts even at limited amounts and consequent transportation into the shoot system eventually raise the salt concentration of leaves to harmful levels. Apparently mangroves and other halophytes accumulate large amounts of Na^+ and Cl^- [73–76], and thus balancing of the salt content in photosynthetically active leaves and growing tissues becomes a necessity to sustain growth. There are several mechanisms by which mangroves and other halophytes maintain the salt balance in the leaves. These include salt secretion, salt accumulation in bladder hairs, retranslocation to other organs, shedding of old leaves, and temporary growth adjustments. The relative importance and efficacy of each mechanism varies among different species of mangroves and different ecological conditions [26,57].

Salt secretion is one of the best-known adaptive mechanisms whereby mangroves regulate the salt content of their leaves [47]. Species of *Acanthus*, *Aegialitis*, *Aegiceras*, and *Avicennia* possess salt-secretion glands in their leaves [46,73,77]. Salt glands are regarded as being highly selective and salt secretion by them as a fast-operating mechanism. However, in *A. marina*, salt glands are nonspecific and secrete a variety of ions [78,79]. The composition of salt gland secretion, nonetheless, is largely a reflection of the ionic composition of the root-bathing medium; thus, Na^+ and Cl^- were the predominant ions present in the secretion of *A. marina* salt glands [78,79].

In spite of its wide occurrence, the structural and functional aspects of salt secretion have been studied only in a limited number of plant species. High rates of secretion of Na^+ and Cl^- were

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shown for different species of mangroves [46,73] and some species of coastal halophytes such as *Spartina anglica*, *Limonim vulgare*, *Armeria maritima*, *and Glaux maritima* [75,80]. In a detailed investigation of salt balance of *A. marina* leaves, Waisel et al. [47] found that salt secretion accounted for the removal of approximately 40% of the salts entering the leaves. Contrary to the expectation, no correlation between the rates of transpiration and salt secretion was noticed in this plant species. Such a correlation would have been expected if the salts brought in by the transpiration stream were the direct and sole source for secretion. The lack of such a correlation indicates that the salts which reach the leaves via transpiration are sequestered in the cells initially, and then they are probably channeled to the salt glands for secretion by some hitherto unknown mechanism. It is suggested that the salt-secretion process in *A. marina* is possibly supported by the higher concentration of salts in the xylem sap [47]. Indeed, in another study [61], the salt content of the gland cells of *A. marina* was decidely lower than that of the mesophyll. In this species, a downhill salt gradient was found to exist from cells near the xylem through the mesophyll to the gland cells. A similar effect of salt concentration on salt secretion also was observed in other salt-secreting plants [80,81]. Although the mechanism controlling salt secretion is not precisely understood, it must be noted that ''ion pumps'' have been proved to participate in salt excretion in *Atriplex* [82] and *Limonium* [83,84], and it is likely that they are a feature of other salt gland–bearing plants as well.

Although highly efficient, salt secretion is not a common salt-adaptive feature of mangrove species. Indeed, most mangrove species lack salt-secretion glands. As Na^+ and Cl^- are continuously transported to the shoot by the transpirational stream, it is imperative that the leaves of nonsecreting species must evolve adaptive mechanisms to maintain the leaf salt concentration to physiologically acceptable levels. Available evidence indicates that the nonsecreting species usually maintain constant concentrations of Na^+ and Cl^- in the leaves. This is often achieved by modulating growth, increasing succulence, and retranslocating ions to older tissues [1,57,74,85]. It is suggested that a considerable amount of accumulated ions will be channeled to actively growing tissues, such as expanding leaves, as the demand for ions will be greatest in those tissues [86]. However, the requirements of salt utilization for cell growth are not sufficient to balance the rate of salt influx to leaves even in fast-growing systems [46].

The development of succulence appears to help maintaining salt balance and osmoregulation in mature nongrowing leaves of some mangroves. For instance, the leaf thickness of *Rhizophora mangle* grown under fluctuating salinity conditions almost doubled when they were exposed to continuous salinity [85]. An increase in leaf succulence with increasing salinity of the growth medium also was observed in seedlings of *A. marina* [24]. In *Laguncularia racemosa*, Biebel and Kinzel [87] reported a fourfold increase in leaf thickness from the youngest to oldest leaves along a shoot. Such an increase in leaf thickness is probably representative of many mangrove species.

Although leaf succulence alleviates the problem of salt accumulation to some extent, it is argued that retranslocation of ions to older leaves/tissues seems to be a more useful strategy to maintain a favorable ion concentration in growing and metabolically active tissues for some mangrove species. As seen from the data presented by Waisel et al. [47], about 25 and 19% of the absorbed Na^+ and Cl^-, respectively, were transported out of the leaves of *A. marina* at the rate of 0.205 mmol NaCl per gram dry weight per 24 h. It is well established that any retranslocation of ions from leaves must take place via the phloem, but there is no convincing evidence that reexport of excess ions are transported via the phloem. Furthermore, in species where retranslocation of excess Na^+ and Cl^- ions were documented, the distances to which ions were transported were short [47]. Taken together, it is apparent that more indepth analysis of ion transport under salt-stress conditions is required before making any definite conclusion about ion retranslocation strategy in mangroves.

Causes of Growth Limitation and Costs of Salt Tolerance

The distribution of mangrove species along salinity gradients is primarily determined by the extent to which a species can tolerate different levels of salinity. Detailed analysis of energy requirements

for various cellular processes associated with salt adaptation [48] indicates that an increase in salt tolerance incurs considerable energy costs to the species. In mangroves, this aspect is best studied in relation to conservative water use and photosynthesis.

Mangroves follow C_3 photosynthetic pathway, and there is no evidence that these plants can shift from the C_3 to C_4 or crassulacean acid metabolism (CAM) pathways even under different environmental conditions [10]. In their elegant studies, Ball and others [6,48,53,68,88] found that mangroves possess an array of functional and structural attributes to maximize photosynthetic efficiency under stressed conditions. For instance, the gas exchange and water-use characteristics of mangroves are unusually conservative for the C_3 pathway [62]. Also, depending on the environmental conditions, the amount of water used for carbon assimilation varies considerably between species. This is exemplified by the water-use characteristics of *Aegiceras corniculatum* and *Avicennia marina*, two mangrove species with different salinity tolerances, grown under different salinity (50, 250, and 500 mM NaCl), and leaf to air vapor pressures (6, 12, and 24 m bar) [48]. With increasing salinity and decreasing humidity, the net water-use efficiency declined in more salt-sensitive *A. corniculatum*. On the other hand, the net water-use efficiency in the more salt-tolerant *A. marina* remained almost constant under similar environmental conditions. Thus, the water-use efficiency was more conservative in the more salt-tolerant species and became increasingly conservative with increase in salinity. Conservative water use may be a consequence of the high-energy cost of water uptake. But it can be viewed as a desirable adaptation to maintain a favorable carbon-salt-water balance, as restricted water uptake limits the entry of undesirable salts into the transpiration stream [48]. Evidently, the slow growth of highly salt-tolerant mangrove species such as *A. marina* in both freshwater and seawater could be probably due to the maintenance of high water-use efficiencies at the cost of carbon assimilation [89]. At this juncture, it is interesting to note that the hydraulic conductance of *A. marina* roots even under freshwater conditions was about two orders of magnitude lower than those of salt-sensitive species and decreased with an increase in salinity [72]. Although the reduced intake of water helps regulate the influx of ions, it necessitates the development of a massive root system to meet the water demands of the shoot. This results in the preferential allocation of photosynthates to root development at the expense of canopy development, as seen in *A. marina*.

Restricted photosynthetic gas exchange and the inability to exploit high irradiance are the other two major factors limiting the productivity of mangroves under saline condition. Conservative use of water imposes severe limitations on leaf functioning under field conditions. For example, high water-use efficiency could be related to the necessity to regulate the salt balance in the tissues and to minimize energy loss in eliminating accumulated salt. High water-use efficiency can be attained only by close coordination between the photosynthetic metabolism and the functioning of stomatal aparatus [90,91]. In a detailed study of the leaf functioning of mangroves in relation to the leaf temperature, Ball et al. [6] observed that stomatal conductances of *A. marina, Bruguiera gymnorrhiza*, and *Rhizophora apiculata* were considerably lower than those typically found in well-watered C_3 species. This reduced leaf conductance restricted the influx of CO_2 and the efflux of water vapor, causing photosynthesis to operate at a low intercellular CO_2 concentration but with high water-use efficiencies (i.e., mmol CO_2 gained per mol water lost). Indeed, Ball et al. [6] reported water-use efficiencies ranging from 3.2 in *B. gymnorrhiza* to 4.6 in *A. marina*; values which are exceptionally high for C_3 plants under salt-stress conditions.

Although restricted stomatal conductance results in very high values of water-use efficiency, it inevitably increases the leaf temperature to inhibitory levels [90]. In mangroves, photosynthetic activities and stomatal conductance are maximal between 25 to 30°C and decline dramatically above 35°C [6,32,90,92]. Considering the fact that photosynthesis in mangroves becomes light saturated at quantum flux densities ranging from 30 to 50% sunlight [88,92,93], and that stomatal conductances are highly restricted, it is conceivable that mangroves must avoid high light intensities if leaf temperatures are to be maintained within physiologically favorable ranges. In all the mangrove species thus far examined, evaporative cooling was found to be insufficient to prevent the leaf temperature from rising above the ambient temperature [10]. Thus, mangroves evolved other additional strategies to overcome leaf temperature build-up to undesirable limits. Ball et al. [6] identified

three properties, leaf angle, leaf size, and heat capacity per unit area, which are involved in the maintenance of a desirable leaf temperature in mangroves. In all the five mangrove species studied, *B. gymnorhiza* (L) Lam., *Ceriops tagal* (Perr.) B. Rob. Var. *australis* (White.), *R. apiculata* Bl., *R. lamarckii* Montr., and *R. stylosa* Griff., increasing the leaf angle (i.e., the inclination of leaf to the horizontal plane) greatly reduced the intensity of heat loading. The leaf angle, considered as a compromise between the requirements for illumination and reduction of temperature, was increased from approximately 0° in full shade to about 75° in fully exposed leaves. Apparently, the response of the leaf angle to irradiance displayed a distinct species specificity, with the leaf angle increasing with increasing salinity tolerance of the species [6]. The effect of leaf orientation in minimizing leaf to air temperature differences was also striking in *R. stylosa* Griff. growing in its natural environment [90].

The leaf size is another attribute shown to influence the maintenance of the leaf temperature in mangroves. Heat transfer between the leaf and the ambient air is determined by the heat-transfer resistance imposed by a boundary layer, the characteristics of which are a function of the leaf size and other environmental variables such as the air temperature and wind speed. It has been shown that a decrease in leaf size enhances the boundary layer conductance and thus helps maintain the leaf temperature closer to that of the ambient air. Interestingly, there is a correlation between the leaf size and the salt-tolerance trait of mangrove species. For example, the leaves of *Ceriops tagal*, the most tolerant of the five species studied [6], were the smallest and most sensitive to a variation in exposure to sunlight. Similarly, the leaves of mangrove species growing in hypersaline coastal environments such as *A. marina, C. tagal var. australis, Excoecaria ovalis, Lumnitzera racemosa*, and *Osbornia octodonta* are much smaller than those dominating low-saline habitats [10].

Another important leaf characteristic regulating the leaf temperature is the heat capacity per unit area. It is shown that leaf succulence increases the heat capacity per unit area and thus minimizes fluctuations in the leaf temperature due to variation in irradiance and other environmental variables [6]. In mangroves, the leaf heat capacity increases with an increase in the leaf dry weight and the water content per unit area. Evidently, the heat capacity showed a positive correlation with the salinity tolerance of the mangrove species [6].

It appears that there is an inverse correlation existing between water-use and salinity tolerance in both salt-secreting [10,53] and nonsecreting species [6]. Under normal field conditions, mangroves usually control the entry of excess Na^+ and Cl^-, regulate the leaf temperature build-up, and capture maximum irradiance to maintain optimal carbon fixation. Excessive levels of transpiration inevitably lead to the accumulation of ions in leaves resulting in several metabolic dysfunctions. It is therefore reasonable to assume that leaf conductance (stomatal transpiration) should be minimized to sustain photosynthetic processes at the required threshold.

WATERLOGGING, SOIL REDOX CONDITIONS, AND GROWTH AND DISTRIBUTION OF MANGROVES

Like soil salinity, waterlogging is a common environmental condition in mangrove swamps. The roots of mangroves in flooded habitats must endure anoxic soil conditions, since waterlogged soils become devoid of oxygen within a few hours after flooding [94]. Mangroves evolved several structural and functional adaptive mechanisms to cope with this stressful condition. These include the development of pneumatophores (*A. marina*), stilt roots (*R. stylosa*), and knee roots (*Bruguiera exaristata, Xylocarpus granatum*) with extensive aerenchyma, numerous lenticels [1], and the occurrence of anaerobic root metabolism [95]. Although the aerenchyma is extensively developed,which is estimated to be as much as 70% of the total root volume in some species [96], its ability to provide sufficient oxygen for complete aerobic metabolism in the roots of mangroves and other wetland plants growing in flooded soils has been questioned [97–99].

Although several studies have been conducted with herbaceous marshy plants and flood-toler-

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ant tree species [100–106], little is known about the root metabolism in mangroves [95,107]. In response to hypoxia, the root metabolism in the black mangrove, *Avicennia germinans* (L.), became anaerobic by increasing the capacity for alcoholic fermentation dramatically [95]. Oxygen concentrations in the roots decreased markedly under anoxic soil conditions, and this low level of oxygen was observed even after 96 h of flooding. The intact roots of flooded plants responded metabolically by increasing the activity of alcohol dehydrogenase and malate dehydrogenase; however, no change in phosphenol pyruvate carboxylase activity was discerned. The build-up of oxygen tension and the increased capacity for alcoholic fermentation in hypoxic roots indicate that, as in many other marshy plants, oxygen diffusion from the aerial parts of the roots of *A. germinans* was not sufficient to maintain complete aerobic metabolism in the hypoxic environment.

Among the other metabolic changes observed, an alteration in adenine nucleotide production was the most pronounced. In the hypoxic roots of *A. germinans*, adenine nucleotide concentrations and the adenylate energy charge ratio were significantly lower than those of the aerobic controls [95]. However, in this system, increased glycolysis and alcoholic fermentation helped maintain sufficient ATP production when aerobic metabolism was limited. These observations clearly suggest that metabolic adaptations will be as important as the enhanced internal oxygen diffusion attained by structural adaptations to tolerate waterlogged conditions.

The functioning of mangrove roots under saline, flooded conditions seems to be highly complex [38,95,107]. There is a pronounced interaction of salinity and flooding on nutrient availability. For instance, the salinity tolerance of all the species studied was found to be markedly decreased under waterlogged conditions. This reduction in salt tolerance has been ascribed to, at least in part, soil hypoxia associated with flooding, as it affected both salt-exclusion and salt-selective absorption of K^+ over Na^+ [108,109] even in highly salt-tolerant mangroves like *A. marina* [22,53,110]. Studies with Australian mangroves [111] found that nitrogen and phosphorus also were limiting under salt-water flooding. In continuously flooded soil, ammonia will be the major form of combined inorganic nitrogen which readily adsorbs onto organic sediments, making it less available for uptake by roots. In contrast, when soil is less frequently flooded, mangrove growth is evidently limited by the availability of phosphorus [111]. At less frequent inundation, soil becomes more aerated and hence more oxidized. Under this condition, a considerable amount of phosphorus may be precipitated with calcium, aluminium, and iron or may be adsorbed onto clay particles.

Waterlogging in mangrove swamps results in various intensities of soil redox potential, which on its own imposes substantial levels of stress, especially nutrient stress, on plants. The redox status of the sediment, which affects both the form and availability of inorganic nutrients, is largely determined by the degree of soil saturation. In a recent study, *A. germinans* and *R. mangle* displayed distinct species specificity in their tolerance to soil redox potential under saline condition [112]. Seedlings of *A. germinans* showed a greater sensitivity to low redox potential than *R. mangle*, and the level of sensitivity was closely related to the intensity of the soil reduction.

In addition to the effect of the soil redox condition, the presence of sulfides in interstitial water also was found to have a significant influence on the growth and succession of mangroves [113,114]. An interesting pattern of sulfide sensitivity was, however, noticed in *A. germinans* and *R. mangle* [115]. *A. germinans* seedlings were more sensitive to sulfide than those of *R. mangle*. But mangrove zones dominated by *A. germinans* were characterized by strongly reducing soils with high sulfide (2–4 mM), whereas the zone dominated by *R. mangle* had moderately reducing soils with low-sulfide concentrations (about 0.3 μM). This is intriguing, as the sensitivity to sulfide reversed almost completely during the adult phase. It is believed that the sensitivity of the root system of *A. germinans* seedlings to sulfide is related to the oxygen status, and the development of various structural and functional adaptations during the adult phase probably alleviates root hypoxia and hence the sensitivity to sulfide. From this example [115], and the other studies reported earlier [116–118], it is apparent that the soil redox conditions and the sulfide concentration in the soil environment have a major role in the growth and species distribution of mangroves under waterlogged conditions. However, it must be noted that the growth of mangrove plants in a flooded environment may be affected by the accumulation of the reduced forms of iron and manganese [119], organic acids [120],

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and gases such as ethylene, methane, and carbon dioxide [121]. Involvement of these factors in the species distribution of mangroves therefore cannot be excluded.

GENETIC VARIABILITY AND CONSERVATION OF MANGROVES

Mangrove forests are valuable natural resources with a unique habitat value. This highly productive salt-tolerant ecosystem is not only a habitat for several unusual plants and animals but also supports the economic life of many coastal communities in the tropics [2]. Mangroves offer an enormous variety of natural products which include timber (species of *Avicennia*, *Bruguiera*, *Ceriops*, *Excoecaria*, *Heritiera*, *Nypa*, *Oncosperma*, *Rhizophora*, *Sonneratia*, and *Xylocarpus*), food (fruits, seeds, and young sprouts of *Avicennia*, *Acrostichum*, *Bruguiera*, *Nypa*, *Rhizophora*, and *Sonneratia*), medicinal plants (species of *Bruguiera*, *Ceriops*, *Excoecaria*, and *Xylocarpus*), fuel wood, tannins, and dyes (mainly members of Rhizophoraceae), and saponins (species of *Barringtonia* and *Dorris*). In addition, mangroves also are utilized for fisheries, agriculture, coastal protection, wildlife management, and sewage treatments [1,122].

The extensive and indiscriminate utilization of mangroves for industrial and developmental purposes resulted in rapid, massive destruction of this potentially renewable vegetation. This is best exemplified by the decline in mangrove forests in Kerala, a coastal state in South India. At the turn of the century, some 70,000 ha of pristine, rich mangrove vegetation bordered the coastal line of Kerala, but owing to rapid urbanization, it is now almost reduced to about 250 ha occurring as small patches of vegetation along the shoreline [2]. Although not so acute, mangrove forests are being cleared in Southeast Asian countries as well. Unfortunately, little effort has been made to prevent the rapid destruction of the mangrove ecosystem until recently. Of late, however, several countries have passed Regulatory Acts to protect mangrove forests. In addition, many national and international programs are now in place for the conservation of this unique ecosystem.

A major stumbling block for effective conservation and afforestation program is the lack of knowledge about the genetic make-up of plant species within the mangrove ecosystem. Conventional genetic analysis is difficult in mangrove species, and so far no detailed studies have been carried out in this group of plants. Further, available information on the reproductive biology and population genetics is rather scanty and restricted to selective species [123–127]. In a recent study aimed at identifying distinct genotypes for long-term conservation. Lakshmi et al. [125] analyzed 48 genotypes of the mangrove *Acanthus ilicifilius* and representing eight distinct populations. In this investigation, the first report on the use of molecular markers in assessing intrapopulation and interpopulation variability in mangroves, they detected a low level of (4–6%) polymorphism at the intrapopulation level through both random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) techniques. However, at the interpopulation level, the polymorphism of RAPD and RFLP was about 28%. In a similar study (see Ref. 125 and unpublished results quoted therein) with *Excoecaria agallocha*, a dioecious mangrove tree species, the extent of polymorphism detected was of much higher magnitude. The intrapopulation polymorphism ranged from 20 to 31%, whereas as much as 65% of the amplification products were polymorphic at the interpopulation level. These examples indicate that considerable genetic variability exists between populations, and that different mangrove species may display varying degrees of polymorphism depending on their edaphic preferences and adaptations, as suggested earlier.

Although the DNA marker–based analysis done by Lakshimi et al. (see Ref. 125 and unpublished results quoted therein) showed considerable genetic variability among different populations of *Acanthus ilicifolius* and *Excoecaria agallocha*, the findings of Lowenfeld and Klekowski [127] revealed very high genetic similarity among three different populations of red mangrove, *R. mangle*. From the segregation ratios for the chlorophyll-deficiency mutation, Lowenfeld and Klekwoski [127] concluded that selfing is very prevalent in the populations studied and that *R. mangle* forests may be essentially single-species ''natural monocultures'' with little genetic diversity. As this study is

soley based on a single genetic marker, a more definite conclusion on the genetic variability of *R. mangle* can be drawn only after an indepth analysis of a large number of populations of this species using modern DNA marker–based techniques.

CONCLUSIONS

Mangroves are one of the much neglected and overexploited ecosystems in the world. Fortunately, owing to the activity of various environmental interest groups, there is now an increased awareness and appreciation of the importance of mangroves in protecting coastal and estuarine ecosystems. It is now recognized that there is an urgent need to devise management practices which optimize the conservation of mangrove resources on a sustainable use basis [12]. As mangroves are nonhomogeneous open ecosystems that are extremely dynamic, a thorough understanding of the interaction of physical and biological processes occurring in this system is essential to develop effective management practices. In the past, we have achieved reasonable success in defining the physiological manifestations of dominant mangrove species under different environmental conditions [5,10]. However, we still do not know how different plant species establish and survive the harsh conditions of a mangrove habitat where they are continuously challenged by different environmental stresses simultaneously. Plants are complex organisms and they possess sophisticated control mechanisms, both at the cellular and organismal levels, to deal with different environmental situations [26]. It appears that integration of the knowledge gathered in the past may help achieve a better understanding of these mechanisms/processes which are required for developing a comprehensive model for mangrove ecosystem dynamics. We hope that a detailed knowledge of the mangrove ecosystem dynamics [10], the genetic diversity [125–127], and the exploitation of biotechnological applications would eventually pave the way for the development of a management program for the conservation and sustainable use of this ecosystem.

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Advances in Water Relation and Moisture Stress Studies of Plants

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INTRODUCTION

Early plant scientists focused on understanding the two main physiological processes in plants—absorption of solutes and water. Water is the major constituent of plants. Like other organisms, life in plants takes place in an aqueous medium. Besides being the primary constituent of protoplasm, water is used as reagent in photosynthesis and hydrolytic proceses. It is a solvent in which salts and gases move into and through the plant and maintains cell turgor. The hydrophilic force between water molecules and the cell wall components like the cellulose, lignins, and pectins has a significant role in maintaining the plant structure. Nevertheless, water is required for the integrity of cells, tissues, and the organisms. The comprehensive work related to plant water studies was documented by workers like Kramer, and by Crafts, Curtis, and Stocking [1]. Since 1949 a number of papers have appeared in this field. In this chapter, an attempt has been made to summarize the work carried out in understanding plant water status, their quantification and possible mechanism by which plants sense moisture deficit.

BASICS OF PLANT WATER RELATION STUDIES

Earlier there were a number of ways to express plant water status; that is, absolute water content, relative water content, water saturation deficit, and so forth. But later on it was suggested that the chemical potential be used as the basis for the property of water in plants as well as in soil systems, [2]. The free energy per unit quantity of a substance, specifically per gram mole, is called as the chemical potential. The chemical potential of a substance is independent of the quantity of the substance. The water potential of a system, or part of a system, that contains water or could contain water is equivalent to the chemical potential of water in that system, or of the part of the system, compared with the chemical potential of pure water at atmospheric pressure and the same temperature [3]. It is suggested that the water potential of pure water be considered as zero. Hence, the

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chemical potential of water in a system will be negative if it is lower than that of reference pure water. The water potential of a system is expressed as

$$\psi_w = \mu_w - \mu_w^0$$

where

ψ_w = Water potential of the system
μ_w = Chemical potential of water in the system
μ_w^0 = Chemical potential of pure water

Here, the water potential is expressed in terms of units of energy; for example, joules per gram, joules per mole, calories per mole, or calories per kilogram. If, however, the chemical potential is divided by the specific volume of H_2O, which is 1 $cm^3\ g^{-1}$, the energy unit of the water potential is expressed in the units of pressure [4]. The current accepted unit of water potential, in terms of pressure, is the megapascal (MPa, 1 MPa = 10 bars= 1×10^6 dynes cm^{-2} or 9.87 atmospheres). The pressure unit is most acceptable one in the field of plant water relationship studies. The water potential of pure water is zero. When a solute is dissolved in water, the water potential of the system becomes negative, as the presence of solutes decreases the free energy of water. Substances in a liquid or gas phase move in response to differences in their chemical potential. Similarly water too diffuses in response to differences in the water potential; that is, from a higher water potential to a lower water potential. The gradient in the water potential generates the driving force for the movement of water between systems.

In plants, the water potential is mainly governed by the osmotic potential (ψ_s) and the pressure potential, or turgor pressure (ψ_p), of the plant and expressed as

$$\psi_w = \psi_s + \psi_p$$

The osmotic potential of plants is always negative and ranges between -1.0 and -2.5 MPa. It is generated by organic and inorganic solutes in the cell cytoplasm and vacuoles. Photosynthesis and ion absorption are the basis of the metabolic control of ψ_s [5].

The pressure potential may be generated in the turgid cell, where it is positive or in water columns under tension where it is negative. In plants, negative turgor may develop in the lumen of the cell, particularly when the intracellular osmotic potential difference (i.e., between the apoplastic space and the cell) is larger than the turgor pressure. Such a condition may occur when transpiration is larger than the absorption of water through the roots [6]. It is argued that reports on negative turgor are fallacious owing to the techniques used [7]. Recently, this aspect has been extensively reviewed [8]. If a cell containing a constant number of solutes reaches zero turgor and the water potential further drops, it will adjust its water potential to the new condition by changing the volume and/or pressure [9]. If the cell walls are elastic, this may cause a shrinkage and maintenance of turgidity [10]. A cell with a rigid wall will resist such a shrinkage and turgor will be reduced to a constant volume [11,12]. In such a case, negative turgor also might be possible, as the environment dries and the tissue water potential falls. Negative turgor in mesophytic tissues has been reported [13].

In all living systems, there is another component of the water potential; that is, the matric potential (ψ_m). This component also is negative in value and very low in magnitude (less than -0.1 MPa). The matric potential arises from the microfibrillar cellulose matrix of the cell walls. Practically it is not possible to separate ψ_m from ψ_s, and it is hardly referred to in the literature. Imbibing seeds and some nonvascular plants like, for example, algae and lichens with large extracellular pools of polysaccharides may represent a matric potential component of some magnitude [5].

QUANTIFICATION OF PLANT WATER STATUS

Earlier studies were restricted to the measurement of the absolute water content on the fresh or dry weight basis of plants. But there is the significant diurnal and temporal variations in the water content of the plant, so it is an unsatisfactory parameter.

The relative water content (RWC), which is expressed as

$$\text{RWC} = \frac{F_w - D_w}{T_w - D_w} \times 100$$

where F_w = fresh weight, D_w = dry weight, and T_w = fully turgid or saturation weight or water saturation deficit (WSD), which is expressed as

$$\begin{aligned}\text{WSD} &= \frac{T_w - D_w}{F_w - D_w} \times 100 \\ &= 100 - \text{RWC}\end{aligned}$$

have been found to be more satisfactory parameters [14].

Quantifications based on the water potential are widely accepted [4]. It is difficult and cumbersome to measure the water potential of the whole plant; hence most of the measurements are made on the leaves, and the leaf water potential has been the primary index of the crop water status [15]. The pressure chamber is the equipment widely used to measure the water potential, although it has some limitations [16]. It is suggested that the presence of mucilaginous substances in the vessels renders the use of the pressure chamber obsolete [17], because the measured balancing pressure may merely reflect the pressure required to squeeze water out of the gel structure and it may poorly correlate with the in situ xylem pressure [18]. In the presence of mucous substances in the xylem, a number of tiny gas bubbles may persist establishing a liquid/air interface and interfacial flow, which is termed Marangoni streaming, and may cause an error in the water potential measurement. The thermocouple psychrometer is another important and standard instrument for the measurement of the total water potential [15].

The osmotic potential of a solution can be measured by changes in the freezing point, boiling point, or vapor pressure of the solution as compared with pure water. The vapor pressure osmometer is the most preferred instrument to measure the osmotic potential of a solution even if the solution is available in very small quantity (few microliters). Before measuring the osmotic potential of a tissue, it is necessary to reduce the pressure potential to zero. This is achieved by freezing the tissue for a sufficient time followed by thawing and squeezing to extract the sap. All these processes cause mixing of the cytoplasmic contents, cell wall water, and other vacuolar substances and result in an erroneous estimation of the osmotic potential of the vacuolar sap, which is much larger in volume as compared with the cytoplasm and, thus, is primarily responsible for the osmotic behavior of plant tissues [3]. Another method of estimating the osmotic potential of a plant organ is by the pressure volume relationship [19]. Besides estimating the osmotic potential of a tissue, this method also provides, for example, an estimation of the water potential at incipient plasmolysis, the volume of free water in the tissue, and the total volume of tissue water. The twig or leaf is cut from the plant and hydrated for a sufficient time, generally overnight, by placing it in water in a closed chamber. The hydrated material is placed in the pressure chamber and the pressure is applied gradually. At a particular pressure the volume of the exudate is determined. This exercise is carried out at several increasing pressures. A graph is plotted between the reciprocal of the balancing pressure (1/P) and the reciprocal of the volume of exuded sap (1/V). A curve obtained for these characters is shown in Figure 1. With a decrease in 1/P, the relationship is curvilinear up to point B, and then it becomes almost linear. If this line is extrapolated, intercepts A and C are obtained on axes Y and X, respectively. Point C represents the reciprocal of the volume of free water in the tissue, whereas point A represents the reciprocal of the water potential of the hydrated tissue. Point B designates the reciprocal of the water potential at which incipient plasmolysis occurs, that is, the reciprocal of the osmotic potential of the tissue. If the same tissue is oven dried and the absolute water content is determined, this amount minus the volume of water in the tissue corresponding to point C gives the amount of bound water in the material.

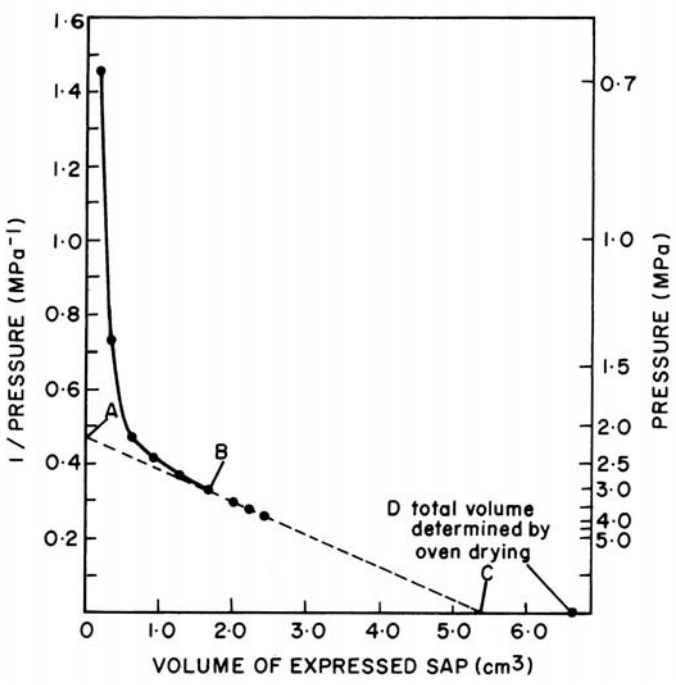

FIGURE 1 Pressure-volume relationship to measure several parameters of plant-water-relationship. (A) Water potential of hydrated tissue; (B) water potential at incipient plasmolysis or osmotic potential; (C) volume of free water; (D) total volume of water; and (D–C) bound water in the tissue. (From Ref. 3.)

The pressure potential is generally calculated from the measured water potential and the osmotic potential values as

$$\psi_P = (-)\psi_w + \psi_s$$

However, ψ_P also can be measured directly by using a pressure probe [20–22].

According to the gas equation

$$P = n\frac{RT}{V}$$

where P = pressure, R = gas constant, T = absolute temperature, V = volume, and n = number of solutes. Any change in P is possible by changes in n, V, or both at a constant temperature. A negative value of P is equal to the osmotic potential. For a hydrated tissue (fully turgid), where V is to its maximum, an increase or decrease in P may be due to a change in the number of solute molecules (n). Thus, a plant sample with a lower osmotic potential at full turgor indicates a higher concentration of solutes in the cell sap. This parameter is taken to ascertain the active accumulation of solutes; that is, the amount of solute molecules over and above the value which could be expected by reduction in the volume of the system [23].

MOVEMENT OF WATER IN SOIL-PLANT-ATMOSPHERE SYSTEM AND DEVELOPMENT OF MOISTURE STRESS IN PLANTS

Water moves in liquid form from soil to the leaves through the root and stem. In the intercellular spaces in the leaves, water is converted to vapor form and crosses the leaf epidermis and air boundary layer and finally to the atmosphere. The major portion of water from leaves is lost through the stomata. The extent of stomatal opening does regulate the water loss from plants as well as the assimilation of carbon dioxide [24].

Absorption of water by plants may take place by three processes: (a) osmotic uptake, which depends on the osmotic potential of the cell sap; (b) metaosmotic uptake, which depends on the binding of water by adsorptive forces in the cell; and (c) non-osmotic or active uptake where water movement is caused by energy released in respiration. The first two processes are passive, whereas the third process is an active. However, the bulk absorption as well as the transport of water is very much dependent on passive movements downhill in terms of the free energy status or the water potential. The leaf and shoot water potentials must be lower than the root and soil water potentials for water absorption and transport to the leaves. The resistance created by the transporting channel also has significant role in regulating the rate of water absorption and translocation. Transpiration, by reducing the leaf water potential, gives rise to the water potential gradient for uptake. As the water uptake is dependent on transpirational losses, the higher evaporative demand from the atmosphere leading to higher transpiration results in a lower leaf water potential [24]. Obviously, the plant water potential is determined collectively by soil, plant, and atmospheric factors, which is given as [25]

$$\psi_{leaf} = \psi_{soil} - T\,(R_{soil} + R_{root} + R_{shoot})$$

where ψ_{leaf} = leaf water potential, ψ_{soil} = soil water potential, T = transpiration rate, and R = resistance of the soil, root, and shoot to the liquid water flow. The equation emphasizes that, for a given leaf water potential the soil water potential must be more positive by a factor of the transpiration rate times the sum of the liquid phase resistance in the soil plant pathway [26]. A low leaf water potential may be caused by soil drying (low soil water potential, high transpiration, high soil resistance, or a combination of two or more of these factors). Plants in general cannot store moisture; hence, a control mechanism is required to regulate the plant water status, which may be achieved by regulating transpirational losses, absorption of water through roots, or a combination of both. When transpirational losses exceed the absorption, plants experience moisture stress [15].

EFFECT OF MOISTURE STRESS ON WATER RELATION PARAMETERS OF PLANTS: POSSIBLE MECHANISM OF SENSING MOISTURE STRESS

A voluminous literature is available to explain the influence of moisture stress on water relation parameters; that is, the relative water content, the leaf water potential and its components, and various plant processes [27–31]. With most crop plants, the maintenance of function and ultimately survival depends on the maintenance of a relatively high water content of the protoplasm.

Under a moisture stress condition, derangement in the leaf water potential and its components takes place [Table 1] [32]. It is reported that the water relation and transpirational parameters are closely correlated [Table 2], and in the laboratory, where equipment to quantify plant water potential and its components are not available, determination of the RWC is still a valid parameter to quantify the plant water status [27,33,34]. In wheat genotypes, a linear, quadratic, or sigmoidal relationship has been reported between the RWC and the leaf water potential [35]. However, in maize, when

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Table 1 Effect of Moisture Stress on Transpirational and Water Relation Parameters of Two Genotypes of Wheat

	Genotype			
	Kharchia 65		Kalyansona	
Parameter	Control	Moisture stress	Control	Moisture stress
Transpiration rate ($\mu g\ cm^{-2}\ sc^{-1}$)	12.59	2.76	7.26	2.43
Leaf conductance ($cm\ sc^{-1}$)	0.72	0.13	0.46	0.21
Leaf water potential (−MPa)	1.23	1.45	1.26	1.45
Osmotic potential (−MPa)	1.67	1.69	2.95	2.59
Turgor pressure (MPa)	0.45	0.24	1.69	1.14

Plants were raised on normal Hoagland solution (osmotic potential − 0.33 MPa). After 25 days, moisture stress was imposed by polyethylene glycol − 6000 (osmotic potential − 0.61 MPa). Observations were made after 14 days of stress.
Source: From Ref. 32.

Table 2 Correlation Coefficient (r) Between Various Components of Water Relation and Transpirational Parameters in Wheat

Parameter	Osmotic potential	Transpiration rate	Leaf conductance	Relative water content
Water potential	0.97	0.55	0.51	0.87
Osmotic potential		0.77	0.69	0.93
Transpiration rate			0.98	0.71
Leaf conductance				0.71

Two wheat genotypes, C306 and Kalyansona, were raised in pots. After 70 days of sowing, irrigation was checked in some of the pots, observations were recorded for above parameters on control, and stressed plants at an inveral of 2 days for 22 days and correlation between various parameters were determined.
Source: From Ref. 27.

the water supply is withheld from only a part of the root system, the leaf expansion rate is sometimes decreased with no apparent change in the leaf water status [36]. In wheat, separation of seminal and nodal root systems resulted in a significant decrease (14%) in the leaf elongation rate of the main stem and first tiller even when both the systems were well irrigated. In the same experiment, stress of the nodal or seminal rootzone increased the nodal root growth [37].

During periods of water deficit, the amount of water lost depends on the way in which the cells respond to the reduction in the water potential. Where cells are turgid, perhaps the most common response is loss of water and a decrease of the turgor and osmotic potentials until a new equilibrium is established. The rate of change of the relative cell volume or of the water content with a change in the water potential then depends on the elasticity of the cell walls and the initial osmotic potential [38]. The potential difference may be partly or fully eliminated by a decrease in the osmotic potential due to an increase in the amount of solute in the protoplasm; that is, due to

osmotic adjustment. The osmotic adjustment may lead to little change in the turgor and water content [35], maintain extraction of water from the soil [39,40], stomatal opening, and photosynthesis [41]. The factor(s) that induces solute accumulation in response to increasing water deficit are not known in higher plants. A reduction in cell volume is one cause [41], whereas other causes might be continuous accumulation of photosynthates and reduced leaf growth under moisture stress, as photosynthesis is less sensitive to moisture stress than the leaf growth [39].

Attempts to correlate plant growth with the water potential indicated that although movement of water in the soil-plant-atmosphere system and transpiration may be dependent on it, but morphological and physiological processes are closely correlated to the osmotic and turgor potentials [15]. There are, however, a number of reports that leaf growth, leaf conductance, and photosynthesis decrease as the soil dries even when the leaf turgor is maintained and before there is any significant change in the total water potential of the plant [31,42–53]. All these observations point to the fact that the leaf turgor and osmotic potential are not the only transducers of water deficit for growth, transpiration, and photosynthesis. It now seems likely that, at least in maize and sunflower, soil drying results in the increased synthesis of abscisic acid (ABA), which moves in the transpiration stream to the shoots to inhibit stomatal opening and leaf growth [50,51,54]. The evidence of the involvement of root signals in the response of plants to soil drying results from field observations and from the use of three experimental systems: i.e., (a) split roots [37], large soil columns [36,50,51], and a whole-plant pressure chamber [46,55]. Root exposure to a drying top soil may cause the induction of a root hormonal signal to the shoot, thereby causing a reduction in plant assimilation and growth [56–62]. As soil moisture is further depleted and/or the atmospheric evaporational demand increases, a hydraulic gradient develops between the leaf and the drying soil. This gradient incites the development of a leaf water deficit followed by turgor loss [31]. ABA accumulates in the shoot as a result of influx from root [63,64] causing stomatal closure, a reduction in assimilation, and reproductive failure [65]. A comprehensive model describing involvement of the soil, plant, and atmospheric variables and incorporating the ABA in regulating the soil–plant–water–relationship is given in Figure 2 [61].

The concentration of ABA in the xylem stream has been found to be a sensitive indicator of the water status of the soil around the roots of the plant. In certain studies, it has been found that the stomata close down before any detectable change in the concentration of ABA. It is observed that, under drought conditions, accumulation of some large molecular weight substances (not ABA) other than normal ones takes place [66]. Involvement of more than one substances in sensing soil moisture stress in plants has been reported [67].

Additional evidence that several chemical components may be involved in chemical signaling comes from studies on plants where mycorrhizal symbiosis occurs [68–72]. It is well known that mycorrhizal association influences the ion balance and hormone balance of the plant and there is an interacting effect of ABA and cytokinins on the growth and stomatal behavior of mycorrhizal plants [69]. It is proposed that probably a reduced supply of cytokinins and perhaps other promoters from the roots in drying soils contribute to the signaling process.

CONCLUSIONS

Plant water relation studies are attaining greater significance in order to explain the movement of water in the soil-plant-atmosphere system, the influence of moisture stress on morphophysiological and biochemical parameters, and the mechanism of moisture stress resistance in plants. Although the quantification of moisture in plants on the basis of the energy status (i.e., water potential and its components) seems to be more realistic, but still there is a need for a method which is rapid, less cumbersome and with the least errors, and suitable for a wide range of experimental conditions. The concept that, under the moisture stress condition, a change in the turgor pressure and the osmotic potential of the leaf leads to derangement in the morphological and biochemical processes of plants, but involvement of ABA and other hormones in sensing moisture stress has shifted attention from

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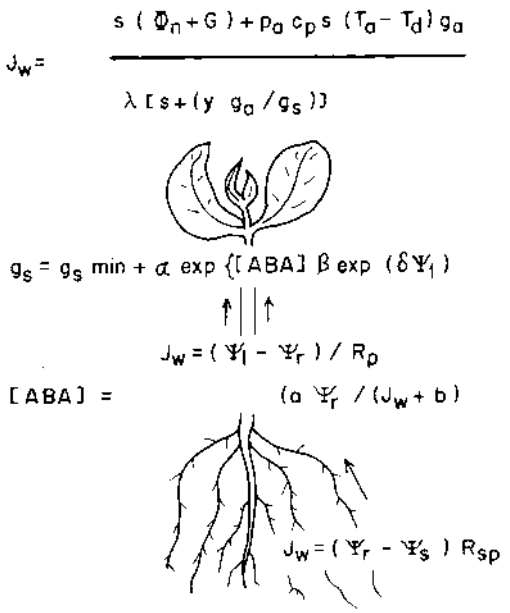

FIGURE 2 A model incorporating soil, plant, and atmospheric variables in describing flow of water in soil-plant-atmosphere system (Φ, net radiation; T_a and T_d, air and dew point temperatures, respectively; ψ_s, soil water potential: R_p and R_{sp} are the plant and the soil-plant resistance to water flux respectively; g_s, stomatal conductance: ψ_r and ψ_l, root and leaf water potentials, respectively: J_w, water flux; [ABA], concentration of ABA in the xylem; other symbols are constants. Arrows symbolize transfers of water and/or ABA. (From Ref. 61.)

the shoot to the root of the plant to investigate the process of sensing and the plant response to moisture stress. Such studies certainly have not eliminated the role of the plant water relation parameters, especially the turgor pressure and the active accumulation of solutes in regulating the physiological and biochemical processes and confering moisture stress resistance in plants. The scenario has changed with respect to the attention of scientists from the shoot to the root of the plant in characterizing the signal(s) and understanding the mechanism of moisture stress resistance in plants.

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Crop Responses to Salt Stress: Seawater Application and Prospects

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INTRODUCTION

Salinity is a significant environmental stress for crop plants. Soil salinization may arise from intrinsic soil components, the excessive use of low-quality water for irrigation, or the excessive use of fertilizer. It was estimated that salinization impacts between 4×10^8 to 9×10^8 ha of land, an area that is three times greater than the land currently used for agriculture [1]. With a decline in the quality of irrigation water, salinization of arable land is increasing [2,3]. Salinity was shown to account for about 70% of the losses in crop yield [1,4,5]. The growing demand for food and plant products to feed the expanding world population with ever-decreasing soil resources and dwindling fresh water supplies warrant the need for biological and technological solutions to overcome the physiological limitations that restrict crop productivity. The technological approach is to use advanced soil management and irrigation technology [1], and the biological approach is to select and improve the species of plants, introgression of desirable agronomic traits [6], if necessary, transfer of salt-tolerant characters into crop plants, and development of halophytes as alternative crop [7].

The majority of crop plants are glycophytes [8–10], and the manipulation of glycophyte plants to adjust and produce under conditions of moderate or low levels of salinity is important [11]. Efforts were made to improve salt tolerance through breeding involving introgression of the genetic background from wild salt-tolerant relatives to cultivated plants [2,10,12–14]. However, the progress is very slow, primarily due to an inadequate knowledge of the genetics and mechanism of salt tolerance [15].

Recent advances in plant biotechnology may play a crucial role in the development of superior crop plants with better environmental stress tolerance and higher yield potential under stressful conditions. Appropriate research strategies are, however, required to harvest the fruits of the recent explosion of information in the field of molecular genetics.

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SEED GERMINATION AND PLANT GROWTH

Salinity, whether natural or induced, is a widespread environmental stress that limits the growth and development of salt-sensitive plants [16,17]. Plants vary greatly in their tolerance to salts. Halophytes can complete their life cycle under saline conditions [18]. Glycophytes, although generally more sensitive to salinity, exhibit differences between species and even varieties [16,19–22]. Such genotypic variation has been explained for certain crops. However, the performance of crops under saline conditions depends on seed germination and establishment and also tolerance at later stages of growth [23,24].

When the seeds are sown in an environment with a high concentration of salt, both the rate and percentage of germination decreases [19,22,25]. The capacity of seed germination relates to the extent of imbibition of solutes by the seed and the resultant activity of the embryo. Thus, water absorption plays a key role. In the growth phase, mobilization of reserve food from the cotyledons and their transportation to the growing embryo becomes a critical factor. Weakening of the physical restraints imposed by the surrounding endosperm tissue on the embryo can play an important role in germination, suggesting that radicle protrusion across the seed relies on weakening the endosperm wall hydrolysis [26–28]. According to Fooland and Jones [29], endosperm additive and testa dominance effects were highly significant and the embryonic genotype played no significant role in germination performance under saline conditions.

The reduction in germination under saline conditions also could be attributed to the increased osmotic pressure of the soil solution, which diminishes the water absorption rate, leading to moisture stress in the seeds [30] and mobilization of food reserves [31] by effecting the enzymes responsible for hydrolysis of reserve food material and its translocation to the growing embryo axis.

Iyengar et al. [19,32] extensively studied the varietal differences in different crop plants under saline conditions and reported the different behaviors of varieties of a particular crop to salinity. Maliwal and Paliwal [33] demonstrated significant varietal differences in the salt tolerance of important crops during germination. Patolia [34], Shannon and Noble [22], West and Taylor [35] also observed significant varietal differences in the salt tolerance of wheat and clover during germination and seedling growth. Differences in the salt tolerance of *Arabidopsis thaliana* mutants was correlated with differences in the absorption of reactive cations (Na^+, K^+) during imbibition [21].

It is known that salt stress interrupts metabolic activity by ion excess, imbalance, and interference of toxic ions on the uptake of essential nutrients [36], which are detrimental for seed germination under saline conditions. The recent work of Thomas et al. [37] on *Arabidopsis* shows that metabolic engineering may be a potential approach for the improvement of salt tolerance. A better understanding of the underlying mechanism involved in seed germination and the subsequent growth under saline conditions is essential to confront this agronomic problem.

SALINITY-TOLERANCE MECHANISM

Salinity in the soils and ground waters has become a major environmental issue [38], and excessive salinity in the soil or irrigation water has been considered as the main limiting factor for the distribution of plants in natural habitats [4,5]. In irrigated areas where decreasing pasture yield due to salinity is a major concern, there is a need for plant species with greater salt tolerance [22]. The presence of halophytes (natural salt-tolerant plants) which can tolerate the 0.5 M NaCl present in seawater [39–41] indicates that there is a potential to increase the salt tolerance of plant species for which understanding the mechanism of salt tolerance is very important. Despite its importance, the physiology and molecular basis of salt tolerance has been investigated less than that of tolerance to water and temperature stresses [15,16,42–47].

Morphological Adaptations

The response of plants to salt stress is complex. It varies with the salt concentration, the type of ions, other environmental factors, and the stages of plant development. The root may be considered

to be the plant's sensor in the soil, since it is through the root that the whole plant is affected by changing soil conditions.

Besides growth inhibition, salinity causes several specific structural changes that disturb the plant water balance or status [45]. These structural changes include fewer and smaller leaves, increased succulence, thickening of the leaf cuticle and surface layers of wax, reduced differentiation and development of vascular tissue, increased development of tyloses, and earlier lignification of roots. These responses vary with plant species and the type of salinity [43]. Information on the causes and benefits of these structural changes in response to salinity is inadequate.

Chloride salinity causes succluence in many plant species [48–49]. Succulence, whcih is manily due to the increased elongation of palisade cells, tends to dilute the interal ionic concentration and reduce the leaf surface significantly.

The salinity effects on vascular tissue seem to be structural adaptation that reduces water conduction, possibly in relation to lower transpiration losses. Reduced transpiration under saline conditions has been reported for many plants [45–48].

Since roots are directly exposed to the saline environment, it seems remarkable that the root growth is usually affected less [45–50] than the vegetative shoot growth. The resultant decrease in the shoot/root ratio presumably improves the water balance by maintaining the potential for water absorption while reducing transpiration. Studies have shown that plants can tolerate high-salinity levels in part of the rootzone if the remaining roots are exposed to low-salinity levels [51].

Effects on Metabolism

Studies on the responses of the growing regions to salt stress have been limited. The results of Munns et al. [52] with barley and Taleisnik-Gertel et al. [53] with tomato suggest that the primary cause of reduced shoot growth under saline conditions is located in the growing tissues, not in the mature photosynthetic tissues. Salt inhibition of cell division or enlargement (or both) in the growing regions may be direct or indirect [16,54]. Salt may affect growth indirectly by decreasing the rate of photosynthesis or by preventing growth factors from reaching the growing regions [16,52]. The amount of photosynthates reaching the growing regions may decrease because of the inhibition of photosynthesis due to stomatal closure [55] or by the direct effect of salt on the photosynthetic apparatus. In addition, the transport of photosynthates in the phloem may be inhibited. Results with barley [16,52] suggest that inhibition of growth involves a water deficit rather than a direct adverse effect of ions on metabolism. The greater inhibition by salt on the growth of salt-sensitive compared with salt-tolerant tomatoes could result from an adverse water balance or from the direct toxic effects of the ions [53]. Mass and Neiman [43] suggested that salt ions can damage growing cells indirectly by depriving them of essential substances. Leopold and Willing [56] reported various symptoms of salt damage that may result from membrane damage by salt ions. Salt also affects the cellular and nuclear volume [57] and inhibits or stimulates nucleic acid and protein synthesis [54–57].

Regulation of Ion Contents

The regulation of transport and distribution of ions in the various organs of the plant and within the cell is an essential factor of the mechanism of salt tolerance. The knowledge on the regulation of ions in the whole plant has been extensively reviewed [16,22,42,45,58].

There are larger differences in ion concentration between different parts of the same plant. Older leaves of glycophytes, grown at high salinity, usually have a higher Cl^- concentration than the younger leaves of bean [59] and soybean [60]. These trends are probably due to a combination of a rapid volume increase in expanding leaves and the prolonged intake of the ions by expanding leaves via the transpiration stream. The latter could account for the large increases in ion (i.e., Cl^-) concentrations in older leaves. Maize plants accumulated 7–10 times more Na^+ in the mesocotyl than in shoots and 2–3 times more in mesocotyl than in roots [61]. Rice, although morphologically

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distinct, concentrated four times more Na^+ in the sheath than in the leaf laminae [62]. Na^+ and Cl^- concentrations were more in older leaves, whereas K^+ was greater in younger leaves [63]. Two rice cultivars differing in salt tolerance differently accumulated Na^+ and K^+. A tolerant variety (Pokkli) used Na^+ exclusion, whereas a sensitive variety (IR29) accumulated significantly more Na^+. However, in the absence of K^+ in the medium, the tolerant variety also increasingly accumulated Na^+ similar to the sensitive variety [64].

In rice, salt tolerance was found to be through Na^+ exclusion and increased the absorption of K^+ to maintain a good Na-K balance [63–65]. In a relatively salt-tolerant variety, the growth is faster than the absorbed Na^+, so that the absorbed Na^+ undergoes a greater dilution than the sensitive variety [66,67].

The capability of plants to maintain an adequate K^+ content under saline conditions is enhanced by an ample K^+ supply. Salt-adapted *Sorghum* plants [68] were able to grow in 0.3 M NaCl in the presence of Hogland solution supplemented with K^+. The plants did not grow in 0.3 M NaCl without half-strength Hogland solution. Salt-tolerant glycophytes, such as *Atylosia sericea* and *Glycin max c.v. Lee*, maintained a constant K^+ content or even increased in the presence of NaCl [69,70], whereas sensitive glycophytes fail to maintain the K^+ content in the presence of high salt concentrations [69–71]. Such a decrease in the K^+ content may indicate damage [71]. However, Leigh and Wyn Jones [72] observed a decrease in the K^+ content in *Lyopersicon esculentum, Solanum pennelli*, and *Sorghum bicolor* with an increase in external salt concentrations without concommitant damage. The maintenance of an adequate K^+ content under saline conditions seems to be dependent on K^+-selective uptake as well as selective K^+ and Na^+ compartmentalization within the cell and distribution in the shoot. Glenn et al. [58] are of the opinion that salt tolerance of *Atriplex canescens* genotypes depends on initial leaf Na^+ levels.

Seemann and Chritchley [73], using x-ray microanalysis, found little difference in the cytoplasm, chloroplast, and vacuolar Na^+Cl^- ion levels in bean plants exposed to 150 mM NaCl even though the growth was reduced to 70%. A study with a halophyte (*Salicornia* species) showed that the compartmentation of ions takes place in different tissues. The ion content was greater in spongy mesophyll cells than in palisade cells [74] where active photosynthesis takes place. This ability to regulate ion concentration through compartmentation is an important aspect of salt tolerance.

The rate of Na^+Cl^- transport are much higher in salt-sensitive varieties at the initial seedling stage causing a greater accumulation of ions in the shoot [63], whereas in *Aster tripolinum*, x-ray microanalysis has revealed that the sodium content of the stomatal guard cells remains much lower than that of other leaf cells when plants were grown at high salinity. Large amounts of sodium did, in contrast, accumulate in the epidermal and subsidiary cells and particularly in the mesophyll cells suggesting that a mechanism exists to limit the extent of entry into the guard cells and that the ability of the guard cells to restrict the intake of sodium ions may be an important component of sodium-driven regulation of transpiration and hence salinity tolerance [75,76].

Many salt-tolerant plants exhibit greater K^+/Na^+ selectivity than salt-sensitive plants. This is usually manifested by a higher K^+/Na^+ ratio in tolerant plants and is thought to be a significant salt-tolerance adaption [77–79].

The mechanisms that are involved in K^+/Na^+ selectivity are not clear, but they may include increased extrusion of Na^+ and increased uptake of K^+. The uptake of K^+ was shown to increase in cells adapted to grow in saline media [80]. In suspension cultures of *Brassica napus* and *Nicotiana tobaccum*, enhanced NaCl tolerance has been attributed to the capacity of the selected cells selectively to take up K^+ [81–82]. These results indicate that at the cellular level an increase in K^+ uptake and a decrease in Na^+ accumulation are involved in K^+/Na^+ selectivity. The enhanced K^+ uptake is an adaptive mechanism that allows the cells to evade K^+ starvation in the presence of higher levels of NaCl. There are indications that a myraid of mechanisms are involved in K^+/Na^+ selectivity, and the mechanisms employed may change with genotypes. A molecular model for Na^+ and K^+ fluxes has been proposed in yeast, and two genes involved in K^+ and Na^+ transport system have been cloned [83].

Table 1 Concentration of Ions in Different Tissue Layers of *Salicornia brachiata*[a]

Tissue layer	Na^+	K^+	Na^+/K^+	Ca^{2+}	Mg^{2+}	Zn^{2+}	Mn^{2+}	Fe^{2+}	Cu^{2+}	Cl^{2-}
Palisade	12.34	11.39	1.08	15.11	13.70	3.76	0.13	0.023	Trace	43.08
	±2.83	±1.60		±0.81	±0.67	±0.33	±0.03	±0.003		±13.79
Spongy mesophyll	30.54	9.74	3.13	17.82	18.43	4.67	0.057	0.145	Trace	50.81
	±1.00	±2.35		±0.67	±0.20	±1.31	±0.008	±0.012		±4.67
Vascular	3.02	4.65	0.64	8.52	5.67	0.22	0.027	0.267	Trace	18.08
	±0.19	±1.32		±0.33	±0.58	±0.05	±0.008	±0.04		±0.84

[a] Mean of three independent replications
Source: From Ref. 74.

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Osmoregulation

When exposed to a saline environment, plants generally accumulate inorganic ions commonly present in the environment, but these become detrimental to cellular biochemistry at high concentrations and must be sequestered into the vacuole. To keep the cytoplasm osmotically balanced, the plants usually accumulate organic molecules such as proline, glycinebetaine, dimethyl sulfonium, propionate, fructanes, trehalose, and polyamines such as mannitol, sorbitol, and myo-inositol, which are correlated with salinity tolerance of plants [84–90] and termed as ''compatible solutes.'' In osmotic adjustment, they act as osmolytes to facilitate the retention of water in the cytoplasm and the protection of membranes, protein complex, and cellular structures [44].

The free proline content in glycophytes is normally negligible compared with halophytes, and their concentration increased markedly in plants subjected to salt stress [90,91]. Simultaneous treatment of salt-tolerant cells of tobacco with high temperature (40°C) and NaCl (170–340 mM) resulted in transient overproduction of proline accompanied by an increase in thermal tolerance [90]. The transient initial raise in the proline content under stress and its further decrease can be explained by protein transmethylation to form derivatives (N-methyl proline, proline betaine, hydroxyproline betaine) capable of protecting the cell under more severe stress [92]. Salt-tolerant cell lines are capable of overproducing methionine and have a high transmethylating activity [93]. Moreover, salt-tolerant cells are capable of accumulating betaines under long-term salt stress, and they could compensate for a dramatic drop in the proline content of NaCl-containing medium at 40°C [90].

The accumulation of proline in response to environmental stress indicated that its synthesis is a nonspecific response to a decreased water potential. The role of proline during stress is a subject of controversy and interesting because it accumulates to very high levels under adverse conditions [94].

Testing of high concentrations of proline and glycinebetaine on isolated enzymes has shown that they have essentially no deletarious effects, and in fact they protect various enzymes against a range of perturbing effects [95]. It appears that osmoregulating substances affect the conformation of enzymes, thereby stabilizing the active conformation and in this way protect enzymes against conformational perturbances caused by ions [86,96]. These metabolites may also replace water at the surface of biopolymers, stabilize macromolecular structure, or act in scavenging of radical oxygen compounds [15,44,97,98]. The protection through mass action has been termed osmotic adjustment [44,99].

Biochemical pathways that lead to the production of some of these osmoprotectant solutes are known [15,100]. Genes encoding several of the relative enzymes have been cloned, and expression of specific genes indicated that tolerance is conferred by genetically encoded mechanisms. Genetic modification of plant species to increase the contents of the compatable solutes mannitol [101], fructans [89], proline [88], and glycinebetaine [102] have revealed that overproduction of these compounds is strongly correlated with resistance or tolerance of plants to abiotic stresses. Thus, the genetic modification of plant species to increase the content of osmotic solutes that is now technically possible may be one of the approaches to increase salt tolerance in plants. However, transfer of individual genes confered only marginally increased stress tolerance strategies are to be developed to transfer several or many genes at once to increase the salt tolerance of plant species reasonably [15].

Membrane Changes

Membranes form barriers between the plant and its environment, organelles, and tissues. They are the major sites for controlling active and passive solute fluxes, and thus membranes may be of special importance to plants for regulating the ion content. Mineral imbalances of the root environment, such as those commonly encountered by plants in salty soils, often affect the structure and chemical composition of root cell membranes [103–105] and may thereby interfere with nutrient aquisition, transport, and compartmentation.

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Sterols and phospholipids are the principal structural components of the lipid matrix of the plasma membrane and tonoplast [106]; the two membrane systems at which salt-tolerance mechanisms are likely to operate [107]. The significance of the quality and quantity of membrane lipids to control ion transport in salt-tolerant plants has been reviewed [56].

The free sterol content was higher in the roots of salt-tolerant grape variety as compared with their sensitive variety [108]. The levels of free sterols and sterol esters in the roots of salt-tolerant *Plantago maritima* and *P. coronopus* was maintained on exposure to salinity and it decreased in salt-sensitive *P. media* [109]. In wheat, salinity increased the free sterol composition (cholesterol, stigmasterol, and brassisterol) and decreased the levels of phospholipids and phosphatidyl choline [110]. In *Kostletzkya virginica*, the relative percentage of sistosterol decreased, whereas that of camposterol increased with an increase in salinity [105]. The enhanced free sterol content in the root of salt-tolerant plants contribute to a higher molar ratio of free sterol/phospholipids. Changes in the free sterol/phospholipid ratio affect the membrane permeability minimizing the potassium leakage from cells and, thus, energy required for efficient transport and subcellular compartmentation [105].

Free sterols also are known to interact with the fatty acid chains of phospholipids and restrain their mobility [111]. Thus, the higher proportion of stigmasterols in plants under stress may have led to lower mobility of lipid molecules. In addition, the looser binding of stigmasterol to phospholipid acyl-chains might alter the lipidic environment of intrinsic membrane enzymes [112], altering the protein-phospholipids interactions, in a similar way as pH affects the solubility of aqueous proteins [113]. Interaction with membrane phospholipids through their constituent fatty acids with sterols and intrinsic membrane proteins [111] may change the activity of intrinsic membranes/proteins such as H^+ ATPase [114,115].

Oxidizing Enzymes

When plants are subjected to environmental stress, the balance between the production of reactive oxygen species such as superoxide ($O_2^{\cdot-}$) hydroxyl radical (—$^{\cdot}$OH), and hydrogen peroxide (H_2O_2) and the quenching activity of antioxidants is upset, often resulting in an oxidative damage [116–119], suggesting active oxygen species play an important role in the mechanism of stress injury. Both enzymatic and nonenzymatic mechanisms have evolved to overcome the potential toxic effects [118,119]. Plants with high levels of antioxidant enzyme activity are reported to have greater resistance to this oxidative damage [116,120].

The role of superoxide dismutase (SOD) in protecting aerobic organisms against oxidative damage is well established [117]. An increase in the activity of oxidizing enzymes increases salt tolerance [121–124]. Singha and Choudhuri [125] have reported that, in *Vigna catjang* and *Oryza sativa*, the $O_2^{\cdot-}$ radical and H_2O_2 could play an important role in the mechanism of salt injury. Gossett et al. [118] examined the relationship between NaCl tolerance and antioxidant enzyme activities in different salt-tolerant and salt-sensitive cotton cultivars and reported that leaves from the NaCl-tolerant cultivers contained significantly greater constitutive levels of catalase and NaCl induced peroxidase and glutathione reductase. In contrast, callus tissues from the NaCl-sensitive cultivars showed no difference from that of nonstressed leaves in the activity of these enzymes. The NaCl-induced enhancement of ascorbate peroxidase in adapted callus indicates that these cells have a higher capacity for the decomposition of H_2O_2 generated by SOD [126]. The increase in the activities of oxidizing enzymes may be essential for the survival of plants under saline conditions to overcome the peroxidation of membrane lipids known to occur in plants under adverse conditions [116,120,127].

Recent attempts to improve salt tolerance through the overexpression of enzymes involved in scavenging reactive oxygen intermediates by gene transfer technology has provided new insights into the role of these enzymes [122,123,128]. Physiological and genetic evidence clearly indicates that the reactive oxygen intermediate scavenging system of plants is an important component of stress tolerance, and raised hopes that in future this approach can be used to improve the stress tolerance of economically important plants [122–124].

Adaptation to Salinity at the Plant Cell Level

Plant cell culture and regeneration of plants from potential cell mutants has led to the expectation that these techniques could be used to generate useful mutant traits from plant cell cultures. Stress-tolerance selection in plant cell cultures has suggested that salt- and water-stress tolerance may be characteristics which are linked in the selection processes at the cellular level and therefore can be manipulated in culture [129–131].

Recent reviews of Tal [132], Dracup [133], Binzel and Revueni [134], and Hasegawa et al., [135] provide extensive summaries of research that has been carried out toward the goal of utilizing cell line selections to obtain salt-tolerant plants. Cell lines were selected for many plant species which can grow in the presence of high concentrations of NaCl [81,136–139]. In many instances, tolerance to salinity was lost when the cells were recultured in the absence of salt, and the plants regenerated from these plants did not show increased salt tolerance [32,140]. However, McHughen [141] in flux, Waskom et al., [142] in barley, and Winicov [130] in alfalfa demonstrated improved tolerance to salt from the plants regenerated from the selected cell lines as compared with the original explant source, whereas Flowers et al. [143] illustrated an example where tolerance in culture cells exceeded that exhibited by the whole plant. Comparison of the salt tolerance of the whole plant and cell cultures derived from them indicated that, for certain species, tolerances were similar for whole plant and cell cultures [144,145], indicating salt tolerance of at least certain species was based on an intrinsically cellular process and established the use of cell cultures as a means to elucidate a cellular mechanism of salt tolerance. However, the results of Smith and McComb [145] and Cherian et al. (personal communication) has shown that tolerance at the whole-plant level is substantially greater than the cell lines, suggesting, in some plants, anatomical organization is responsible for salinity tolerance. Plants regenerated from salt-tolerant cell lines did not show increased tolerance to NaCl stress [80,146] even though cell suspensions obtained from these regenerated plants retain their tolerance to NaCl [80,146], indicating both cellular and whole-plant traits contribute to NaCl tolerance, and the adopted cell lines can contribute to our understanding of the physiological and biochemical mechanisms that are the basis for salt tolerance. These results point out the necessity of integrating information regarding the mechanism of salt tolerance derived from studies at the cellular level with those from the whole plant.

Adaptation of Cells in Suspension Culture to Salinity

Salt-sensitive cells in suspension culture can, in some cases, be adapted to grow at higher levels of salinity by a stepwise increase in NaCl and can therefore serve as useful models for studying the mechanisms of salinity resistance at the cell level. Using this technique, tobacco cells were adapted to grow in a medium containing 500 mM NaCl [80,81,146]. These cells retained their adapted character even if grown for many generations in the absence of salt. The process of adaptation of the cells to salinity is accelerated by the addition of abscisic acid (ABA) to the medium.

Maintenance of intracellular K^+ concentrations that are not growth limiting in an environment of high Na^+ is characteristic of the NaCl-adapted cells of tobacco. Potassium uptake into NaCl-adapted cells was 1.5 times higher than NaCl- unadapted cells at 0 NaCl and 3.5-fold greater when cells were exposed to 160 mM NaCl. At lower NaCl levels, salinity caused an increase in intracellular Na^+, whereas intracellular K^+ was decreased. Above 200 mM NaCl in the medium, the intracellular K^+ remained constant, whereas intracellular Na^+ increased gradually. According to Lerner [96], there seems to be a mechanism limiting the K^+ and the Na^+ accumulation in these cells. The difference in the net K^+ uptake between adapted and unadapted cells is primarily due to the higher rate of entry than the reduced K^+ uptake into adapted cells [80,146]. K^+ uptake and K^+ secretion have been shown to be influenced in some systems by turgor pressure [147,148].

An alteration in the cell wall structure [81]; composition [149]; differences in the accumula-

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tion of amino acids, sugars, and organic acids [150,151]; changes in the activity of enzymes; expression of new isoenzymes [152]; and plasma membrane transport [153] of salt-adapted cells was reported.

Integrating results from cell studies with those emerging from research with the whole plant should result in a more comprehensive understanding of the complex array of mechanisms that together enable a plant to tolerate salinity. Cell culture studies enable us to focus on the influence of environmental stimuli on genetic expression without the added complexity of developmental and tissue-specific controls.

Proton Pump

Plants that grow successfully in saline soils must maintain a much higher K^+/Na^+ than generally present in the surrounding medium [16,154]. Transport of ions at the plasma membrane and tonoplast is thought to play an important role in the process by which certain plant cells maintain a very high ratio of K^+/Na^+ in the cytoplasm [154]. Minerals enter the root through transport proteins located in the root plasma membrane. The detailed mechanism of transport of K^+ and Na^+ across the plasma membrane and tonoplast are not fully understood. However, the net effect of the ion transport process is selectively to take up K^+ into the cytoplasm and to extrude Na^+ both into the vacuole and to external medium.

The plasma membrane H^+ ATPase is the primary pump of plant cells that drives all secondary transport systems [155–157]. Cloning, expression, the structure-function relationship of the plasma membrane P-type H^+ ATPase [157,158], the vacuolar H^+ ATPase [159], and vacuolar H^+ ATPase [160,161] have dramatically increased the knowledge about the transport proteins which energize secondary ion transport, regulation of cell turgor and cell wall extension, and intracellular pH regulation [157]. H^+ ATPase has been shown to be involved in salinity tolerance [162]. Plant cells exposed to a high ionic environment increased proton fluxes [147,148,163] accompanied by changes in the activity and kinetic properties of the plasma membrane H^+ ATPases [164,165]. In suspension cultures of *Atriplex nummularia*, the steady-state transcript levels of P-type H^+ ATPase increased only when adapted cells were expressed to NaCl, and the induction was dependent on the developmental stage of the cultured cells [162]. The in vivo activity of this ATPase, as assessed by proton flux measurements, seems to increase during osmotic adaptation in tobacco [163] and carrot [148] cell cultures. However, salt stress has no effect on in vivo activity of the ATPase in sunflower roots [166]. In citrus cells adapted to grow in 200 mM, NaCl the activity decreased [167]. Proton efflux from plant cells has not been rigorously demonstrated to correspond to the activity of plasma membrane H^+ ATPase [156]. However, Braun et al. [164] have demonstrated that salinity during growth increases the in vitro activity of plasma membrane H^+ ATPase in *Atriplex nummularia* (halophyte). Apparently this phenomenon has not been reported in nonhalophytes.

The transport of Na^+ across the tonoplast and its accumulation in the vacuoles is an important mechanism of plant tolerance to salinity [47,168,169]. The Na^+/H^+ antiport mechanism catalyzes the accumulation of sodium into the vacuole, and other antiport systems may accumulate, for example, sugars, amino acids, and phosphates [156,170]. The functionality of the Na^+/H^+ antiport system depends on the establishment of a proton gradient generated by the vacuolar electrogenic pumps [171,172]. An Na^+/H^+ antiport activity has been detected in the tonoplast vesicles from the leaves of halophytes such as *Atriplex gmelini* [173] and *Mesembryanthum crystallinum* [174], as well as in roots of *Atriplex nummularia* [175]. In glycophytic plants, it has been reported to occur in tonoplast vesicals isolated from salt-tolerant species such as sugar beet in the storage tissue [176], the root of barley [177], cotton [169,175], *Plantago maritima* [178], and *Cathrantus roseus* [179].

Comparmentation of Na^+ Cl^- at the vacuole also has been investigated using x-ray microanalysis and efflux kinetic analysis [180] in different plant tissues, and it has been reported that the capability for vacuolar compartmentation correlates with the NaCl tolerance. The vacuolar ATPase

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seems to be induced by salt stress in barley roots [181]. In tobacco, the specific activity of this enzyme is increased during NaCl adaptation, but the amount of protein quantified by antibodies decreased [152]. According to DuPont [172], the regulation of vacuolar ATPase during salt stress is due to increased coupling between proton transport and ATP hydrolysis. The induction was not dependent on protein synthesis, and activation by posttranslational modifications has been suggested. However, activation of the antiport mechanism by salt in sugar beet correlated with the enhanced synthesis of a 170-kDa tonoplast polypeptide. Antibodies against this protein inhibited antiport activity, suggesting that it is a component of the system [182]. A polyclonal antiserum raised against the denaturated 170-kDa protein inhibited antiport activity in vitro, indicating that the 170-kDa protein is identical with a part of the Na^+/H^+ antiport.

The tonoplast H^+ ATPase activity increased substantially in a facultative halophyte within 8 h after treating 4-week old plants in the C_3 state with 400 mM NaCl. This rapid effect was confined to the roots and young leaves, whereas in fully expanded leaves, only subunit C was increased, and this increase was transient suggesting that like P-type H^+ ATPase, the V-type H^+ ATPase also depends on the plant developmental stages [183,184].

The driving force for the vacuolar accumulation of Na^+ in salt-stressed plants is provided by the H^+ electrochemical potential difference generated across the tonoplast by H^+ pumps. The salt-induced increase in tonoplast H^+ ATPase activity ensures the existence of a sufficient driving force for significant accumulation of Na^+ through the Na^+/H^+ antiport. An increased H^+ transport activity was considered to be homeostatic mechanism to cope with the salt stress, since it can provide the energy necessary for the operation of the Na^+/H^+ antiport in the vacuolar membrane required for the sufficient control of the cytosolic concentration of Na^+ [152,169,174].

Salt-Inducible Gene Expression

The identification of genes whose expression enables plants to adapt or tolerate salt stress is essential for development of salt-tolerant species. The recent focus on identification of genes induced under environmental stress led to an overexpanding list of cDNAs that detect upregulation of mRNA under conditions of drought/salinity [44,185–187]. Such genes with a known function broadly fit into the categories of energy metabolism ion transport, osmoprotectant synthesis, and macromolecules that contribute to the structural stability of cellular components. Many more other genes have been identified to date, although they lack functional identity. Second, minor components which may have important catalytic or regulatory role would be missed using the usual strategies of differential screening of cDNA libraries and two-dimensional protein gels by which only relatively abundant mRNAs and proteins can be isolated. It is becoming increasingly clear that these genes are responsible for only minor stress-tolerance effects. Strategies to identify regulating molecules that coordinately regulate these genes are required to elicit the greater effects on salt tolerance [44].

Serrano and Gaxiola [47] are of the opinion that most of the current research on salt tolerance can be considered as being ''phenomenological,'' because it concentrates on phenomena occurring during salt adaptation without establishing a hierarchy of physiological significance.

A new approach for identifying genes that function in plant salinity-stress tolerance is based on the use of a model, cellular-based, molecular genetic system (e.g., yeast, *Chlamydomonas*) to isolate plant genes that cause functional sufficiency for salt tolerance or complement the phenotype for salt-sensitive mutants [188–190]. Because this approach utilizes a screen for genes that can ''function'' in stress tolerance rather than screen for genes, whose expression is regulated ''in response'' to stress imposition. Further, the use of appropriate mutants in these systems makes it possible to dissect salt adaptation into more precise mechanistic entities from components of the salt-stress signal cascade to tolerance determinants that are regulated by this cascade; for example, the Na^+ transport system. The only problem to this approach is the need to generate a random collection of genetically modified organisms that need to be screened for salt tolerance.

Calcineurin (CaN) is a calcium-dependent protein phosphatase that a focal intermediate of the principal salt-stress signal cascade in yeast that regulates Na^+K^+ homostasis through the modulation of plasma membrane influx and efflux transport systems [188–191]. The expression in yeast of a constitutively active recombination form of CaN substituted for Na^+ stress signaling to induce salt-adaptation responses mediated through the regulation of ion homeostasis [189]. CaN-deficit mutants fail to convert the K^+ transport system to the high-affinity state that facilitates better discrimination for K^+ over Na^+ [83,188] resulting in substantially greater Na^+ accumulation and consequently a salt-sensitive phenotype. Similarly, expression of activated yeast CaN in transgenic tobacco plants resulted in increased NaCl tolerance [192]. Certainly these and similar studies will increase our underatanding of the basic cellular mechanisms contributing to salt tolerance.

FERTILIZER MANAGEMENT AND SALINITY TOLERANCE

With the increasing use of saline water or saline soils for agriculture, fertilizer application under saline conditions has been the subject of considerable interest, because salt damage to crops involves not only the osmotic effect but also specific ion effects [193]. It has been postulated that crop salinity tolerance can be improved by the use of suitable nutrients [194,195].

Salinity and fertility, because of their economic implications, have been the subjects of many greenhouse and field studies [194–197]. These studies were conducted to evaluate improved fertilization management as a means of alleviating growth inhibition by salinity. The effects of fertilization on nutrient uptake by plants, on the chemical composition of plant tissues, and on crop yield were studied under various salinity conditions and soil and crop interactions, since salinity and soil fertility are determined by the concentration of various ions in the plant rootzone.

The availability of N, P, and K in soil is too low for an economical yield of agricultural crops. The addition of fertilizer to maintain an adequate soil fertility level is therefore a standard practice in agriculture. The optimal concentration of nutrient elements in the soil is usually well below the level needed to cause a salinity effect. However, excessive application of fertilizer, particularly N and K because of their high solubility, may result in a salinity build-up in the soil. This is typically reflected in decline in yield when the fertilizer exceeds the optimum level.

The simultaneous presence of salts and nutrient elements in the rootzone can influence ion uptake by plants and affect the plant's chemical composition. Synergistic and antagonistic effects can increase or decrease the intensity of this process [198,199]. For instance, Award et al. reported [200] a positive effect of P on the yield of foxtail millet (*Setaria italica* L.), clover (*Trifolium alexandrinum* L.), and tomato (*Lycopersicon esculentum* L.) grown in saline soils, whereas Bernstein et al. [196] detected a reduction in the yield of corn (*Zea mays* L.) grown in sand cultures when a high salinity level was coupled with a high concentration of P. Champagonal [198] reported that 34 of 37 crops studied responded positively to P fertilizer. Application of higher dose of N fertilizer to crops growing under a saline environment has provided a beneficial effect by minimizing salt-induced damage [201].

INTERACTIVE AND ANTAGONISTIC EFFECTS OF SALINITY AND FERTILITY

Some cations influence the uptake of other cations by plants. Such antagonism occurs between K^+ and Na^+ and Ca^{2+} or Mg^{2+}. These effects may be involved in the occurrence of nutritional disorders in plant tissues. There is abundant evidence that Na^+ and the Na^+/Ca^+ ratio can affect K^+ uptake and accumulation within plant cells and organs [202]. Salt tolerance appears to be correlated with the selectivity of K^+ uptake over Na^+. Ben-Hayyim et al. [202] and Lauchli and Stelter [203] found

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that the growth of cultured citrus cells in various NaCl and $CaCl_2$ concentrations was a function of the internal K^+ concentration independent of the NaCl concentration.

Ben-Hayyim et al. [202] reported that K^+ application can reduce the deleterious effect of salinity on plant growth and development. However, contradictory results on the effects of K^+ fertilization under saline conditions on field crops have been reported. Potassium uptake by plants is affected by high salinity and the Na^+ concentration in the soil.

Under saline conditions, a high Ca^{+2} supply alleviated the inhibition of NO_3 uptake [204] and increased Na^+/K^+ selectivity. Cramer et al. [205] and Martinez and Lauchli [206] showed that P translocation from roots to young shoots increased in the presence of an additional supply of Ca^{2+}. The effect of salinization on P nutrition depends on the available P in the substrate. A low supply of P to young tissues could become a limiting factor to their growth under saline conditions. An increased Ca^{2+} supply to the plant could be more efficient than P fertilization in restoring the P supply to young tissues under saline conditions.

High levels of $CaCl_2$ in the nutrient media resulted in a greater increase in the Ca concentration and reduced in the K^+ and Mg^{2+} levels in the tissues of bean plants. On the other hand, the addition of K as KCl increased K^+ and decreased Ca^{2+} and Mg^{2+} concentrations in maize plants [207]. Corn (*Z. mays* L.) plants tested in the same study responded differently to different Cl salts. Plant growth was better in the presence of $CaCl_2$ than with any combination of NaCl, $MgCl_2$, or KCl at a comparable osmotic pressure. A high Ca^{2+} content depressed the K^+ and Mg^{2+} levels; however, a mixed solution (Ca + K + Mg + Na) corrected the imbalance. Elevated Ca^{2+} levels may protect the plant from NaCl toxicity by reducing the displacement of membrane-associated Ca^{2+} [208] by reducing, Na^+ uptake and transport to the shoots [209] or by a combination of these effects. The Ca^{2+} also improves K^+ uptake under NaCl salinity, effectively increasing the Na^+/K^+ ratio in the tissues [205,208].

An increase in the Cl^- concentration in the nutrient media led to a reduction in the NO_3 content of tomato plants [194]. However, an increase in the concentration of NO_3 in the nutrient media, from 7.5 to 20 meq L^{-1}, in the absence of Cl^- had no effect on the NO_3 concentration. It seems unlikely that the composition between H_2PO_4 and Cl ions is important because of the greater differences in the physical and physiological properties of these ions [198].

The effect of salinity and fertilizer on grains and several vegetables is independent and additive when stress imposed on them when nutrient deficiency and salinity are moderate [196]. When either of these factors severely limit growth, the other has little influence on yield. Okusanya and Unger [210], with two halophytes and a glycophyte, also reported similar results. Nutrient application increased the growth of the halophyte under saline conditions, presumably because salinity was moderately growth limiting. On the other hand, nutrient application did not improve the growth of the glycophyte under saline conditions, presumably because salinity was severely growth limiting. Under low-salinity stress, nutrient deficiency limits plant growth more than salinity and a positive interaction or increased salt tolerance response occurs. Under moderate salinity, nutrient deficiency and salinity stress may equally limit plant growth and no interaction occurs. Under highly saline conditions, salinity limits growth more than fertilizer. According to Grattan and Grieve [211], the plant performance would always exhibit a negative interaction or a decreased salt-tolerance response if nutrient element was limiting growth under saline conditions and the upper salinity treatment was lethal or severely growth limiting.

Salinity tolerance under suboptimal conditions is important only under dry land conditions, where high levels of fertilizers are not economical or the availability of fertilizer is limited [210]. The disagreement between some of the publications dealing with the salinity-fertility relationship may stem from the use of different salinization techniques and the use of different chemicals to change the level of salinity. The difference between species varieties, the duration of the experiments, and growth conditions also could explain the variations in crop responses. Standardization of methodology, when feasible, could reduce variations between experiments. Chemical analysis of plant tissues and studies of the physiological and biochemical process involved in salinity fertility relationship are essential to explain salinity-fertility interactions.

SELECTION AND BREEDING FOR SALT TOLERANCE

Breeding programs to improve salt tolerance are particularly important in countries that would benefit from stress-tolerant crops [212–216]. Improvement of crop plants for salt tolerance depends on the existence of biological variability for salt tolerance. Variation to salinity tolerance has been observed not only between species but also between the cultivars of species [32,215,216].

Genetic variability of salt tolerance has been characterized for certain agronomic crops [2,32]. The work of Abel [217] on *Glycine max* was one of the first attempts to analyse salt tolerance genetically. The tolerance in the Lee variety was associated with the exclusion of chloride from the leaf, and exclusion is controlled by the root [60,70].

Breeding for improved salt tolerance has been reviewed extensively [218–221]. The transfer of genes that can improve salt tolerance from wild species to crop plants has been attempted by traditional techniques [8,13,14,222]. However, in spite of the presence of potential donors of salt tolerance and successful hybridization, no cultivar suitable for commercial growing has been developed so far [15,223]. Wild hybridization has been successful in the quest of characters such as insect and disease resistance where a single gene may be introgressed and selection to maintain that character is relatively straightforward in the backcross generations which are needed to dilute the rest of the alien gene from the resulting hybrid. The situation for salt tolerance is different. The limiting factors in developing salt-tolerant plants appears to be the quantitative nature of salt tolerance [66,214,224], the lengthy backcrosses which are required to recover useful agronomic features together with the halotolerant genes [223], and the difficulties involved in detecting the introduced variation [225]; and not all crop plants have salt-tolerant relatives capable of cross fertilization [47].

Richards [212,213] infers that there is no need to breed for salt-tolerant crops because of the complexicity of the problem and patchiness of salt-affected fields. It is preferable to concentrate on yield potential, plant the highest yielding cultivar, take the yield from the areas with lower salt concentration, and accept any losses on the more salt-affected patches rather than accept any losses in potential yield that might be associated with breeding for stress resistance (Disregard tolerance per se and select instead only for yield as the only ultimately interesting parameter [212].)

In contrast, Blum [226] supported the theme of selection of plant species to breed for salinity tolerance. Usually, the variety with superior yield, under optimum conditions, also yields relatively well under suboptimal conditions, assuming that the same genes control both high and stable yield. The advantage of this approach, if valid, is that selection for yield is more efficient under optimal conditions. However, the more commonly accepted approach in breeding for salt tolerance is that the maximum potential yield and the stability of yield are largely independent of each other. Stress-tolerant cultivars should thus be selected under the stress to which tolerance is desired [5]. This assumes that yield stability in various environments, with many attributes desired by the breeder, can be transferred from wild stress-tolerant plants independently of their low growth rate and metabolism.

Neverthless, tolerance of salt, water or other kinds of stress is still considered ''complex'' and can be resolved through a coordinated physiological genetic approach. In the breeding of multicellular organisms, genetics cannot be separated from epigenetics, which includes the biochemical and physiological aspects of gene action. These factors, along with environmental factors, determine the final phenotype.

AGRICULTURAL PRODUCTION OF HALOPHYTES

Boyko [227] drew attention to the possibility of crop production using seawater as an irrigant. Even though this idea was supported and experiments undertaken by many workers, the fact remained that no conventional agricultural crops are grown with undiluted seawater on dune sand without a significant reduction in yield. Successful seawater irrigation depends on the use of halophytes. In

recent years, several investigators have promoted the use of halophytes for the seawater irrigation problem [228–229]. Mudie [228] explains the potential of halophytes and the transfer of halophytic germplasm to glycophytic crops. Somers [230] promoted the cultivation of wild halophytes directly with seawater.

DRAINAGE WATER REUSE FOR GROWING PLANTS

Water is a limited natural resource in many arid and semiarid regions of the world and in populated metropolitan areas, large quantities of sewage effluents are discharged making the groundwater polluted. Drainage water reuse also has been promoted as an environmentally sound method for the disposal of saline drainage water [231]. Screening and cultivation of suitable plant species can not only prevent the groundwater pollution but productivity can be obtained. Plant species like *Atriplex, Medicago, Trifolium heymus, Puceinellia, and Truf* were grown using drainage water [232]. Plant species respond to different ions differently. For example, *Chemopodium ruburum* grew better with chloride salinity, whereas *Kochia* performed better with sulfate salinity [233]. *Portulacia oleracea*, a valuable nutritive vegetable for human consumption, can selectively accumulate selenium, and it has been suggested that the species can be included in a saline drainage water-reuse system where the selenium concentration is very high [234].

Halophytes irrigated with seawater have a remarkable potential as crop plants despite the high salt content in their tissues. It was reported that *S. bigelovii* could be substituted for conventional safflower oil and its seed cake is a good poultry feed, and that *Portulaca oleracea* could be a source of food for human consumption and fodder for livestock.

SEAWATER APPLICATION—CROP PRODUCTION

The need for salt-tolerant crops increases each year, as the growing population seeks to feed itself with ever-decreasing soil sources and dwindling freshwater supplies. Besides humanitarian reasons, there are economic reasons for developing salt-resistant crops. Recent work in the field of plant physiology and breeding has pointed out the possible utility of underground saline water for growing salt-tolerant plants on sand dunes.

The basic approach to seawater irrigation is to develop a range of new crops from wild halophytes that will result in economically worthwhile yields with seawater irrigation and/or to acclimatize the glycophytic crop plants through forced selection to take up seawater as an irrigant [32–34].

The use of water with higher salt levels and even of seawater for irrigation of various food, feed, fiber, and fodder crops has been reported [7,8,19,36,68,229,235] and has produced grains and oil seeds, vegetables, fodder, fuel and fibers, pharmaceuticals, and other products using highly saline water.

The World Health Organization (WHO) [236] and Norleyn and Epstein [237] reported that selected barley strains grown with full-strength seawater yielded at least 50% of the yield obtained under irrigation with freshwater.

The responses of crop plants to seawater irrigation are being extensively studied by scientists at the Central Salt & Marine Chemicals Research Institute in Bhavnagar, India, to investigate the potential of using seawater on coastal sand dunes as a supplemental irrigation for the production of food grains and oil seeds. Germination, a critical stage of plant growth, was evaluated for different varieties of crops irrigated with seawater. The crops tested were cereals (wheat, barley, rice, and maize), millets (bajra, jowar, and ragi), pulses (red gram, green gram, and lentil), and oil seeds (sesame, peanut, sunflower, and mustard). Many of the crop varieties tested tolerated up to 10,000 ppm seawater without a significant reduction in the germination percentage. The tolerance to seawa-

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ter salinity is in the order cereals > pulses > millet > oil seeds and grass > legumes. The growth development, biomass production, and final yield also differed considerably for different crops and for different varieties (Table 2). Depending on the genotypic variation, crop species such as wheat variety Karchia (local) and the bajra variety Babapuri (local) were selected for improvement for salinity tolerance and for productivity.

Acclimatization

Most of plants are capable of tolerating a certain range of salinity and the range varies for different species, varieties, and ecotypes. In glycophytes, the range is rather narrow, whereas in halopytes, it is wide. A large part of the research on salinity is carried out with the intention of accommodating crop plants to grow in salinities outside the natural range of tolerance and neverthless obtain an appropriate agriculture yield called ''adaptation or acclimatization.'' Acclimatization is achieved during a specific treatment and involves changes in plant behavior and expression of certain properties which are not evident before the treatment. A plant is considered adapted when the mean growth rate of salt-treated plant increases or when the plant has acquired the capacity to complete its life cycle in a saline environment in which the nonacclamitized plants fail to do so [235,238].

When *Phaseolus vulgaris* was exposed to NaCl (48 mM), at first the growth was inhibited and leaf Na^+ Cl^- concentrations increased rapidly. However, after 25 days of continuous salinization, the relative growth rate was restored similar to control, suggesting that the plant had adapted to salinity [239]. Similarly, *Sorghum bicolor* was acclimatized to grow at 300 mM NaCl without a reduction in the relative growth rate, and Na^+ Cl^- concentrations also were stable and controlled [68].

The period required for adaptation was shortened by ABA treatment, and the process of acclimatization was inhibited by exogenous CK and/or GA [68,235], and adaptation to salinity by ABA pretreatment was developmentally regulated. The defined period of time required for adaptation of a particular species was considered as the developmental window [240]. However, Greenway [241] is of the opinion that the response of plants to salinity is often not by adaptation but rather through the preexisting tolerance mechanisms.

A gradual acclimatization from lower to a higher grade of salinity is considred an inherent characteristic of the species combating the stress. Using the technique of forced selection, Karchia wheat and Babapuri bajra were acclimatized to grow with seawater (20,000 ppm for Karchia wheat) and direct seawater (35,000 ppm for Babapuri bajra) with little reduction in the yield. The addition of nutrient elements (N, P, and K) improved the growth, biomass production, and yield under seawater irrigation. Although a reduction in the growth and yield was apparent, there was no change in nutritive values of seeds (Table 3). Seawater utilization has been a recent effort to explore the possibility of obtaining a reasonable yield and quality of products from the plant species.

PROSPECTS

The salinization of soil and water is becoming an increasingly serious constraint for crop production, particularly in the arid and semiarid regions of the word. With the human population expected to reach over 10 billion by the year 2050, to meet the requirements, increasing areas of land in arid and semiarid regions are to be brought into production. Overirrigation with inadequate drainage facilities and the use of low-quality water for irrigation are causing concern for secondary salinization [242]. The long-term survival of the present agricultural system depends on tackling the problem in a more integrated manner using management and biotechnological approaches. Improved salt tolerance in crop plants appears to be a desirable trait in view of the large percentage of agricultural land that are saline and or salinized by local irrigation practices.

The biological options open the way for the novel concept of using halophytes as an alternative

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TABLE 2 Percentage Reduction in Crop Varieties Under Seawater Irrigation

Crop	Total no. of varieties	Seawater tolerance in varieties: Tolerant (15,000 ppm & above)	Moderate tolerant (up to 10,000 ppm)	Susceptible (up to 5000 ppm)	% Reduction over control	Remarks
Cereals						
Wheat	15	Karchia	NP-324, J-1-7	J-18, J-40, Sona Lok-1, Sonalika, A-206, JU-34, Raj 1781	30	Pot, sand bed, and field studies
Barley	7	BG-131	BG-1, BG-7 BG-137 and BG-138	BG-161, BG-24	40	Pot and sand bed studies
Millets						
Bajra	38	Babapuri (local selection)	HB-3, IJ-1934, NHB-5, GHB-14, GHB-12, GHB-11	Remaining inbrid and hybrid varieties	25	Pot, sand bed, and field studies
Sorghum	16	Swarna, S-105-1 S-105-2 and S-100-1	604, Chandisor	Remaining varieties	45	Pot, sand bed, and field studies
Kodomillet	2	—	—	CO-2	75	Sand bed studies
Oil Seeds						
Safflower	3	—	NP-30	2-3-P1, B-38-5	40	Pot studies
Castor	9	—	Junagadh-1	Remaining varieties	50	Pot and sand bed studies
Fiber crop						
Cotton	3	—	1AN-579-189	Remaining varieties	38	Sand bed studies
Sugar crop						
Sugar beet	20	—	Dobroivica C and USH-9	Remaining varieties	4	Pot and field studies
Sugarcane	2	—	CO-577	CO-419	2	Pot studies
Vegetables						
Onion	8	—	Local-1 (white selection)	Remaining varieties	4	Sand bed and field studies
Garlic	2	—	Local-1	Anand-I	4	Pot studies
Topioca	8	—	H-97, H-226,A-2304	Remaining varies	35	Sand bed studies
Chillies	5	—		Highly susceptible	—	Pot studies
Knol-khol	2	—		Highly susceptible	—	Pot studies
Raddish	5	—		Pusaresham	28	Sand bed studies
Cabbage	5	—		Early wonder	80	Pot studies

Source: From Ref. 238.

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TABLE 3 Nutritive Value of Bajra and Wheat Grown with Seawater (percentage constituents per 100 g seeds)[a]

	Bajra[b]		Wheat[c]	
Constituent	Seawater	Normal sample	Seawater	Normal sample
Moisture, %	7.7	12.4	8.6	12.8
Protein, %	9.5	12.6	17.3	11.8
Fat, %	5.0	5.0	1.5	1.5
Minerals, %	2.1	1.3	1.5	1.5
Fiber, %	1.2	1.2	2.1	1.2
Carbohydrates, %	74.5	67.5	69.0	71.1
Calorific value, %	381.0	361.0	359.0	346.0
Calcium, mg	51.0	42.0	51.0	41.0
Phosphorus, mg	384.0	296.0	248.0	306.0
Iron, mg	20.0	5.0	10.3	4.9
Thiamine, mg	4.46	0.33	0.52	0.45
Riboflavin, mg	0.17	0.25	0.18	0.17
Nicotinic acid, mg	1.8	2.3	4.9	5.5

[a] Samples were tested by the National Institute of Nutrition (ICMR), Hyderabad, India.
[b] Direct irrigation with seawater (18,000–24,000 ppm).
[c] Diluted seawater (10,000–20,000 ppm).
Source: From Ref. 238.

crop [6,7] and making use of seawater as an irrigant for the production of food and plant products using relatively salt-tolerant varieties along the coastal line [238], which at present lie idle for lack of crops that can be grown in these regions.

With the low number of varieties released for agricultural production on saline soils or using seawater as an irrigant [2,220], it appears that the criteria followed for salt tolerance is not appropriate. Salt tolerance appears to be a quantitative trait and is controlled by many genes [67,214].

Today, we know many biochemical mechanisms that form the basis for salinity tolerance [86,88,89,101] and tools are available to accomplish metabolic engineering of crop plants [44]; although not enough to speak of generation of salt tolerance for growing in the field but significant enough to recognize the underlying mechanisms.

Sodium transport across the tonoplast and its accumulation in the vacuole by the sodium proton antiport system have been described biochemically [186]. Certain plants use Na^+ as a signifying molecule to stimulate the activity of the transport protein which sequesters Na^+ away from the salt-sensitive metabolic machinery through the sodium proton antiport system. Plant genes are not yet available to test how sodium efflux at the plasma membrane or increased sodium influx at the vacuolar membrane can provide advantages to plants under stress.

The success in molecular engineering of improved salt tolerance in crop plants depends on understanding not only the contributions that the individual genes make to tolerant phenotype but also the control exerted by the molecular regulating circuits that integrate endogenous program for development and differentiation with those of exogeneous stress stimuli.

More concerted attempts should be made toward understanding the molecular mechanisms by which crop plants could acquire improved salt tolerance. We hope that wild relatives of crops will play a more prominent role than hitherto in the development of salt-tolerant crop varieties, since in naturally tolerant plants, the mechanisms are fully developed and functional to make the species productive under stress. However, much remains to be done, and an active collaboration between plant breeders, genetists, molecular biologists, and plant physiologists is essential to make significant advances in this field of research.

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Strategies and Scope for Improving Salinity Tolerance in Crop Plants

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INTRODUCTION

Salinity is a condition of excess salts in the soil, which affects plants by increasing the osmotic pressure of the soil solution, interfering with normal nutrient uptake, and inducing ionic toxicity and associated nutrient imbalances. Osmotic stress under saline conditions, termed physiological drought [1], subjects plants to dehydration. Ionic toxicity resulting from the accumulation of specific ions, such as Na and Cl, in the cytoplasm or apoplast interferes with plant metabolic functions [2].

Under low to moderate salinity (actual salinity levels may vary from low to moderate depending on the crop species), plants adjust osmotically by using a portion of their photosynthates to increase internal solute concentrations and thus do not show dehydration symptoms. Also, plants regulate their ionic balance to maintain normal metabolism. For example, uptake and translocation of toxic ions, such as Na and Cl, are restricted, and uptake of metabolically required ions, such as K, is maintained or increased.

Although plants may not show water-deficit symptoms and metabolize normally under low to moderate salinity levels, the additional energy requirements for maintaining normal metabolism demand substantial photosynthate diversions from growth [3]. This leads to a reduction in leaf area, light interception, light utilization efficiency (due to partial stomatal closure and the resultant decrease in CO_2 fixation), and, ultimately, a reduction in growth and yield. Plants die when salinity levels exceed a certain critical level (which varies from crop to crop). Death is the result of physiological mechanisms breaking down and consequent ionic toxicity. Poor plant stand is one of the factors causing low yields under saline conditions, as salinity is nonuniform in its distribution under field conditions.

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The main objective of this chapter is to present an overview of the current status of the knowledge and approaches to the genetic improvement of salinity tolerance in crop species. Management aspects that could alleviate salinity problems for crop production also are discussed, however, because genetic improvement cannot be considered in isolation in confronting a salinity problem. The chapter is focused on giving a conceptual framework for the genetic improvement of salinity tolerance. This demands an interdisciplinary team approach; to our knowledge, there is little evidence of this in present-day research efforts.

SALINITY AND CROP PRODUCTION

The expected yield losses under different levels of salinity for various crops are given in Table 1. Data on regional yield losses for various crops due to salinity are not readily available. Irrigated agriculture contributes substantially to crop production in arid and semiarid regions of the world. Secondary salinization, which is associated with irrigated agriculture, is becoming a serious concern in these regions. Nearly 40% of irrigated lands are affected by some degree of salinity [4]. Considering that nearly 240 million ha land worldwide is under irrigated crop production [5], the economic impact of secondary salinization on crop production could be astronomical. Rain-fed agriculture also can be affected by salinity through the effects of deforestation and other vegetation changes in altering underground movement patterns of water and salts.

We discuss in this section the various aspects related to the understanding of crop response to salinity, including the growth-stage response, the role of environmental factors in modifying the salinity response, and the management options that could alleviate the crop tolerance to soil salinity.

Measurement of Soil Salinity

It is important to quantify and characterize salinity distribution in a production area to make decisions regarding the selection of a crop and the management practices necessary to minimize yield reduction. Appropriate sampling techniques and salinity measurement methods are necessary to assess salinity levels properly and map their distribution in the production area during a cropping season.

Soil Sampling

Generally, major root activity occurs in less saline strata of the soil profile [6], and this should be taken into account when relating plant growth and yield to soil salinity status. Soil samples should be taken from the active rootzone and should not be contaminated by surface salt encrustations. Since salt concentrations can vary markedly with soil depth, samples are best collected at several depths, such as 0–15 and 15–20 cm, depending on the rootzone [7].

Determination of Salinity

The electrical conductivity of a saturation extract, EC_e, expressed in dS m^{-1} at 25°C, is recommended for correlating salinity level with growth [8]. The electrical conductivity of the saturation extract is directly related to the soil soluble salt concentration. The relationship between EC_e and osmotic potential Ψ_o is $\Psi_o = -0.36\ EC_e$. Use of EC_e is recommended by the U.S. Salinity Laboratory, because the saturation percentage is easily determined and is accurate for soils that vary widely in texture [8]. For most soils, the soluble salt concentration in the saturation extract is about one half the concentration of the soil solution at field capacity and about one fourth the concentration at permanent wilting point [9].

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TABLE 1 EC_e at Which 10, 25, and 50% Yield Reduction Can Be Expected for Various Agricultural Crops

	% Yield reduction		
	10	25	50
Field crops			
Barley	11.9	15.8	17.0
Sugar beet	10.0	13.0	16.0
Cotton	9.9	11.9	16.0
Safflower	7.0	11.0	14.0
Wheat	7.1	10.0	14.0
Sorghum	5.9	9.0	11.9
Soybean	5.2	6.9	9.0
Sesbania	3.8	5.7	9.0
Rice	5.1	5.9	8.0
Corn	5.1	5.9	7.0
Broadbean	3.1	4.2	6.2
Flax	2.9	4.2	6.2
Bean	1.1	2.1	3.0
Vegetable crops			
Beets	8.0	9.7	11.7
Spinach	5.7	6.9	8.0
Tomato	4.0	6.6	8.0
Broccoli	4.0	5.9	8.0
Cabbage	2.5	4.0	7.0
Potato	2.5	4.0	6.0
Corn	2.5	4.0	6.0
Sweet potato	2.5	3.7	6.0
Lettuce	2.0	3.0	4.8
Bell pepper	2.0	3.0	4.8
Onion	2.0	3.4	4.0
Carrot	1.3	2.5	4.2
Bean	1.3	2.0	3.2
Forage crops			
Bermuda grass	13.0	15.9	18.1
Tall wheatgrass	10.9	15.1	18.1
Crested wheatgrass	5.9	11.0	18.1
Tall fescue	6.8	10.4	14.7
Perennial rye	7.9	10.0	13.0
Beardless wild rye	3.9	7.0	10.8
Alfalfa	3.0	4.9	8.2
Orchard grass	2.7	4.6	8.1
Meadow foxtail	2.1	5.5	6.4
Clovers, alsike and red	2.1	2.5	4.2

Source: From Ref. 39.

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Crop Tolerance to Salinity

There are different ways of defining crop salinity tolerance, depending on the context in which it is used. Some of these are as follows:

1. ''The capacity to persist in the presence of increasing degree of salinity'' [10]: a given species may make little or no growth at higher salinity levels but does survive. This is the criterion generally used by ecologists in evaluating halophytic environments. Ecologists maintain that the species most capable of persisting in a saline area become the climax vegetation of that area.
2. ''The degree to which osmotic adjustment can be made without sacrifice in growth'' [11].
3. ''The absence of negative effects on growth in plants that accumulate salts in their tissues'' [1].
4. ''Yield decrease expected for a given level of soluble salts in the root medium as compared with yield under non-saline conditions'' [9].
5. ''The sustained growth of plants in an environment of excess salts in the growth medium'' [12].

In the context of crop production under saline conditions, definitions 4 and 5 are more relevant. Crop salt tolerance has usually been expressed as the yield decrease expected for a given level of salinity in the root medium compared with yield under nonsaline conditions [8]. Therefore, salt tolerance is a relative value based on the growing conditions of the crop.

Growth Stage Response

Information on the growth-stage response to salinity within a crop is important in adopting suitable genetic and management strategies for saline soils. For example, if a crop is more sensitive during one stage than another, there is an opportunity to regulate the salinity of irrigation water during the season to minimize salt injury at the sensitive stage.

Ontogenetic drift, a change in genotypic expression with plant development, is one of the factors that can modify the relationship between phenotype and environment. During plant growth, the form and function of various organs change. The plant's ability to respond to salt stress depends on the genes that are functioning at the stage of development during which the stress occurs [13]. Thus, salinity effects may vary depending on the growth stage at the time of stress. One example, often cited, is that salt tolerance at germination is not consistently related to tolerance during emergence, vegetative growth, flowering, or fruiting. Sugar beet, barley, and cotton are among the most salt-tolerant agricultural crops, but each is relatively sensitive during germination or early seedling growth [14,15]. On the contrary, corn, pea, gram, and beans are more sensitive during later stages of development [15,16].

Relative sensitivity could change from one developmental stage to another. Rice is tolerant during germination [17] and becomes very sensitive during the seedling stage and again somewhat sensitive during fertilization of florets [18]. Corn is more salt sensitive during emergence and seedling growth but becomes more salt tolerant by the flowering stage [19]. Salt resistance is low in young tomato plants, becomes much higher by the bud stage, and decreases during flowering [20].

Sensitivity to salinity in durum and bread wheat decreases with age, indicating the importance of keeping soil salinity levels low during germination and seedling emergence [21]. Similarly, cowpea becomes increasingly more salt tolerant as plants develop during the growing season [22]. One of the reasons for the decreasing sensitivity with age could be a gradual acclimation of the crop to salinity. This indicates that if cowpeas or wheat are irrigated with water containing salt levels below the threshold, before the flowering stage, higher levels of saline irrigation water could be used at later growth stages without any deleterious effect on yield [21,22].

Within a species, varietal rankings could change with the growth stage, and this has been observed with rice [23]. For barley, varietal differences increased with the plant development stage

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[24]. Changing varietal differences (i.e., relative tolerance rating) over time were also reported in sugar cane [25]. This would complicate the screening and selection process, if it is based on a single growth stage.

Environmental Interactions

Interactions between salinity and soil, water, and climatic conditions change the plant's ability to tolerate salinity. A basic understanding of the interactions between salinity and the environment is necessary for an accurate assessment of salt tolerance. In addition to precipitation, temperature and atmospheric humidity can markedly influence salt tolerance. Many crops are less tolerant when grown under hot dry conditions than under cool humid conditions [9]. This is mainly due to decreased ion accumulation and/or improved plant water relations [26,27].

Rice suffered more salt injury at 30.7°C and 64% relative humidity (RH) than at 27.2°C and 73% RH [28]. High humidity overcame lethal levels of salinity on *Phaseolus vulgaris* L. [26]. In wheat, a higher transpiration rate occurred at low RH and high temperatures, thus increasing the mass flow of salts into the transpiration stream and their accumulation to toxic levels in the shoot [29]. Further, salts may accumulate in the rhizosphere with increased transpiration [29].

Suboptimal soil conditions also can affect the apparent salt tolerance of crops. For example, plants grown on low-fertility soils may appear to be more salt tolerant than those grown with adequate fertilization [30]. A reason for this could be that soil fertility, not salinity, is the prime limiting factor for crop growth. In this case, proper fertilization would increase yields under saline as well as nonsaline conditions but proportionally more under nonsaline conditions.

Comparative Effects of Different Salts

Specific ion toxicity is the primary cause of plant mortality at higher levels of salinity [1]. Different salts have different threshold osmotic concentrations for injury, and the relative toxicities of specific salts are not constant for all crop plants under all conditions [1]. For example, cotton, rice, and wheat are less resistant to NaCl than Na_2SO_4 salinity [31–33], but *Phaseolus*, guayule, flax, and chickpea show the reverse relationship [34,35]. Alfalfa is more affected by Na_2SO_4, K_2SO_4, and NaCl salts than $MgCl_2$ and $MgSO_4$ salts [36], whereas the reverse is the case with mung bean and red kidney bean [37,38]. Beans and wheat are more affected by $CaCl_2$ compared with NaCl salinity [39,40], whereas the response is the opposite with corn [34]. Mung bean and red kidney bean were equally affected by NaCl, Na_2SO_4, KCl, and K_2SO_4 [37,38]. For many crops, carbonates are more toxic than Cl and/or SO_4 [34].

Protective Effects of Calcium

The importance of Ca in maintaining membrane stability and for selective ion uptake by plants is well documented [41]. Under saline conditions, the ratio between required ions (e.g., K) and unessential ions (e.g., Na) is reduced, and thus selective ion transport by plant roots becomes crucial for survival. Low levels of Ca (<1 mM) in the absence of NaCl salinity support normal growth in most crop plants [2]. Under saline (NaCl) conditions, however, such levels of Ca in the medium result in Ca deficiency in many crop plants [42,43]. Under NaCl salinity, a decrease in the membrane-associated Ca content due to the displacement of Ca by Na leads to the disruption of membrane integrity [43]. This causes an increase in passive Cl and Na transport and results in ion toxicity [44]. The NaCl salinity (at low Ca levels) also inhibits Ca transport from roots to shoots by interfering with the active loading and release of Ca into xylem vessels [45].

Several reports indicate that supplemental Ca (usually up to at least 5 mM) may alleviate the reduced growth caused by NaCl salinity. In *P. vulgaris*, dry weights increased with increasing Ca levels up to 3 mM at 50 mM NaCl in the ambient solution, and there was no further improvement at higher Ca levels [46]. A positive growth response to increasing Ca under NaCl salinity was also

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reported for barley [47]. The germination and seedling growth of Wimmera rye grass under NaCl and $MgCl_2$ salinity improved with the increasing Ca concentration in the growth medium [48]. Some crops, however, including rice and lettuce, do not respond positively to Ca addition under NaCl salinity [49,50].

Supplemental Ca, under NaCl salinity, normally improves Ca absorption of the plants [51]. Calcium also protects NO_3 transport under saline conditions [52]. In pigeon pea (*Cajanus cajan*), a positive growth response to a decrease in the Na/Ca ratio was observed at a constant salinity of 6 or 8 dS m^{-1} [53]. A decrease in the Na/Ca ratio in the medium improved K/Na in the shoot and thus improved plant growth. With a decrease in Na/Ca ratio, however, tissue Cl levels increased and to some extent counteracted the positive effects of improving the K/Na ratio.

Management Practices that Minimize Yield Reduction Under Saline Conditions

Although the main objective of this chapter is to document the scope of genetic options to improve salinity tolerance in crop plants, this topic cannot be considered in isolation from various management options that reduce salinity damage. Further, we emphasize that a practical approach to alleviating salinity effects is a close integration of genetic and management options. Management practices that can be used to minimize yield reduction under saline conditions are mostly related to the control of rootzone salinity and reduced damage to the crop plants [54]. Control of rootzone salinity can be achieved by irrigation and leaching. For example, intermittent leaching can be more advantageous than leaching at each irrigation [55,56]. Similarly, by increasing the irrigation frequency, the salinity effect on crop growth can be considerably minimized [57]. The control of rootzone salinity in the initial stages of germination and early seedling growth could play a major role in plant stand establishment.

Several cultural and management practices have been developed to enhance plant stand establishment under saline conditions [54,58]:

1. Irrigate lightly each day after seeding with a sprinkler system until the stand is established, and then convert to furrow irrigation.
2. Leach salts from the soil surface before planting to allow stand establishment before salts can accumulate at levels that would interfere with germination or damage seedlings.
3. Prepare seed beds in such a way that salts accumulate at the top of ridges, and then sow seeds in the furrow or on the slope between the furrow bottom and ridge top [54].
4. By applying a mulch to reduce evaporation, increase water uptake by plants and increase leaching of salts [59].

Although soil salinity reduces the plant growth potential, this may not necessarily reduce the total field yield, the field yield is the product of stand density and yield per plant. Using the crop growth model of Maas and Hoffman [9], the predicted reduction in individual plant growth due to salinity can be estimated. Therefore, plant populations could be adjusted to compensate for reductions in individual plant growth [60].

Salinity and Fertilizer Use

By changing the fertilization regimens (type and quantity of fertilizers and method of application) from those considered to be appropriate for nonsaline conditions, it is possible to alleviate the effects of salinity on agricultural crops [61]. Salinity interfered with P translocation in cotton [62] and the uptake of NO_3 in barley [63]. Reduced P translocation is caused by inadequate Ca levels in the roots, and thus the primary response is on Ca uptake [62]. This can be corrected by either foliar P fertilization or Ca fertilization. The latter is more desirable, because it corrects the primary effect and thus improves the salinity tolerance. Different crops and genotypes are known to have differences in

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their ability to take up Ca during NaCl salinity [2]. Tolerant genotypes are able to maintain Ca uptake, whereas sensitive genotypes are not. Therefore, depending on the crop or genotype used in a particular fertilizer trial, different responses can be expected. Positive growth responses to fertilization under saline conditions are reported in clover [64], wheat [65], tomato [66], bean [67], and pepper (*Capsicum annuum* L.) [68]. On the other hand, negative growth responses were reported in cotton [69], rice, barley [70], corn [68–70], and soybean [71].

GENETIC IMPROVEMENT IN SALINITY TOLERANCE

Several points must be considered before initiating a breeding program to improve salinity tolerance. In the first instance, alternative cropping strategies should be evaluated. Selection of a different crop that is more salt tolerant may result in productivity far exceeding the genetic limits of the crop originally targeted for salinity-tolerance breeding. For example, by changing the cropping system from wheat to barley, the necessity of genetically improving wheat salinity tolerance can be avoided: Considerable genetic improvement in wheat is needed to raise its tolerance to the level already existing in barley.

This strategy of expanding the use of salt-tolerant species without going through selection within a species could be sufficient to circumvent salinity problems to some extent. However, economic considerations, food habits of the region's population, and cropping systems that have evolved based on these crops and that fit well into existing agroecological niches may not allow replacement of existing crops with a more salt-tolerant crop. For instance, salinity problems that confront lettuce, tomato, and other vegetable growers in California could be eliminated if these vegetable crops were replaced by barley. Vegetable production is a highly commercialized system and the economic backbone of California's agriculture, which does not permit such an option [12].

Legumes are very sensitive to salinity in comparison to cereals [72]. The semiarid regions, which include a large proportion of the world's irrigated agriculture, are now under threat from secondary salinization. Legumes play an important role in these production systems, which are largely based on cereal-legume cropping patterns. Such patterns contribute to the maintenance of soil fertility and soil structure and long-term sustainability of these production systems. For example, in rice-based cropping systems, such legumes as green gram and black gram play an important role in this cereal-legume cropping pattern in the Krishna and Godavari delta regions of peninsular India. This production system has recently been threatened by secondary salinization, and the legume component is being affected first because of its higher susceptibility to salinity. Although the long-term sustainability of this production system requires the development of suitable management practices to arrest the further build-up of salts, this process could be enhanced by the use of legume genotypes with higher levels of salinity tolerance than are now available.

For many biotic and abiotic stresses, the feasibility of a genetic approach in improving crop tolerance has been demonstrated convincingly. For certain abiotic stresses, however, such as drought and salinity, genetic improvement remains a challenging task because of the difficulties in defining precisely the target environment, which is a prerequisite to focusing genetic improvement. Further, serious obstacles to genetic improvement of salinity tolerance are the diversity of physiological mechanisms that determine the level of tolerance to salinity or drought, their multigenic nature of inheritance, and the lack of appropriate screening methodology, appropriate selection criteria for evaluation of germplasm, and segregating material. These points are discussed in detail in this section.

Screening Methodology

Field Environments

Field salinity is inherently variable (levels can vary from <4.0 to >40 dS m^{-1}); variation occurs both horizontally and vertically and changes temporarily within and between growing seasons (depending

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mainly on the amount of precipitation and evapotranspirational demands) [73]. Spatial variation in a saline soil can be enhanced further by irrigation [73]; on the other hand, an insufficient moisture supply exacerbates the variability in plant growth by the development of variable moisture-stress conditions in addition to variable salinity effects. Plant roots avoid more saline soil areas and take up water and nutrients from less saline areas [74]. Plant growth under such variable saline conditions may be more a result of escape than of genetic differences in tolerance [75].

Because of the natural field variability in salinity levels, it is very difficult to evaluate germplasm lines under field conditions. Environmental variance effects are likely to exceed those of the genetic component, thereby making selection for genetic improvement difficult.

An alternative approach is field testing under relatively controlled conditions, as done by the U.S. Salinity Laboratory at Riverside, California [9]. Using a nonsaline, sandy loam soil and by irrigating with different levels of saline irrigation water (usually by adding NaCl + $CaCl_2$ wt/wt), relatively uniform salinity levels, within a given salinity treatment, can be created. By increasing irrigation frequency and by applying excess irrigation water, the build-up of salts can be prevented. A nonsaline control treatment for all genotypes is usually used to determine inherent differences in the growth and yield potential. Therefore, genotypes can be evaluated at different salinity levels on a relative yield basis [76].

Controlled Environments

Most researchers use controlled environments, such as greenhouses or growth chambers, for the preliminary evaluation of germplasm lines. This helps to reduce the number of lines to more manageable levels for more rigorous testing at a later stage under controlled-environment or field conditions. Also, selection of breeding materials in early generations involves exposure of plants to salinity in a relatively controlled environment to minimize environmental variance and maximize genetic variance. Plants are then grown in containers with a salinized media. Salt concentrations for selection vary with species sensitivity. For most glycophytic crop plants, the concentrations used for screening range between 50 and 300 mM NaCl (representing rice and barley, respectively) [75].

In most large-scale screening of germplasm lines for salinity tolerance, an aerated and salinized hydroponic system is used. The principles to observe in any hydroponic system are (a) balanced supply of nutrients, (b) proper aeration, (c) control of salt concentration and solution pH over time, and (d) gradual increase in salinity level in several increments over time until the desired treatment salinity level is reached to avoid osmotic shock to the plants.

Salinity Tolerance Criteria

Genotypes may be evaluated for vigor, leaf damage, survival, and ability to grow under saline conditions. A salinity level is chosen to select about 10% of the material for further evaluation over a range of salinity levels. Sand culture under greenhouse conditions may be used to determine growth response curves at various salinity levels. The parameters that might be used in assessing the effect of salinity on a particular species include survival, leaf damage, and vegetative growth and yield. All are of course interrelated: There can be no yield without survival, although a species may survive vegetatively and yet fail to produce yield. Therefore, knowledge of all these parameters contributes to the assessment of the effects of salinity on a particular crop species.

Based on Germination

Selection on the basis of germination tests shows little promise as a means of improving salinity tolerance in subsequent growth stages [77]. However, lack of association does not mean that germination tests are not useful in a salt-tolerance breeding program. In many situations, the ability to germinate and establish a good plant stand in saline soils is an important factor in crop production. However, this depends on the crop under consideration and the agronomic practices associated with it. In rice, tolerance at germination and initial seedling growth is not important, because this crop

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is mostly transplanted. Development of genotypes with tolerance at all growth stages requires selection at several points in the life cycle.

Based on Survival

Plant survival at high salt concentrations, irrespective of their growth rate and productivity under moderate salinity levels, has been proposed as a selection criterion for tomato, barley, and wheat [78–80]. The philosophy behind this is to focus on tolerance per se, thereby separating yielding ability from salinity tolerance; considering that these two are independent attributes. The ability of a genotype to survive and complete its life cycle at very high salinity levels, irrespective of its yield potential at moderate salinity levels, is considered tolerance in the absolute sense. Also, yield is regulated by a number of genetic factors not contributing directly to salinity tolerance. Once sources of very high levels of salinity tolerance are identified, attempts can be made to combine these with high-yield potential through standard breeding procedures. This is similar to the approach adopted in disease-resistance breeding, in which the initial selection emphasis is on identifying the sources of disease resistance rather than the yield ability in disease environments.

Based on Leaf Damage

Most crop plants are glycophytes and, unlike halophytes, cannot tolerate high-salt levels (mainly Na and Cl) in their leaf tissues. Therefore, one important factor in the physiological mechanisms operating in glycophytes is preventing Na and Cl ions from translocation to the shoot. Beyond a certain critical level of salinity stress, this regulation breaks down, resulting in the translocation of large amounts of Na and Cl to the shoot, causing ionic toxicity. Critical levels vary among genotypes, varieties, and crops and usually determine the differences in the level of tolerance. Leaf damage (bleaching or necrosis) is a symptom of a breakdown in ionic regulation. Therefore, selection against leaf damage should lead to the identification of genotypes that have more efficient ionic regulation and other physiological mechanisms that contribute to higher tolerance levels. In alfalfa (lucerne), selection criteria based on leaf damage of less than 10% resulted in rapid improvement in selection for salinity tolerance [81].

Based on Growth and Yield

Salinity tolerance is usually assessed in terms of absolute and/or relative growth or yield. Although absolute yields have an obvious practical application, they often reflect qualities other than tolerance

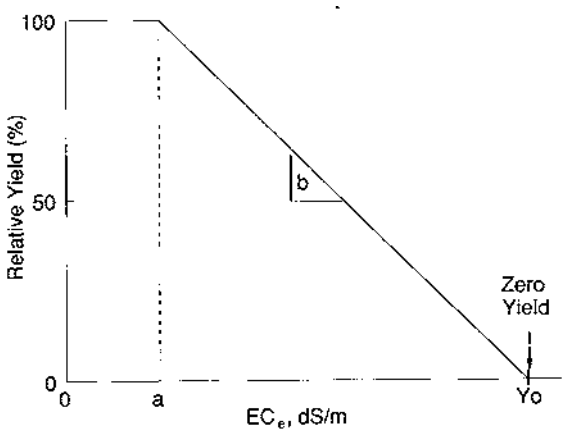

FIGURE 1 Response curve to salinity. (From Ref. 54. Reprinted by permission of Kluwer Academic Publishers.)

to salinity and can lead to illogical conclusions if considered alone. Inherent differences between genotypes in their growth rates or habits does not permit a valid assessment of their relative salinity tolerances using absolute yield or growth criteria at a particular salinity level. For example, a genotype may suffer a severe yield reduction at a given level of salinity and yet yield more than another genotype whose yield is unaffected by salinity [82].

The performance of a genotype under saline conditions in comparison with that under nonsaline conditions provides a measurement of salt tolerance stripped of extraneous influences. Also, this approach provides a means to compare crops whose yields are expressed in different units or that differ widely. However, the reliability of relative salt tolerance data depends on the degree to which yield reductions are unaffected by extraneous interactions [9]. If reductions in relative yield are independent of differences in absolute yield caused by irrigation, climate, fertility, or other variables, the relative yield-salinity relationship permits a useful expression of plant tolerance to salinity [83].

The crop response to salinity is usually described as a decreasing function with an increase in the EC_e of the soil solution. It has been suggested [9,84] that a reduction in crop yield due to salinity can be linearly related to the EC_e of the soil solution after a certain threshold value of EC_e is reached (Fig. 1). This can be expressed as

$$\frac{Y}{Y_{\max}} = 1 - b(EC_e - a) \tag{1}$$

where:

Y = yield
$Y_{\max}$ = yield of nonsaline control
a = salinity threshold value, EC_e units (dS m^{-1}), that is, the maximum soil salinity that does not reduce yields below those produced under nonsaline conditions
b = slope, the relative reduction per unit salinity increase from threshold

Based on the salinity threshold level a, slope value b, and salinity level at which yield becomes zero Y_0, Maas and Hoffman [9] grouped most important crops into four categories: (1) sensitive, (2) moderately sensitive, (3) moderately tolerant, and (4) tolerant.

Genotypes or germplasm lines could be evaluated for their salinity tolerance using this linear growth-response model. However, many data points above and below the threshold level are required to define the threshold level accurately and to measure the slope value [85]. This kind of evaluation should mainly be used to assess the production capacity of selected, contrasting genotypes in saline environments, not for the initial evaluation in which many germplasm lines must be screened.

Conceptual Framework for Genetic Improvement Under Salinity Stress

The linear growth model of Maas and Hoffman [9] could be used as a conceptual framework for the genetic improvement in salinity tolerance. To improve crop performance genetically under saline conditions, it is necessary to shift the threshold value a to the maximum extent possible and to reduce the slope value b to give stability in crop performance across a range of salinity levels and an increase in Y_0. Genetic improvement in these three components would involve screening, selection, and recombination through breeding. This should result in better crop performance under saline conditions.

The three components of the model (a, b, and Y_0) may be considered to the independent crop attributes, because each component refers to a crop response at a given range of salinity (i.e., the a value refers to the crop performance at low-salinity levels, the b value to moderate-salinity levels, and Y_0 to high-salinity levels). Considering the principles of quantitative genetics, Falconer [86]

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proposed that a characteristic in two different environments may be regarded as two characteristics rather than one.

The criteria for evaluating crop salinity tolerance vary, depending on the level of salinity stress. In a low- to moderate-salinity range, the production capacity of the genotype is the main criterion, whereas survival ability is the main criterion at higher salinity levels [87]. It is likely that the physiological mechanisms that play a major role in maintaining the production capacity of a genotype are not the same as those that contribute to tolerance at extremely high-salt concentrations [88].

Assuming that these three components are independent crop attributes, independent genetic improvement should be sought for each component. Once improved sources of genetic materials are identified for each component, these could be combined into a single genotype through breeding. However, the decision to breed for improved salinity tolerance for a given crop should be carefully considered. The plant breeding approach, although remarkably successful in some instances, is very time consuming and labor intensive when conventional breeding methodologies are used. Also, it must be realized that salinity tolerance is a finite attribute, and genetic improvement through selection and recombination can improve tolerance only up to a certain level within a given crop species. The degree of improvement depends on the availability and extent of the variability for salinity tolerance and the existing tolerance level of the species. Also, higher levels of soil salinity could place considerable pressure on the plant's photosynthetic capability, because physiological defense mechanisms that permit survival and production under saline conditions demand a larger portion of available photosynthate [3]. This leads to a decline in the production potential. If production falls below a certain level, the economics of cultivation of the crop under consideration comes into question.

Potential gains from a breeding program should be realistically estimated. Gains from improving stress resistance may be offset by adverse correlated responses that are inevitable because of the physiological interconnections of plant growth processes. This can result in developing varieties that are salinity resistant and suitable only for saline soils but not for nonsaline soils, since their yield ability may be low and unable to compete with existing commercial varieties that can be grown in these nonsaline soils.

The various management options discussed earlier can also improve crop performance to a greater extent than may be realized through breeding. The physiological requirements for a given crop to perform under saline conditions should be evaluated. Careful assessment of energetic and assimilatory requirements for growth under various degrees of stress can reveal whether it is physiologically feasible to expect an improvement in production in stress environments. Ideally, fundamental growth processes should be well enough understood that crop growth can be modeled at various degrees of salinity stress. The results of such modeling exercises could provide guidance about the extent of physiological improvement required for the known or anticipated level of salinity [89].

The following aspects should be considered in initiating a program for the genetic improvement of salinity tolerance in a given crop:

1. Define the target environment.
2. Define the level of improvement necessary.
3. Define the growth stage response.
4. Choose the screening methodology to be adopted.
5. Choose the selection criteria.
6. Assess the genotypic variation for the various traits under consideration that may have a functional role in improving salinity tolerance.
7. Identify genetic sources for the various components (traits) of salinity tolerance.
8. Determine the genetic basis for traits under consideration and estimate their heritability.
9. Initiate breeding programs that combine various traits from different sources into a locally adapted variety or genotype for the ultimate development of a salt-tolerant variety.
10. Test evolved genotypes in multiple locations, in a range of saline soils within a production environment, to assess their potential adaptability as new varieties.

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Strategies for Genetic Improvement

Define the Target Environment

This is one of the most crucial requirements for the success of a genetic improvement program: It is unrealistic to attempt to develop a single variety that can be grown universally in all types of saline soils. The type of salinity (i.e., salt composition) in the target environment and the anticipated salt dynamics during the growing season should be assessed. This should help in designing genetic improvement programs specifically aimed at developing varieties that best fit given target environments. Laboratory or greenhouse studies should reflect the specific ion toxicities (and proportions) in the area where the crop is intended to grow. Even in a specific environment, the concentration of soluble salts changes depending on the soil structure and composition and its equilibrium with a variable moisture content. The amount of salt carried by irrigation water also may vary throughout the growing season. Such changes must be monitored and taken into consideration when developing appropriate breeding strategies to alleviate salinity problems.

Screening and Selection

Once a target environment is well defined, appropriate screening methodologies should be adopted to test the available germplasm for genetic variability in the salinity response. Analyses of variability are needed to establish that genetic variability exists and that it can be utilized in breeding. This requires formal studies on the heritability of the stress response and related physiological and morphological characteristics.

Varietal testing for salt tolerance often reveals only small differences among the limited numbers of varieties examined, such as lettuce [90], muskmelon [88,91], and grapevine [92]. A greater variation for salt tolerance is more likely to occur among species of halophytic origin, such as sugar beet [93]. Based on germination and early seedling growth in barley with 75% seawater, large differences among genotypes were reported [94]. Systematic large-scale screening of available gene pools of wheat and barley using hydroponic systems has been attempted with the specific aim of selecting genotypes suitable for seawater culture [80,95,96]. Nearly 7200 barley genetic lines synthesized from a composite cross (involving a number of lines) were evaluated [97]. Of these, only 22 lines were able to survive, grow, and complete the life cycle by setting seed at 75–90% seawater salinity in a hydroponic system. Tolerance here refers to the ability to germinate, establish seedlings, grow, flower, and set seed at 75–90% seawater supplied throughout the life cycle of the plant [80]. Further, these lines were evaluated under field conditions for their yield ability by irrigating with undiluted seawater. Some of these lines could yield up to 1.58 t ha^{-1}. This shows the feasibility of this approach in developing barley lines or varieties that can be grown with seawater-based irrigation [80]. Therefore, the basic concept of irrigating barley with seawater is at least a ''biological success,'' and the selection approach based on tolerance throughout the life cycle appears to be feasible in identifying lines capable of producing under saline conditions. Similar attempts have been made in rice [98].

There is scope for the selection and development of rice varieties that are high yielding under saline conditions. The IRRI (International Rice Research Institute) has developed a number of salt-tolerant varieties, such as IR 50. This was reported to yield an average of 3.0 t ha^{-1} in multilocational yield evaluation trials in saline fields, where the traditional high-yielding varieties could not survive [99]. It also was demonstrated that, using cumulative crosses involving a number of tolerant cultivars, one could develop varieties with higher levels of tolerance than their parents. Crosses using two of the IRRI most salt-tolerant cultivars have demonstrated overdominance for salt tolerance in F_1, and many progeny lines of F_3 are far more tolerant than either of the parents [100].

Screening plants from germination to maturity using large-scale solution culture systems is the best option for identifying genotypes or genetic materials that are tolerant to salinity at all growth stages. If different genotypes respond differently at different growth stages, however, this suggests that salt tolerance is under separate genetic control at each of the developmental stages. If this is

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so for the crop under improvement, then genetic sources may need to be identified that possess higher levels of tolerance for each of the growth stages, with the assumption that tolerance at each growth stage could be an independent attribute. Jones and Qualset [89] proposed that by reducing tolerance to similar developmental units, the genetic components of this tolerance potentially also will be simpler. Analysis may therefore be facilitated by reducing the number of segregating loci in crosses, thereby simplifying genetic segregation ratios and identifying the underlying physiological basis of adaptation. It might then be possible to integrate differential tolerances at specific stages into a single highly tolerant cultivar with a high-yield potential.

Role of Wild Relatives

Wild relatives of plants have been used as sources of disease, insect, and nematode resistance, to widen adaptation, to provide alternative cytoplasms and develop cytoplasmic sterility systems, to improve quality, to alter modes of reproduction, to induce short stature, to increase crossability between species, to improve resistance to stress, and to increase yield [101]. The use of wild relatives in crop improvement accelerated after systematic efforts by the CGIAR (Consultative Group for International Agricultural Research) centers to collect, maintain, and make this material available to researchers. Many breeders are reluctant to use wild germplasm in their breeding programs, however, because it takes a long time and much backcrossing to remove the undesirable traits that are linked with the desirable traits.

Several studies have shown that, in many crops, wild relatives can offer higher levels of tolerance to salinity that can be transferred to cultivated crops through breeding. In the tomato, the lack of variation in the cultivated germplasm prompted Epstein and his colleagues to test various wild relatives of tomato [102–104]. *Lycopersicon cheesmani*, a wild tomato collected from the Galapagos Islands, was found to be highly salt tolerant and could survive and produce with 50% seawater, a saline level toxic to the cultivated tomato. Further studies with interspecific hybrids of cultivated tomato demonstrated that the higher level of tolerance is a dominant genetic factor. Recurrent selection for salt tolerance of the hybrids resulting from backcrosses to a domestic cultivar gave plants that survived in up to 70% of the concentration of seawater. Fruit size, quality, and yield increased with successive backcrossing.

In barley, preliminary studies with a limited number of accessions of *Hordeum spontaneum*, an immediate progenitor to cultivated barley and the only wild relative in the primary gene pool, did not show any additional sources of tolerance compared with cultivated barley (G. V. Subbarao and S. Jana, unpublished results). However, a large number of collections are available in this species that could offer higher levels of tolerance than cultivated barley. Other species of *Hordeum*, such as *H. jubatum* and *H. marinum*, have substantially higher levels of tolerance to salinity than that available in cultivated barley [105]. Utilization of this tolerance depends on the development of techniques to overcome incompatibility barriers.

Several wild species related to wheat have shown substantially higher levels of salinity tolerance than cultivated wheat [106]. *Elytrigia elongata*, a wild wheatgrass, had a higher salinity tolerance than cultivated wheat (*Triticum aestivum*). The salinity tolerance trait was expressed in the amphidiploids of *T. aestivum* × *E. elongata*, indicating that the tolerance trait is a dominant genetic factor [107]. By transferring five chromosomes and a telosome from *E. elongata* to *T. aestivum* in the BC_2F_4 derivative, it was found that the tolerance trait was expressed in these derivatives. These derivatives grew to maturity even at 35 dS m^{-1} salinity, similar to the tolerant parent *E. elongata* [108]. *Oryza coarctata*, a wild rice species, tolerates salinity up to 40 dS m^{-1} [109]. The cultivated rice (*O. sativa*) could tolerate only 5 dS m^{-1}. Some pigeon pea wild relatives were found to have higher levels of tolerance to salinity than the cultivated pigeon pea [110].

FUTURE OUTLOOK

The salinization of soil and water is becoming an increasingly serious constraint for crop production, particularly in the arid and semiarid regions of the world. These areas are under immense pressure

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to produce more food per unit area of land because of ever-increasing human populations and expectations of economic improvement. Increasing areas of land in arid and semiarid regions are being brought into production through the introduction of canal irrigation, without taking into account the salt balance of these production systems or providing suitable drainage [111]. Secondary salinization, which is usually associated with irrigated agriculture, is becoming a serious problem in many areas of the world, threatening the long-term sustainability of these production systems. Nearly 1.5 million ha of prime farmland in the world is going out of crop production each year because of secondary salinization [112]. The long-term survival of present agricultural production systems based largely on irrigation depends on tackling salinity problems in a much more integrated manner. This is suggested to be through a proper balance between the management approach in containing further salinity build-up in these soils, coupled with the biological option of genetic improvement in salinity tolerance.

The biological option, apart from contributing to the survival of present production systems, also opens the way for the novel concept of using seawater irrigation for food production along coastlines at present lying idle for lack of crops that can be grown in these regions. Early attempts by Epstein and his colleagues with barley demonstrated the feasibility of this approach. However, much more needs to be done to realize this dream. Not all barley germplasm collections have been systematically evaluated for their potential to grow with seawater irrigation. Apart from this, wild relatives of barley have not been thoroughly explored for their potential to contribute to the genetic improvement in salinity tolerance. The *Hordeum* species, such as *H. spontaneum, H. jubatum*, and *H. marinum*, could provide the necessary ''genetic means'' to develop barley cultivars that could be grown with seawater to give reasonable yield levels. We hope that future efforts will be directed toward realizing this goal.

Improving salinity tolerance in many crops whose production systems are being threatened by secondary salinization is of immediate importance to the continuation of these crops in their present production environments. ''Genetic support'' should be recruited from wild species should sufficient variation not be found among cultivated germplasm collections.

Traditional breeding approaches can be used for genetic improvement in salinity tolerance in a target crop species, and these may have a higher level of success if integrated with physiological research. Biotechnological approaches, such as using somaclonal variation in tissue culture for generating salt-tolerant cell lines and, finally, plants, have been projected to have much promise [113]. Consistently, however, no salt-tolerant plants have been regenerated from these so-called salt-tolerant cell lines [114]. Salt tolerance is much more of a whole-plant phenomenon. It depends on a number of physiological processes that need to coordinate at the whole-plant level to provide the necessary stable ionic environment in the cytoplasm and the required osmotic adjustment for the turgor-driven water uptake under saline conditions. It is thus not surprising that plants regenerated from the tolerant cell lines have not shown the same level of tolerance as the original cell lines [114].

However, other aspects of biotechnology show promise for use in the genetic enhancement of salinity tolerance. For example, RFLP (restriction fragment-length polymorphism) or RAPD (random amplified polymorphic DNA) markers could be used for tagging the physiological components of salinity tolerance. These methodologies could be effectively integrated into breeding programs for the genetic improvement in salinity tolerance in crop plants [115].

More concerted attempts should be made to integrate physiological research in plant salinity tolerance with genetic aspects so that a combined physiological-genetic approach may be realized. We hope that wild relatives of crops will play a more prominent role than hitherto in the development of salt-tolerant crop varieties.

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Plant and Crop Response to Trends in Climatic Changes

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INTRODUCTION

The scientific community is considerably concerned with the increasing CO_2 concentration [CO_2] in the atmosphere. There is clear evidence that the rise is linked to industrial emissions and is strongly correlated with the rapid increase in the global consumption of fossil fuels. Deforestration is considerably concerned with the increasing CO_2 concentration [CO_2] in the atmosphere. The level of atmospheric CO_2 in Europe has increased from about 280 μmol CO_2 mol^{-1} before the industrial era to 358 μmol CO_2 mol^{-1} in 1995 [1]. This trend is expected to continue and result in an increase to over 700 μmol mol^{-1} by the end of the next century if no steps are taken to limit emissions [2]. In parallel with the recent trend of rising atmospheric [CO_2], ozone (O_3) also has increased and is regarded as being one of the most phytotoxic of the air pollutants commonly encountered in the developed countries of the Northern Hemisphere [3]. Owing to industrial emissions of nitrogen oxides and volatile organic compounds, background concentrations of tropospheric O_3 have roughly doubled during the last century from the rural mean concentration of less than 20 nmol mol^{-1}. The current trend of the increase is about 1–2% per year [4]. Owing to their ability to trap terrestrial radiation and so warm the atmosphere, CO_2, O_3, and other radiatively active gases—methane, nitrous oxide, chlorofluorocarbons—contribute to the ''greenhouse'' effect which is predicted to affect the global pattern of temperature and precipitation and have direct impacts on plant physiology and crop production [5,6]. The increase in the global mean temperature is likely to be of the order of 1.5–4.2°C. It is generally considered that the increase in temperature will be greatest at high latitudes and minimal at low latitudes [7]. Although models suggest that increasing [CO_2] may result in a

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slight increase in the global mean precipitation, large uncertainties exist on the regional scale due in part to the large spatial and temporal variability inherent in precipitation events and their intensity. Some regions might receive increased precipitation, whereas others might receive less. However, these projected changes in climate are uncertain [8,9]. The resulting precipitation shifts could have considerable agricultural impacts, especially in regions that become drier [10].

Plants respond to elevated levels of atmospheric CO_2 in a wide variety of ways [11]. Many aspects of the plant life cycle depend on the importance of the photosynthetic response to elevated [CO_2], with notable examples being growth and yield, structure and anatomy, photosynthetic and respiratory gas exchange, and C and N assimilation [12]. Among other factors for plant growth to be sustained by photoassimilate, turgor must be maintained [13]. In addition to enhancing photosynthesis and growth, elevated [CO_2] also affects stomatal conductance which in turn affects transpirational water loss and water-use efficiency [14]. The various aspects of the effects of elevated [CO_2] on plant water relations include gas exchange, morphology, and stomatal and internal water stress regulation. Accordingly, [CO_2] and water vapor pressure will have direct and possibly interactive effects on leaf expansion and biomass accumulation by virtue of their combined impact on transpiration and assimilation.

Multidisciplinary studies are necessary to understand the impact of changes in the atmosphere on plant life. A large number of papers, reports, and reviews have been devoted to the impact of elevated [CO_2] on several aspects of plant physiology, biochemistry, and photosynthetic carbon metabolism. Moreover, various studies have recently been initiated to investigate the effects of high [CO_2] on the regulation of photosynthesis-associated genes, PAGs [15], with the aim to develop a comprehensive understanding of the mechanism of the plant response to changes in [CO_2]. Some other studies were concerned with elevated O_3, or high temperature, or drought. The objective of this chapter is to gather the current knowledge on the different aspects of the effects of the expected climatic changes which plants will very likely have to face during the course of the next century.

This chapter describes the action and interaction of elevated [CO_2], [O_3], temperature, and drought on some aspects of plant physiology; mainly carbon and water acquisition and use. It deals with major crop plants (e.g., wheat, rice tomato, tobacco) and also herbaceous plants which are components of the grazing ground. In addition, because of the standing biomass they represent, their significant role in the global carbon balance and their economical importance, trees also are mentioned.

EFFECTS OF ELEVATED CONCENTRATIONS OF ATMOSPHERIC CO_2 ON PLANT PHOTOSYNTHESIS AND RELATED PHYSIOLOGICAL PROCESSES

Experimental Approaches

Field conditions are variable in time and space. Interactions with other environmental factors occur. Species can differ in their response to CO_2 concentration [16] and strong year-to-year variations in weather are found [17]. To study the responses to elevated [CO_2], experimental plants have been exposed to enriched [CO_2] in different ways. Initially, phytotronic chambers were used; subsequently, in order to reproduce field conditions as closely as possible, open-top chambers (OTCs) were used in the field [18], and more recently an open-field method was developed called Free air CO_2 enrichment (FACE) [19]. In OTCs, a ''chamber effect,'' mainly due to an increase in temperature compared with the real field conditions, was often observed. Thus, an ''absolute'' growth effect could not be determined with high confidence with this technique [20]. The FACE approach is preferable, because both absolute and relative responses are reliable. However, it must be mentioned that, because the FACE high technology is very costly, OTCs are still very widely used. The recent addition of a temperature-control system [21] to the OTC technique will probably remove the major flaw limiting its usefulness for global change research.

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Growth Response

Carbon dioxide is the substrate for photosynthesis for all terrestrial higher plants. It was formerly assumed that photosynthesis was usually limited by other environmental variables such as temperature, water, and nutrient availability, so that plants did not respond to increased atmospheric CO_2; however, it is now well recognized that increased atmospheric CO_2 concentrations, $[CO_2]$, above normal levels increase the photosynthetic rate of plants and therefore potentially their growth.

CO_2 Fixation and Productivity

Among the wide range of C_3 species that have been examined, including virtually all crop and forest species of northern latitudes, the photosynthesis of some 95% is not saturated by the present $[CO_2]$. C_3 plants require 800–1000 μmol mol^{-1} CO_2 for saturation of photosynthesis. Almost all show significant increases in photosynthesis and dry matter production in response to an increase in $[CO_2]$ of between 500 and 1000 μmol mol^{-1} [22,23]. A doubling of $[CO_2]$ from 330 to 660 μmol mol^{-1} increases the productivity of crops and C_3 plants by 33–41% [24–26].

Although the net photosynthesis assimilation increases by an average of 50% in the short term, the plant weight increases by only 40% over a long period [27]. Such stimulation is modest compared with what might be expected on the basis of short-term increases in carbon fixation at high $[CO_2]$. Also, there is much evidence that the initial CO_2 stimulation of photosynthesis is not maintained and that downregulation of photosynthesis caused by acclimation occurs after prolonged exposure to high CO_2 concentration [6,23].

Because C_4 and crassulacean acid metabolism (CAM) species use phosphoenolpyruvate carboxylase (PEPCase) for their initial fixation of CO_2 and PEPCase can be saturated at current atmospheric $[CO_2]$, a little influence of elevated $[CO_2]$might be expected for these plants. Indeed, elevated CO_2 concentrations have only a small effect on the net CO_2 uptake by C_4 plants [6,28] The effects of CO_2 enrichment and irradiance on the growth and gas exchange of the two tropical grasses *Panicum laxum* (C_3) and *Panicum antidotale* (C_4) were compared. Elevated CO_2 enhanced the plant dry weight at low and high irradiances in the C_3 species but only at high light in the C_4 species.

Elevated atmospheric $[CO_2]$ has varied effects on the net CO_2 uptake and productivity of CAM plants. Some are not affected, but a doubling of CO_2 concentration stimulated growth in two highly productive CAM species, *Agave salmiana* Otto ex Salm. var. *salmiana* and the widely cultivated prickly pear cactus *Opuntia ficus-indica* (L.) Miller [29]. In *Agave salmiana* grown for 4.5 months in open-top chambers, 55% more unfolded leaves and 52% more fresh weight mass was produced at 730 than at 370 μmol CO_2 mol^{-1}.

Temporal and Space Factors

$[CO_2]$ experiments are mostly done in controlled environments. However, there are substantial variations in the absolute values of environmental parameters and in their variability and coupling. Field conditions are variable in time and space, and strong year-to-year variations are observed [16]. Species can differ in their response to CO_2 concentration. This makes necessary the confirmation that the responses to $[CO_2]$ observed in experiments apply in agricultural or ecological situations.

The effect of elevated $[CO_2]$ on the productivity of spring wheat, winter wheat, and faba beans was studied in experiments in temperature-regulated crop enclosures [11]. At an external $[CO_2]$ of 700 μmol mol^{-1}, the maximum canopy CO_2 exchange rate (CERmax) was stimulated by 51% for spring wheat and by 71% for faba bean. At the end of the growing season, the aboveground biomass increase was 35% for spring wheat and 58% for faba bean, whereas the harvest index did not change. This differential effect in the CO_2 response was shown to be at least partly due to differences in the daily air temperatures during comparable stages of growth of these crops. Simulations also showed that variations between years in the CO_2 response can be largely explained by differences in weather conditions between growing seasons.

Results for well-fertilized and irrigated *Lolium perenne* swards grown at elevated (700 μmol

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mol^{-1}) and ambient atmospheric CO_2 in open-top chambers in Ireland for a 3-year period showed marked seasonal variations in the aboveground stimulation in yield (Fig. 1) [22]. The largest increases in yield were recorded in the early and late growing season, with an overall stimulation in yield for the elevated CO_2 treatments of about 20 (1992), 28 (1993), and 42% (1994).

The effect of CO_2 enrichment on yield is dependent on the stage of growth at which it is applied, as reviewed by Lawlor and Mitchell [30]. Enrichment from emergence to anthesis or from anthesis to early pod development did not increase soybean yield, whereas enrichment from early pod development until maturity and from emergence to maturity increased yields by 27 and 36%, respectively.

Biomass Allocation

The pattern of biomass allocation is a key factor determining a plant's potential for early vegetative growth and yield [31]. Stulen and den Hertog [32] provided a detailed review of the effects of elevated [CO_2] on biomass partitioning in vegetative plants. Rogers et al. [33] also reviewed the subject, focusing on the effect of elevated [CO_2] on the root/shoot ratio of the crop plant.

Plants growing under CO_2 enrichment develop differently from plants growing under ambient CO_2 levels. In cucumber, longer internodes and more male flowers are found than under ambient [CO_2] conditions [34]. It is not clear whether these developmental changes are directly due to an enriched supply of photoassimilates or an altered hormonal signal reflecting changes in the forms and amounts of carbohydrates and amino acids in the transport system.

Increases in yield due to CO_2 enrichment are often more due to an increase in the number of harvested structures rather than to an increase in their mean size [30]. In soybean, yields increased almost entirely as a result of greater numbers of seeds, and this in turn was due to an increased number of pods rather than the number of seeds per pod, which decreased [35]. Similarly for bean, rice, wheat, and sweet potato, increases in yield are entirely the result of more pods, grains, or tubers. Presumably the availability of energy and substrates determines how meristematic tissue develops. The relative insensitivity of seed or tuber size to additional carbohydrates during filling suggests that there is a genetically determined upper limit [30].

A stimulation of root system development has often been found; in a review of the literature, Rogers et al. [36] reported that 87% of the species studied showed a root dry weight increase with elevated [CO_2]. Roots often exhibit the greatest relative dry weight increase among plant organs under these conditions. The most striking effect concerns the root architecture. CO_2 enrichment

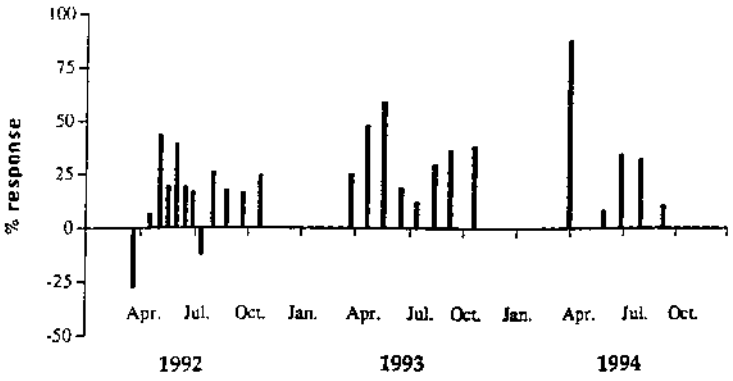

FIGURE 1 The seasonal change in yield stimulation over a 3-year period for *Lolium perenne* swards grown in Dublin, Ireland, in open-top chambers at elevated (700 μmol mol^{-1}) CO_2 compared with an ambient CO_2 treatment. (From Ref. 22.)

appears to increase the length of tap roots and the number of laterals [37]. A study using cotton in the FACE system [38] led to the conclusion that the spread of cotton roots through the soil profile is faster and more prolific under elevated [CO_2]. A change in root architecture and development also was highlighted in the study of Berntson and Woodward [39]. The stimulation of root system development is very likely to be associated with changes in the rhizosphere microbiology which will alternatively act on soil formation processes [40].

No clear trend has been observed in controlled-environment studies on the effect of CO_2 enrichment on the distribution of dry matter between plant organs, which allowed for a greater proportion of plant dry weight at high CO_2. Elevated [CO_2] was found to cause a 24% increase in spring wheat total biomass, with a 25% decline in root mass, and a 29% increase in grain mass [4]. Although earlier papers have demonstrated that [CO_2] favors investment of the biomass in roots relative to that in leaves [41], it has become clear that these are indirect effects due to the more rapid depletion of nutrients in the root environment as a consequence of enhanced growth [32,42]. If the nutrient supply is maintained at an optimum level, there is no effect on the fraction of the biomass allocated to the root. However, if nutrients cannot be absorbed in proportion to the enhanced growth, then CO_2-enriched plants show an increased allocation to roots at the expense of that to leaves [43]. This is the normal plant response found at a suboptimal nutrient supply [44]. The question remains controversial because of the highly variable experimental factors interacting with CO_2.

Controlled-environment studies have shown that CO_2-induced increases in leaf area are largely due to more extensive branching in dicotyledonous plants [36] and tillering in grasses [45], although there also is an increase in expansion rates and a small increase in maximum leaf size [25]. In some species or conditions, elevated [CO_2] produces substantial carbohydrate accumulation within the leaves. The leaf morphology can be changed [46]; massive starch granules can distort chloroplasts [47] and possibly disrupt function by distending the thylakoid membranes and imposing constraints on the diffusion of gases or metabolites.

The leaf mass per unit area (LMA) has been found to increase in response to elevated [CO_2] in soybean but not in maize [30]. The general increase in LMA is presumably mostly due to the increase in starch content [48], but the leaves of bean plants grown at high [CO_2] also are thicker owing to an increase in the number of palisade cells [49], an effect which again does not occur in maize.

Mechanisms which might explain the effect of environmental variables on the pattern of biomass allocation have been discussed by Lambers et al. [44]. Although greater allocation of biomass to leaf area (high leaf area ratio, LAR) has a positive effect on a plant's potential relative growth rate (RGR), Van den Boogaard et al. [50] and Veneklaas and van den Boogaard [51] found no significant correlation between the RGR and LAR in a comparison of wheat cultivars grown well-spaced in soil. The lack of a correlation between the RGR and LAR is due to the negative correlation of the LAR with the net assimilation rate (NAR), which counteracts the positive effect of the LAR.

Chemical Composition of Plants

Several field studies have addressed the question of whether CO_2 enrichment affects the composition of plants [33,52]. No change was found in the N content per unit dry matter of leaves, stems, or grain due to CO_2 enrichment in winter wheat [53]. Crop products consumed by humans have generally not revealed any difference in quality in elevated [CO_2]. However, a recent paper by Blumenthal et al. [54] indicated a loss of protein in wheat grain and poorer quality of bread.

In leaves, the bulk of the dry weight increase is due to massive starch accumulation [34]. High atmospheric CO_2 levels also enhance the synthesis of transport sugars, primarily sucrose [55], or the raffinose series of sugars, such as stachyose, in some species like cucumber [48]. At first glance, sugar synthesis and export seem to compete with starch synthesis and storage, but the two processes are merely components of a general strategy, plants having evolved mechanisms for fixing maximal amounts of CO_2 when optimal environmental conditions for leaf photosynthesis prevail.

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Elevated CO_2 was found to increase the carbohydrate status (starch, sucrose, and glucose) of soybean under field conditions [35]. Similarly, wheat plants doubled their amounts of sucrose and starch accumulated when grown in high [CO_2], and sweet potato showed increased starch in the storage tuber and leaves [56]. Huber et al. [48] concluded from the above data that most of the additional net photosynthesis was accumulated as starch, as there was little increase in sucrose phosphate synthase or in carbon export.

CO_2-induced changes in chemical composition have been investigated in the leaves of 27 C_3 species, including those of crop plants and fast- and slow-growing wild herbaceous plants, as well as tree seedings [27]. There were two constituents on which CO_2 had a major effect. The most important effect was the increase in the concentration of total nonstructural carbohydrates (TNC) from 137 to 211 mg g^{-1} dry weight at ambient and doubled [CO_2], respectively, with considerable variation in the response of different species, ranging from almost zero to over 100%. The increase also was evident when results were expressed in terms of relative total nonstructural free carbohydrate (Fig. 2). This increase may indicate that at elevated [CO_2] carbon is not the limiting factor. The capacity for accumulation of soluble carbohydrates depends on the leaf development stage [15].

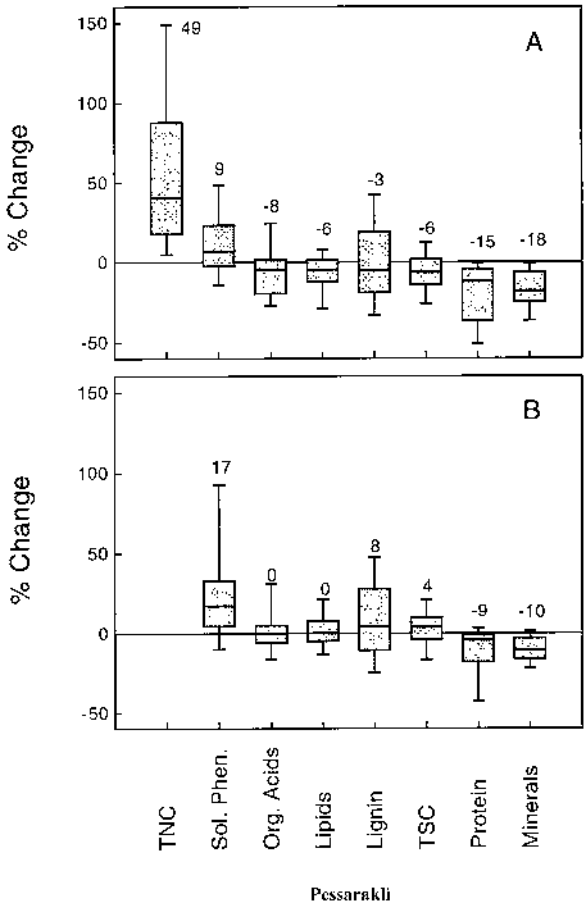

FIGURE 2 Proportional changes in leaf chemical composition of 70 Pa CO_2-grown plants relative to values for plants grown at 35 Pa CO_2 (A) Values on a total dry weight basis; (B) values on a TNC-free basis. (From Ref. 27.)

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There was no significant difference in the glucose and fructose content in the plants grown at high CO_2 at 60% leaf expansion. At 95% leaf expansion, hexose and sucrose levels significantly increased in response to exposure to elevated CO_2. Starch levels of the control plants remained unchanged from 2 to 60% leaf expansion and then increased up to the 95% expansion stage and declined by maturity. Overall starch significantly accumulated in the plants grown at high [CO_2], especially in the older leaves (Fig. 2). The second largest change induced by high [CO_2] was a decrease in the average protein concentration from 270 to 219 mg g^{-1} dry weight for ambient and doubled [CO_2] [27]. Since the latter was largely a consequence of the decrease in N, the C/N ratio showed increases of up to 80% in some species (Fig. 2).

A differential effect of high [CO_2] on tomato chloroplast proteins has been observed by Van Oosten and Besford [15]. No effects of elevated [CO_2] on the levels of various proteins associated with thylakoïds (D1 and D2 of the photosystem II [PSII] core complex, cytf, and PS I core protein) were detected up to 22 days. However, in the fully expanded and mature leaves, thylakoïd protein levels were observed to decrease as the growth CO_2 level increased. However, major soluble polypeptides (large subunit and small subunit of ribulose bisphosphate carboxylase oxygenase (Rubisco) and Rubisco activase protein) encoded by chloroplast or nuclear genes declined under elevated [CO_2] earlier in the leaf's development than was the case for the thylakoïd proteins derived from chloroplast genes. Thus, prolonged exposure to increasing doses of CO_2 changes the stoichiometry between thylakoïd and soluble photosynthetic proteins. Chlorophyll was found to decline in parallel with the thylakoid proteins only in fully mature leaves.

The only other class of compounds for which the average (negative) change was larger than 10 mg m^{-1} was the minerals [57].

With respect to C_4 plants, it must be borne in mind that no effect of CO_2 enrichment on the element composition nor on the moisture, fiber, oil, protein, or fatty acid composition of maize plants was found [52].

As a consequence of the chemical changes plants have to withstand, plant-insect-herbivore interactions must be given careful consideration, because their consumption, growth, and fitness may be affected by the lower quality of plants grown under high [CO_2] [58].

Respiration

A topic that has raised discussion in the literature is the effect of CO_2 on respiration. Many reviews have been published on this important subject [59–63]. Direct (or short-term) and indirect effects of elevated [CO_2] have been found, although their significance is not yet fully understood.

The short-term effect is characterized by an immediate reduction in the apparent respiration rate. This is observed in any organ and any kind of tissue of C_3, C_4, or CAM plants [63]. Dark CO_2 fixation through PEP carboxylase activity has been invoked in some cases as an explanation [63], and the most recent hypothesis describes a mechanism involving the direct inhibition of enzymes involved in mitochondrial electron transport [12]: Elevated [CO_2] has been shown to reduce the activity of cytochrome oxidase and succinate dehydrogenase in isolated mitochondria [64]. The long-term response to elevated [CO_2] is mainly of a biochemical nature.

The observed decline in the respiration rate is correlated with both an accumulation of total nonstructural carbohydrates (TNC) and a reduction in protein. The accumulation of TNC has a direct effect on growth respiration, as compounds like starch can be formed with little CO_2 production. The reduction in protein has an effect, as protein synthesis is accompanied by a large CO_2 production, whereas protein maintenance is less costly.

The ''construction cost'' was defined by Penning de Vries et al. [65] as the amount of carbohydrate required to synthesis 1.0 g of plant dry mass (carbon skeletons and energy necessary for biosynthetic reactions). The equation quantifies the substrate and oxygen demand as well as carbon dioxide evolution. It accounts for all substrate molecules required for biosynthesis of the carbon skeletons and to provide the energy required for the nonsynthetic processes such as transport of molecules and maintenance of RNA and enzymes. Given that C concentration did not change,

whereas growth respiration decreased on average by 11%, Poorter et al. [27] calculated that the construction cost at high [CO_2] decreased by 3–4%. Their schematic representation of the most important changes in the chemical composition of plants grown at elevated [CO_2] and the consequences for leaf construction cost is shown in Figure 3. The reduction in protein concentration is more important than the increase in TNC in explaining the decreased growth respiration per unit biomass formed. However, it must be remembered that the growth stimulation resulting in larger plants often compensates for all of the above-described reduction in respiration per unit dry weight, and that the overall plant respiration is eventually mostly enhanced after long-term CO_2 enrichment.

Net CO_2 Uptake

The two essential steps of assimilation of CO_2 into organic photosynthetic products are the uptake or diffusion of the gas into the leaves followed by its fixation into a usable reduced form during photosynthesis.

Stomatal Response

Stomata in the epidermis of leaves are the main route if not the only one for CO_2 influx. Photosynthesis is increased in most species when stomata are open, a condition normally favored by high air humidity and low CO_2 partial pressures within the leaf. High CO_2 partial pressures within the leaf, such as occur during CO_2 enrichment, cause the stomata to close and conductance is strongly reduced [66,67]. In some tree and crop species, stomatal frequency was found to decrease as [CO_2] increased from preindustrial levels [68]. But the observed reduction in conductance is more likely to be due to the direct effects of [CO_2] on the stomatal aperture. At high CO_2 levels, the net result of stomatal

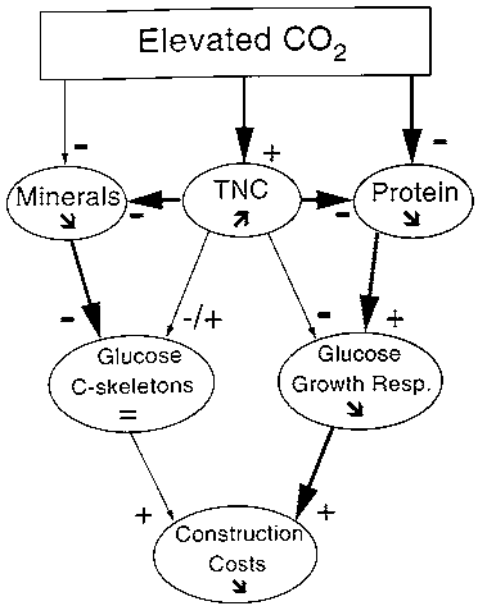

FIGURE 3 Schematic representation of the most important changes in chemical composition of plants at elevated CO_2 and the consequences for leaf construction costs and its components. Thick arrows represent strong effects and thin arrows smaller effects. + indicates a positive effect and − a negative one. The net result of all the effects on the various constituents and glucose costs is indicated by the up and down arrows in the boxes. (From Ref. 27.)

closure is a reduction of the transpiratory flux and therefore an improved water-use efficiency (WUE = dry matter gained/water loss). Nevertheless, with CO_2 enrichment, the gradient of $[CO_2]$ remains steep enough to maintain the net carbon gain.

Morison [69] showed that the stomatal conductances of C_3 and C_4 leaves respond similarly to elevated $[CO_2]$. The two tropical grasses *Panicum laxum and Panicum antidotal*, of the C_3 and C_4 carboxylation types, respectively, respond like most plants to CO_2 enrichment by a strong reduction (50%) in stomatal conductance [70]. The mechanism of stomatal regulation by $[CO_2]$ involves Ca^{2+} ions, cytokinine, and abscisic acid [66]. Since the work of Farquhar et al. [71], an empirical relationship which links the atmospheric CO_2 concentration to the intercellular CO_2 concentration has been used, which shows the interdependence between the assimilation rate and stomatal conductance.

The 30% yield increase reported for wheat and many other C_3 species grown under doubled $[CO_2]$ [24] results primarily from an increase in the net photosynthesis rate but also by the improvement of the whole-plant water-use efficiency resulting from this partial stomatal closure [72].

The increase in production of tall prairie grasses, belonging to the C_4 species, whose photosynthetic mechanisms were originally thought to be ''insensitive'' to increased $[CO_2]$, also has been attributed to this improvement in WUE [73,74].

Net Photosynthesis and Photorespiration

Our current knowledge of the mechanisms of photosynthesis leads us to interpret the effect of a $[CO_2]$ increase in terms of an increased carboxylase activity and a decrease in the oxygenase function of Rubisco. As a direct consequence of CO_2 enrichment, the oxygenase reaction of Rubisco and hence photorespiration is suppressed [75]. This is another direct consequence of CO_2 enrichment that affects the efficiency of the Calvin cycle, phosphate exchange, starch synthesis, and carbohydrate export within a typical leaf. Some temperate crops growing under low irradiance have been found to respond more to CO_2 than those growing at higher irradiance. This is thought to be due to the suppression of photorespiration [76].

When other processes limit photosynthesis, the response to elevated $[CO_2]$ will be smaller. In extreme cases where the rate of photosynthesis is limited by an end-product synthesis, rising $[CO_2]$ has no effect at all on photosynthesis [77].

Rubisco

Rubisco plays a leading role in the response to elevated $[CO_2]$ owing to the fact that this enzyme is both the primary regulatory site for CO_2 fixation and a major storage site of leaf N [78]. There are reports for a number of species with reduced Rubisco activity at elevated $[CO_2]$, as reviewed by Bowes et al. [23]. Rowland-Bamford et al. [79] analyzed the activity, amount, and activity ratio of Rubisco along with other leaf parameters in the C_3 plant rice grown under various $[CO_2]$ concentrations that ranged from that corresponding to the Ice Age to predicted values for the next century. Expressed on the basis of leaf area, Rubisco protein, or chlorophyll content (Table 1), total leaf Rubisco activity decreased with increasing $[CO_2]$. A decrease in Rubisco activity may be caused by a reduction in Rubisco protein concentration, which is consistent with the hypothesis that N is being reallocated. The Rubisco protein content did indeed decline linearly with increasing $[CO_2]$; dropping by as much as 60% (Table 1). Rubisco protein (RuBP) may still decline with seemingly adequate N supplies, possibly because the C/N ratio during growth is unbalanced. [23].

Since the publication of Farquhar's model of leaf photosynthesis [80], photosynthetic responses to CO_2 have needed to be evaluated in terms of potential limitations from RuBP regeneration and P ion (Pi) supply in addition to Rubisco and CO_2 [81].

The substrate RuBP is a potent inhibitor of the activation process. This phenomenon takes place via Rubisco activase, a regulatory enzyme found in the chloroplast stroma that catalyzes the carbamylation of Rubisco and modulates its activity. The substrates for Rubisco activase are the

Table 1 Rubisco Activity and Content in Rice Leaves Grown Under a Range of CO_2 Concentrations for 75 Days

$[CO_2]$ (μmol mol^{-1})	Rubisco protein content (% total soluble protein)	Total Rubisco activity	
		A	B
160	62	0.77	1177
250	59	0.75	1173
330	54	0.75	1097
500	49	0.76	948
660	43	0.68	738
900	42	0.65	693

A: μmol mg^{-1} enzyme min^{-1}; and B: μmol mg^{-1} chlorophyll h^{-1}.
Source: From Ref. 79.

RuBP-inactivated Rubisco complex and CO_2. Some species show a decrease in Rubisco activation as the CO_2 concentration for growth increases; this does not occur with the activation state of soybean or rice Rubisco, which is not affected by $[CO_2]$ enrichment [16].

An endogenous inhibitor of Rubisco, 2-carboxyarabinitol 1-phosphate (CA1P) [82], is synthesized in the dark. Measurement of Rubisco activities from dark-sampled leaves of rice and soybean indicated that CO_2 enrichment may enhance its synthesis in these two species [23]. The activity ratio (initial/total activity) was used to determine the proportion of the non–inhibitor-bound enzyme which was carbamylated and catalytically competent. The activity ratio declined by 24% in rice leaves grown at 900 μmol mol^{-1} $[CO_2]$ (see Table 1).

Tomato plants are a very well-characterized model system for the plant response to elevated $[CO_2]$ [15]. A regression analysis indicates a differential linear response to $[CO_2]$ at different times. There was little response at 60% leaf expansion, with almost no difference in Rubisco large subunit (LSU), small subunit (SSU), and Rubisco activase (rca) protein level. But there was increasing loss of Rubisco activity as the leaf expanded and was exposed to high levels of $[CO_2]$. At full expansion, the levels of LSU, SSU, and rca decreased as the CO_2 growth levels increased.

For their initial fixation of atmospheric CO_2, CAM plants use PEPCase which can be saturated at normal atmospheric $[CO_2]$. However, the Michaelis constant (HCO_3) for PEPCase was shown to be 15% lower for *Agave salmiana* and 44% lower for *Opuntia opuntia* when the CO_2 concentration was doubled [29]. Doubling the CO_2 concentration increased the daily net CO_2 uptake in another common CAM species *A. deserti*. The increase was up to 49% and lasted throughout the long-term experiment [22]. The substantial increase in the net CO_2 uptake and biomass production that occurs in these three CAM species when the ambient CO_2 concentration is doubled results mainly from higher inorganic carbon levels available to their carboxylating enzymes, a greater substrate affinity for PEPCase, and a greater percentage of Rubisco in the activated state. Also, in C_4 plants, the enzyme responsible for the initial fixation of CO_2 is PEPCase. The major differences between C_3 and C_4 species in response to CO_2 is the use by C_3 plants of Rubisco as the initial carboxylase, and its association with photorespiratory activity [83]. A number of important studies have allowed the observation of actual growth differences associated with C_3 and C_4 *Panicum* species. Growth of the C_3 *Panicum* was increased by CO_2 enrichment, whereas the C_4 *Panicum* was much less affected, as would be predicted from the enzymatic responses of Rubisco compared with PEPCase [84]. High CO_2 strongly reduced the stomatal conductance in both species, whereas it affected the Rubisco content of only the C_3 species exposed to high light [70].

Molecular Biology Studies

A few studies have been carried out on the effects of high [CO_2] on the regulation of photosynthesis-associated genes (PAGs). An upregulation of carbonic anhydrase gene expression was observed in *Arabidopsis* plants exposed to 700 μmol CO_2 mol^{-1} for 3 weeks compared with ambient-grown plants [85]. Studies using very high CO_2 concentrations demonstrated that nuclear PAGs were more sensitive than plastid PAGs in tomato plants exposed to elevated [CO_2] [86]. The model predicts that plants acclimated to high [CO_2] progressively accumulate sugars as a result of insufficient sink strength.

The relationship between aspects of molecular biology (PAG gene transcript accumulation), biochemistry (Rubisco activities, chloroplast protein composition, and sugar content) and photosynthetic gas exchange was investigated by Van Oosten and Besford [15], with respect to leaf development in tomato plants exposed to three CO_2 concentrations (350, 700, 1050 μmol CO_2 mol^{-1}). There was a significant decrease in *rbc*L transcripts (coding for LSU protein) and *psaA-B* transcripts (coding for the A_1 and A_2 proteins of the PSI core complex) throughout leaf expansion in all of the [CO_2] treatments. Exposure of the plants to elevated [CO_2] did not cause any further significant decreases in the *rbc*L and *psaA-B* transcript levels in leaves at any stage of leaf development (Table 2).

According to the ''molecular model'' [87], which takes into account the source-sink status of a plant and the metabolic regulation of nuclear PAG expression by hexoses, it should be possible to predict how and when protein, enzyme activities, and transcript levels of some of the PAGs should respond to elevated [CO_2]. Overall, the transcript levels of PAGs were strongly decreased after 60% leaf expansion. There was a coordinated pattern of expression of *rbcs* and *rca* transcripts and corresponding protein levels during leaf expansion and exposure to elevated CO_2 concentrations. Besford et al. [88] and Van Oosten et al. [89] have shown (using Rubisco kinetics, total activities, and the equation of von Caemmerer and Farquhar [90]) that there is a causal link between transcript levels and the reduction of the amount and activity of Rubisco. Although there were significant changes in the levels of mRNA of nuclear genes caused by the elevated CO_2 concentrations, the levels of nuclear rRNA transcripts were not affected, which indicates the specificity of the molecular effect of [CO_2] on nuclear gene expression. Finally, the model predicts that the chloroplast genes should be much less sensitive to elevated [CO_2]. Indeed, it is only in the mature leaves that some

TABLE 2 Levels of *rbc*L Transcripts (Coding for LSU Protein) and *psaA-B* Transcripts (Coding for A1 and A2 Proteins of the PSI Core Complex) of Tomato Plants Exposed to Different CO_2 Concentrations During Leaf Development

mRNA measured (SED)	Day(s) after exposure	Growth CO_2 concentration (μmol CO_2 mol^{-1})			
		350	700	1050	1400
*rbc*L (17–80)	0	320.4	—	—	—
	11	295.9	305.2	296.3	289.3
	22	175.2	181.5	174.4	160.9
	31	102.4	91.4	81.8	62.4
psaA-B (13–29)	0	214.2	—	—	—
	11	178.1	181.6	182.8	180.1
	22	62.0	66.3	58.6	66.0
	31	56.1	56.7	43.4	26.3

Data are from dot-blot analysis of total RNA in the fifth leaf of tomato plants exposed to 350, 700, 1050, and 1400 μmol CO_2 mol^{-1} for 11, 22, or 31 days and then hybridized with the appropriate ^{32}P-labeled probe. SED, standard errors of different means.
Source: From Ref. 15.

transcript levels of chloroplast genes are sensitive to high CO_2. When the levels of transcripts of chloroplast genes examined in this work are expressed on a *16S* rRNA basis (which is a marker of chloroplast number), no difference due to elevated [CO_2] was observed in the mature leaves. The mature leaves of plants grown in high [CO_2] might have lower chloroplast numbers, as the level of *16S* rRNA transcripts was lower than in the control plants. This might explain why the level of thylakoïd proteins associated with the PSI or PSII core complexes of cyt*f* and the level of soluble LSU protein were all lower in the mature leaves exposed to elevated [CO_2] than in the control plants. The low responsiveness of chloroplast genes to other environmental factors as compared with nuclear genes has been reported by several investigators [91,92].

Van Oosten et al. [87] found some evidence that the regulatory metabolites responsible for the effect of CO_2 on nuclear PAGs are the hexose glucose and fructose. Jang and Sheen [93] have recently demonstrated that plant hexokinases are involved in the sensing of the level of hexoses. A molecular model invokes the metabolic regulation of gene expression, with glucose providing a regulatory signal to repress the transcription of photosynthetic genes, including those encoding the small and large subunits of Rubisco [94]. This concept is consistent with findings for CO_2-enriched rice [23]. Although Rubisco concentration and activation were found to be downregulated, the activity of sucrose phosphate synthase was found to increase by about 20% at 600 vpm as compared with 330 vpm [CO_2]. A similar situation was found to occur in the sink-limited regions of leaves of transgenic tobacco overexpressing invertase in the cell walls [23].

Acclimation

In a wider perspective, we need to address the problem of photosynthesis rate acclimation: Is the increase in rate maintained throughout the life of the leaf or the plant? As an example, the light-saturated rate of photosynthesis, *A*, of tomato plants measured at growth CO_2 is significantly stimulated by elevated CO_2 concentrations [15]. However, the initial stimulation of *A* by high levels of CO_2 seen during the expansion phase of the leaf is lost at full expansion after 31 days of acclimation. When measured after full leaf expansion, *A* is actually negatively correlated to the CO_2 levels in which the plants were grown (Table 3) [88]. No significant difference was observed between the rate of nonphotosynthetic CO_2 evolution in the light for the plants grown at ambient CO_2 and for those fully acclimated to their elevated CO_2 growth concentration.

Acclimation can be defined as the physiological changes which occur when plants are grown in high [CO_2] [12]. The role of acclimation is presumably to optimize carbon acquisition and utilization [23]. In most instances, downregulation of CO_2 assimilation probably reflects a restricted capacity to handle the extra carbon, because other environmental resources are insufficient, or the plant has inherent metabolic limitations. According to this view, acclimation is an optimization process that reallocates resources from nonlimiting components, such as carbon acquisition, into limiting

TABLE 3 Light-saturated Rate of Photosynthesis, P_{max}, Measured at 350 μmol CO_2 mol^{-1} (1450 μmol m^{-2} s^{-1} PAR) of the Unshaded Fifth Leaf of Tomato Plants at Various Stages of Development and Exposure to Various Concentrations of CO_2

Leaf expansion/% (days exposure)	Growth CO_2 concentration (μmol CO_2 mol^{-1})			
	350	700	1050	1400
2% (0)	16.3	—	—	—
60% (10)	18.9	19.5	17.8	16.2
95% (21)	15.0	10.6	8.5	7.6
100% (30)	9.3	4.1	3.4	1.9

P_{max} is expressed in μmol CO_2 mol^{-2} s^{-1}.
Source: From Ref. 15.

components, such as electron transport and carbohydrate handling [95]. The availability of N would appear to be a primary factor, because CO_2 enrichment increases the C/N ratio of plants.

Acclimation of assimilation during extended periods of growth under conditions of elevated [CO_2] has been documented [96]. There is abundant evidence that, in the long term, the photosynthetic properties of leaves which developed at elevated [CO_2] differ from those which developed at normal [CO_2]. Growth in elevated [CO_2] commonly results in decreased photosynthesis relative to controls when measured at normal atmospheric [CO_2]. Arp [97] drew attention to the strong correlation between the rooting volume and the acclimation of the photosynthesis of plants in elevated [CO_2]. In a survey of 163 species, the stimulation of net photosynthesis was about 50% for large rooting volumes and field experiments but reduced by about half of this amount when the rooting volume was limited. The acclimation of photosynthesis involves various aspects of metabolism. It is accompanied by higher carbohydrate concentrations and lower concentrations of soluble proteins, especially Rubisco [12]. This is often attributed to the small rooting volume [97] or to a low nitrogen availability [42], as if the plants were compensating for the change in the rate of their photosynthetic activity by modifying their storage and allocation strategies. In some species or conditions, because the plant may be unable to use all the additional carbohydrate that photosynthesis in elevated [CO_2] can provide, substantial carbohydrate accumulation within the leaves is produced. The leaf morphology can be deformed; massive starch granules can distort chloroplasts and possibly disrupt function by distending the thylakoid membranes and imposing constraints on the diffusion of gases or metabolites [95].

Downregulation in photosynthesis may occur in the assimilation rate over the intercellular [CO_2] (A/Ci) curve, with changes in the initial slope and/or RuBP-limited region. The underlying causes of acclimation in the A/Ci curve are only partially resolved. Potentially it could be a stress response indicating physiological disfunction in plants adapted to low [CO_2]. Alternatively, it may be an optimization process as resources change. In the most recent review published on the effect of elevated [CO_2] on plant photosynthesis, Drake et al. [12] concluded that rising [CO_2] will lead to more efficient plants either in shade or in dense canopies.

There are many reports of a decrease in respiration in elevated [CO_2] [12]. It can be reduced to about 20% for a doubling of the atmospheric [CO_2]. Acclimation of the rate of respiration in wheat was shown to be correlated with reduced activity of enzymatic complexes of the mitochondrial electron transport chain (cytochrome oxidase and complex III) and resulted in a diminished capacity for tissue respiration [98].

For some species, acclimation of photosynthesis resulting from long exposure to elevated [CO_2] is concomitant with downregulation of the amount of Rubisco protein [79]. This coarse control of the amount of Rubisco protein probably serves to optimize CO_2 acquisition with utilization of the fixed carbon [99]. A survey by Drake et al. [12] shows an average reduction in the amount of Rubisco of 15% in eight studies including 11 species and a reduction in the Rubisco activity of about 24%. As Rubisco protein can constitute 25% of leaf N, these reductions affect a major component of the foliage tissue N (15–19%). In wheat grown with an adequate supply of N and water under elevated [CO_2], there was a significant loss of Rubisco followed by other photosynthetic proteins relative to controls at the completion of flag leaf development [100].

Regulation of the expression of photosynthetic genes may underlie acclimation to growth in elevated [CO_2]. Acclimation of photosynthesis to elevated [CO_2] has frequently been suggested to be more marked when N supply is limiting. At high N, the stimulation of net photosynthesis by elevated [CO_2] is about 50%, but this stimulation drops to about 25% when available N is low. Rubisco and Rubisco large subunit rbcL mRNA expression in *Pisum sativum* and *Triticum aestivum* were shown to be unaffected by growth in elevated [CO_2] when the N supply was abundant but showed marked decreases in response to elevated [CO_2] when N was deficient [101].

Interspecific and Intraspecific Differences

Poorter [26] has reviewed the response to elevated [CO_2] in 156 species. It was found evident that large differences exist between different functional types. We have already mentioned large differ-

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ences due to the photosynthetic pathway used by the plant, with C_3 species having a larger growth stimulation than C_4 species. This will alter the competitive strength for C_4 crops like corn, sugar cane, or sorghum, a possibly significant aspect in weed control. Differences between C_3 species are largely determined by their developmental strategies, with sink size being the major determinant of the maintenance of a higher photosynthetic rate and increased leaf area production having the opposite effect owing to mutual shading of leaves. Crop species usually respond stronger than wild growing species, a response which may be linked to mineral nutrition, which is generally controlled in many crops [102]. In this connection, it must be remembered that nitrogen-fixing species have generally higher growth stimulation than non–N_2 fixers, and that legumes will probably benefit the most from rising [CO_2] levels [103–105]. Woody crops seem to be less responsive than herbaceous crops. This has been attributed to the limitation of the net assimilation rate of tree leaves by higher internal resistance to [CO_2] transfer [106]. Recently, the occurence of intraspecific variations in the response to elevated [CO_2] has been described [107,108]. The conclusions are that intraspecific variations in CO_2 responses are often comparable or even greater than interspecific differences. Thus, in all cultivated crops, there is a genetic potential which could be selected in order to maximize the productivity as [CO_2] concentrations increase. However, additional work is required to identify the best cultivars in each species.

INTERACTIONS BETWEEN ELEVATED [CO_2] AND WATER

Many climate models predict that as atmospheric concentrations of CO_2 increase, the frequency of drought also will increase, and that associated with this higher [CO_2], water-use efficiency will rise owing to reduced stomatal conductance.

The relationship between CO_2 and water is complex, because water interacts at an instantaneous and microscopic scale in the mass flux of molecules in the stomatal pore during gas exchange; at a larger organ scale in the determination of the plant photosynthetic capacity by plant water status; and at the whole-plant scale because of the influence of the history of CO_2 fixation on the leaf area, plant stature, development stage, root mass, and distribution in the soil [109].

Elevated [CO_2] and Water-Use Efficiency

Elevated [CO_2] has been shown to have a direct effect on the plant transpiration rate through stomatal closure. For example, the average daily transpiration was reduced in sorghum (C_4 plant) and in soybean (C_3 plant) under elevated [CO_2] [110], but the transpiration reduction was higher for the C_3 than for the C_4 plant. In C_4 as well as C_3 grasses of the Kansas prairie [74], the relative stomatal closure in high [CO_2] induces as much as a 50% reduction in CO_2 leaf conductance. Moreover, the kinetics of stomatal responses to changes in light were more rapid in elevated [CO_2] [111]. The overall result was an improved water status for plants exposed to elevated [CO_2]. At the canopy level, evapotranspiration was reduced by 22%. This reduced water use under elevated [CO_2] extended the active period when water became limiting and was beneficial to the prairie during drier years. A similar result was found by Samarakoon and Gifford [112] on maize. In wet soil, the transpiration rate was reduced by 29% at high [CO_2]; when the soil was drying, plants in high [CO_2] used about 30% less water, and plant growth accumulated 35% more leaf area and 50% more dry matter owing to the fact that the soil had a greater water content in high [CO_2].

These results are amplified by the fact that elevated [CO_2] very often decreases the stomatal density [113]. Decreased stomatal density with high [CO_2] has been observed on longer experimental time periods of exposure (up to 12 months or several years) even at the historical scale [114] essentially on tree species; they concern differences in stomatal density [113] as well as the stomatal index [115]. But it must be mentioned that, in perennial rye grass, this effect has been shown to depend on temperature and to vary with the season and the anatomy of the leaf being considered [116].

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As the primary effects of elevated [CO_2] on C_3 plants result in an increase in the rate of assimilation and a decrease in leaf conductance for water vapor, a combination of these two effects often leads to an increase of the instantaneous water-use efficiency (CO_2 assimilation rate/transpiration rate) or the more long-term expression of it; that is, biomass accumulation over water consumption [109].

An increase in WUE may be either the result of a decreased leaf conductance and transpiration with no effect on photosynthesis (this is precisely the case of the majority of C_4 species tested) or, on the contrary, the result of a substantial increase in photosynthesis without any effect on leaf conductance and transpiration [105,117] or a combination of the two.

When plants are well watered, the increased yield is essentially due to the increase of photosynthesis. The result of a reduction in the stomatal aperture is a smaller water loss per unit leaf area and consequently an increase in the leaf water potential provided there is no change in the leaf area and root distribution. However, as stated in the above, this is very rarely the case: The improvement of the water status of the plant is often confounded with differences in the plant leaf area and the consequent evaporative demand and there is often a compensation between the increase in the leaf area brought about by elevated [CO_2] and stomatal control of transpiration [118,119]. In cotton, Samarakoon and Gifford [112] reported that, in wet soil, the approximately 15% reduction in transpiration per unit area owing to CO_2 was only half that for other species, whereas effects on growth and leaf area were relatively larger. Consequently, the rate of water use per plant was higher in elevated [CO_2] plants compared with other species where it was reduced. This greater water use caused the soil to dry faster in elevated CO_2. This contrasts with both maize and wheat species which conserve water at high [CO_2] when wet [120].

From the fact that instantaneous WUE is invariably enhanced in elevated [CO_2], it is often thought that elevated CO_2 will alleviate the impact of drought constraints in C_3 plants and increase drought tolerance [109,121,122]. However, this may not necessarily be the case [123,124]: Resistance to drought may also depend on a large number of factors affecting the water evaporative demand such as the leaf area already cited or the ability of stem and root systems to transport water [125].

The comparison of drought-avoiding or non–drought-avoiding species shows that this effect of elevated [CO_2] and drought is very species specific [118,126]. For example, in *Pinus pinaster*, a drought-avoiding species, the stomatal function was not affected but internal [CO_2] was increased. In contrast, in *Quercus petraea,* a drought-tolerant species, drought avoidance was increased in elevated [CO_2] as a result of stomatal closure. Despite these differential responses of stomata and photosynthesis to elevated [CO_2], intrinsic WUE was increased in both species [118]. In the species *Lotus corniculatus*, elevated [CO_2] ameliorates some of the effects of drought, including reproductive capacity, but reduces flowering time, with the final result of shortening the vegetative period and, in fact, the reproductive capacity [127].

Drought stress is a major environmental limitation for crop growth and is common in rainfed rice production systems. Baker et al. [128,129] showed that elevated [CO_2] significantly increased the P_N and WUE of rice, whereas reducing evapotranspiration by about 10%. This water savings allowed photosynthesis to continue 1 or 2 days longer during drought and should promote growth and yield in rice.

Elevated [CO_2] and Performance of Plants Under Drought

Before analysis of the global response of droughted plants to an increasing atmospheric CO_2 level, the effects of water stress and elevated [CO_2] on photosynthesis will be examined.

Resistance of the Photosynthesis Mechanisms to Drought

The net leaf CO_2 assimilation measured in normal air on many C_3 plants declines rapidly with increasing water deficit, being negligible at around 30% LWD [130,131]. Stomatal closure and leaf

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net CO_2 uptake decline in parallel during drought [132]. Data strongly suggest that stomatal control explains most of the observed decrease in leaf photosynthesis in plants subjected to mild drought. They also suggest that drought affects photosynthetic mechanisms through an effect on the relative water content. Demonstration of the drought effect on the nonstomatal component of leaf photosynthesis relies on our capacity to know whether the CO_2 concentration in the chloroplast remains high or not during a water shortage [132].

Obviously, the operation of the photosynthetic carbon reduction cycle, including ribulose 1,5-bisphosphate regeneration, is not impaired in spinach leaf disks subjected to a mild drought [133]. By analyzing the metabolite pool size on French bean maintained in normal air and subjected to a mild drought, Sharkey and Seemann [134] found no evidence for a lesion in the chloroplast biochemistry necessary for photosynthesis and came to the conclusion that Rubisco is not a prime target of water deficit.

The maximum apparent quantum yield measured at high CO_2 on several different plants does not vary much over a 40% range of leaf water deficit showing that whole-chain electron transport and related processes also are very resistant to dehydration [132]. As a whole, the photosynthetic apparatus of C_3 plants appears to be very resistant to desiccation with the maximum quantum yield and maximum capacity of photosynthesis decreasing significantly only when the leaf relative water content is reduced below 70% [135]. Tourneux and Peltier [136] demonstrated that the rate of photorespiration increases relative to that of net CO_2 uptake as the leaf net CO_2 assimilation decreases in a plant maintained on a drying soil.

It is clear that the photosynthetic apparatus of C_3 plants is very resistant to dehydration. It was shown that water deficit induces expression of particular genes, and this is associated with the adaptive responses of stressed plants [133]. Nevertheless, the photosynthetic apparatus is eventually damaged as dehydration of leaf tissue increases and, after rehydration, the photosynthetic activity of a leaf which has been severely dehydrated does not resume the rate it showed before the drought. The nature of the inhibition of the photosynthetic mechanism during severe desiccation is not yet well understood.

Less is known about the effect of drought on mechanisms of C_4 plant photosynthesis. In contrast to what is usually observed for C_3 photosynthesis [132], the ability of the mechanisms of C_4 photosynthesis to withstand dehydration depends on the speed of the desiccation.

Combined Effects of Elevated [CO_2] and Drought on Plant Photosynthesis

It was examined whether or not these responses of the photosynthesis mechanisms to drought were likely to be modified under elevated atmospheric CO_2 levels.

The mean variation of the maximum apparent quantum yield measured at high CO_2 molar fraction on different plants does not vary much over a 30% LWD range showing that whole-chain electron transport and related processes are very resistant to dehydration under high as well as under low [CO_2] [133].

Quick et al. [137] have shown that the ratio of 3-phosphoglyceric acid to triose phosphate was decreased and that the ratio of triose phosphate to ribulose 1,5-bisphosphate increased in osmotically shocked spinach leaf disks maintained at a high [CO_2] molar fraction in spinach leaf disks.

Using fluorescence techniques, it is possible to show that, in dehydrated bean leaves (LWD of about 30%), a [CO_2] molar fraction as high as 10–12% is necessary to inhibit the oxygenase function of Rubisco [133].

Global Response of Plants Exposed to Elevated [CO_2] and Drought

In light of the possible precipitation shifts caused by global warming [10] and the importance of rain-fed crop production to the world's food supply, determining the direct and possible interactive

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effects of CO_2 with water management cultural practices and drought stress becomes extremely important.

The atmospheric concentration of CO_2 and water vapor pressure will have interactive effects on leaf expansion and biomass accumulation by virtue of their combined impact on transpiration and assimilation [13].

There are at least two reasons why a rise in atmospheric CO_2 levels should allow better plant performance under drought or on soils with high impedance [138]. First, as the rate of carbon fixation increases, the amount of carbohydrates available for growth and maintenance of osmotic pressure should be greater. Second, lower stomatal conductance should allow maintenance of higher water potentials at a given soil water content.

The improvement of water relations occurs because of a fall in the stomatal conductance with increasing $[CO_2]$. This fall is often more pronounced for water-stressed plants than well-watered ones, which led Pearcy and Björkmans [83] to refer to the protective effects of high $[CO_2]$.

Under elevated $[CO_2]$, C_3 species usually use less water per plant even if the plants are bigger [139]. Under drought, the correspondingly smaller flow of water through the soil to the roots may reduce the uptake of nutrients. Reduction of plant water use under elevated $[CO_2]$ affects the acquisition of nutrients by the plant in different ways, because the smaller flow of water to the root surface might reduce nutrient uptake. But the faster diffusion of nutrients in wet than in dry soil might offset the reduction in uptake caused by smaller mass flow. Finally, elevated $[CO_2]$ also might increase nutrient uptake via increasing the root length.

Crop Response to Interactive Elevated $[CO_2]$ and Drought

Owing to their importance to the world's food supply and their economic interest, the responses of wheat, rice, maize, cotton, grasslands, and trees to interactive elevated $[CO_2]$ and drought are considered below.

The available data from investigations on the growth response to elevated $[CO_2]$ under edaphic stress show that droughted plants do grow faster at elevated $[CO_2]$ and more so, in relative terms, than well-watered plants [138]. Under a mild or late water deficit developing toward the end of the plant development cycle, elevated $[CO_2]$ may allow plants to overcome totally water-stress–induced growth reduction [140].

Wheat

For drought-stressed spring wheat, elevated $[CO_2]$ increased dry matter production owing to increased water-use efficiency and resulted in adaptation to water stress through osmoregulation [141].

FACE experiments were conducted on wheat at both ample (wet) and limiting (dry) supplies of water [142]. For both wet and dry treatments, the relative increase in the biomass resulting from elevated $[CO_2]$ showed a progressive increase with time. The relative effects of elevated $[CO_2]$ on the root biomass were somewhat larger than on the aboveground biomass for the wet treatment but, for the dry treatment, the effects were larger for the aboveground biomass than for the roots. Elevated $[CO_2]$ not only increased the amount of root biomass but also changed its vertical distribution in the soil [139]. More roots were present at high than at low $[CO_2]$ and in the wet than in the dry treatments. The $[CO_2]$ and watering treatments did not significantly affect the maximum root length.

Elevated $[CO_2]$ accelerated plant development by 2.3 and 1.5 days to mid anthesis for wet and dry conditions, respectively, and shorten time to maturity [142]. The accelerated rates of development were associated with higher plant canopy temperatures under elevated $[CO_2]$. As the crop senesced, there was a dramatic decline in green leaf area, with the dry plot declining first, then the wet, followed by the control.

Under the well-watered regimen, elevated $[CO_2]$ caused a modest but statistically significant average increase in grain yield [142]. On the other hand, under the dry treatment, elevated $[CO_2]$ caused a highly significant increase in final grain yield. Elevated $[CO_2]$ also caused a small but

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significant increase in the harvest index (ratio of grain biomass to total aboveground biomass) for both wet and dry conditions.

Although CO_2 enrichment had positive effects on growth and development of winter wheat at tillering, these were insufficient to counterbalance the debilitating effects of water limitation, and drought stress was shown to have a large negative effect on leaf development in winter wheat [143]. There was only limited compensation for this decrease when the atmospheric CO_2 level was doubled. Thus, if increased drought accompanies the predicted increase in atmospheric [CO_2], the beneficial effect of the latter on production of winter wheat is expected to be minimal.

In an attempt to investigate how [CO_2] and soil water availability, both singly and interactively, affect nutrient uptake by spring wheat, Van Vuuren et al. [139] grew plants at 350 (low CO_2) and 700 (high CO_2) μmol mol^{-1} CO_2 and with frequent (wet) and infrequent (dry) watering. The total amount of water used by the plants differed significantly between the treatments. Plants grown at low [CO_2] used about 1.25 as much water as plants grown at high [CO_2]. ''Wet'' plants used about 1.4 times as much water as plants from the ''dry'' treatments. This was probably due to the smaller stomatal conductance at elevated [CO_2], as has been shown in C_3 species [144,145]. The reduction of plant water use at elevated [CO_2] left the soil wetter between watering both at frequent and infrequent watering. This affects the acquisition of nutrients by the plant.

The interaction between the effects of [CO_2] and soil water availability differ between nutrients, because nutrients differ in their amounts and mobility in the soil. Thus, the total plant N, P, and K contents increased significantly with watering frequency [139], but only the total P content increased with [CO_2]; for the total plant K, the increase with [CO_2] was only marginally significant. The total plant N contents did not differ significantly between [CO_2] treatments. A difference in the plant N content was indicated by the dry treatment at the last harvest. The plant N contents in the dry treatment were greater at high than at low [CO_2]. In the wet treatment, the plant K content was greater at low than at high [CO_2], possibly reflecting the difference between [CO_2] in water use.

Rice

Excluding irrigated rice, about half of the world's rice land area depends on rainfall and is often subjected to drought stress. In terms of final seed yield, the vegetative phase of development is far less sensitive to drought than reproductive development [146]. The sensitivity of rice reproductive growth stages in terms of yield reduction or sensitivity to drought was ranked in the following order: (a) flowering, (b) gametogenesis, (c) panicle initiation, and (d) grain fill [128]. The effects and possible interactions of elevated [CO_2] and drought stress imposed at panicle initiation, anthesis, and both panicle initiation and anthesis on rice growth, grain yield, and yield components have been quantified [128].

As expected, drought stress reduced biomass accumulation in both ambient and elevated [CO_2] treatments [128]. [CO_2]-enriched plants grown under drought stress have increased growth compared with plants grown under drought stress at low [CO_2]. The [CO_2] enrichment increased stomatal resistance sufficiently to maintain high leaf water potentials and avoid drought. In both CO_2 treatments, the anthesis drought treatment reduced the aboveground biomass far more severely than the panicle initiation or panicle initiation and anthesis treatments. The large reduction in the biomass of the anthesis treatment suggests that the panicle initiation drought, in some way, acclimated or hardened the plants to withstand the subsequent anthesis drought.

In both [CO_2] treatments, the effect of drought stress on final seed yield was small except for the anthesis drought, which reduced grain yield. This decline was due to the significant reduction in individual seed mass [128].

Mild drought can accelerate development, whereas more severe drought stress often delays flowering relative to well-watered controls [147]. The drought at panicle initiation delayed the onset of panicle appearance and anthesis by about 3 days in both [CO_2] treatments.

Baker et al. [129] quantified the effects and possible interactions of [CO_2] at 350 and 700 μmol mol^{-1} and drought stress on rice (*Oryza sativa*, L.) photosynthesis, evapotranspiration, and

water-use efficiency. Carbon dioxide enrichment significantly increased both the canopy net photosynthetic rate and water-use efficiency, whereas reducing evapotranspiration by about 10%. This water saving under [CO_2] enrichment allowed photosynthesis to continue for about 1–2 days longer during drought in the enriched compared with the ambient [CO_2] control treatment. These results indicate that, in the absence of other potential climate change factors, such as increased air temperature, rice grown in the next century may use less water, use water more efficiently, and become better able to avoid drought in some situations.

Maize

The C_3 cycle in bundle sheath cells of this C_4 species is naturally exposed to high CO_2 concentrations, but the extra carbohydrates do not accumulate, because the rate of assimilation export is greater than in C_3 leaves. This higher capacity for sucrose translocation in maize may be supported by the closer proximity of mesophyll cells to the vascular system. It is thus believed that maize plants will cope with elevated CO_2, because they have mechanisms to deal with high rates of carbohydrate synthesis and transport [148]. High [CO_2] was shown to counteract most of the inhibitory effect of drought on *Zea* leaves' photosynthesis. It was shown that, in soil that was drying from field capacity, plants in high [CO_2] used about 30% less water than those in ambient [CO_2]; this resulted in higher soil water content at high [CO_2], and the plant accumulated 35% more leaf area and 50% more dry matter [149]. In drying soil, the increase in WUE was both due to increased dry matter and reduced water use, with the contribution from each depending on the stage of soil drying.

Cotton

In cotton plants, the enhanced dry matter yield due to doubled [CO_2] was 1.6-fold greater at low humidity than at high humidity [13]. Apart from the direct effect of the elevated CO_2 level on photosynthesis, the greater effect of doubled [CO_2] on the dry matter yield at low humidity was probably due to (a) increased leaf water potential caused by reduction of transpiration resulting from the negative response of stomata to increased [CO_2], the consequence being greater leaf area expansion; or (b) reduction of the CO_2 assimilation rate at low humidity and normal CO_2 concentration as a result of the humidity response of stomata causing reduction of the intercellular CO_2 concentration.

Sunflower

Despite the differences in the rate of change of conductance and relative water content during drought, photosynthetic electron transport activity, inferred from measurements of chlorophyll *a* fluorescence in vivo and PSII activity of isolated thylakoids, remained functional until desiccation occurred [150].

Grasslands

Grasslands that regularly undergo drought may profit from reduced evaporation under elevated [CO_2], especially if they contain a majority of C_4 species [74,151], and this effect may be more important than the direct effects of enhanced carbon uptake in prairies with C_3 plants. Field et al. [152] found that soil moisture remained higher under plots receiving CO_2 enrichment in a natural annual grassland. This resulted in an extension of the period of active photosynthesis by about 10 days [145]. In the precise case of *Lolium* and *Trifolium* canopies [105], it was shown that *Lolium* was more sensitive to drought stress in its initial response but divided the available water more proportionally over the stress period than *Trifolium*. WUE was roughly doubled and was affected later by drought stress in high CO_2 for both species. In general, the drought and CO_2 act both directly on the productivity and on the water use of grass swards and indirectly through changes in soil moisture content [153].

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Trees

To investigate the combined long-term effects of increased [CO_2] and drought, it is necessary to examine all the processes which take part in the complexity of the tree physiology (Fig. 4). Dixon et al. [154] exposed red oaks and Norway spruce to elevated [CO_2] and followed the effects throughout the vegetation period as natural drought developed. During the first year of growth, drought caused photosynthetic reductions and stomatal closure in both species. During the second year of growth, there were large interspecific differences. The net photosynthesis results showed statistically significant increases in CO_2-treated red oak before drought developed. The relative photosynthesis increase was gradually lost as drought developed. In contrast to red oaks, there was no apparent photosynthetic enhancement under elevated [CO_2] in undroughted Norway spruce. However, as drought developed and caused restrictions in photosynthesis, the trees grown at elevated [CO_2] had relatively higher net photosynthetic rates. From these different results, it appears that the presence of a continued photosynthetic enhancement was dependent on plant water relations.

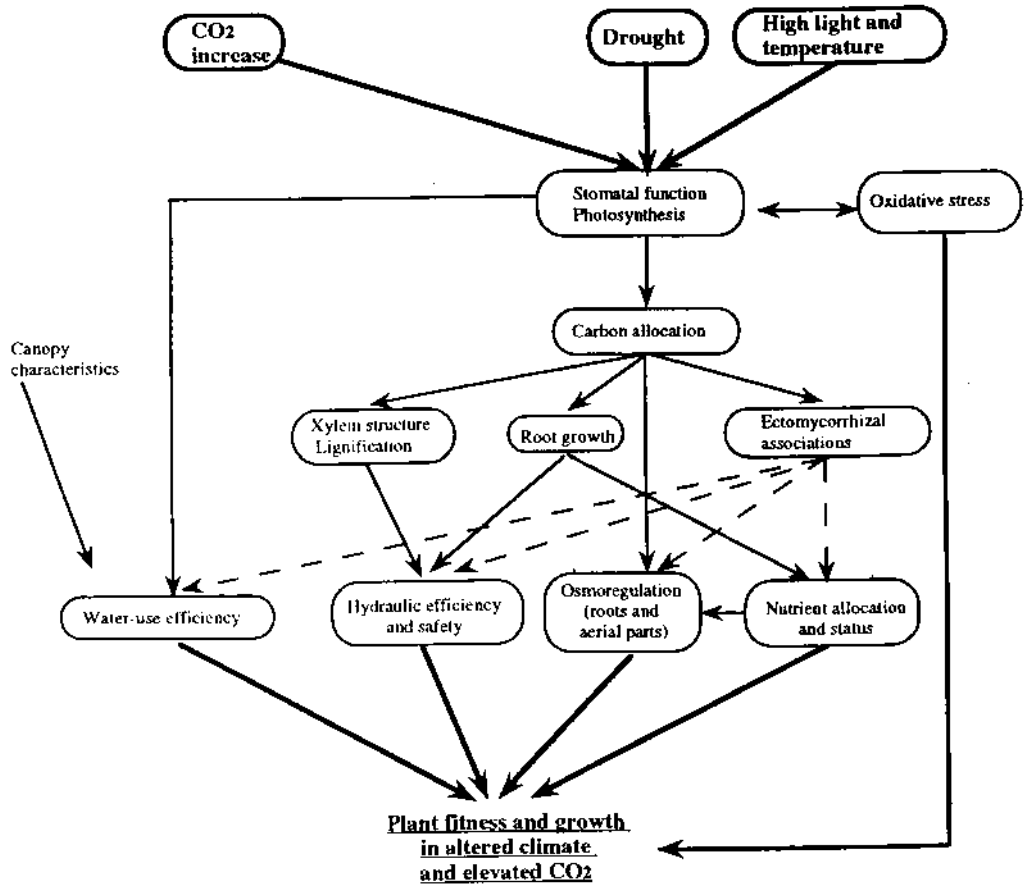

FIGURE 4 Schematic presentation of the structural and functional characteristics investigated for assessing the mechanisms determining plant performance in the context of climate change and elevated CO_2. For sake of clarity, direct effects of drought on growth and allocation processes and the nutrient ''dilution'' effect linked to increased carbon supply to the different plant components have not been represented. (From Ref. 211.)

As the drought develops, there is a decrease in leaf conductance to water vapor under elevated [CO_2], but this level of conductance allows relatively higher rates of photosynthesis than the ambient air. Because of this, the droughted trees under elevated [CO_2] have greater water-use efficiency values compared with droughted trees under ambient air. All trees had increased rates of photosynthesis as stomatal conductance increased. No typical relationships were observed between photosynthesis and intercellular CO_2 concentration.

The only significant difference concerning the stomatal density was an increase in the number of stomata in red oaks exposed to elevated [CO_2] [154]. Changes in stomatal density are important, as they can affect both the uptake of CO_2 and the rate of water loss. The results allowed Dixon et al. [154] to state that the role of any stomatal density effect in influencing the gas exchange measurements is primordial.

A moderate reduction of stomatal conductance has a significant effect on tree transpiration, which is roughly proportional to stomatal conductance. In the studies conducted under the European Collaboration on CO_2 Responses Applied to Forests and Trees program, this reduction of stomatal conductance ranged from 20 to 90%: WUE increased markedly in high CO_2, especially when the plants were droughted (150% in *Quercus ilex* in spring and from 200 to 300% in droughted poplars). Increased CO_2 may thus alleviate moderate water stress and allow some extension of forests in drier areas.

Vivin et al. [155] did not observe any growth increase of *Q. robur* seedlings in response to elevated [CO_2] under drought, although there was a stimulation in net photosynthesis. However, in addition, the respiration rate of the root system was slightly lower in the elevated than in the ambient [CO_2]. These results together with the results from short-term ^{13}C labeling suggest enhanced relative carbon loss (root or aerial respiration) under elevated [CO_2] in the drought conditions. It was shown that osmotic potentials at full turgor were lowered in response to water stress in leaves by 0.4 MPa for the elevated [CO_2] treatment only. In roots, osmotic adjustment (0.3 MPa) occurred in both of the [CO_2] treatments.

INCREASED TEMPERATURE AND RISING [CO_2] LEVEL

It is taken as axiomatic that future elevated atmospheric CO_2 concentrations will probably be associated with warmer temperatures. A rise in temperature lowers the ratio of [CO_2]/[O_2] in solution and higher global temperatures are an important consideration in the rising [CO_2] debate because of interacting effects on photosynthesis [12].

The need appeared for complementary equipment to modify temperature and elevated [CO_2], allowing studies of the interaction of rising temperature and elevated [CO_2] in natural conditions of light, humidity, and wind speed. Just as the FACE method had been introduced to simulate future CO_2 levels and validate responses obtained in the unnatural conditions of chambers, the free air temperature increase (FATI) technique was developed. This system simulates global warming in small ecosystems [156].

C_3 species differ in response to [CO_2] and temperature. In some plants, the stimulatory effect of elevated [CO_2] on plant growth is temperature dependent. Thus, for a 3°C increase in the air temperature at which *Gossypium hirsutum* was grown, the average growth-enhancement factor resulting from increasing [CO_2] to 640 μmol mol^{-1} was an increase from 1.3 to 1.56 [157]. In wheat, the adverse effects of elevated temperature on photosynthesis was moderated by CO_2 enrichment [23]. Long [76] has shown that elevated [CO_2] could alter both the magnitude of the response of leaf and canopy carbon gain to rising temperature and sometimes the direction of response. There is some evidence that elevated [CO_2] may lower the minimum temperature at which some plants grow and complete their life cycle [158].

Conversely, in some cases, the effects of CO_2 enrichment can be moderated by adverse effects of elevated temperatures. The high-temperature treatment was shown to reduce the net photosynthetic rates of rice and soybean by 25 and 38%, respectively. For winter wheat (determinate crop), yield decreased with an increase in temperature, and this decrease could completely negate the

increase attributable to elevated [CO_2] [159]. An increase in yield resulting from enhanced [CO_2] can be canceled by a 2–3°C increase in temperature [160]. This is because, as the elevated [CO_2] causes partial stomatal closure, the resultant decrease in transpirational cooling increases the foliage temperature [157] and the leaf transpiration, thereby counteracting the effect of the CO_2-induced stomatal closure [153]. In contrast, in potato (indeterminate crop), the growth response was found to increase in response to an increase in both temperature and [CO_2]. The effect of elevated [CO_2] on the dry matter yield of perennial rye grass swards also increased with air temperatures above 14.5°C and was promoted by a larger soil moisture in elevated compared with ambient [CO_2] [153].

Because it is an important tropical plant, a major food crop, and the only cereal used almost exclusively for human consumption, rice has been the subject of a number of studies which examined and quantified the effects of elevated [CO_2] and air temperature on its growth and yield. Previous studies were concerned with rice grown in glasshouses or in soil-plant-atmosphere-research chambers [161]. They were complemented by studies which examined the interactions of increased [CO_2] and air temperature for rice under irrigated field conditions in a tropical environment under the wet and dry seasons similar to the conditions where most rice is grown [162]. Overall, increasing the atmospheric [CO_2] resulted in a significant increase in growth, total biomass at maturity, and grain yield for rice over two different growing seasons. The observed increase in the biomass was primarily due to increases in tiller number and stem, root, and panicle weight. Many of the current high-yielding semidwarf rice cultivars are sensitive to high temperatures, with yields decreasing in direct proportion to day temperatures above 33°C [163]. The decline is primarily due to increasing floral sterility associated with decreased viability of pollen and to limited carbon translocation to the developing grain. If both [CO_2] and air temperature increase simultaneously, however, any potential benefit of CO_2 on the biomass and grain yield is negated, but plant development is accelerated at the higher growth temperature. Thus, the biomass and grain yield appear to be insensitive to the [CO_2] at the higher growth temperature [162]. Moreover, a simultaneous increase in [CO_2] and temperature also was found to alter grain quality. Protein content was decreased and overall nutritional quality was reduced. These described effects of a concomitant increase of [CO_2] and temperature on the quantitative and qualitative characteristics of rice crops might hopefully stimulate plant breeders to take such factors into account when developing future breeding strategies.

Interactions between rising [CO_2] and increased temperatures are not simply additive. That the combined effects of elevated [CO_2] and elevated temperature are less than additive indicates a decline in response to elevated [CO_2] as temperature increases. Rye grass growth rates declined as the temperature increased from 10/4°C (day/night) to 22/16°C. This decline was greater at elevated [CO_2]. In contrast, the white clover growth rate increased with temperature and was stimulated by elevated [CO_2] [22]. The grain yield of CO_2-enriched rice showed a 10% decline for each 1°C rise above 26°C [161]. Similar scenarios have been reported for soybean and wheat [159]. This is because growth and reproduction reflect the integrated temperature response of the metabolism and developmental stage.

The effect of temperature is primarily exerted through Rubisco [76]. Rubisco specificity (ratio of carboxylation to oxygenation activity when the concentration of CO_2 and O_2 at Rubisco are equal) declines at elevated temperature, because the affinity of Rubisco for CO_2 relative to O_2 decreases. A rise in temperature shifts the specificity of Rubisco toward oxygenase [23]. With a concomitant increase in mean growth [CO_2] and temperature, the proportion of fixed carbon entering the photorespiratory pathway increases, but as respiratory CO_2 decreases on elevated [CO_2], it is finally the balance between assimilatory carbon fixation and global respiratory losses that determines the net flux response to elevated [CO_2] and temperature. Fine control of Rubisco activation was shown to be influenced by both elevated [CO_2] and temperature [76]. In rice and soybean, there was an interplay between elevated growth temperatures and [CO_2] on the Rubisco parameters [23]. For both species, the Rubisco protein concentration and the activation of Rubisco declined with increasing temperature as well as with elevated [CO_2]. Both temperature and elevated [CO_2] enhanced the Rubisco catalytic turnover in soybean and had no effect in rice. For soybean, the temperature regimen has more effect on the Rubisco protein content than [CO_2], whereas for rice, both environmental

factors exert coarse control effects on Rubisco [164]. Postsunset declines in Rubisco activities were accelerated by elevated [CO_2] in rice and by high temperature in soybean, suggesting that [CO_2] and growth temperature influence the metabolism of 2-carboxyarabinitol-1-phosphate and that the effects might be species specific.

In addition to effects on photosynthetic and Rubisco activity, elevated temperatures influence carbohydrate metabolism. In CO_2-enriched rice plants, sucrose phosphate synthase activity was increased by temperatures up to 34°C but thereafter declined. Total nonstructural carbohydrates declined with increasing growth temperature, but the decline in the starch content was much greater than sucrose [23]. Consequently, the sucrose to starch ratio increased with temperature. Data suggest that high temperatures not only influence the amount of carbohydrate produced but also its composition, possibly shifting the amylose to amylopectin ratio in favor of the former. However CO_2 enrichment moderates the differences. Although ADP–glucose pyrophosphorylase activity was not greatly altered by temperature or CO_2, the starch-branching enzyme activity is enhanced by CO_2 and considerably decreased at high temperature.

Qualitative and quantitative changes also were observed in the lipid fraction (both nonstarch and starch lipid) of wheat plants grown under regimens combining two temperatures with two concentrations of [CO_2] and two nitrogen fertilizer applications [159]. Temperature was by far the most influential growth factor. Growth at elevated temperature had the general effect of reducing the amounts of accumulated lipids, particularly nonpolar lipids. There were changes in the proportions of the major nonstarch (membrane glycosylglycerides and phosphotidylcholine) as well as the starch lipids (mainly lysophosphatidylcholine and lysophosphatidylethanolamine). Significant changes in the acyl composition of individual lipids also were observed; most often in the proportions of palmitate, oleate, and linoleate. The observed alterations in wheat lipids are likely to affect the properties of any flours derived from grain grown under climatic change conditions.

Understanding how cellular processes such as cell expansion and cell division are affected by temperature and elevated [CO_2] is crucial for debate on how plants will react to predicted global environmental change [165]. The subject was addressed by Taylor et al. [166], using the leaf lamina of herbaceous angiosperms and the lateral root primordia in *Populus euramericana*. They have identified cell wall loosening as a critical component of increased cell expansion as a result of elevated [CO_2] treatment. Circumstantial evidence indicates a stimulation of cell division by elevated [CO_2], increased cell number in the leaf lamina, and an increase in the number of lateral root primordia. Measurements of cell division in the meristems of plants exposed to ambient or elevated [CO_2] and incremental temperature treatments were undertaken on two natural populations of the perennial grass *Dactylis glomerata* originating in Portugal or Sweden [165]. Cell division was assessed in the shoot meristem, since it is from the latter that all aboveground tissues form. Elevated [CO_2] resulted in substantial decreases in cell division time compared with the corresponding measurements at ambient [CO_2] in all zones of the meristem and at all temperatures. Differential responses of the two populations were observed. The cells in the young primordia of the Swedish population were much less affected by elevated [CO_2] and temperature than the Portuguese population. It is concluded that the cell division time in the shoot apex of the Swedish population is relatively buffered against temperature change, whereas in the Portuguese population, the cell division time shortens progressively with increasing temperature.

Relationship between alterations to cell division at the shoot apex and the overall growth response of the plant are certainly complex. Cell division in the plant shoot apex is regulated by a number of factors, among which is the provision or withdrawal of sucrose which may sustain or regulate signal transduction, which in turn regulate the expression of cell cycle genes. Faster rates of cell division at elevated [CO_2], most notably in the Portuguese population, may be a response to a greater supply of sucrose at the apex. It is suggested that elevated [CO_2] ameliorates nonoptimal temperatures for cell division [165].

Because of the warming, the winters may become milder, which will predispose the trees to increased risks of frost damage [167]. Frost hardening and dehardening are the result of a multitude of physiological, biochemical, and biophysical changes in cells. Although the elevated [CO_2] in

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summer increases photosynthesis, plant metabolic activity and accumulation of high-energy compounds in cells, the processes regulating frost hardiness may also be affected. Increased activity during the dormant season, especially at elevated temperatures, predisposes trees to frost damage. Although there were significant differences between treatments and significant variation between trees in frost hardiness, the results suggest that the risks of frost damage are marked in the predicted climatic conditions in the boreal zone.

Finally, it appears that the responses of plants to climatic change variables are the results of both the direct effects of increasing [CO_2] and the indirect effects of rising temperature [22].

EFFECTS OF ELEVATED [O_3] AND [CO_2] LEVELS

Ozone was first described by Richards et al. as a toxic air pollutant originating from reactions between constituents of photochemical smog [168]. Industrial emissions of nitrogen oxides and volatile organic compounds and interaction with ultraviolet (UV) radiation have led to rising background concentrations of tropospheric O_3, in areas of intensive urbanization but also in rural areas [169]. Models predict that tropospheric ozone will increase 0.3–1.0% per year over the next 50 years [170].

O_3 in an aqueous environment can generate OH^-, O_2^{2-}, and H_2O_2. O_3 generation of OH^- is accelerated by Fe^{2+}, thiol groups, amines, and phenolics like caffeic acid [171]. Interactions between plants and ozone (O_3) have all been shown to be associated with the production of other chemical species (e.g., hydroxyl radicals (OH^-), superoxide (O_2^-) anions, and hydrogen peroxide [H_2O_2]), which have even higher oxidizing potentials.

Ozone enters the leaf through the stomata and diffuses within the apoplast where it rapidly decomposes to hydroxyl and superoxide anion radicals, hydrogen peroxide, and other reactive oxygen species. Recent research on the impact of ozone on trees indicates that ozone uptake is an important physiological link in the understanding of differences in the ozone response between tree species as well as between trees of different sizes within species [172].

Ozone Impact on Plants and Crops

A number of reviews are available on ozone phytotoxicity, dealing with visual symptoms and growth effects, as well as with the problem of leaf–internal ozone dose, effects on stomatal regulation, photosynthetic functions, and assimilate allocation [173].

The significant effects on growth and yield are primarily related to changes in the photosynthetic physiology and stomatal conductance. Recent studies have shown that, in combination, the effects of [CO_2] and [O_3] are not simply additive [4]. It was previously shown that elevated [CO_2] protects against the deleterious effects of elevated [O_3] on photosynthesis in spring wheat [174]. The hypothesis that the protective effect of elevated [CO_2] against ozone damage also will be exerted on the biomass and yield of spring wheat was tested [4].

The factors underlying modifications in photosynthesis were investigated with *Pinus sylvestris* L. [175]. Elevated [O_3] led to a significant decline in the CO_2 compensation concentration, maximum ribulose-bisphosphate–saturated rate of carboxylation, maximum rate of electron transport, maximum stomatal conductance, and sensitivity of stomatal conductance to leaf-to-air vapor pressure difference. Calculations showed that elevated [O_3] decreased the apparent quantum yield by 18–35% and the maximum rate of photosynthesis by 21–29%. The interactive effects of O_3 and CO_2 on the maximum ribulose-bisphosphate–saturated rate of carboxylation and the maximum rate of electron transport were significant and closely related to the regulation of the stomatal conductance and stomatal sensitivity induced by elevated [CO_2].

It is expected that the progressive increase of tropospheric trace gases such as CO_2 and O_3 will have a significant impact on agricultural production. Increasing tropospheric O_3 concentrations can appreciably alter the nutritive value of herbaceous legumes that are presently an important source

of N and energy for grazing ruminants [176]. White clover, for instance, is a major perennial pasture legume. Its nutritive value has important implications to herbivore production. Its changes due to O_3 regimens at ambient and enriched [CO_2] was explored. Although in vitro dry matter disappearance of laminae declined linearly with increasing exposure to O_3, the laminae neutral detergent fiber and total nonstructural carbohydrates increased. But at enriched [CO_2], O_3 lacked influence on the nutritive value of white clover. The single and combined effects of CO_2 enrichment and tropospheric O_3 on grain quality characteristics in winter wheat were examined [177]. Milling and baking quality were not significantly changed. Flour yield was increased by elevated [CO_2], but this increase was counteracted when elevated [CO_2] was combined with chronic O_3 exposure. Flour protein contents were increased by greater [O_3] exposure and reduced by elevated [CO_2]. In conclusion, although the single effect of either [CO_2] enrichment or chronic [O_3] exposure had some impact on the grain quality characteristic, it was noted that the combined effect of these gases was minor, and it is concluded that the concomitant increase of [CO_2] and [O_3] in the troposphere might have no significant impact on wheat grain quality [177].

Harmful Effects of Atmospheric O_3 on Vegetation

O_3 sensitive/tolerant bioindicator surveys have used symptoms of visible injury to assess the extent of possible O_3 injury on vegetation over large areas [178]. The phytotoxicity of ozone can be divided into chronic and acute damage, reflecting different defense strategies of the plant [173]. The acute damage resembles the hypersensitive response which occurs after a pathogenic attack or elicitor treatment, leading to lesion development and eventually including cell death. Chronic damage leads to a general reduction of growth that is somewhat similar to a premature senescence.

Necrotic Lesions

Pell et al. [179] have reviewed the mechanism by which O_3 induces necrotic lesions and/or accelerated foliar senescence. Symptoms of acute damage have been observed on a wide range of species [180]. In addition to small or large, reddish or whitish colored necrotic lesions, foliage under acute injury may exhibit accelerated senescence. An early event leading to lesion formation is the loss of semipermeable function in the plasma membrane [181]. Components of the cell wall and membrane become oxidized during the initial O_3 exposure. Given that the cell wall contains phenolic groups, oleic compounds, proteins, and lipids, some of which are unsaturated, there are clearly numerous sites for primary oxidation events. Reactions between O_3 and any of these compounds could result in the production of active oxygen species. To date, we can only speculate that active oxygen species will be produced as a result of primary reactions between O_3 and constituents of the cell.

A central question is whether lesions are a result of rampant oxidation and subsequent unregulated rapid cell death or alternatively a result of some type of programmed cell death. There is little direct information regarding the nature of the reactions which lead to altered plasma membrane function. ATPase activity of plasma membranes from O_3-sensitive *Phaseolus vulgaris* foliage was shown to be severely inhibited owing to a decrease in the K^+-stimulated component [182]. It also was found that O_3 can influence calcium transport in plant membranes [183]. Lipid components of membranes might also be the target of attack by O_3 [184].

Induction of Accelerated Foliar Senescence

An accelerated foliar senescence is a common response of many ozone-treated plants [185]. Multiple oxidation events are responsible for the accelerated foliar senescence (Fig. 5). Even if necrotic responses are absent or of minor importance, foliage begins to exhibit signs of accelerated senescence after a period of weeks of exposure to O_3. More ozone-tolerant species such as potato, cereals, or conifers typically respond with symptoms of chlorosis and also exhibit accelerated senescence. It was determined that O_3-induced foliar senescence is closely associated with the loss of Rubisco

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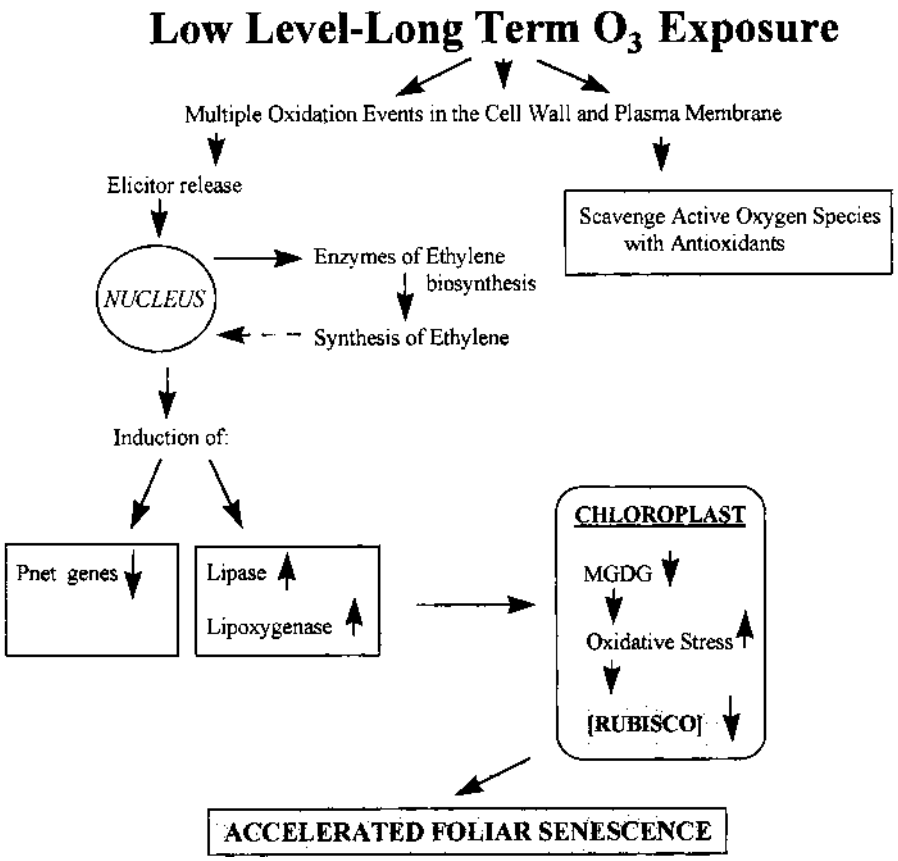

FIGURE 5 Model to explain potential mechanism whereby low level ozone exposures induce accelerated foliar senescence. MGDG refers to monogalactosyl diglycerides. (From Ref. 178.)

and some other less prominent enzyme activity. The decline in the net photosynthesis rate correlates in potato with a loss of Rubisco activity. In vitro and in vivo experiments and the observation that *rbc*S mRNA dropped earlier in the development of potato leaves subjected to chronic O_3 exposure support the notion that the major loss of Rubisco reflects enhanced degradation of the Rubisco protein. Transcript coding for other chloroplastic proteins such as glyceraldehyde-3-phosphate dehydrogenases and a PSII chlorophyll *a/b*–binding protein were found to decline in parallel. In contrast, the cytoplasmic isoform of glyceraldehyde-3-phosphate dehydrogenase, which is involved in glycolysis, increased in response to ozone. Since Rubisco is central to leaf longevity, O_3-induced acceleration in loss of this protein may contribute significantly to the increased rate of leaf loss observed in plants subjected to chronic exposure to the pollutant. Leaf lipid analyses have revealed that plastidic monogalactosyl diglycerides (MGDGs) decline and triglycerides increase. It has been suggested that lipase activity might be induced by O_3 and would react with the MGDGs to provide free fatty acids which would then be the substrate for lipid peroxidation ultimately leading to increased active oxygen species in the chloroplast [186]. Lipid hydroperoxides reduce the membrane fluidity. They are intermediates in the biosynthetic pathway of jasmonic acid. Located within chloroplasts, methyl jasmonate was shown to increase the potential of leaves for the production of volatile compounds found in ozone-treated tobacco [187].

Foliar Emission of Ethylene

Foliar emission of ethylene is an early event associated with O_3 exposure [188]. It has been correlated with visible ozone lesion development in many herbaceous plant species. An explanation of ozone toxicity is related to ethylene emission, a well-known senescence-promoting hormone of plants [189]. Ozone may react with volatile compounds emitted by the plant into the apoplast. Interaction of ozone with ethylene or more complex hydrocarbons such as isoprene and α-pinene are thought to be part of the mechanism leading to injury. Ethylene production in the plant occurs enzymatically through conversion of l-methionine via S-adenosyl methionine (AdoMet) to l-aminocyclopropane-1-carboxylic acid (ACC). This key step in ethylene biosynthesis is catalyzed by the enzyme ACC synthase. The differential expression of ACC synthase may be an important mechanism by which ethylene production is regulated in many physiological processes. Several investigators proposed that ethylene reacts nonenzymatically with O_3 to produce O_2 and reactive aldehydes that might be responsible for plant damage [190]. It also is likely that ethylene acts directly on plant metabolic responses to O_3 through a number of mechanisms, including gene regulation [189].

Potential Mechanism for O_3-Induced Accelerated Foliar Senescence

The model presented by Pell et al. [179] to explain the potential mechanism by which prolonged exposure to O_3 induces foliar senescence is presented in Figure 5. Ethylene is frequently associated with senescence. The amount of visible injury caused by O_3 correlates to the rate of ethylene production. Studies with transgenic tomato plants, which produce low levels of ethylene and have delayed loss of chlorophyll and delayed leaf senescence [191], support the model that the photosynthetic decline is coupled to senescence and that ethylene plays an important role in leaf senescence [192]. Active oxygen species may provide the signal(s) to the nucleus leading to induction of a suite of responses which lead to increased oxidizing stress in the chloroplast. As the leaf ages, the production of antioxidants declines and this stress increases. The role of ethylene may be by facilitating the progress of senescence rather than serving as a necessary signal [193].

Rubisco normally degrades after oxidative modification, and in the O_3-treated foliage, the process will occur more rapidly. In ozone-sensitive species, changes in sugar metabolism are indicated by an accumulation of starch along the veins and the formation of starch granules in epidermial cells. Starch and hexoses inhibit photosynthesis at the level of several Calvin cycle enzymes, including Rubisco. Reduced CO_2 fixation in the light increases the pool of reduction equivalents, such as NADPH in chloroplast. These changes in primary metabolism even have a strong impact, as crop yield is reduced and the whole life cycle of the plants appears to be influenced [194]. In both acute and chronic attacks, the pattern of gene expression changes significantly. However, no genes that are specifically and solely regulated by ozone have been described so far.

Ozone-Dependent Induction or Suppression of Genes

Damage does not seem to be the direct result of ozone toxicity, but ambient ozone concentrations might rather affect signaling pathways within the plants. Ozone induces the genes of several pathogenesis-related proteins [173]. In tobacco, the accumulation of β-1,3-glucanase mRNA is correlated with ethylene formation [195]. The increase in the expression of genes for β-1,3-glucanase and chitinase in response to O_3 is supported by an increase in the activities of these enzymes. These proteins are known to be associated with loosening of the cell wall during development [196].

There have been reports of O_3-induced reductions in the level of transcript for the small subunit of Rubisco (*rbc*S), chlorophyll *a/b* protein (*cab*), and glyceraldehyde-3-phosphate dehydrogenase (*gap*A and *gap*B) [189]. Whether transcription is regulated in response to O_3 or specific Rnase activity is regulated by the pollutant is not known.

Considerable effort has gone into the purification and cloning of the genes for this important

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regulatory enzyme. Stimulation of ACC synthase activity noted in many developmental and inducible systems results from an increase in the levels of mRNA for ACC synthase [197]. Since O_3-induced ethylene production is closely correlated to the accumulation of ACC, it is likely that O_3 promotes increased ACC synthase activity. Transcripts for ACC synthase were found to be detectable 1 h after the onset of acute O_3 exposure. It increased dramatically after 2 h, with high levels of expression up to 4 h. No ACC synthase mRNA was detected in nontreated plants. Evidence was provided that the increase in ACC synthase mRNA was due to gene transcription. The isolation of a second ACC synthase cDNA whose transcript accumulated very quickly in response to O_3 led to the hypothesis that there are at least two ACC synthase genes expressed in response to O_3, and the timing of expression may reflect differences in the mechanisms of signal transduction and/or gene regulation [198].

Plant Defense Reactions and Acclimation

The probability of disease in plants is determined in large part by defensive and antioxidative reactions of secondary metabolism [173]. These reactions include a local oxidative burst, cell wall reinforcement (lignin, callose, extensins), and the induction of phytoalexins, antioxidative systems, and pathogenesis-related (PR) proteins.

Several ozone-sensitive and ozone-tolerant cultivars, clones, or populations of various species are known which are useful tools in comparing different strategies of plants. These strategies deal with the detoxification of radical species or with signal cascades, leading to common plant defense reactions. Ozone-induced plant responses are probably mediated by interference of at least three different signaling pathways depending on ethylene, reactive oxygen species/lipid hydroperoxide, and salicylate. Reactive oxygen species production and lipid peroxidation take place either on the plasma membrane or in the chloroplastic membrane, and lipid hydroperoxides and derivatives, such as jasmonate, can act as signals for subsequent plant reactions. They react with proteins or lipids of the plasma membrane. Alternatively, they may be detoxified by radical scavengers, ascorbate, polyamines, and tocopherol located within the apoplast. Reactive oxygen species induce detoxifying systems, as demonstrated in cell suspension cultures [199]. Transcripts were found to be elevated after treatment with hydrogen peroxide in a narrow range of 1.8–4.0 mM. Application of polyamines to tobacco roots was found to reduce the level of ozone-induced lesions [200]. Reactive oxygen species–detoxifying systems such as isozymes of dismutases or ascorbate peroxide also are present in the chloroplast. A prominent role for glutathione is indicated by the increase of glutathione during ozone treatment; in beech, the level of glutathione increases in response to high ambient ozone concentrations [201]. Some evidence was provided that the levels of glutathione and glutathione reductase as well as the rates of the transmembrane transport of ascorbate and dehydroascorbate may moderate the ozone susceptibility of plants, although there is no direct correlation between enzyme levels and stress tolerance.

The assessment of the harmful effects of atmospheric O_3 on vegetation has been greatly improved by comparisons between O_3-sensitive and O_3-tolerant cultivars or selections of the same species. A range of species, including poplars and other hardwood trees, conifers, tobacco, corn, soybean, legumes, clover, and plantains, has been used in a number of comparisons [178]. Controlled-environment studies using lower concentrations of O_3 have shown that the O_3-sensitive partner of a comparison pair may also exhibit several signs of invisible injury, whereas the O_3-tolerant cultivar shows evidence of acclimation. For example, Bel-W3 tobacco readily produces stress ethene [202], which can react with O_3 to form harmful radicals [190], whereas Bel-B forms polyamines faster [203] which can function as protective free radical scavengers [204]. Consistent differences in the rates of ethene release between acclimated and nonacclimated plants also were found [202].

Evidence was shown for common differences across different plant species in (a) ethene release (ethene, putrescine, spermidine, polyamines, phenols) and (b) dependence on similar free radical scavenging systems (reduced glutathion, reduced ascorbate) for protection on exposure to O_3. The combined assays with six pairs of O_3-sensitive/O_3-tolerant cultivars, families, and so on (to-

bacco, plantain, clover, radish, poplar, and loblolly pine) clearly indicated that rates of ethene formation after O_3 exposure were always significantly higher in O_3-sensitive selections but were unchanged or lower in O_3-tolerant selections [178]. O_3-tolerant cultivars clearly show several patterns of response. In some cases, adequate endogenous levels of potential antioxidant already exist, whereas in others, they are rapidly expressed and this affords protection against O_3. Conversely, some of the weaknesses of the O_3 cultivars have been detected but not always in the same area. If one were to attempt to specify tolerance to O_3, the following characteristics should be taken into account: (a) reduced ethene emissions; (b) the ability to form putrescine rapidly; (c) enhanced levels of one or more phenylpropanoid, flavonoid, or lignin components; and (d) the ability to form sufficient reduced glutathione and ascorbate quickly [178].

A new concept was recently developed that ozone could act as a powerful and ubiquitous abiotic elicitor [173].

CONCLUSIONS

There is much concern over the possible impact on agriculture of climatic changes. As breeding programs for crop species need to take climatic change into account, the risk of a global increase of atmospheric CO_2 concentration and associated climatic change and their influence on agriculture need to be assessed [205]. Although the broad-scale prediction is for a smooth increase in global temperature, there may be rapid warming in some regions and possibly even periods of cooling in others [206]. Marked drought stress may occur in regions where there is no accompanying increase in precipitation. One important question is whether agricultural systems can adapt to the predicted rates of changes. The use of manipulative experiments to study directly the effects of climatic change on natural and managed systems is expensive if performed on a large scale; it also relies on predictions of future climatic conditions, the accuracy of which is doubtful. Predictive modeling is less expensive and permits a range of scenarios to be considered [207].

In short, the effects of the main climatic changes predicted to occur during the next century (i.e., elevated $[CO_2]$ and $[O_3]$, elevated temperature, and shortage of water supply on plants and crops) can be stated as follows: As current ambient CO_2 levels are suboptimal for most crops, at least for C_3 plants, agricultural productivity will benefit from both direct (fertilizer) or indirect (climatic) effects of increased atmospheric $[CO_2]$ concentration. The significant effects of $[CO_2]$ on crop growth and yield are primarily related to changes in photosynthetic physiology and stomatal conductance, whereas temperature affects metabolic reactions. By increasing the growth rate, elevated temperature shortens the required growing period resulting in a decreased yield. High temperature is a factor of sterility. The pollutant O_3 has deleterious effects on photosynthesis. Water stress alters transpiration rates which in turn disturbs the flow of solutes within the plant and affects the translocation of nutrients.

However, there is clearly no general rule for the response of plants and crops to the predicted changes in climate. In combination, the effects of elevated $[CO_2]$, $[O_3]$, temperature, and water stress are not simply additive. Studies on the interaction of temperature and $[CO_2]$ do not necessarily demonstrate a synergistic effect [162]. It is possible that biochemical phenomena might not always be expressed at the whole-plant or canopy level. Considering the possible interactions which might take place, it is important to allow for the effects of temperature and water supply on the carbon partitioning of wild and cultivated plants when predicting the effects of elevated $[CO_2]$ on growth and productivity. When water is limiting, the effects of a temperature rise and higher $[CO_2]$ levels on crop production are different from the potential production [208]. Both temperature rise and an increase in $[CO_2]$ concentration reduce the water requirements of the crop.

The intensity of plant responses also depends on other environmental conditions, plant species, the age of the plant, and so forth. The effects of $[CO_2]$ enrichment and higher temperatures are variable from year to year and they depend on the weather, N availability, and the variable annual pattern of plant development [209]. They may also be masked by the interannual climatic fluctuations

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that cause such a high degree of variability in agricultural production in most parts of the world. Any factor which increases environmental stress on crops may make them more vulnerable to attack by insects and plant pathogens and less competitive with weeds [210]. There is a large species imbalance in the data, with some plants being well represented, some less, and totally inadequate information for certain crops, particularly C_4 cereals and tuber crops. Programs concentrating on the most economically important crops are vital.

A detailed understanding of the responses of field-grown crops to elevated [CO_2] and interactions with other factors is essential to assess the impact of the predicted changes in the environment on agriculture. With this knowledge, it may be possible to modify the genetic constitution of plants, by conventional breeding or by genetic engineering techniques, in order to improve their efficiency in the predicted future conditions. It is important to increase substantially the range of current research into how agriculture can best adapt to such changes. It is suggested and recommended that we (a) improve methods for the estimation of probable changes in climate by developing modeling techniques; (b) closely link field studies to the modeling techniques so that specific predictions can be tested; (c) confirm that plant response to climatic factors observed in the large number of controlled environment experiments apply in agriculturally or ecologically realistic conditions; (d) concentrate programs on the most economically important crops, particularly cereals, legumes, and tuber crops (which are actually less represented in this field of research); (e) extrapolate the results of experiments to the population level; (f) compare the response of different genotypes of the same crop with well-established genetic characteristics with the aim of optimizing cultivars to future climates using biotechnological approaches; and (g) consider the type of action required to adapt to climatic change through adjustments at the field level to issues in regional and national policy.

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Leaf Development and Acclimation to Elevated CO_2

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INTRODUCTION

Acclimation to Elevated CO_2

Atmospheric CO_2 levels have changed dramatically since the Industrial Revolution, increasing by almost 40% to a present-day concentration of approximately 350 ppm [1]. It is thought that consumption of fossil fuels is the main contributor to this increase. The outlook for the future is much the same, with the current level of CO_2 expected almost to double by the beginning of the twenty-second century if existing conditions are not altered drastically. How such changes in the atmosphere will affect the growth and development of plants, both on an individual and global scale, is an important but unanswered question.

In many C_3 plants under normal environmental conditions, the photosynthetic rate is limited by the rate of initial CO_2 fixation into the Calvin cycle. This rate-limiting step is catalyzed by Rubisco, which carboxylates ribulose-1,5-bisphosphate (RuBP), generating two molecules of 3-phosphoglycerate (3-PGA). Some 3-PGA molecules are exported into the cytosol for use in sucrose biosynthesis, whereas others are responsible for RuBP regeneration and starch synthesis in the plastid. Rubisco is an inefficient enzyme with a low affinity for CO_2, and it is substrate limited under present atmospheric concentrations [2].

One of the most important responses of plants to growth in elevated CO_2 is the phenomenon of ''acclimation'' (reviewed in Refs. 2–4). Because elevated CO_2 represents an increase in substrate availability, increased rates of carboxylation in elevated CO_2 should result in higher net photosynthetic rates. In many experiments, this is observed in the short term. However, the enhancement of photosynthetic performance is not maintained in plants that ''acclimate'' to high CO_2, and in these plants, photosynthetic rates fall below those predicted on the basis of Rubisco kinetics. It is this loss of the predicted benefit of high CO_2 growth that is referred to as acclimation.

Acclimation has been observed in many agronomically important C_3 species, including tomato, wheat, pea, soybean, sugar beet, cotton, tobacco, and several tree species (reviewed in Ref.

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4). However, C_3 plants differ in the severity of the acclimation response. Also, C_4 and crassulacean acid metabolism (CAM) plants do not acclimate, because they have biochemical carbon-concentrating mechanisms and are therefore constantly under an enriched CO_2 regimen.

Molecular Mechanisms of Acclimation

Several mechanisms have been proposed to explain the downregulation of photosynthetic rates that occurs during acclimation (reviewed in Ref. 2). One mechanism is that enhanced starch accumulation in high-CO_2–grown plants results in large grains that disrupt chloroplast membrane structure and function. A second mechanism is that growth in enriched CO_2 results in a reduction in stomatal conductance, which restricts the amount of CO_2 entering the leaf; photosynthetic rates consequently fall (e.g., see Ref. 5). Yet a third mechanism suggests that the decreases in photosynthesis during acclimation are a consequence of enhanced rates of sucrose synthesis that accompany increased CO_2 uptake. Enhanced sucrose production results in feedback regulation of sucrose phosphate synthase (SPS) and a sequestering of Pi pools in the cytosol [2,6,7]. Without sufficient levels of Pi returning to the chloroplast, RuBP regeneration, and hence photosynthesis, is restricted. Finally, it has been suggested that a limitation in nitrogen may be a causal factor of acclimation [8]. According to this mechanism, nitrogen assimilation is not able to keep pace with enhanced photosynthetic rates under high CO_2; that is, the photosynthetic mechanisms are nitrogen limited.

Although all of these mechanisms may contribute to the acclimation response, recent evidence has suggested that none can fully explain the long-term decreases in photosynthetic rates that characterize this phenomenon. Rather, evidence favors the hypothesis that long-term exposure to elevated CO_2 results in a downregulation of photosynthetic gene expression. One of the early pieces of evidence in support of this hypothesis was that declining photosynthetic rates in high-CO_2–grown plants are accompanied by a loss of Rubisco protein (e.g., see Refs. 1, 2, 7, and 9–15). In a few cases, it has been further demonstrated that the loss of Rubisco protein is accompanied by coordinate decreases in Rubisco small subunit (*rbc*S) and large subunit (*rbc*L) transcript levels in the nucleus-cytoplasm and chloroplast, respectively [7,15].

Although alterations in *rbc*S and *rbc*L transcription may explain, at least in part, why photosynthetic rates decrease during acclimation, there still remains the question of what factors control the changes in transcription of these genes. These factors are poorly understood, but may include a variety of environmental factors such as nutrient status, water supply, mineral availability, and temperature [16], which have been demonstrated to influence the sensitivity of the acclimation response. There also have been suggestions that acclimation can be influenced by the leaf and plant developmental stage [14–16]. The latter is the focus of the rest of this chapter.

ELEVATED CO_2 AND TOBACCO LEAF DEVELOPMENT

Regulation of Photosynthesis During Leaf Development

Under ambient CO_2 conditions, leaf development can be separated into two distinct phases [17]. The first stage is associated with leaf growth and expansion. During this stage, leaf photosynthetic rates increase over time. There follows a transient peak of maximal photosynthetic rates correlated with the attainment of full expansion, and then rates begin to decline. This is the second stage of development, which is referred to as senescence. As the senescence phase progresses, leaves yellow as chlorophyll is broken down, and resources are reallocated to different parts of the plant, such as reproductive structures. In many species, the changes in photosynthesis that occur during these phases are largely due to changes in the levels of Rubisco protein and activity (e.g., see Refs. 18 and 19). The alterations in Rubisco protein, in turn, are due to coordinate changes in *rbc*S and *rbc*L mRNA amounts. This finding emphasizes the notion that senescence involves a modulation of anabolic processes as well as catabolic processes (cellular breakdown).

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Previous experiments in our laboratory have demonstrated that genetic manipulation of sink/source balance profoundly impacts plant growth and developmental processes [19,20]. These experiments were performed with Rubisco antisense mutants of tobacco, in which Rubisco levels are decreased up to 90% of those found in the wild type. Source strength (carbohydrate production) also is impaired in these plants. We also have examined growth and development under conditions where source strength is increased by growing tobacco plants under elevated CO_2 conditions. These studies led to some novel observations on the phenomenon of acclimation.

Photosynthetic Parameters

To investigate the effect of increased source strength on leaf development, we examined tobacco plants under ambient CO_2 levels (approximately 350 μL/L) and enriched CO_2 concentrations (950 μL/L) [21]. Leaf 10 (counting up from the base), which is a middle canopy leaf, was chosen for analysis because of its large final size. The elevated CO_2 regimen was initiated at the time of visible leaf 10 emergence. Measurements were taken at varying time points throughout leaf development. We first performed gas exchange analyses and examined the CO_2 exchange rate (CER), stomatal conductance (Cs), and internal inorganic carbon concentration (Ci). As illustrated in Figure 1, ambient CO_2-grown leaves exhibited increasing CERs to day 12, a transient maximum, then a steady decline from day 14 onward; rates fell below zero at day 40. Relative to their ambient-grown counterparts, the high-CO_2–grown leaves displayed a similar pattern of CER change over time as well as a similar photosynthetic maximum. However, these leaves reached their photosynthetic maximum and initiated their photosynthetic decline at day 4; significantly earlier than in the ambient-grown leaves. The rate of photosynthetic decline following this maximum was comparable to that in the ambient-grown leaves except that CER reached zero at day 25. These results suggest that the magnitude and onset of maximal photosynthetic rates and their subsequent decline is similar in plants with enhanced source strength, but that in these plants, the onset of the decline is temporally shifted to an earlier initiation point.

To determine whether stomatal aperture was responsible for the changes in photosynthesis in high CO_2 versus ambient-grown leaves, Cs and Ci were plotted versus relative leaf age [21]. The data indicated that there were no significant differences in Cs between the two treatments, but that

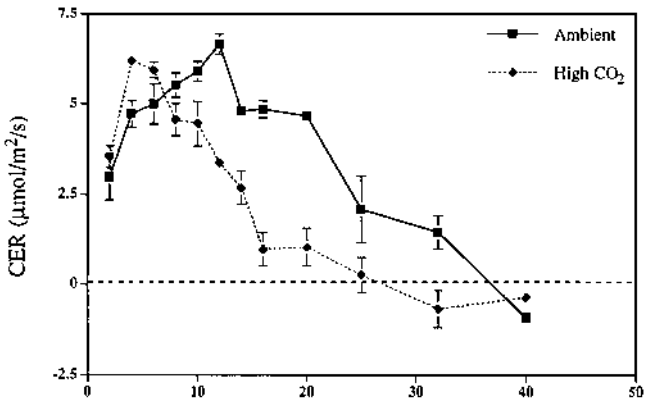

FIGURE 1 CERs during development of tobacco leaves grown under ambient (circles) or elevated CO_2 conditions (squares). Plants were moved into high CO_2 when leaf 10 reached 1 cm in length; day ''1'' status was assigned when the leaf reached 3 × 5 cm in length several days after transfer. Each point represents the average (± SE) of multiple measurements on leaves from at least 4 different plants. (From Ref. 21.)

the levels of Ci were generally higher in the elevated CO_2-grown leaves throughout the developmental time course. Clearly, stomatal conductance did not cause the decline in photosynthesis observed in the high-CO_2–grown plants, since internal CO_2 levels were not reduced. Considered together, these data demonstrate that the CER was not limited by CO_2 availability.

An examination of other photosynthetic parameters supported the conclusion that photosynthetic rates undergo a temporal shift to an earlier photosynthetic maximum in high-CO_2–grown leaves. In the first place, chlorophyll concentrations were similar between the two CO_2 concentrations early in development, but by day 6, chlorophyll amounts had already begun to decline in high-CO_2–grown leaves; levels in ambient-grown leaves remained relatively constant until about day 16. The rates of chlorophyll loss during senescence were comparable between the two treatments. Second, the CER profiles were generally mirrored by similar changes in Rubisco activity and content in both sets of leaves. This suggests that photosynthetic rates may be determined primarily by Rubisco throughout leaf development.

NEW PARADIGM TO UNDERSTAND ACCLIMATION

Temporal Shift Model

We propose a new model to explain the acclimation phenomenon. This model should be applicable to plants like tobacco whose major sinks are developing leaves. Much research over the years has supported the notion that there is a process that initiates the downregulation of photosynthesis via reductions in photosynthetic proteins after a certain length of exposure to elevated CO_2. The end result is a loss of potential photosynthetic gain under favorable substrate conditions. This is especially evident when photosynthetic rates are measured under normal CO_2 conditions. Figure 2 illustrates this process.

To explain this downregulation, we have proposed a ''temporal shift'' model, which suggests that the lower photosynthetic rates observed after prolonged exposure to enriched CO_2 are due to an earlier onset of the natural ontogenic decline of photosynthesis associated with the senescence phase of development [21]. Figure 3 illustrates how our model differs from a photosynthetic downregulation model based solely on a change in the magnitude of photosynthetic rates that would occur during all leaf developmental phases.

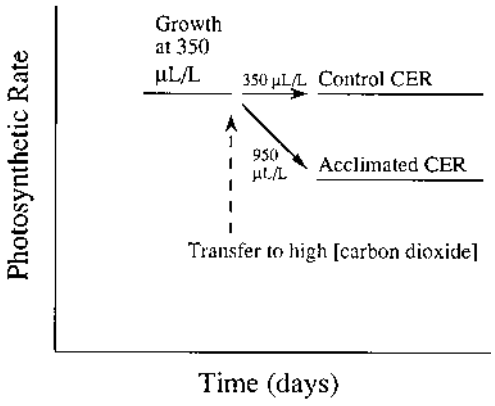

FIGURE 2 Downregulation of photosynthetic rates during a typical ''acclimation'' experiment. Plants in ambient conditions (350 µL/L CO_2) are shifted to high CO_2 (950 µL/L); controls are retained under ambient conditions. Measurements of photosynthetic rates are conducted under ambient conditions after growth for varying amounts of time.

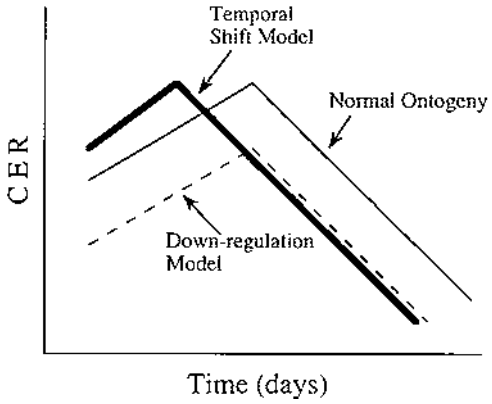

FIGURE 3 The temporal shift model of acclimation. (From Ref. 21.)

Testing the Temporal Shift Model in Reduced Source Strength Conditions

Elevated CO_2 provides an easy method for increasing the source strength of a tobacco leaf. One way to test the validity of the temporal shift model is to decrease the source strength and examine the impact on photosynthetic rates during leaf development. One way that decreased source strength leaves has been achieved genetically in tobacco has been through antisense repression of Rubisco holoenzyme levels [22]. Rubisco is composed of eight large subunit (LS) and eight small subunit (SS) proteins encoded by genes in the chloroplast (*rbc*L) and the nucleus (*rbc*S), respectively. To generate the antisense mutants, a highly expressed member of the tobacco *rbc*S gene family was introduced into tobacco in the antisense orientation behind the highly expressed ''constitutive'' CaMV 35S promoter. The resulting transgenic plants had reduced *rbc*S mRNA and SS protein levels, and the reductions in SS were matched by corresponding reductions in LS and Rubisco holoenzyme amounts in the plastid, indicating that stoichiometric reductions occurred in the accumulation of these proteins in the mutant plants [22]. However, in contrast to *rbc*S mRNAs, *rbc*L mRNA levels were unperturbed in the mutants; it appears that LS accumulation is regulated primarily at the level of *rbc*L mRNA translation initiation in these plants [23]. The reductions in Rubisco range from 10 to 90% of the wild type, and these reductions correlated with antisense copy number—the more *rbc*S antisense DNA present, the greater the repression of Rubisco. The reductions in Rubisco were accompanied by depressed photosynthetic rates, indicating that carbohydrate production (source strength) also was severely reduced in the mutant plants [24].

To test the temporal shift model we have examined CERs and various other photosynthetic parameters during leaf development in the Rubisco antisense plants. We found that CERs are lower throughout development in antisense leaves than in leaves from either wild-type or high-CO_2–grown plants (as expected). However, the onset of the decline in CERs associated with senescence occurred temporally later in leaf development in the mutants (data not shown). This is consistent with the temporal shift hypothesis.

MECHANISM OF THE TEMPORAL SHIFT

As illustrated by the above data, changes in leaf source strength appear to result in a temporal shift in the onset of the decline of photosynthetic rates associated with senescence. Increased source strength caused a shift in photosynthesis to an earlier onset, whereas decreased source strength resulted in a delayed onset. This shift explains why, at a given leaf age, leaves from high-CO_2–grown

plants have lower photosynthetic rates than their ambient-grown counterparts, such as observed in the tobacco acclimation studies of Sicher et al. [12].

The temporal shift model is consistent with the notion that photosynthetic output is determined by the sink status of the plant (sink regulation of photosynthesis) (reviewed in Ref. 2). According to this theory, high photosynthetic rates are maintained in a source leaf as long as there is sufficient sink tissue (sink demand) to consume the carbohydrate that is produced by the source. However, once the demand for photosynthate falls, surplus carbohydrate begins to accumulate in the source. It is thought that this results in a long-term decline in photosynthetic rates due to feedback inhibition of photosynthetic gene expression. Our temporal shift model is entirely consistent with the sink regulation hypothesis. Under increased source strength conditions (as long as the sink status remains unchanged), carbohydrate would accumulate more quickly, resulting in an earlier onset of the photosynthetic downregulation associated with sink limitation. Decreased source strength would have the opposite effect. One possibility is that the changes in source strength are mediated by alterations in gene expression that occur by a sugar-signaling system similar to the catabolite-repression system of yeast (e.g., see Refs. 2, 25, and 26).

There have been suggestions that the acclimation response is modulated by leaf developmental factors (e.g., see Refs. 14–16). The question arises whether the temporal shift in photosynthetic rates is due to changes in photosynthetic gene expression. Our data also raise the question whether the effects of source strength might be more broad and encompass other aspects of leaf developmental programming. We are currently investigating these questions.

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Changes in CO_2 Levels and Their Stress Effects on Photosynthetic Carbon Fixation

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RAISING OF CO_2 IN THE ATMOSPHERE

As the global carbon dioxide concentration rises, we need to understand the combination of direct stress effects of this gas and the anticipated effects of climatic change, including drought, on the physiology and growth of all crops [1]. The current increase in the atmospheric carbon dioxide concentration along with predictions of possible future increases in global air temperatures have stimulated interest in the effects of CO_2 and temperature on the growth and yield of food crops [2] (Fig. 1). The rise in atmospheric CO_2 has been documented continuously since 1958 by Keeling et al. [3], and currently the concentration of CO_2 in air is about 360 μL L^{-1}. The concentration could increase to about 670–760 μL L^{-1} by the year 2075 mainly because of the burning of fossil fuels [4,5]. General circulation models predict that global warming will result from rising CO_2 and other greenhouse gases [6–11].

The stress effects of rising CO_2 and elevated temperatures on tropical plants have received less attention than the effects on temperate species [12]. Because both CO_2 and temperature have large effects on plants, especially those with the C_3 photosynthetic pathway, it is important to quantify the effects of these climatic variables on C_3 food crops [10]. Concern over the well-documented increase in the concentration of carbon dioxide in the earth's atmosphere has stimulated research on the response of plants to this aspect of global change. Much of this research has focused on the response of photosynthetic carbon dioxide fixation, because the process is often dramatically and directly affected by the carbon dioxide concentration, and it is of fundamental importance both to plant growth and to ecosystem carbon storage. The concentration of carbon dioxide in the atmo-

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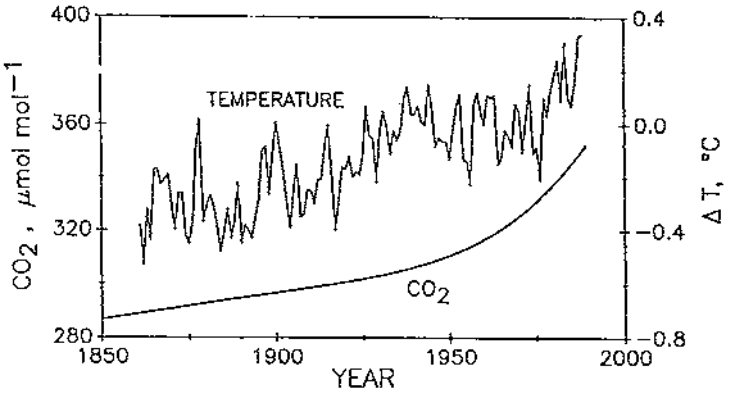

FIGURE 1 Atmospheric CO_2 concentration and estimated mean annual surface temperature changes. (From Ref. 41.)

sphere has increased from about 280 cm^3 m^{-3} prior to the industrial revolution in Europe to approximately 355 cm^3 m^{-3} currently [13]. Depending primarily on future changes in land use and fossil fuel consumption, the concentration may double by the end of the next century [14,15]. As the rate of photosynthesis in C_3 plants is strongly dependent on the CO_2 concentration, this should have a marked effect on photosynthesis, and hence on plant growth and productivity [16].

There is legitimate concern over how the predicted doubling of the CO_2 concentration during the next century will influence the earth's climate, ecosystems, and agricultural production; but substantial fluctuations in CO_2 have occurred in the past [11]. When plants made the transition to land, some 420 million years ago (MYA), the atmosphere may have contained as much as 4000–6000 μmol CO_2 mol^{-1} [17–20]. The rise of vascular plants, and their attendant photosynthesis, was a major factor in the decline of the atmospheric CO_2 concentration, which by 300 MYA may have dropped to near present values [20,21]. Thus, the atmospheric CO_2 concentration has fluctuated perhaps by as much as 20-fold over geological time; so higher values are not a new phenomenon for plants [21]. As CO_2 rises, general circulation models predict increases in mean global temperatures of between 1.5 and 4.5°C and increased, but variable, precipitation patterns [7–9]. However, at regional levels, the magnitude of these changes is uncertain [21].

The principal change to date is in the balance of gases that form the earth's atmosphere. These naturally occurring ''greenhouse gases,'' including carbon dioxide (CO_2), methane, nitrous oxide, and water vapor, keep ground temperatures at a global average of 15°C [22]. Without this natural blanket, the earth's surface would be about 30°C colder than it is today, making the planet a freezing, barren, lifeless place similar to Mars. The greenhouse gases keep the surface warm, because as incoming solar radiation strikes the earth, the surface gives off infrared radiation, or heat, that the gases temporarily trap and keep near ground level. The effect is comparable to the way a greenhouse traps heat. The problem is that human activity may be making the greenhouse gas blanket ''thicker.'' For example, burning coal, oil, and natural gas spews huge amounts of carbon dioxide into the air; the destruction of forests allows carbon stored in the trees to escape into the atmosphere; and other activities such as raising cattle and planting rice emit methane, nitrous oxide, and other greenhouse gases [22].

This chapter examines the stress response of plants to rising atmospheric CO_2 and to the various climatic factors that have been investigated within the last 10–15 years. Within the last 15 years, there have been numerous reports and reviews on stress responses of plants to elevated CO_2 [11,23–42]. Most published studies on the increasing CO_2 concentration in the atmosphere and temperature effects on plants have dealt mainly with temperate crop species, whereas tropical plants

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have received less attention [12,43]. Since both [CO_2] and temperature can have large effects on plants, it is important to quantify the effects of these climate variables on food crops [43].

BIOMASS PRODUCTION

Above- and Belowground Biomass

The growth responses of crops to elevated CO_2 have been reviewed by Acock and Allen [44] and Acock and Pasternak [45]. They suggest that the order of priorities for the use of photoassimilates by plants is (a) survival, (b) reproduction, (c) growth of existing organs, (d) increase in the number of existing organs (mostly by branching or tillering), and (e) storage. When these needs are met, the plant may decrease CO_2 fixation in the face of an imbalance of the source/sink ratio for photoassimilates. As with photosynthetic rates, growth and yield show a wide range of responses to the increasing CO_2 [41]. Most of the studies that show a lack of response of plants to elevated CO_2 are probably due to the inadvertent limitations imposed by experimental conditions, such as restricted rooting volumes [41].

Allen et al. [46] used a nonlinear model to predict photosynthetic, final biomass, and seed yield responses of soybean to CO_2 relative to a CO_2 concentration of 330 μmol mol^{-1}. Table 1 shows that the rise of CO_2 from preindustrial values to present values should cause a 13% increase in seed yield [47]. Furthermore, a doubling from 315 to 630 μmol mol^{-1} could cause a 32% increase in seed yield; an increase that is in close agreement with the survey of plant responses to a doubled CO_2 increase [41] (Table 1). The seed yield responses were less than biomass responses because of greater vegetative growth and less efficient conversion to seed under high levels of stressing CO_2. Plant breeding and selection may be able to provide more efficient conversion of photoassimilates to seed yield in the future [41,48].

Growth and final yield of rice increased across the subambient to the superambient range of CO_2 treatments from 160 to 900 μmol mol^{-1} but tended to flatten out above 500 μmol mol^{-1} more than soybean [49–51] (Fig. 2). Leaf appearance rates were increased as the CO_2 concentration was increased [52]. Furthermore, the final number of mainstem leaves decreased with increasing CO_2. Imai and Murata [53] found small increases in leaf appearance rates for rice plants exposed to elevated CO_2 at 1000 μmol mol^{-1} versus 300 μmol mol^{-1} controls, but Gifford [54] found no effects of CO_2 on the phyllochron interval of wheat [41]. These results indicate that future increases in [CO_2] are likely to benefit rice production by increasing photosynthesis, growth, and grain yield (Fig. 2) and reducing water requirements. As shown in Table 2, in warmer areas of the world, possible future increases in air temperature may result in yield decreases and increased water requirements [42].

Brakke [55] and Brakke and Allen [56] reported that dense canopies of Carrizo citrange and Swingle citrumelo seedlings had a twofold greater photosynthetic rate when exposed to 840 μmol mol^{-1} CO_2 in comparison with 330 μmol mol^{-1}. The elevated CO_2 alleviated midday depression of

TABLE 1 Soybean Responses to Rising CO_2 Predicted by the Nonlinear, Hyperbolic, Modified Michaelis-Menten Model

Soybean responses	CO_2 change	Photosynthesis	Biomass	Seed
Year	(μmol mol^{-1})	% increase		
1800–1958	276–315	12	10	8
1800–1986	276–345	20	17	13
1958–2058	315–630	53	43	32

Source: From Ref. 46.

TABLE 2 Effects of Plant Physiological Responses to Rising Atmospheric CO_2 Concentration and Temperature

Elevated CO_2 concentration
enhances photosynthetic CO_2 uptake, especially by C_3 plants
decreases stomatal conductance
results in increased partitioning to roots
directly inhibits plant respiration and may reduce stress tolerance
enhances biomass accumulation of woody plants
enhances C_3 plant photosynthesis at higher temperature
Increased temperature is likely to increase plant and ecosystem respiration.
Higher temperature in combination with elevated CO_2 concentration promotes vegetative biomass accumulation.
Rising CO_2 concentration and increasing temperature are likely to result in a net carbon sequestration by plants.
Under high CO_2 levels, stress effects may be delayed for a few days in some crops because of the higher level of leaf starch build-up and lower stomatal conductance to water loss.
In warm areas of the world, however, possible future global warming may result in both substantial yield decreases and increased water requirements.

Source: From Ref. 42.

photosynthetic rates due to either atmospheric evaporative demand stresses or low available soil water stresses [41]. Jurik et al. [57] also found that species of northern hardwood forests had leaf photosynthetic rates about 2.5-fold higher when exposed to 1900–2500 μmol mol^{-1} CO_2 in comparison with ambient CO_2 [41].

Grain yield increases in both wheat (*Triticum aestivum* L.) [50,54,58–60] and rice [53,61–63] have been shown to result from [CO_2] enrichment. This increased grain yield is often associated with increased tillering and the production of more spikes in wheat [54,58–60] or panicles in rice [50,63]. Yields of rice cultivar IR-30 declined by 10% for each 1°C rise in day-night temperature above 28/21°C, and elevated CO_2 had little effect in ameliorating this temperature response [51]. Sharp decreases in the number of filled grains per panicle accompanied these yield decreases [10]. Total growth duration of rice was shortened by 10–12 days across a CO_2 concentration treatment range of 160–500 μL/L because of a shortened vegetative phase of development and a reduction in the number of mainstem leaves formed during this period [10]. Carbon dioxide enrichment from 330 to 660 μL/L increased grain yield mainly by increasing panicles per plant; whereas increasing temperature above 28, 21, and 25°C resulted in decreased grain yield largely because of a decline in filled grains per panicle. Grain yields were highest at a weighted mean temperature of 26°C and declined by about 10% per each 1°C rise in temperature above 26°C [10].

Net Assimilation Rate

No differences between crops and weeds or between cool- and warm-climate species were found in the responses of growth or photosynthetic acclimation to elevated carbon dioxide [64]. The photosynthetic response and acclimation to elevated carbon dioxide differed depending on the photosynthetic photon flux density (PPFD) used to measure photosynthesis. This could greatly complicate predictions of the relative stimulation of the net assimilation rate (NAR) by stresses at elevated carbon dioxide in variable PPFD environments [64]. In soybean, reduced photosynthesis after acclimation to elevated carbon dioxide assayed at high PPFD was accompanied by a reduction in the quantum yield of photosynthesis [64,65].

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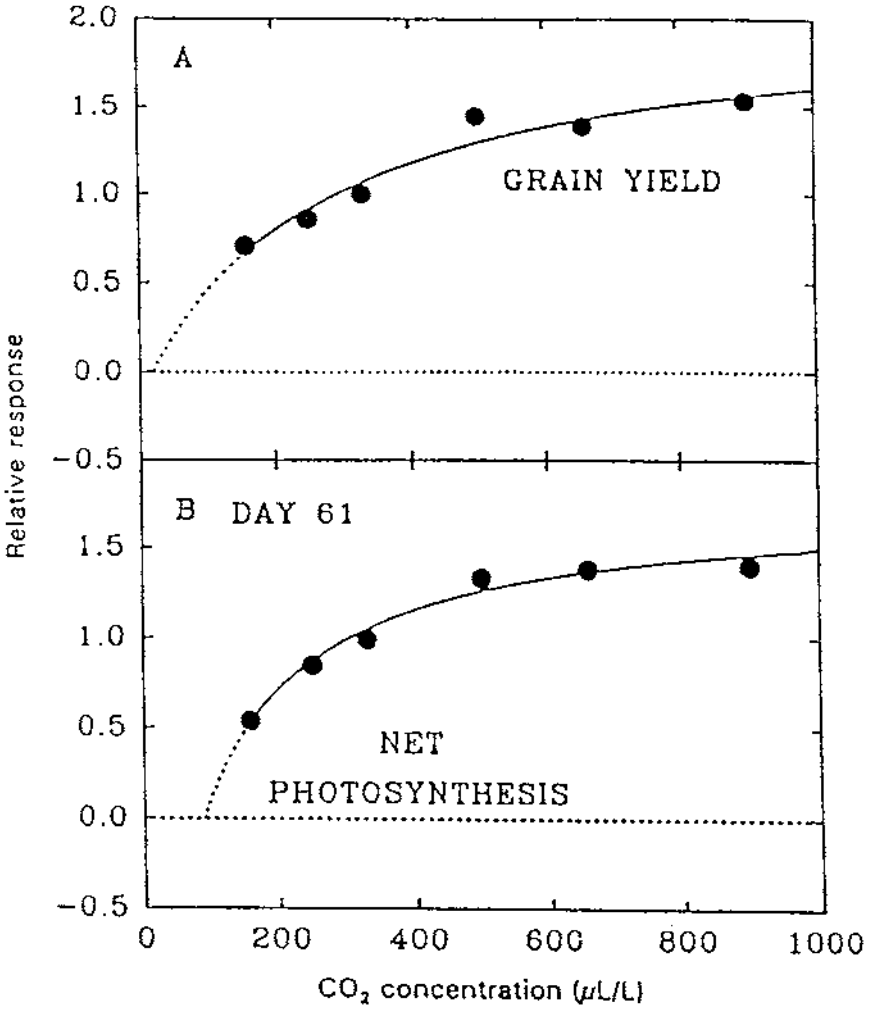

FIGURE 2 (A) Rice grain yields and (B) net photosynthesis of canopy in response to CO_2 concentration normalized to the values obtained from the 330 μl/L CO_2 treatment. (From Ref. 51.)

Relative Growth Rate

Increases in the relative growth rate (RGR) at elevated carbon dioxide were more highly correlated with the relative increase in NAR at elevated carbon dioxide than with the response of the leaf assimilation rate (LAR) [64]. Response curves of the photosynthetic rate to temperature at ambient and elevated CO_2 concentrations indicate that the photosynthesis and biomass accumulation of many C_3 plant species could increase with stresses imposed by increasing CO_2 concentration and increasing temperature [66], at least up to a threshold value or temperature optimum [42].

We do not know how universal the enhancement of photosynthesis and vegetative growth may be when plants are exposed to a combination of elevated CO_2 levels and high temperatures. Nevertheless, higher temperature in combination with CO_2 enrichment appears to enhance vegetative biomass accumulation in C_3 plants, and this relationship might contribute to a negative feedback

on the atmospheric CO_2 increase [42]. Reproductive growth of plants appears to respond quite differently to temperature increases than does vegetative growth [67]. Baker and Allen [51] reported that in a cultivar of tropical lowland rice (cv. IR-30) grain yield decreased linearly about 10% for each 1°C increment across the range of 26 to about 36°C under CO_2 treatments of both 330 and 660 ppm [42].

Atmospheric carbon dioxide is known to affect plant yield. Kimball [24] reviewed 430 observations of carbon dioxide–enrichment studies conducted prior to 1982 and reported an average yield increase of 33% plus or minus 6%, for a doubling of the carbon dioxide concentration [68]. This value has been generally confirmed by many other studies since that time. The yield increases seem to apply for both biomass accumulation and grain yield. Thus, plants may grow larger and use more water as the global carbon dioxide concentration increases. Soybean seed yields and biomass yields are predicted to increase 31 and 41%, respectively, from a doubling of carbon dioxide. A small decrease in the soybean harvest index under elevated carbon dioxide conditions has been commonly observed.

STOMATAL CONDUCTANCE AND WATER-USE EFFICIENCY

The responses of photosynthesis to the stress effect of increasing carbon dioxide concentration in terrestrial C_3 gymnosperms and angiosperms are presented here. Photosynthesis is the only one of several plant processes affected by an elevated carbon dioxide concentration [11,15]. For example, stomatal conductance to water loss is often reduced as carbon dioxide concentrations increase, and this may alter plant resposes to drought [70]. At strongly limiting carbon dioxide concentrations, carbon fixation is thought to be limited by ribulose bisphosphate carboxylase oxygenase (EC 4.1.1.39; Rubisco) activity. Since the pathway of carbon dioxide movement includes the stomatal pore, changes in pore size caused by changes in the carbon dioxide concentration in the substomatal air space [71] also often occur. Thus, in order to predict the response of photosynthesis to carbon dioxide concentration when the concentration is limiting, it is necessary to know the response of stomatal resistance [15].

Although stomatal conductance may be decreased about 40% for doubled CO_2, water use by C_3 crop plants under field conditions will probably be decreased only up to 12%. If the leaf area increases due to doubled CO_2 are small, then the transpiration reductions may be meaningful, albeit small. If the leaf area increases due to doubled CO_2 are large, then no reductions in transpiration are to be expected, and even increases in water use may be possible [41].

Long-term studies show only a small effect of elevated CO_2 on reducing transpiration per plant, because the plants tend to have a larger leaf area with slightly greater foliage temperature. Water-use efficiency (WUE) is increased by elevated CO_2, but the effect on C_3 crops is mediated primarily by enhancing photosynthesis and growth and only secondarily by decreasing transpiration [41]. Stomatal conductance is inversely related to the CO_2 concentration [72,73]. Atmospheric water vapor is the most important greenhouse gas [74], and the effects of stomatal closure elicited by CO_2 stresses on climate via hydrological cycles are unclear [42].

The link between CO_2 and stomatal conductance appears to be at the level of intercellular CO_2 [71]. Because an increase in the CO_2 level accelerates photosynthesis, slows photorespiration, and decreases leaf surface conductance, the instantaneous WUE on a leaf area basis is positively related to the CO_2 concentration [75]. Although decreased stomatal conductance owing to an elevated CO_2 concentration can decrease transpiration on a leaf area basis, an increased leaf area resulting from an elevated CO_2 concentration may offset the leaf-level transpiration decline when transpiration is considered at the level of the canopy [68,70]. Increased WUE under elevated levels of CO_2 may be due to increased CO_2 assimilation rather than to decreased transpiration, especially for C_3 species [75,76]. Decreased stomatal conductance and leaf-level transpiration will tend to increase the leaf and canopy temperature. The increase in the canopy temperature has the potential to accelerate the

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developmental rate at a given air temperature. Finally, decreased stomatal conductance under elevated CO_2 levels may contribute to a small positive feedback to increasing surface temperatures [42]. Increased carbon dioxide concentrations are known to cause smaller stomatal apertures and hence to decrease the leaf conductance for water vapor [73]. This is a second mechanism whereby stresses caused by increased carbon dioxide concentrations may affect plant transpiration [68]. Kimball and Idso [77] calculated a 34% reduction in transpiration in response to a doubled carbon dioxide concentration in several short-term plant growth chamber experiments. Morison and Gifford [78] also showed that doubling carbon dioxide will cause a more rapid development of the leaf area for many plants and hence an equal or greater transpiration rate in the early stages of plant growth due to a more rapid development of transpiring surfaces. Therefore, increased rates of development of transpiring leaf surface offset the reduced stomatal conductance for water vapor [68].

An increase in WUE of carbon dioxide–enriched crops is largely due to sizable increases in photosynthesis, growth, and yield. Louwerse [79] reported that differences in stomatal behavior are only partly species specific and depend mainly on growing conditions [1]. Allen, et al. [1] reported that leaflets at high CO_2, either water stressed or well watered, had higher photosynthetic and lower transpiration rates, and therefore higher water-use efficiencies than those at control CO_2 levels. Leaf conductances are goverened by CO_2 assimilation rates under water-stressed as well as unstressed conditions [1]. Leaves adapted to high CO_2 had higher WUE than leaves adapted to ambient CO_2 mainly because of a twofold increase in carbon exchange rate [80]. Increases in WUE with increasing CO_2 were realized by both substantial increases in CO_2 uptake and somewhat smaller decreases in water loss. Increasing temperatures greatly increased water use and decreased photosynthetic WUE [10]. The cause and effect relationships can be summarized as follows: Any reduction in stomatal conductance due to increasing carbon dioxide concentration will restrict transpiration rates per unit of leaf area. A reduction in transpiration rates will result in less evaporative cooling of the leaves and the leaf temperatures will rise [68].

PHOTOSYNTHETIC RESPONSES TO CO_2 STRESS

Sites of Action of CO_2

Atmospheric O_2 also interacts with Rubisco; as a competitive inhibitor with respect to CO_2 and as a substrate for the monooxygenase activity of this bifunctional enzyme to produce phosphoglycolate [21]. Through O_2 inhibition and photorespiration, the present atmospheric CO_2/P_2 ratio causes about a 35% reduction in the photosynthesis of C_3 plants at 25°C, and higher temperatures amplify this inhibition. Because of the competitive interaction, as CO_2 rises, it will diminish the inhibitory effects of O_2; a doubling of the present CO_2 concentration should more than halve photorespiration. Thus, increasing the CO_2 supply for C_3 species not only provides more of a limiting resource (carbon) but also has the potential to improve the use of other resources and raise the optimum temperature for photosynthesis [38]. This effect is analogous to the CO_2-concentrating mechanism (CCM) of C_4 species [21]. Somewhat surprisingly, a stress of doubling in CO_2 concentration also reduces dark respiration in a number of species [32].

Another interesting facet of increased CO_2 enrichment is the apparent action of CO_2 as a growth modulator. A doubling in CO_2 can cause changes in anatomy, morphology, and phenology [29,31]. These indirectly influence photosynthesis by altering various plant characteristics, including branching, leaf area, duration of assimilation, and sink capacity [11,21]. Several CO_2-dependent promoters have been detected in the cyanobacterium *Synechococcus*. These may contain regulatory regions that respond directly to CO_2 concentration or indirectly via metabolites in the photorespiratory carbon oxidation (pCO) pathway whose concentrations change with the CO_2/O_2 ratio [81,82]. The CO_2-concentrating mechanism of C_4 plants allows CO_2 to compete more effectively with O_2 for binding sites on the enzyme, ribulose biphosphate carboxylase oxygenase (RuBP; Rubisco) [41]. Therefore, C_4 plants have a more efficient photosynthetic apparatus than C_3 plants (at temperatures above 25°C) under recent atmospheric concentrations of CO_2 (270–355 μmol mol^{-1}) and do not

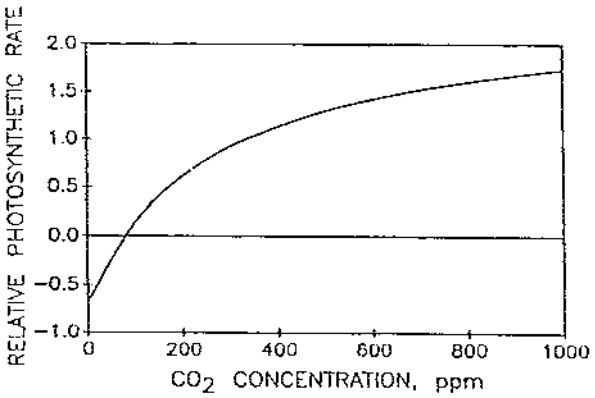

FIGURE 3 Photosynthesis carbon dioxide uptake rate responses of soybean canopy exposed to various carbon dioxide concentrations. (From Ref. 46.)

increase photosynthetic rates as much as C_3 plants in response to increasing levels of CO_2 (Fig. 3); in fact, C_4 photosynthetic rates are near CO_2-saturation under current levels of atmospheric CO_2. As the CO_2 concentration continues to increase, C_3 crop photosynthetic rates should approach or exceed those of C_4 crop plants, because CO_2 can compete with O_2 for binding sites on Rubisco more effectively, and because the C_3 plants would not have to expend energy for the CO_2-concentrating process [41].

Effects of Increasing CO_2 on Crop Photosynthesis and Productivity

An increasing CO_2 concentration confers a selective advantage on the C_3 plants and puts them into an increasingly favorable competitive position. An increased carbon gain by C_3 plants would allow them either to increase root growth and compete more successfully with their C_4 neighbors for nutrients or increased foliage production to compete more successfully for available light. The continuously improving photosynthetic performance of C_3 plants should put great competitive pressure on neighboring C_4 plants, especially in warmer regions where the improvements in the performance of C_3 plants should be most marked [16]. Mechanistic formulations of the direct CO_2 effects on photosynthesis have been incorporated in some physiology-based models, whereas modifications incorporating direct CO_2 effects in nutrient-driven models have usually been more empirical. Physiology-based models predict considerable CO_2-fertilizer effects, whereas nutrient-driven models tend to be less sensitive to an elevated ambient CO_2 concentration [83].

Weed growth was consistently increased by carbon dioxide enrichment, but weed species' composition was unaffected. Canopy carbon dioxide uptake was slightly higher in the elevated carbon dioxide treatments, which was consistent with the increased weed growth. In alfalfa, elevated carbon dioxide significantly reduced canopy carbon dioxide efflux at night for the same daytime uptake rate and temperature [84]. The optimum temperature for photosynthesis is increased at elevated carbon dioxide [15].

Even at limiting photon flux, the photosynthetic rate of C_3 plants responds positively to increases in carbon dioxide concentration [85]. It gradually became recognized that part of the syndrome of the C_4 pathway of photosynthesis was saturation of the rate of carbon dioxide fixation at lower carbon dioxide concentrations than it occurs in C_3 plants. Photosynthesis in C_4 plants is often near or at saturation for carbon dioxide at the current atmospheric concentration even at high photon

flux. This explains why the growth rate of C_4 plants is often relatively insensitive to increases above that concentration [86] and no stress effects whatsoever are observed. Although we now understand the basis of much of the observed variation in the short-term response of photosynthesis to carbon dioxide concentration, the long-term response is still largely unpredictable [15]. It has been recognized for nearly 20 years that long-term exposure to elevated carbon dioxide concentrations can induce metabolic stress and reduce photosynthetic capacity [87]. In spite of considerable research effort and significant progress, the ability to predict the occurrence and magnitude of ''photosynthetic adjustment'' to the stress effects caused by carbon dioxide concentrations remains elusive. In the basic C_3 photosynthesis models, carbon dioxide saturation of photosynthesis indicates limitation by RuBP regeneration. Long and Drake [88] found no long-term reduction in quantum yield after years of exposure of a salt-marsh species to double the ambient carbon dioxide concentration but rather a continued stimulation when leaves were measured at the elevated carbon dioxide concentration. A common symptom of negative photosynthetic adjustment to elevated carbon dioxide is the low nitrogen content of leaves. However, Bunce [89], in soybean and sugar beet, and Wong [90], in cotton, have found that negative photosynthetic adjustment could not be overcome by high nitrogen application rates [15].

Several reviews and assessments of the response of plants to rising levels of atmospheric CO_2 have been published within the last 15 years [11,23–42]. However, decreased rates of photosynthesis have sometimes been reported when plants are exposed to elevated CO_2 for prolonged intervals [41,91].

Direct Effects of Carbon Dioxide on Plants

Growth of C_3 plants at elevated CO_2 levels has resulted in reduction of the photosynthetic capacity and Rubisco activity [92,93], but photosynthetic capacity was maintained [94] or even increased in other cases [33,95–97]. Photosynthesis of C_3 plants generally increases nonlinearly with increasing CO_2 [41] (see Fig. 3). This type of nonlinear response appears to be universal for C_3 plants. Many early studies tended to focus on the negative responses of plants to prolonged exposure to elevated CO_2 [23], but recently the numerous findings of positive responses have gained greater recognition [33,96,97]. A wide range of positive responses of leaves and plants to elevated CO_2 was reviewed by Allen [41] and suggested some inadvertent limits that experimental techniques may have imposed on plant responses to elevated CO_2.

Starch accumulation increased at a greater rate as the CO_2 exposure level increased [98] imposing a stress condition on the photosynthetic apparatus. More starch accumulates in leaves under high CO_2 treatments, with much of the starch being digested and exported at night [98–101]. However, if low temperature, lack of sink, or some other limitation to growth prevents mobilization and translocation of photoassimilates, starch can accumulate in leaves up to 40% or more of dry weight [87,102–104]. The probable cause of decreases in photosynthetic rates after prolonged exposure to CO_2 is an excess of photoassimilate production in leaves with respect to photoassimilate utilization by the plant or crop system [87,105,106]. The process of photosynthetic rate reduction under conditions where source exceeds sink has been called endproduct feedback inhibition of photosynthesis. Endproduct feedback inhibition has been invoked as a concept for downregulating leaf photosynthetic rates and is considered as a marked stress effect to elevated CO_2 concentration [41].

Biochemical feedbacks on enzymes such as inhibition of RuBP carboxylase activity by phosphorylated sugar via competition with RuBP for binding sites [107] and inhibition of sucrose-phosphate synthase would favor starch formation at the expense of sucrose synthesis [108]. Another suggested mechanism is that triose phosphate is inhibited from forming sucrose in the cell cytosol and prevents the normal cycling of inorganic phosphate back into the chloroplast, thus slowing photosynthesis [109]. Loss of capacity for sucrose synthesis is a more likely mechanism involved in downregulation of leaf photosynthetic rates under the duress of the increasing concentration of CO_2 than is a direct effect of endproduct inhibition [41,110].

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The bonsai syndrome certainly would call into question any attempt to extrapolate quantitatively growth chamber work conducted with small rooting volumes to field agronomic conditions. Furthermore, the changes in photosynthetic capacity that appear to be downward regulation in many studies may be an artifact of the imposed experimental conditions. The downward acclimation of rice canopy photosynthetic rates was accompanied with a 66% decrease in the total Rubisco activity [41]. The flattening out of the canopy photosynthetic rate in response to CO_2 above 500 μmol mol^{-1} was attributed to the reduction in Rubisco activity [51,111].

Crassulacean acid metabolism (CAM) species respond positively to elevated CO_2 [112,113], but they generally display lower photosynthetic rates than either the C_3 or C_4 plants. CAM plants fix CO_2 (actually HCO_3^-) into organic acids such as malate at night. During the day, malic acid is decarboxylated by the similar mechanisms present in C_4 bundle sheath cells and the resulting CO_2 is assimilated by the C_3 pathway. Thus, all three plant types (C_3, C_4, and CAM) use the C_3 pathway during some stage of photosynthesis, but C_3 plants show the greatest potential for response to elevated CO_2 levels. Because most plants are C_3 species, we would expect that on a global scale photosynthesis is likely to increase with increasing atmospheric CO_2 [42].

Many of the early studies on the effects of an elevated CO_2 concentration on plants reported initial increases in the leaf or canopy photosynthetic rates followed by a decrease after exposure for a number of days or weeks as the photosynthetic rates acclimated [42]. Other studies however, have shown no decreases or even increases in photosynthetic rates during long-term exposures to elevated CO_2 levels [11,33,76,80,114–117]. In cases where decreases in photosynthesis have been observed, starch tended to accumulate in leaves [118,119] rather than being translocated to sinks of photoassimilate such as growing leaves, roots, and fruits. Root growth restriction might have played a role in several observed declines in the photosynthetic capacity following the long-term CO_2-enrichment stress effect [104]. Starch accumulation is not, however, always associated with inhibited leaf photosynthesis [101], and the mechanism responsible for the potential feedback inhibition of photosynthesis is likely to involve insufficient organic phosphate for sucrose formation and export rather than direct effects of starch accumulation in leaf chloroplasts [35,110,120].

The response of leaves to an elevated CO_2 concentration may depend on the inherent sink strength of a species [35]. The upregulation or downregulation of photosynthesis may involve anatomical as well as biochemical responses [42]. For instance, photosynthetic capacity per unit leaf area may be increased as a result of increasing leaf thickness accompanied by production of an additional layer of palisade cells, as observed in soybean (*Glycine max* L.) [87,121,122]. On the whole, present data show that CO_2 enrichment enhances net photosynthesis in C_3 plants, and this situation might contribute to a negative feedback on atmospheric CO_2 increase at least in the short term [42].

Valle et al. [115] concluded that during seed fill, leaflets adapted to high CO_2 environments exihibited a capacity to utilize CO_2 and radiation more efficiently at elevated CO_2 and throughout all light levels than leaflets grown at low CO_2.

Response curves of the photosynthetic rate to temperature at ambient and at elevated CO_2 concentrations indicate that photosynthesis and biomass accumulation of many C_3 plant species could increase both with increasing CO_2 concentration and increasing temperature at least up to some maximum value (Fig. 4). However, the reproductive growth and grain yield of crops like tropical lowland rice may decrease steadily with increasing temperature above their temperature optima regardless of the concentration of CO_2 [41].

Increasing atmospheric carbon dioxide levels have caused increasing photosynthetic rates, biomass growth, and seed yield for all of the globally important C_3 food and feed crops [29,44,123]. Some plants, such as cucumber, cabbage, and perhaps tomato, have shown a tendency first to increase leaf photosynthetic rates in response to elevated carbon dioxide concentrations and then to decrease photosynthetic rates after several days. This behavior is called endproduct inhibition of photosynthesis, and it is caused by the failure of translocation of photoassimilates to keep up with the photosynthetic rates [118].

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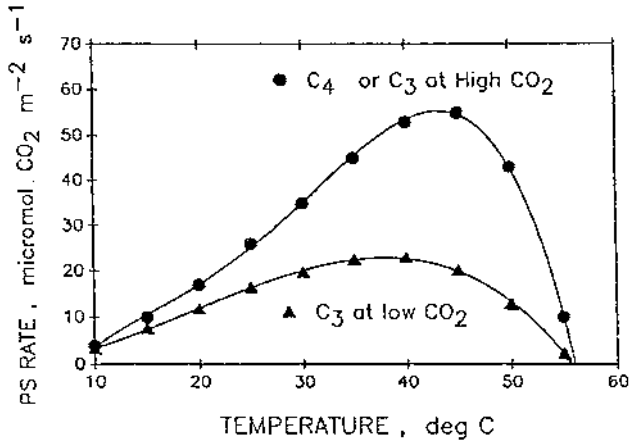

FIGURE 4 Photosynthetic rate versus temperature for C_3 and C_4 leaves. (From Ref. 91.)

Diurnal Changes in Response to Canopy Photosynthetic Rate to Elevated CO_2 in a Coupled Temperature–Light Environment

Photosynthesis is significantly more sensitive to CO_2 at higher temperatures than at lower temperatures. Consequently, growth responses to CO_2 would also be expected to be much more pronounced at higher than at lower temperatures. The interaction between CO_2 and temperature also needs to be taken into account in the interpretation of experimental data [16]. Galtier et al. [124] reported that sucrose phosphate synthase (SPS) activity is a major point of control of photosynthesis, particularly under saturating light and CO_2.

At high CO_2 and saturating light, photosynthesis is limited by the electron transport capacity and the rate of regeneration of the the substrate for carboxylation, RuBP [125–127], which is determined by the rate of electron transport that provides the NADP and ATP to drive the Benson-Calvin cycle. ATP synthesis, in turn, may be restricted by the availability of Pi. The relative importance of each of the above factors in limiting the overall photosynthetic rate depends largely on the prevailing environmental conditions. Increasing irradiance decreases the limitation by the RuBP regeneration phase, whereas increasing the atmospheric CO_2 concentration shifts the limitation away from Rubisco [124].

We do know that leaf photosynthetic rates of C_3 plants are enhanced more by elevated CO_2 at high temperatures than at low temperatures [26,44,128]; therefore, it would seem logical to expect increased growth rates when elevated CO_2 is accompanied by higher temperatures at least up to some maximum temperature. Reproductive growth of plants appears to respond quite differently to temperature increases than vegetative growth [41]. For example, Baker et al. [129] and Baker and Allen [51] reported that rice (cv. IR-30) grain yield decreased linearly about 10% for each 1°C increment across the range of 26 to about 36°C under CO_2 treatments at both 330 and 660 μmol mol^{-1}. The steady decline in rice grain yield with increasing temperatures was accompanied by a sharp decline in the number of filled grains per panicle, small declines in grain mass per seed, and small increases in the number of panicles per plant [41,51,129]. Photosynthetic rates of C_4 plants tend to increase with temperature to a greater extent than those of C_3 plants at current levels of atmospheric CO_2 [42].

All crops will respond more to the combination of increasing temperature with elevated carbon dioxide than to elevated carbon dioxide levels alone. However, during reproductive growth of soybean plants, the opposite trend was found. The complex responses of various kinds of plants to

interactions of carbon dioxide, temperature, water supply, light, and photoperiod (day length) need further research [68]. Temperature excerts a major influence on rice growth and yield. Biomass accumulation increases with increasing paddy water temperature from 18 to 33°C [130,131]. Tillering is similary stimulated with increasing temperature across a temperature range from 15 to 33°C [132]. Rice grain yield is negatively correlated with air temperature during the reproduction phase of growth [133]. Studying grain filling responses to temperature, Yoshida and Hara [134] found that the optimum temperature range for maximum grain weight was 19–25°C for an indica cultivar (cv. IR-20) and 16–22°C for a japonica cultivar (cv. Fujisaka 5) [43].

Raising CO_2 Levels and Their Potential Significance for Carbon Flow in Photosynthetic Cells

Crop biomass accumulation and grain yield are positively related to CO_2 concentration. Fruit trees and some vegetable crops appear to be just as responsive as soybean to increases in atmospheric CO_2 levels [42,55,56,135–140]. Forest tree leaf photosynthesis responds positively to increases in CO_2 concentration [57,117]. Growth and biomass accumulation of woody species under conditions of increasing atmospheric CO_2 will contribute to a negative feedback on CO_2 accumulation [42]. Only a limited amount of work concerning interactions between rising temperature and CO_2 has been conducted [42,66,70].

Adaptation to Changes in Atmospheric CO_2

For the past 20–30 million years, terrestrial vegetation has had to cope with stresses associated with a CO_2-poor atmosphere, and it has become progressively more adapted to such conditions. In contrast, within just the last 200 years, the planet's flora has experienced about a 28% rise in CO_2 concentration. This raises questions as to what extent readaptation is occuring and whether an increase in CO_2 concentration is stressful for plants adapted to CO_2-depleted conditions [21]. By examining herbarium specimens of temperate arboreal species from AD 1787 to the present, Woodward [141] first reported a 67% decline in stomatal density. Concomitantly, Woodward [141] calculated WUE to be improved by twofold during that time period. CO_2 was affecting stomatal initiation and not just epidermal cell expansion [142–144]. From such data it appears that stomatal conductance and atmospheric CO_2 concentration are negatively correlated over the past 16,500 years [142]. Along with decreases in stomatal density, herbarium specimens are suggestive of improvements in WUE with increasing CO_2 concentration [142,145]. Not all studies have reached the same conclusion. Korner [146] was unable to detect significant differences in stomatal density for over 200 lowland and alpine species from literature measurements of the past 100 years [21].

An interesting geological phenomenon that is being exploited to study the adaptation of plants to high CO_2 concentration is geothermal gas vents, whose emissions contain as much as 96% CO_2 [21]. So plants in the vicinity can be exposed to 10,000 μmol CO_2 mol^{-1} potentially over hundreds of years [147]. *Quercus pubescens* growing among natural gas vents in central Italy showed no differences in stomatal density or index as a function of distance from the vents; however, near the vents, the mean guard cell sizes and pore lengths were reduced [21,147,148]. Underground limestone springs in Florida are supersaturated with dissolved CO_2 concentrations of several hundred micromolars [149]; where they exit the ground, they become a source of CO_2 for submersed and terrestrial vegetation in the vicinity. When grown under a CO_2-enrichment regimen, *Boehmeria cylindrica* seeds collected from naturally enriched populations produced plants with significantly greater stem and root dry weights than seeds from a nonenriched population [21].

More studies with naturally enriched populations or with a ''fast plant'' such as *Arabidopsis thaliana* are required to establish unequivocally that photosynthetic adaptations to rising CO_2 occur in a short period (decades) [21].

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Diversity in Photosynthetic Responses to CO_2-Enrichment Species Differences and Acclimation Mechanisms

A major recent development in the understanding of photosynthetic responses to carbon dioxide concentration is the proposed phosphate limitation mechanism of feedback inhibition of photosynthesis [150]. Under this hypothesis, chloroplast phosphate concentration limits the short-term photosynthetic response to increased carbon dioxide concentration when the rate of photosynthate production would exceed the rate of photosynthate use in starch and sucrose synthesis. Since phosphate limitation is determined by the photosynthetic rate, it may not occur at lower PPFD [150]. Therefore, photosynthesis at cooler temperatures early or late in the day is not likely to be phosphate limited, because low PPFD would reduce the photosynthetic rates below those capable of causing feedback inhibition [21]. Methodologies to elevate the CO_2 around plants under field conditions, including soil-plant-air research (SPAR) units, open-top chambers, and free-air CO_2-enrichment systems, have drawbacks [151,152]. So possible artifacts have to be taken into account when evaluating responses to elevated CO_2 [21].

The capacity of C_3 species to respond to CO_2 does differ, and it may be related to the species' ecological niche. For an approximate doubling of the CO_2 concentration, growth of C_3 species was stimulated on an average by 41%. Crops, which tend to be C-strategy species, increased more in dry weight than wild species (58 vs 35%) Fast-growing wild species were stimulated more than the slow-growing ones (54 and 23%, respectively). Webber et al. [153] suggested that the differences may be related to sink capacity [21]. Long-term exposure to a doubling in CO_2 concentration leads to a variety of acclimation effects that directly or indirectly influence the photosynthetic capacity of the plant. In addition to changes in photosynthetic biochemistry and stomatal physiology, acclimation has been observed in the leaf area, leaf area index, leaf area duration, leaf thickness, branching, tillering, stem and root dry weights, fruit size, timing of developmental events, and life cycle completion [29,31,38]. Consequently, even if photosynthesis per unit leaf area declines, changes in parameters such as leaf area or duration can result in greater biomass and yield [21, 29,38,154,155].

Downregulation is a common CO_2-enrichment response in the literature, although whether it would be so in nature is still an open question. No downregulation and even upregulation have been reported for C_3 species in these circumstances, including crop plants: cotton, soybean, and kidney bean. In some field situations, acclimation does seem to occur; there are reports of C_3 species that failed to maintain photosynthetic or growth enhancements under natural field conditions; notably the sedge, *Eriophorum vaginatum*, in an arctic tundra ecosystem, and *Poa pratensis* in a tall-grass prairie [156,157]. Low temperature and competition for light, water, and nutrients may have restricted the CO_2-enrichment response.

Is downregulation a stress response indicating physiological dysfunction in plants that over millennia have become adapted to low CO_2? or is it an optimization process in reaction to a change in resources? In some species or conditions, elevated CO_2 produced substantial carbohydrate accumulation within the leaves, which could be stressful. The leaf morphology can be deformed; massive starch granules can distort chloroplasts and possibly disrupt function by distorting the thylakoid membrances and imposing constraints on the diffusion of gases or metabolites [35,158,159]. However, in most instances, downregulation of CO_2 assimilation probably reflects a restricted capacity to handle the extra carbon because of either insufficiency in other environmental resources or inherent metabolic limitations. Photosynthetic acclimation is an optimization process rather than a stress response [21]. According to this concept, acclimation involves the reallocation of resources away from nonlimiting components such as carbon acquisition and into more limiting components such as light harvesting, electron transport, and carbohydrate handling, thereby minimizing single limitations [158–160]. The resource reallocation would predominantly involve N, because CO_2 enrichment increases the C/N ratio of plants [90,161].

Various biochemical components have been implicated in acclimation, with Rubisco having the leading role. Rubisco is modulated by growth at elevated CO_2, with reports of reduced activity

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in a number of species [90,93,154,162–166]. A decrease in Rubisco activity may be manifested by a decline in the Rubisco protein content, a lowered activation state, an inhibition of the carbamylated enzyme, lower specific activity, or altered kinetics; but not all apply to CO_2 enrichment [158]. Decreases in the Rubisco protein content are observed in some cases as much as 60%; indicating that N is being reallocated [93,164–166]. However, Rubisco protein may still decline in the presence of adequate N supplies, and the reduction is not always sufficient to account for the lower N concentration [89,161,165]. Also, some species show no decline in Rubisco content [88,89,154,167]. Consequently, changes in the Rubisco content alone cannot always account for the acclimation phenomena that have been observed [21].

Perhaps the most often cited rationale for acclimation and the downregulation of Rubisco is that CO_2 enrichment causes an imbalance in the source-sink capacities; especially insufficient sink capacity for the excess carbohydrate production [35,168–171]. Studies on plants with large sinks and manipulations of the sink capacity have led to this conclusion, and there is much evidence to commend it. Accordingly, N would be reallocated to upgrade the sink capcity and/or reduce the source capacity to bring the two into confluence [21]. The mechanism by which the imbalance is sensed, at least in part, is likely to involve feedback effects via endproduct accumulation [35,170]. This was indicated by a number of sugar-feeding studies which resulted in reduced photosynthesis and the Rubisco activity and its content [21]. Similarly, the overexpression of acid invertase in transgenic plants, and the resultant hexose accumulation, decreased photosynthesis and the polymerase chain reaction (PCR) cycle enzyme activities [21,170,172].

How is the feedback exerted? In CO_2-enriched cotton and kidney bean, the presence of O_2-insensitive photosynthesis points to a Pi limitation of the RuBP regeneration capacity, because carbohydrate accumulation ties up Pi [21,167,173]. However, this cannot be the sole reason, as it does not explain changes in extractable enzyme activities and amounts. A molecular model invokes the metabolite regulation of gene expression [35,170]. Glucose provides a regulatory signal that represses the transcription of photosynthetic genes, including those encoding the small and large subunits of Rubisco, *rbc*S, and *rbc*L [21,170,174].

In addition, genes involved directly with carbohydrate metabolism can be positively or negatively regulated by sugars [170]. This could be a means to upregulate enzymes that process carbohydrate and thereby assist in balancing the sink capacity with the source [21]. This concept is consistent with the findings that, in CO_2-enriched rice with increased sucrose and starch, the activity of SPS is upregulated about 20%, whereas the Rubisco activity and its content are downregulated [165,175]. A similar situation occurs in the sink-limited regions of transgenic tobacco leaves which have invertase overexpressed in the cell walls; Rubisco and fructose bisphosphatase activities decline but SPS increases [172]. By way of contrast, CO_2 enrichment of kidney bean causes some reduction in the SPS activity [21,167]. More work is required to determine how CO_2 enrichment influences the enzymes and allocation of carbohydrates in plants that are predominantly starch or sucrose accumulators [21].

In C_3 species, elevated CO_2 not only reduces the amount of carbon entering the PCO cycle but concomitantly the flux of N through the associated photorespiratory N cycle [22]. Photorespiratory N flux is large, being up to 10-fold greater than N assimilation [161]. A high-CO_2–induced reduction in this flux should have a substantial impact on N metabolism, but it has received minimal attention. Leaf N concentration is generally lowered by CO_2 enrichment, as are the nitrate reductase activity and critical nitrate concentrations [176,177]. It has been suggested that nitrate may act as a metabolic signal for protein kinases to regulate the flow of carbon between sucrose and amino acids [178]. If so, a lowering of the leaf nitrate concentration by high CO_2 could divert carbon from amino acids to sucrose biosynthesis in a mechanism ancillary to the glucose repression of gene expression [22].

In regard to C_4 species, the presence of a CCM (carbon dioxide concentration mechanism) would lead one to anticipate little or no increase in photosynthesis or growth from a doubling of atmospheric CO_2. However, reported responses are often positive, although less than for C_3

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plants. In the survey by porter [179], the average stimulation in dry weight for 19 C_4 species, both cultivated and wild, was 22% as compared with 41% for the C_3 species. What causes stimulatory effects of CO_2 on C_4 species? Several factors that indirectly impinge on Rubisco may be involved. First, elevated CO_2 reduces the stomatal conductance of C_4 as well as C_3 species. In the case of *Eragrostis orcuttiana*, this resulted in a 50% improvement in WUE [22,180]. In water-stressed environments, a CO_2-induced improvement in WUE could enhance growth. This has been proposed as a factor in the increased production by C_4 species in a tall-grass prairie ecosystem and for the improved photosynthesis of the C_4 salt-marsh community [33,157]. Second, a rise in the CO_2 concentration can enhance tillering and increase the leaf area and its duration [29, 38,180], so that total plant photosynthesis is greater even without an improvement in CO_2 assimilation per unit leaf area. There is concern as to how the different responses of C_3 and C_4 photosynthesis will affect competitive interactions in the ecosystems of a higher CO_2 world [31,36]. In several studies, the competitive abilities of C_3 species were enhanced relative to C_4 [21,181–183].

A higher atmospheric CO_2 concentration will not just influence C_3 and C_4 interactions; C_3 species do not all respond alike, so competition among plants within this category may also be modified. The competition studies indicate that the rise in CO_2 will alter the species composition of communities and species distribution. The exact changes cannot be predicted, although it appears that C_3 species are more likely to be favored than C_4 species [21].

The few CO_2-enrichment studies of CAM plants that have been undertaken have yielded mixed results. During the day, CAM plants close their stomates and raise CO_2 to more than 10,000 μmol mol^{-1} by the decarboxylation of malate that was accumulated the previous night via the activity of phosphoenol pyruvate carboxylase (PEPC). Thus, a doubling in the atmospheric CO_2 concentration should have little effect, and this seems true for pineapple [184]. However, if stomates open in late afternoon and fixation by Rubisco occurs, this provides an opportunity for CO_2 enrichment to stimulate photosynthesis. It may explain the 36% stimulation of dry weight reported for one CAM plant and a mean of 15% for six other species [113,179,184,185]. The possibility of greater nocturnal fixation via PEPC needs further examination, as does the photosynthesis of facultative CAM plants operating in the C_3 mode [21].

For marine and freshwater environments, approximately 50,000 submersed photosynthetic species are taxonomically far more diverse than the estimated 300,000 species of vascular plants which constitute the major photosynthetic organisms of terrestrial habitats [186]. The latter are believed to be derived in the past 450 million years from just one division and one class (Chlorophyta: Charophyceae). In contrast, submersed species include cyanobacteria, several algal divisions, bryophytes, lower vascular plants, and angiosperms, and some have histories that date back 2–3 billion years in environments with very variable CO_2 and O_2 concentrations. The diversity and long history gives them a greater potential for variability in carbon-acquisition mechanisms than terrestrial species [38,186]. This, together with the fact that little is known about many of the individual species, makes predictions about stress responses to rising CO_2 tenuous at best [21].

In waters where HCO_3^- predominates, species with a high capacity for HCO_3^- should be less affected by air enrichment than those using only free CO_2. This hypothesis was recently tested using the submersed freshwater angiosperms *Callitriche cophocarpa* and *Elodea canadensis*, a CO_2-only user and a HCO_3^- user, respectively. They were grown in water with 0.2 or 1.0 mM HCO_3^- and sparged with air containing 350 or 800 μmol CO_2 mol^{-1} [21]. The dry weight gain of *Callitriche* was doubled by the elevated CO_2 treatment, but that of *Elodea* was stimulated only about 20% irrespective of the HCO_3^- concentration in the water. Conversely, the higher HCO_3^- concentrations substantially increased the growth of *Elodea* but had only a minor effect on *Callitriche*. Elevated CO_2 caused little photosynthetic acclimation of either species. These data raise the possibility that, for waters low in free CO_2, the species composition may change as atmospheric CO_2 rises, with CO_2 users alone being favored [21].

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TRANSPORT OF ASSIMILATES

Partitioning of Dry Matter

Effects of an elevated CO_2 concentration and a nonstructural carbohydrate status on partitioning are poorly understood from a mechanistic perspective. Evidence exists suggesting that elevated CO_2 levels, that is, greater photosynthesis and elevated nonstructural carbohydrate levels, can result in a relatively greater partitioning of carbon to roots versus shoots [39,187]. Increased root growth under elevated CO_2 conditions might permit water extraction from a greater soil volume, which could be important in a future warmer and drier environment [42].

Low temperature results in enhanced storage of nonstructural carbohydrates at the apparent expense of growth. Warming also tends to result in relatively greater partitioning of carbon to shoots compared with roots [169], and this process can enhance photosynthesis by providing increased leaf area and promoting interception of photosynthetically active radiation. Farrar and Williams [169] point out that an increasing CO_2 concentration and an increasing temperature may have opposite effects on partitioning. Warm plants have lower levels of nonstructural carbohydrates and lower ratios of root to shoot than cool plants [42]. Plants in high-CO_2–level environments have large nonstructural carbohydrate stores and increased ratios of root to shoot. The combined effects of warming and an increased CO_2 level on partitioning are less clear. Warming can increase the specific respiration rate, whereas an elevated CO_2 level can slow it. Increased photosynthesis resulting from an elevated CO_2 level in combination with the increased sink metabolism (growth) allowed by warming has the potential to produce larger plants and increase carbon storage in terrestrial ecosystems as the CO_2 concentration and temperature increase [42].

Nutrient Assimilates and Partitioning

Ferrario-Mery et al. [188] speculated on the effects of elevated CO_2 on the composition of plant materials used for human nutrition or animal feed. Their results confirmed that N deprivation of plants grown at high CO_2 can be overcome by optimization of the fertilization regimen and allowing unrestricted growth. Their results support the concepts of gene manipulation to improve the nitrogen assimilation capacity and achieve an improved nutritional quality for sustainable agricultural production in a changing global environment.

PHOTORESPIRATION

With carbon dioxide saturation, insensitivity to oxygen concentration also occurs. A new hypothesis also explains the decrease in the photosynthetic rate at high carbon dioxide concentrations, which is sometimes observed, by decreased photorespiration causing a further decrease in the chloroplast phosphate concentration [15,189]. Oxygen in the choloroplasts can interfere with the photosynthetic reduction of CO_2. As the CO_2 concentration increases, CO_2 would more likely than oxygen bind to the active site of RuBPC, because more CO_2 molecules would be present there. Indeed, in some experiments, photorespiration was reduced by 50% when the CO_2 concentrations increased to 600 $cm^3\ m^{-3}$. Limiting photorespiration means that plants can use more of their energy to build tissues [190].

DARK RESPIRATION

Bunce [191] and Amthor [32] reported that the dark respiration rate is sensitive to the instantaneous concentration of CO_2 and decreases as CO_2 increases (Fig. 5). Growth of plants under conditions of elevated CO_2 often results in a decrease in the respiration rate [89,192,193]. Gifford et al. [194] observed a 45% reduction of the specific respiration rate in roots of CO_2-enriched wheat (*Triticum*

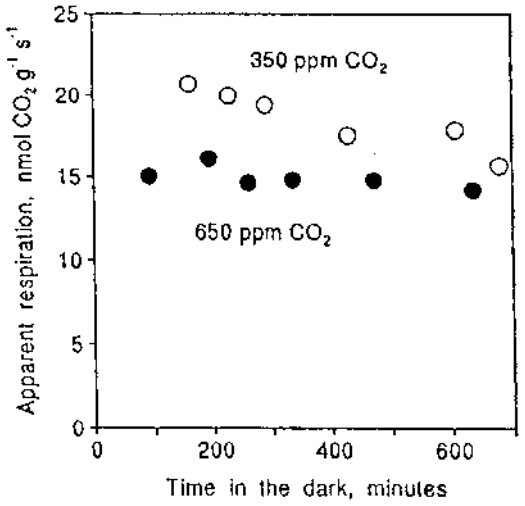

FIGURE 5 Effects of ambient CO_2 levels on apparent respiration rate in leaves of *Rumex crispus.* (From Ref. 192.)

aestivum L.). Long-term effects of CO_2 on respiration are mediated through effects on plant composition, and in particular by an increase in the C/N ratio as CO_2 concentration increases [193,195,196]. Concomitant measures of the growth rate, tissue composition, and respiration rate are required in order to unravel the links between long-term elevated CO_2 levels and respiration and growth [42]. A direct inhibition of respiratory metabolism by CO_2 has the potential to increase susceptibility to various stresses, because it is respiration that supplies much of the energy, reductant, and carbon skeletons used in repair and detoxification processes. The slowing of specific respiration rates with long-term CO_2 enrichment may be greatest at low compared with high temperatures [42,196]. There is a need for measurements of growth and respiratory responses by plants and ecosystems to long-term changes in temperature. Although information is scanty and variable, the effects of increases in global temperature on plant and ecosystem respiration are likely to contribute to positive feedback on increasing atmospheric CO_2 levels [42].

MODIFICATION OF CO_2-ENRICHMENT RESPONSES BY ENVIRONMENTAL CONSTRAINTS

The fact that CO_2 is a greenhouse gas makes higher global temperatures an important consideration in the rising CO_2 debate. Temperature and CO_2 have interactive effects, because a rise in temperature lowers the ratio of CO_2/O_2 in solution, shifts the specificity of Rubisco toward oxygenase, enhances photorespiration and dark respiration, and increases the sink response relative to the source [21]. These photosynthetic gains may or may not be realized in long-term growth and yield owing to an interplay of factors that complicate the issue. For example, leaves compensate for increased air temperatures by greater transpiration, whereas CO_2 enrichment tends to raise foliar temperatues by reducing transpiration [29,197]. Temperature and CO_2 can have greater interactive effects on the net leaf area production than photosynthesis per se [198]. Furthermore, species in the C_3 category differ markedly in the temperature regimens to which they are adapted, and also in their tolerance of the low and high extremes where temperature becomes stressful. Even with a single plant, temperature regimens that enhance CO_2-stimulated vegetative growth can negatively impact reproductive

growth. Thus, the grain yield of CO_2-enriched rice showed about a 10% decline for each 1°C rise above 26°C, and similar scenarios have been reported for soybean and wheat [199], as already discussed. This is because growth and reproduction reflect the integrated temperature response of metabolism and developmental processes and not just photosynthesis alone. As a consequence, species, developmental stage, light regimen, nutrient status, and the temperature range all modify the interactive temperature and CO_2 responses [21,66].

In nature, photosynthesis occurs in both high- and low-light environments; in the latter situation, the processes involved in RuBP regeneration are a greater limitation than the CO_2 supply or Rubisco capacity. Several growth studies demonstrate that CO_2 enrichment enhances light-limited C_3 photosynthesis [80,88,198,200,201], because it reduces O_2 inhibition. A rise in the CO_2 concentration results in a higher quantum yield [21]. Thus, assimilation versus irradiance (A/I) response curves for leaves of soybeans grown and measured at 660 μmol CO_2 mol^{-1} not only showed greater light-saturated rates but also had steeper initial slopes, that is, higher apparent quantum yields, and lower light compensation points than those grown at 330 μmol mol^{-1} [80]. Thus, CO_2 enrichment does not seem to downregulate the photosynthetic electron transport capacity, and limiting light conditions do not eliminate positive enrichment responses [21].

The upward trend in atmospheric CO_2 probably has already enhanced the photosynthesis, WUE, and growth of many of the earth's plants, especially C_3 species, and potentially will continue to do so. However, not all species have the capacity to respond, and those that do can be constrained by environmental parameters. Consequently, debate centers around the degree to which this greater photosynthetic potential will be realized during long-term growth of natural and agroecosystems whether these systems can continue to sequester carbon and how adaptation, competitive interactions, and survival will be influenced [21,29,31,36]. Much of the current data pertain to agricultural systems, and there can be little doubt that most crops will perform better in a higher-CO_2 world and may even require fewer subsidies. Less favorable C/N ratios may diminish crop quality, and higher temperatures may reduce grain yields, but breeding or molecular manipulation should correct these problems. Major changes in precipitation patterns would seem to have the most potential to disrupt current agricultural systems [21]. The situation is less clear with regard to ''natural'' ecosystems. Examples can be cited that both do and do not respond to enrichment; where nutrient availability and/or temperatures are low, responses will probably be constrained. However, many terrestrial and some aquatic ecosystems are likely to encounter marked shifts in species composition and distribution. But just as increases in atmospheric CO_2 are not novel, so continual changes in vegetation patterns are a fact of life on this planet irrespective of whether humans regard them as being beneficial [21].

Interactions Between CO_2 and Air Pollutants

Increased atmospheric CO_2 levels have the potential to mitigate some air pollution injury by virtue of increased photosynthesis (hence increased supply of photoassimilate to be used in various repair and detoxification processes) as well as reduced stomatal conductance (hence slower uptake of the pollutants) [11,68].

CONCLUSIONS

Increased photosynthetic rates are a widely observed response of plants to elevated atmospheric CO_2 [24,202,203]. The photosynthetic enhancement that occurred at elevated CO_2 partial pressures either persisted indefinitely [204] or was partly to fully reversed after weeks of CO_2 enrichment [119,205]. Changes of photosynthetic rates after prolonged exposure to elevated CO_2 were either positive or negative and were variable both among and within species [15,93,183]. The biochemical basis for photosynthetic acclimation to elevated CO_2 is unknown, although a source/sink imbalance

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[35,158] and carbohydrate accumulation and negative feedback mechanisms have been proposed [202,206,207]. As photosynthesis was far from being saturated at the current ambient CO_2 concentration, considerable further gains in photosynthesis are predicted through continuing increases in CO_2 concentration. The strong interaction with temperature also leads to photosynthesis in different global regions experiencing very different sensitivities to increasing CO_2 concentrations [16].

When growth is limited by water availability, plant growth should be relatively more responsive to CO_2 concentration than under well-watered conditions [208,209]. Under water-limited conditions, growth is essentially determined by the rate of diffusion of CO_2 into the leaf, which in turn is limited by the availability of water for diffusion out of the leaf [16]. On the other hand, growth is dependent not only on carbon gain but also on the availability of nutrients to turn the initial carbon gain into the growth of fully functional plant parts. The availability of nutrients may therefore introduce additional limitations which may reduce growth responses [16,83,210]. This appears to be part of the reason why no sustained growth response was observed in CO_2-enrichment experiments in the tundra [16,112,156].

The C_4 species respond much less than C_3 species. In the case of the long-term photosynthetic response to increased CO_2, there is as yet no firm explanation of what determines whether a species will have either positive or negative photosynthetic adjustment in a given environment. The phosphate-limitation hypothesis of feedback inhibition of photosynthesis helps explain variation in the short-term response to carbon dioxide concentration [15]. Plants with substantial sink capacity, such as crop and competitive-strategy species, have the greatest response to CO_2 enrichment, with an average of 30–40% stimulation of biomass, whereas those with small sinks have the least. Among submersed species, photosynthesis and growth of CO_2 users is only enhanced, but HCO_3^- users show a minimal response. Enrichment can stimulate photosynthesis and growth when water, nutrients, or light are suboptimal and temperatures are high, although extreme conditions can abolish the benefits. The photosynthetic CO_2 stress response is not always the major factor influencing competitive interactions among species, but directly or indirectly rising CO_2 concentration will alter the species distribution and composition of ecosystems [21].

Crop plants have been exposed to large increases in atmospheric CO_2 concentration over the last 200 years from about 270 to 280 μmol mol^{-1} to more than 355 μmol mol^{-1} today. Recent research has led to the following general conclusions concerning crop response to a doubling of atmospheric CO_2 [41]:

1. Production from C_3 crop plants is likely to increase by 30% or more. Production increases from C_4 plants will be less than 10%.
2. Although stomatal conductance may be decreased by about 40%, water use of C_3 plants will be decreased only about 10% or less.
3. Water-use efficiency will increase substantially owing to increases in CER with minor contributions from decreases in TR.
4. The interaction of high temperatures (at least up to a point) with elevated CO_2 should improve photosynthesis and vegetative growth but not necessarily reproductive growth.
5. Decreases in rainfall or disruptions of rainfall patterns, as predicted by some GCMs, would have a greater impact on crop production than would an increase in temperature.
6. How is the photosynthetic rate regulated by CO_2. What are the mechanisms? Why do some plants appear to downregulate, whereas others appear to upregulate it? How should this phenomenon be upscaled to whole canopies throughout the crop life cycle?
7. Elevated CO_2 reduces plant respiration. What are the mechanisms? What are the long-term consequences; that is, does reduced respiration enhance or inhibit plant responses to CO_2? Is there an impact of high nighttime CO_2 exposure level on crop performance?
8. How do plant canopies modify their microenvironments under changing CO_2 levels and climates and how do the modifications affect water use and the physiological response to CO_2 and climate?

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9. How can we assess the potential for genetic adaptability of crop materials to new ranges of climatic conditions? What are the ranges of crop adaptation that already exist among the present-day climates over the surface of the earth?
10. How can information from the above sets of questions be incorporated into crop models?
11. How can crop physiologists best participate with other disciplines to assess consequences, mitigations, and adaptive responses to rising CO_2?

Increasing atmospheric CO_2 and potential climate change challenge our understanding of the physiology and determination of crop yield. However, the range of climates where agriculture can flourish in the future lies mainly within the range of climates that exist today. Increasing our understanding of processess governing yield will be best served by developing a consciousness and expertise in global environmental plant physiology and crop ecology [41].

In general, the relative enhancement of photosynthesis and plant productivity owing to elevated CO_2 is greatest under stressful conditions, whereas the absolute enhancement is greatest under favorable conditions (i.e., for plants well supplied with water and nutrients at temperatures favorable for growth) [42]. The effects of plant physiological responses to rising atmospheric CO_2 concentration and temperature are summarized in Table 2 [42].

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Effect of Increased Atmospheric CO_2 Concentration on Water-Use Efficiency of Plants

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GLOBAL CLIMATE CHANGE

Carbon is a principal element for life, as it comprises a major part of the dry mass in living organisms. Carbon is required by green plants, taking up CO_2 from the surrounding air. The average atmospheric CO_2 concentration is around 350 μmol mol^{-1} and has increased by about 70 μmol mol^{-1} in the last 200 years, but it is still not saturating for C_3 photosynthesis. In the future, owing to antropogenic processes (mainly fossil fuel combustion and forest destruction), the CO_2 concentration is expected to continue to rising (the recent rate of the increasing CO_2 concentration is about 1.8 μmol mol^{-1} $year^{-1}$ [1]). Other trace gases, such as methane, oxides of nitrogen, and the synthetic chlorofluorocarbons, also have been increasing in concentration. Together, these gases may have the effect of raising the earth's temperature and increasing aridity in some parts of planet. However, much uncertainty remains about the exact magnitude of the change in the climate.

The global climatic change will have important implications for plant photosynthesis. The rate of photosynthesis depends on over 50 individual reactions, each of which potentially has a unique response to an environmental variable. The ability of plants to compensate for environmental effects on photosynthesis is critical for their performance and survival. Moreover, an ineffective response of the photosynthetic apparatus depresses yield, resulting in substantial economic cost [2].

The upward trend in the atmospheric CO_2 concentration probably has already enhanced the photosynthesis and growth of many plants, especially C_3 species, and potentially will continue to do so. CO_2 enrichment is a powerful tool to enhance the production in greenhouses. However, one of the central issues is whether long-term exposure of plants to an elevated CO_2 concentration results in a downregulation of photosynthesis; that is, that the photosynthetic rate at elevated CO_2 is lower than it would be expected based on short-term assessment of photosynthetic rates as a function of CO_2 concentration. In addition, an increased rate of photosynthesis and growth at elevated CO_2

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seems to be only maintained if the acquisition of other resources—soil nutrients and water—is sufficient as it is usually in agrosystems but not in natural ecosystems. Plant species differ in their responses to CO_2 and these differences can be very large, even among co-occurring species of a community. A CO_2-stimulated increase in plant growth could be of benefit if the stimulated species are economically valuable, but it could be a serious problem if the stimulated species are weeds [3]. If plants are growing more rapidly, a greater amount of organic matter could be stored in vegetation and soils, moderating the increase in atmospheric CO_2 and in the potential for global warming [4–7]. If temperature increases as predicted, plants will be helped or harmed depending on whether they are presently growing at temperatures below or above their optimum [8]. Further details can be found in several books and reviews (e.g., see Refs. 9–13).

The literature on plant responses to elevated CO_2 contains thousands of reports. The growth, photosynthetic rate, stomatal density and conductance, transpiration rate, water-use efficiency, for example, were found to be affected by an enhanced CO_2 concentration.

Many of the ecosystems are highly sensitive to changes in the water supply, because water is a key variable driving their composition and productivity. The growing human population has brought about a continuous effort to increase agricultural production and thus water use. As most of water used in irrigation is lost by transpiration, extensive research is being done on how to increase the efficiency of water use. This practical problem has stimulated the investigation of many theoretical questions; for example, the interrelationships between the stomatal conductance (g_s) and the transpiration rate (E) or photosynthetic rate (P_N), the physical and biochemical basis of the functioning of guard cells, and the effect of changes in metabolic processes in leaves on stomatal opening. In spite of great progress reached in this field, many questions still remain unanswered. Plant traits that increase water-use efficiency (WUE) may conflict with those that promote the growth rate. Plants or cultivars with a high WUE might be most suitable for use in drought conditions, whereas those with a low WUE for use in irrigated conditions [14].

WUE usually means the ratio of P_N to E, but sometimes it also means biomass production per amount of water used. The P_N/E ratio is affected by all environmental factors to which the response of P_N and E is not the same. In addition, every change in g_s brings about the change in the P_N/E ratio, as the effect of g_s on E is usually more marked than that on P_N owing to differences in the transport pathway of water vapor and CO_2. Thus, the P_N/E ratio is usually higher at a lower g_s than at a higher one. Therefore, an increased ambient CO_2 concentration is an ideal antitranspirant, as it positively influences P_N owing to an increased gradient for CO_2 transfer and simultaneously decreases g_s and thus E (for reviews, e.g., see Refs. 15–17). WUE more than doubles after short-term doubling of CO_2 [18].

The chapter focuses on the recent advances made in elucidating the long-term effects of an elevated CO_2 concentration on WUE and on development of water stress. In connection with this, a brief survey of the long-term effects of elevated CO_2 concentration on the stomatal density and conductance, transpiration rate, and net photosynthetic rate is presented. We have selected important up-to-date references from a voluminous body of literature to make the chapter comprehensive and current. As drought is one of the most limiting environmental constraints and global climatic changes may increase the frequency of drought in some areas, the possibility of increasing efficiency of water use ranks uppermost in the hierarchy of advantages brought about by an elevated CO_2 concentration.

STOMATAL DENSITY AND CONDUCTANCE AS AFFECTED BY ELEVATED CO_2 CONCENTRATION

Stomatal conductance (g_s) usually decreases when the ambient CO_2 concentration increases; the stomata closing effect of CO_2 is smaller at high than at low irradiance, and it is affected by air humidity, temperature, water stress, and plant hormones. Stomata from growth chamber-grown plants may have enhanced sensitivity to CO_2 in comparison with greenhouse-grown plants (e.g., in

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Vicia faba; see Ref. 19). The magnitude of stomatal response varies greatly among species; nevertheless, g_s often changes in such a way that the ratio of ambient and internal CO_2 concentration remains more or less constant. The mechanism of action of CO_2 on guard cells remains uncertain. It might be linked to malate synthesis, which regulates anion channels in the guard cell plasma membrane [20]. However, Esser et al. [21] report that malate does not function as a primary CO_2 signal in stomatal regulation. Probably also the change in cytosolic calcium ion concentration is a component of the CO_2 signal transduction pathway (e.g., see Ref. 22).

Long-term increased atmospheric CO_2 concentration often, but not always, leads to a large decrease in g_s, which is especially important in water-limited areas (for reviews see, e.g., Refs. 23–26). A common response to a doubling of CO_2 is a 30–60% reduction in g_s in C_3 and C_4 species, although there are cases of insensitive stomata [23,24]. Besides contraction of stomatal pores, leaf stomatal density may decline. However, a larger reduction in g_s than in stomatal frequency indicates that stomatal closure predominates [10,27]. Exposure to elevated CO_2 concentration often results in a short-term, reversible decline in g_s as a result of decreased stomatal aperture and a long-term, irreversible decline in g_s as a result of a decreased stomatal density [28]. According to Donoso et al. [29], the effects of elevated CO_2 on stomatal characteristics are very species specific (comparison of *Alternanthera, Ipomoea, Jatropha*, and *Talinum*).

In *Olea europea*, a decrease in stomatal density from the year 1327 BC was observed [30]. Also in *Acer pseudoplatanus, Carpinus betulus, Fagus sylvatica, Populus nigra, Quercus petraea, Q. robur, Rhamnus catharcticus*, and *Tilia cordata*, the stomatal density has decreased in the past 200 years [31]. Woodward and Kelly [32] followed the effect of an elevated CO_2 concentration on stomatal density in 100 species grown in nature. They found a reduction of stomatal density in 74% of tested species. These investigators did not find any significant dependence on growth form (trees vs shrubs or herbs) or stomatal distribution on the leaf (amphistomatous vs hypostomatous). However, in species grown in air-conditioned chambers, they observed the reduction of g_s in 60% of species, lower average reduction, and greater changes in g_s in amphistomatous than in hypostomatous leaves. A significant reduction in stomatal density was found in expanding *Populus* leaves but not in middle and lower leaves [33] (Fig. 1). Plants of *Nardus stricta* from higher altitude showed a greater decline in stomatal density with elevated CO_2 concentration than plants from lower altitude [34]. No differences in stomatal density due to elevated CO_2 concentration were found in *Citrus aurantium* [35], *Phaseolus vulgaris* [36], *Rumex obtusifolius* [37], *Trifolium repens* [38], and *Triticum aestivum* [35,39]. In *Lotus corniculatus* and *Sanguisorba minor*, stomatal density increased at both leaf surfaces, whereas in *Plantago media* and *Anthyllis vulneraria*, it decreased [40]. In *Andropogon gerardii*, reduction of stomatal density was found especially on the abaxial side [41]. In *Vicia faba*, stomatal density increased but significantly only on the adaxial surface [42]. In *Tradescantia fluminensis* plants grown at an elevated CO_2 concentration, the stomatal density did not differ, but the number of subsidiary cells in stomatal complexes was increased and substomatal cavities were enlarged [43].

Very slight or no changes in stomatal density and size in *Avena sativa, Prosopis glandulosa, Schizachyrium scoparium*, and *Triticum aestivum* were found at subambient CO_2 [44].

As was mentioned above, long-term CO_2 enrichment (usually to double the present ambient concentration) decreased stomatal conductance in both C_3 and C_4 species; for example, in *Abies fraseri* [45], *Andropogon gerardii* [46], *Atriplex canescens* [47], *Bellis perennis* [48], *Brassica oleracea* [49], *Capsicum annuum* [50], *Cucumis sativus* [51], *Fagus sylvatica* [52], *Glycine max* [53], *Gossypium hirsutum* [54], *Liquidambar styraciflua* [55], *Lycopersicon esculentum* [51], *Maranthes corymbosa* [56], *Panicum antidotale* and *P. laxum* [57], *Phaseolus vulgaris* [58], *Picea abies* [59], *Picea sitchensis* [60], *Pinus sylvestris* [61,62, Fig. 2], *Platanus occidentalis* [55], *Prosopis glandulosa* [63], *Prunus avium* [64], *Quercus petraea* [65], *Q. pubescens* [66], *Q. robur* [67], *Q. suber* [68], *Schizachyrium scoparium* [47], *Solanum melongena* [69], *Trifolium repens* [70], and *Triticum aestivum* [39,71]. For 41 observations covering 28 species, the average reduction of g_s was 20% [20].

No changes in g_s were found in *Acer saccharum* [55], *Fagus sylvatica* [67], *Pinus taeda*

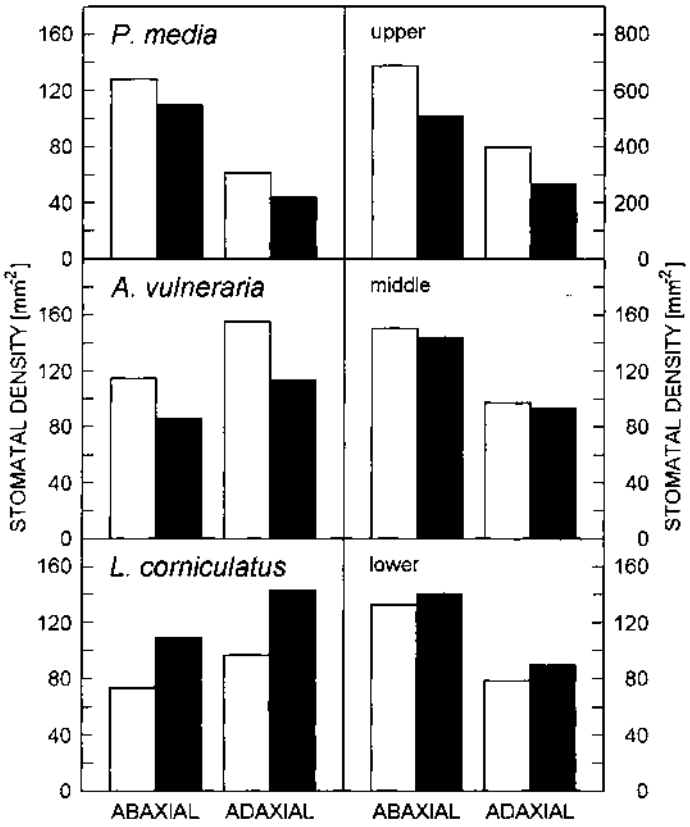

FIGURE 1 Effect of elevated CO_2 concentration (full columns) on stomatal density on abaxial and adaxial epidermes. Comparison of different plant species (*Plantago media, Anthyllis vulneraria* and *Lotus corniculatus*) (on the left) and comparison of poplar (*Populus deltoides* × *P. nigra*) leaves of different insertion (upper, middle and lower) (*on the right*). (Adapted from Refs. 33 and 40 by J. Solárová.)

[72,73,74], and in *Pinus pinaster* [65]. Higher g_s under CO_2 enrichment was observed in *Alnus rubra* [75] and *Quercus robur* [64]. Increased g_s was also found in *Solanum tuberosum* at very high CO_2 concentrations (3 or 14 times higher than ambient [76]). In *Glycine max*, the response of g_s was dependent on CO_2 concentration; g_s decreased, did not change, or increased at CO_2 concentration elevated to 3, 6, and 14 times of ambient, respectively [77]. Stomatal opening in *Lycopersicon esculentum* was enhanced by 4-week enrichment, and the enhancement decreased with time [78].

However, when plants are transferred from an enriched to a normal CO_2 concentration, the g_s recovers over a period of several days to the value typical for plants grown under normal CO_2 concentration [79].

The effect of long-term CO_2 enrichment also is dependent on CO_2 concentration during measurements; for example, in *Trifolium repens*, g_s under saturating irradiance was found to be the highest when plants were grown and measured at ambient CO_2 concentration, and the lowest g_s was in plants grown and measured at elevated CO_2 concentration [60] (see Fig. 3).

In *Rumex obtusifolius*, an elevated CO_2 caused a much greater reduction in g_s for the adaxial surface than for the abaxial surface [37]. As concerns the interactive effects of other environmental factors, the decline in g_s (e.g., in *Glycine max*) was a function of both the leaf temperature and the

leaf to air vapor pressure difference; a relative stomatal sensitivity to air humidity was decreased with an increase in CO_2 concentration and leaf temperature [80]. In *Pinus sylvestris*, long-term elevated CO_2 concentration decreased g_s at almost all levels of irradiance, temperature, vapor pressure deficit, and internal CO_2 concentration [61] (Fig. 2).

During June, when water availability was high, elevated CO_2 resulted in decreased g_s in 10 of 12 species measured. The greatest decrease in g_s (about 50%) occurred in species with the highest potential growth rates. During a dry period in September, the reduction in g_s was found in only two species, whereas increased g_s at elevated CO_2 levels was measured in *Amorpha canescens, Baptisia australis*, and *Symphoricarpus orbiculatus*. These increases were attributed to the enhanced leaf water potential [81]. In *Platanus occidentalis*, reduced g_s was observed only in well-watered trees [82]. In *Triticum aestivum*, CO_2 enrichment caused a higher reduction in g_s under sufficient irrigation than under a low one [83]. In *Acer rubrum*, a reduction of g_s occurred under sufficient water supply but not under water stress [84]. Similarly, in *Mangifera indica*, CO_2 enrichment caused a higher reduction of g_s in the wet than the dry season, and in this way it moderated seasonal changes in g_s [85].

Stomatal conductance in *Andropogon gerardii* reached new steady-state levels more rapidly after abrupt changes in irradiance at elevated CO_2. This was due to the reduction in g_s at elevated CO_2 and also by a more rapid stomatal response [46]. Stomatal sensitivity to internal CO_2 concentration (c_i) was decreased in *Chenopodium album* grown in elevated CO_2 [18]. In *Maranthes corymbosa*, but not in *Eucalyptus tetrodonta*, g_s was more sensitive to the leaf water status (but not to

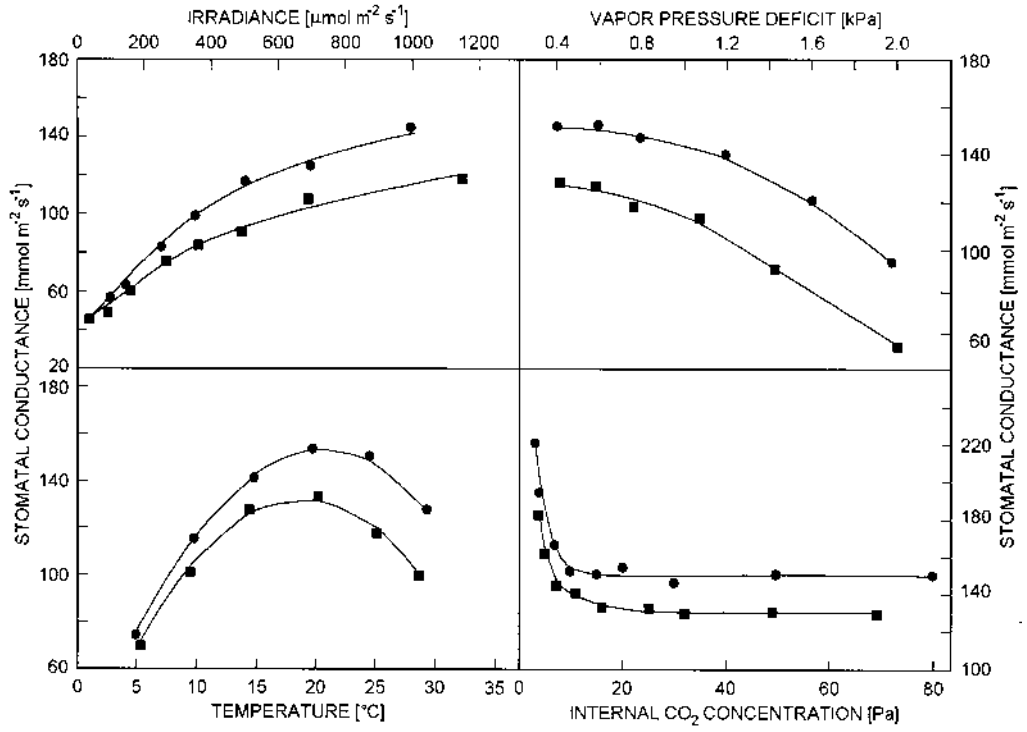

FIGURE 2 Stomatal conductance of *Pinus sylvestris* needles as a function of irradiance, temperature, vapor pressure deficit, and internal CO_2 concentration. Comparison of trees grown under ambient (circles) and elevated (squares) CO_2 concentration for 5 months. (Adapted from Ref. 61 by J. Solárová.)

the addition of abscisic acid) under CO_2 enrichment [28]. The stomata of elevated CO_2-grown *Quercus suber* seedlings were less responsive to high temperature [67]. The response of g_s in *Acer pseudoplatanus* to air humidity was not affected by the CO_2 concentration [31], but in *Pinus sylvestris*, sensitivity of g_s to low air humidity was increased in trees grown at elevated CO_2 concentration [62].

LONG-TERM EFFECTS OF CO_2 CONCENTRATION ON TRANSPIRATION RATE

The transpiration rate (E) depends on the supply of energy and vapor pressure gradient between the evaporating surfaces and the ambient air. E is modified by plant factors such as leaf structure and stomatal behavior. CO_2 concentration mainly affects E through changes in g_s. As g_s often decreased in consequence of CO_2 enrichment, a simultaneous decrease in E was observed (Fig. 3) (for reviews, see, e.g., Refs. 26, 86, and 87).

Under long-term CO_2 enrichment, decreased E per unit leaf area was found in both C_3 and C_4 species; for example, in *Andropogon gerardii* [88], *Begonia* × *hiemalis* [89], *Betula pendula* [90], *Capsicum annuum* [50], *Cucumis sativus* [51], *Dactylis glomerata* [91], *Fagus sylvatica* [52], *Festuca rupicola* [91], *Glycine max* [53,92–94] (Fig. 4), *Gossypium hirsutum* [54], *Lycopersicon esculentum* [51], *Prosopis glandulosa* [63], *Prunus avium* [64], *Quercus robur* [95], *Rosa hybrida*

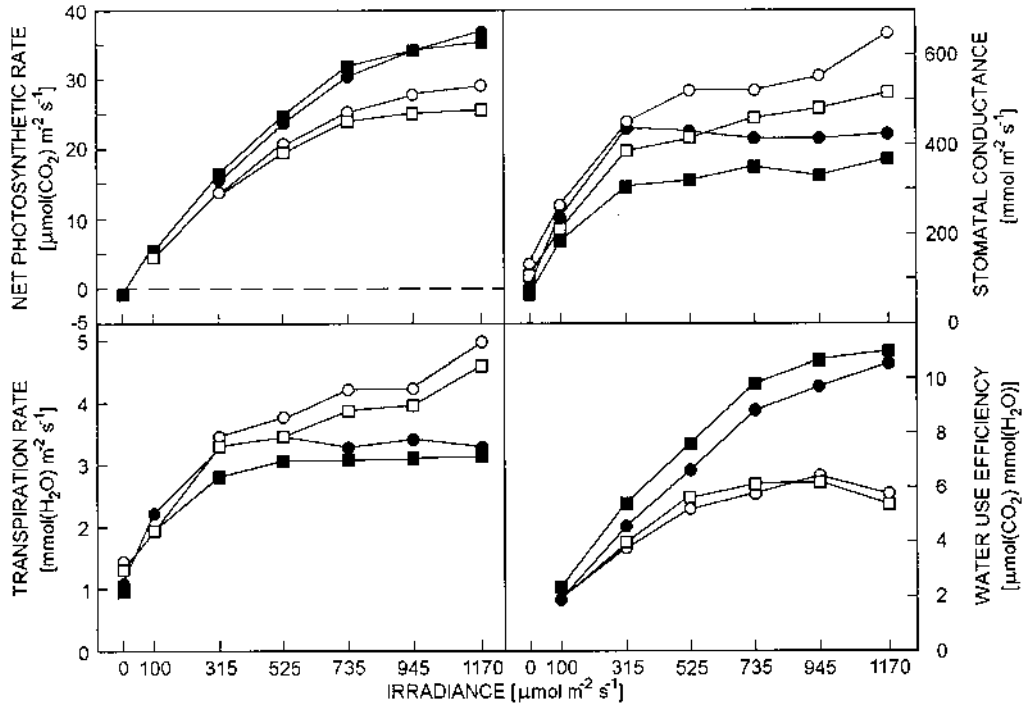

FIGURE 3 Net photosynthetic rate, transpiration rate, stomatal conductance, and water use efficiency of young, fully expanded leaves of *Trifolium repens* as a function of irradiance. Plants were grown at ambient (circles) and elevated (squares) CO_2 concentration and measured at ambient (empty symbols) or elevated (closed symbols) CO_2 concentration. (Adapted from Ref. 69 by J. Solárová.)

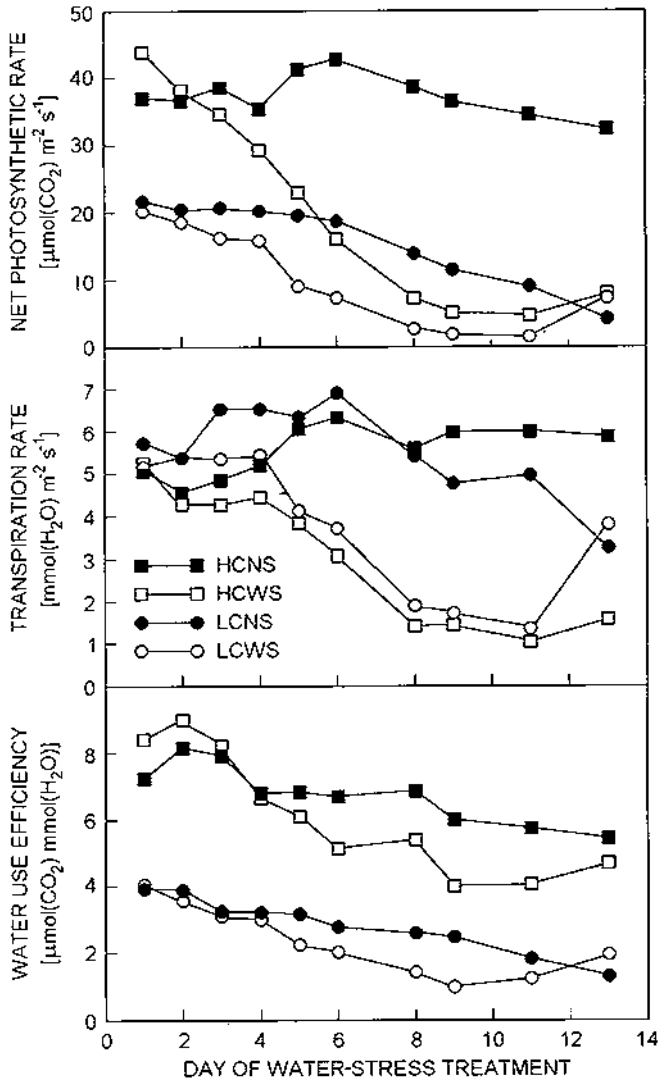

Figure 4. Net photosynthetic rate, transpiration rate, and water use efficiency of leaflets of *Glycine max* plants grown under ambient (LC, circles) or elevated (HC, *squares*) CO_2 concentration. Plants were measured during development of water stress (WS, *empty symbols*) or under sufficient water supply (NS, closed symbols). (Adapted from Ref. 93 by J. Solárová.)

[96], *Rumex obtusifolius* [37], *Solanum melongena* [68], *Sorghastrum nutans* [88], *Sorghum bicolor* [94], *Triticum aestivum* [70,97,98], and *Vernonia baldwini* [88].

On the contrary, in other plant species or as reported by other investigators on the same plant species, no significant differences in E were found; for example, in *Fagus sylvatica* [99] (Fig. 5), *Filipendula vulgaris* [91], *Gossypium hirsutum* [100,101], *Salvia nemorosa* [91], and *Zea mays* [97]. In *Quercus robur*, a slight increase in E was observed [64].

Reduced transpiration was accompanied by reduced sap flow in *Andropogon gerardii, Quercus ilex, Q. pubescens, Q. robur, Sorghastrum nutans*, and *Vernonia baldwini* [67,66,88]. Small

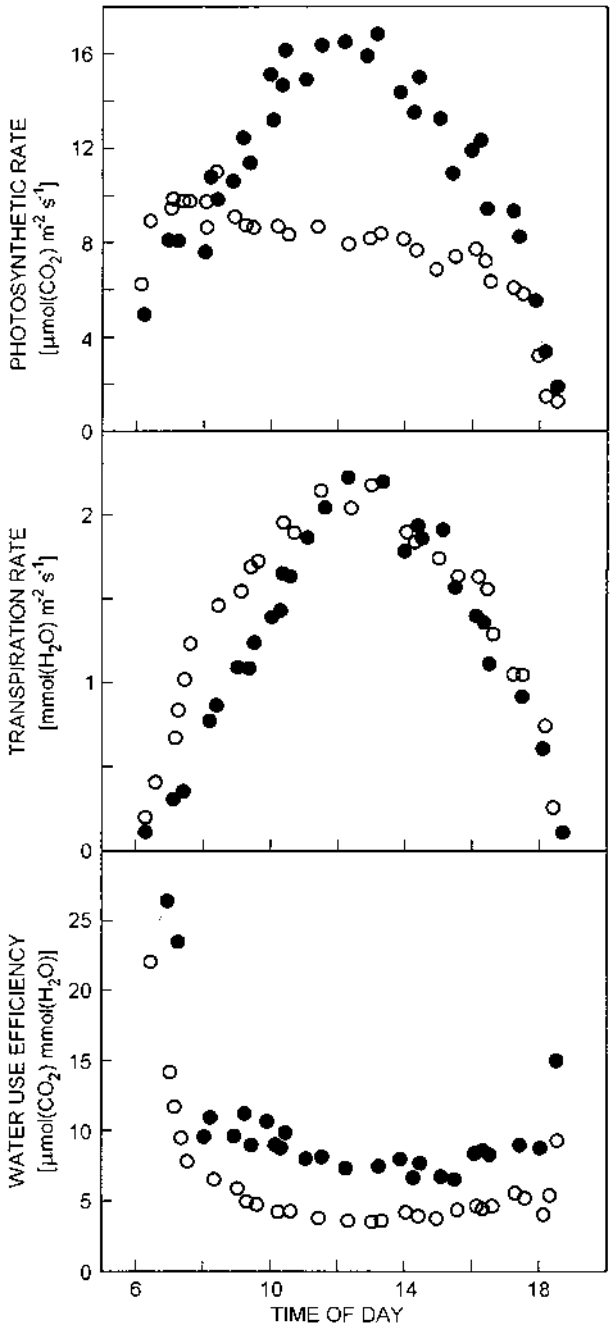

FIGURE 5 Daily course of net photosynthetic rate, transpiration rate and water use efficiency of *Fagus sylvatica* branches grown under ambient (open circles) and elevated (closed circles) CO_2 concentration. (Adapted from Ref. 99 by J. Solárová.)

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differences in sap flow were found in *Triticum aestivum* [102] and *Fagus sylvatica* [67] and no effect in *Gossypium hirsutum* [101].

At the ecosystem scale, the magnitude of the response of evapotranspiration to CO_2 enrichment is lower in comparison with transpiration at the leaf scale. This difference arises from the effect of decreased g_s on leaf temperature and air humidity in the boundary layer which might increase the driving gradient of water vapor concentration and so the evapotranspiration rate [16,17,26,103–105].

The reduced g_s also can improve the leaf water potential, which can accelerate leaf expansion. Therefore, E per plant may or may not decline because of an increased leaf area. Decreased g_s and no differences in E due to larger leaf area were found, for example, in *Glycine max* [106,107]. Similarly, owing to the leaf area increase, a small effect of elevated CO_2 on evapotranspiration in a grassland ecosystem [26] and a *Lolium perenne* stand [108] was found. Canopy conductances were lower by as much as 20% in *Medicago sativa* and by 60% in *Dactylis glomerata*; however, the evapotranspiration rate never differed by more than 3% in *M. sativa* or by 8% in *D. glomerata* [109].

A reduced E is favorable under insufficient soil moisture; however, it may increase the leaf temperature, particularly under high irradiance, and decrease the transport of those nutrients that are translocated with the transpiration stream [110].

LONG-TERM EFFECTS OF CO_2 CONCENTRATION ON NET PHOTOSYNTHETIC RATE

Carbon dioxide is of primary importance as a substrate in photosynthesis. A low CO_2 concentration lowers P_N, whereas its elevation enhances P_N. Since the natural CO_2 concentration is not saturating for the photosynthesis of C_3 plants, a short-term enhancement of CO_2 concentration increases P_N (approximately 95% of terrestrial plants are C_3 species, about 1% are C_4 species, and 4% use the crassulacean acid metabolism (CAM) pathway [24]). A decrease in CO_2 concentration in dense canopies (due to an insufficient rate of CO_2 transport from the air above the canopy) might contribute to a midday depression of photosynthesis [111].

At low CO_2 and saturating irradiance, the capacity of ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco) to carboxylate RuBP is limiting for photosynthesis, and the slope of the initial response of P_N to c_i (often termed carboxylation efficiency) is directly dependent on Rubisco activity. In addition to directly increasing the rate of carboxylation, the increased CO_2 concentration increases the rate of CO_2 fixation by depressing RuBP oxygenation and thus photorespiration. The slope of the P_N/c_i response progressively declines as c_i increases (usually above 200 μmol mol^{-1}). P_N is enhanced by increasing c_i to a value (about 1000 μmol mol^{-1}) when the capacity for RuBP regeneration is limiting (for reviews see, e.g., Refs. 20, 24, and 112).

The biochemical pathway of photosynthetic carbon metabolism influences the responses of plants to CO_2: C_4 plants have a more efficient photosynthetic apparatus than C_3 plants at the natural CO_2 concentration and do not increase P_N as much as C_3 plants in response to elevated CO_2 concentration. In general, C_3 plants show increased P_N at the leaf level and whole canopy level when grown at CO_2 enrichment (for recent review, see, e.g., Ref. 87). However, the long-term exposure to increased CO_2 concentration did not always increase the photosynthetic rate. On the contrary, increased CO_2 concentration often lead to downregulation of photosynthesis, as was mentioned in the introduction to this chapter (for reviews, see, e.g., Refs. 2, 17, 24, 113, and 114).

The downregulation of photosynthesis may be in a consequence of (a) decreased stomatal density and partial stomata closure reducing the CO_2 transport to the sites of carboxylation, (b) rapid production of photosynthates leading to an excess amount of starch in the chloroplasts and feedback inhibition of P_N; this inhibition is dependent on the availability of sinks in the plant and also on

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the availability of nitrogen to balance enhanced availability of carbon, and (c) reduction in the amount or activity of Rubisco.

The species showing reduced g_s at CO_2 enrichment usually had lowered P_N [87,113]. However, according to Drake et al. [20], stomata may not limit photosynthesis with elevation of CO_2 concentration more than they do at normal CO_2 concentration. Downregulation of the Rubisco content and/or activity at elevated CO_2, reported in many species, is not a universal phenomenon; a number of species show either no decline or even an increase in the Rubisco content under this condition (e.g., see Refs. 24 and 79) or an increased ability to regenerate RuBP (e.g., see Refs. 48 and 115). Similarly, the source-sink status of plants is not only species specific but it also depends on the developmental stage and environmental conditions. No downward regulation of photosynthesis during long-term exposure to elevated CO_2 was found in some tree species; for example, *Fagus crenata* and *Quercus crispula* [116] or in the CAM plant *Opuntia ficus-indica* (in the latter species, the enhanced saccharide production was accompanied by higher source-sink photosynthate transport [117]).

Nevertheless, despite acclimation responses, the rate of P_N at saturated irradiance (P_{Nsat}) is usually higher in C_3 plants grown and measured at elevated CO_2 concentration than in those grown and measured at normal CO_2 concentration [24,96]. However, in *Picea abies*, P_{Nsat} was not stimulated by a 4-week exposure to elevated CO_2 and decreased by 24-week exposure [118,119].

The response of P_N is species specific. Under similar conditions, the acclimation of P_N was found to be somewhat downward in *Festuca rupicola*, fully downward in *Dactylis glomerata*, and upward in *Salvia nemorosa* and *Filipendula vulgaris* [91]. It was dependent on CO_2 concentration; in *Glycine max*, P_N increased in the whole range of increased CO_2 concentration (from half to three times of ambient), whereas in *Oryza sativa*, the greatest increase in P_N occurred at CO_2 concentration one and a half of ambient [24]. Also, differences between fast-growing *Populus* clone Beauprè and slow-growing clone Robusta were found: In clone Beauprè, elevated CO_2 resulted in an increase in quantum yield of photosystem 2, P_{Nsat}, chlorophyll content, and Rubisco activity, whereas in clone Robusta, primary reactions of photosynthesis were depressed and Rubisco activity decreased [120]. Acclimation of P_N was dependent on the leaf insertion level in *Pisum sativum* and *Glycine max* [121].

Differences between P_N in plants grown under ambient and elevated CO_2 concentration depend on CO_2 concentration during measurement (see Fig. 3). In *Trifolium repens*, the differences in P_N were found only when measured at the same CO_2 concentration at which they were grown [69]. On the other hand, P_N was similar in the ambient and elevated CO_2-grown *Panicum antidotale* (C_4) and *P. laxum* (C_3) plants when measured at the same CO_2 concentration at which they were grown; however, P_N of elevated CO_2 grown plants was lower when measured at ambient CO_2 concentration [57]. In *Oryza sativa*, comparison of P_N at a wide range of CO_2 concentrations (160–1000 μmol mol^{-1}) indicated that long-term treatments of 350 and 700 μmol (CO_2) mol^{-1} [122] resulted in very little photosynthetic acclimation [123].

P_N is usually more stimulated by long-term CO_2 enrichment at higher than at lower temperatures [87,124,125]. On the contrary, in *Betula platyphylla* grown at elevated CO_2, quantum yield, P_{Nsat}, and carboxylation efficiency were decreased more at higher than at lower temperature [126]. Also, in *Lolium perenne*, the negative effect of elevated CO_2 concentration was accentuated by high temperature, but it was observed under high irradiance but not under low irradiance. P_N was more stimulated by enhanced CO_2 during midday hours than in the morning or evening in *Fagus sylvatica* (see Fig. 5) [99], under water stress than under an adequate moisture supply in *Acer rubrum* [84], *Arachis hypogaea* [27], *Bouteloua gracilis, Pascopyrum smithii* [127], *Picea mariana* [128], and *Triticum aestivum* [129], and similarly in *Mangifera indica* during the dry than during the wet season [85]. In *Glycine max*, however, stimulation of P_N by elevated CO_2 concentration disappeared under severe water stress [93] (see Fig. 4). Downregulation of the photochemical efficiency of photosystem II (F_v/F_m) at CO_2 enrichment was observed in well-watered but not in water-stressed *Eucalyptus macrorhyncha* and *E. rossii* [130], whereas in *Quercus ilex*, it was much higher at severe than at moderate water stress [131].

During leaf senescence, elevated CO_2 concentration mostly decreased P_N in *Acer pennsylvani-*

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cum, A. rubrum, Betula alleghaniensis, B. populifolia, and *Fraxinus americana* [132]. In *Rumex obtusifolius*, acceleration of the ontogenetic decline in P_N but not the reduction in the leaf life span was observed under elevated CO_2 concentration [133].

Photosynthetic capacities of leaves can be analyzed from the relationship between P_N and c_i and both the initial slope and the CO_2-saturated photosynthetic rate are often affected by CO_2 enrichment (for reviews, see Refs. 2 and 17). The P_N/c_i ratio can be used in order to eliminate the effect of g_s. Under elevated CO_2, an increased P_N/c_i ratio was found; for example, in *Andropogon gerardii* [134] and *Helianthemum nummularium* [48]. However, in some plant species, for example, in *Chenopodium album, Phaseolus vulgaris, Pinus taeda*, and *Zea mays* [58,124,135,136], growth at different CO_2 concentrations had no discernible effect on the short-term response of P_N to c_i. Stomatal limitation of P_N was increased in *Betula platyphylla* grown under elevated CO_2 [126] but decreased in *Bellis perennis, Helianthemum nummularium, Poa alpina, P. annua*, and *Plantago lanceolata* grown and measured at elevated CO_2 concentration [48].

No change or a reduction in dark respiration per leaf area unit with CO_2 enrichment is more prevalent, but the canopy respiration rate increased in association with the greater biomass and the increase in the relative growth rate [20,24,87].

WATER-USE EFFICIENCY

Under long-term elevation of the CO_2 concentration, the increase in WUE is the most common positive effect. An increased P_N/E ratio was observed not only in plants with increased P_N but also in plants where downregulation of P_N was observed, because, in these plants, a decrease in P_N was usually accompanied with a decrease in g_s. The range of the increase in WUE induced by CO_2 enrichment is dependent on the plant species and interactions with other environmental factors, especially with water stress.

Enhanced WUE under CO_2 enrichment was found; for example, in *Acacia smallii* [138], *Betula platyphylla* [126], *Citrus sinensis* [107], *Dactylis glomerata* [91], *Fagus sylvatica* (see Fig. 5) [52,91,99], *Festuca rupicola* [91], *Ficus benjamina* [139], *Filipendula vulgaris* [91], *Glycine max* [92,106,107,140], *Gossypium hirsutum* [141], *Lolium perenne* [108,142,143,144], *Oryza sativa* [107,123,125,145], *Pinus sylvestris* [61], *P. taeda* [73,136], *Salvia nemorosa* [91], *Trifolium repens* [142,143,144], *Triticum aestivum* [71], and *Zea mays* [140]. For many other plant species, see comprehensive tables in reviews elsewhere [10,104,115,146]. According to carbon isotope discrimination, an increased WUE during the last 240 years was found in herbarium specimens [147]. Bert et al. [137] calculated a 30% increase in WUE between the years 1930 and 1980 from changes in carbon discrimination ($\delta^{13}C$) in tree rings.

WUE was increased under both well-watered and drought treatments; for example, in *Glycine max* (see Fig. 4) [93] and *Picea sitchensis* [60]. The combined effect of CO_2 enrichment and drought stress on WUE was significant in *Alnus firma* [148]. In *Anthyllis vulneraria* and *Sanguisorba minor*, WUE increased at elevated CO_2, with a higher average increase under water stress [149]. On the other hand, WUE was greater for trees grown at elevated CO_2, but when subjected to drought, the relative enhancement in WUE was reduced in *Quercus rubra* or even disappeared in *Picea abies* [150]. In *Phaseolus vulgaris*, CO_2 enrichment doubled WUE at a high nutrient supply and tripled at a low nutrient supply [58].

WUE in *Chenopodium album* more than doubled after a short-term doubling of CO_2 concentration. However, WUE of plants grown and measured at elevated CO_2 was only about one and a half times that of plants transiently exposed to elevated CO_2 owing to stomatal acclimation [18]. Similarly, in *Trifolium repens*, the increase in WUE was much more dependent on CO_2 concentration, irradiance, and leaf temperature during the measurement than on CO_2 concentration during growth (see Fig. 3) [69].

However, WUE was not increased under elevated CO_2 concentration in *Abies fraseri* (due to strong downregulation of P_N [45]) and in *Quercus robur* [64], and it even decreased in *Prunus avium* [64].

CO_2 enrichment also increased biomass accumulation per water consumption (WUE_m); for example, in *Atriplex canescens* and *Schizachyrium scoparium* [47], *Pinus pinaster* and *Quercus petraea* [151], *Pseudotsuga menziesii* [152], *Quercus robur* [95], *Sinapis alba* [153], *Triticum aestivum* [98], *Zea mays* [154], and halophytic species (for review, see Ref. 155) or grain production per water consumption in *Triticum aestivum* [156]. In *Gossypium hirsutum, Triticum aestivum*, and *Zea mays*, WUE_m increased under high CO_2 for both wet and dry conditions; however, *Gossypium hirsutum* exhibited a very large leaf area response under wet soil leading to much greater water use per plant [157]. Similarly, in *Lolium perenne*, WUE_m increased at elevated CO_2 with no significant interaction with soil moisture or N supply [158]. Under supraoptimal CO_2 concentration, WUE_m was decreased in *Glycine max* [77] and *Solanum tuberosum* [76].

INCREASED CO_2 CONCENTRATION AND DEVELOPMENT OF WATER STRESS

As mentioned above, the nutrient and water limitations restrict the responses of P_N to increased CO_2; however, the elevated CO_2 would allow plants to cope more successfully with stressful habitats [3,97,159].

In most species and under most circumstances, stomatal conductance is the main limiting factor to the photosynthetic rate under mild water deficit, because the photosynthetic apparatus is usually affected only under severe water stress. Elevated CO_2 concentration may compensate decreased stomatal conductance by an increased gradient of CO_2 concentration between the exterior and interior of the leaf (for review, see, e.g., Ref. 160). In connection with this, an increased P_N/g_s ratio was found in *Quercus petraea* and *Pinus pinaster* [65] but not in *Zea mays* [135].

However, another possible consequence of elevated CO_2 and water stress (and high temperature) might be the change in susceptibility to photoinhibition. The probability of photoinhibition occurrence might be increased owing to a reduction of photorespiration or decreased by a better supply of CO_2 (for review, see Ref. 160).

Many studies have shown that plants at elevated CO_2 concentration tend to dry more slowly as water is withheld, which is consistent with their lower stomatal conductance and slower transpiration rate (Fig. 6) (for reviews see, e.g., Refs. 104 and 159). Decreased water use observed (e.g., by *Oryza sativa* [122], *Triticum aestivum* [161] or by grassland [3]) allowed photosynthesis or growth to continue for some days longer during drought in the enriched CO_2 concentration compared with ambient CO_2 treatment. However, if the leaf area increases, we may expect even higher water use per plant, and so water stress may develop more rapidly [26,162–165]. *Fagus sylvatica* may substantially increase whole-plant water consumption at elevated CO_2 [71]. Similarly, elevated CO_2 concentration in well-watered *Quercus petraea* and *Pinus pinaster* increased water consumption but decreased it under water stress [151]. Increased water uptake also was observed in *Solanum tuberosum* [76]. CO_2-enriched *Triticum aestivum* plants use less water per day during the first 30 days of soil drying but more water per day during the further 10 days [98]. Similarly, under high irrigation, a slight reduction in seasonal water use by *Triticum aestivum* was observed, but under low irrigation, there was even a slight increase in the water use [83]. In *Acacia smallii*, the total water loss was not affected by CO_2 enrichment in spite of declined stomatal conductance and increased WUE [138].

Higher leaf water potentials at elevated CO_2 [134,159] could increase leaf expansion and carbon dioxide fixation and thereby contribute to the stimulation of growth [63,164]. At elevated CO_2, the root/shoot ratio, important in the balance of saccharide allocation and water use by plants, is usually altered in favor of roots (for review, see, e.g., Refs. 24, 63, 87, 104, 159, and 163), which might increase water uptake. However, no changes in the root/shoot ratio in *Betula pendula* and *Picea abies* were observed [90].

Hydraulic conductance at elevated CO_2 concentration increased in *Quercus robur* and *Prunus avium* × *P. pseudocerasus* [64] but decreased in *Glycine max* and *Medicago sativa* [164].

Water-stressed seedlings of *Eucalyptus macrorhyncha* (Fig. 6) [130], salt-marsh plants *Scirpus*

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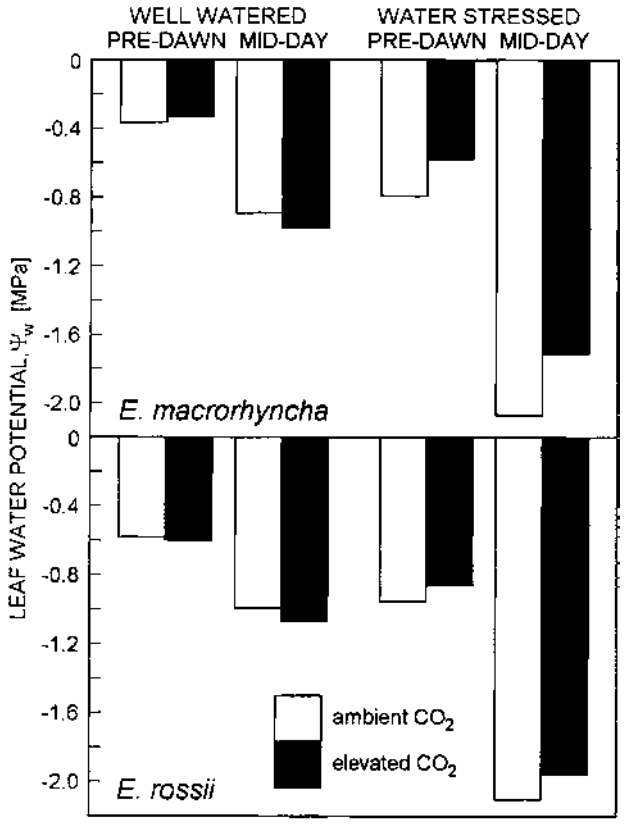

FIGURE 6 Pre-dawn and mid-day water potential in leaves of well-watered and water-stressed seedlings of *Eucalyptus macrorhyncha* or *E. rossii* grown under ambient (empty columns) or elevated (full columns) CO_2 concentration. (Adapted from Ref. 130 by J. Solárová.)

olneyi and *Spartina patens* [166], C_4 grass *Andropogon gerardii* [134], and *Triticum aestivum* [97] had a higher leaf water potential when grown in elevated CO_2, but in *Lolium perenne* [167] and *Zea mays* [97], the leaf water potential did not differ. In *Lolium perenne*, the pressure potential increased at elevated CO_2 in spring and remained similar in summer, and osmotic potential decreased in spring and increased in summer [167]. Leaf water and osmotic potentials in *Lotus, Sanguisorba, Plantago*, and *Anthyllis* decreased and the pressure potential increased at CO_2 enrichment [149,169]. In *Phaseolus vulgaris*, water and osmotic potentials increased and the pressure potential was not affected by elevated CO_2 treatment at cell division and/or cell expansion phases [168]. The increased osmotic potential at zero pressure potential was determined in *Acer saccharum* but not in *Platanus occidentalis* and *Liquidambar styraciflua* [55]. The osmotic potential was not affected by CO_2 treatment in *Prosopis glandulosa* [63]. In *Cucumis sativus, Phaseolus vulgaris*, and *Zea mays*, chilling at elevated CO_2 induced a less decrease in transpiration rate, relative water content, and leaf water potential than chilling at normal CO_2 concentration [170].

Both drought and high CO_2 resulted in osmotic adjustment in *Helianthus annuus*, with drought having a greater effect than CO_2, and their combination being more effective [171]. On the contrary, no indication of enhanced osmotic adjustment under CO_2 enrichment was found in *Pinus taeda* [172]. In *Quercus robur*, a water-stress–induced osmotic adjustment in leaves was found only under

elevated CO_2 in roots under both natural and elevated CO_2 [173]. *Betula populifolia* had a lower osmotic potential and a lower modulus of elasticity at full hydration under xeric conditions and elevated CO_2 concentration than under ambient CO_2 concentration and mesic conditions which enabled it to maintain a positive pressure potential at a lower water potential [174]. At elevated CO_2, the decreased osmotic potential, symplasmic water fraction, and rate of water transport, increased the modulus of elasticity and no changes in the formation of xylem embolism were found in *Quercus pubescens* and *Q. ilex* [66].

Elevated CO_2 concentration had no effect on the rate of rehydration nor on the de novo photosynthesis in desiccated *Xerophyta scabrida* [175].

CONCLUSIONS

Physiological responses to elevated CO_2 are different at the leaf, plant, and stand scale. They depend on CO_2 concentration (subambient, ambient, double, triple, or supraoptimal) and the duration of elevated CO_2 treatment (short-term treatments, long-term treatments lasting for weeks, months, or years). The range of the effects of long-term treatment with elevated CO_2 on individual physiological parameters is further dependent on other environmental factors. In addition, long-term treatment with elevated CO_2 affects not only the absolute values of physiological parameters but also modifies the responses to other environmental factors [61,176].

Greater carbon assimilation per unit of water loss, per unit nitrogen content, or per unit absorbed radiant energy is usually found in plants exposed to elevated CO_2 [20]. Simultaneously, stomatal conductance and transpiration rate often decrease. Thus, WUE is usually enhanced in elevated CO_2; however, this may not necessarily lead to an increased drought tolerance [71]. Similarly, the decrease of long-term water use is uncertain, because CO_2 enrichment may increase the leaf area and thereby increase the water use. However, some plant species seem to cope better with drought stress at elevated CO_2 concentration, and these species may be able to extend their biotope into less favorable sites in the future [115]. Under certain conditions, plants at elevated CO_2 concentration either deplete soil water more slowly at the same growth rate or grow faster for the same rate of soil water depletion. Under water-limiting conditions, this can lead either to a prolongation of the growing period or to a larger biomass accumulation per unit of water used [177].

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Beneficial Aspects of Stress on Plants

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INTRODUCTION

More than any other field known to this author, research into plant stress has been curiously biased. A plant that had never been subjected to stress of any kind would probably be as vulnerable as a human being who had somehow been raised in a completely stress-free environment. Every living thing, plant or animal, is the end result of various forms of stress from early evolution, through ontology, to individual development. Nevertheless, plant-stress research has been almost totally focused on combating various ill effects of stress to the almost total exclusion of the logical approach of also acknowledging, and when possible utilizing, potential beneficial aspects. This has been so much so that the previous principal text in this field [1] lacks even a single index entry relating to the beneficial aspects of stress.

How has this bias come about? Two adverse influences are apparent, not that there may not be others. Like it or not, in today's complex society, funding controls the direction in which virtually every field of science develops. As local funding sources continue to shrink, research is increasingly confined to those areas favored by the few individuals who control grant funds. As pointed out elsewhere [2], this often severely distorts what might otherwise be logical development of scientific research. Certainly within the United States, policies of agencies administering grant funds have been totally slanted toward merely alleviating the adverse effects of stresses of various kinds to the total exclusion of support for research on how to utilize beneficial aspects when possible. This is particularly unfortunate in that it has excluded grant support for stress utilization in various commendable efforts to develop nonchemical methods for modern agriculture.

A second, more minor, but increasingly powerful factor is the modern reliance on computer-based literature searches. The computer is a marvelous servant but a poor master. Increasingly, we have research workers who rely almost exclusively on computers for literature search, and usually their computers tend to find only those entries identified by title or by ''key words.'' This is only one small aspect of an increasing pressure toward conformity that this author has strongly decried

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[3]. Such limitation to computer searches can exclude much ''precomputer'' literature. Indeed, it is not ''literature search'' in the classic sense of Dr. Samuel Johnson's (1709–1784) excellent eighteenth century advice: ''The first task is to search books, the next to contemplate nature'' [4]. Almost any text on horticulture (and sometimes on agronomy also), ancient or modern, amateur or professional, can yield examples of beneficial aspects of various forms of deliberately applied stress even though the word *stress* may not itself be used in the text.

This brings us back to ''key words,'' which are all too often limited to the ''buzz words'' currently predominant in a particular field of research. Key words deserve more respect! They are authors' means of access to the attention of research workers (and perhaps funding agencies) who may integrate the reported research into quite unexpected fields of endeavor. The ''ripple effect'' of well-chosen key words should never be underestimated. Too often, research workers do not think to include stress, beneficial or otherwise, among their key words. A very recent example is a paper [5] cited later in a discussion of certain beneficial aspects of applied mechanical stress. Unfortunately, the author did not include the word *stress* in either her title or her key words (although the phrase ''mechanical stress response'' occurs in the first sentence of her paper). Thus, this very worthwhile review will probably be missed in any computer search for literature on stress-related research. In a review of the beneficial aspects of physiological stress [6], my coauthors and I cited 128 papers, texts, and so on. I do not recall any of them being located through ''key words.''

Examples cited in this chapter are heavily slanted toward horticulture, this having been the author's lifelong field of study. Workers in other disciplines can probably find equally cogent examples in their own fields of expertise. Most particularly, research workers in the field of stress alleviation are invited also to consider possible *benefits* of natural or applied plant stresses. Authors in almost any aspect of plant physiology, agronomy, and forestry, indeed almost any biological science, have the possibility of unusually eclectic recognition of their research whenever such key phrases as ''stress alleviation'' or ''stress utilization'' are appropriate.

Definitions

Stress

Stress is here considered to be any external factor that results in a less than optimal growth rate (i.e., any factor that interrupts, restricts, or harmfully accelerates the normal metabolic processes of a plant). When growth is not involved (as with living, harvested plant parts), stress is considered here to be any factor that, if applied in excess, kills the organ or tissue involved.

The benefits of various forms of plant stress can be either biological or economic.

Biological Benefits

These are plant responses to stress that enable the plant to grow, survive, and propagate itself regardless of any interaction with humankind.

Economic Benefits

These are responses to stress that make plants more valuable to humankind. Such benefits are typically in terms of increased monetary yield from crops. However, there also can be other economic benefits. As I watched a documentary film of the building of the replicas of Columbus's ships, I wondered what natural stresses those oak trees had endured to make them grow into the irregular shapes carefully selected for ''knees'' to secure the vessels' ribs to their keelsons.

Stress Research Related to Beneficial Aspects

As noted, there is an increasing volume of valuable research on the beneficial aspects of stress that is never recognized as such in the literature. Much of this has already been reviewed [6], but reports

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continue to be published that are never collated into the increasing body of research on stress, because they are not so indexed. Such a recent review paper is Latimer's report, ''Mechanical conditioning for control of growth and quality of vegetable transplants'' [5]. As its 81 literature citations attest, this is an extensive review of the beneficial effects obtained by application of various mechanical stresses (bending, brushing, and shaking) on a range of species. Of great importance is her observation that, in some instances, such mechanical stresses can substitute for treatment with growth regulators, such as daminozide (B-Nine or Alar), which are increasingly faced with legal restrictions on their use on food crops.

Until such research is included in the rubric of stress research, plant stress will continue to be a curiously unbalanced field of study.

STRESS AS AN INEVITABLE COMPONENT OF ANY ENVIRONMENT

Stress In Evolution

Overwintering

Seeds of tropical plants usually germinate as soon as they are mature, often immediately after separation from the plant that bore them. If seeds of Temperate Zone plants behaved similarly, their newly sprouted seedings would be destroyed by the first hard frost. Temperate Zone seeds must initially be dormant; dormancy being later broken by the hard but beneficial stresses of winter temperatures. Exceptions to this principle are seeds of Temperate Zone plants that bloom early enough in the spring to be able to establish mature plants before the onset of winter. The dandelion (*Taraxacum officinale*) is a familiar, and usually unwelcome, example.

The dormancy of perennial plants affords an outstanding example of beneficial responses to the gradually increasing stresses exerted by decreasing temperature, shortening day length, and decreased light intensity. With deciduous trees, this effect is particularly obvious as leaves change color and then fall. Under the stresses of autumnal climatic changes, however, even conifers undergo considerable, although invisible, metabolic modifications enabling them to adapt to winter temperatures that would be fatal if experienced during full growth flush [7]. A chapter, or perhaps a book, could be written on such natural responses to beneficial stress. This account, however, must give priority to manipulation of plant stresses for economic benefit. However, first let it be noted that, in all fields, exhaustive, seemingly interminable biochemical and biophysical studies of what happens during stress-induced changes are now giving way to investigation of the growth regulators that mediate and control such phenomena (e.g., see Ref. 10). Understanding such specific mechanisms is increasingly placing them within the reach of control for economic benefit.

Mechanical Stress

Nearly 100 years ago, when Captain Joshua Slocum prepared to build his little sailing vessel, *Spray*, which he was to sail around the world, he looked for an old solitary oak tree that had been wind stressed throughout its long life. As builders of wooden ships have known through the ages, such ''prestressed'' trees were the best source of tough, reliable timber.

When the heavily timbered shores of Canada's British Columbia began to be developed after World War II, planners and builders sought to save some of the magnificent great fir, cedar, and hemlock trees for landscape purposes. Solitary trees that, for one reason or another, had grown alone served well. However, it was soon found that leaving occasional trees as the forest was cleared could be disastrous. Deprived of the shelter provided by their fellows, such unstressed trees tended to fall in the next Pacific gale. This strengthening effect of mechanical stress is not confined to trees. As described here, it can be utilized in improved handling of various crop plants.

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Aridity

Beneficial responses to various forms of stress have been critical in the evolution of plants adapted to growing in apparently hostile areas. Untold generations subjected to severe drought conditions have selected desert plants capable of surviving by virtue of their various, sometimes elaborate, adaptations for water conservation.

More than simple conservation of moisture is involved in the evolution of the ''desert ephemerals,'' annual flowering plants whose seeds germinate only when there has been a single rainfall sufficient to carry the plant from seed germination through bloom to seed maturation. Before knowledge of growth regulators, how this could work was a mystery. We now know that seeds of desert ephemerals have a growth inhibitor that is leached out by rainfall sufficient to carry the plant through its life cycle. Such a mechanism was obviously developed by natural selection under the severe stresses of advancing desertification. Plants whose seeds germinated with trivial rainfall were selectively discarded; those with increasingly high levels of growth inhibitor survived, which is an elegant example of the beneficial response to stress.

Fire

There can be no greater stress than fire. Nevertheless, fire has played an essential role in the evolution of some plants, providing them with particularly favorable ecological niches.

A number of species of pine trees (*Pinus* spp.), referred to by foresters as ''serotinous,''have cones that require fire to open their scales to release their seeds. Probably the best known of these is the lodgepole pine (*P. contorta*) common in the northern Rocky Mountains of Washington and British Columbia. In such areas, with repeated forest fires, the lodgepole pine increasingly becomes the ecological climax species.

Another, less well-known, example of a pine with serotinous cones is the jack pine (*P. banksiana*) of the U.S. Great Lakes States and adjacent Canada. Other pines benefitting from fire for reseeding, although not completely serotinous, are the pond pine (*P. serotina*) and the pitch pine (*P. rigida*) [8].

A recently discovered, quite different, mechanism for ''propagation by fire'' involves seed germination [9]. In some areas of California subjected to wildfires, it is commonly observed that after dense perennials such as chaparral are burned away, the annual wild flower ''whispering bells'' (*Emmenanthe penduflora*) promptly flourishes. Research workers at Occidental College, Los Angeles, recently found that seeds of (*E. penduflora*) are totally dependent on nitrogen dioxide (at levels that occur in wildfire smoke) for seed germination. There is an irony here. NO_2, so widely decried as an atmospheric pollutant when it comes from automobile exhausts or gas cooking stoves, is essential for the life cycle of at least one plant species and perhaps others also. For that matter, it is ironic to read the endless denunciations of CO_2 as a ''greenhouse gas'' with no acknowledgment that CO_2 is the essential feedstock for photosynthesis on which all higher life forms are dependent.

Other Stresses

There is not space enough here to attempt to cover all the natural stresses that have shaped the evolution of useful plants. Salinity is an obvious example—from strains of barley that have selectively adapted to brackish conditions in which other feed grains cannot survive, to the mangroves that grow in seawater, protecting and extending tropical shorelines. Such selection by chemical stresses, although benefiting the plant, are not necessarily beneficial to people. Within living memory, imported woody asters have invaded selenium-rich soils in parts of Wyoming. Not only do they survive the stress of levels of selenium toxic to most plants, they accumulate it and become highly poisonous to livestock that may graze on them, a property that is definitely protective for the asters but disastrous for ranchers and their herds [11].

There are surely many other examples of plants selectively benefiting from their specific responses to various forms of plant stress.

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Mechanisms Involved in Stress Adaptation

Abscisic Acid

It is only since World War II that improved instrumental analysis has made possible the identification and quantification of the growth regulators (GRs) that control plant physiological activities as surely as the endocrine hormones control the physiology of vertebrates [12].

Attention is drawn to two publications. In March 1944, the remarkable horticultural scientist W. H. Chandler presented the University of California annual faculty research lecture on the topic of winter hardiness of trees [13]. He reviewed physical and biochemical changes in detail. Growth regulators were not mentioned. In an August 1969 symposium 25 years later on cold hardiness, dormancy, and freeze protection of fruit crops [14], four of seven authors commented on the emerging role of growth regulators in winter hardiness. Today, consideration of GRs would be almost implicit in such a symposium. The use of controlled stress to manipulate GRs is the logical next step.

One of the first such GR studied in detail was abscisic acid (ABA), named because of its visually dramatic effects in the autumnal abscission of the leaves of deciduous trees. It is now known that ABA has many other functions (some of which are discussed below). Dormancy is an obviously beneficial response to climatic stresses at the approach of winter. Dormancy and bud breaking are controlled at least in part by the balance between ABA and gibberellins. The role of ABA in dormancy is still being studied but appears to be related to the synthesis of RNA and protein [15].

Other Growth Regulators

Cytokinins also are associated with autumnal leaf dehiscence [15], but for which deciduous trees would not survive the winter.

Possibly the most ubiquitous GR associated with stress is ethylene, which over the ages has been involved in many traditional uses of stress physiology even though its role was assuredly not understood by the biblical prophet Amos (see later discussion of fig ripening).

Other Mechanisms

Until recently, the various benefits obtained by such deliberately applied stresses as pruning, ''hardening off,'' bending fruit tree limbs, and girdling of fruit trees have been explained in terms of the carbohydrate nitrogen ratio, a mechanism originally proposed to account for the physiological responses of the tomato [16]. It is quite likely that hormonal regulation also is involved.

INTENTIONAL MANIPULATION OF STRESS

There is nothing new in the concept of deliberately applying stress in various ways for the enhancement of crops of various kinds (even though such practices have been ignored in the ''stress literature''). Today, however, imposing controlled stress for crop improvement is greatly facilitated by our increasing understanding of the GR mechanisms involved. Moreover, manipulation of endogenous GR levels is a considerable step in decreasing the overreliance on chemicals for which modern agriculture is so generally criticized.

Tree and Vine Crops

Today, almost all tree fruits and many grapes are grown as a rootstock-scion combination. A single bud or short graft piece is taken from a plant of the desired scion variety and inserted against the cambium layer of a seedling (or rooted cutting) selected for such qualities as vigor of growth, adaptability to soil type, and disease resistance. As soon as the scion piece is growing strongly, the seedling is cut off, an extremely stressful start to a long productive lifetime. From then on, the tree

or vine (now of the scion variety) is often subjected to a further series of applied mechanical stresses to produce a profitable crop.

Various forms of mechanical stress are commonly applied to fruit trees to bring them to bear. One such is tying down branches of nonflowering trees (particularly certain varieties of apples). Early in the season, upward growing branches are bent down and tied in place, thus subjecting them to severe mechanical stress. Another such application of a mechanical stress is ''ringing,'' in which a narrow strip of bark is removed all around the circumference of the trunk. (If done properly, the ''ring'' heals completely before the tree goes dormant in the fall. If not properly done, the ring does not heal and the tree dies). In either case, the bent branch or the ringed tree is usually forced into initiation of flower buds.

Forestry (Silviculture)

Fire, the ultimate stress, has long been used to *prevent* calamitous forest wildfires. Use of frequent controlled burns to remove flammable undergrowth was initially developed in Florida and has become a very general practice across North America. Frequent controlled minor burns prevent the build-up of the dense flammable undergrowth that fosters destructive forest fires [17–20].

Annual Crops

Quite drastic stresses are routinely imposed in the production of many annual crops, particularly those for which individual plants are transplanted from seedling beds to the field (i.e., many types of vegetables, florists' stocks, and tobacco). From time immemorial, growers have known that seedlings transferred in full growth flush often do not survive transplanting. Experience has shown that seedlings need to be ''hardened off'' before transplanting. This normally involves reducing the water supply almost to the wilting point; temperature reduction also is involved for greenhouse-grown seedlings. Such properly stressed plants survive transplanting far better than those in full growth flush. Vague explanations were accepted in the past (''hardening taught the plants to adapt''). We now know that adequate (but not excessive) stress induces the production of protective levels of ABA [21–24].

Fairly recent research has shown that even the act of handling such seedlings, although harmful if done incorrectly, can be highly beneficial. Latimer's review [5] is highly recommended to those interested in the possible use of mechanical conditioning (e.g., by bending, stroking, or shaking) instead of applying exogenous GRs, such as daminozide (B-Nine or Alar), to such seedlings.

A striking example of applied stress to benefit an annual crop is the grazing of winter wheat in the fall. In such climates as Canada and the northern United States, ''winter wheat'' is sown in late summer or early fall. It grows to a height of 25 or 30 cm before going dormant at the onset of winter. As a young farmhand well over 50 years ago, I was incredulous when the very wise farmer for whom I was working told me to turn the dairy herd in to graze on the winter wheat. ''It's good feed and it's good for the wheat.'' Grazing on young plants that are expected to produce a crop of wheat the following summer is a very severe form of stress. But he was correct: ''stooling'' (production of basal shoots to form additional plants) is stimulated by grazing and by the trampling of the cattle.

Pasture Crops

Grazing of winter wheat leads to consideration of the possible beneficial effects of stress on pasture grasses. A thoughtful paper [25] compared continuous overgrazing of U.S. western rangelands with the apparent overgrazing by vast herds of herbivores, such as wildebeest, zebra, and gazelle, on the African savannahs. A good case is made that when the apparent overgrazing is seasonal (as with these strictly migratory animals), the trampling and close cropping before the grasses go dormant for the dry season is, although temporarily stressful, ultimately strongly stimulating to the next

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season's growth; an example of natural beneficial stress that awaits possible exploitation by cattle ranchers.

Induced Rest Period

As the costs of refrigerated transportation rise (and when access to foreign exchange becomes limiting), an interesting development is that of applying ''false winter stress'' to grow deciduous fruits completely out of their normal ranges. An example is using severe water stress, plus manual or chemical defoliation, to force apple trees grown in a tropical climate, such as that of Java, to go into the rest period necessary for initiation of fruiting buds [26]. This can be so successful that the writer has observed apples and bananas growing side by side in Colombia's Valle del Cauca.

Differential Heat Stress for Pest Control

Enough heat stress can kill any living tissue, but advantage can be taken of the differences in susceptibility to heat stress between host and pathogen. This is not new, as shown by a 1948 report [27] that *Penicillium* mold of citrus fruits can be inhibited by prolonged exposure to temperatures (30–32°C) tolerated by Florida citrus fruits. With increasing public resistance to the use of chemicals for pest control, the use of differential heat stress for pest control is increasingly popular. Some typical recent reports include postharvest heat treatments for leafroller insects infesting avocados [28], *Penicillium* mold of grapefruit [29], anthracnose decay of mangos [30], and control of various fruit flies infesting papayas, carambolas, mangos, and grapefruit [31].

All the above examples (and many more could have been cited) are postharvest treatments. A preplanting heat-stress treatment provided essential control of the root-infesting nematode *Radophilis similis* that in the 1950s threatened to wipe out the Florida citrus industry. Before planting out in the grove, roots of nursery-grown saplings were exposed to water hot enough to kill all stages of the nematode life cycle but which were tolerated by the citrus roots [32,33].

IMPROVEMENT IN PRODUCT QUALITY

Controlled stress, either pre- or postharvest, can be used to improve the postharvest quality of various crops. Although this is more general for horticultural crops, examples are cited here for products as varied as oil seeds, medicinals, and tobacco.

Pruning and Training

Even after many orchard crops are well established, for many types of fruits, product quality must be maintained by pruning, training, and/or thinning. Although such practices are intrinsically stressful, they improve the market grade (and hence monetary value) of the resultant crop as assuredly as the considerable stress involved in castration of a bull calf results in a superior meat animal. Most varieties of apples and grapes, if spared such stressful treatments, ultimately produce heavy crops of small fruit that are virtually worthless on the fresh fruit market.

Applied Water Stress

Kramer [34] cites various authors who have reported that moderate water stress can improve the quality of apples, pears, peaches, and prunes, increase the rubber content of guayule, improve the aromatic constituents of Turkish tobacco, increase the alkaloid content of *Atropa belladonna* and *Hyoscyamus muticus*, and raise the essential oil content of mint and the oil yields of olive oil and of soybeans. (Water stress of cotton plants, by omitting irrigation in August, results in uniform and early opening of the bolls with increased prospects of completing harvest before the first frost [M. Pessarakli, personal observation].) An account from India reports that water stress improved the

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quality of peanut oil (*Arachis hypogaea*) by increasing the proportion of unsaturated fat [35]. In view of the current emphasis on decreasing saturated fats in the U.S. diet, this latter finding might merit further investigation of other crops yielding edible oils. Current Canadian research indicates that the dry matter content of cannery tomatoes (and hence their monetary value) may be increased by judicious application of water stress (M.A. Dixon, personal communication).

Enforced Delay of Ripening

It can be advantageous to delay ripening of fruits when refrigeration is not available or when the product tolerates refrigeration poorly. (See comments on chilling injury below.) Ripening is initiated by the production of endogenous ethylene. The ethylene-forming enzyme (EFE) system in papayas can be inhibited by closely controlled heat stress [36]. Similarly, ripening of guavas can be delayed by hot-water treatment [37].

Enhancement of Citrus Fruit Color Through Stress

Citrus fruits are more or less fully green at maturity when grown in the fully tropical lowlands where they first evolved in Southeast Asia. Centuries of growing citrus fruits in far more stressful ''Mediterranean-type'' climates have led the world's consumers to expect, and pay for, certain typical stress-induced brilliant peel colors [38]. In particular, orange fruit and orange color are so closely associated that the buying public (anywhere but in the tropics) instinctively resists buying oranges that are not orange in color. Ethylene is a clearly demonstrated product of fruits and vegetables under stress [39]. Cold nights during fruit maturation cause sufficient stress to produce enough ethylene to remove chlorophyll and stimulate the production of the carotenoid pigments resulting in typical ''varietal colors'' [40–42]. In a climate such as Florida's, however, it is necessary to have at least five nights below 10°C to produce the requisite color change [43]. Such conditions often do not occur until the harvesting season is well advanced, so lacking this beneficial preharvest stress, ethylene is applied postharvest to destroy the chlorophyll whose green color is almost invariably considered an indication of immaturity by the buying public [44].

Induced Maturation of Figs

The earliest recorded example of the deliberate use of stress-induced ethylene concerns the edible wild fig (*Ficus sycamorus* L.), the natural maturation of which is dependent on a diminutive wasp (*Sycophaga sycomori* L.). The sycamore fig was endemic in Biblical lands, but at that period, the *Sycophaga* wasp was not. In modern times, Galil [45] showed that the sycamore fig sets parthenocarpically and ripens to edibility in about 4 days only if wounded enough to stimulate the production of endogenous ethylene. Examples of the small curved knives that herdsmen (such as the prophet Amos) used to nick the immature figs have been found in excavations in Egypt. Since then, Maxie and Crane [46] have shown that the same effect can be obtained by sprays of the synthetic growth regulator 2,4,5-trichlorophenoxyacetic acid, which causes sufficient stress to induce the production of enough endogenous ethylene to result in parthenocarpic fruit set.

Astringency Masking in Persimmons

Many varieties of persimmons are too astringent to eat until they are very close to being overripe. Many hundreds of years ago, the Japanese found that sealing mature (but still astringent) persimmons in used sake (rice wine) barrels for a few days removed (more correctly masked) the undesirable astringency. This effect used to be attributed to lingering alcoholic fumes from the sake. It has since been shown to be due to mild stress from short-term exposure to the high carbon dioxide levels accumulated from respiration [47]. Too long an exposure or too high a concentration of carbon dioxide can cause excessive stress, ruining the persimmons.

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Irradiation for Varietal Improvement

Gamma irradiation can exert a drastic stress on any living tissue, as the writer and his colleagues found in an unsuccessful search for a radiation dosage high enough to control decay pathogens but low enough not to damage the peel of citrus fruits [48,49]. Nevertheless, gamma irradiation is being used with considerable success in the improvement of various crop plants. The modern consumer of grapefruit prefers red flesh, and the more highly pigmented the better, even though the red pigment (lycopene) has no effect on flavor. However, the buying public has an antipathy to seeds. The Hudson variety has very desirable flesh color but is very seedy. R. A. Hensz of Texas subjected the seeds of the Hudson variety to a mild dose of irradiation. This provided stress enough to produce a seedless mutant with highly pigmented flesh that is now being widely planted in Texas and Florida as the Star Ruby variety [50].

Star Ruby grapefruit is just one of many agricultural products for which improved strains have been obtained by irradiation, a method being widely used all around the world. The 1974 *Encyclopedia Britannica* lists no fewer than 45 improved strains of various crop plants produced by irradiation [51]. Undoubtedly many more have been (such as Star Ruby grapefruit) produced since then. Irradiation is a deadly stress in excess but a very useful tool when used in moderation. How many of these examples would be found in a modern computer search for ''beneficial uses of stress''?

As this chapter is being written, the professional scaremongers, a thriving and prosperous group, are raising a clamor with regard to a recent U.S. Supreme Court ruling that ''genetically improved'' food crops do not have to be submitted to exhaustive (and exceedingly expensive) toxicological testing before release. Let it be noted that, ever since irradiation was first used to improve a food crop, long before the advent of gene manipulation by biotechnology, ''genetically altered'' food plants have been in general use without evidence of any toxicological problems. Fortunately, this beneficial use of stress achieved general worldwide acceptance without the professional alarmists noticing.

Hypovirulent Virus Inoculation

Infection of a crop plant by a virus is usually high on the list of forms of stress to be avoided. Increasingly, however, benefits are being found from inoculation with hypovirulent strains of plant viruses. One such has been known, but not understood, for hundreds of years. In the quest for new and striking color combinations, tulip breeders have long known that the color break factor (giving striking bicolor blooms) did not follow a Mendelian inheritance pattern. Not until 1928 was it recognized that the ''color break factor'' is due to infection with a hypovirulent virus. Today, such hypovirulent viral strains are being used for benefits as diverse as control of animal and plant pests, weed control, and limiting the size of citrus trees. Interested readers are referred to a review by Cohen [52] that covers not only historical and present developments but also future trends and possibilities for the use of these hypovirulent viruses.

Mitigation of the Chilling Injury Syndrome

Many plants and detached plant parts, such as harvested fruits, are susceptible to chilling injury; that is, low-temperature injury at temperatures somewhere below 10°C but well above 0°C. Anyone who has ever placed a banana in a household refrigerator and had it blacken overnight has observed chilling injury (CI). After 25 years of studying and trying to control chilling injury, the writer conducted a series of studies that showed that judiciously applied stress could be a very valuable tool in mitigating CI.

Chilling injury is typically a problem of plants originating in the tropics, although all plants originating in the tropics are not necessarily CI susceptible. For example, we found no evidence of chilling susceptibility with the Golden Star variety of carambola (*Averrhoa carambola* L.) [53],

definitely a tropical fruit. However, Arkin, a new variety released after that report was published, proved to be susceptible. Susceptibility has to be determined species by species, variety by variety, and district by district. Chilling injury also can be a problem in the field, as with cotton seedlings in the spring and maturing tomatoes in the fall, but this discussion is confined to postharvest aspects, for which CI can place severe constraints on the storage and marketing of susceptible fruits (including those vegetables that are botanically fruits).

Even within a given species or cultivar, CI susceptibility can vary considerably with the growing district. Thus, California-grown Valencia oranges are susceptible to CI at temperatures below 5°C [54], but the same variety grown in Florida's very different climate is CI resistant. Thus, Florida Valencia (late season) oranges can be stored and shipped at the same temperatures as those commonly used for apples and other produce not susceptible to CI. However, Florida grows about 75% of the world's grapefruit and it is CI susceptible, thus sharply limiting export shipping temperatures throughout the harvesting season and storage in the 4 summer months when grapefruit are not being harvested. The storage life of grapefruit is terminated either by fungal attack or by CI (which so disfigures the peel as to make the fruit unsalable). Considerable storage life can be obtained at temperatures just above 10°C by using fungicides to the maximum legal limit. However, at such temperatures, the peel turns an atypical deep yellow, which is rejected by the market. Moreover, some of the more effective fungicides being used elsewhere (e.g., Panoctine or Guazatine) are not approved in the United States, and those that are approved are under constant attack by well-meaning but often misinformed consumer groups. The problem, to which the writer and a series of graduate students applied over 25 years of research, becomes that of finding a way of storing and shipping grapefruit at temperatures commonly used for nonchilling susceptible products. This became even more urgent when the federal ban on ethylene dibromide (EDB) fumigation for fruit fly quarantine made ''low-temperature sterilization'' necessary for shipments of grapefruit from quarantined districts to citrus-growing areas, such as Japan. Since this involved research into the basic causes of CI, there was an excellent prospect that research findings would be applicable to other CI-susceptible products.

First, a comment on grapefruit, which is in many ways an ideal test material for such studies: Marketable fruit from a single bloom (usually in late March) can be picked for as long as 8 months (typically from mid September to mid May). Thus, experiments on fruit from a single bloom can be replicated over a period of 8 months. Moreover, since fruit can hang on the tree for as long as 15 months, it is possible to conduct experiments comparing the response of new crop, immature fruit with that of very mature fruit from the previous bloom on the same tree. Additionally, trees being perennials, fruit from the same plants can be used in successive years. One series of experiments involved over 100 harvests of random pickings from the same 28 trees taken over a period of 8 years. Readers interested in the details of such research over many years are referred to various published reports and doctoral dissertations [55–63]. To summarize our findings very briefly:

1. Every season included a mid winter period of remarkable resistance to CI, but the exact dates varied from year to year.
2. Such resistant periods were clearly correlated ($r = 0.93$, $P = .01$) with prevailing temperatures during the dry season [61].
3. Susceptibility to CI often varied more with time between picking and storage than with the postharvest treatments under study.
4. Spraying trees with various growth regulators caused marked (but unpredictable) changes in the susceptibility of the grapefruit to CI [56].

Another line of investigation was inspired by an observation by visiting South Africans that, although grapefruit grown on the Transvaal high veldt was susceptible to CI, after shipment across the Great Karroo Desert in steel, unrefrigerated railcars to the port at Cape Town, it could be ''cold sterilized'' against fruit fly larvae at temperatures a little above 0°C. To simulate this, we added a biweekly treatment in which samples were placed in a metal shed on the roof of our building for

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2 days. The results were remarkable in late summer, which was the end of the rainy season, with noon shade temperatures always over 32°C and late afternoon temperatures in the roof shed exceeding 40°C. The ''Karroo Desert treatment'' did the grapefruit no harm and extended successful storage at 4.5°C from 10 to 90 days. This effect decreased sharply when the summer rains ceased and noontime temperatures decreased in the fall. The conclusions were obvious: Fruit picked from trees in good growth flush and stored immediately were very susceptible to CI unless severely stressed before storage. Fruit from trees stressed by the annual winter dry season also were resistant to CI. A painstaking graduate student showed that, just as for seedlings hardened before transplanting, endogenous ABA was involved in the development of resistance to CI [57]. Subsequent research in cooperation with the U.S. Department of Agriculture and the Florida Department of Citrus showed that such postharvest conditioning treatments did not need to be as drastic or as short. With longer periods (e.g., 7 days) and more moderate temperatures (e.g., 15–16°C), grapefruit can be ''cured'' to enable it to withstand storage and transit temperatures low enough to cold sterilize fruit fly larvae (0–2°C) [64]. This protective effect is not peculiar to grapefruit, as shown by a recent report on prestorage conditioning of squash (*Curcubita pepo* L.) to inhibit chilling injury [65]. It appears that conditioning-induced protective levels of ABA are as general for harvested fruits as they are for seedlings of cucumber [66] and cotton [67].

Prestorage conditioning is now becoming general for products as diverse as summer squash [65] and *Opuntia* ''cactus pears'' [68].

Thus, research on the *benefits* of applied stress can undermine apparent truths such as the old precept that perishable products should all be refrigerated as promptly as possible after harvest. Obviously the benefits of prompt refrigeration do not necessarily apply to CI-susceptible products which often need to be preconditioned by precise application of postharvest stress.

CONCLUSIONS

Stress is not merely something to be combated: Stress is one of nature's essential tools. Intelligently employed, stress can be used to obtain many benefits for both research workers and plant industries. In particular, the attention of two groups must be drawn to this dual role of plant stress. The first such group consists of the individuals, committees, and administrators who control grant funds for research on plant stress. The second group involves virtually all those who teach, or otherwise advise, on the handling of horticultural products. For these, it has long been holy writ that a fruit, vegetable, or flower should be refrigerated as soon as possible after harvest. This otherwise excellent principle needs to be reconsidered when chilling-susceptible products are to be stored for any considerable period.

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