HORMONAL REGULATION OF PLANT GROWTH AND DEVELOPMENT

ADVANCES IN AGRICULTURAL BIOTECHNOLOGY

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Hormonal Regulation of Plant Growth and Development

edited by

S.S. PUROHIT

Post Graduate Department of Botany
Dungar College, Bikaner 334 001
India

1985 **MARTINUS NIJHOFF/DR W. JUNK PUBLISHERS**
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DORDRECHT / BOSTON / LANCASTER

Distributors

for the United States and Canada: Kluwer Academic Publishers, 190 Old Derby Street, Hingham, MA 02043, USA
for the UK and Ireland: Kluwer Academic Publishers, MTP Press Limited, Falcon House, Queen Square, Lancaster LA1 1RN, UK
for India, Pakistan, Burma and Sri Lanka: Agro Botanical Publishers (India), Old Ginnani, Bikaner 334 001 India
for all other countries: Kluwer Academic Publishers Group, Distribution Center, P.O. Box 322, 3300 AH Dordrecht, The Netherlands

Library of Congress Cataloging in Publication Data

Main entry under title:

Hormonal regulation of plant growth and development.

(Advances in agricultural biotechnology)
1. Plant hormones. 2. Growth (Plants) 3. Plants--Development. I. Purohit, S. S. II. Series.
QK731.H64 1985 582'.03'1 85-11634

ISBN-13: 978-94-010-8773-5 e-ISBN-13: 978-94-009-5139-6
DOI: 10.1007/978-94-009-5139-6

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PREFACE

Plant hormone research is the favorite topic of physiologists. Past three decades have witnessed that this subject has received much attention. The inquisitive nature of human mind has pumped much in literature on this subject and this volume is the product of such minds. In the following pages various hormonal-controlled physiological processes like, flowering, seed dormancy and germination, enzyme secretion, senescence, ion transport, fruit ripening, root growth and development, thigmomorphogenesis and tendril thigmonasty have been included. The volume also contains a review paper on 'Growth Regulating Activity of Penicillin in Higher Plants' and has been presented for the first time. The vast contents of each review paper have been written by erudite scholars who have admirably carried out their evangelic task to make the text up to date. This volume, I am sure, would stimulate the appetite of researchers of peripheral disciplines of botany and agricultural sciences and they will continue to enjoy the fun and adventures of plant hormone research.

Save one, my most outstanding debts are due to the rich array of the contributors and other plant physiologists specially to Prof. Thomas Gaspar (Belgium), Prof. E. E. Goldschmidt (Isreal), Prof. H. Greppin (Switzerland), Dr. K. Gurumurti (India), Prof. M. A. Hall (U.K.), Prof. H. Harada (Japan), Dr. M. Kaminek (Czechoslovakia), Dr. J. L. Karmoker (Bangla Desh), Prof. Peter B. Kaufman (U. S. A.), Dr. V. I. Kefeli (U. S. S. R.), Dr. M. Kutaoek (Czechoslovakia), Prof. S. Lewak (Poland), Dr. K. Lurssen (West Germany), Prof. S. Mukherji (India), Dr. Cl. Penel (Switzerland), Dr. K. G. Purohit (India), Dr. V. Rajagopal (India), Prof. B. Sabater (Spain), Prof. G. Sambdner (D. D. R.), Prof S. P. Sen (India) Prof. K. Shimokawa (Japan), Dr. H. S. Srivastava (India), Prof. A. Szweykowska (Poland), and Dr. S. Tanimoto (Japan) to whome this volume belongs. They have inspired me for the preparation of this volume. In editing and production I have received assistance from many persons who cheerfully embraced the onerous task, I am thankful to them.

I also express my thanks to Mrs. Saraswati who have taken keen interest in publication of this volume.

Bikaner July 1984

S. S. Purohit

LIST OF THE CONTRIBUTORS

DAYANANDAN, P., Department of Botany, Madras Christian College, Tambaram, Madras 600 059 (India).

GASPAR,THOMAS, Hormonologie, Fundamentale et Appliquec, Institut de Botanique (B 22) Univ. de Liège-Sart, Tilman B-4000, Liegè (Belgium).

GERPPIN H., Laboratoire de Physiologie Végétale, 3 Place de l' Université, CH- 1211, Genève 4 (Switzerland).

GURUMURTI, K., GUPTA B. B., KUMAR, ADARSH, Plant Physiology Branch, Forest Research Institute, Dehra Dun 248 006 (India).

HARADA, H., Institute of Biological Sciences, University of Tuskuba, Sakura-mura, Ibaraki, 305 (Japan).

JAFFE, MORDECAI, J., Department of Biology, Wake Forest University, Winston-Salem, North Carolina 27109 (U. S. A.)

KARMOKER, J. L., Department of Botany, Dacca University, Dacca (Bangla Dash).

KAUFMAN, PETER B., Division of Biological Sciences, The University of Michigan, Ann Arbor, Michigan 48109-1048 (U. S. A.)

LEWAK, STANISLAW, Institute of Botany, The University of Warsaw, Warszaw (Poland).

LURSSEN, KLAUS, Bayer A. G., Sparte Pflanzenschutz, Anwendungy stechnik, Biologische Forchung. 5090 Leverkusen, Bayerwerk (West Germany).

MUKHERJI, S., Department of Botany, University College of Science, Calcutta University, Calcutta 700 019 (India).

PENEL, C., Laboratoire de Physiologie Végétale, 3, Place de l' Université, CH-1211 Genève 4 (Switzerland).

PUROHIT, S. S., Laboratory of Plant Physiology, P. G. Departement of Botany, Dungar College, Bikaner 334 001 (India).

SABATER, B., Dpto. Fisiologia de Vegetal, Facultad de Ciencias, Univ. de Alcala de Henares, Apdo 20-Alcala de Henares, Madrid (Spain).

SEN, S. P. Department of Botany, University of Kalyani, Kalyani, 741 235 (India).

SHIMOKAWA, K., Faculty of Agriculture, Miyazaki University, Miyazaki 880 (Japan).

SRIVASTAVA, H. S., Department of Life Sciences, University of Indore, Khandawa Road, Indore 452 001 (India).

TANIMOTO, S., Institute of Biological Sciences, University of Tuskuba, Sakura-mura, Ibaraki, 305 (Japan).

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The Molecular Basis of Hormone Action

S. P. Sen

Introduction

Several chapters in this volume will describe the various physiological effects of plant hormones and some of the current views regarding the mechanisms of action of plant hormones. The progress in this field has been quite spectacular in recent years but probably it would have been more so, if workers in this field were less fascinated by hormone effects on extension growth and had paid equal emphasis to other aspects which are not generally considered as growth processes. An understanding of hormone action is dependent on the location of the sites of action and the multiplicity of such sites is explicit in the large variety of the effects which the hormones evoke. The mechanisms of hormone action proposed so far, though based on considerable experimental evidence with respect to one or two aspects of hormone action, are quite often oversimplifications. Generalisation is certainly useful as a working hypothesis but if they are carried too far, truth recedes further in the background. Although there are considerable similarities between plant and animal life, in searching for unity, we should not forget the diversity. Thus, analogy between plant and animal hormone effects is certainly useful and sometimes it has speeded up development in this field but this may not be the case always. Plant hormones differ from animal hormones in that they are small molecules not synthesized in a gland, target organs are quite often several or many and sometimes the hormones are relatively immobile even though the physiological effects are quite striking. Animal hormones like ecdysone or the steroid hormones either have no effect in plant systems or elicit responses which cannot be considered unequivocally as a response characteristic of the hormones.

Three of the major plant hormones-IAA, gibberellic acid and kinetin-were first discovered from fungi or are derived from some fungal products, but there is no conclusive evidence to indicate that any one of

them acts as a hormone in the source organism. The literature concerning auxins has been reviewed by Gruen (1959). Neither auxin nor gibberellic acid increases the dry weight of *Rhizopus nigricans* or *Gibbrella fujiukuroi* at any of the concentrations tried (Mitra, 1968). IAA is known to be produced by several bacteria and Libbert Silhingst (1970) was of the opinion that much, if not all, of the IAA found in plants is produced by epiphyllous microorganisms. In prokaryotic systems hormonal activity has not been detected for any known hormone of plant or animal origin; cAMP, which is considered to be a second messenger for several plant and animal hormones, however, has important regulatory roles to play in microbial life. Why hormones are inactive in prokaryotic system has not been explained yet ? One possibility which should be considered is that bacterial DNA is not associated with histons and chromatin as is found in the eukaryotic systems does not occur in the prokaryotes.

The Implication of the Kinetics of Hormone Action

James Bonner, some thirty years ago had applied the principles of enzyme kinetics to auxin action in growth. The interaction of an enzyme with the substrate may be considered to be similar to the interaction of a hormone with the reactive site in a cell forming a hormone-reactive site complex synonymous with the enzyme-substrate complex formation in enzyme action. Physiological effect was considered to be the product of this interaction. The dose response curves of hormones are suggestive of saturation of the reactive site at which optimum physiological effects are observed (Fig. 1.1 and 1.2) when the physiological effect of a hormone is studied at different concentrations, what is actually done is a sort of titration of these sites with the hormone molecules, the optimum concentration providing an approximate measure of the number of such sites in a cell. The dose response curve of gibberellins has considerable similarity with that of the animal hormones which are active over a wider concentration range. The two phase concentration curve characteristic of auxin action is of a rather different nature. The Lineweaver–Burk plotting of the reciprocal of the hormone–induced effect versus the reciprocal of hormone concentration provides Vmax and Km values from which the degree of maximum observable effect and the affinity of the hormone for the receptor site respectively can be calculated (Fig. 1.1). Using dwarf TN–1 rice seedlings it has been observed in the author's laboratory (J.L. Das, unpublished) that the Vmax for several

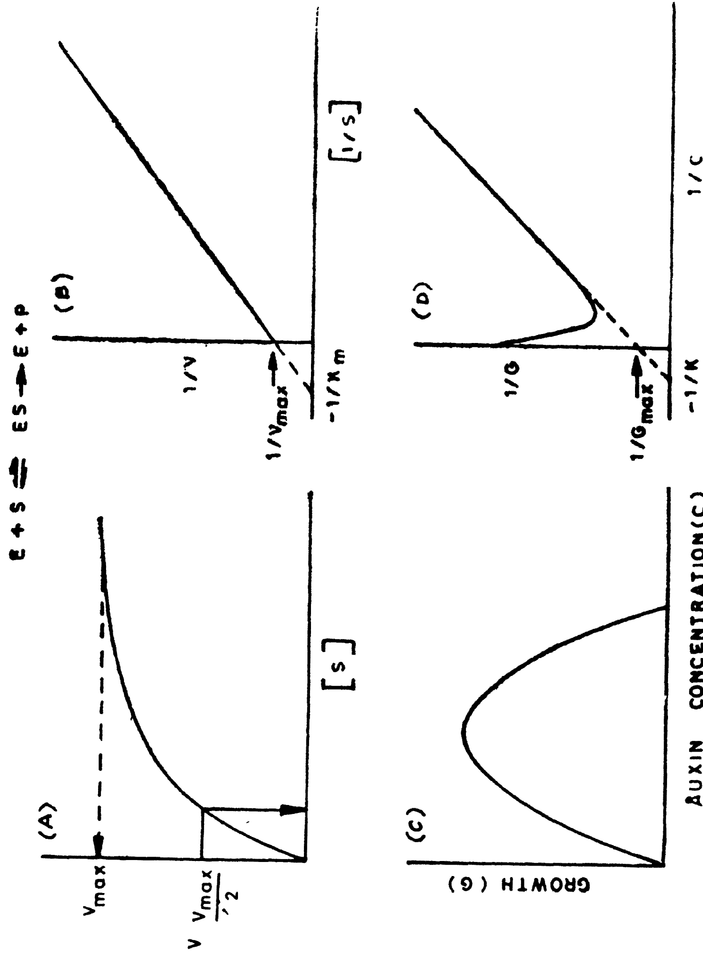

Fig. 1.1. A comparison of the Kinetics of enzyme and hormone action (A) the relationship between the velocity of an enzyme catalyzed reaction and substrate concentration; (B) the reciprocals of the same values plotted by the Lineweaver-Burk method; (C) the typical curve of an auxin effect; (D) the same plotted by the Lineweaver-Burk method; E- enzyme; S- Substrate; ES- enzyme substrate complex; P- product; G- growth; V- velocity; Vmax- maximum velocity; Km- Michaelic constant; G- growth or other hormone effect; Cmax- maximum growth or other hormone effect. Note that the reduction in the increment of growth or other physiological process at higher hormone concentrations gives a bell-shaped curve and hence the Lineweaver-Burk plot is not identical to that of an enzyme action, although the nature is similar.

gibberellins tested was identical; this is suggestive of either the site of action of different gibberellins being the same on that the different

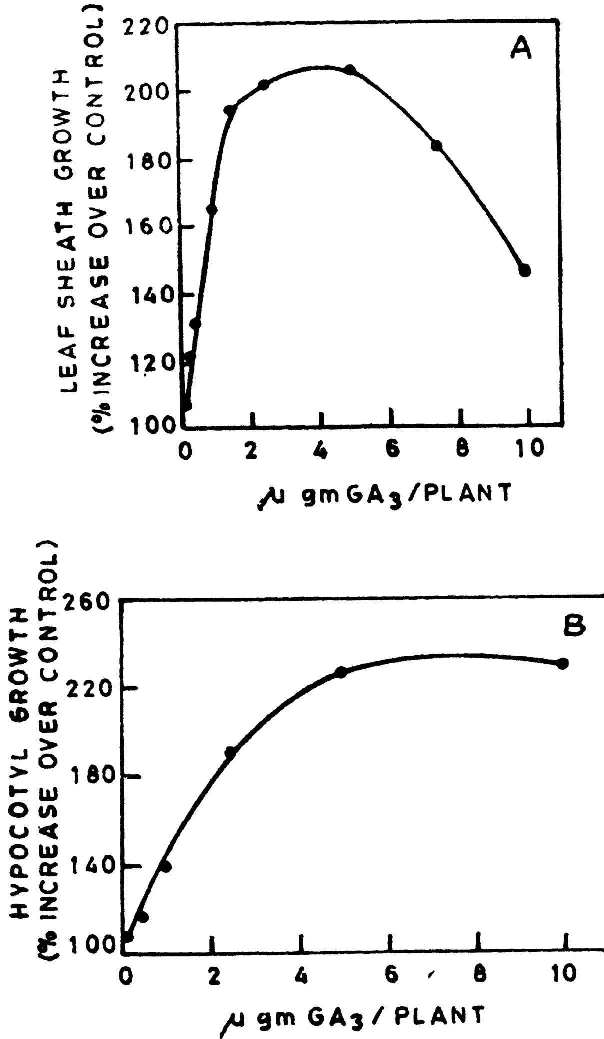

Fig. 1.2 The relationship between amount of GA_3 applied per plant the growth of leaf sheath of TN-1 rice (A) and cucumber hypocotyl (B). Note that the range of concentration for hypocotyl growth is much greater than that for growth of leaf sheath. In the latter case the "growth active sites" with which the effective form of GA_3 interacts becomes saturated at a much lower concentration of applied GA_3 than in the former case (J. Guha, unpublished).

gibberellins, before they act are converted to one common form, which reacts with a particular site. There is evidence for the interconversions of several and the actual conversion of GA_9 , and GA_{20} to an active hydroxylated form. In the case of auxin Bonner's group had earlier observed that for molecules in the auxin series Km values vary considerably; however, these values agree very well with the relative biological activity of the hormones utilized (Foster *et al.*, 1952).

Time course experiments have clearly revealed that while hormone effects on extension growth can be demonstrated in a few minutes, other effects take several hours or days indicating dual action with different sites. The kinetic experiments indicate that plant hormones interact with some site (s) in the cell for a particular physiological effect and provide information regarding their affinity for such sites but do not tell us anything about the nature of these sites. The elegant studies by workers in different laboratories on the relationship between molecular structure and biological activity of the hormonal substances have brought out the minimum structural requirements for the biological activity of such molecules and provide some indirect information regarding these sites. A summary of the available information follows:

Relationship Between Molecular Structure and Biological Activity

Auxins

In the case of several auxins this requirement appears to be a ring structure, certain degree of unsaturation in the ring, an acidic side chain at least one carbon removed from the ring and a free *ortho*-position. However, several synthetic compounds considered as auxins e.g., the thiocarbamates and some benzoic acid derivatives do not meet these requirements. Porter and Thimann (1965) showed that for many auxins including the thiocarbamates which do not possess the structural features, the distance between the fractional positive and negative charge densities of molecules which qualify for the role of an auxin is 5.5 Å; more recent and precise estimates put this value close to 5Å. If an auxin molecule has to attach to a reactive site through its positive and negative charges, then the region of the reactive site which would be involved in this interaction, should have a complementary pattern having opposite charges the same distance apart. Although, sites which would satisfy these requirements have not been isolated or characterised and charge distribution data of such auxin molecules as α–naphthalene acetic acid (NAA) and several other auxins are not available, these studies provide an excellent

theoretical background for formulation of the nature of the reactive sites. Depending on pH, the major cellular polymers–proteins and nucleic acids- would be differentially protonated providing a point of attachment for the negative charge on the carboxyl group of the auxin molecule; the small negative charge density due to the lone pair of electrons on the 0 atom of the carboxyl or hydroxyl group or the phosphate residues may attract the fractional positive charge centres of the auxin molecule. This would provide several regions of the macromolecule for selective association with the auxins. If the auxin molecule interacts with a receptor protein, which then undergoes allostearic modification, a larger region of the chromatin will be involved in the interaction. Some membrane components or cell wall polysaccharides may also satisfy this requirement.

Gibberellins

In the case of gibberellins, struture–function relationship studies have been less rewarding. About 60 gibberellins of natural origin have been reported already. Most active gibberellins possess an ionizable carboxyl function at the 7-position and masking of this group by methylation drastically lowers the biological activity indicating that the carboxyl group probably reacts with the positively charged element of the reactive site. The 19, 10-lactone function also seems to be important for biological activity. Hydroxylation of the 2 beta and 12α- positions abolishes the biological property. High activity is observed when the molecules are hydroxylated at 3 beta and 13-position. In many plant tissues GA_1 is converted to GA_8 the practically inactive form, by 2 beta hydroxylation and the enzyme has also been isolated from *Phaseolus vulgaris*. Stoddart and Venis (1980) have observed that in presence of this enzyme only those GA molecules would survive in an unsubstituted state, if it is attached to the active site or protected by compartmentalization. If the response to GA depends on a reversible association between GA and a finite number of active sites, then the extent of response would depend on the rate of removal of the active molecules by hydroxylation at the 2-position. Short term requirement for GA's can be met by release from the protected compartments like organelles bounded by lipid membranes. Halogenation of GAs has not increased the biological activity very much, unlike auxins, in spite of the enhancement of electronegativity and the H-bonding capacity of the molecules. In the O-gluosyl ethers or glucosyl esters which probably become active only after hydrolytic cleavage, the attachment of a glycosyl

group may interfere with the functioning of GA at the aqueous lipid interphases e.g., a membrane surface.

Cytokinins

Although naturally occurring cytokinins are few in number, about 500 derivatives have been synthesized so far, only few of which are biologically active. The biologically active forms are usually purine and pyrimidine derivatives with the exception of N, N'-diphenyl urea and the 8-azapurine. The natural ocurrence of diphenyl urea has been questioned. In the purine ring exchage of positions by C and N in the 7-and 8-positions reduces activity by two orders of magnitude and the N^6 position. The pyrimidine derivative, benzimidazole, is quite active in the deferral of leaf senescence indicating that for these effects a purine nucleus is not essential. The length of the N^6 side chain and the degree of unsaturation is important for biological activity. The inclusion of furfuryl or benzyl group in the side chain is advantageous for bioactivity. Substitution at 1- or 3-positions in the purine ring reduce biological activity. Halogenation of 2-positions of zeatin enhance its activity and the same is also true for a 9-methyl derivative.

Isopentenyl adenosine and zeatin have been detected in several tRNAs of plants and bacteria. However, all species of tRNA do not contain a cytokinin base which are constituents of the anticodon loop which associate with the ribosome or mRNA-binding sites. The cytokinins are not incorporated in tRNA; the adenine residues receive the isopentenyl pyrophosphate as a part transcription modification.

Ethylene

In ethylene the unsaturated double bond is important for biological activity which varies inversely with the molecular size. The terminal carbon atom should be electrophilic and any substitution which causes electron delocalization reduces biological property. Methylacetylene is highly active but acetonitril is inactive. Thus, nitrogen substitution of a carbon atom at the end of a double bond is deleterious to the biological effectiveness (Stoddart and Venis, 1980).

Abscisic Acid

A large number of analogues of this sesquiterpene has been reported (Milborrow, 1974; Bittner *et al.*, 1977) and it appears that small deviations in molecular structure lead to very marked changes in biological activity indicating that the molecule fit in some receptor site may be precise (Stoddart and Venis, 1980). Thus, activity is almost

completely lost when Δ^2 -*cis* ABA is isomerized to Δ^2 -*tarns* ABA. The related compound phasic acid is biologically much less active xanthoxin, the product of lipoxygenase mediated or oxidative degradation of violaxanthin in light is a strong germination inhibitor. All *trans* farnesol, like ABA, is a very potent antitranspirant. The same is also true for romifoliol, but it has no inhibitory effect on growth. Hormonal regulation of stomatal movement has been discussed by Purohit (1983)

Terpenoids and steroids

Most terpenoids have growth inhibitory properties and some of them have allelopathic activity. They include the monoterpene essential oils, camphor, cineole, several α-methylene lactones of Compositae, several sesquiterpene lactones, sesquiterpenoid ketones, a number of unsaturated lactones e. g., podolactone E and momilactone A, the non-lactone diterpenoids portulal and the tetracyclic and pentacylic triterpenoids like cucurbitacius, nimbin, taraxasten *etc.* Generally the unsaturated lactones of diterpenoids become more inhibitory with increase in polarity. Howere, the α, beta-unsaturated γ-lactone strigol has pronounced stimulatory effects on seed germination; the α-pyrones nagilactones A-D have growh promoting property. The cucarbitacins and the pentacyclic triterpenes also act as gibberellin antagonists (Guha and Sen 1973, 1975).

The tetracyclic terpenoid limonene which has a unusual chemical structure behaves both as a gibberellin antagonist and a cytokinin. Studies on structure and function relationships of molecules like this will be fascinating. Grunwald (1975) has suggested that plant sterols might act as hormones, or more likely be precursors to steroids which act as hormones. However, this contention is not generally accepted (Thimann, 1973) since effects of growth, reproduction and sex expression though reported in several plants, have not been observed in other plants (Grunwald, 1975; Mitra and Sen, 1975). Some steroidal substances of *Coleus* show auxin-like activity in the *Avena* curvature test. Young growing tissues of *Phaseolus* have been found to be rich in sterol content and oestrogens increase at the time of flowering. Oestradiol-17 beta induces the flowering of *Cichorium intylus* under non-inductive conditions. SK&F 7993 an inhibitor of steroid biogenesis inhibits both GA_3-induced growth and flowering of several plants, the interaction with GA_3 is probably non-competitive. Steroid effects may be brough about indirectly through their effects on auxin and gibberellin levels. Both steroids and gibberellins are derived from mevalonic acid.

Brassins

Brassinolide is a steroidal lactone (22R, 23R, 24S)-2α, 3α, 22, 23-tetrahydroxy-24 methyl-6,7-seco-5 α-cholestans-6,7-lactone) isolated from rape pollens. Structurally it has some resemblance to the ecdysones. Brassins cause elongation, swelling and spletting of the bean second internode. The α-orientation of the OH-groups in the 2-and 3-positions and 22-and 23-hydroxyl and 24-methyl (or ethyl) substitutions appear to be essential for biological activity but stereochemical variations in the positioning of these substituents do not affect biological activity. Brassins evoke responses similar to auxin, gibberellins and cytokinins in many respects, but they do not promote the growth of dwarf maize and surprisingly light appears to be required for the response (see West, 1980). Alnusin isolated from *Alnus* pollens have properties similar to Brassins in several respects (Mandava and Mitchell, 1971).

Lipids

A veriety of lipids (oleanimins) sromote the growth of pea plants in presence of red light, sugars or auxins (Stows and Obreiter, 1962; Stowe and Dotz 1671; Lethan, 1978). Stowe aud Dottz (1971) have deduced that the lenght of the lipids to be active should exceed 20 Å reaching an optimum near 28-30Å. The lipids become active by forcing apart leacithin molecules, changing the charge distribution or chelaling properties of a regulatory membrane. Long chain acids, alcohols, aldehydes and ketones have inhibitory activity, the inhibition increasing with lipophilicity. 1-docosanol has auxin like activity and triacantanol promote the growth and yield of rice, maize and barley. Several fatty acids antagonize gibberellin action (Stoddart and Venis, 1980).

Cyclic nucleotides

Cyclic nucleotides like cAMP are believed to be second messengers of hormones. Whether it also acts as a second messenger for plant hormones is a matter of controversy (see Amrhein, 1977). 2′,3′-and 3′,5′-cGMP are also effective in some systems. Kessler (1972) considers that the cyclic mononucleotides probably act as primary inducers at the genome level leading to the biosynthesis of gibberellins and probably other regulators. The use of dibutyryl derivatives, caffeine and theoply-lline may help in studying the specificity of the effect.

Polyamines

The diamines and polyamines which are of wide occurrence in plants have remarkable growth regulatory properties. The common diamines are putre scine and cadaverine, and spermidine are tri and tetramines,

respectively. Prokaryotes have more of putrescine than spermidine and spermine is absent. In eukaryotes both spermine and spermidine are present in relatively large amounts and putrescine is detected only in traces. Active growth is quite often associated with increase in polyamine content. Stimulation of the growth of potato tuber by polyamines is comparable to that by auxin. Auxin application results in the accumulation of polyamines. The contribution of polyamines to growth may be considerable. Polyamines also help in the proliferation of normal and callus tissues. They behave as cations at physiological pH or bind strongly to negatively charged group of nucleic acids and proteins. They then stabilize the secondary structures of nucleic acid and proteins.

Non-protein amino acids

The non-protein amino acid azatidine-2-carboxyle acid, pipecolic acid, mimosine, methylene cyclopropylglycine or β-pyrazol-1-glalanine as also canavanine or N, N-dimethyl-L-tryptophan, inhibit growth, presumably by inhibiting protein synthesis or by disruption of the protein function after incorporation (Stoddart and Venis, 1980).

Alkaloids

Most alkaloids and cyanogenic glucosides are inhibitory to growth. These include amygdalin, mustard oil glycosides, colchicine, caffeine, strychnine etc. Delcosine, the diterpenoid alkaloid interferes with gibberellin action. Camptothecin at 10^{-4} M completely inhibits the growth of tobacco but sorghum and beans are unaffected. However, 2-hydroxy cinchoninic acid and zeanic acid stimulate plant growth. Very little is known about the nature of this inhibition.

Phenolics

Phenolics generally inhibit germination and growth. Some phenolic acids like salicylic acid and gallic acid inhibit flowering (Khurana and Maheshwari, 1978). Dihydroconiferyl alcohol synergises gibberellin action in hypocotyl elongation. Phenolic compounds are presumed to act indirectly though their effects on IAA oxidase. Thus monophenols are inhibitory because they promote the decarboxylation of IAA, whereas the diphenols are growth promoters because they inhibit IAA-oxidation. High concentrations, however, are always inhibitory. The betalains also interfere with IAA oxidase. Some flavonoids interact with IAA oxidase and light alters the ratios of flavonoids like kaempferol or quercetin. Sayanedine acts as a cytokinin and naringenin as a gibberellin antagonist. Many coumarins act as inhibitors of growth and germination.

Some promote root growth and act as gibberellin antagonists.

Miscellaneous

There are microbial metabolites which have apparently no structural similarity with any other plant hormones but have pronounced biological activity. Thus fusicoccin effects are very similar to auxin effects including the acid growth effect; helminthosporal resembles rings C and D of gibberellin but penicillin has nothing in common with giberellins, yes both of them behave as gibberellins. How penicillin induces α-amylase activity in cereal endosperms (Biswas and Mukherji, 1980), increases leaf area (Purohit and Purohit, 1983), and prevent monocarpic senescence (Purohit and Chandra, 1983)? Are GA_3 and penicillin degraded to the same compound which actually induces α-amylase ?

The Probable Sites of Hormone Aation

The centres of fractional positive and negative charges in molecules other than auxins have not yet been located experimentally, although, theoretical predictions are possible. Consequently the nature of association with the reactive sites is a matter of speculation. Among the macromolecules DNA, RNA and proteins are the major candidates for the effective sites.

DNA

Auxins

In the case of auxins there is considerable evidence for association with DNA. Mitra *et al.* (1970) have determined the affinity constants of auxin molecules binding with DNA and the maximum number of auxin molecules which could be bound to DNA were found to be 5000, 5000 and 1670, respectively for IAA, NAA and phenylacetic acid per molecule of DNA. Subsequent experiments revealed that IAA binds to apurinic DNA but not with apyrimidinic DNA. Since it also binds with dAT co-polymer, thymine residues of DNA would appear to be the binding site (Mitra *et al.*, 1970). Since the auxin induction of growth is prevented by phleomycin, which binds with 2-position of thymine residues, auxin may associate with DNA at the 2-position of thymine of DNA. IAA also fails to bind to DNA, if it is pre-treated with phleomycin (Sen and Das, 1982).

Since IAA binds to poly dC, even though to a lesser extent than to poly dT, auxin may also bind to cytosine residues. High concentration of IAA interferes with binding of actinomycin D to DNA, indicating that

guanine residues may be close by the thymine or cytosine residues which bind IAA, as actinomycin -D is known to bind with the 2-position of guanine of DNA. Higher concentrations of IAA also erase the actinomycin inhibition of RNA (Roychoudhury *et al.*, 1965) and protein (Datta *et al.*, 1965a) syntheses and growth (Datta *et al.*, 1965b), Fellenberg (1969) and Bamberger (1971) have also shown that IAA interacts with DNA and nucleoprotein and the Tm to both was decreased.

Other hormones

ABA, GA_3 , Kinetin and cAMP also bind with DNA (Mondal and Biswas, 1972; Fellenberg, 1969; Bamberger, 1971; Sen and Das, 1982). The binding of kinetin to DNA was first demonstrated by Roychwdhury (1964a). Kessler and Snir (1969) reported that GA_7 binds with plant DNA but GA_3 does not. This is surprising, since GA_3 and GA_7 are almost equally active in several plant systems. Mondal and Biswas (1972) reported that ABA interacts with DNA, increasing its stability to alkali and heat denaturation. Fellenberg (1969) and Bamberger (1971) reported binding of GA_3 and kinetin to DNA or nucleoprotein. The Tm was decreased except in the case of kinetin when there was an increase. The different hormones interact with each other in varying degrees for binding to DNA. Thus, GA_3 decreases the binding of both IAA and kinetin at relatively high concentrations. cAMP at a concentration of 10^{-5} M binds more efficiently with DNA than IAA at the same concentration. Both IAA and kinetin markedly decraese the binding of cAMP to DNA and at 10^{-4} M, the interference is complete. Kinetin binds to DNA practically to the same extent as cAMP (Sen and Das, 1982). The exact site of binding of kinetin, cAMP and GA_3 are not known; however, such sites would appear to be in proximity to the IAA binding sites, since they interact with each other. These observations may explain the differential physiological responses observed with different ratios of the hormones e. g., the effect of auxin and cytokinin in differentiation of callus cells.

RNA

IAA also binds with RNA and 10^{-5} M IAA binds pea RNA, practically to the same extent as DNA. IAA binds to same extent with apurinic RNA but not at all with apyrimidinic RNA or poly U. Probably, the appropriate base sequence required for binding of IAA to RNA is available in apurinic RNA only to some extent but not at all in poly U and the appropriate sequence includes a cytosine residue but there is no direct

evidence for this. The *in vivo* binding of IAA to RNA has been questioned (Davies and Galston, 1971; Davies, 1973). Hormone binding to an aminoacyl tRNA may regulate protein synthesis by affecting polypeptide chain elongation due to aminoacyl tRNA conservation being rate limiting. It may be of interest to mention here that the steroid hormones progesterone, oestradiol and testosterone bind to aminoacyl tRNA but not to deacylated tRNA; the binding however, is restricted to only few species of tRNA and the singlestranded loop sequence of tRNA in a particular conformation is the binding site (Chin and Kidson, 1971).

Protein

Auxin

IAA also binds to a variety of proteins, both enzymic and structural. The extent of binding with bovine serum albumin fraction IV or the histones of pea and calf thymus is no less remarkable than that of the binding of IAA to DNA. Hexokinase also binds IAA appreciably. It does not however, bind significantly with collagen, elastin, DNase and RNase. The binding of IAA to histone is of interest in view of the regulatory role unless which has been ascribed to it by several workers. Hydrocortizone binds with rat liver histone mole for mole.

Considerable work has been done with hormone receptors which are protein in nature (Evans, 1983; Stoddart and Venis, 1980). Protein fractions capable of binding with auxins have been detected in pea, coconut endosperm, corn coleoptiles, *Avena* coleoptiles and roots, tobacco cells and soybean cotyledons. These proteins have been isolated from membrane fractions, cytosols and nuclei. The molecular weights of these proteins vary between 20,000–200,000 and the K_D values of the auxin receptor complex show a range of 10^{-8} M–7.5 x 10^{-6} M (Matthysse and Phillips 1969; Mondol *et al.*, 1972; Roy and Biswas 1977;Bhattacharya and Biswas 1982). Mondal *et al.* (1977) showed that the IAA-receptor complex enhances DNA dependent RNA synthesis in presence of RNA polymerase, DNA and an initiation factor B, probably a non-histone protein. In *Avena* thc coleoptiles contain both high and low affinity binding sites, while the roots possess only the low affinity once.

Cytokinins

Cytokinin-binding protein were first reported by Matthysse and Abrams (1970) from the chromosomal protein of pea. This is a mediator protein which stimulates RNA synthesis with pea DNA as template in presence

of *E. coli* RNA polymerase. More recently, cytokinin-binding proteins have been isolated from wheat embryos with a molecular weight of about 155,000 and possessing 4 non-identical subunites. One molecule of cytokinin binds with one molecule of cytokinin-binding protein (Reddy and Datta, 1982; Reddy *et al.*, 1983). The binding is specific for benzyl adenine, is inhibited by kinetin and benzyladenosine but by cAMP, and zeatin, the natural cytokinin- only slightly inhibits the binding. Several other workers have reported the isolation of cytokinin-binding proteins ranging in molecular weights from 4,000 to 50,000 from higher plant ribosomes. According to Erion and Fox (1981) the concentration of cytokinin–binding factors in wheat germ is 2.2 mg/gm fresh weight and occurs in both native ribosome as also in the cytosol with about 3 copies of the latter for one of the former and one molecule binds with each 80 S ribosome particle. It has a strong affinity for isopentenyl adenine but very little for *cis* or *trans* zeatin. Tobacco suspension cultures containing a heat-labile factor with a molecular weight of 80,000 (Sussman and Kende, 1978); a polypeptide with a molecular weight of 4,500 has also been isolated from tobacco cells having a moderate affinity for both benzyladenine and kinetin (Takegami and Yoshida, 1975). The binding proteins isolated from cabbage ribosome have a low affinity.

Gibberellins

Stoddart *et al.* (1974) using high specific activity detected the presence of soluble macromolecules in epicotyl hooks which could associate with GA_1 . Biologically inactive gibberellins like GA_8 did not reduce the overall levels of GA_1 binding and could not be recovered in any of the macromolecular associations.

ABA

Abscisic acid has also been found to bind with membranous preparations of bean leaves, the binding increasing with increasing concentrations. Two classes of binding sites were detected, the higher affinity site had a dissociation constant of 3.5×10^{-10}M (Hocking *et al.*, 1978).

Ethylene

There is some evidence that several plant species have mechanisms for compartmentation of ethylene (Jeric *et al.*, 1979). In *Phaseolus* there is a high affinity binding site which has a dissociation constant of the order of 10^{-10}M (Bengochea *et al.*, 1980). The relative physiological effectiveness of the structural analogues of ethylene was comparable to their capacity to compete with ethylene for the binding sites.

Biological Significance of Receptors

The significance of the binding of plant hormones to receptor proteins has been critically analysed by Stoddart and Venis (1980). Not a single plant hormone receptor system has so far been characterised for which a causal relationship to its physiological effects has been unequivocally established. The specificity of the reactions has not always been adequately tested, and the evidences are quite often indirect. In some cases e.g., for cytokinin-binding proteins the dissociation constant of the high affinity site equals or exceeds the concentration at which most physiological processes become saturated. It is generally believed that the plant hormones in some unknown manner release the binding proteins from some membranous structures -endoplasmic reticulum, plasmalemma or the tonoplast the hormone receptor complex then finds its way to the genome where a specific interaction takes place between the hormone receptor complex and the chromatin. How the hormonal substances at such low concentration dissociate the binding proteins from the membranous structures is not understood, neither do we have any information regarding the nature of association of such proteins with the membranous structures. Such proteins are apparently solubilized during the experimental procedures which are quite often not very mild. The association of such factors with the membranous structures or their presence in the cytosols may well be antefacts produced during the extraction procedure. The migration of the hormone-receptor complex to the genome has also been rarely demonstrated. While analogy with steroidal hormone action may well be valid, particularly in the case of gibberellins, the situation may well be different and one should not be biased either way. The nature of interaction between the hormone-receptor complex and the chromatin is also not known. Does it involve one part of the hormone and a part of the protein or the protein alone is involved ? Is there an allosteric modification of the receptor protein by the hormone ? These are questions which have to be answered before the contribution of the hormone-receptor complex to the physiological response is assessed.

Lipids

Current theories of auxin action visualize an interaction of auxin with membranes. Since membranous strutures are lipoproteins, an interaction with the lipid components has been suggested. Veldgtra (1953) had observed that the lipophilic part of the auxin molecule may associate with the lipid component, with the side chain protruding outwards. Weigl (1969) has shown with his model membrane, that there is a good specificity for the active auxins; he attributes the case of this binding to

the spacing of the positive and negative charges on the auxin matching opposite charges on the lecithin moiety of the membrane. In pea the most common membrane lecithin is 1-Palmitoyl-2-linoleoyl-3 Sn-phosphatidyl choline (Tremoleres and Lepage, 1971). The choline moiety is at the aqueous interphase and the hydrocarbon tail back up against the lipophilic surface of another monolayer facing the other direction. The plant sterols associated with the membranes may hydrogen bond with the carboxyl or a negative charge centre of the hormone molecule and with the phosphate hydroxyl of the lecithin in the other side (Stowe and Dotts, 1971),

In the case of gibberellins Jones (1969a,b) has shown that in GA_3 treated cells the endoplasmic reticulum and rough endoplasmic reticulum became prominent. GA_3 enhances the activity of enzymes concerned with lecithin synthesis several fold and choline incorporation is enhanced (Johnson and Kande, 1971; Evins and Varner, 1971). Using artificially prepared liposomes, Wood and Paleg (1972) have shown that GA_3 enhanced the leakage of glucose from the liposomes, only when crude lecithins of soybean were used but not with purified preparations. GA_3 thus affects the permeability of liposome membranes. It apparently interacts with lecithin.

Although ethylene is suspected to interact with membranes, there is no conclusive evidence that there is a modification of membrane structure.

Carbohydrates

Several hormones conjugate with carbohydrates. Although their biological activity has not been unequivocally demonstrated, several of them have been found to be as active or more active than the free compounds, presumably after hydrolysis. It is generally believed that they help as storage reservoirs helping in the detoxification of relatively high concentrations of hormones and thereby regulating hormone levels; they also help in the translocation of the hormones. IAA has been found to conjugate with glucose, arabinose and rhamnose. The carboxyl group of IAA is involved in the esterification with sugars. However, the cytokinins are usually ribosylated in the N–9 position; glucosylation involves N-3, N-7 or the N-9 position. The O-4' position is also involved in the formation of several cytokinin-O-glucosides. In the case of gibberellins glucose is usually conjugated to the hydroxyl or the carboxyl group. Abscisic acid has been reported to occur as its β-D glucopyranosyl ester in several plants.

Hormones have also been found to be bound to polymeric carbohydrates (Davies and Galston, 1974). Sen and Das (1982) observed that IAA does not bind significantly to cellulose or pectin- which are cell wall components. These polymers were not isolated from growing tissues and the active conformation may well be different.

Myoinositol

Auxins have been reported to form conjugates with myoinositol also. Indole-3-acetyl-2'-myoinositol has been isolated from several tissues; inositol has been found to bind more than one molecule of IAA and such conjugates also occur in nature. Such conjugates can be glycosylated additionally, the sugars being arabinose, or galactose. IAA-glucosyl ester appears to be the precursor of IAA-myoinositol. These complexes are highly active in tissue culture growth. In the complexed form IAA is probably protected from oxidative degradation. Biswas (1982) has described the occurrence of a metabolic cycle involving glucose-6-phosphate and myoinositol phosphate during formation and germination of mungbean seeds. Since during these processes active auxin metabolism occurs and the operation of the cycle is associated with synthesis, the relationship between plant hormone and myoinositol metabolism should be investigated. Seeds are rich in phytin and the enzyme phytase is active in germinating seeds.

Amino acids

Auxins, cytokinins and gibberellins also form conjugates with amino acids. Indole acetyl aspartate has been most intensively studied. The enzyme responsible for its formation is an inducible one and other synthetic auxins are also active. In some tissues ethylene helps in its formation. Conjugates with other amino acids like glutamic acid, lysine, glycine, alanine and valine have also been reported. Glycine, proline and glycyl glycine have been found to conjugate with GA_1. Lupinic acid is β-(zeatin-9-yl)-alanine. The biological importance of the conjugates of hormones with amino acids is probably the same as in the case of sugars mentioned above.

Hormones have also been reported to form conjugates with several other compounds.

Hormone Action and the Synthesis of RNA and Proteins

The biological significance of the hormone-receptor complex has quite often been assessed in relation to the transcription process. Auxin stimulation of nuclear RNA synthesis was first demonstrated by

Roychoudhury and Sen (1964a, b) and Roychoudhury *et al.* (1965). Gibberellin stimulation of nuclear RNA synthesis was shown by Varner and Chandra (1964). Roychoudhury *et al.*, (1965) also demonstrated that kinetin stimulates nuclear RNA synthesis. The initial site of auxin action, in so far as promotion of RNA synthesis is concerned, is localized within the nucleolus ; this is soon followed by the nuclear ribosome fraction (Das *et al.*, 1980). Template activity of chromatin is enhanced by the hormones but the melting temperatures of the chromatin or of DNA is unaffected. Viscosity of DNA was also unaltered (Das *et al.*, 1980). Abscisic acid inhibits RNA synthesis even in the presence of GA_3, the effect of which was usually counteracted by ABA. ABA according to some workers blocks transcription by inhibiting RNA synthesis (Chrispeels and Varner, 1967 ; Mondal and Biswas, 1972). Polyamines promote the synthesis of DNA, RNA and protein. It has been suggested that they stimulate all steps of polypeptide chain synthesis. At low concentration polyamines promote RNA polymerase activity but DNA polymerase activity is not much affected. However, spermidine and spermine usually inhibit RNase although promotion of RNase activity has also been reported. Polyamines prevent senescence of both excised leaves and protoplasts, which become more stable to lysis and show high macromolecular synthesis. RNase activity is inhibited by spermine, spermidine, cadaverine and putrescine in that order. This may be due to either repression of enzyme synthesis or protection of the substrate from enzymatic degradation through electrovalent attachment of the positive charged amines to the negatively charged phosphate groups of RNA (Kaur–Sawhney and Galston, 1982).

For the hormone effects to be observed, the presence of the hormone during isolation of nuclei or chromatin is usually considered to be essential. Washed chromatin preparations do not exhibit the hormone effect. These observations have been interpreted to imply the formation of the hormone-receptor complex, the receptor being supplied by cell membrane or nucleoplasm on the chromatin and it is the hormone-receptor complex which is responsible for the stimulation of RNA synthesis. A non-histone protein is believed to be essential to trigger RNA synthesis on a chromatin template. Both specific and nonspecific binding of hormone-receptor complex to chromatin and DNA has been acknowledged by all workers ; the distinction has scarcely been achieved experimentally. It is also believed that the specific

nteraction of hormone-receptor complex with the genome leads to the opening up of new initiation sites for transcription. According to Biswas and Roy (1978) for a single acceptor site in the chromatin at least 10-12 molecules of IAA-nuclear receptor complex are associated in order to obtain the saturation of binding. For oestradiol action, Yamamoto and Alberts (1976) have suggested that the receptor proteins bind in clusters to specific sites in the genome to initiate the signal for transcription. In Chick DNA, although there are only 1-2 ovalbumin genes per haploid genome, oestrogen increases the number of new initiation sites for RNA synthesis on the oviduct chromatin (Tsai *et al.*, 1975). Mondal *et al.* (1972) have observed that both qualitative and quantitative changes in RNA synthesis occur in presence of IAA-receptor complex when a homologous non-ribosomal RNA polymerase transcribes DNA. In the growing tissue where no morphogenetic alteration takes place, for the *status quo* to be maintained no qualitative change in the mRNA molecules is required. Since no specific protein has so far been detected in a growing tissue, it is doubtful that new mRNA molecules are actually synthesized in such cases. In fact Das *et al.* (1980) could not detect any such qualitative change in the nuclear preparations. However, for active growth to continue proteins have to be synthesized at an accelerated rate which implies continued synthesis of rRNA, tRNA and mRNA of the same variety. Either several copies of the genes are activated by the hormone-receptor complex or the rate of transcription and translation is maintained at a steady rate. However, when hormones evoke morphogenetic changes e.g., in the initiation of adventitious roots, formation of epiphyllous buds, induction of flowering or alteration of sex, qualitative alterations in the mRNA molecules is a prerequisite. These observations imply the existence of several receptor proteins for the different plant hormones and for different physiological events. Although several receptor proteins have been isolated, they have not yet been characterised sufficiently so that the relevance of the hormone--receptor complex concept to the variety of hormonal effects can be adequately assessed. Differentiation involves both repression and derepression of genes and how the hormone-receptor complexes achieve this dual effects has to be explained in molecular terms with adequate experimental data. Since both free hormones and the hormone-receptor complexes bind with DNA, there will be a competition between them, the overall effect being determined by their

relative concentrations. The nature of the interaction would also be complex.

The sequential synthesis of the different types of RNA molecules in response to the appearance of hormones in the vicinity of genome has not yet been studied, This would have helped in the detection of genes which are activated first by the hormones as such or associated with its receptor. In germinating seeds there is usually no mRNA synthesis in the early stages and the conserved mRNA species are used for translation. The hormones ABA, gibberellin, IAA and cytokinin are released (from bound forms) or are synthesized, in a sequential manner. In the case of gibberellin action that α-amylase synthesis is an early event in seed germination is now well documented (Varner and Chandra, 1964 ; Chrispeels and Varner, 1967) ; this is preceded by the formation of certain species of polyadenylated RNA, the exact function of which is not understood (Jacobsen and Zwar, 1974). The mRNA for α-amylase has been isolated by Higgins *et al.* (1977) and it is now possible to locate the genes for these RNAs though the use of reverse transcriptase and DNA-DNA hybridization.

Hormone effects on protein synthesis are generally achieved throngh enhanced RNA synthesis, formation of polyribosomes or through an interaction with the t-RNA and ribosomes as in the case of cytokinins. Hormonal stimulation of nuclear protein synthesis was first shown by Datta and Sen (1965). Chromosomal acidic protein synthesis was most markedly stimulated by IAA but histone synthesis was inhibited by both IAA and GA_3 . Chloroplastlc and mitochondrial protein synthesis was unaffected (Das *et al.*, 1980). It has recently been observed in the author's laboratory that IAA at growth-inhibitory concentrations inhibit the activation of tryptophan and thereby inhibit the synthesis of all proteins which contain tryptophan, including those which are concerned with the biogenesis of IAA from tryptophan (A. Jana, unpublished data).

Hormones, apart from influencing the synthesis of enzyme proteins, directly or indirectly, may also influence their activity. This aspect has been dealt with extensively by Barendse (1983) and will not be discussed here any further. It is evident from this account that enzymes concerned with all aspects of plant metabolism are influenced by the

plant hormones. The effects may be both promotive or inhibitory, the extent depending on the concentrations, higher concentrations being inhibitory, in most cases. Both synthetic and degradative enzymes are affected. Apparently the totality of such effects determine the overall physiological response. Hormones may also modify the conformation of enzyme proteins e.g., RNA polymerase. Prolonged treatment of Lentil roots with auxin has been reported to increase the transcriptional ability of rRNA polymerase. According to Teissere *et al.* (1975) this is due to a synthesis of a specific γ-factor for rRNA polymerase.

Apart from gene transcription hormone effects on isoenzyme formation may also be exercised through their effects on oligomerisation of protein subunits. The stimulation and appearance of multiple forms of *o*-diphenolase in wheat embryos was inhibited by ABA and this inhibition was countered by 2, 4-D which brought about a molecular rearrangement of multiple forms by association-dissociation. Since actinomycin-D inhibited both callus growth and activity of certain multiple forms transcription is apparently required for them (Taneja and Sachar, 1977). Berry and Sachar (1982a) showed that there is an activation by GA_3 of preformed monophenolase molecules and oligomerisation of forms which move fast during gel electrophoresis, helps the enzyme molecules to function at varying temperature, pH and ionic strength during germination. GA_3 not only induces poly (A) polymerase but also regulates the expression of conserved mRNA for poly (A)+ RNA in aleurone layers. The GA_3 stimulation of enzyme activity is completely inhibited by ABA ; the control thus, is exercised at the post transcriptional level (Berry and Sachar, 1982b). IAA, however, represses activity of this enzyme in pea epicotyls resulting in a decrease of poly (A)+ RNA (Berry and Sachar, 1983). In mungbean seedlings auxin, ethylene and GA_3 differently activate growth and activity of peroxidase, multiple forms of which appear during germination (Dendsey and Sachar, 1982). In cowpea seedlings both GA_3 and cAMP not only stimulate the activity of RNase but also augments the RNase isoenzymes ; this process requires both RNA and protein synthesis. In the same seedling GA_3 and cAMP counteract the glucose inhibition of starch hydrolysis (Kapoor and Sachar, 1979).

The dependence of hormone action on the Cenral Dogma involving DNA, RNA and Protein is necessary for both qualitative and quantitative changes in Plant function due to hormone action. Since all

enzymes are destroyed after a certain period of time and their mRNA usually decays with relatively short half lives, the synthesi; of RNA and protein have to continue all the time (Fig. 1.3); the changing environment (physical/chemical) however, may necessitate both qualitative and quantitative changes which can be brought about only by selective repression

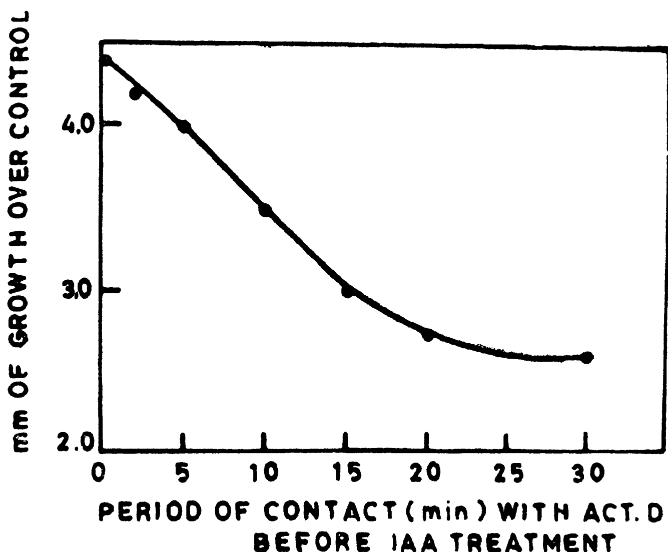

Fig. 1.3. Auxin-induced growth of wheat coleoptile as affected by Actinomycin-D pretreatment. Sonalika wheat coleoptiles (6 mm) treated with actinomycin-D (Act. D) for different time periods and washed in water until IAA treatment, which in all cases began 35 min. after commencement of Act. D treatment. After 22h contact with IAA growth was measured. Note that growh decreases as the period of pretreatment with Act. D increases (From J. Das, 1974).

and derepression of the genome. That RNA synthesis is an important link in the chain of events originating from hormone application and culminating in the expression of physiological response, is evident from the fact that the dual effects of hormone concentrations on growth are also exhibited by RNA synthesis, protein synthesis, respiration, CO_2 fixation and other metabolic processes (Datta *et al.*, 1965 a, b). Of the various substances present in a cell, the dual effects on RNA synthesis is exhibited only by the hormonal substances and the B vitamins, thiamine and pyridoxine; cellular metabolites like sugars, organic acids and amino acids and the vitamin ascorbic acid increase RNA synthesis with increasing concentrations. Auxin-stimulation of RNA synthesis usually shows a proportionality with its biological effects. Thus, IAA,' indole butyric

acid (IBA) and indole propionic acid (IPA) promote RNA synthesis in this order, reminiscent of their relative biological activity. The Q_{10} values of auxin-induced growth and RNA synthesis are also close. The auxin antagonists 2,3,5 tri iodobenzoic acid and maleic hydrazide inhibit RNA synthesis and this inhibition is countered by increasing concentrations of IAA; similarly the growth retardant cycocel inhibits RNA synthesis and the inhibition is erased by higher concentration of GA_3 (Mitra and Sen, 1968; Mitra, 1968). That RNA and protein synthesis are essential requirements for auxin-induced growth were shown by Nooden and Thimann (1963), Key and Shannon (1964), Datta *et al.* (1965) and others, using inhibitors of nucleic acid and protein synthesis. These inhibitors are not completely specific, but they are active at very low concentration when non-specific effects are very little, if at all. But they are also expected to inhibit all types of RNA and protein synthesis and only stable mRNA molecules are expected to be translated.

The nature of cell growth

Cell growth in the simplest analysis involves a loosening of the cell wall, expansion of the cell volume bounded by the plasmalemma by increased water uptake, increase in the dry weight of total cell matter largely due to import of inorganic and organic constituents and increase in cell number due to higher frequency of cell division, which in turn implies a duplication of DNA and synthesis of cell polymers. Most of these processes require a supply of energy which must be provided by substrate level, oxidative or photophosphorylation. Since enzyme molecules are not long-lived, continuity of cellular processes involves a replenishment of all such molecules necessitating continued RNA and protein synthesis. Responses involving morphological changes involve turning on new and turning off of some old genes, and new RNA molecules have to be synthesized or their synthesis stopped depending on the conditions. This in turn imlies an alteration of nucleic acid and protein synthesis. It usually takes at least about 10 minutes for a RNA or protein molecule to be synthesized and consequently hormone actions of these types are relatively time-consuming and are, therefore, considered as delayed actions. The primary reactions in cell elongation are, however, very rapid and can be demonstrated experimentally to take about only 1-10 minutes. Such actions are very fast and utilize preformed molecules which are relatively stable.

The primary and "fast" reactions

About fifty years ago Heyn in Netherlands and Soding in Germany obse-

rved that extensibility of the cell wall had both reversible and irreversible components. The former is responsible for the elastic and the latter for the plastic property of the cell wall; these properties were altered in a similar manner under applied tensions and in presence of auxins. This was found to be so both in *Avena* seedlings and in dicotyledonous flower stalks, although the latter was less sensitive. These workers suggested that auxin modifies cell wall properties, resulting in elongation. A few years later Ruge suggested that the middle lamella and not the wall proper are the main sites of auxin action. Recent studies of Masuda, Cleland, Ray, Evans and others have emphasized the loosening of the cell wall as a primary requirement for the early events in growth (see Evans, 1983). Changes in the activities of several polysaccharidases acting on cell wall components are being intensivly studied. The pectin of middle lamella has been found to swell in presence of several acids including IAA. Strong acidification of the medium in which coleoptile segments grow in presence of auxin was first reported by Bonner about 50 years ago; Yamaki's (1954) observation concerning the importance of dark CO_2 fixation or auxin stimulation of respiration (Commoner and Thimann, 1941; Bonner, 1949) in auxin-induced growth can also be interpreted in terms of H^+ geneartion. However, no marked acidification occurs in green tissues and thus, the growth of green tissues in light are difficult to explain.

The 'proton pump' theory has several other limitations also. In order that electroneutrality is maintained protons must be exchanged for other cations or at least be balanced by cations. The proton pump concept actually implies the movement of an electric current in the opposite direction which has not been unequivocaliy shown (Burström, 1981). The acidification of the medium results as soon as unequal amounts of anions and cations are taken up by a plants tissue although, such a differential uptake does not have any specific effect on growth if the nutritional uptake is ignored. It is doubtful whether this macroscopic mechanism is comparable to the exchange of protons and electrons across organelle membranes according to the chemiosmotic model of Michel for energy in ATP formation (Burström 1982). If growth is the result of acidification then the pH should be lowered thrhughout the entire tissue where the cells are growing, leading to tissue tensions in the sections of the organs under study. How this can be brought about is difficult to visualise. In the intact plant the apical meristem receives

nutrients, hormones etc. through unidirectional streams in the stele. It is difficult to understand how this could be a specific growth-inducing mechanism. The suggestion that H^+ anb OH^- ions are both excreted across the tonoplast is also untenable since the charged ions cannot pass undisturbed through the cytoplasmic layer which is also changed. It is also known that auxin produces acidification in roots aiso, even though auxin decreases root growth· In fact root growth is maximum int he most acidic medium. In recent studies dissociation of malic acid has been utilized to explain proton transport across membranes. Although, there is good evidence for this in stomatal cells, the role of malic acid in the operation of proton pump in relation to growth is doubtful (Burström, 1982).

Much less is known about the effect of other hormones on the cell wall. In lettuce hypocotyl sections which exhibit cell elongation in response to GA there is no detectable wall acidification and H^+ efflux (Stuart and Jones, 1978). ^{3}H-GA accumulates in a cell wall fraction sedimentable at 2000 g. The ^{3}H-activity could be removed largely by KOH. The rate of ^{3}H-GA incorporation in the fraction correlated highly with growth rate and inhibitors of protein synthesis inhibited the formation of this cell wall fraction (Stoddart and Williams, 1979); Stoddart and Venis, 1980). The role of this fraction in wall softening is not known.

The nature of interaction between auxin and gibberellin with the cell membrane is also not understood. Auxin effects on membrane permeability have been demonstrated by several workers, but the phenomenon has not been explained at the molecular level. The nature of association between protein, lipids, sterol and phytochrome in the cell membrane also has not received the attention, it deserves. It is now well established that phytochrome controls cell permeability. It is largely through this property that phytochrome achieves its various effects, through fast and delayed actions, reminiscent of auxin effects. Phytochrome effects on growth and development are well known and the interactions between hormones and red-far-red irradiation are also well documented. Nevertheless, the relationship between the two has so far been elusive. Das (1974) Sen and Das (1972) have observed that the polyene antibiotic filipin which binds with membrane- bound steroids increase the permeability of *Rheo* cells and sugar beet root discs; this effect is marked at 10^{-3} M IAA in the former and 100 μg/ml GA_3 in the latter. The effect of IAA and GA_3 on the induction of growth of wheat coleoptiles

and TN-1 rice seedlings respectively, is also inhibited markedly by filipin (Fig. 1.4). Spectrophotometric studies have revealed significant interaction between IAA and GA_3 on the one hand and cholesterol, β-sitosterol, γ-sitosterol and stigmasterol in the other, in the region of maximum absorption of filipin. IAA and GA_3 effects on permeability could be demonstrated in a very short time and thus interaction between hormones and membrane-bound steroids may constitute a primary interaction concerned with the so-called fast reaction (Sen and Das, 1982).

Energy Implications of Hormone Action

The profound physiological effects of a small amount of a hormonal substance indicates considerable consumption of energy and an activation of metabolic processes to an extent which is not easy to explain. In

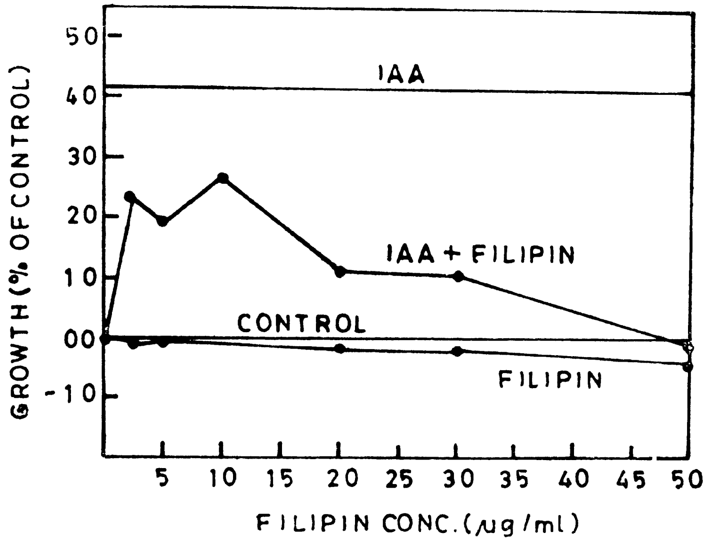

Fig. 1.4 Effect of the polyene antibiotic filipin on IAA-induced growth of Kalyansona wheat. Coleoptile sections (6 mm) were treated with different concentrations of filipin and 10^{-5} M IAA, alone or in combination at 22° C in the dark. Growth was measured after 20 hours in darkness (From J. Das, 1974).

non-green tissues the main source must be respiratory energy. More than four decades age Commoner and Thimann (1941) had reported the auxin stimulation of respiration. How this is specifically achieved is not understood. Part of this must be due to the stimulation and continued synthesis of respiratory enzymes but the energy relations are difficult to explain. Inhibitors of respiration also inhibit growth. Auxin

stimulation of ATP synthesis was demonstrated by Sen Gupta and Sen (1961b). Following Yamaki's (1954) observation that auxin stimulation of growth cannot be demonstrated in absence of dark CO_2 fixation, Sen Gupta and Sen (1961c) fed $^{14}CO_2$ to coleoptile tissues and detected a massive accumulation of ^{14}C in malate . Since NADH is required for reduction of oxalacetate to malate in dark CO_2 fixation, these workers suggested that auxin application may result in an alteration of NADH/ NAD ratio. Marre and Bianchetti (1961) have emphasized the importance of NADPH/NADP ratio in growing tissues. Reduced pyridine nucleotides thus may play an important role in auxin-induced growth. The involvement of ATPase in auxin action is now recognised. The auxin binding sites are distinct from the electrogenic ATPase associated with the plasma membrane. However, auxin-induced H+ -excretion is inhibited by inhibitors of ATPase and ATP synthesis. Since cycloheximide also inhibits H+ -excretion, continued ATP and protein synthesis appear to be required. In that case it would be doubtful if the primary site of auxin action could be a membrane-bound ATPase (Cleland 1979).

The inhibition of nucleotide synthesis by the uncoupling agent 2, 4-dinitrophenol can be counted partially by relatively higher concentration of IAA (Sen Gupta and Sen 1961 b). How auxins may stimulate nucleotide synthesis is not well understood. While this may be achieved partly by oxidative phosphorylation. Rhodes and Ashworth (1952) has suggested the formation of indole acetyl phosphate, a high energy compound having a carboxyl phsphate bond. While such a compound may be utilized directly, or via ATP synthesis, there is no evidence as yet that indole acetyl phosphate actually occurs in plant tissues. Water uptake, ion uptake and growth are all promoted by auxin and actinomycin D inhibits such promotions (Mitra and Sen, 1965). Apparently some carrier poteins or permease type of enzyme are involved, the synthesis of vairous types of nucleic acids and proteins, a large amount of nucleoside triphosphate would be required. Hormone effects on enzymes of photosynthesis have been discussed by Barendse (1983).

Practically nothing is known about the energetics of the physiological effects elicited by other plant hormones. The stimulation of various metabolic processes by the growth substances may provide the energy required at the expense of oxidation of reduced compounds.

The Problems and Paradoxes of Hormone Action

As is evident from the above discussions, we are just beginning to understand the molecular basis of hormone action, and are far from comprehending te entire situation. Whatever we know is the result of application of one hormone at a time (Thimann, 1973; Leopold, 1982) and the results usually provide some information regarding the *in vitro* situation which may be much different from what actually happens *in vivo*. This is equally true for the physiological effects ascribed to a particular hormone applied on intact plants, where a large number of naturally occurring hormones and inhibitors of diverse chemical nature are present and are capable of interacting with the applied regulartory substance, directly or indirectly. This is evident from the examples collected in Table 1. The situation is further complicated by the fact that hormone effects are concentration-dependent. Thus, a hormone concentration promotes root growth will inhibit stem growth and the optimum for stem growth is also different from that for bud growth in the same plant. If hormone action depends on its interaction as such or in association with a receptor with the genome, then the functional status of the chromatins of different organs of the same plant would appear to vary. To all an extreme example Sumac pollen extract promote the growth of second internode of bean by 430 per cent, while the growth of the 3rd internode is inhibited by 20 per cent (Mandava and Mitchell, 1971). If we visualise the existence of the hormone-receptor complex for one physiological effect in one organ than we have to assume the existence of a large number of hormone-receptor complexes which would interact in a manner the complexity of which is difficult to comprehend at the present moment.

Even the members of the same class of hormones do not behave similarly in the same test system. Thus, 2,4-D widely regarded as an auxin cannot be substituted by IAA for the growth of a dedifferentiated tissue; however, for redifferentiation even in appropriate ratios with cytokinin 2,4-D usually cannot replace IAA. For rooting of cuttings IBA and NAA are much more effective than IAA. In several test systems where kinetin the synthetic cytokinin - is highly effective zeatin the naturally occurring by cytokinin - is completely ineffective. Transformation into one active form should have the same results.

Another aspect which is not adequately realized is that for a hormone effect to be realized non-hormonal cellular metabolites may also make

Table-1 Summary of the variety of physiological effects evoked by plant hormones.

Effect	Auxin	Cytokinin	Gibberellin	Brassin[a]	Ethylene	ABA
Extension growth	+		+	+	—	—
Cell division	+	+			—	
Lateral bud development	—	+				
Leaf growth						
In length			+			
In breath		+				
Chlorophyll formantion		+				
Vein elongation	+					
Leaflet movement	+					
Root formation	+					
Fibre growth in cotton seeds	+	—	+			
Abscission	—		—		+	+
Sex expression						
Maleness	—		+		—	
Femaleness	+				+	
Fruit growth and development	+	+	+		+	+
Xylem formation	+		+			
Flower	+	+	+		—	—
Dormancy break						
Seed dormancy		+	+		+	—
Bud dormancy		+	+		+	—
Senescence		—	—		+	+
Tropsim	+		+		+	

+ promote, — inhibits

(a) Enhances responses to auxins other than chlorinated compounds. In several assay systems the effects resemble those of gibberrellin and cytokinins-brassin effects are most similar to gibberellin effects but dwarf maize is unaffected by brassins. (b) The presence of auxin is essential for the GA effect to be evident in a few plants; there are also plants when the effect is additive. (c) Synergistic effects with auxin. (d) In the apical hook of pea both auxin and ethylene are inhibitory. Auxin and gibberellin interact in cambium. In lateral buds both auxin and ethylene inhibit cell division, and both types of inhibition are counterd by cytokinin. Once the inhibition is renewed bud elongation is promoted by gibberellin. (e) Auxin and gibberellin promote in the early stages, cytokinin controls nuclear division in the liquid endosperm and embryo growth, and ethylene and abscisic acid promote ripening and abscission. (f) Auxins promote phototropic and geotropic curvature by differential distribution. Gibberellins induce the heliotropic movement of sunflower stalks and ethylene induces diageotropic movement, a vertical plant becoming horizental.

important contributions. Thus for auxin effect on xylem formation sucrose has to be supplied, and even glucose is ineffective. For redifferentiation in addition to hormones casein hydrolysate or a mixture of amino acids is essential. Sugars are important for differentiation of fern gametophytes. Sucrose and malic acid may induce respeetively the flowering of short day *Xanthum* and long day wintex barley plants, even under non-inductive conditions. Non-hormonal cellular metabolites have also been found to influence not only the transcription but also the translation process even in completely *in vitro* systems (Jana, 1982). Interaction of hormones with the cellular metabolites at the genome level further complicate the situation.

One hormone may influence the biogenesis, transport and inactivation of another hormone, thereby altering the effective concentration of the other hormone (Leopold, 1982). Thus, relatively high levels of auxin may stimulate the biogenesis of ethylene which may be further enhanced by cytokinins; abscisic acid has exactly opposite effects. Ethylene in turn not only inhibits the transport of auxin but also favours its degradation. Thus, the effects observed by the application of one hormone may actually be due to the effect of the altered levels of several other hormones. Auxin induction of flowering in pine apple or promotion of femaleness is now krown to be achieved via alteration in the level of ethylene. How ethylene promotes flowering or alter sex expression had not been explained in molecular terms but the relationship between sex hormones sex expression in animals has also not yet been established (Thimann, 1973).

The concentration of the applied hormone is quite often much different from the actual concentration of the hormone in the microenvironment of the genome or the cell membrane. The actual levels have not been determined. The physiological effect of applied hormone are interpreted to imply the need for boosting up of the level of the hormone to the optimum value but the levels of gibberellin, cytokinin and ABA sometimes are even quite high in dwarf plants, senescing leaves or dormant buds (Leopold, 1982); the effect thus must be dependent on other factors also. Correlation between hormone concentration and growth rates are not always discernible. The ratio of the free and bound forms may well be important.

There are several instances in which the different stages of a physiologi-

cal response are controlled by several hormones in a sequential manner. Thus, the growth of *Avena* coleoptiles or the bean hypocotyl hook sequentially regulated by gibberellin, cytokinin and auxin. For fruit growth auxin and gibberellin act in the early stages, cytokinin in the liquid endosperm stage and embryo growth while ripening and abscission is the result of the activity of ethylene and abscisic acid in the last stage (Thimann, 1973). We have already referred to the sequential effect of different hormone during seed germination. How is this sequential regulation actually achieved ? Apparently one hormone unfolds the gene responsible for biogenesis or action of another hormone. In seed germination gibberellins promote the synthesis of several hydrolases which are required for hydrolysis of polymers to monomers, which are then utilized as energy sources, carbon skeletons for biosynthesis of other compounds or reassembly into polymers of another monomer sequence. Many of these hydrolases are present in the lysosomes providing a package of these enzymes. If these hydrolases are all synthesized simulianeously the intersting possibility is suggested that one operator gene with which GA_3 or a product of its metabolism interacts as such or associated with a receptor protein regulates the biosynthesis of several hydrolases; alternatively, the gene for each hydrolase has to be activated by the hormone-receptor complex; Hydrolases are also synthesized in relatively large amonts in senescening tissues; the formation of these enzymes is prevented by cytokinins in many plants, and auxins and gibberellins in several others. Obviously, these hormones in senescing tissues repress the genes controlling the hydrolases. In senescing tissues there is degradation of many proteins but the synthesis of the basic protein histone, which is believed to regulate gene function, is synthesized at an enhanced rate, howevre, auxin application inhibit the biosythesis of histones and promotes the synthesis of non-basic proteins (Seal and Sen 1968). How these dual effects could be achieved is again a mystery.

There is some evidence that plant hormones may mimic phytochrome effects. The 'fast action' of both auxin and phytochrome has considerable similarities and involve the so-called proton pump. The locale of action of both of them may be organelle membranes or the plasmalemma concerned with control of permeability of solutes. Phytochrome control of nuclear RNA synthesis has also been demonstrated (Sen Gupta and Sen, 1982). Kinetin has been reported to potentiate the effect of Pfr in activating NAD kinase. Gibberellin substitution of red light or cold requirement has been reported in several cases. Although gibberellins

generally promote the flowering of long day plants, several short-day plants like *Impatiens balsamina* also respond to gibberellin treatment. The relationship between gibberellin action and phytochrome which controls the photoperiodic behaviour of photoperiodsensitive plants still reamins to be explained. The flowering hormone which so far appears to be elusive, is believed to be synthesized in the leaves and transported to the growing points where floral primordia are initiated. RNA synthesis in both leaves and buds is inhibited by inductive photoperiods and there are alterations in the template availability for RNA synthesis; this is accompanied by extensive repression and derepression of genes (Sen Gupta *et al.*, 1981a, b). Among plant sterols Oestradiol-17 β stimulates nuclear RNA synthesis but progesterone and α-spinasterol are inhibitory and β-sitosterol had no effect. Cucurbitacin B, the tetracyclic triterpenoid which antagonises gibberellin action (Guha and Sen, 1973) also inhibits RNA synthesis. Various other plant growth inhibitors which belong to diverse classes of chemical compounds also antagonise hormone action but the molecular mechanisms are unknown, some of them have been reported to inhibit RNA synthesis.

It is the integrative actions of the active levels of different hormones, inhibitors metabolite or phytochrome effects which are responsible for the orderly performance of each organ and the plant as a whole. For redifferentiation of a differentiated tissue, the ratios of only two hormones-auxins and cytokinins are important. For the growth of a single organ like the leaf, the growth in length is controlled by gibberellin, that in breadth by cytokinin and the frame work provided by the veins is the act of auxin (Thimann, 1973). The structure and function of each organ thus, is the cooperative effort of several regulatory substances. Hormone effects on initiation and plane of cell wall growth, so important for differentiation, again are aspects about the molecular basis of which we konw almost nothing. During reproductive differentiation phytochrome achieves the same presumably in collaboration substances in an unknown manner.

Inaugurating the 8th Plant Growth Substance Conference and the first meeting of International Plant Growth Substances Association in Japan, a decade ago Thimann (1973) introduced the Law of Duplicate Function and observed that "if in the course of evolution a substance or an organ is developed which has 2 functions-usually interrelated-rather than one, it is correspondingly much more likely to become established and

widespread". While it is possible that several substances can be metabolised into one common substrate, a second messenger like cAMP may carry the message of different hormones, but the function of cAMP or any other common messenger in plant systems is not established. When the diversity of the physiological effects produced by not only the hormones of different classes, but also hormones of the same class, when are analysed minutely the multiplicity of the modes and the sites of action of the hormones become evident. In fact it is quite likely that such hormonal substances may have more than two functions and when we consider the large number of probable interaction among them, there would appear to be multiple modes of action.

An integration of the concepts can only be achieved when we have really understood the individual events in molecular terms so that they can be put together into a meaningful whole. This may take many more years to come but the rate of progress in this field certainly provides enough reasons for us to be hopeful for the future.

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Hormonal Regulation of Flowering

S. Tanimoto and H. Harada

Introduction

Flower initiation in higher plants marks an important transition from vegetative growth to reproductive development, which is one of the most crucial events in the life cycle of plants. Numerous studies on the physiology of flowering have been conducted by many investigators, and their valuable contributions were presented in several review articles such as those by Lang (1961, 1965), Searle (1965), Evans (1971) and Zeevaart (1976), and also in books edited by Evans (1969b), Bernier (1970) and CNRS (1979), for example.

According to classical theories, certain environmental factors such as photoperiod and temperature would be responsible for the production of flower inducing substances; florigen, in the case of photo-inducing flowering (Chailakhyan, 1937), and vernalin, in the case of thermo-inducing flowering (Melchers, 1939). Unfortunately, attempts to isolate these flower inducing substances have so far been unsuccessful.

Several known chemicals including plant hormones may play a key role on flowering. From this point of view, many research workers have attempted to induce flowering by externally applied plant hormones under non-inductive environmental conditions. Although extensive informations concerning the relation between plant hormones and flower formation have been accumulated with many plant species, comparably less effort has been made in order to understand fundamental mechanisms of various hormonal actions during flowering process. In 1978, Zeevaart made an intensive survey on numerous reports (about 200 articles) to review the effects of externally applied plant hormones on flower formation and the changes in endogenous levels of plant hormones in relation to flowering. Since his review, many important papers dealt with hormonal regulation of flowering have been published. Thus, the recent advances in this field are reviewed in the present section.

Techniques of *in vitro* culture of plant tissues and organs may provide useful means for the research in the physiology of flowering. When *in vitro* techniques are applied, certain difficulties related to the use of intact plants can be avoided. For example, the effects of hormonal treatments given to a certain organ of an entire plant are largely influenced by the presence and state of other organs on the same plant, making the interpretation of experimental results difficult. The experimental approaches based on *in vitro* culture techniques also serve for a better understanding of fundamental mechanism of flowering in higher plants. Therefore, a number of significant studies dealt with hormonal regulation of *in vitro* flowering are also surveyed in this chapter.

Hormonal Regulation of Flowering in Intact Plants

Short-day Plants

Xanthium strumarium

Hamner and Bonner (1938) were the first to examine the effects of auxin on flowering in *Xanthium*, but did not find any promotive effects. Inhibitory effects of IAA and NAA applied to the cuttings or leaves during inductive photoperiod were observed by Bonner and Thurlow (1949), and they also reported that the inhibition caused by auxin treatment could be removed by simultaneous application of an auxin antagonist, 2,4-dichloroanisol. The inhibition caused by auxin was quite evident when IAA was applied to the leaves at the beginning or during an inductive dark period (Salisbury, 1955). Although a single treatment with TIBA, one of the auxin transport inhibitors, induced some flower formation under a low light intensity and LD condition in which the controls remained vegetative (Bonner, 1949), auxin transport was not affected by photo–induction, even with as many as seven inductive cycles (Jacobs, 1978).

The inhibitory action of auxin on flowering was also observed when

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Abbreviations : ABA : abscisic acid, Amo-1618 : 2'-isopropyl-4'- (trimethylammonium chloride) -5'- methylphenyl piperidine -1- carboxylate, Ancymidol : α-cyclopropyl -α- (4-methoxyphenyl) -5-pyrimidine methanol, BA : N^6-benzyladenine, B-995 : N-dimethylamino succinamic acid, CCC : 2-chloroethyltrimethylammonium chloride, CHI : cycloheximide, 2, 4-D : 2, 4-dichlorophenoxyacetic acid, Ethrel : 2-chloroethylphosphonic acid, GA : gibberellin, GA_3 : gibberellic acid, IAA : indole-3yl-acetic acid, IBA : γ-(indole-3yl) butyric acid, 2iP : N^6- (2-isopentenyl) adenine, LD : long day, LDP : long day plant(s), NAA : α-naphthaleneacetic acid, PBA : 6- (benzylamino) -9- (2-tetrahydropyranyl) -9H- purine, Phosphon-D : 2,4-dichlorobenzyl-tributylphosphonium chloride, SA : Salicylic acid, SD : short day, SDP : short day plant(s), SD-8339 : 6-benzyl -9- tetrahydropyrane adenine, TIBA : 2,3,5,- triiodobenzoic acid.

auxin was applied after an inductive period (Salisbury, 1955; Salisbury and Bonner, 1956). Salisbury (1969) suggested that auxin caused the inhibition of transport and/or destruction of floral stimulus. Endogenous level of auxin was revealed to be lower when plants were grown under SD conditions than under LD conditions, and auxin contents dropped as soon as flowers appeared (Cooke, 1954).

It is possible that inhibitory action of auxin on flowering is mediated by ethylene production which is stimulated by auxin treatment. Abeles (1967) reported that the plants treated with ethylene during an inductive night remained vegetative, and the amount of ethylene produced in IAA--sprayed plants was 4 times greater than that in control plants. On the other hand, Khudairi and Hamner (1954) obtained some promotive effects of ethylene chlorohydrin under LD conditions. They speculated that the treatment with ethylene chlorohydrin resulted in a decrease in endogenous auxin contents.

Application of GA did not induce flowering under absolutely non-inductive conditions (Lang, 1956b, 1957), but promoted floral development in the plants which were given some inductive cycles (Greulach and Haesloop, 1958). When GA was applied before the end of an inductive dark period, slight promotion of flowering was observed (Salisbury, 1969).

More cytokinin was found in aphid honeydew (represents phloem sap) collected from reproductive plants than that from vegetative plants (Phillips and Cleland, 1972). On the contrary, the contents of endogenous cytokinin in leaves rapidly decreased after SD treatment (Van Staden and Wareing, 1972). After a single inductive night, the cytokinin levels in extracts from roots, leaves and buds decreased to between 10 and 25% of the controls, but no satisfactory explanation relating the decrease in cytokinin contents and flower induction was made (Henson and Wareing, 1974, 1977).

Pharbitis nil

Ogawa (1962) reported that flower initiation in *Pharbitis* was inhibited by IAA when applied to the cotyledons before or during the first 6 hr of an inductive dark period, but endogenous levels of auxin in the cotyledons were not significantly different under SD and LD photoperiods. When the plants were grown at relatively low temperature, however, IAA treatment slightly induced flowering even under LD conditions (Bose and Harada, 1970).

Inhibitory effects of ethylene at 100 ppm or higher applied during an inductive dark period was also reported (Suge, 1972). In the case of ethylene treatment, a complete inhibition of flowering was obtained when applied to the seedlings only during the latter half of a 16 hr inductive dark period, and, no inhibition was observed when given earlier (Suge, 1974). These results may suggest that ethylene does not effect the translocation of floral stimulus produced by photo-induction, but suppresses inductive processes taking place in the cotyledons.

Application of different kinds of GA did not induce flower initiation in the plants grown under non-inductive light conditions, but promoted flowering under a slight inductive photoperiod (Ogawa, 1961a). The application of GA to shoot apices and to cotyledons showed similar effects. When grown at a temperature of 27° C (15 hr) and 17° C (9 hr) under LD, the treatment with GA_3 did not induce floral bud formation on the main stems, but produced some floral buds on the axillary stems (Bose and Harada, 1970). Stimulating effects of GA_3 on flower formation were also observed in the seedlings which were repeatedly decapitated (Harada, 1979). Takimoto (1969) postulated that GA seemed to promote flower initiation by increasing the mitotic activity in the apical meristem, rendering it more responsive to floral stimulus. Under an inductive dark period, GA_3 applied to the plumule before or shortly after the start of the dark period promoted flowering, but the latter treatment showed less promotion (Ogawa, 1981). This variation with time in the response to GA_3 indicates that only initial process of flowering seems to be stimulated by GA_3.

When a growth retardanant, Ancymidol, was applied during an inductive dark period via cotyledons or roots, flower formation and plant growth were strongly inhibited (Suge,1980). Other growth retardants such as CCC, Amo-1618, Phosphon-D and B-995 were also inhibitory for flowering (Zeevaart, 1964, 1966, 1967). These inhibitory effects of growth retardents could be completly reversed by simultaneous application of GA_3. Therefore, the action of these growth retardants seem to modify endogenous levels of GA. In fact, the treatment with ethylene or Ancymidol reduced endogenous GA contents (Suge, 1974, 1980). On the other hand, El-Antably *et al.* (1967) reported that the tretment with CCC and B-995 caused flower formation under LD conditions. They also described some stimulatory effects of ABA applied to the roots of the seedlings grown under LD (El-Antably and Wareing, 1966; El-Antably *et al.*,

1967), but their experimental conditions were not clearly stated. Harada *et al.* (1971) and Nakayama and Hashimoto (1973) failed to induce flowering with ABA under strictly non-inductive conditions. When the plants were slightly induced, however, ABA stimulated flower, initiation (Harada *et al.* 1971.) It seems that slightly promotive effects of ABA may become evident when vegetative growth is hindered.

Kinetin supplied to the cotyledons during the first 4 hr of an inductive dark period stimulated flower formation (Ogawa, 1961b). The inhibition of flowering by far-red irradiation and IAA treatment, both given at the beginning of an inductive dark period, and the inhibition by red light interruption in the middle of a dark period, were greatly reduced by subsequent application of kinetin (Ogawa, 1961; Nakayama *et al.*, 1962). Under slightly inductive light or temperature conditions, phenylurea derivatives having some cytokinin activity also stimulated flower formation (Bose and Harada, 1970; Harada *et al.*, 1971). On the contrary, the removal of roots from young seedlings could induce floral bub initiation, probably by decreasing endogenous cytokinin contents (Wada, 1973, 1974).

Under non-inductive photoperiod, BA (10mM) applied to the cotyledons of seedlings induced flowering (Ogawa and King, 1980). They also examined the interaction between the application of BA and brief red light irradiation on the flowering of dark-grown seedlings. In the presence of BA, a single and brief red light irradiation resulted in flowering of 3 day-old seedlings, but the effect of BA was not obsereved in 6 day-old seedlings (Ogawa and King, 1979a). However, when BA was applied to photo-indued cotyledon, the flowering was inhibited in association with reduced export of assimilate. Conversely, when BA was applied to a non-induced cotyledon, flowering was promoted with an enhanced export of assimilate from another induced cotyledon (Ogawa and King, 1979b). They postulated that cytokinin had an indirect effect on photoperiodic induction by altering the translocation of assimilate and floral stimulus to the shot apex.

Perilla

Application of 2, 4-D or NAA to the leaf under SD conditions caused slight delay in flowering time (Chailakhyan, 1948), but the application of IAA or NAA to the shoot apices in either SD or LD did not affect flower formation (Chailakhyan and Khlopenkova, 1959). NAA treat-

meuts to light-induced leaves grafted onto stock plants growing under LD, did not show any effectiveness in the transmission of flowering stimulus (Zeevaart, 1951). Auxin seems to affect some photo-susceptible processes of the induction, but not the transport of flowering stimulus. Some experiments showed that the leaves and apices under LD contained more endogenous auxin than those in SD (Chailakhyan and Zhdanova, 1938; Zhdanova, 1945; Harada, 1962).

Photo-induced flower initiation in this species was completely inhibited by the simultaneous treatment with ethylene without causing any damage to plauts (Zhdanova, 1950).

Under non-inductive light conditions, GA application did not induce flower initiation but stimulated stem growth (Chailakhyan, 1957; Horavka et. al., 1962; Lona and Fioretti, 1962). However, in sub-optimal photoperiod for flowering, the minimal number of SD cycles was reduced from 12 to 9 by a single spray with GA_3 (Razumov, 1960). Harada (1972) reported that endogenous levels of substance E which showed GA-like physiological properties were higher under LD than under SD.
Cytokinin caused slight promotion of flower formation in this species. By daily spraying with kinetin, the number of SD cycles required for flower induction was reduced (Lona and Bocchi, 1957; Chailakhyan and Khlopenkova, 1961). The contents of endogenous cytokinin in xylem sap taken from reproductive plants was 5 times higher than those from vegetative plants (Beever and Woolhouse, 1973).

Chenopodium rubrum

In this plant, an action site of auxin seemed to be localized in the apical meristem (Krekule and Privratsky, 1974). The increment of mitotic activity caused by photo-induction in the lateral meristems was inhibited by auxin applied to the shoot apex (Seidlova and Khatoon, 1976). Therefore, auxin may promote flower initiation by suppressing axillary growth and stimulating the development of shoot apex. Seidlova (1980) reported that auxin exerted an inhibitory or stimulatory effect on flowering depending on the time of its application. When auxin was applied during the first two inductive cycles, it inhibited the transition from vegetative to reproductive state, but the application of auxin after two SD showed a stimulatory effect on flowering (Khatoon *et al.*, 1973).

A number of studies were also conducted regarding the effects of ABA on

flowering. When ABA was applied to the seedlings maintained under continuous illumination at 20°C, about a half of treated plants produced flowers (El-Antably *et al.*, 1967). Conversely, the treatment with ABA could not induce flower initiation under absolutely non-inductive light conditions, but it stimulated the flowering of plants which were slightly induced (Krekule and Ullmann,1971; Krekule and Horavka, 1972). According to Krekule and Kohli (1981), the promotive effects of ABA on flower formation was observed only under the conditions which were favourable for the growth and branching of the meristem, but the treatment with ABA alone did not induce full flowering. The weakening of apical dominance may be a prerequisite for the promotion of flowering by ABA. Lozhnikova *et al.* (1981) also reported that ABA did not influence flower formation under continuous illumination, but enhanced flowering in the plants growing under LD and inhibited it in the plants induced by SD. They concluded that ABA could not substitute for photo-inductive treatment but its action might be additive to intiate reproductive stage as evoked by LD. Under three sub-critical photoperiodic cycles, the formation of lateral organs was promoted by ABA and was followed by stimulated inflorescence formation (Seidlova, *et al.*, 1981).

GA_3 brought about a similar effect on flower formation as ABA (Seidlova, *et al.*, 1981), and rapid elongation of all components of shoot apices took place, usually leading to more conspicuous growth of bud primordia (Seidlova, 1980).

Cytokinin inhibited the flowering of plants grown under inductive conditions (Krekule and Seidlova, 1977) and the treatment given to induced plants caused the enhancement of leaf initiation (Seidlova and Krekule, 1977). Seidlova (1980) assumed that cytokinin level was high when the plants were grown under continuous light and in vegetative state, and that the decrease in cytokinin contents was induced by SD. Root removal from the plants which were at the threshold of induction stimulated the flowering of these plants under continuous light, but this promotion was nullified by BA application (Krekule, 1979). Similar results on the promotion of flowering by root removal and the inhibition of flowering by cytokinin treatment in the plants without roots were obtained in *C. polyspermum* (Sotta and Miginiac, 1975 ; Sotta, 1978 ; Miginiac, 1978). Miginiac (1978) indicated that the inhibitory action

played by roots was probably hormonal nature and the production of cytokinin by roots might be involved.

Lemna paucicostata 6746

Flower formation in this strain of duckweed under non-inductive LD could be induced or stimulated by some chemical substances such as copper, silver, mercurry and ferricyanide which were known to inactivate SH-enzymes (Hillman, 1962 ; Takimoto and Tanaka, 1973, 1974 ; Tanaka and Takimoto, 1978), as well as salicylic acid (SA) and its derivatives (Khurana and Maheshwari, 1978 ; Cleland and Tanaka, 1979 ; Watanabe and Takimoto, 1979). However, studies on the effects of plant hormones were rather limited.

Gupta and Maheshwari (1970) examined the effects of various plants hormones on flowering. Addition of cytokinins such as zeatin, BA, kinetin, SD-8339 and 6-(γ,γ-dimethylallylamino)-purine to culture media could induce flowering under non-inductive photoperiod,zeatin and BA having been most effective. High concentrations of IAA, GA_3, ABA and CCC were inhibitory for flowering, but at low concentrations, they were ineffective. IAA and GA_3 nullified inductive effects of cytokinins. On the other hand, a slight flowering response was obtained by the treatment with ABA at a low concentration (Higham and Smith, 1969) or with CCC and ABA at a low concentration (Kandeler and Hugel, 1973).

The inhibitory effects of GA_3 also was reported by Hillman (1960). Hodson and Hamner (1971) reported that autoclaved GA_3 was inhibitory but filter-sterilized GA_3 showed no inhibitory action. Pryce (1973) identified breakdown products of autoclaved GA_3 as allogibberic acid and showed its strong inhibitory action on flowering.

Recently, Fujioka *et al.*, (1983) reported the effects of plant hormones on flowering in *L. paucicostata 151 and 381*. In these strains, high flowering response was obtained by the treatment with benzoic acid even under continuous illumination, but simultaneous addition of GA_3, IAA or ABA strongly inhibited the flowering induced by benzoic acid. Although the treatment with zeatin alone induced no flowering, zeatin applied together with benzoic acid which was even at the concentration too low to induce flowering by itself, acted synergistically and could elicited high flowering response. They suggested that the balance between endogenous levels

of benzoic acid and plant hormones contributed to the regulation of flowering in *Lemna*.

Glycine max

Hamner and Nanda (1956) examined the effects of IAA applied to the entire tops of Biloxi soybean at the end of each photoperiod of 7 SD. The IAA treatment decreased flowering rates with rising concentrations of IAA. A low concentration of NAA applied before an inductive SD had a pronounced promotive effect on flower formation (De Zeeuw and Leopold, 1956).

Plants of cv. Peking could not be induced to flower by TIBA under LD, but the number of floral buds in photo-induced plants increased ten-fold by the treatment (Galston, 1947). Promotive effects of TIBA also reported by Fisher and Loomis (1954). Endogenous levels of auxin were higher in the plants grown under LD than in those raised under SD, but a transient increase in auxin contents was observed when plants were transferred from LD to SD (Cooke, 1954). Maleic hydrazide inhibited to some degree the development of flower primordia in photo-induced Biloxi soybean (Klein and Leopold, 1953). Application of GA (Lang, 1957) or ABA (El-Antably *et al.*, 1967) did not affect flower formation in Biloxi soybean.

Kalanchoe blossfeldiana

In this species, all of the plant hormones examined showed inhibitory effects on flower formation. IAA treatment given to leaves which were located above photo-induced ones was inhibitory for flowering, and the treatment given to the leaves located below induced ones reduced flower numbers and caused increased leafiness of the inflorescence (Harder and Van Senden, 1949 ; Van Senden, 1951). Suppression of endogenous auxin transport resulted from TIBA treatment was also inhibitory for flower formation accompanying with malformation of leaves (Harder and Oppermanh, 1952).

Under strictly non-inductive conditions, GA_3 could not induce flower initiation, but GA treatment to the induced-plants greatly reduced flower numbers and caused elongation of internodes and leaves (Harder and Bunsow, 1956, 1957, 1958 ; Schmalz, 1960 ; Schwabe, 1972). ABA injection to an photo-induced leaf provoked strong inhibition of flower

initiation and a similar treatment with xanthoxin was somewhat less inhibitory than ABA (Schwabe, 1972).

Chrysanthemum morifolium

Individual treatment with BA or GA showed slight promotion of flowering in SD variety Pink Champagne grown under LD, and application of BA together with GA_5 was very effective in inducing the inflorescences, although they could not develop to anthesis (Pharis, 1972). In the var. Luyona, the transfer of plants grown in SD to LD suppressed the development of flower, but the injection of BA into a leaf caused normal flower development under above conditions (Bennink, 1974).

Ethylene treatment caused complete inhibition of flower formation accompanied by the elongation of lateral buds and lowering of plant height in var. No. 3 Indianapolis White grown under SD (Tjia *et al.*, 1969). The treatment was more effective when given during dark periods than in light periods. Sengupta *et al.* (1974) reported that endogenous level of ABA in apices of the same variety decreased by spraying ethrel or by transferring from LD to SD.

Impatiens balsamina

Although GA caused no flowering under strictly non-inductive day-length in many SDP, flower induction of *Impatiens* maintained in LD was achieved by GA_3 treatment (Lona and Fioretti, 1962; Nanda *et al.*, 1967; Lona, 1972). When GA_3 was applied to the apex on alternate days under continuous illumination, five treatments with GA_3 were needed for flower induction, and three treatments with GA_3 together with SA or β-naphthol were sufficient for floral bud initiation (Sood and Nanda, 1979). Guanosine monophosphates also could induce floral buds under continuous light and increased the number of floral buds under SD (Sharma *et al.*, 1978). Kumar and Nanda (1981) examined the changes in electrophoretic pattern of amylase in the leaves during flower induction. A new band of amylase was found in the sample prepared from the leaves of GA_3-induced and photo-induced plants. They postulated that it might be associated with floral bud initiation.

Other Short-day Plants

In *Ribes nigrum* (Schwabe and Al-Doori, 1973) and *Fragaria* (Guttridge and Thompson, 1964), flower formation was completely inhibited and

vegetative growth was promoted by GA applied before photo-induction. Besides, ABA and CCC caused flower initiation under LD in both species (El–Antably *et al.*, 1967; Schwabe and Al-Doori, 1973). The suppression of vegetative development seems effective for flower initiation in strawbery and black currant.

In SD duckweed *Wolffia microscopica*, various cytokinins such as zeatin, kinetin, BA, SD-8339 and 6-(γ,γ-dimethylallylamino) purine were highly effective in inducing flower formation under LD, but IAA, GA and CCC showed no effect in both LD and SD. ABA exerted strong inhibitory action on flowering (Venkataraman *et al.*, 1970).

In *Panicum miliacem* and *P. miliare*, the treatment with GA_3 could elicite formation of flowers under continuous illumination, and hastened the emergence of ears in *Setaria italica* (Kumar *et al.*, 1977). The GA_3-induced inflorescences, however, remained short, had only few spikelets, and remained sterile.

Cannabis sativa is a historically important species used in the studies on sex expression and flower formation. In fact, working with this plant on sex expression, Tournois firstly discovered and demonstrated in 1912 the importance of photoperiodism in the flowering of higher plants. Hemp is one of the typical dioecious plants and a number of investigations have been conducted with this species from the viewpoint of hormonal control of sex expression in higher plants (reviewed by Heslop-Harrison and Heslop-Harrison, 1969 ; Zeevaart, 1978).

Long-day Plants

Silene armeria

Konishi (1956) systematically investigated the effects of auxin and auxin metabolism in relation to flower formation and stem elongation. The amount of IAA or NAA transported from apical cut surfaces of stem cuttings to the basal parts was larger in bolting plants than in rosette plants. The production of auxin and the amount of auxin diffused to basal part showed gradual increase when plants were exposed to LD. The activity of IAA-oxidase declined soon after LD treatments. He concluded that the contents of auxin in bolting plants were greater than in rosette plants due to decreased IAA-oxidase activity. He also reported that flower initiation and bolting in rosette plants were induced by spraying IAA every day for abaut 2 months. A similar but a weak flowering

response also obtained by IAA application under a threshold long-photoperiodic conditions (Liverman and Lang, 1956).

In many LDP, GA could induce flower formation which was preceded by stem growth under SD. *Silene* plants also responded to GA, but a relatively long period of the treatment and a large dose of GA were required for flower induction (Lang, 1957). Lang (1957) suggested that the relatively weak reaction to GA might be due to the difference in specificity of various GAs. Michñiewicz and Lang (1962) compared the effects of nine different GAs on flowering in the plants grown under non-inductive photoperiod. Flowering was obtained only with GA_7 but not with GA_3.

The GA contents in the plants transferred to LD increased up to two-fold over that in the plants grown under SD, while the level of diffusable GA increased as much as ten-times. Amo-1618 reduced the endogenous level of GA, and inhibited bolting but not affected flowering (Cleland and Zeevaart, 1970). In a dwarf strain induced to flower by LD treatment, the increase in plant height (bolting) was very little, and GA_3 application partially stimulated and Amo-1618 suppressed the bolting, but the period required for the anthesis was not affected by the treatments with both chemicals (Suttle and Zeevaart, 1979). Thus, the mechaism of GA action in inducing flowering seems different from that in causing bolting. Wellensiek (1972, 1976) reported that GA_3 application in SD promoted the bolting but did not induce flower formation in a normal strain, and caused neither enhanced bolting nor flowering in a dwarf mutant. He also examined the function of applied GA_3 using two selected lines; one reacted to GA_3 with partial flower induction in SD and another was fully induced to flower by the treatment with GA_3 in SD (Wellensiek, 1978). According to his interpretation, the function of GA_3 in flower induction seemed as follows; GA_3 may directly act on the blocking mechanism for floral expression, and/or GA_3 always indirectly favours flowering by changing the critical daylength.

Lemna gibba G3

The treatment with IAA at a high concentration (10^{-4} M) completely inhibited flower formation under LD (Oota, 1965) and that at a low concentration (10^{-9} M) slightly promoted the flowering in photo-induced plants (Oota and Tsuzuki, 1971). By contrast, Cleland and his co-workers reported that IAA had no effect on flowering at low concentra-

tions and showed slight inhibition at high concentrations under both SD and LD (Cleland and Briggs, 1967; Cleland, 1979; Cleland *et al.*,1982). Ethylene did not induce flower formation in SD and was inhibitory for flowering under continuous illumination, but greatly stimulated frond gibbosity which was normally accompained with the onset of flowering (Pieterse, 1976; Elzenga *et al.*, 1980). This result indicates that the flowering and frond gibbosity may be regulated independently.

Oota (1965) reported a slight stimulation of flowering by 10^{-5}M GA_3 under sub-optimal photoperiodic conditions, and strong inhibition by 10^{-4}M GA_3 in LD. Similar inhibition at high concentrations was also reported by Cleland and Briggs (1969). The treatment with CCC completely inhibited flowering which was induced by LD, and the addition of GA_3 in culture medium partially reversed the inhibition (Cleland and Briggs, 1969). Peterse (1976) reported that GA_3 affected neither flowering nor frond gibbosity, but break-down products of GA_3 specifically inhibited both responses.

Kinetin treatment suppressed the flower formation of photo-induced plants and promoted vegetative growth (Oota, 1965). In slightly inductive conditions, kinetin was slightly stimulatory for flowering, but under SD or continuous illumination, it was inhibitory for flowering (Cleland *et al.*, 1982). The application of BA alone had no effect on the flowering, but in strictly non-inductive SD, SA caused the flowering only when BA was applied together with SA (Pieterse and Muller, 1977).

In the case of plants grown under continuous light, the treatment with ABA at a low concentration caused complete inhibition of flowering without any decrease in frond number (Cleland *et al.*, 1982).

Lolium temulentum

Flowering response in slightly induced plants was reduced by IAA application, but a little promotive effect was obtained by the same treatment with the plant held in SD with a light-break (Evans, 1964). The inhibitory effects of auxin seemed time-dependent, showing its effects with different degrees depending on the time of application during a photoperiodic cycle.

A single application of GA_3 induced the initiation of inflorescence under SD, and greatly promoted flowering in the plants exposed to a single

LD. The effectiveness of GA_3 was most evident when applied at the end of light period of a single LD, but the treatment with GA_3 at the beginning or in the second half of a light period of LD treatment had little effect. The order of effectiveness of various GAs was $GA_3 > GA_5 > GA_1 > GA_4, GA_8, GA_9$ (Evans, 1964, 1969a).

Injection of Amo-1618 or CCC to shoot apices showed no effect on LD induction or on the promotion by GA_3 (Evans, 1964). As CCC applied simultaneously with GA_3 showed a synergistic rather than an antagonistic effect on flowering, inductive effect of LD may not be due to an increase in endogenous level of GA (Evans, 1969a).

Floral initiation induced by a single LD was significantly inhibited by foliar spray of ABA or by the injection of ABA near the shoot apices (Evans, 1966). The flowering of plants exposed to 15 LD was also completely inhibited by ABA application to the leaves (El-Antably *et al.*, 1967). Evans (1966) assumed that ABA interfered with the action of flowering stimulus in the shoot apices. However, it is not obvious whether or not ABA effects flower induction itself or merely delays floral development.

Hyosyamus niger

Under sub-optimal LD conditions, IAA elicited flower buds in an annual variety of *Hyosyamus* (Liverman and Lang, 1956). However, the interpretation of this effect was difficult, because some anti-auxins such as 2, 4-dichlorophenoxyisobutyric acid and TIBA could also induce flower formation under the same conditions (Lang, 1961). Besides, auxin did not cause flower initiation under non-inductive conditions and did not show any inhibitory effect in pre-induced plants (Warm, 1980). Endogenous level of auxin in induced leaves by long photoperiod continuously increased reaching its maximum on the 15th day, and the changes in ABA contents showed a similar tendency. A large amount of cytokinin was accumulated during the flower evocation (Kopcewicz *et al.*, 1979).

The treatment of leaves with GA_3 caused flower initiation preceded by considerable stem elongation under non-inductive photoperiods (Lang, 1957). Although the treatments with appropriate concentrations of GA_3 induced the bolting in all of the treated plants, only 60% of the treated plant were induced to flower (Warm, 1980). Total amount of

GAs in leaves remarkably increased during the flower evocation (Kopcewicz *et al.*, 1979). Far-red irradiation at the close of LD promoted flowering of the bolting and flowering (Downs and Thomas, 1982).

When CHI, an inhibitor of protein synthesis, was infiltrated into leaves, the flowering was induced under sub-critical SD (Klautke and Rau, 1973 ; Warm and Rau, 1979).. With the CHI treatment, stem elongation did not occur; the transport of applied GA_3 from leaves to apices was inhibited; and GA_3- induced bolting was apparently suppressed (Warm and Rau, 1979)

Rudbeckia

GA applied to a shoot tip caused rapid flower formation with stem elongation in *R. bicolor* and *R. hirta* (Bunsow and Harder, 1957). Nitsch and Harada (1958) applied GA to the apex of *R. speciosa* and succeeded to induce bolting and flowering in this plant under non-inductive SD. Appreciable elongation of petioles by the GA treatment was also observed. Chailakhyan (1957) also obtained similar promotive effects of GA in *R. bicolor.* The treatment with furfuryl alcohol also induced to a certain degree the stem elongation and flowering in *R. speciosa* under SD (Nitsch and Harada, 1958).

Chailakhyan (1958) presumed that the action site of applied GA for the bolting and flowering in rosette plants of *R. bicolor* was the shoot tip. Jacqmard (1967, 1968) observed that GA applied to *R. bicolor* plants under SD caused a persistent synchronization of cell divisions in the shoot apices, and GA increased mitotic activity both in the subapical region and in the central zone.

According to the studies made by Konishi (1965), the activity of IAA-oxidase was much greater in rosette state then in bolting state with *R. bicolor.*

Harada and Nitsch (1959a) examined the contents of growth substances in *R. speciosa* during LD treatment using *Avena* mesocotyl bioassay which responds to both auxin and GA. Among several substances detected, one substance named E after chromatographical position, appeared after 3 weeks of LD treatment. The substance E was thought to be responsible for bolting as the plants just started to bolt when the

level of substance E reached at its maximum. The application of substance E extracted from photo-induced *R. speciosa* provoked stem elongation and flower initiation in the same species under SD (Harada and Nitsch, 1959b). Harada (1962) also showed that the substance E extracted from shoot tipes of thermo-induced chrysanthemum variety caused the flowering in *R. speciosa* under non-inductive conditions.

Endogenous auxin level in the leaves of *R. bicolor* was low under SD but became much higher when the plants were placed under LD or SD with night interruption (Chailakhyan and Lozhnikova, 1966). In this species endogenous ABA level was high regardless of growing conditions, namely, under SD or with 11 LD (plants bolting) (Kochankov, 1971). Application of cytokinin to *R. bicolor* grown under LD inhibited flower formation (Chailakhyan and Khlopenkova, 1961).

Sinapis alba

In many caulescent LDP, GA is usually uncapable of inducing flower initiation under non-inductive SD. A single treatment with GA_3 did not affect flowering of *Sinapis* plants grown undar SD, but when it was applied at the beginning of an inductive LD, a slight stimulation was obtained (Bernier, 1969). Phosphon-D also showed a slight promotive effect on flowering but greatly reduced stem growth (Bernier, 1969).

Application of ABA to the shoot tips of plants exposed to a single LD reduced flowering response and prevented early mitotic activation which was generally accompanied with an increase in DNA synthesis (Kinet *et al.*, 1975).

Although cytokinin was unable to induce flower formation, Bernier *et al.* (1977) thought that the mitotic component of the floral stimulus in *Sinapis* was a cytokinin, since a single application of BA or zeatin to apical buds of vegetative plant caused a mitotic wave which was very similar to that found in induced plants.

Spincia oleracea

Flower induction of spinach kept under SD could be achieved by GA treatment (Wittwer and Bukovac, 1957, 1958). Spraying of GA_3 caused a certain change in peroxidase activity as was observed when plants were photoperiodically induced (Karege *et al*,, 1979, 1982). El-Antably *et al.* (1967) reported that photo-induced plants could be remained in vegetative state if ABA was sprayed.

Arabidopsis thaliana

Many ecotypes including cold-requiring and LD types, are known to exist in this species. In LD varieties, GA could induce flower initiation under SD conditions (Langridge, 1957). Besnard-Wibaut (1981) also examined the effects of GA and BA on flower formation in these varieties. The treatment with GA_3, $GA_{4/7}$ or BA given during vegetative stage induced no flowering and caused stimulation of phylogenetic activity in lateral meristems. When the meristems in an intermediate stage were treated with GA_3 and BA, they were transformed into reproductive state. Application of kinetin or vitamine E to the tips of plants maintained under SD also induced flowering, but stem elongation was less than that obtained with GA treatment (Michniewicz and Kamienska, 1965).

Other Long-day Plants

It is well known that the treatment with GA could induce flower initiation in many LDP under non-inductive SD. Among various GAs examined, GA_3 was generally the most effective in inducing flowering in many LDP. However, Michniewicz and Lang (1962) reported that the flowering in *Silene* could be induced by the treatment with GA_7 but not GA_3. In *Hydrophyllum virginicum*, GA_5 was the most effective for flower induction among various GAs tested and the greatest bolting was obtained by GA_7 (Lona and Fioretti, 1962; Lona, 1963, 1972).

On the other hand, in several LDP in rosette state such as *Blitum virgatum* (Jacques, 1968), *Hieracium auranticum* and *H. pratense* (Peterson and Yeung, 1972) GA treatment induced only stem elongation; but not flower induction.

In the following caulescent LDP, GA application could not induce flower initiation under non-inductive light conditions: *Urtica* spp., *Sedum telephium*, *S. hispanicum*, *Anagallis arvensis*, *Calamintha grandioflora*, *Isnardia palustris* (Chouard, 1960) and *Trifolium pratense* (Stoddart, 1962).

Inhibition of flowering by GA treatment was obtained in following two cases: GA_3 applied to shoot tips of *Fuchsia hybrida* completely suppressed flower initiation (Sachs *et al.*, 1967) and GA application to *Hieracium floribundum* suppressed both bolting and flowering under inductive LD (Peterson and Yeung, 1972).

Cold-requiring Plants

Chrysanthemum morifolium

Development of inflorescence in vernalized plants (var. Indian Summer)

was inhibited by IAA applied to terminal buds (Schwabe, 1951). In var. Sunbeam, thermo-induced flower formation also was partially inhibited by IAA or NAA treatment, and the treatment suppressed stem elongation rendering plants into non-vernalized shape (Schwabe, 1970). In this veriety, endogenous auxin contents in shoot apices of non-vernalized plants was very low and a remarkable increase in auxin and IAA-oxidase activities were noted after the end of thermo-induction (Tompsett and Schwabe, 1974).

Using three cold-requiring varieties, Shuokan, Kinkazan and Shinmisono, Harada and Nitsch (1959c) showed that GA_3 applied to the apices of plants could induce flower formation with stem elongation, which was similar to the responses induced by cold treatment. Similar results were obtained with var. Sunbeam (Schwabe, 1959). Endogenous level of GA steeply rose about 2 weeks from vernalization and declined several weeks latter. Phosphon–D treatment prevented thermo-induced flowering and lowered GA contents (Tompsett and Schwabe, 1974). Harada (1962) compared the levels of substance E (see above) extracted from shoot tips of vernalized and non-vernalized plants of var. Shuokan. During vernalization, the contents of the substance E increased about ten times comparing to that in non-induced plants. The substance E extracted from vernalized plants caused the flowering and bolting in vegetative plants without any cold treatment.

Althaea rosea

Harada and Nitsch (1961, 1963) extracted several biologically active substances from shoot tips of this species. Among them, the substance E (named after chromatographic position) in a biennial veriety drastically increased after thermo-induction. The application of the substance E as well as GA_3 to non-induced plants provoked stem elongation but no flower formation. Other substances extracted from shoot tips of thermo-induced plants were identified to be GA_1, GA_3 and GA_9 (Harada and Nitsch, 1967a, 1967b). Harada and Yokota (1970) also isolated GA_8-glucoside from the same material which was hervested just before the plants began to bolt. During the stem elongation, the amount of GA_8-glucoside decreased while those of free GAs increased. They postulated that such a behavior might indicate a gradual transformation of GA_8- glucoside into active free GAs during cold treatment and the stem elongation.

Other Cold-requiring Plants

The first discovery that GA could induce flowering under non-inductive conditions was reported by Lang (1956a, 1956b), using a cold-requiring biennial variety of *Hyosyamus niger*. Since his discovery, many investigators reported that the application of GA caused flower induction in non-induced cold-requiring plants : these were *Petrosilenum crispum*, carrot, *Brassica napus* (Lang, 1957), *B. oleracea*, *B. napobrassica*, *B. rapa*, *Digitalis purpurea*, *Bellis perennis*, *Matthiola incana*, *Viola tricolor* (Wittwer and Bukovac, 1957), *Apium graveolens*, *Beta vulgaris* (Wittwer and Bukovac, 1958), *Centaurium minus* (McComb, 1967), *Arabidopsis thaliana* (Sarkar, 1958), *Cichorium intybus* (Michniewicz and Kamienska, 1964) and *Myosotis alpestris* (Michniewicz and Lang, 1962).

On the other hand, GA application caused only stem elongation, but not flower formation in several cold-requiring plants : *Eryngium variifolium*, *Genum urbanum*, *Saxifraga rotundifolia*, *Scrophularia vernalis* (Chouard, 1957), *Althaea rosea* (Harada, 1962). Michniewicz and Lang (1962) reported that GA_7 applied to *Myosotis alpestris* could induce both bolting and flowering but GA_3 caused only stem elongation. This result suggests that the effectiveness of GA varies depending on plant species.

In many cold-requiring plants which can be induced to flower by GA treatment, the bolting occurs preceeding to flower initiation. An exceptional case was observed in a biennial variety of *Arabidopsis* (Sarkar, 1958). In this case, when plants were treated with GA, flower buds appeared before stem started to elongate. Working with the same species Besnard-Wibaut (1981) observed, however, that GA_3 applied to non-induced plants could not cause flower formation. In vernalized radish, CCC application inhibited the bolting and decreased GA contents, but did not affect flower formatio (Suge, 1970). The mechanisms of GA action in inducing flower initiation under non-inductive conditions may be different from that in stimulating stem elongation.

The treatment of *Arabidopsis* plants with GA_3, $GA_{4/7}$ or BA during cold treatment accelerated flowering, but BA application after a cold treatment did not show any effectiveness (Besnard-Wibaut, 1981). GA application provoked the flowering and stem elongation in non-vernalized *Cichorium intybus*, and kinetin and vitamine E also showed similar effects (Michniewicz and Kamienska, 1964). Cytokinin may play a certain role in the bolting and/or flowering in cold-requiring plants. In

fact, endogenous cytokinin in roots of *C. intybus* increased during thermo-induction (Joseph and Paulet, 1973).

Other Plants

Ananas comosus

Pineapple, *Ananas comosus*, is a quantitative SDP, but the requirement for SD for flower initiation was very weak, and the flowering occurs throuh-out the year in tropical farms (Gowing, 1961). In pineapple farms, wood smoke has been used during past half a century to simultaneously induce flower formation and to produce fruits in the off-season. Traub *et al.* (1949) examined histologically the effects of ethylene on the initiation and development of inflorescence. After 3-4 days of a 24 hr ethylene treatment, the apices began to enlarge, and flower primordia appeared in the axils of the bracts after 10-14 days. The effectiveness of wood smoke for flower induction in pineapple plants was revealed to be due to ethylene contained in it.

Calcium carbide also was effective for flower induction (Gowing and Leeper, 1961), because when this chemical was moistened, it released acetylene (Aldrich and Nakasone, 1975). In the shoot apices of plants treated with acetylene, the cells in apical zones were less vacuolate and contained a large amount of RNA (Gifford, 1969). Propylene also could induce flowering (Gowing and Leeper, 1961). Other chemicals which caused flower induction were Ethrel (Cook and Randall, 1968) and β-hydroxy-ethylhydrazine (Gowing and Leeper, 1955; Dollwet and Kumamoto, 1972), and they were known to release ethylene.

Application of auxin also stimulated or induced flower formation in pineapple plants. Several auxin such as NAA (Clark and Kerms, 1942), 2, 4-D (van Overbeek, 1945) and IAA (Gowing, 1956) were effective. In the plants placed horizontally for at least 3 days, rapid flower formation was observed (van Overbeek and Cruzado, 1948). This response seemed to be due to auxin accumulation in lower sides of shoot apices. Burg and Burg (1966) was the first to showed that the effectiveness of auxin in inducing flower formation was due to the action of ethylene, the production of which was stimulated by auxin treatment. The auxin treatment resulted in the release of ethylene within 24 hr, and the release continued for 7 days, provoking flower formation 6 weeks after the treatment.

Bryophyllum

Several species belonging to *Bryophyllum* produce flower buds in response

to gradual changes in natural daylength from LD to SD, thus they are classified as long-short-day plants (Zeevaart and Lang, 1962).

Application of GA to plants grown under SD enhanced flower initiation, but the application to plants maintained in LD could not cause flower induction (Harder and Bunsow, 1958; Penner, 1960). The amount of GA which was capable of inducing flower formation differed depending on plant species. In *B. crenatum*, 0.75 μg of GA_3 was enough to produce flower, but in *B. daigremontianum* and *B. tubiflorum*, 5 to 10 μg of GA_3 was required for flowering (Penner, 1960). Most effective GAs in causing flower formation in *B. crenatum* were GA_3, GA_4 and GA_7 (Michniewicz and Lang, 1962). In *B. daigremontianum*, native GA_{20} was 20 times less active than GA_3 (Gaskin *et al.*, 1973).

Although photo-induction was not effective in juvenile plants, GA treatment could induce flowers in young plants (Chailakhyan *et al.*, 1968). Old plants of *B. calycinum* responded to less amount of GA than young ones (Wadhi and Mohan Ram, 1967). Therefore, endogenous amount of GA seems to increase during plant growth, and it may reach a certain level which is sufficient to substitute for LD requirement.

The treatment of induced-plants with CCC completely suppressed flower formation and further application of GA_3 could restore floweing response (Zeevaart and Lang, 1963). Endogenous level of GA_{20}, a native GA in *B. daigremontiana*, increased when plants were transferred from LD to SD (Gaskin *et al.*, 1973).

Short-long-day Plants

GA treatment to one strain of *Scabiosa succisa* induced rapid flower formation under LD and showed much less stimulatory effect in SD conditions, but many other strains which required stronger vernalization did not respond to GA treatment (Chouard, 1960). In *Campanula medium* grown under LD, GA caused only stem elongation but not flower initiation (Wellensiek, 1960). Application of GA_3 to *Coreopsis grandiflora* caused flower induction in SD, but under LD GA_3 caused only stem elongation (Ketellapper and Barbaro, 1966). In this species and in *Bryophyllum*, GA was able to substitute for the requirement of LD.

Pisum sativum

Flower initiation in an early variety of pea was delayed by NAA treatment but auxin application together with a cold-treatment was rather promotive

for flower initiation (Leopold and Guernsey, 1953, 1954). The suppressing effects of auxin may be related to the production of ethylene. In an early flowering variety, Ethral also delayed flower initiation and increased the number of leaves preceeding the first floral bud (Reid and Murfet, 1974). GA also delayed flower initiation and decreased the promotive effects of cold-treatment (Barber *et al.*, 1957).

Hormonal Regulation of *In Vitro* Flowering

Apex culture

In 1946, Loo was the first to obscrve *in vitro* floral bud formation in excised stem tips of *Cuscuta compestris.* Since his observation, many research workers examined physiological and/or environmental factors controlling *in vitro* flowering using apex culture of a number of plant species including *Helianthus annuus* (Henrickson, 1954), *Perilla frutescens* (Raghavan and Jacobs, 1961). *Cuscuta rcflexa* (Baldev, 1962), sugar-cane (Coleman and Nickell, 1964), *Ranunculus sceleratus.* (Konar and Nataraja, 1964), *Viscaria candida, V. cardinalis* (Blake. 1966), *Pharbitis nil* (Bhar, 1970), *Xanthium pensylvanicum* (Jacobs and Suthers, 1971, 1974), *Chenopodium rubrum* (de Fossard, 1973a).

Hormonal control of *in vitro* flowering was firstly investigated by Chailakhyan and Butenko (1959) using apex cultures of a SDP *Perilla ocymoides* var. *nankinensis.* Vegetative apices formed flower buds when cultured under 9 hr light or continuous dark conditions. Under 15 hr light conditions, flower formation was not obtained but addition of kinetin (1 mg/1) to a culture medium could induce floral buds. In *P. frutescens,* application of IAA to a culture medium caused a progressive delay in the appearance of floral primordia in the apices cultured under SD, and the transition of flowers to sterile cone structures by higher doses (Raghavan, 1961). However, photo-induced explants with the 1st and 2nd pairs of unfolded leaves flowered even with any of the concentrations of IAA applied (0. 1 to 100 mg/1), while those kept in LD remained entirely vegetative.

Harada (1967) reported the effects of plant hormones on flower formation in apex cultures of *Chrysanthemum morifolium* and *Pharbitis nil,* both SDP. Vegetative shoot apices excised from the plants grown under LD were able to initiate flower buds when cultured in SD conditions. Addition of IAA or GA_3 to a nutrient agar medium retarded

flower initiation and development, but kinetin had little effect on flowering in both species.

Addition of IAA, GA_3 or kinetin to a liquid medium could not replace inductive dark treatment for flower development in *P. nil*, but GA_3 increased the rate of flower formation in the cultured shoot apices (Matsushima *et al.*, 1974). Using excised plumules from *P. nil* seedlings subjected to a single inductive dark treatment, Choshi (1979, 1980) examined the effects of IAA and GA_3 applied to a liquid culture medium on flower formation. IAA treatment inhibited the flowering of the plumules excised after an inductive dark period. The inhibition was intensified with rising concentrations of IAA (0.0001 to 10 mg/l). Although complete inhibition of flowering by a high concentration of IAA (10 mg/l) was observed when the plumules were excised immediately after a dark-induction, slight flowering response was obtained in the plumules excised 2 days after the dark-induction (Choshi, 1979). In the case of GA_3 treatment, flowering was promoted at low concentrations (0.0001 to 0.01 mg/l), and inhibited at high concentrations (0.1 to 10 mg/l) (Choshi, 1980).

When flower buds of a LDP *Viscaria candida* were excised and cultured *in vitro*, the development of flower parts such as calyx and corolla was promoted by GA_3, and pollen production was also stimulated by GA_3 but inhibited by kinetin (Blake, 1969). Blake (1972) reported that extracts from vegetative *Kalanchoe blossfeldiana*, a SDP, caused strong inhibition of flowering in cultured apices of partially photo-induced plants of *V. candida*.

The presence of cotyledons appeared to inhibit epicotyl development in the cultures of shoot tips excised from photo-induced seedlings of *Chenopodium rubrum*, a SDP, and addition of GA_3 to a culture medium overcame this inhibition (de Fossard, 1972). Although 10^{-7}M GA_3 hastened flowering in the stem tips with cotyledons, GA_3 (10^{-8} to 10^{-6} M) did not affect the flowering response of cultured stem tips devoid of cotylenons (de Fossard, 1972). ABA and CCC inhibited or delayed flowering in excised shoot tips of photo-induced seedlings (de Fossard, 1973b). He suggested that the inhibition of flowering was mainly due to inhibited leaf development caused by ABA and CCC treatments. Ethrel also inhibited flowering.

Tendrils were formed from shoot tips of *Vitis vinifera*, when cultured *in vitro*, and the tendrils developed into inflorescences if BA or 6-(benzyla

mino) -9-(2-tetrahydropyranyl) -9H- purine (PBA) were applied directly to the tips of tendrils (Srinivasan and Mullins, 1978). If BA, PBA or zeatin riboside was applied to a agar medium, inflorescences were not formed on the tendrils, but cytokinin applied to a liquid medium were effeetive. Calyx, corolla, stamens and pistils developed normally in the presence of both zeatin riboside and PBA, but micro-and macrosporoge nesis were absent.

As an ontogenetic sequence in a developing inflorescence, *Lamium amplexicaule* produces both cleistogamous and chasmogamous flowers. Load and Mayers (1982) investigated the effects of plant hormones on the 2 flower types using *in vitro* cultures. When floral primordia which appeared to be of undeterminated floral type, were grown *in vitro* in the presence of kinetin, it grew to maturity as cleistogamous flowers. Further addition of GA caused anthesis but no true chasmogamous flower was produced.

In 1974, de Fossard suggested some advantages of apex cultures as adequate bioassay systems for testing plant extracts and other substances which might possess flower-inducing activity.

Callus Culture

Chailakhyan and his co-workers investigated *de novo* flower differentiation in callus tissues derived form stem segments of several varieties of *Nicotiana* (Aksenova *et al.*, 1972; Konstantinova *et al.*, 1969, 1975, 1974; Chailakhyan *et al.*, 1974, 1975). The ability of callus tissues to differentiate reproductive and vegetative buds was variable depending on the origin of explants and/or culture conditions. The calli obtained from the floral stalks of certain varieties could produce flower buds and ones derived from stem segments of vegetative plants formed only vegetative buds. Moreover, the differentiation of floral buds occurred when glucose concentration in a medium was sufficiently high, and it became moree pronounced with increasing concentration of kinetin and some purine and pyrimidine bases. When glucose content was low, vegetative buds were formed on the calli, and a high auxin content accelerated vegetative bud differentiation. They also observed that floral bud formation in tobacco callus could be stimulated by increasing the concentration of kinetin and lowering the concentration of NAA in a culture medium. The ability to differentiate reproductive buds was slightly decreased during several passages of culture.

In tobacco callus tissues which were subcultured successively for prolonged periods, regeneration capacity of the callus tissues greatly reduced, but the application of IAA increased the capacity (Syono and Fruya, 1973). Almost all of the flowers differentiated on the callus tissues were more or less abnormal. They were sterile and had abnormal immature pollen grains. These abnormalities were not found in callus tissues subcultured for a relatively short period.

Except for the case of tobacco callus, studies on the flower formation initiating from callus cells were rather limited. When young flower buds of *Phlox drummondii* were cultured on a nutrient medium, callus was formed from the pedicels, and flower buds were differentiated in the apices of shoots regenerated from callus (Konar and Konar, 1966). In the case of *Crepis capllbairis*, shoots were differentiated in the callus derived from the hypocotyls which were cultured on a nutrient medium containing IBA and kinetin, and the capitula were developed if the medium contained kinetin (Jayakar, 1970).

Chang and Hsing (1980) obtained *in vitro* flower formation in embrioids induced from root callus of ginseng (*Panax ginseng*). First, they obtained callus tissues from cultured piths of mature ginseng roots using nutrient medium supplemented with 2,4-D and cytokinin. Then, somatic embryos were induced by reducing the concentration of 2,4-D and omitting cytokinin. When the embryoids were isolated and cultured on a medium containing BA and GA_3, flowers were directly differentiated on the embryoids. Floral parts were minuscule but easily recognisable and almost 100% of pollen grains were fertile.

Organ Culture

Nicotiana tabacum

Since Skoog (1955) observed for the first time occasional *de novo* floral bud formation in *in vitro* cultured stem segments of *N. tabacum* cv. Wisconsin 38, many investigators started to use *in vitro* culture techniques of organ fragments (having no meristems) in order to elucidate the mechanism of flower induction. Most of published works dealt with the effects of environmental, physiological, nutritional and hormonal factors regulating *in vitro* flowering.

Chouard and Aghion (1961) demonstrated the presence of a "floral gradient" along the stem of reproductive plants ef tobacco cv. Wisconsin 38. The explants excised from the floral stalks formed more floral

buds than those excised from the basal part of stems. The stem segments from vegetative plants produced only vegetative buds regardless of original position on the stems of mother plants. They suggested that this "floral gradient" reflected the gradient of floral stimulus existing along the stems of tobacco cultivar used. Flowers were occasionally differentiated from the calli derived from basal stem segments, when glucose or sucrose was present in a medium at high concentrations (Aghion-Part, 1962, 1965a). The "floral gradient" was disturbed when kinetin and/or auxin were added to a medium (Aghion-Prat, 1965a). Kinetin at high concentrations stimulated vegetative bud differentiation and that of auxin promoted adventitious root formation. The treatment with GA_3 completely inhibited floral bud formation.

When *Agrobacterium tumefaciens* was injected into the stems of reproductive tobacco plants, floral buds were formed on the crown-gall tumours induced in apical part of stems (Aghion-Prat, 1965b).

In vitro flower formation was also obtained with *N. suaveolens*, *N. glauca* × *N. langsdorffii*, and 12 varieties of *N. tabacum*. all of which belong to day-neutral type, but not with *N. sylvestris* (LDP) and *N. tabacum* cv. Maryland Mammoth (SDP) (Aghion-Prat, 1965a).

Using apical internodal segments of cv. Wisconsin 38, Wardell and Skoog (1969a) studied the effects of plant hormones on *in vitro* floral bud formation. The presence of IAA at low concentrations was required for normal development of flowers, but IAA at high concentrations tended to inhibit flowering and to transform buds from reproductive to vegetative state. High doses of kinetin greatly increased the number of vegetative buds but had no significant effect on the number of floral buds neo-formed. GA_3 inhibited strongly both floral and vegetative bud formation, but its application after florol buds had formed, promoted their elongation.

Hillson and LaMotte (1977) also described the effects of kinetin and IAA. High doses of kinetin inhibited the formation and development of floral buds regardless of simultaneously applied IAA concentration, but stimulated vegetative bud formation. IAA inhibited vegetative and reproductive bud differentiation when applied with kinetin at a low concentration, but did not affect the development of once initiated floral buds.

Application of the analogues of RNA base such as 2-thiouracil, 6-azauracil and 8-azaguanine, slightly increased the number of floral buds formed per explant, and when these analogues were applied in combination, they were more effective (Wardell and Skoog, 1969b). The treatments with these analogues induced floral bud formation even in the explants taken from basal parts of the stems, and partially removed inhibitory effects of IAA but not the inhibition by GA_3.

The relation between *in vitro* flowering and DNA contents or synthesis in internodal explants was also investigated (Wardell and Skoog, 1973). DNA contents in apical stem segments excised from reproductive plants was 10 times higher than that in the explants taken from lower parts of the stems. Wardell (1975, 1976) showed some qualitative differences between DNA extracted from the stems of flowering plants and that from vegetative plants. A purified DNA solution prepared from flowering plants could induce flower formation in vegetative plants of the same species (Wardell, 1976, 1977).

Tran Thanh Van and her co-workers examined some physiological, chemical, physical and genetic factors influencing *in vitro* flowering using small explants composed of epidermal and sub epidermal cell layers prepared from the internodes of *N. tabacum* cv. Wisconsin 38 (reviewed by Tran Thanh Van, 1980a, 1980b, 1981). The explants grown on a nutrient agar medium containing 10^{-6}M IAA and kinetin produced floral vegetative buds (Tran Thanh Van, 1973a). The "floral gradient" along the stem was clearly observed, namely, all of the explants excised from flower stalks formed flowers, and lower the position of explants on the stem of mother plants, the less was the flowering rate.

The contents of peroxidase was much larger in the explants excised from basal parts of the stems than in the explants taken from apical parts, and the specific activity of peroxidase was about 60 times higher in the basal parts than in the apical parts of the stems in reproductive plants (Thorpe *et al.*, 1978). During floral bud differentiation in the explants, its activity tremendously increased, suggesting possible changes in auxin requirement during floral bud differentiation.

Both IAA and cytokinin (kinetin or BA) were required for flower formation and their most favourable concentration was 10^{-6}M (Tran Thanh *et. al.*, 1974a, 1974b). Physiological state of mother plants was very important, and explants excised from the plants in which terminal buds

were in green fruit stage formed floral buds most readily and at the highest rate (Tran Thanh Van *et al.*, 1974a).

When the explants composed of epidermal and a few subepidermal cell layers were allowed to float on the surface of a liquid medium, no flower was produced, even when they were cultured under the same favourable condition which was use for the culture with agar medium (Cousson and Tran Thanh Van, 1981),

De novo flower differentiation in the epidermal explants was also observed with other day-neutral species or hybrids of *Nicotiana* such as *N. tabacum* cv. Samsun, cv. Xanthi, cv. Lacerata, *N. plumbagintfolia*, *N. tabacum* cv. Samsun × *N. sylvestris* or × *N. tomentosiformis* (Tran Thanh Van and Trinh, 1978; Tran Thanh Van, 1980a; Kamate *et al.*, 1981). However, no flower formation was observed with LD species such as *N. alata* and *N. sylvestris* and SD species such as *N. tabacum* cv. Maryland Mammoth, *N. otophora*, *N. tomentosiformis* and *N. plumbaginifolia* × *N. tomentosiforlis*. The explants from haploid plants of *N. tabacum* and *N. plumbaginifolia* could form flowers and they had a larger number of flowers and required a shorter time for flower differentiation than diploid explants (Trinh and Tran Thanh Van, 1981).

Tran Thanh Van and her co-workers also made studies on histological and cytochemical changes occurring during *in vitro* floral bud differentiation in tobacoo epidermal explants (Dien and Tran Thanh Van, 1974; Tran Thanh Van and Chlyah, 1976).

Cichorium intybus

This species belongs to cold-requiring type. Root explants excised from thermo-induced plants could differentiate floral buds when cultured under LD conditions. IAA completely inhibited both vegetative and reproductive bud differentitation but addition of p-coumaric acid or chlorogenic acid in a medium significantly promoted both responses (Paulet and Nitsch, 1964). Thirty to 50 day cold treatment of the roots was required for high flowering response in cultured root segments. During cold treatment, endogenous contents of zeatin riboside and dicafeylquinic acid in the roots increased significantly (Mialoundama and Paulet, 1975a, 1975b; Paulet, 1979). The contents of chlorogenic acid and dicafeylquinic acids in cultured explants were increased when zeatin or 2ip was added to a medium, but decreased when IAA was applied (Paulet, 1979).

The presence of a meristem in a root segment during cold treatment was revealed to be unnecesary for flower formation (Pierik, 1966a).

In vitro floral bud differentiation in this species was also observed when leaf fragments (Nitsch and Nitsch, 1964) and flaral stalk sections (Margara, 1965; Harada, 1966; Margara and Bouniols, 1967) were cultured. In these cases, LD during culture and relatively high concentration of sugar were required for *in vitro* flowering at a high rate. Application of IBA or 2,4–D strongly suppressed floral initiation and GA_3 stimulated it (Margara, 1974).

Plumbago indica

Internodal segments excised from vegetative plants of *Plumbago indica*, an absolute SDP, could be induced to form adventitious buds when cultured on a nutrient medium containing cytokinin (Nitsch and Nitsch, 1967a). Vegetative bud formation was stimulated by addition of adenine and inhihited by LD treatment or application of GAs.

Generally, cultured explants produced inflorescences or normal flowers only under SD (Nitsch and Nitsch, 1965). The minimum duration of SD treatment required for floral initiation was of the order of 4 weeks (Nitsch and Nitsch, 1967b). Since cultured explants did not bear any leaf, it seemed that photoperiodic treatment was perceived by stem stgments.

The treatments with some of auxins and GAs inhibited the production of floral buds in SD. ABA enhanced the formation of inflorescence under SD but had no effect under LD (Nitsch and Nitsch, 1967b). Addition of CCC in a medium was slightly stimulatory (Nitsch, 1967). Ethylene (0.1% in air) treatment given during the 2nd week of culture and addition of methionine to a culture medium could induce flower formation in a certain degree under LD (Nitsch, 1968).

Streptocarpus nobilis

In this SD species, flower buds were formed in leaf explants excised from non-induced mother plants, when the explants were cultured on a medium without plant hormone under SD. Rossini and Nitsch (1966) reported that application of cytokinins such as kinetin, BA and SD-8339 stimulated flower formation but not zeatin. Simultaneous application of IAA at low concentration together with cytokinin significantly increased the percentage of cultures with floral buds, but that

of GA completely inhibited *in vitro* flowering. Floral buds were initiated from meristematic cells formed around a cut surface and/or arised directly from epidermal and subepidermal tissues (Handro, 1977). *In vitro* flowering response in this plants changes depending on nutritional factors. Simmonds (1982) reported that explants from photo-induced plants could not form floral buds in SD when cultured on a high nutrient medium without hormone, but if BA-was added to the medium, 50% of cultures produced flowers. Simultaneous addition of IAA to the medium strongly inhibited flowering. In the presence of BA (10^{-6}M), if the concentration of KNO_3 was lowered to 1 to 5mM, 100% of explants formed flowers.

Torenia fournieri

In 1903, Winkler reported that flowers were formed on midribs of excised leaf-cuttings of *T. asiatica*. Working with *T. fournieri*, a quantitative SDP, Chlyah and tran Thanh Van (1971) also obsreved flower formation in excised leaves grown on vermiculite. They reported that SD culture conditions were favourable for flower initiation from the explants. Using excised leaves of *T. fonrnieri* cultured on a nutrient medium containing IAA (2 ppm) and kinetin (2 ppm), Bajaj (1972) observed the formation of vegetative and floral buds.

Working with excised stem and leaf fragments, Chlyah reported that floral bud differentiation was delayed by the treatment with high temperature (36°C) (Chlyah, 1973a), and that both NAA and BA strongly inhibited flowering of explants excised from vegetative mother plants (Chlyah, 1973b).

Detailed examinations of environmental, physiological, nutritional and hormonal factors controlling *in vitro* flowering were carried out by Tanimoto and Harada (1979, 1981a, 1981b, 1981c, 1982b), using stem segments cultured *in vitro*. In this material, adventitious bud differentiation was strongly promoted by the addition of cytokinin and inhibited by simultaneous addition of auxin (Tanimoto and Harada, 1982c, 1982d). However, occasional differentiation of floral buds were obtained when the explants were cultured on a high nutrient medium which contained NAA together with BA (Tanimoto and Harada, 1981a).

The dilution of the Murashige and Skoog's medium (Murashige and Skoog, 1962) and/or the elimination of NH_4NO_3 from the medium increased the differentiation of adventitious buds which subsequently

developed and formed floral buds in the absence of plant hormones. Increasing the concentration of sugars in a culture medium increased the rate of cultures with floral buds, and stimulated the development of floral buds to anthesis (Tanimoto and Harada, 1981a). The inhibitory effects of NH_4NO_3 and the promotive effects of a high concentration of sucrose were noted only in the development of floral buds, and flower initiation itself was not affected by these 2 treatments (Tanimoto and Harada, 1981c).

In stem segments excised from 12 week-old reproductive plants, zeatin inhibited floral bud differentiation, but IAA did not (Tanimoto and Harada, 1981b). IAA, when applied only for the first 2 weeks of culture (adventitious bud forming phase), stimulated the initiation and development of floral buds, most of cultures having produced floral buds and the majority of them having reached anthesis (Tanimoto and Harada, 1981c). Application of GA_3 or ABA did not influence floral bud formation in stem segments excised from 12 week-old plants (Tanimoto and Harada, 1981b).

Physiological factors such as the size of explants, the age of mother plants and stem portions from which explants were taken, largely influenced *in vitro* floral bud formation. Two to 8 mm long segments formed floral buds, but 10 to 12 mm long segments produced only vegetative buds. The highest rate of flowering was obtained when the explants were prepared from the mother plants which were just differentiating flowers. A gradient of floral bud forming capacity along the stem was noted. The segments taken from upper portions of stems showed high rate of floral bud formation than ones taken from lower portions of stems (Tanimoto and Harada, 1979).

Tanimoto and Harada (1981b) also examined the effects of plant hormones on explants taken from mother plants at different ages. IAA stimulated floral bud initiation and development in the explants taken from 2 to 20 week-old mother plants. The sestimulative effects of IAA were more clearly seen in the explants taken from 18 to 20 week-old plants in which flower abscission was taking place, but not very evident when explants were prepared from younger materials. ABA acted in a reversed way to auxin, greatly promoting floral bud initiation and development in originally vegetative explants. Zeatin was inhibitory or ineffective for floral bud formation. In the

explants taken either from basal parts of stems or from 18 to 20 week-old plants, however, floral bud initiation and development were promoted by zeatin application.

Therefore, the action of plant hormones on floral bud initiation and development was clearly related with physiological state of stem segments used as explants. These differences in the response of explants in various physioiogical states to phytohormones may be due to different endogenous levels of, and sensitivity to plant hormones and/or other substances in the explants.

Other cases

Pierik (1966a, 1967) examined the relation between vernalization and IAA treatment on flower formation using petiole explants of *Lunaria annua*, an absolute cold-requiring plant. Flower formation was occurred only when explants were cultured at 26°C after a cold treatment of 16 weeks. Application of IAA during vernalization completely suppressed both sprout and flower formation, but IAA applied after cold treatment did not prevent both responses.

Flower buds could be produced *in vitro* when the explants were taken from leaves or flower stalks of *Begonia socotrana* and *B. teuscheri* × *B. coccinea*, but not with the explants excised from petioles (Ringe and Nitsch, 1968). The differentiation of adventitious buds was stimulated by IAA applied with adenine, and simultaneous addition of kinetin, BA or 2iP to a medium stimulated further this response. For the formation of flowers, the incorporation of IAA, BA and adenine into a medium was necessary. The most favourable concentration for obtaining the highest flowering response were 1 mg/l IAA, 5×10^{-7}M BA and 3×10^{-4}M adenine.

In *Kalanchoe blossfeldiana*, a SDP, *in vitro* flower formation was observed only with the explants taken from floral parts and not with the explants taken from peduncles, flower stalks and leaves (Margara and Piollat, 1981). Application of TIBA or B-995 completely inhibited *in vitro* flowering : that of CCC was more or less inhibitory ; and GA_3 caused no effect on the formation of flowers.

Occasional floral bud formation was observed in leaf discs excised from vegetative plants of *Perilla frutescens*, an absolute SDP, when the explants were cultured on a nutrient agar medium containing BA and NAA

under LD (Tanimoto and Harada, 1980). The *in vitro* flowering was depended on original leaf positions on mother plants from which the leaf explants were excised. The gradient of floral bud forming capacity along the stem existed in *Perilla* as seen in the cases of tobacco and *Torenia* (see above).

In *Rudbeckia bicolor*, a LDP, stem and leaf explants excised from photo-induced plants could produce floral buds when the explants were cultured on a medium containing GA_3 (0.1 to 1 mg/l) under LD, but did not form flower buds in the absence of GA_3 or under SD (Tanimoto and Harada, 1982a). Cotyledonary explants taken from vegetative mother plants could not differentiate floral buds even when the explants were cultured under LD and on the medium containing GA_3.

In vitro flower differentiation was also observed in several plant materials including foliar epidermal explants of *Nautilocalyx lynchei* (Tran Thanh Van, 1973b), leaf discs of *Crepis capillaris* (Brossard, 1979), floral stalk pieces of *Allium sativum* (Tizio, 1979), anthers of *Dianthus caryophyllus* (Villalobos, 1981) and perianth explants of *Haworthia arachnoidea* and *H. cymbiformis* (Konishi *et al.*, 1982). However, detailed studies on hormonal action in relation to flowering were not conducted with these plant materials.

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Hormones in Seed Dormancy and Germination

Stanislaw Lewak

Introduction

Dormancy of the seed is a very peculiar phenomenon. First, it occurs at a very early stage of development. Successful fertilization, incorporation of other nuclei in to zygote, intensive cell divisions and differentiations, accumulation of food reserves- all this seems to ensure prosperous growth and development. All this stops, however, for some obscure reasons. No similar phenomenon occurs in animals; even the diapause among insects, if it takes place, appears at a much later stage of development.

Another peculiarity of seed dormancy is its persistency. The deep dormancy consists in partial anabiosis of the living organism. It is extremely hard, however, to imagine what could be the mechanism of "reduced life" persisting over centuries and even thousands of years (case of Lupinus seeds in northern Canada, Porsild *et al.*, 1967). Already we know something about the control mechanisms responsible for dormancy, but we know nothing about the control mechanisms that ensure essential life processes in a differentiated organism under extremely unfavourable conditions.

When all, or almost all, vital functions of a dormant organ are restored, the dormancy is broken. Elimination of dormancy leads to growth of tissues; release of the seed from dormancy allows its germination. There are, however, several phenomena between the start of dormancy removal and the visible germination (growth axis). It is still disputable whether these phenomena are part of the germination process or whether they present a separate set of events subordinated to the mechanisms involved in dormancy maintenance.

There are several classifications of seed dormancy, proposed by different authors (for recent review see Bewley and Black, 1982). All of them reflect the mechanisms involved in reduction of vital embryonal processes. The main types of dormancy are :

The imposed dormancy (quiescence)

Germination is prevented as a result of unfavourable environmental conditions. Such dormancy is caused by the lack of water, improper temperature etc. It is sometimes considered as inhibition, but not as a true dormancy. Under favourable conditions this kind of dormancy disappears and the seed germinates immediately.

The innate (deep) dormancy

The inability of seed to germinate is connected with structural, chemical or physiological properties of the seed itself. We can distinguish physical, chemical, morphological and physiological types of dormancy. These terms describe the main mechanisms that are responsible for the inability of embryo to grow : physical barrier preventing water and / or oxygen access, presence of growth inhibitors, morphological or physiological immaturity of the embryo. These mechanisms can operate separately or in various combinations. They are clearly described and classified by Nikolaeva (1977).

Conditions involved in removal of the innate dormancy are, as a rule, different from those favourable for germination. This point to the most evident difference between the imposed and innate dormancies.

The initiation of growth processes in the seed is usually considered as germination. Germination is often described as transformation of the seed into the seedling. There are different criteria of germination; the commonly accepted one is the protrusion of the seed coat by the embryo radicle, but sometimes another one is required, namely the full development of the photosynthetic capacity (autotrophy) of the seedling. As in many other areas, the criterion depends on the needs. Often the germination process is differently understood from the point of view of an agriculturist, a biochemist or a physiologist.

The latter point of view, obviously is the most interesting for the purposes of this review. It seems that germination can be considered as a set of processes preceding the embryo growth. The visible symptoms of growth, such as embryo axis elongation, protrusion of seed coat by the radicle etc. indicate the end of germination and the beginning of the next developmental phase-growth and development of seedling.

It is much more difficult to precise the time of the beginning of germination. For all seeds the substantial prerequisite of germination is a sufficient

water supply, imbibition being the first event enabling the subsequent processes of germination. For seed of many species the limited water access is the only cause preventing germination, and hence the only cause of impose dormancy. One could pose the question: is imbibition the beginning of germination or is it the process of dormancy elimination ? In author's opinion there is no necessity to answer this question. It seems to be more justified to consider the dormancy removal as a Part of germination process *sensu lato*. This seems evident in a case when the removal of dormancy is caused by water supply. It is however less obvious for dormancy types caused by physiological immaturity and for those consisting in morphological underdevelopment of embryo or in structural hindrancies preventing growth. Nevertheless we shall use this approach. It allows some generalisation and eliminates semantic problems e. g., how to classify a given phenomenon; as part of dormancy removal or as part of germination ? Taking into account the above remarks we can also define the beginning of germination: the mature seed begins to germinate when the processes that lead to dormancy removal and growth are initiated.

Summarizing, germination is a separate deveploment phase in life-cycle of the higher plant. This phase is preceded by embryogenesis and seed development and it is followed by seedling growth and development. Thus, a seed has a specific programme for germination consisting in differential gene activation, distinct from programmes for embryogenesis and for seedling development. In that programme the elimination of dormancy is included. Full understanding of the genetic programme of germination will enable to answer both questions: what is germination ? and what is dormancy ?

The Phasic Character of Germination

Several succesive phases can be distinguished during germination of all seeds. Generally, three main phases make up the process of germination. They are denominated in different manner by various authors (Evenari, 1975; Katoh and Esashi, 1975; Lewak *et al.*, 1975; Tissaoui and Come, 1975). The distinction of phases is based upon recognization of processes dominating during a given period of germination.

During the first phase, imbibition of seed takes place. The colloids became hydrated and their structure rearranged. Several enzymes regain their activity. Hydration of cell colloids allows the diffusion of solutes inside

of the seeds. Imbibition also affects the non-colloidal structure of the seed; the structure of cell membranes undergoes rearrangement that assure their semipermeable properties.

Imbibition enables the occurrence of processes belonging to the second phase of germination. During that phase, the true restoration of life in the seed takes place. The dominant processes are hydrolysis and degradation of food reserves. For this reason this phase is often called the catabolic phase of germination. However, some syntheses, mainly of hydrolytic enzymes do occur in parallel to degradation of storage proteins, fats and carbohydrates. Respiratory processes and energy generation sharply increase at the beginning of that phase.

Accessibility of low molecular products coming from storage material degradation as well as a high level of energy-rich compounds allow the processes of the last, anabolic phase of germination to start. During this phase, (considered as the phase of induction of growth) massive synthesis of cell wall material, as well as increased synthesis of protein and ribonucleic acids occur. It seems that in the course of this phase all essential capacities of a living plant are restored.

The distinction of the phases of germination is arbitrary. It is obvious that the processes that are typical for one phase can start during the preceding phase and can continue during the subsequent one. As a rules the phases of germination overlap one another.

It was proposed above, that the removal of dormancy can be considered as a part of the germination process. The removal of the dormancy imposed by the lack of water obviously takes place during the first phase of germination. Similar situation seems to exist for dormancies caused exclusively by the lack of appropriate light conditions or by the presence of inhibitors in the seed coat. Nevertheless, other mechanisms maintaining dormancy might act at another phase of germination or even before it start. The mechanisms involved in physiological immaturity of the embryo are released as a result of a relatively long chilling of the imbibed seed, when intense catabolic processes already occur. Therefore, total elimination of dormancy in seeds of this type takes place in the second phase of germination. On the other hand, imbibition of the so-called hard seeds is only possible after softening, or impairement of the seed coat, which is the main barrier preventing germination. In such seeds germination starts by softening, which requires temperature and humidity

regime different from other parts of germination process, and precedes imbibition.

Complex types of dormancy are caused by several different mechanisms which may make germination more difficult or hinder it altogether. We can consider these mechanisms as a set of barriers which prevent germination. They can act upon whichever particular process, belonging to any phase of germination.

Hypotheses and Generalizations Concerning Involvement of Hormones in Dormancy and Germination Control

The general role of plant hormones seems to lie in shifting a tissue from one physiological state to another one. Thus, the entrance to a new development phase is supposed to be triggered by a hormonal factor. The mechanisms of hormone action rely upon their control of differential gene activation (transcriptional or translational control) or in alteration of the properties of cell membranes. Taking into account the concept of specific receptors required for the action of hormones (Trewavas and Jones, 1981), one can assume that the primary event caused by a hormone is a conformational change of a specific macromolecule. Depending on character and location of that macromolecule (in membranes, cytosol, or in nucleus) further events may lead to different kinds of responses.

Germination is a developmental stage clearly distinct from the preceding one. Hormones have been early recognized as agents triggering processes that lead the seed into germination. The major classes of hormones associated with seed physiology are gibberellins (GA) abscisic acid (ABA) and cytokinins (CK). Auxins (mainly IAA) and ethylene were also considered as involved in germination. Moreover, there are various regulatory substances as coumarins, phenolics, sterols and cyanide that play a role in germination of some seeds.

In order to attribute to a native hormone an active role in the control of a given physiological phenomenon one should obtain consistent results from several different experimental approaches : (i) exogenous hormone has to provoke the specific effect when applied at concentrations similar these to present in the plant material under study, (ii) changes in the content of the endogenous hormone should correlate with the course of the studied process, (iii) inhibitors of hormone biosynthesis, action or translocasion have to inhibit the processes thought to be under control of the hormone.

During the early studies on hormone involvement in seed germination, and even in more recent works, the above rules were not always observed. Moreover, in numerous studies, the final effect was recorded late after the beginning of imbibition, of ten several days after the protusion of the radicle, so that it is sometimes hard to distinguish between involvement of the hormone in a process related to germination or in growth of the seedling.

We have, therefore, to interpret with caution the date on hormone involvement in dormancy and germination. Nevertheless, many authors arrived to similar conclusions using different approaches; so, the question: do the hormones play any role in the control of germination ?- ought to be answered positively.

The inhibitor hypothesis

The early hypotheses postulated that the dormancy of seeds is caused exclusively by the presence of germination inhibitors. The substances such as coumarins (Evenari, 1949) or phenolics (Murphy and Noland, 1981; Van Summere *et al.*, 1972) present in seed coats limit the oxygen access to the embryo, thus reducing cellular oxidation and energy generation needed for reassumption of growth. Leaching of these substances out of the coat, (rain, imbibition conditions) enables immediate germination.

The concept of germination inhibitors as principal agents responsible for seed dormancy was supported by the discovery of embryo-located substances of this kind (Bohamy and Dennis, 1977; Dennis *et al.*, 1978; Luckwill, 1972). They were chromatographically isolated from different species and the term 'B-inhibitor complex' came into use. This complex included several phenolics and mainly abscisic acid. The embryo-located inhibitors are metabolized during removal of dormancy and it was postulated that their level is controlled by the factors affecting dormancy (temperature, light, humidity) (Kefeli and Kadyrov, 1971).

The discovery of abscisic acid in seeds (Milborrow, 1968), gave a new powerful argument to the inhibitor concept of dormancy. ABA is the only inhibitory substance that is commonly present in dormant tissues (Addicot and Lyon, 1969; Milborrow, 1968). It is active at concentrations of several orders lower than those of phenolics and other germination inhibitors. It has all features of a plant hormone and it was postulated

to be a universal dormancy hormone (its first denomination was 'dormin').

Abscisic acid was found in every plant species examined (Milborrow, 1974). It was demonstrated for numerous seeds that the content of ABA is positively correlated wiih the depth of dormancy. During the removal of dormancy in *Prunus avium* (Lipe and Crane, 1966), *Juglans regia*, *Fraxinus americana* (Sondheimer *et al.*, 1968), apple (Rudnicki, 1969; Balboa-Zavara and Dennis, 1977), *Acer saccarum* (Webb *et al.*, 1973, a), *Acer pseudoplatanus* (Webb *et al.*, 1973 b) and *Acer platanoides* (Tomaszewska, 1976) a step wise decrease of ABA content was observed down to amounts undectectable with bioassays. ABA is located in different parts of seed; embryo axis, cotyledons and seed coats (Enu-Kwesi and Dumbroff, 1977; Balboa-Zavala and Dennis, 1978). Its inhibitory action is supposed to be exerted upon different mechanisms and its decrease may also result from different causes such as catabolism, conjugation and leaching.

In seeds of some other species the decrease in ABA content during removal of dormancy, however is less pronounced. Non-dormant, after ripened seeds of *Fraxinus excelsior* contain considerable amounts of ABA (Villiers and Wareing, 1965; Kentzer, 1966). Similar situation has been stated for seeds of *Acer saccharinum* (Rudnicki and Suszka, 1969). Morever, in some seeds the decrease in ABA content during storage was independent of conditions enabling dormancy removal (*Fraxinus americana*-Sondheimer *et al.*, 1974; lettuce–Braun and Khan, 1975) and in seeds where ABA disappeared during dormancy removal its disappearance preceded the full achievement of germination capacity (e. g. apple, *Acer saccharum* loc. cit). All these observations indicated that despite of its universal presence in dorment seeds and its pronounced inhibitory activity, ABA is not the exclusive factor involved in physiological dormancy.

In seeds of some woody speices relatively high amounts of other germination-inhibiting hormone, indolylacetic acid, were found. It has been postulated by Nikolaeva (1967), that IAA, in parallel to ABA, plays a role in onset and maintenance of embryonal dormancy. She observed that the level of endogenous IAA gradully decreased during dormancy removal in *Acer tataricum*. Exogenous IAA not only inhibited germination of non-dormant embroys but, at lower concentrations, caused

anomalies in seedling development similar to those observed during germination of embryos isolated from fully dormant seeds. Attempts to detect free auxins in dormant seeds of many other species were, however, without success. In contrast, in some cases auxin-like compounds were found in germinating or ready-to-germinate seeds (apple-Kopecky *et al.*, 1975; *Acer platanoides*-Tomaszewska, 1976). It seems that the role of IAA in dormancy may be restricted to some species (including *Acer tataricum* but it is not as common as that of ABA.

Inhibitor-promotor equilibrium

Numerous observations indicate that seed germination is promoted by a number of chemicals. Among these promotors special attention must be given to hormones- gibberellins and cytokinins, because of their pronounced germination- stimulatory effect and their common occurrence in seeds during dormancy removal and germination.

It has been suggested that the seed can not germinate, *i.e.*, it is dormant, not necessarily because of the presence of inhibitor (s), but also because of the absence of aproppriate stimulators (Luckwill, 1952; Amen, 1968).

The strongest arguments speaking for the involvement of hormonal stimulators in dormancy removal were the observations that gibberellins are able to replace light requirement in breaking the photodormancy in lettuce seeds (Khan, 1960) and that cytokinins (benzyladenine, kinetin) are indispensable in elimination of thermodormancy in the same material (Khan, 1971). Morever, both these hormones alleviate or remove dormancy in seeds requiring the after-ripening period. In cereal grains, which need several weeks of dry storage in order to achieve full germination ability, gibberellin treatment markedly shortens this period (Lang, 1965). Germination of isolated haselnut embryos, that require a long-lasting moist chilling, is cosiderably stimulated by gibberellins (Bradbeer, 1968). Similarly, the dark germination of isolated, dormant apple embryos (requiring a three months cold stratification) is stimulated by gibberellins (Smolenska and Lewak, 1971) or by cytokinins (Lewak and Bryzek, 1974). In this last case, however not all symptoms of embryonal dormancy, that disappear in result of chilling, are removed by hormonal treatment. In the case of complex dormany in *Fraxinus excelsior*, composed from anatomical underdevelopment of the embryo (requiring warm stratification) and its physiological immaturity (requiring cold

treatment) it has been demonstrated that gibberellin treatment can effect only the physiological, chilling sensitive type of dormancy (Wcislinska, 1977).

Further support for the promotor concept has been obtained from determinations of changes in endogenous gibberellin and cytokinin levels during the release of seed dormancy. Gibberellin content constantly increases in seeds of *Coryllus avellana* during stratification (Frankland and Wareing, 1966; Bradbeer, 1968). In *Acer saccharum* and in apple seeds the contents of both GAs and CKs rise during stratification, but after reaching a maximum they decrease to a very low level. In apple the maximum contents of both hormones fall into approximately the the same period around the 30th day of stratification (Sinska and Lewak, 1970; Borkowska and Rudnicki, 1975) whereas in sugar mapple the maximum of CKs content precedes those of GAs for about 20 days (Webb *et al.*, 1973a).

Similarly as found for ABA, the content of gibberellins and cytokinins may very in differnt parts of the seed. Thus, the inhibitor-promotor equilibrium may be different in each of these parts and the regulatory effect of that equilibrium may be of various character. Moreover, the mechanisms determining that equilibrium may work differently in various parts of the seed. Some of these mechanisms are evidenced, but their location is far from known. These are : selective leaching or translocation of hormones (Rudnicki and Czapski, 1974; Jarvis, 1975; Dennis, 1977; Jarvis and Wilson, 1977), hormone biosynthesis (for GA–Sinska and Lewak, 1975; Arias *et al.*, 1976), reversible formation of inactive hormone conjugates (for CKs – Borkowska and Rudnicki, 1975; Barthé, 1979; for GAs – Halinska and Lewak, 1978; Isaia and Bulard, 1978).

The fact that gibberellin (GA_4) biosynthesis occurs during apple seed after-ripening provides further support for the concept of involvement of these hormones in dormancy release. Endogenous GA_4 level increases during the first 30 days of seed after-ripening and afterwards it gradually drops. Gibberellin biosynthesis takes place during the first 50 days only. Sensitivity of isolated embryos to exogenous gibberellin diminishes during stratification, being the highest in dormant embryos. Treatment of the gibberellin-sensitive, GA-synthesisng embryos with AMO – 1618, which acts as a gibberellin biosynthesis inhibitor, hinders germination, but treatment of emryos isolated from seeds stratified for 50 days or longer is without effect on germination (Sinska and

and Lewak, 1977). It seems that these results univocully confirm the involvement of a hormonal promotor in dormancy removal.

On the other hand, ABA added to stratification medium delays the rise in gibberellin content in apple seeds (Rudnicki *et al.*, 1972). This seems to indicate that the inhibitor-promotor interactions may relay upon regulation of one hormone level by another one, rather than on an opposite action at the same regulatory point.

Changes in the inhibitor-promotor ratio during dormancy removal are obviously under more or less direct environmental control. In this respect an interesting hypothesis coming from Bulard's (Nice, France) group is worth being mentioned: low temperature, necessary for deep dormancy removal in seeds of many species, differently affects the enzymes involved in formation and hydrolysis of inactive, bound forms of different hormones, so that it favours the formation of ABA conjugates and simultaneously promotes the release of GAs and CKs from their inactive forms. This hypothesis requires a full experimental confirmation.

Ethylene is probably another hormone that plays a role in the inhibitor-promotor equilibrium. Its involvement in dormancy removal and germination of seeds was postulated by Toole *et al.* (1964), but the first data on the germination-stimulating activity of ethylene were reported by Haber as early as in 1926. Some years ago a comprehensive review on ethylene in seeds was published (Ketring, 1977); therefore, we shall only shortly discuss that problem. Ethylene increases the germinability of seeds with different types of dormancy. Also its increasing emanation from seeds during dormancy removal has been observed. Factors affecting ethylene biosynthesis, when applied to the seeds, also affects their germination. On the other hand, conditions that cause elimination of ethylene from atmosphere surrounding the seeds also decrease germination ability. It is, however, doubtful weather ethylene is really involved in germination control or it affects the early growth of the seedling. Moreover, there are no data on the actual ethylene concentration in the seeds; all investigations concern the rate of ethylene production rather than its level in the tissue. Recently, the accumulation of ^{14}C-ethylene in *Phaseolus* cotyledons was described (Hall *et al.*, 1980; Evans *et al.*, 1982); nevertheless, for the time being, these data do not help in understanding the involvement of ethylene in seed physiology.

According to the present knowledge, ethylene seems to play a supplementary role in the first two phases of germination, as compared with the substancial role of ABA, GA or CK. Its involvement in the last period of germination, the growth initiation, appears to be more evident but still requires further studies. Apart from the hormones listed above, several other factors are also included in the regulatory equilibrum that determines the onset, maintenance and removal of dormancy.

Seeds of some species contain considerable amounts of cyanogenic glucosides and cyanolipids, which release free hydrogen cyanide as a result of hydrolysis. The production of HCN takes place during the removal of dormancy (in apple - Dziewanowska *et al.*, 1979b). Moreover, application of cyanide to various seeds markedly stimulates germination (Taylorson and Hendrieks, 1973; Rollin, 1975; Dziewanowska *et al.*, 1979a; Perino and Come, 1981; Dziewanowska and Lewak, 1982). There is, therefore, a serious probability that HCN may contribute to the complex regulatory mechanism involved in dormancy removal, at least in those seeds where its presence is proved.

Hypothesis on the selective roles of hormones

The involvement of a considerable number of hormones and other regulatory agents in the maintenance and removal of seed dormancy as well as in seed germination provoked an attempt to attribute a specific role to each agent in the control of these processes.

The inhibitor-promotor concept considered only two classes of factors. moreover the term 'promotor' designated a large group of substances with various physiological activities and properties. On the other hand, in some seeds dormancy was broken without elimination of endogenous ABA (Williers and Wareing, 1960; Kentzer, 1966); the effect of exogenous ABA or other inhibitors could be reversed by application of cytokinins, but not by gibberellins (Khan, 1967; Khan and Downing, 1968); cytokinin could synergize the effect of GA. From these observations Khan (1971, 1975) concluded that dormancy and germination could be determined in one of several ways, and not always by an excess of inhibitors or an excess of promotors. He attributed to gibberellins the primary role in the regulation of germination and in the release of dormancy whereas the roles of inhibitors and cytokinins would be of secondary character: preventive and permissive, respectively. According the Khan, the hypothesis of the selective action of hormones would be also valid for other

physiological phenomena, but the particular roles in such cases would be played by different hormones. For example, in apical dominance, auxin would act as the primary factor, whereas cytokinins and gibberellins as preventive and permissive ones, respectively (Khan, 1975).

Khan's hypothesis assumes a very limited number of regulatory sites (points) affected by hormones that control dormancy. The competitive nature of ABA and CK interaction implies a common site for these two hormones. In contrast, the non-competitive character of ABA and GAs effects suggests that they act at different regulatory points. The above conclusion from Khan's hypothesis, however does not fully comply with the current views on basic mechanism of hormone action.

The plant hormone, in order to act, has to be linked with a specific receptor (Trevawas and Jones, 1981). In other words : the primary reaction of the hormone is its binding with a specific receptor. The next events are mediated by the hormone-receptor complex. The variety of specific receptors can explain the variety of physiological response to a hormone. Changes in the number of the available receptor molecules in the tissue during ontogenesis reflect changes in sensitivity towards that hormone. There is a good evidence for the existence of such specific receptors, for hormones cf all known classes (for review, see Kende and Gardner, 1976). Nevertheless, evidence concerns, as yet, solely the occurrence, specificity and structure of receptors; there are no convincing reports on the physiological activity of a hormone-receptor complex.

The known early (not primary) responses to hormones in the seed may be of different nature. There are well documented examples of induction or repression of different enzymes at the level of transcription as well as at that of translation (for reviews see Higgins *et al.*, 1976, 1977) numerous enzymes are activated in result of hormone treatment (Tao and Khan, 1977). It is also known that hormones can affect the structure of cell membranes (Wood and Paleg, 1974). It would be an unlikely assumption that in the control of all these phenomena only a few types of receptors are involved.

Since the only physical meaning of Khan's 'regulatory site' of the hormone is a specific receptor molecule, we have to consider the hypotheses of selective roles of hormones only as a useful simplification of the much more complex relations between hormones that control dormancy

and germination. Other arguments supporting that opinion will be discussed later.

Metabolic Events During Dormancy Removal and Germination

Metabolic activity in the dormant seed is extremely low, independently of the mechanism of dormancy. In contrast, a ready-to-germinate, non-dormant seed is metabolically very active. During germination *sensu lato* (including dormancy removal) the entire metabolic apparatus of the seed becomes operative in a controlled, sequential fashion. This activation of ring the last stages of seed development and dessication; the sequence of appearance of particular events and the character of these events are subordinated to the main objective of germination- to produce a vigorous plant.

Membranes

This sites of metabolic processes in the living cell are its compartments separated from each other by membranes. It is obvious that the integrity of cell membranes, their structure and properties play˙a decisive role in the control of all metabolic activities. It seems that the most important properties of membranes are: the semipermeability, the activity of membrane-bound enzymes and the membrane potential.

There is some indirect evidence that during the very early stages of germination structural rearrangement of membranes takes place. The leakage of amino acids and other solutes, that occurs during the first hours of imbibition indicate some injuries. This leakage decreases in the cours of germination, thus indicating membrane rearrangement and the existence of repair mechanisms (Simon and Raja-Harun, 1972; Hendricks and Taylorson, 1976, 1979; Murphy and Noland, 1982). This last suggestion has been confirmed by demonstration of phospholipid turnover during early germination (Simon, 1974).

There are some indications that gibberellins may affect the state of plant membranes thus changing the permeability (Wood and Paleg, 1974; Wood *et al.*, 1974). Moreover, it was demonstrated that ABA acts upon membrane potential and prevents the proton extrusion from cells (Lado *et al.*, 1975; Ballarin-Denti and Cocucci, 1979). Gibberellin affects also the synthesis of some essential membrane constituents (phosphatitylcholine, Ben-Tal and Varner, 1979). This observation suggests the hormonal control of membrane repair mechanisms as well as the control of membrane development during germination.

Although the role of cell membranes in germination seems to be unquestionable, there are some problems that require further studies with the use of more direct approaches. These are: (i) which cell membranes are important at the early stage of germination ? (ii) how far do the changes in membrane structure and function during germination affect the metabolic events related to germination ? and (iii) do some membranes play the role of sensors towards germination-triggering factors such as temperature and hormones ?

Nucleic acid and protein synthesis

Numerous authors have convincingly demonstrated that in the dormant seed the synthesis of protein and nucleic acids is blocked. The genetic activity of such a seed is repressed. During the release of dormancy a gradual derepression of genes tekes place. At the time being it is not possible to establish whether the mechanism and the sequence of activation of processes involved in protein synthesis are identical for all kinds of seeds and all types of dormancy, since a number of controversial data is still published.

Some years ago an interesting generalization concerning the sequence of appearance of different types of RNA during germination of cereal grains was described (Dobrzanska *et al.* 1973; Rajman and Buchowicz, 1973). The authors proposed a 'cascade' model of genome activation after the elimination of seed dormancy.

According to that model the derepression of genes coding mRNA occurs first, thus allowing the onset of synthesis of some proteins. It is followed by the synthesis of ribosomal RNA and the last synthesized kind of RNA is the tRNA. This sequence is roughly in agreement with other data, except for two observations. The authors of the 'cascade' model have located the initiation of DNA synthesis (replication) just after activation of mRNA-coding genes, whereas according to the convincing data coming from an elegant experimental series, DNA replication takes place late during germination (Osborne, 1981). It does not occur until dormancy is completely broken. Another example of incompatibility of the 'cascade' model with experimental data is the synthesis of some enzymes frequently observed very early in the course of germination (for a recent review, see Mayer and Marbach 1981).

The presence in the dormant seed of the mRNA formed before the onset of dormancy and stored in an inactive form until imbibition gives

an explanation of this early protein synthesis. The early works (Marcus and Feeley, 1966) indicated the presence of longlived mRNA in dry wheat embryos and peanut cotyledons and than the occurrence of such preformed mRNA was intensively studied (for review, see Dure 1977). It is now commonly accepted that the preformed mRNA is responsible for early protein synthesis during germination; the open question is how important for germination process is the synthesis of protein coded by that mRNA. It seems, that it may be involved in rapid resumption of growth of the embryo, but does not control its further development.

The mRNA already present in a dry seed has to be activated in order to rusume its function; hydration of seed colloids enabling structural rearrangements and diffusion of solutes was shown to be effective in activation of a small fraction of preformed mRNA. It seems that this fraction, called residual mRNA, is engaged in the synthesis of protein involved in seed development during maturation or in the general cell activity, but is doesnot play any specific role in the processes of germination. The bulk of preformed mRNA is stored in dry seed in a form which is not activated simply by a contact with water.

The stored, masked mRNA is unfunctional because it is non-adenylated this prevents its translation (Dure and Haris, 1977). Thus, polyadenylation has to precede the initiating of protein synthesis, as, in fact, observed during germination of seeds of different species (Nazus and Brodkiewicz- Proba, 1976; Delsney *et al.*, 1977; Seliwanowicz *et al.*, 1977). The stored mRNA upon polyadenylation rapidly disappears during germination. It is replaced by a freshly formed poly mRNA. The intensive synthesis of polyadenylated mRNA has often been observed in germinating seeds, but it starts relatively lately.

Fig. 3.1 shows schematically the sequence of appearance of new protein, mRNA and DNA and the changes in the rates of their syntheses. It ought to be underlined that synthesis of some enzymes (including proteases) precede synthesis of mRNA. Protein content changes in the seed result from the intensities of new protein synthesis and reserve protein hydrolysis. It is also worth noticing that DNA replication directly precedes growth and proliferation of cells, in parallel to increased RNA and protein synthesis.

The involvement of hormones in the control of processes that lead to protein biosynthesis has often been investigated (Teissere *et al.*, 1973;

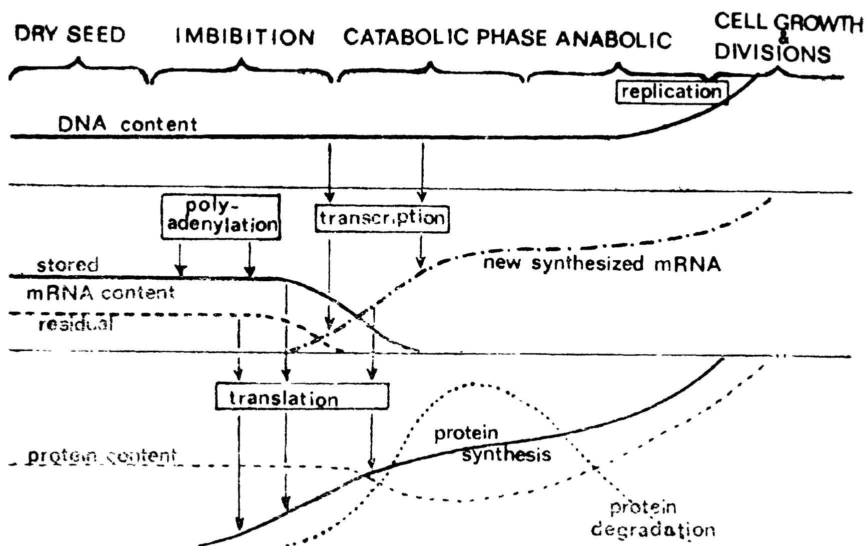

Fig 3.1. Representing the sequence of protein, RNA and DNA synthesis during germination.

1975; Tarantowicz-Marek *et al.*, 1975; Wasilewska and Kleczkowski, 1976). The synthesis of amylase in aleurone cells of germinating cereal grains is stimulated by GA (Varner, 1964; Varner *et al.*, 1965). ABA is able to fully reverse that stimulation (Chrispeels and Varner,1966). It is of some interest that the effect of ABA can be overcame by cyto-kinin, but not by GA treatment (Khan, 1975). It was demonstrated that hormones controlling the synthesis of α-amylase (also protease, ribonuclease and other enzymes) act at the level of transcription, regulating more or less directly the genome activity (forreview-Tao and Khan, 1977). There are, however, some data that can be interpreted as indications of a parallel hormonal control of the translation process (Davis and Larkins, 1982).
There is no evidence for the hormonal control of polyadenylation of preformed mRNA during early germination. One can not, however, exclude such a possibility.

Enzyme activation

A great number of enzymes involved in metabolism of the germinating seed are synthesized *de novo*. Nevertheless, many enzymatic activities

appear early during germination without concomitant protein synthesis. To this group chefly belong the enzymes involved in RNA and protein synthesis (e.g., RNA polymerases) and enzymes engaged in mobilization of reserves (mainly hydrolases). There are several possibilities of such non-synthetic rise in enzyme activity. Some of them can be active in the immature seed, survive dehydration and regain their activity in result of rehydration. Other enzymes are present in the dry seed in an inactive form that cannot be activated simply by the contact with water. There is convincing evidence for activation of numerous enzymes during seed germination, coming mainly from experiments with the use of inhibitors of protein synthesis. The mechanism of activation is however known in a few cases only. Generally, there are the following possible ways of activation : (i) removal of an inhibitor, (ii) release from a subcelluar compartment, (iii) release from a precursor by partial degradation, (iv) aggregation of enzyme subunits and (v) conformational changes of an inactive protein. The problem of enzyme activation has been reviewed a few years ago by Mayer and Shain (1974).

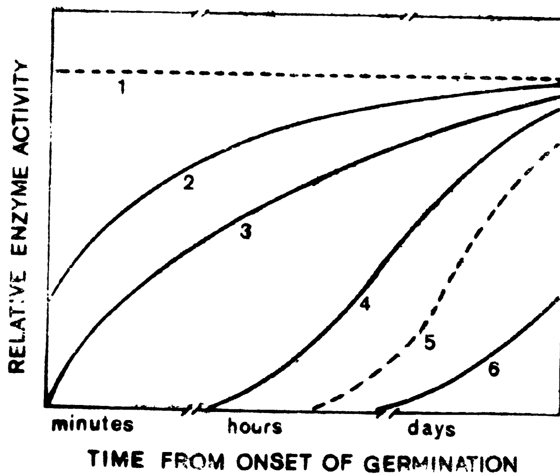

Fig 3.2. The sequence of appearance of enzyme activities during germination. 1: enzymes continuousely active. 2: activity rising during germination, but residual activity present in dormant seed. 3: as (2), but no residual activity. 4: enzymes activated in result of imbibition (dormancy breaking). 5: enzymes synthesized with the use of preformed mRNA. 6: enzymes synthesized with the use of newly formed mRNA. (Mayer, 1977; modified).

The sequence of appearance of enzyme activities, depending on their origin, is schematically presented in Fig.3.2. When a block involved in

dormancy maintenance is removed later during the imbibition, the rise in enzyme activity is adequately delayed.

All the possible mechanisms of enzyme activation might be affected by hormones. Nevertheless, there are no convincing data, for such interactions. It has been suggested that activity of some hydrolytic enzymes increases in seeds in result of gibberellins treatment, are activated by that hormone (e. g., β-amylase, Varner, 1964; Varner and Chandra, 1964). There are aboundant other indirect indications for hormonal activation of enzymes (e. g., data reviewed by Ashton, 1976). The inactive proenzyme was not, however identified for such enzymes, nor were other persuasive experiments *in vitro* performed.

The process of enzyme activation during seed germination is at least as important as nucleic acid and protein synthesis. It seems, therefore, that it still deserves much more attention than it has been paid until now.

Mobilization of storage materials

The sequence of metabolic events during germination results from sequential appearance of different enzymic activities and complies with the needs of an awakening seed. The first mass process is the mobilization of stored material; this consists in such a transformation of reserves that they can be used for energy generation and for building of the new compounds. The first step of the reserve mobilization is hydrolysis of more complicated compounds into simpler low-molecular units.

For the majority of seeds the main storage materials are lipids (triacylglycerols) and proteins. Other seeds, (some of them having a primary economic value) store mainly polysaccharides (starch). The oily, as well as the starchy seeds contain reserve proteins and cell-wall hemicelluloses that also are utilised during germination. The course of reserve mobilization differs in seeds depending on the character of storage materials, environmental requirements and further developmental pattern of the plant. Nevertheless, some of the features of onset of hydrolytic processes seem to be general.

The molecule of triacylglycerol bears much more energy that can be utilized for syntheses and eventually for the process of growth, than a sugar molecule. On the other hand, sugars are much more easily available for energy-yielding reactions and as substrates for syntheses of

protoplast and cell-wall material. The strategy of storage material mobilization results from the features of both classes of reserve substances.

It was noted in the preceding sections that some energy-requiring processes, such as protein synthesis, start by the end of imbibition. The source of that energy is oxidation of monosaccharides originating from stored oligosaccharides (sucrose, raffinose and other). Thus, hydrolysis of those sugars presents one of the first events in early germination, considerably preceding the breakdown of bulk reserve materials. It is of some interest that the beginning of degradation of galactomannans and other cell-wall polysacchrides also takes place much earlier than starch hydrolysis. It seems possible that the same enzymes are involved in hydrolysis of storage oligosaccharides and cell-wall polysaccharides.

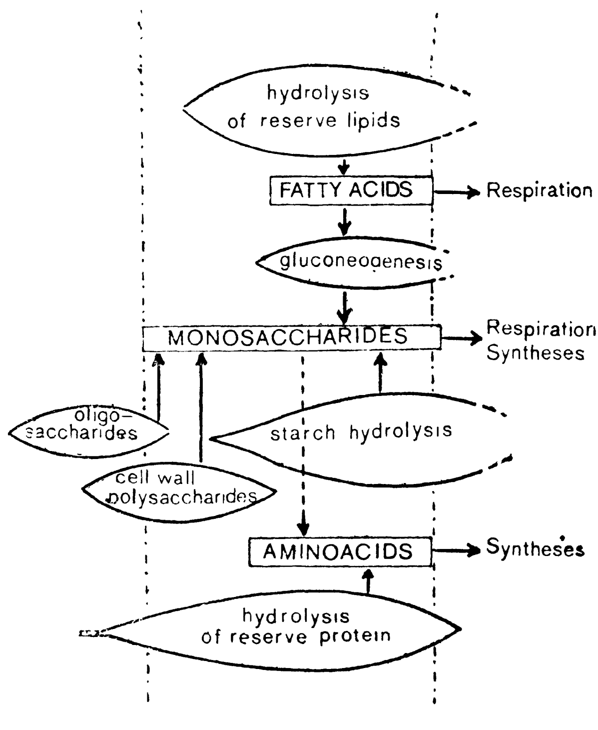

Fig. 3.3. Schematic presentation of the mobilization of reserve materials during the catabolic phase of germination.

The biological sense of that early breakdown of cell wall material is evident: it produces material available for energy generation; moreover, it causes a weakening of walls that enables the diffusion of enzymes from the sites of their production into reserve-containing cells and subsequent translocation of the products of hydrolysis into the embryo axis.

The sequence of hydrolytic processes during the catabolic phase of seed germination is schematically presented in Fig 3.3. The reserve materials are accumulated in specialized cell compartments: lipids -in spherosomes (lipid bodise, oleosomes), proteins- in protein bodies, whereas starch forms granules originating from desintegrated plastids. Spherosomes and protein bodies are closed in a single membrane envelope. The enzymes involved in the hydrolysis of lipids and protein are operating in these organelles, the breakdown products being translocated outside. The lipid bodies disappear in result of hydrolysis of stored substances, whereas the protein bodies transform into vacuoles. Starch seems to be hydrolysed by cytoplasmic enzymes and the granules disappear when its hydrolysis is completed.

Some of the products of reserve material hydrolysis can be directly used for syntheses (amino acids, glucose), but most of them has to be first transformed into appropriate compounds. In the germinating seed there function almost all systems that enable interconversions of sugars and aminoacids, synthesis of purines and pyrimidines etc., described for other developmental phases. Probably the catabolic pathways of triacylglycerols are most specific for the germinating seed. The fatty acids produced in spherosomes are translocated into glyoxysomes where they undergo β-oxidation. In the same organelles acetyl-CoA enters into the glyoxyllate cycle and is converted into succinate. Succinate, after translocation into mitochondria enters the tricarboxylic acid cycle. The part of oxal–acetate that forms in mitochondria is exported and converted in cytosol into sugars by the gluconeogenesis pathway. In some oily seeds such a transformation of lipids into carbohydrates plays an important role. It can be considered as a secondary mobilization, yielding a product more useful for intensive growth processes terminating germination.

There is aboundant number of data concerning hormonal control of reserve mobilization, collected until now. Most of these data reflect the involvement of different hormones in hydrolytic enzyme activation

and/or biosynthesis. It is, however, impossible to do any generalization; in seeds of different species the hormonal control of reserve hydrolysis seems to operate according to particular needs of these seeds.

Energy generation

During the whole period of germination of the seed the only source of energy is the oxidative breakdown of storge materials. It seems worth noticing that in spite of limited reserves, the effective utilization of that energy (under the form of ATP) does not exceed 10% (Maleszewski, 1965). Carbohydrates are the main direct substances for oxidative catabolism in seeds.

The conventional pathway of oxidative glucose breakdown including glycolysis, tricarboxylic acid cycle and cytochrome electron transport chain has been demonstrated in germinating seeds in many reports. Moreover, it has been shown that during germination also oxidative pentose phosphate pathway is operating. Both are involved in oxygen uptake, production of ATP and reduced nicotinamide nucleotides (NADH and NADPH, respecttvely). The ratio between intensities of these two paths is not constant during germination; generally glycolysis is the dominant process at the beginning of germination, whereas the contributian of pentose phosphate pathway increases later on. Roberts (1973) postulated an important role of pentose phosphate pathway in dormancy release of the seed. That suggestion resulted mainly from observations that inhibitors of the conventional pathway (cyanide, azide, CO, H_2S, iodoacetate or malonate) increase the participation of pentose phosphate pathway in sugar catabolism and simultaneously stimulate germination.

Moreover, anaerobic conditions applied for a relatively short time to an imbibed seed stimulate its further germination, eliminating dormancy (Tissaoui and Come, 1973).

The mechanism of this dormancy-eliminating effect is still obscure; the involvement of changed NADH/NADPH ratio was hypothesized Hendricks and Taylorson, 1975) or the participation of some unidentified intermediates of pentose phosphate path (Roberts, 1977). The germination–stimulating effect of inhibitors of cytochrome oxidase was also interpreted as the involvement of the alternative, cyanide insensitive electron transport in dormancy breaking. It seems that we are still far

from understanding the role of oxidative catabolism in seed germination and there is a necessity of further studies.

Respiration of the seed, measured as oxygen up-take, sharply rises during imbibition; during the catabolic phase its increase is slow if any, and afterwards a second sharp rise is noted. The increase in ATP level (3-10 fold) during early imbibition precedes the rise in respiration, and despite the early observed glycolysis it cannot be considered as a result of classical oxidative phosphorylation. It has been recently demonstrated that cell-free extracts from dry seeds contain an enzyme system able to produce ATP from AMP and phosphoenolpyruvate (Perl, 1980/81). On the other hand, according to the not yet fully evidenced suggestions, the intense productions of ATP during early germination is related to its *de novo* synthesis and/or to the transphosphorylation of membrane located phosphoproteins (Mayer, 1977). In this last case the driving force for ATP formation would be the gradient of water potential during imbibition.

Further increase in ATP level and in energy charge (EC) results from the stepwise increase in activity of oxidative catabolic processes, deve-

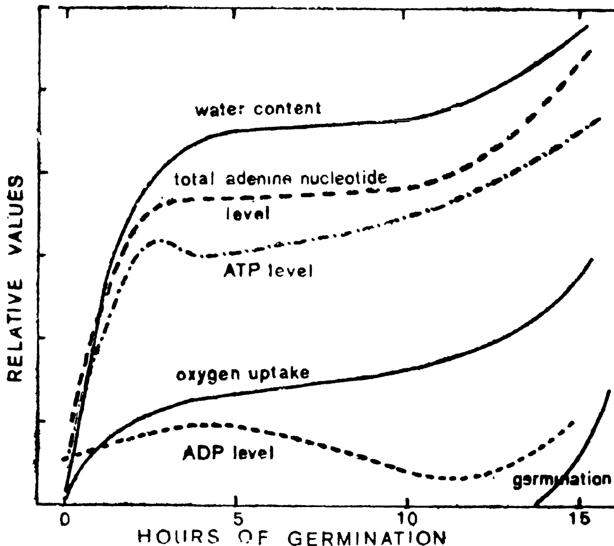

Fig 3.4. Time-course of increases in water uptake, oxygen consumption and adenine nucleotide levels during germination of lettuce seeds (Pradet *et al.*, 1968; modified).

lopment (maturation) of mitochondria that occurs already in the dry seed and synthesis of new mitochondria (relatively late during germination). The synthesis *de novo* of adenine nucleotides increases when supplied with aminoacids coming from storage protein hydrolysis.

The energy metabolism during germination is illustrated with a diagram presenting changes in water uptake, oxygen consumption and in adenine nucleotide levely Fig 3.4. Note the phasic character of these changes. There is only indirect evidence for hormonal control of ATP level in germinating seeds (Yomo and Varner, 1971). Similarly, only a correlation was found between the effects of exogenous hormones (GA_3, kinetin) on the depth of dormancy and on the energy charge level in cold-requiring seeds (Simmonds and Dumbroff, 1974).

Metabolic regulation

The moderately high level of ATP ensures the anabolic reactions during germination of the seed. It also has a regulatory effect upon the activity of numerous enzymes, including those involved in catabolic reactions (for review, see Ching, 1972). Moreover, the high energy charge has been shown to control various metabolic sequences involved in energy utilization and production. The level of energy charge has been correlated with germination ability of seeds of different species; it increases during the breaking of dormancy in *Pinus ponderosa* (Ching, 1972) and in *Acer saccharum* (Simmonds and Dumbroff, 1974). On the other hand, it was demonstrated that, although EC increases under conditions favouring dormancy release, it is not sufficient to overcome all barriers preventing germination; similar rise in EC was also observed under conditions that do not eliminate dormancy (warm stratification versus cold stratification).

In germinating seeds the same mechanisms operate as in other developmental phases, mainly involved in control of enzyme activities. Numerous examples of induction by a substrate, inhibition by an end-product, as well as effects of inducers, effectors and modifiers were described (for review, see Ching, 1972). These effects are involved in regulation of cell catabolic and anabolic processes and maintain their sequences.

Another control mechanisms relies upon regulation of translocation from cell to cell or from one part of the seed to another. Since the catabolic reactions take place mainly in storage organs (endosperm, cotyledons) and the syntheses occur most intensively in the embryonal

axis, the main stream of translocated substances is directed into the axis. The active transport of sugars from endosperm through scutellum into axis of cereal grains, connected with several transformations of the sugar molecule is a well described example of such translocation. Another extensively investigated example presents the secretion of hydrolytic enzymes (e. g., α-amylase) from aleurone cells into endosperm in cereal-grains (Yomo and Varner, 1971; Jones, 1973). It seems that the hormonal control of seed dormancy may be considered in the same categories. The translocation of gibberellin from the embryo into the aleurone layer results in triggering the synthesis of hydrolytic enzymes. In apple seeds, abscisic acid moves from cotyledons into the embryo axis, thus reinforcing dormancy (Balboa-Zavala and Dennis, 1977).

Also the translocation may be affected by a uncomplete development of structures in the seed. This phenomenon seems to be of special meaning for dormancy cause by anatomical underdevelopment of seeds. During dormancy removal differentiation of the vascular bundles has been observed in the cotylednons (Dawidowicz-Grzegorzewska, 1981); thus translocation of mobilized storage materials into the embryo axis becomes possible.

The interaction between different parts of the seed has the character of a physiological correlation. The presence of cotyledons affects the functioning of axis and vice versa. This effect may change depending on environmental conditions and on the physiological state of the seed. A convincing example of such a correlation in apple embryos was given by Thévenot (1982). It is obvious that in correlation of this type the translocation phenomena are involved. The translocated substances might be the nutrients, hormones and probably other, so far unknown factors. The problem of hormone involvement in correlative interactions between different seed parts deserves much more attention than it has been paid until. An indirect regulation of metabolic activity of the seed during the course of germination may be related to the underdevelopment of structure during seed maturation. In most dormant seeds mitochondria lack certain important constituents that are incorporated into mitochondrial membranes during early germination following imbibition- In result the stability of mitochondria increases and some of their activities develop (cytochrome oxidase, malic dehydrogenase, electron transport) (Narva and Asahi, 1971, 1973; Wilson and Bonner, 1971). In parallel. new mitochondria are formed (Solomos *et al.*, 1972).

This stepwise appearance of functional, energy generating machinery affects the regulation of metabolic processes through ATP level.

Environmental Control of the Dormancy Removal and Germination

Supply of water to the dry seed is the essential condition for the dormancy removal. Hence, humidity is the first environmental factor affecting germination. Other main factors are temperature and light. The seed reaches the optimum ability to germinate under conditions of a very specific combination of these factors; it responds to a precisely defined environmental complex. The failure in the optimal fulfilment of one factor causes the change in requirement for another one, thus changing the environmental complex that allows germination. This is illustrated by the following example: lettuce (var Grand Rapids) achenes germinate within 24 hrs at 17°C in the dark. An increase of the temperature up to 25°C inhibits germination completely, unless exposure to light is applied (Evenari 1952).

The environmental complex for optimum germination may remain constant or the environmental requirements may change during the period of germination. These changes reflect the complexcity of dormancy in the seed. The different sets of environmental conditions alleviating the depth of dormancy correspond to different barriers, or complexes of barriers that prevent germination, and are eliminated under these conditions.

A general example of changing environmental needs present in the seed that exhibits the innate, true dormancy. In order to germinate and to produce healthy plants the dormant seeds of many herbaceous plants need first to be stored dry at a relatively high temperature during several weeks or months (after-ripened, optimum humidity ca 15% optimum temperature for cereals 48-40°C). These seeds have to be then imbibed and germinated at much lower temperature than that of after-ripening (optimum around 20°C). Seeds of many woody plants of moderate climate require cold stratification conditions (full imbibition, low temperature) prior to conditions optimum for germination (as a rule, they germinate at temperatures higher by 10-15°C than those of stratification). Other examples are the diurnal fluctuations of temperature, necessary for optimum germination, observed for numerous cultivated herbaceous plants.

Also light requirements may change during germination. The photosensitivity appears in the non-photoblastic seeds under different stress

conditions such as heat (the cited above example of lettuce seeds), osmotic stress, or in result of treatment with some germination inhibitors (e. g., coumarin). The photosensitivity of seeds may appear as a result of dry storage (Koller, 1972; Vidaver, 1977) or it can disappear after chilling (Bryzek and Lewak, 1974).

The perception of stimuli affecting germination is the first event that enables their action. Only the receptor of light stimulus is well known. The phytochrome system has been first described in photosensitive seeds (Borthwick *et al.*, 1952), ann it is now commonly accepted that phytochrome plays the role of receptor in all responses of the seed, that are influenced by light, including the photoperiodic ones.

The mechanism of perception of other environmental stimuli is still disputable. It is very probable that some cell membranes could play the role of temperature sensors. The hypothesis has already mentioned. Also, there are some indications of the involvement of some enzymic proteins in perception of the thermal stimulus; this problem will be discussed later.

Assuming the crucial role of hormones in the control of dormancy and germination, one could expect that a change in hormonal balance is an immediate response to the environmental complex that provokes the release of dormancy. The data collected so far do not give an unambiguous confirmation of that assumption. In some cases the light stimulus causes a rapid increase of hormone level (GA_4 in apple embryos, Smolenska and Lewak, 1971, GA_9 in lettuce seeds, Bianco and Bulard, 1981), but in other cases changes in the content of hormones are not observed (ABA in lettuce seeds, Khan and Braun, 1975; GA_4 and GA_7 in the same material; Bianco and Bulard, 1981). Similarly, chilling of imbibed apple or sugar mapple seeds results in pronounced rise in GAs and CKs levels. whereas in hazel seeds no such changes have been stated (Ross and Bradbeer, 1971).

The above remarks are aimed at prooving that a change in hormonal equilibrium is not necessarily the first event following the perception of an environmental stimulus and may be it is not at all an obligatory event in removal of seed dormancy. In view of the data presented previously it seems that such a conclusion is premature. The methods of quantification of hormones do not allow to determine their changes in different cell compartments. It was suggested that a physiological phenomenon may

be triggered by a hormone not necessarily in result of the total increment of that hormone level in the cell, but rather as the effect of its increased accessibility at the site of its action (Thoumas, 1977). Phytochrome is involved in altering the selective permeability of cell membranes (Fondeville *et al.*, 1966) and hydration or temperature stresses also may affect the permeability. These findings allow to propose that the first response of some the seed to an environmental stimulus would be a release of hormones from the cell compartments, where they were stored, into the compartment where they can be active or from which they can be exported into the target cells and tissues (e.g., translocation of GA from the embryo to the aleurone layer in cereal grains).

It seems, thus, that the synthesis of some hormones, observed during germination of the seeds of some species (e. g., Sinska and Lewak, 1977) is not the primary response for the environmental agents; but rather one of the later events in the causal chains that link the stimulus and the end of germination. In this respect one still unanswered question seems to be most interesting: are the enzymes involved in hormone biosynthesis, degradation and metabolism (reversible formation of conjugates) just activated during seed germination or are they synthesized *de novo* (stored mRNA) ?

The fact that there are no single agents responsible for the elimination of the individual barriers maintaining dormancy, but usually a set of factors, makes the study of involvement of hormones in dormancy and germination control difficult. The sequences of events starting from an environmental stimulus and contributing to the germination processes are entangled and interrelated. Therefore, only few of them are known.

Chains of Events During Dormancy Removal and Germination

The causal chains of events involved in germination *sensu lato* have been studied with the use of different approaches and different seed material, which exhibited different kinds of dormancy, differences in its depth and which required different environmental conditions in order to eliminats dormancy. Therefore, the generalization of the aboundant data collected until now is impossible; only some similarities can be indicated.

Probably apple seeds are most widely investigated in this respect. This is not only because of the economical importance of the apple, which is the most cultivated fruit in moderate climate countries. Apple seeds

show the physiological dormancy located within the embryo which is consolidated by the presence of thin endosperm and integument. The seeds are large enough for experimental manipulations and they are sufficiently small to be handled in quantities adequate for large scale experiments. In these seeds the coat can be easily removed and the embryos excised. The cotyledons contains sufficient amounts of storage materials (lipids and protein) which allows the culture of isolated embryos without an exogenous supply of nutrients.

Moerover, experimentation with the apple has already given results of a crucial importance for the development of human ideas. As examples can be given the curiosity of Adam and Eve towards the paradise apple or the penetrating Newton's observations of the apple falling from a tree.

For these reasons numerous laboratories over the world are interested in the physiology of apple and many of them, including ours, in the problems of apple seed dormancy. Because of the wealth of data obtained for this material, and of our personal involvement, we shall try to present the known chains of events leading to dormancy removal and germination, using the apple seed as a model.

Dormancy in apple seeds is routinely eliminated by horticulturists by means of cold stratification - relatively long chilling of fully imbibed seeds. Optimum temperature and period of cold treatment depend on the variety, but usually fall within 4-10°C and 5-20 weeks. During this period the after-ripening is accomplished and then the seeds are able to germinate. The temperature optimum of germination is higher than that of stratification; it amounts to 15-20°C.

The removal of seed coat (with endosperm) eliminates one of the barriers that prevent germination of apple seeds. Naked embryos need a shorter period of chilling to acquire the full germination ability. The embryos isolated from dormant seeds are photosensitive; they germinate much better in light than in dark (Smolenska and Lewak, 1971).
The germinability of embryos isolated from partly stratified seeds increases with the time of stratification, inversely to the depth of dormancy, whereas photosensitivity simultaneously decreases (Lewak and Bryzek, 1974).

Germination of dormant embryos is stimulated by gibberellins and cytokinins almost to the same extent as by light. Germination of non–

stratified embryos can also be improved by previous exposure for 7 days to completely anaerobic conditions (Tissaoui and Come, 1973) or by pretreatment of intact seeds with a relatively high (30-35°C) temperature (Thévenot and Come, 1978). Nevertheless, the speed of germination and its final percentage is always considerably lower for dormant embryos isolated from fully stratified seeds. Moreover, the dormant embryos during germination as well as seedling grown from such embryos show numerous developmental deformations and anomalies (Come, 1970). They gradually disappear when the seeds are stratified before excision of the embryos.

The above observations indicate that (i) the innate, deep dormancy in apple seeds is caused by at least two sets of barriers : one is related to the presence of seed coat and another one presents typical, cold-requiring physiological dormancy of the embryo, (ii) embryonal dormancy is fully eliminated only in result of moist-cold treatment and (iii) it is partly released by light or hormone treatment; hence, the embryonal dormancy is caused by several interacting mechanisms (barriers).

From what was said above the following questions arise : (i) what is the extent of identity of the chains of events initiated by two different environmental factors, low temperature and light, that lead to a full or partial release of embryonal dormancy ? and (ii) in the case of the basic identity of both chains what element (s) is lacking when the chain is initiated by light ?

Metabolic events during the cold mediated removal of apple seed dormancy

The phases of cold-mediated after-ripening of apple seeds were distinguished on the basis of hormonal equilibrium changes and alterations in the intensity of some metabolic activities during the period of stratification (Lewak *et al.*, 1975; Lewak and Rudnicki, 1977). Changes in sensitivity towards some factors affecting germination were also taken into account (Lewak and Bryzek, 1974; Rudnicki, 1969; Sinska and Lewak, 1977). Three phases of after-ripening were also distinguished by demonstrating their different temperature optima (Come and Thévenot, 1982). The first phase showed the lowest one (5°C) hence being considered as the true phase of dormancy removal. The second phase, with temperature optimum around 15°C, was called by Come's group (Paris, France) the phase of germination *sensu stricto*, being actually the typical cata-

bolic phase of germination, and the third phase, that of growth induction (anabolic phase) showed the temperature optimum of 25-30°C. Moreover, the dynamics of oxygen uptake was distinctly different during each of the above phases (Tissaoui and Come, 1975; Bogatek and Lewak, 1978).

By introducing and additional barrier limiting the progress of after-ripening, namely incomplete imbibition of seeds inside of apples treated by cold, it has been shown in the laboratory of Come that under these conditions of release of dormancy is stopped between the first and the second phase (Come, 198/81). This approach allowed to investigate in more detail some of the processes belonging to the last phases of germination *sensu lato.*

In the apple variety Antonowka, studied in our laboratory, the first phase of seed after-ripen'ng lasts until approximately the 20th day of stratification in the cold. It is characterized by a very low metabolic activity. In spite of a significant rise in oxygen uptake (Bogatek and Lewak, 1978),. the activity of reserve hydrolysing enzyme (proteases and lipases) is low and starts to increase as late as the end of this phase only. An exception present acid phosphatses (Lewak *et al.*, 1975) and catalase activities (Duczmal, 1963) relatively high during that period. A small amount of starch present in dormant seeds disappears around the 10th day of after-repening and starts to accumulate again at the end of the first phase of dormancy removal (Dawidowicz-Grzegorzewaska and Lewak, 1978). A similar lack of changes is observed in the content of free growth stimulators (GAs and CKs). In contrast, the level of ABA markedly decreases during that phase (Rudnicki, 1969). May be the most interesting metabolic event of the first phase of the after-ripening is the peak of free hydrogen cyanide level, as well as the maximum HCN evolution (Dziewanowska *et al.*, 1979b). Nevertheless, the contribution of cyanide-resistant respiration in total oxygen uptake by apple embryos is independent of the progress of after-ripening (Bogatek and Rychter, 1983).

In the next period of apple seed after-ripening the most dramatic changes in the molecular composition and in enzymes activities are observed. During this phase, which lasts approximately up to the end of the second month of after-ripening, protease reaches its maximum concomitant with the maximum content of soluble protein and free amino acids (Kepczynski and Rudnicki, 1977; Rudnicki *et al.*, 1975; Dawidowicz-Grzegorzewska *et al.*, 1982).

Although protein synthesis increases only slightly during this phase of after-ripening (Lewak *et al.*, 1975) it is accompanied by a steep increase of RNA synthesis (Dawidowicz-Grzegorzewska and Zarska-Maciejewska, 1979). Also, the activity of acid lipase, that shows the temperature optimum at 5°C, rises during this phase (Zarska-Maciejewska and Lewak, 1976); this rise is reflected in the advancement of decrease in storage lipid content (Kawecki, 1970) and digestion of lipid bodies (Dawidowicz-Grzegorzewska, 1981). Many other enzymes such as acid phosphatas and peroxidase reach their maximum activity between the 20th and 60th day of after-ripening. During this catabolic phase the content of ABA is negligible, whereas that of free cytokinins is the highest. From the two main gibberellins of apple seeds, the level of GA_7 does not show any significant change during the whole period of after-ripening, whereas that of GA_4 considerably increases around the 30th day.

The activity of hydrolytic enzymes during the third and last phase of seed-ripening is much lower than the preceding phase. One exception is the activity of alkaline lipase. However, this enzyme operates in a temperature range far from the optimum for after-ripening and its activity can be attributed to growth, rather than to the after-ripening process (Zarska-Maciejewska and Lewak, 1976). On the other hand, there is a rapid increase in new protein synthesis during that period, and a high level of RNA synthesis is maintained, accompanied by a marked increase in nucleolar volume (Dawidowicz-Grzegorzewska and Zarska-Maciejewska, 1979). Starch accumulated during the second and third phase after-ripening begins to be degraded at the end of the last phase (Dawidowicz-Grzegorzewska and Lewak, 1971).

It seems that hormones are not directly involved in the processes of the third phase of after-ripening. This can be concluded from (i) the lack of changes in the relatively low levels of studied hormones during that period and (ii) lack of hormone effects upon the germination of embryos isolated from seeds stratified for periods longer than 60 days.

The changes in hormonal equilibrium during apple seed after-ripening are shown in Fig 3.5. It should be stressed that all determinations of hormones were only performed with the use of bioassays except gibberellins that were also quantified by the gas-chromatography mass-spectrum method (Sinska *et al.*, 1973). The absolute values of particular gibberellin concentrations have recently been questioned by Dennis *et al.*,

(1980); nevertheless, the character of above changes was repeteadly confirmed not only in our laboratory (e. g., Isaia and Bulard, 1978; Balboa, 1980).

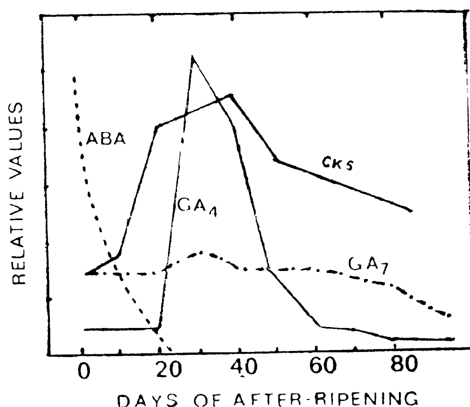

Fig. 3.5. Changes in hormonal equilibrium during after-ripening of apple seeds. Pattern drawn on the basis of data of Rudnicki (1969). Sinska and Lewak (1971) and Borkowska and Rudnicki (1975).

Reviewed above phenomena occuring during the after-ripening of apple seeds, proceed in a phasic fashion and in the described sequence only when seeds are subjected to stratification at the low temperature. Exposure of imbibed seeds at 20°C (warm stratification) does not lead to dormancy release. Among changes observed under conditions of cold stratification only few occur during warm stratification. There are: increase of peroxidase activity (Thévenot *et al.*, 1977), decrease in ABA level (Balboa-Zavala and Dennis, 1977), maximum content of free HCN (Dziewanowska *et al.*. 1979b). The pattern of other changes under conditions of warm stratification is quite different (e. g., the involvement of pentose phosphate pathway, Bogatek and Lewak, 1978); some others, are not observed at all. Thus, it seems, that the conditions of cold stratification enable the essential processes of germination to occur at optimum intensities and at the right moment. This sequence of events ensures the elimination of all barriers preventing germination, that is, the elimination of dormancy. The elevated temperature causes a disorder in that sequence rendering impossible some crucial processes that are required for dormancy release and germination.

Metabolic events during germination of isolated apple embryos stimulated by light

As already mentioned, the removal of the coat from the dormant seed eliminates one of the barriers involved in the dormancy maintenance. Exposure of the naked embryo to light eliminates another barrier, thus making the embryo able to slowly germinate under conditions of unlimited water supply at about 20°C.

A series of experiments on isolated embryos of apple cv. Antonowka, performed under standardized conditions of culture (12 hrs photoperiod, 20-24°C) allowed to establish several essential differences between metabolic events that occur in embryos in such a culture and in intact seeds during cold stratification.

First of all, the localization of activation within the embryo is clearly distinct in both cases. Fig. 3.6 shows schematically the histological patterns of cellular activation in apple embryos during cold stratification (3.6 A) and during the culture of dormant embryos for 12 days at 12 hrs photoperiod (3.6 B). In the first case degradation of protein is followed by degradation of lipid bodies and then by the increase in starch content followed by its degradation. All these processes start at the periphery of the hypocotyl and proceed inwards and longitudinally up and down in the embryonic axis. The mobilization and degradation of storage materials starts at the cotyledonary node relatively late during stratification and spreads acropetally. In dormant, cultured embryos (Fig. 3.6 B) a small activation of reserves can be observed in the cells lying in close proximity to the apical root meristem. However, the degradation of bulk reserves depleted in the cotyledons proceeds from their apical to the basal part (Dawidowicz-Grzegorzewska, 1981).

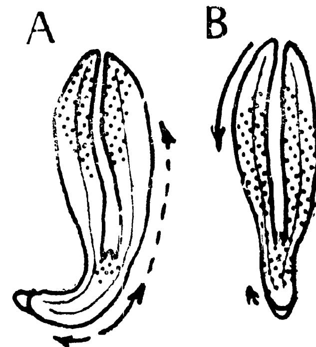

Fig 3.6. Diagrammatic presentation of the activation of apple embryo during cold-mediated after-ripening of seeds (A) and during light-stimulated germination of isolated, dormant embryos (B). Shaded fielsnon-activated area (Dawidowicz - Grazegorzewska, 1980).

Another essential difference between the embryos treated in both ways is that light-cultured, isolated embryos develop photoynthetic activity,

on the contrary to the cold-treated embryos kept under seed coats. The photosynthetic activity appears first in the apical parts of cotyledons, spreading out gradually over all these organs. The formation of photosynthetic apparatus preceeds for several days the beginning of growth (expansion of embryo axis) in the naked embryos in culture. The development of several features of the photosynthetic apparatus during the culture of dormant embryos was investigated : the ability to assimilate CO_2 (Maciejewska and Maleszewski, 1976), formation of chlorophyll (Maciejewska *et al.*, 1974), activities of glyceraldehyde phosphate dehydrogenases (Ryc and Lewak, 1977), ribulose-bis-phosphate carboxylase (Ryč and Lewak, 1980), and phosohoenolpyruvate carboxylase (Ryc and Lewak, 1975). All these features were affected by the same hormones that affect germination of isolated embryos (Ryé and Lewak, 1982). Nevertheless, different elements of the photosynthetic apparatus differently respond to hormonal treatments. Studies on interactions between ABA, GA and CK in the control of photosynthetic apparatus formation during the culture of isolated apple embryos demonstrated that the effect of ABA may be reversed by CK or GA or may be enhanced by the same hormones depending on the dormancy status of the embryo. This finding is inconsistent with the hypothesis of selective roles of hormones, which indicates that in addition to simple hormonal equilibria other mechanisms are involved in the onset, maintenance and removal of dormancy (Ryc and Lewak, 1982). The metabolic character of these mechanisms has been postulated.

Almost all the studied enzyme activities increase during the culture of the isolated, dormant embryos. The increase is, however as a rule markedly smaller than that during cold-mediated after-ripening of seeds. This observation indicates that the activities of phosphatases, glucosidases, peroxidases and phenyalanylammonia lyase (Lewak *et al.*, 1975, Szkutnicka and Lewak, 1975; Podstolski and Lewak, 1973) are involved in the germination process, whenever it is initiated by light or by cold, and the mechanisms involved in dormancy maintenance are not related to activation of these enzymes. On the other hand, the enzymes that hydrolyse the main seed reserves show a quite different activity pattern. After 5 days of light-culture of embryos at 20°C, protease activity reaches the same high level as after 50 days of cold stratification of seeds (Lewak *et al.*, 1975). Although the activity of acid lipase, the main lipolytic enzyme during stratification, rises only slightly during the culture of dormant embryos in light (Zarska Maciejewska and Lewak, 1976), the activity of

alkaline lipase rapidly increases achieving at the 6th day of culture the value similar to that in intact seeds after 80 days of cold stratification. This activity is increased under light and gibberellin control (Smolenska and Lewak, 1974). Thus, it seems justified to consider the proteolytic and lipolytic enzymes as activated by the same factors that initiate the release of embryonal dormancy. Activation of these enzymes may present an essential step in dormancy removal.

Unfortunately, among endogenous hormones only the gibberellins have been investigated during the culture of naked apple embryos. The content of GA_4 increases ten-fold during the first 3-4 days of culture in light, whereas in the dark it does not change at all. The level of GA_7 does not during the culture, independently of the light conditions (Sinska and Lewak, 1970; Smolenska and Lewak, 1971).

Causal chains leading to growth initiation

The events that occur during germination follow each other, composing time-arranged sequences. In order to attribute to such a sequence the character of a causal chain the cause-result relationships have to be demonstrated for each pair of subsequent events; also such a relationship must be shown for all events that precede the studied phenomenon (the final result) in the postulated chain.

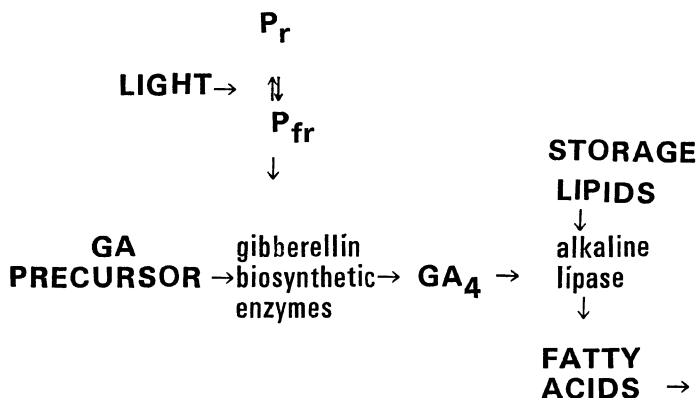

Fig. 3.7. Scheme of initiation of storage lipid mobilization by light.

Fig. 3.7 presents one of such casual chains that operate in the light-induced germination of dormant apple embryos. The phytochrome

involvement in stimulation of GA_4 biosynthesis has been evidenced by demonstrating that (i) GA_4 accumulates during the culture of embryos in the dark only if a short red-light exposure is applied, (ii) the effect of red light is reversed by the subsequent far-red exposure, (iii) incorporation of $2^{14}C$ mevalonate into GA_4 takes place only during the light-culture of embryos or in the dark after a red-light exposure and (iv) the treatment of embryos with AMO 1618 (inhibitor of GAs biosynthesis), independently of the light conditions of culture, prevents GA_4 accumutation and mevalonate incorportion (Smolenska and Lewak, 1971; Sinska and Lewak, 1977). The GA_4 involvement in alkaline lipase activation was shown by proving that (i) the maximum content of GA_4 in light-cultured embryos precedes the increase in alkaline lipase activity, (ii) the enzyme activity during the dark-culture of embryos rises in the presence of GA_4, but is not affected by GA_7 treatment, whereas GA_3 acts with a considerable delay and (iii) the factors that affect the endogenous GA_4 level (light conditions of the embryo culture, AMO treatment) similarly affect the activity of alkaline lipase. Moreover, the same factors that affect the GA_4 level ond alkaline lipase activity, influence in similar manner the germination of embryos (Smolenska and Lewak, 1974). This last observation allows to attribute to the sequence shown in the Fig. 3.7, an important role in the germination process.

A similar sequence although differing in several essential points, was demonstrated for lipolytic activity induction during the cold-mediated after-ripening of apple seeds (Fig. 3.8). The main enzyme hydrolysing storage fats, acids lipase, operates during the second phase of apple seed after-ripening. Under conditions of cold stratification this enzyme, with a temperature optimum of 5°C, becomes active (Zarska–Maciejewska and Lewak, 1976). The maximum activity of acid lipase during after-ripening is preceded by the maximum of endogenous GA_4 level; this suggests that activation of the enzyme is caused by gibberellin. The activation has been demonstrated by culturing the embryos isolated from partly stratified seeds in the presence of gibberellin, under conditions stimulating those of stratification (cold and darkness); the activity of acid lipase is significantly stimulated as compared with a control untreated with gibberellin (Zarska-Maciejewska *et al.*, 1980). The biosynthesis of GA_4 during cold-mediated after-ripening of apple seeds has already discussed in this review. It has been confirmed in the experiments with the dark-cold culture of partly stratified embryos : the treatment of embryos with AMO 1618 simultaneously inhibited GA_4

accumulation and acid lipase activity. Thus, the causal relation : low temperature - GA_4 biosynthesis and accumulatian-acid lipase activity

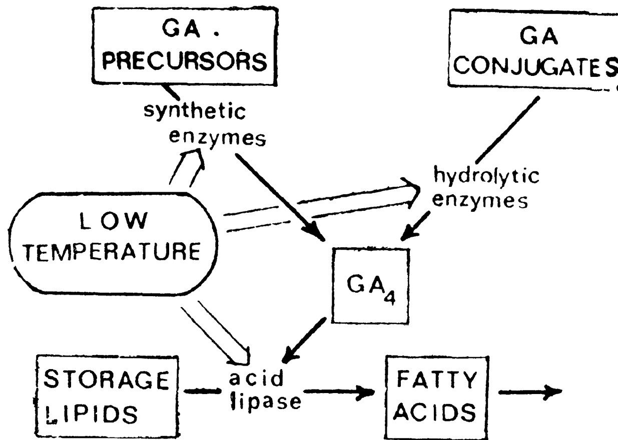

Fig. 3.8 Scheme of initiation of storage lipid mobilization by low temperature of stratification.

has been evidenced. On the other hand, it was shown that AMO treatment does not completely prevent the rise in GA_4 level during the cold-culture of embryos, indicating that it is also due to other processes besides biosynthesis. The simultaneous involvement of GA_4 conjugate hydrolysis in the accumulation of free hormone during apple seed after-ripening was postulated (Halinska and Lewak, 1978; Isaia and Bulard, 1978).

Reserve protein is the next to lipids storage material located in apple cotyledons. As mentioned earlier, both protein and lipid hydrolysis is an essential process during the cold-mediated removal of seed dormancy. Contrarily to lipid hydrolysis, the initiation of proteolysis occurs without the direct involvement of the hormonal control. None of the hormones checked (GA, CK, ABA) did affect the activity of protein hydrolysing enzymes in apple seeds (Rudnicki, 1968, Zarska-Maciejewska and Lewak, 1983).

The probable sequence of events initiated by low temperature and leading to hydrolysis of storage protein is shown in Fig. 3.9. The masive hydrolysis of reserve protein occurs during the second, catabolic phase of apple seed after-ripening. It is not directly cold-mediated, as demonstrated in

experiments with partly stratified in cold and in the dark; no increase in proteolytic activity has been observed as compared with the culture at 25°C. The study of the particalar proteolytic enzymes, with the use of the synthetic substrates, naphtylaminoacid derivatives, or short peptides, indicates that the activity towards almost all the substrates tested is not

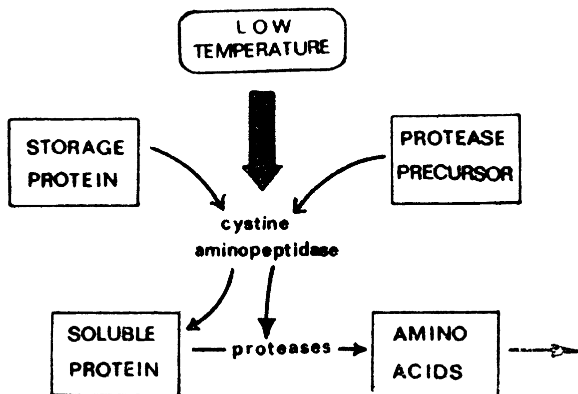

Fig. 3.9. Hypothetic scheme of initiation of reserve protein mobilization by low temperature of stratification. Cystine diaminopeptidase plays a dual role: solubilization of proteins and proteolytic activation of enzymes hydrolysing bulk reserve protein.

under the direct temperature influence. The aminopeptidase that hydrolyses L cystine-di-β-naphtylamide is an exception. This enzyme shows a temperature optimum at 5°C, its maximum activity occurs at a very early stage of seed after-ripening (around the 20th day of stratification) and it is stimulated by cold treatment of isolated embryos (Zarska–Maciejewska and Lewak, 1976). It has been proposed, that the cystine aminopeptidase initiates the massive storage protein hydrolysis by (i) solubilization of reserve protein due to its partial hydrolysis, that enables the action of other proteases and/or (ii) activation of other proteases through partial hydrolysis of the corresponding proenzymes. Both these possibilities are shown in Fig. 3.9.

The examples shown in Figs. 3.7, 3.8 and 3.9 illustrate an approach to understanding the causal relations between the processes that occur during apple seed germination. Fig. 3.10 shows in a simplified way some of the known chains of events that take place during the cold-mediated after-ripening of apple seeds. All the causal relations are evidenced, but not all proofs come from the data for apple seeds (for references, see Lewak,

1981). In the author's opinion the diagram presented in Fig. 3.10 illustrates that is still a working hypothesis; it omits a number of events that are not understood enough. Also, some yet unknown facts might be included in the future.

In diagram in Fig. 3.10 the sites of hormone action are not indicated. Some of these sites are well evidenced as for example the involvement

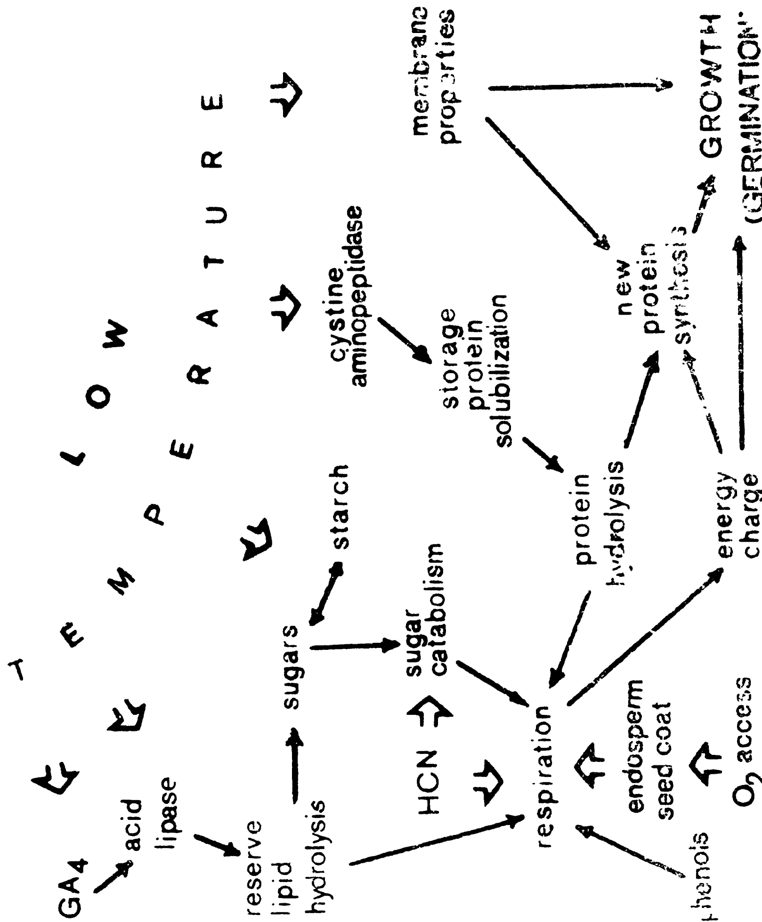

Fig. 3.10. Schematic presentation of some known causal chains during removal of apple seed dormancy under condition of cold stratification.

of hormones in the control of protein synthesis or in regulation of several catabolic activities. Evidence for hormonal control of other regulatory points is less convincing. Nevertheless, in few cases only the involvement of hormones can be excluded (e.g., protein hydrolysis).

On the other hand, in the seeds of other species than apple, other regulatory points can be under hormonal control (e.g., proteolysis in cereals. Moreover, the same processes can be controlled by different hormones during germination of different seeds (e. g., amylase activity in cereals- by GAs and in Papilionaceae- by CKs). It seems that hormonal control of germination is exerted upon different points of similar pathways in seeds of different species. It is tempting to assume that this control is related to specific obstacles preventing germination, that are responsible for particularities in dormancy of these seeds.

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Hormonal Control of Enzyme Secretion by Plant Cells

Claude Penel, Thomas Gaspar and Hubert Greppin

Introduction

Enzyme secretion is a common feature of most of the living cells. By this process, cells may act on their environment by modifying its chemical composition and ensure their defence againt external agressions. In pluricellular organisms, groups of cells (glands) become specialized in the secretion of enzymes which are utilized in a function useful for the whole organism. The control of such a release of enzyme is often mediated by chemical substances which migrate from one tissue to another one composed of secreting target cells. This coupling between stimulus and secretion is widely distributed in animal organisms. Its mechanism is described with many details (Putney *et al.*, 1978; Rubin, 1982).

The notion of hormone in plants as well as their mode of action do not necessarily correspond to their counterpart in the animal kingdom (Trewavas, 1981). Phytohormones do not originate from specialized glands; they are apparently synthesized by any type of living cells even in the supposed target cells. The question of specific receptors for the different types of hormones is still under debate. Each plant cell however progressively builds up a cell wall which necessitates a continuous transport and secretion of material during its whole life. Auxins, gibberellins ethylene, ... are already known to affect the release of several enzymes by plant cells. In a few systems, they apparently can be assimilated to triggers which induce the release of an enzyme. In most cases, however, these substances exert a modulating action on enzyme secretion.

The aim of this chapter is to recapitulate some generalities on enzyme secretion in higher plants and to describe with details the regulation by phytohormones of some enzymes secreted by plant cells either in whole organisms or in cell suspension cultures.

The Mechanism of Protein Secretion

The synthesis, intracellular transport, and release of secretory proteins is a basic cellular function common to most eukaryotic cells (Chrispeels, 1976). In the pancreatic exocrine cell for example, the secretory proteins are the object of six steps or operations, which are: *synthesis*, *segregation*, *intracellular transport*, *concentration*, *intracellular storage and discharge* (Palade, 1975). Another operation may be added to this list; the post-translational modifications of the protein molecule, such as glycosylation, phosphorylation or proteolytic cleavage.

Proteins for export are generally synthesized on polysomes, attached to the membrane of the rough endoplasmic reticulum. A study of the digestive glands of *Dionaea muscipula* by autoradiography has shown that upon stimulation, radiolabel is lost from the endoplasmic reticulum and found in vacuoles before being visualized in the cell wall (Robins and Juniper, 1980). In etiolated radish seedlings submitted to far red light, which is known to induce the synthesis of β-fructosidase, the enzyme activity is found associated to endoplasmic reticulum in a first time, then in Golgi apparatus and in cell wall (Zouaghi *et al.*, 1979). Stephan and van Huystee (1980, 1981) succeeded in obtaining peroxidase biosynthesis by *in vitro* translation on membrane-bound and free ribosomes isolated from cultured peanut cells. This *in vitro* peroxidase molecule has a higher molecular weight than the molecule isolated from the medium where cells are cultured. The authors interpreted this difference by the existence of a signal peptide which is the code for transport of protein to a site other than the cytoplasm. This segregation of proteins is the second step of the processing of secretory molecules (Campbell and Blobel, 1976; Suominen and Mäntsälä, 1983).

The next operations are the transport, concentration, storage and finally discharge by exocytosis of the secreted proteins. Such a pathway may be evidenced in the case of peroxidase by following the localization of the endogenous enzyme. Herzog (1979) has reviewed the data obtained on several different animal tissues. Peroxidase activity is found in cisternal space of rough endoplasmic reticulum and in stocked Golgi cisternae. It is also present in transport vesicles which probably are issued from the endoplasmic reticulum and fused with Golgi cisternae. Peroxidase are stored in secretion granules. Release of peroxidase occurs by exocytosis. Peroxidase reaction product remains membrane-bound in all compartments participating in the secretory process until

its release by exocytosis. Endogenous plant peroxidases exactly exhibit the same subcellular localization suggesting that they follow the same route in plant cells (Gaspar *et al.*, 1982).

However, this cisternal packaging-exocytosis theory which supposes a continuous isolation of the secretory protein from the cytoplasm was questioned by Rothman (1975) who proposed an 'equilibrium model'. In this model, the cytoplasm is an important enzyme-containing compartment acting as reserve pool to secretion. The enzyme molecules would migrate through membrane. In a complete review of the enzyme secretion by pancreas, Case (1978) discussed these two opposing views.

It is therefore possible that more than one mode of transport of secretory proteins could exist in plants as it seems to be the case in animals. Moreover, the information available concerning plant cells is rather scant. One explanation is the difficulty to obtain good separation of the various cell membranes (endoplasmic reticulum, Golgi apparatus, tonoplast, secretory vesicles or plasmalemma) (Quail, 1979). It is, therefore, difficult to perform a detailed biochemical work to follow the transfer of a molecule from one compartment to the other. Secretory tissues are wide spread in plants (Fahn, 1979). For experimental reasons, the study of secretion in plants was mainly devoted to specialized tissues, such as outer root cap cells, digestive glands of carnivorous plants (Juniper *et al.*, 1977) and aleurone layers. However each plant cell has a secretory function, since it builds up its own wall. Fundamentally, there is no difference between these cells and animal cells as far as the cellular secretory apparatus in concerned.

It is generally accepted that membrane migrates from endoplasmic towards Golgi apparatus and then to plasmamembrane (Morré and Outracht, 1977). This membrane flow implies a progressive biochemical transformation of membranes from endoplasmic reticulum-like to plasmamembrane-like (Mollenhauer and Morré, 1980; Morré and Mollenhauer, 1983). Presumably vesicles from endoplasmic reticulum and juxtaposed to the forming pole of the dictysome fuse to form new cisternae. At the opposite pole, secretory vesicles develop progressively. When they are mature, they separate from the cisternae and then fuse with the plasmamembranes. It seems that vesicles from the Golgi apparatus are partially differentiated into plasmalemma before they fuse with the plasmamembrane (Binari and Racusen, 1983). By this way, the secre-

tory products enclosed in vesicles or bound to their membranes are moved to the cell exterior.

Such an intracellular route, going from endoplasmic reticulum to plasmamembrane via the Golgi apparatus, was recently confirmed for hydroxyproline containing glycoproteins in carrot root discs (Wienecke *et al.*, 1982). The involvement of the Golgi apparatus in this process was already found by Gardiner and Chrispeels (1975). Numerous ultra-structural studies of root cap cells also confirm the membrane flow theory (Volkmann and Czaja, 1981).

For Dauwalder and Whaley (1982), who studied by radioautography the transfer of secretory products within root cells, the precise interrelationship between the endoplasmic reticulum and the Golgi apparatus remains to be unequivocally demonstrated in most higher plant cells. In fact, several authors have presented experimental evidences showing that proteins synthesized on endoplasmic reticulum are directly exported outside the cells. Pickett-Heaps and Northcote (1966) observed that the organization of the growth and development of the wall is controlled to some extent by the endoplasmic reticulum which is often situated in the cell at positions relative to particular regions of cell wall development. Gland-cap cells of *Dionaea muscipula* contain stores of secretory proteins which are released often an external stimulation. The discharge occurs by direct fusion of endoplasmic reticulum to plasmalemma or through vesicles directly derived from the endoplasmic reticulum. These experiments using autoradiography, do not show an involvement of the Golgi apparatus (Rubins and Juniper, 1980).

In *Phaseolus vulgaris* cotyledons, newly synthesized α-mannosidase is sequestred in the lumen of the endoplasmic reticulum before its transport to the cell wall. There is no indication that the Golgi apparatus is involved in the transport of this cell wall enzyme (Van der Wilden and Chrispeels, 1983). Reviewing our knowledge on glycoproteins and enzymes of the cell wall, Lamport and Catt (1981) concluded that the site of synthesis of these macromolecules is certainly rough endoplasmic reticulum but that there is no definite proof that the Golgi apparatus is involved in the packaging and transport of these molecules.

Very recently, it was shown in mung bean cotyledons that there are numerous tubular connections between the cisternae of the endoplasmic reticulum and the Golgi apparatus. Therefore, the structure exist for a

direct transport between these two membranous systems, without the migration of vesicles (Harris and Oparka, 1983).

It is generally accepted that the Golgi complex is the site of numerous covalent modifications of transported materials in eukaryotic cells. It also may act as a sorting centre, discriminating between those proteins which are to be secreted and those to be delivered to other parts of the cell (Farquhar and Palade, 1981; Tartakoff, 1982). Secreted proteins often contain a carbohydrate moiety which is added in a post-translational step by a glycolysation process catalyzed by glycosyl transferases. The function of the carbohydrate part is not known but seems to confer a greater stability to the protein molecule. The use of inhibitors of the glycolysation such as tunicamycin does not necessarily inhibit the secretion of glycoproteins, thus showing that the presence of sugars is not involved in the transport of the glycoproteins (Elbein, 1981; Berger *et al.*, 1982). In general, glycosylation is initiated in the endoplasmic reticulum during the synthesis of the polypeptide or just after, the nascent glycoprotein is transported to the Golgi where terminal glycosylation is completed (Hubbard et Ivath, 1981). The process implies the association of glycosyltransferase either to reticulum or/and to Golgi.

In fact, glycosyltransferases were found associated to membrane fraction derived from the Golgi apparatus in carrot root (Gardiner and Chrispeels, 1975) and onion stem (Powell and Brew, 1974). In *Pisum sativum* cotyledons however, the glycosyltransferases involved in glycoprotein biosynthesis are mainly associated with the endoplasmic reticulum (Nagashi and Beevers, 1978).

In conclusion, there is no evidence for the existence of a single intracellular route leading to enzyme secretion. Although the migration of proteins synthesized in endoplasmic reticulum, through Golgi towards the exterior of cell after exocytosis is likely to occur in some cases, direct transport from reticulum to plasmalemma and transfer of cytoplasmic enzymes accross plasmalemma cannot be excluded. Plant growth regulators could directly interact with these cellular processes. It is known that the main binding site for auxin is located on endoplasmic reticulum and one possible function of these receptors may be the control of the production, the processing and the migration of secretory proteins (Ray, 1977). Golgi apparatus was also proposed as possible site for auxin binding (Batt and Venis, 1976).

The Control of Secretion

The secretory process is a complex mechanism. Although the details concerning its regulation are only partially known, one may say that at least two types of mechanisms are found in animal cells. Tartakoff and Vassalli (1978) have compared these two types. The first one comprises cells which accumulate their secretory products and discharge them following the appropriate stimulation. Examples of such regulated cells are pancreatic acinar cells and cells from several exocrine glands. The second type consists of "non-regulated" cells such as plasma cells, fibroblasts and mecrophages. They do not require an external stimulus to secrete their products and exhibit a more or les steady rate of secretion.

It is known that calcium is essential for the control of the secretory process (Rubin, 1982). Calcium plays a critical role in the exocytosis process, which is a fusion-fission response involving the interaction the of the plasmamembrane and secretory vesicle membrane. By this mean, the content of the vesicle is discharged outside the cell. In adrenal medullary tissue, a protein, synexin, was isolated which may be the intracellular receptor for calcium in the process of membrane fusion and exocytosis (Pollard *et al.*, 1979). With respect to calcium, Tartakoff Vassalli (1978) remarked that regulated cells secrete only if it is present in the extracellular medium, when non-regulated cells secrete their products independently of its presence. But the presence of intracellular Ca^{2+} is an almost general requirement for the secretion by both kinds of cells. A second requirement for the occurrence of secretion is metabolic energy (Rubin, 1970). An adequate intracellular ionic equilibrium is also necessary. A perturbation of the Na^{+}/K^{+} ratio within cells by the use of monensin, a Na^{+} ionophore, abolishes the secretion by non-regulated cells, but does not suppress the discharge by regulated ones. Monensin interferes with the transport of secretory product by the Golgi apparatus (Tartakoff, 1983). It has been reported to inhibit slime secretion by maize root cap cells (Robinson, 1981). It also blocks the transport of fucose or galactose labeled macromolecules to the cell walls of bean cotyledon (Chrispeels, 1983), and increases the number of cisternae per dictyosome in carrot cells (Mooré *et al.*, 1983). The transfer of protein from the cytoplasm to the cell wall is an energy-dependent mechanism, inhibited by uncouplers and inhibitors of electron transport (Doerschung and Chrispeels, 1970; Sticher *et al.*, 1981; Moll and Jones,

1982). These examples suggest that plant cells, or at least some of them, exhibit analogies with non-regulated animal cells for the control of their secretory functions.

Cyclic AMP is a mediator that acts in concern with Ca^{2+} in the stimulus secretion coupling. In several secretory systems, it was shown that the excitation of cells results in a rise of c-AMP which is followed by an increased uptake of Ca^{2+} into the cells (Rasmussen, 1970). The role of c-AMP in many secretory systems is well established (Rubin, 1982).

The occurrence and possible role of c-AMP in higher plant cells has been a matter of debate for several years; It seems now generally accepted that it is present in these cells and the two key enzymes regulating its synthesis and destruction, namely adenylate cyclase and phosphodiesterase, were detected and isolated from several plant materials (Brown and Newton, 1981). By analogy with its second messenger role in animals, c-AMP was tentatively assayed as substitute of plant growth regulators. For example, Duffus and Duffus (1969) reported that c-AMP stimulates the gibberellic acid-controlled releasc of α-amylase by barley endosperm slices and is able to slightly promote this release when it is given alone. Similar results were found, concerning the ability of c-AMP to induce the release of protease and acid phosphatase by embryoless barley half-seeds (Nickells *et al.*, 1971). It seems however unlikely that c-AMP substitutes entirely to gibberellic acid (Pollard, 1971), although dwarf maize seedling treated with gibberellic acid contained more c-AMP than untreated shoots (Tarantowicz-Marek and Kleczkowski, 1978). It was also reported that an auxin treatment caused a rapid increase in c-AMP concentration in *Avena* colepotiles (Brewin and Northcote, 1973). Nevertheless, the speculation that this nucleotide is a second messenger of the phytohormones seems premature and requires further experimental evidences.

As mentioned above, Ca^{2+} is considered as a second messenger in animal cells, able to trigger the discharge of secretory products by regulated cells. In addition, non-regulated cells often requires Ca^{2+} for their secretion (Tartakoff and Vassalli, 1978). It would be, therefore, necessary to explore the knowledge concerning the regulation of Ca^{2+} in plant cells. It is known that Ca^{2+} in involved in the secretion of some plant enzymes, including peroxidase (Sticher *et al.*, 1981), α-amylase (Moll and Jones, 1982), and phosphatase (Ueki, 1982). This effect of Ca^{2+} will be

examined hereafter while discussing some hormonal effects on enzyme secretion. Recently, several articles have been published, which described modes of Ca^{2+} transport across plant membranes. An ATP-dependent Ca^{2+} uptake by isolated membranes vesicles was reported (Gross and Marmé, 1978). The Ca-ATPase in thought to be localised on inside-out vesicles from plasmalemma. Its properties are comparable with the Ca-ATPase from red blood cells (Vincenzi and Larsen, 1980). This means that it is activated by calmodulin (Dieter and Marmé, 1980). It may be concluded that plant cells have a mechanism that is able to maintain at a low level the cytosolic Ca^{2+} concentration. In addition to this Ca-ATPase there is a H^{+}/Ca^{+2} antiporter, which allows the transport of Ca^{2+} against a proton gradient. The cellular localisation of this antiporter is still uncertain (Hager and Harnsdorf, 1981; Zocchi and Hanson, 1983). There are until now very few reports on the possible effect of phytohormones on the cell Ca^{2+} level or on the regulation mechanism of Ca^{2+} concentration. Indoleacetic acid and zeatin were shown to have an effect on the ATP-dependent Ca^{2+} transport in a plasmalemma enriched fraction of etiolated soybean hypocotyls (Kubowicz *et al.*, 1982). A 2hr treatment of hypocotyl segments with growth-promoting concentrations of indoleacetic acid induces greater activity in the ATP-dependent Ca^{2+} transport and, conversely, zeatin inhibits the Ca^{2+} transport. The effect of the two growth regulators on Ca^{2+} transport parallels their effeet on hypocotyl growth. An opposite effect of another cytokinin, benzylaminopurine, was described by Olah *et al.* (1983) on wheat root. In that case, it appeared that roots treated with the hormone contained less Ca^{2+} and that the affinity of the ATPase towards Ca^{2+} and calmodulin increased after the hormonal treatment. Another mode of control of Ca^{2+} by hormones was described by Buckhout *et al.* (1981) who showed that auxin promotes the release of this ion from soybean membranes. Inhibition of auxin-induced cell elongation by calcium is a well-known effect (Cleland and Rayle, 1977).The relationship between calcium and gibberellic acid was also reported (Moll and Jones, 1981). It was proposed that gibberellin controls extension growth by regulating the uptake of calcium by hypocotyl cells.

Therefore, some elements exist for substantiating the hypothesis that phytohormones regulate protein secretion through the mediation of second messenger such as c-AMP or Ca^{2+}/calmodulin (Means and Dedman, 1980). However, the existence of an exchange mechanism

between H^+ and Ca^{2+} suggests that plant growth regulators especially auxin (Hager *et al.*, 1971) could modify secretory process by a modification of the distribution of protons which indirectly affect Ca^{2+} compartmentation. More generally, it was often reported that hormones affect the ionic status of plant cells (Marré, 1978; Neuman and Janossy, 1977; Behl and Jeschke, 1981). On the other hand, auxin treatments change the membrane potential of plant cells (Cleland *et al.*, 1977) and this may constitute a signal for modifying their secretory properties. It was also demonstrated that auxin rapidly activates the Golgi apparatus in oat coleoptile (Gawlik and Shen-Miller, 1974). Morris and Northcote (1977) studying the influence of Ca^{2+}, K^+ and Na^+ at the plasmamembrane in controlling polysaccharide secretion from Sycamore suspension cells concluded that these ions probably induced secretion by causing a depolarization of the cell surface. According to these authors, auxin-stimulated cell wall deposition could be a result of a stimulated influx of Ca^{2+} causing the fusion of secretory vesicles with the plasma membrane.

Hormonal Effects

There are a lot of works showing that treatment of whole plants, isolated plant organs or tissues with phytohormones of biological origin or with synthetic plant growth regulators results in changes in the activity of several enzymes (Barendse, 1983). A considerable number among these enzymes are of an exocellular nature but the process and control of their secretion has not been investigated in depth until now. On the other hand, there are much less papers establishing a correlation between endogenous hormonal status and enzyme levels or activities. It thus can be said that our knowledge of the hormonal control of enzyme secretion by plant cells is for from beeing well known. We hereafter shall discuss only two of the most studied examples of enzyme release under appraent hormonal control.

Alpha-amylase from cereal aleurone cells

The action of gibberellin in cereal seeds (barley and wheat principally) is the most throughly investigated of all the effects of phytohormones on enzyme secretion. In these seeds, surrounding the starchy endosperm, are a few cells constituting the so-called aleurone cell layers which, upon the addition of gibberellic acid, synthesized and release several hydrolytic enzymes. A non exhaustive list of these enzymes includes α-amylase, protease, phosphatase, β-glucanase, ribonuclease, pentosanase,

peroxidase esterase and glucosidase (See Jacobsen and Knox, 1974). This process, which is intensively studied *in vitro* using isolated aleurone layers, is presumed to occur in the intact germinating grains. In that case, gibberellins are produced by the embryo and the enzymes are released by the aleurone layers in the endosperm causing its digestion. This interpretation was critically discussed by Trewaves (1981). One of his arguments was that, in germinating barley, the maximum level of α-amylase is measured before the greatest level of gibberellin is observed (Groat and Briggs, 1969). According to Chem and Jones (1974a), about 70 per cent of the protein released by barley aleurone cells is α-amylase. This probably explains why α-amylasee is the most studied enzyme in this system. Gibberellic acid also enhances α-amylase secretion by sugarcand cell suspensions (Maretzki, 1971). α-Amylase is released by isolated aleurone layers at linear rate after a long period of 6 to 8 hours following the treatment by gibberellic acid. The hormone is continuously required during the period of enzyme production (Chrispeels and Varner, 1967). Abscisic acid has an antagonistic effect (Jacobsen, 1973). Four α-amylases were isolated from barley aleurone layers which differ by their stability at acidic pH and their resistance to EDTA treatment (Jacobsen *et al.*, 1970).

Enzyme release is inhibited by inhibitors of protein and RNA synthesis. Higgins *et al.* (1976) have shown that GA increases the level of translatable m-RNA for α-amylase. Mozet (1980) showed that the m-RNA for α-amylase is the dominant m-RNA in GA-treated aleurone layers. When such a m-RNA from wheat aleurone is translatad by a cell-free translation system a precursor of α-amylase which is 1.500 daltons larger than the secreted form is obtained (Okita *et al.*, 1979; Boston *et al.*, 1982). This larger molecule probably contains a signal peptide. This signal peptide can trigger the attachment of the ribosome to the membrane and direct the growing peptide chain vectorially across the membrane. The signal is then removed from the precursor by proteolytic cleavage (Blobel and Dobberstein, 1975). Most of the secretory proteins are processed in this way and become trapped in the lumen of the endoplasmic reticulum. An extra-peptide was also found after *in vitro* translation of germinating rice m-RNA. In addition, it was shown that rice α-amylase, like barley α-amylase, is a glycoprotein bearing a 2.900 daltons oligosaccharide (Miyata and Akazawa, 1982). The use of tunicamycin, which inhibits glycosylation *in vitro*, does not reduce the rate of α-amylase secretion. On the contrary, this antibiotic, considerably

inhibits the secretion of barley α-amylase (Schwaiger and Tanner, 1979) thus suggesting a role of the carbohydrate moiety in the secretory process. Although, it was claimed that aleurone α-amylase is mainly under a soluble form in cells (Jones, 1972), there is now good evidence that a great part of enzyme activity is bound to endomembranes. Locy and Kende (1978) have shown that at least 40 to 60 per cent of the α-amylase activity in homogenate of aleurone layers occur in a membrane-bound, latent from. A treatment with triton X-100, ethanol, sonication or osmotic shock is necessary to reveal this latent activity. An examination with electron microscopy shows that α-amylase is associated with smooth and rough endoplasmic reticulum. These authors suppose that the enzyme molecules found in membrane vesicles derived from the endoplasmic reticulum are in the way to be secreted. This view was already held by Vigil and Ruddat (1973). On the opposite, Chen and Jones (1974b) concluded that α-amylase is synthesized by the polyribosomes of the endoplasmic reticulum, released into the cytoplasm and secreted from the cell as a soluble enzyme (Jones, 1972). Recently, Jones and Jacobsen (1982) have found α-amylase to be mainly associated with endoplasmic reticulum following purification by isopyenic sucrose gradient centrifugation. It can be concluded that secreted α-amylase passes through the endoplasmic reticulum on its way to the cell exterior. This is consistent with the existence of a signal peptide in the α-amylase precursor. Whether the newly synthesized α-amylase is exported outside the cell directly from the reticulum or is transported by the Golgi apparatus is not known. Thus, it seems that the endoplasmic reticulum plays a crucial role in the production and secretion of α-amylase. Gibberellic acid induces qualitative changes and increase the amount of endoplasmic reticulum in barley aleurone layer (Jones, 1980) but Buckhout *et al.* (1981a) did not find this increase following a GA treatment in wheat aleurone layers.

Once synthesized, α-amylase molecules are exported towards endosperm. Varner and Mense (1972) made a distinction between the secretion itself, namely the outward movement of molecules across the plasmalemma and the release, which is the migration of the secreted α-amylase through the walls into the surrounding medium. The secretion is an energy-dependent process that is not directly dependent on protein or RNA synthesis. Release is diffusion limited process dependent on the presence of ions. Gibberellic acid induces a degradation of barley aleurone cell walls (Ashford and Jacobsen, 1974) may be through the increase

and secretion of xylanase (Eastwell and Spencer, 1982). Ethylene enhances both the xylanase activity and the release of α-amylase without affecting the enzyme activity remaining in the cell (Ho *et al.*, 1982). The two phytohormones thus exert an indirect control of α-amylase release by promoting the degradation of wall. In addition gibberellic acid modifies the ionic composition of the medium bathing the aleurone layers. Potassium, calcium, magnesium and phosphate ions increase (Eastwell and Spencer, 1982). The release of potassium, magnesium and phosphate is a gibberellic acid dependent process, requiring metabolic energy (Jones. 1973). Ethylene enhances the effect of gibberellic acid on the calcium release (Eastwell and Spencer, 1982). It is well khown that α-amylase requires calcium which confers to the molecule the structural properties required for effective catalytic activity (Hsiu *et al.*, 1964). The presence of calcium in the medium increases the amount of α-amylase activity measured either in the absence or in the presence of gibberellic acid (Jones, 1973). In germinating barley grains, extracellular calcium could be provided by the degradation of phytin-a calcium or magnesium salt of inositol hexaphosphate. Such a process would ensure a favourable environment for the action of α-amylase -which requires calcium - and would be a source of calcium for the regulation of the secretory process (Jones, 1973). Using an antomated flow-through apparatus which can measure the α-amylase output of a single aleurone layer at intervals of 1 min, Moll and Jones (1982) were able to damonstrate that the release of α-amylase from GA treated aleurone layers shows marked dependence on calcium. In the absence of calcium, the rate of enzyme release is 20 to 30 per cent of the rate observed in the presence of 10 mM calcium. Withdrawal of the cation from the medium results in inhibition which lasted for the duration of the withdrawal period. In the presence of ruthenium red, which blocks the release of calcium at the plasmalemma and prevents calcium uptake by mitochondria, the secretion of α-amylase becomes independent of the presence of external calcium. This result is explained by a ruthenium-red enhanced cytosolic calcium concentration. Uncouplers and inhibitors of oxidative metabolism induce a rapid increase of the α-amylase secretion followed by a large decrease. It may be supposed that these inhibitors trigger a release of calcium from mitochondria and that this calcium is then available for secretory process. Put together, the data of Moll and Jones (1982) suggest that the release of α-amylase by aleurone cells is a calcium-controlled secretion. However, the incidence of the absolute calcium-requirement by the enzyme molecule itself should be care-

fully examined to eliminate the possibility that calcium acts by activating pre-existing molecules either inside or outside the cells.

In summary, gibberellic acid enhances the biosynthesis of α-amylase by aleurone layer cells. It follows that this increased amount of α-amylase molecules are secreted by a mechanism which is not clearly defined but which could be under the control of cytoplasmic calcium. The presence of extracellular calcium is also required either as source of ion for the intracellular calcium or to maintain the released α-amylase activity. Gibberellic acid could also control the distribution of calcium ions and by this mean the secretory process itself.

As already mentioned, many other hydrolytic enzymes are released from aleurone cells in the presence of gibberellic acid. For example, ribonuclease is formed after addition of the regulator. During the first 24 hr the enzyme is retained within the calls and it is then rapidly released (Chrispeels and Varner, 1967). Protease is also made *de novo* in response to gibberellic acid and is released from the aleurone cells (Jacobsen and Varner, 1967). This is also the case of β-1, 3-glucanase which is released upon the effect of gibberellic acid (Jones, 1971). Acid phosphatase, which remained trapped between plasmalemma and wall, is released after addition of gibberellic acid which promotes the digestion of the wall (Ashford and Jacobsen, 1974).

Peroxidases

Peroxidases are enzyme which fufill many functions in plant cell walls : biosynthesis of hydrogen peroxide, polymerization of lignin, defence against pathogens,..... (see Gaspar *et al.*, 1982). In some tissues, especially those formed by aged cells, up to 95 per cent of the total peroxidase activity may be localized in walls and intercellular spaces (Birecka and Miller, 1974). Peroxidases are glycoproteins)Darbyshire, 1973). They generally exist in a same plant under several molecular forms which can be separated by electrophoretic techniques. Some differences in the electrophoretic pattern of endocellular and wall peroxidases are generally observed (Darimont *et al.*, 1973; Mäder *et al.*, 1975).

In the cell, electron microscope studies have shown in several tissues that peroxidases are associated with endoplasmic reticulum. Golgi apparatus, secretion vesicles and tonoplast (Zaar, 1979; Catesson, 1980). Moreover, they were often found directly linked to the membranes themselves. Biochemical studies, after cell fractionation, revealed that

one part of the enzymes is tightly bound to membranes (Darimont *et al.*, 1977) and another part requires the presence of calcium ions to be associated to membranes (Penel and Greppin, 1979; Penel *et al.*, 1979).

Cells in suspension cultures (Olson *et al.*, 1969; van Huystee and Turcon, 1973) but also whole organs (Gaspar and Xhaufflaire, 1967; Bredemeijer, 1977) have the property of releasing peroxidases in the surrounding medium. In lentil roots, root tips, hair regions and the aged parts release peroxidases in proportion to their endogenous level of activity. Auxin and cytokinin, which increase this activity, do not significatively affect the release of enzymes (Bouchet *et al.*, 1980). It is known that auxin considerably stimulates the *de novo* biosynthesis of two isoperoxidases having a basic isoelectric point in lentil root (Penon *et al.*, 1970).

There are such numerous reports on the effect of plant growth regulators on peroxidase activity (see Gaspar *et al.*, 1982) but it is very difficult in most of these studies to discriminate the direct and causal relationships hormone-enzyme from the enzyme changes appearing as a consequence of previous hormonal effects. The investigations dealing more particularly with the hormonal control of peroxidase secretion are much less numerous.

In several plants, a treatment with gibberellic acid decreases peroxidase activity (Mc Cune and Galston, 1959; Runkova and Gaspar, 1976). Treatments of spinachs cellu spensions have revealed that gibberellic acid suppresses peroxidase secretion into the medium and gibberellic acid is hypothesized to prevent cell wall rigidification by inhibiting peroxidase secretion (Fry, 1979). Fry (1980) proposed that the hormone blocks the transfer of the enzyme through the plasmalemma. According to the author, there is an accumulation of a peroxidase-like polypeptide within the cells, which could represent a precursor of the secreted peroxidase. In his discussion, Fry proposed that gibberellic acid exerts its action through a cellular redistribution of calcium, which modifies peroxidase secretion.

Evidence that peroxidase secretion is dependent on the presence of calcium was provided by studies with cultured cells from spinach and from sugarbeet. In spinach suspension cultures, the addition of 1 mM $CaCl_2$ induces a rather rapid increase of this peroxidase activity measured in

the medium; EGTA, a calcium chelator, decreases the rate of release of the enzyme by cells. Magnesium cannot replace calcium. Celles previously frozen and cells pretreated with sodium azied or sodium hydrogenarsenate present a diminished response to calcium (Sticher *et al.*, 1981). In addition, calcium which cannot activate the preoxidases already secreted in the medium, has an activatory effect on peroxidases extracted from the cells. Peroxidases, like α-amylase, need calcium to be enzymatically active (Haschke and Friedhoff, 1978). The effect of calcium on peroxidase secretion was also verifed on sugarbeet cell suspensions (Kevers *et al.*, 1982). This effect is reduced by vitamins D which interact with the cellular permeability to calcium (Kevers *et al.*, 1983).

The sugarbeet cells used for these experiment are issued from three different types of callus. One is a normal callus, requiring auxin and cytokinin. Another one does not require the supply of exogenous growth regulators, and the third one, also independent of exogenous growth ragulators, exhibits a spontaneous organogenesis (Kevers *et al.*, 1981). These three cell types differently respond to calcium. Normal auxin-requiring cells, and auxin-non-requiring organogenic cells are extremely responsive to calcium addition, while auxin-non-requiring non organogenic cells secrete less peroxidase and are less responsive to calcium in organogenic cells is enhanced by the presence of calcium ionophores such as A 23187 and Ro-20-0006/006, but these ionophores are ineffectivc on non-organogenic cells (Penel *et al.*, 1983). There is a clear relationship between the effect of calcium and auxins. This is demonstrated by the following experiments on auxin-requiring and auxin-non-requiring non-organogenic cells (Gaspar *et al.*, 1983). Six auxins of different chemical structures given together with calcium enhance the effect of the cation on auxin-requiring cells but are ineffective on auxin-non-requiring cells. It is demonstrated that these different responses to the addition of calcium and auxin are dependent on the presence or the absence of auxin in the culture media. Indeed, auxin-requiring cells subcultured during five weeks in an auxin deprived medium partly loose their ability to secrete peroxidase upon calcium addition and their diminished calcium-mediated peroxidase secretion cannot further be activated by auxins. On the contrary, auxin-non-requiring cells, subcultured during five weeks in an auxin–contaning medium acquire the property to secrete much peroxidases after calcium addition and this secretion is enhanced by the presence of auxins. From these data, it

can be hypothesized that auxins, through the mediation of calcium, rapidly control the secretion of peroxidases, enzymes which are capable of destroying indoleacetic acid (Gaspar *et al.*, 1982). This short term action of auxins is only possible in cells which were previously in contact with exogenous auxins. The best explanation for such a situation is the induction of auxin receptors by a pre-culture in an auxin-containing medium. Then, the complex hormone-receptor, would have a promoting effect on the calcium-mediated peroxidase secretion.

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Hormonal Regulation of Senescence

B. Sabater

Introduction

Senecence involves deteriorative changes of mature cells which led them to death. Senescence processes can affect to different plant structures and under very different circumstances. The old and basal leaves of a plant may be senescent while the young and more apical structures are actively growing. In monocarpic senescence (Noodén and Lindoo, 1978), the entire plant senesces at the period of reproductive development. Leaf senescence preceedes to the fall leaf dead of deciduous trees. The maturation to xylem of cells derived from vascular cambium may be considered as a senescence process which is under hormonal control (Wareing and Phillips, 1978). In this last example, senescence results in the production of structures which are truly functional when dead. Fruit ripening is, in many aspect, a senescence process (Sacher, 1973). Senescence must be distinguished from aging (Beevers, 1976; Leopold, 1980). The term aging is used with different significances. Sometimes aging refers to the functional decay of perennial plants. This, in contrast with senescence, appears as the result of an accumulative process of hazardous circumstances and not as an apparently regulated and ordered process (Thomas and Stoddart, 1980). Many other senescence processes may be described, but here we mostly will treat with leaf senescence or with senescence of structures which as cotyledons are related with the leaves. Leaf senescence has been mostly studied in the above mentioned circumstances of monocarpic senescence, basal senescence (mostly in gramineous) and senescence preceeding the fall leaf dead in deciduous plants.

The hormonal regulation is an important aspect of the mechanism of senescence but not an isolated aspect. It seems clear that comprehension of the mechanism of hormone action in senescence would require a better knowledge of many other molecular processes occurring in senescence.

There are important mechanistic differences among the different senescence types in plants. As we will see, hormones affect through very different ways to the senescence of the different structures. Including in leaves, the hormonal mechanisms seem to vary from one to other plant and with the circumstance of senescence. Leaf senescence is one of the most studied types of senescence in plants in any of its different circumstances: basal leaves, monocarpic senescence, deciduous trees and, under some aspects, cotyledons senescence. Although leaf senescence may be under different control mechanisms in the different circumstances mentioned, the senescence symptoms and the role of senescence in the plant are very similar in the four circumstances.

Some Methodological Problems

The most commonly considered symptoms of senescence in leaves are the increased degradations of chlorophylls and proteins. The degradation products, at least the amino acids derived from proteins, move to young or to reproductive structures of the plant to support their growth or move to reserve structures in roots or stems in deciduous plants (Kang and Titus, 1980). The chlorophyll and protein degradation is only the most apparent manifestation of a complete change in the metabolism of a leaf when it senesces. The metabolic change also affects to photosynthesis, protein synthesis, nucleic acid metabolism, etc... and it results in a higher rate of the degradative processes than of the biosynthetic processes. The relation among the different metabolic processes of senescence and of them with the associated ultrastructural changes is not clear. These facts make difficult a knowledge of the mechanisms of the hormonal actions in senescence.

Any mechanism which may be proposed for the hormonal regulation of senescence must be inserted within a chain of causistic relations among all the known manifestations of senescence. We still are far from the possibility to draw such a chain of causistic relations. Moreover, the relevance of the different experimental approaches is discussed. Many experiments on senescence are carried out with detached leavel (Simon, 1967) which show a faster development of the symptoms of senescence than intact attached leaves. Thus, detached leaves provide a more rapid experimental approach and allows an easier study of the hormone and environmental effects on senescence. Moreover, detached leaves may be placed in solutions of prefectly known composition, which makes easier the interpretation of the experimental results of hor-

mone action on senescence. However, the detachment of the leaves may introduce changes in the course of senescence in respect to the natural conditions of attached leaves. The fraction of wounded cells in segments of detached leaves is very low and its contribution to the total evolution of the components of the leaves during senescence is small. But the altered metabolism of the cells near to the wound area and the altered hydric state of the vascular system of detached leaves, can strongly affect the behaviour of the non-wounded cells of the leaf. In fact, differences in the sequence of organelle degradation have been described for the senescence of detached and attached leaves (Hurkman, 1979). In spite of the above objections, the similarity of the senescence symptoms in attached and in detached leaves and, in many cases, the parallel hormonal action in both systems, point to the validity of the study of sensscence in detached leaves. It is evident that a hormonal action on senescence is more easily studied, free of interferences, in detached leaves than in intact leaves. Thus, with caution in the interpretation of the results, the hormonal effects on senescence of detached leaves can give important informations on the mechanism of senescence.

However, it remains some problems with the use of detached leaves for the experimental study of senescence. We still do not know why detached leaves show a quick development of senescence symptoms. Thus, if we find a hormonal effect on senescence (acceleration or retardation) in detached leaves, we are not sure if that hormonal action is a true effect on senescence *in vivo* or on the unknown factor which accelerates senescence in detached leaves. Sometimes, a comparision between the hormonal effects *in vivo* and in detached leaves may decide on the relevance of a hormonal effect. However the results in detached leaves may be different of that in attached leaves due to other causes than the nature of the process of induction of senescence in detached leaves. Problems of hormone transport and metabolism or of the relation between hormone dose for leaf response and the endogenous hormone level, may also be involved in the differences observed between the hormonal effects in attached and in detached leaves.

The same biological material is complex, it includes mesophyll, epidermis (some of them guard cells) and different vascular cells. It is difficult to ascertain if a hormonal action on mesophyll cells senescence is direct or due to a primary effect on other leaf cells.

Some insights in the mechanism of senescence processes can be obtained by studying the order of appearance with time of the different structural and metabolic changes associated with senescence. If cause-effect relations may be drawn among the different processes which occur during senescence, the level at which hormones control senescence may be deduced from a consideration of the senescence processes affected and the senescence prosses not affected by hormone treatment. However this possibility is far to be achieved. Moreover, sometimes, hormones can produce effects in senecent leaves not necessary related to the senescence process. e. g., cytokinins stimulate protein synthesis in mesophyll cells (Kulaeva, 1981; Péaud-Lenoél and Alexos, 1981). If this effect is also found in senescent leaves, it does not necessary mean that increasesd protein synthesis is the way through which cytokinins retards senescence.

Hormonal vs. Nutritional Theories of Senescence

The general simultaneity between monocarpic senescence and reproductive processes, led to Molisch (1938) to postulate that senescence of aged organs is produced by a competence between these and the reproductive structures for nutrient uptake. At the end the competence would produce a withdraw of nutrients from vegetative to reproductive organs and an exhaustation dead of the first ones. The theory can easily be extended for other senescence processes. e. g., the senescence of the basal leaves would be induced by a competition for nutrient incorporation between the young structures and the old structures. It is now clear the insufficiency of the Molisch theory to explain senescence (Noodén and Lindoo, 1978; Noodén and Leopold 1978). Surgical experiments and other observations clearly demonstrate the necessity of hormone-like factors in the manifestation of senescence symptoms and the reader interested in this aspect is submitted to the above mentioned references. On the other hand there are many evidences for hormone effects on senescence in different experimental models. The question is to know the exact role of hormones and the relation of hormone changes observed in senescence with the development of other structures in the plants.

It must be clear that although nutrient redistribution may be a function of senescence, it is not necessary the cause of senescence. It is possible that plants have evolved mechanism for the control of senescence by hormones as they have evolved mechanism for the hormonal control of

their correlative development, according to their genetic potencialities and to the changing necessities of the plant. For example, it is probably that in a dioecious plant such as spinach the male plant has no nutritional motivation for monocarpic senescence. However, the space and nutrient requirements of the future plants, make adventageous the senescence and death of both male and female parents plants. Obviously, it would more complex for the species to have different hereditary mechanisms of sencscence for male and female plants. · On the other hand, although deflowering or depodding usually retards senescence; this is not always the case. Defloration in cocklebur (Krizek *et al.*, 1966) or defruiting in corn (Allison and Weinmann, 1970) and in pepper (Hall and Brady, 1977) do not retard (in some cases they do accelerate) senescence. It must also be noted that nutritional competence can hardly explain the senescence preceeding the fall death of the leaves of deciduous trees. Probably, although with different details in the mechanism, hormones, sometimes together with nutritional factors, control senescence which developed in all plants according with a genetic programme (Thomas and Stoddart, 1980). As we will see later, phytochrome function probably is also an important factor controlling senescence. In this regard, it must be noted the independence of the genetic determinants for floweing and fruiting in peas with that of apical senescence. Proebsting *et al.* (1978) reported the existence of different genetic lines of peas in relation with flowering and apical senescence. One line, G2, requires long days together with flowering for apical senescence. The long days requirement for apical senescnce is determined by the dominant alleles at two genetic loci. Lines I_1, I_2 and I_3 without one or the two dominant alleles at the two loci show apical senescence independently of the photoperiod. , A line, G, which only differs from G2 by the presence of a dominant allele in a first loci also requires long days for flowering . In short days G2 can form flowers without apical senescence, which again rule out any nutritional theory for the control of senescence. Line G requires long days for flowering and for senescence, but a genetic analysis of the other related lines, show the independence of at least some genetic determinants for flowering and for apical senescence.

There are reasons to think that a redistribution of nutrients is not the only function of senescence. A study of the composition of mature and dead leaves, led of Waughman and Bellamy (1981) to conclude that during the senescence, the leaves of many species export to other structures of the plant N, K and in some cases Mg. Whereas senescent

leaves import from other structures of the plant massive amount of Pb, Zn, Cd, Co, Cu, Ni, Fe, Mn, Al, Cr, Na, and Ca. Probably, depending on the ions present in the plant, senescence is also a mean to eliminate ions in excess. This function would be important for the elimination of toxic elements out of the plant which moreover would make difficult the growth and competence by other plants in the soil near to the plant with senescent leaves.

As usefull for different functions in the plant, senescence must be under different control mechanisms and it is reasonable to think a hormonal role in these mechanisms.

In relation with the nutrient redistribution associated with plant senescence, the degradation of proteins and the export of nitrogen by senescing leaves is, probably, the most studied process. However, only a small number of quantitative data are known on the nitrogen supply by senescent leaves to reproductive structures in relation with the nitrogen supply by assimilation *de nove*. By comparing the seed yields in nitrogen and in weight in different crop species. Sinclair and De Wit (1975) concluded that in gramineous, and probably also in leguminous, the seed development requires a withdraw of nitrogen from leaves to reproductive structures. In forage gramineous, Salette and Lemaire (1981) found a continuous diminution in the percentage of nitorgen in dry matter during the growth which requires a continuous mobilization of nitrogen from old to young organs. When nitorgen is abundant in the soil, almost all of the final nitrogen content of the forage plant is uptaken early in the plant development (Salette and Lemaire, 1981). According to Simpson and Dalling (1981) field grown cereals stem contributes 40%, leaves 50% and the roots 10% to the grain nitrogen, which is transported by way of the xylem and phloem.

In soybeans, Abu-Shakra *et al.* (1978) reported that during the first days of flowering there is an increase in nitrogen assimilation (both as N_2 and as nitrate) but this assimilation quickly diminished at the latest steps of seeds development. This behaviour seems to be a very general phenomena (Bethlenfalvay and Phillips, 1977; Pate, 1973; Harper and Hageman, 1972). Abu-Shakra *et al.* (1978) suggest the potential use of soybeans variants with delayed leaf senescence in improving the yield in nitrogen and weight of the crop. Both nitrate and N_2 assimilation may be impaired by the declining of photosynthesis (Bethlenfalvay and Phillips, 1977) in senescing leaves. Probably here, the question is if

senescence is triggered by the increased nitrogen demands of the reproductive structures, which in turn reduces nitrogen assimilation, or if senescence is triggered by other factors than nitrogen demands. Intermediate possibilities may be proposed.

The contribution of a possible ammonia volatilization from senescing leaves (Farquar *et al.* 1979) must also be considered in a balance of nitrogen fluxes in the plant. A batter quantification of the different demands and supplies of nitorgen in the plant is required to elucidate a possible control, direct or indirect, of leaf senescence by reproductive or young organs. Being an autonomous process or a process controlled by other organs, the increased protein degradation in senescent leaves is one of the best characterized symptoms of senescence (Noodén and Leopold, 1978; Thimann, 1978; Frith and Dalling, 1980; Thomas and Stoddart, 1980). In next pages we will study the mechanism of hormone action in senescence, which will need to reconsider the possible causistic relation of nutrient fluxes in the plant with the process of senescence.

Senescence is considered (Woolhouse, 1967; Sacher, 1973; Thomas and Stoddart, 1980; Leopold, 1980) as a genetically programmed process with culminates the development and differentiation of plant structures and which serves to a specific function in the plant. Probably its main functions be the recovery of nutrients for young structures of the same plant or for the descendants, together with a mean of detoxification and defense against competitor plants. As we will discusss, hormones act by controlling the development of the senscence programme.

Probably, in monocarpic crop plants, the human selection has affected the pattern of senescence. The selection of a plant by its weight yield in seeds or by the protein yield in the seeds, probably results of a compromise between, respectively, a continuous grain filling with lipids and carbohydrates and a sacrifice of leaf photosynthesis which would result in a quickly withdraw of nitrogen from the leaves to the developing seeds. The control of senescence and its relation with seed development may be another factor determining the known opposite relation between grain weight yield and protein grain yield in seeds of related species (Byers *et al.*, 1977; Sinclair and De Wit, 1975). On the other hand, it is possible (Noodén, 1980) that as crop plants have been selected for optimal yield in well fertilized soils, they have a great sensitivity to nutrient deficit. Thus, in these plants, nutrients have a great mobility,

they quickly move from one to other part of the plant according to their demands.

When dealing with the hormonal control of senescence two levels of study must be considered. Firstly, it must be identified those hormones which affect senescence symptoms. These are not a definitive criteria for the identification of a mechanism for the control *in vivo* of senescence by a hormone. It is also necessary to demonstrate a change in the concentration of the hormone considered during senescence or, at least a change in the sensitivity to the hormone during senescence. Still, in this first level of knowledge, it must be established that the cause of the change in hormone concentration in the senescent organ (a change in the rate of synthesis, in the rate of degradation, in the supply or in the export in that organ,...) and the relation of that hormone level change with other processes occurring in the plant. In a second level, it must be established the causistic relations in senescent organs leading from hormone change to the observed symptom of senscence. This last level is the mechanism of the hormone action in senescence. Frequently an experimental approach gives insight in the two levels of knowledge. In general, hormone effects and mechanisms are more easily studied in detached leaves. But the important changes in hormone level and correlative effects among organs, usually require studies at the level of the whole plant. Here we first revised some recent evidences on the participation of hormones in senescence and in a second place we revised some probable mechanism of hormone action in senescence. We are still far from satisfactory explanations for the two levels of study of hormone regulation.

Retardants of Senescence

Cytokinins

Since the pioneer discovery by Richmond and Lang (1957) that very low amounts of kinetin (5 ppm) retard protein loss (and in a lesser extent chlorophyll loss) in detached *Xanthium* leaves, cytokinins became the most used hormone in studies of senescence control. Similar results were found by Person *et al.* (1957) for the protection against senescence in detached wheat leaves by benzimidazole. Purohit (1982a) could reverse ethylene-induced senescence in *Helianthus annuus* by kinetin. Today the number of reports dealing with the retardation of leaf senescence symptoms by cytokinins are so impressive that to select some of them here should be arbitrary. The exceptions to the protective effect of cytokinins

against senescence (Bata and Neskovic, 1974; Osborne and Hallaway, 1960; Wollgiehn, 1961), although not in a great number, are indications of a certain diversity of the hormonal mechanisms controlling senescence. Sometimes, cytokinins are not only able to retard senescence, but they also restore the chlorophyll of yellowed detached leaves (Cursanov *et al.*, 1964; Purohit, 1982a).

Frequently, cytokinins have a less marked or no effect on senescence of intact attached leaves (Muller and Leopold, 1966). The probable implication of the roots in this low effect of cytokinins has been reported (Kulaeva, 1962) and will be discussed later. However, many cases are today known on the protection of intact leaf senescence by cytokinins (Biswas and Choudhuri, 1980) (for discussion see Noodén, 1980; Purohit, 1982b). Related to the action of cytokinins in senescence, cytokinins levels have been found to decrease during senescence (van Staden, 1973; Lindoo and Noodén, 1978). Sometimes, an accumulation of inactive zeatin derivatives have been found in parallel with a decrease of free zeatin. The clear implications of cytokinins in the retardation of senescence, will apper more evident when later we study the correlative changes of hormone levels in the plant and when we will consider the molecular control mechanisms of senescence.

Gibberellins

In leaves of *Taraxacum* (Fletcher and Osborne, 1966) and in fronds of *Lemna trisulca* (Bata and Nescovic, 1974) gibberellic acid powerfully retards senescence, but kinetin has no or only a low effect on senescence. In leaves of some other plants, both cytokinins and gibberellins retard senescence (Beevers and Guernsey, 1967; Whyte and Luckwill, 1966). Parallel decrease in the content of gibberellins in senescence has been reported in *Tropaeolum* leaves (Chin and Beevers, 1970) in which senescence is delayed by gibberellins. Apical senescence is delayed in peas by gibberellins (Proebsting *et al.*, 1978).

Auxins

Although not as strong as cytokinin, auxins have a retarding effects on senescence in some assay systems (Sacher, 1957). Correspondly auxin level decrease with age in leaves of several species (Shoji *et al.*, 1979; Sweetser and Swartzfager, 1978).

Other retardants of senescence

The amino acid arginine reverses the senescence promoting effect of

serine in oat leaves (Shiboaka and Thimann, 1970) but the effective concentration is probably too high for an *in vivo* significance of the experiments. The retention of chlorophyll in barley leaf discs is enhanced by the polyamines-putrescine, spermidine and spermine, but spermidine and spermine stimulate soluble protein loss (Cohen *et al.*, 1979). Concluding with senescence retardant, it seems clear that cytokinins are the most frequently found retardants of senescence in leaves. Gibberellins also retard senescence in some plant. The role of auxin is not so clear, probably auxin can sometimes interacts with other retardants in retarding senescence.

Promotors of Senescence

Abscisic acid

The most studied promotor of senescence in leaves is abscisic acid (ABA). At concentrations in the range of 10^{-8} M, ABA promotes the loss of chlorophyll and other symptoms of senescence in detached leaves (Noodén and Leopold, 1978) but its effect is not clear in intact attached leaves. In general, ABA promotes senescence in aged leaves more than in young leaves. In attached barley leaves (Rodriguez personal communication), ABA promotes senescence in the dark but it has no clear effect in light. ABA accelrates senescence in normal soybean plants, but not in depodded soybeans (Noodén, 1980). Frequently ABA or substances with ABA like activities increase in senescent tissues (Böttger, 1970; Chin and Beevers, 1970). But in attached rice leaves (Oritani and Yoshida, 1973), bean leaves (Colquhoun and Hillman, 1975), cocklebure leaves (Raschke and Zeevaart, 1976) and some other cases no conclusive incrase in ABA levels were found during senescence.

Increasing levels of ABA in leaves under water stress are well documented (Wright, 1978). However this stress-induced increse in ABA levels has been reported to be less pronounced as the leaf aged in bean (Eze *et al.*, 1981) and wheat (Quarrie and Henson, 1981). Samet and Sinclair (1980) reported that the ABA increases in senescent leaves of soybean occur very late. Indeed, ABA increases after substantial amounts of chlorophyll and proteins have been loss. Kao (1981) found important metabolic differences between the senescence of water-stressed and turgid excised leaves of rice. In turgid leaves, the increase in proline is less pronounced. Today it is difficult to establish a clear role of ABA in senescence. Its action will be discussed, together with other hormones.

Ethylene

Ethylene seems the main hormonal factor promoting senescence in flowers (Mayak and Halevy, 1980) and ripening of fruits (Rhodes, 1980; Shimokawa, 1983). The involvement of ethylene in leaf senescence is not so clear. Usually ethylene leavels increase in leaves more in abscision than in senescence (Noodén, 1980). Aharoni and Lieberman (1979 a, b) reported that ethylene production decreases at the first phases of senescence and then there is a transitory increase of ethylene production during the phase of rapid chlorophyll loss. In fact ethylene enhances chlorophyll loss in tobacco leaves (Aharoni and Lieberman 1979b) and sunflower leaves (Purohit, 1982a, b,) which suggest the role of ethylene accelerating senescence in its late stage. Little attention has been paid to the possible effect in senescence of the well known high synthesis of ethylene after cutting (Abraham and Reinhold, 1980).

As mentioned earlier that a few polyamines retard some symptoms of senescence but accelerate some other symptoms. Although those results may indicate a role of polyamines in the intracellular mechanism of senescence, there are no reason to think that polyamines may function as interacellular signals of senescence. Methyl jasmonate has been reported as a powerful promoter of senescence (Satler and Thimann, 1981).

Correlative changes in the levels of retardants and promotors of senescence in leaves

It is usally accepted (Manos and Goldthwaite, 1975) that a variation in the sensitivity to hormones during senescence, is accompained with a change in the levels of hormones which affect senescence.

Of the growth regulators considered above, cytokinins seems to most clearly implicated in senescence, due to their effect in intact and in detached leaves and due to their changes in concentration in parallel with senescence. Althogh more discussed, ABA probably is a senescence promotor *in vivo*, at least in some phases of senescence or/and at least in some plants. Gibberellins also seem a probable group of hormone which control senescence. The implication of auxin and ethylene is also not so clear, at least for auxin. Probably, more than by one hormone. Senescence is controlled by the balance among the different growth regulators. As discussed earlier evidences for hormone level changes are associated with senescence in plants. Before to consider the

hormonal interaction and mechanisms in senescence, we will discuss here the hormone level changes as influenced by the developmental stage of the leaves and by the other structures of the plant. Obviously the most of the works have been carried out in relation with cytokinins.

Reports are available indicating decrease in cytokinins leavels in parallel with senescence. As the formation of roots haltes senescence in detached leaves starts. It was suggested (Chibnall, 1954) that roots produce some senescing-retarding factors. Later, Kulaeva (1962) demonstrated that root exudates of substances which retard senescence. It seems (Torrey, 1976) that root apices are the major sites of cytokinins synthesis. Cytokinins are produced in the roots ascent, through the xylem, to aerial parts of the plants. Probably, cytokinins synthesis in the roots is higher in growing roots, (Sachs, 1972). As cytokinins stimulate the growth of aerial structures, cytokinins would function as a factor controlling the correlative growth of roots and aerial parts of the plants. Indeed, by studying the influence of phosphorus supply, Dhillon (1978) found a posative correlation between growth and cytokinins levels in xylem sap of sycamore seedlings. On the other hand, it is well known the posative role of cytokinins stimulating the growth and development of photosynthetic tissues (Parthier *et al.*, 1981). As the leaves are the main sites of transpiration, they may be a place of cytokinins accumulation. Transpiration does not seem to diminish as quickly as photosynthesis during senescence, or at least at the beginning of senescence (Peisker and Václavick, 1980). Thus, if the roots are the main sources of cytokinins for the leaves, a decrease in the level of cytokinins during leaf senescence must be due : (1) to an accelerated cytokinins metabolism in the leaves before their senescence, (2) to an accelerated transport from leaves to other structures, and (3) to a decrease in the supply of cytokinins by the roots. Of these three possibilities the last is the most studied. However no definitive conclusions are still available. In tomato, cytokinin decreases in root exudate early during flower development, but then increases when the flower open (Davey and van Staden, 1976). In tomato and tobacco, disbudding (which retards chlorophyll loss from leaves) increases levels in root exudates (Colbert and Beever, 1981). In *Perilla* (Beevers and Woolhouse, 1975) and in sunflower (Sitton *et al.*, 1967) cytokinins increased in root exudates during flowering. One can think that in a varient of the nutrients competition theory, reproductive and vegetative structures compete for cytokinins from roots. The cytokinins content, in turn, determinates if a structure will be a sink or

a source. However, the fate of root-cytokinins during the reproductive phase is not clear. At early fruit development, its cytokinins content is high, and then decreases (Noodén, 1980). In soybean fruit development no correlation was found between fruit cytokinin decrease and leaf senescence (Lindoo and Noodén, 1978). Noodén and Lindoo (1978) concluded that root-cytokinins reduction is not the primary causal factor in monocarpic senescence in soybean. In this line, from experiments based on the effect of the temperature and of cytokinins on the senescence of intact attached leaves. Kao (1980) concluded that in soybean seedling, leaf senescence is caused by a competition among leaves for root cytokinins, whereas in podded soybean plant, senescence is triggered by a senescence hormone possibly ABA, in developing pods or seeds. Probably, the leaves can export through the phloem cytokinins synthesized *de nevo* in the leaves or cytokinins imported from roots (Noodén, 1980). Cytokinins can be destroyed in leaves to urea and ureides or can be stored as inactive glucosides (Palmer *et al.*, 1981). It is possible that leaves export cytokinins previously stored as glucoside derivatives. The final balance of cytokinins in the leaf may be the results of multiple factors and would be of interest for better knowledge of how the different factors considered here are influenced during vegetative and reproductive development.

Both auxin and gibberellins levels also change during development but the correlative changes among different organs and their relation with leaf senescence, are not as known as those of cytokinins. Again, the metabolism and the storage of hormones in the course of development may be critical. In a line of peas, Proebsting *et al.* (1978) found that probably a polar gibberellin formed from GA_9 in short day plants inhibits senescence. Other lines of peas produce other inactive metabolites of GA_9.

ABA level changes in leaves can easily be produced by synthesis in the same leaves under different stress conditions. Thus, little attention has been paid to possible export and import of ABA by leaves during senescence. However, ABA or an unknown substance with ABA-like activity is a clear candidate if a senescence signal migrates from reproductive structures to leaves (Lindoo and Noodén, 1978). In fact, reporductive structures show high increases in ABA content (Noodén, 1980). ABA can also be produced in roots, but ABA of roots probably does not translocate to leaves (Hartung, 1977).

Ethylene can hardly be a specific signal which translocates among organs in senescence. On the other hand, ethylene produced in leaves seems more related to abscision than senescence. However, some reports are available related ethylene changes in leaves with senescence.

Summarizing, still there are no unambiguous proofs that any of the known plant growth regulators act as translocating signal in senescence. In relation with the most studied hormones in senescence, cytokinins, important differences may be deduced depending on the cause of the change of cytokinins levels in leaves. In spite of, if attractive, the hypothesis of a competence between reproductive structures and leaves for root cytokinins is far to be proved. The fact that debudding, in many cases, increases the level of cytokinins in root exudates, is compatible with both a negative modulation by bud of the production of cytokinins in roots and a competition among different plant structures for root cytokinins. On the other hand very little is known on the control of motabolism and export of cytokinins in leaves.

In relation with the level of ABA in leaves, we have mentioned the possible influence of ABA synthesis in the leaf which make unclear if the leaf imports ABA. Precissely one of the stress circumstances which increases ABA synthesis in leaves the scarcy of nitrogen is a very common circumstance in senescing leaves (Milborrow, 1974). Taken this possibility together with the possible cytokinins level control by its metabolism in the leaves, they question if the hormone level changes in senescing leaves are due to changes in translocation from other structures or are due to secondary consequences of other initial events of senescence. As a decrease in cytokinins and an increase in ABA (promote senescence) the hormonal changes can act as autocatalytic triggers of senescence. However, it remains the question of how other organs influence the senescence of the leaves. The retarding effect of roots in senescence is clearly due the their role as suppliers of cytokinins. More complex is the effect of the buds and in general of the young structures in senscence. Nutrient competition is not the main mechanism of senescence (Noodén and Lindoo, 1978). One can think for a still unknown factor which move from bud to roots or to leaves. That factor may determinate an inhibition of cytokinins synthesis or export by roots. Alternatively that factor may induce senescence directly in leaves. However the interpretation of the debudding experiments does not nece-

ssary led to the implication of senescing factors produced in buds. Debudding can affect: (a) to the import by leaves of senescing factors produced in the same bud, (b) to the competence between buds and leaves for senescence retardants produced in the root, (c) though a nutrient competition effect, to the hormonal metabolism in the leaves, and (d) to an indirect nutrient competition effect by changing the hydric state of the plant, and ofcourse of the leaves, which in turn can affect to the hormonal metabolism in the leaves. Today it is not possible to decide among the different possible mechanisms for the effect of bud in the senescence of leaves and the possibility that a mixture of effects operate *in vivo* can not be excluded. Additional problems need to be considered when more information be available on the change in the level during senescence of other hormones than cytokinins and ABA.

Hormone level changes may be a primary cause or a secondary effect of senescence in leaves. Those changes affect the course of senescence. When studying the mechanism of the hormonal control of senescence, it must have in mind that, it is possible that, one is only studying a late process of senescence.

Phytochrome and senescence

Phytochrome function affects the hormonal levels. As senescence is affected by hormone levels, it is probably that phytochrome affects senescence. We have before cited the results of Proebsting *et al.* (1978) which point to a control of apical senescence in pea by the lenght of the day. This control seems mediated through a day-lengh dependent metabolim of gibberellins. It is reasonable to think that here, the receptor of the light stimulus is phytochorme although no red and far-red assays were carried out.

The effect of phytochrome and in general of the light, on senescence may be complex. Sometimes it is difficult to separate phytochrome-mediated effect of other light effect. In respect to leaf senescence, it is difficult to distinguish the light effect on the degradative processes of senescence of the well known effect of the light stimulating the formation of photosynthetic mesophyll structures. On the other hand, light can affect not only to the senescent organs, but also to other organs implicated in the correlative control of senescence in plants. For example, light has been identified as the sink promoting factor, probably through phytochrome, in rose shooots (Mor and Halevy, 1980a and 1980b). Through this sink effect, we have just discussed how it can

affect to leaf senescence. The effect of the light absorbed by the senescing structure is also of interest. As this last effect of the light retarding senescence is one aspect of the mechanism of senescnce, we will discuss this problem later.

Photoperiodic control of senescence is usually assumed to be mediated through phytochrome. Most of the works on phytochrome control of leaf senescence have been carried out in intact plants. Thus, red light has been found to retard senescence induced by dark in barley (Pfeiffer and Pleudgen, 1980). The effect of red light was reversed by far-red light treatment. Although not clearly related to senescence, long days increase the levels of cytokinin-like substances in leaves of *Allium cepa* L. (Lercari and Micheli, 1981). In these examples as in those of the photoperiodic control of senescence in peas (Proebsting, 1978), is difficult to know if the phytochrome receptor molecules are those of the same senescent structure. Red light has been described to retard senescence in detached rice leaves (Mishra and Pardhan, 1973) in *Marchantia* (De Greef *et al.*, 1971) in cucumber and tomato (Tucker, 1981), in detached flower stalks of *Limonium* (Steinitz *et al.*, 1980) and in detached barley leaves (Biswal and Sharma, 1976). In these cases, far-red treatment reversed the effect of red light. The mechanism of these effects are still not clear.

Mechanism of Hormonal Control of Senescence

The question of the receptor cell

The parallel in the action of several chemical and physical agents on stomatal closure and on leaf senescence (Thimann and Satler, 1979a and 1979b; Thimann *et al*., 1979) leds to the suggestion of a causal relation between the two phenomena. Frequently, it has been found that cytokinins stimulate stomatal aperture which may explain the accumulation of substances in the leaf areas treated with kinetin (Mothes and Engelbrecht, 1961). In fact, the increased transpiration in that areas, pulls the nutrients there. However, the relation of these phenomena of nutrient accumulation in areas treated with cytokinins with process of senescence is not clear. We have mentioned that transpiration disminishes more slowly than other symptoms of senescence(Peisker and Vácalávick, 1980). Guard cell choroplast senesces later than mesophyll chloroplasts (Zeigler and Schwastz, 1982). One possibility (Thimann, 1980) is that the response of stomatal closure implicates a mechanism in guard cells very similar to that of senescence in mesophyll cells.

In general, hormonal effects on senescence are supposed to be due to a primary action in mesophyll cells, a possibility which will be assumed in the following, although caution must be kept in mind of possible intermediary cells playing a role in the hormonal response.

Hormonal interactions

It has been suggested that more than one hormone– the hormonal balance– which controls senescence. The experimental study of hormonal interactions is difficult because an effect obsereved *in vitro* not necessary occurs *in vivo*. Thus, actually, only preliminary conclusions may be obtained on hormonal interactions in senescence.

In cut rose flowers, ethylene treatments produce increasad levels of ABA (Mayak and Halevy, 1972). On the other hand, IAA and kinetin (although they retard senescence) synergistically stimulate ethylene production in leaf discs of tobacco (Aharoni *et al.*, 1979). Cytokinins also stimulate the formation of bound ABA from free ABA (Even Chen and Itai, 1975). This last effect could explain the reversion by kinetin of the stimulating effect of ABA on senescence processes in *Pennisetum* leaves (Eder and Huber, 1977). From experiments with detached lettuce leaves Aharoni and Richmond (1978) concluded that kinetin stimulates the transformation of bound GAs to free GAs. These, with cytokinins, determinate low levels of ABA. In the same report it is suggested that ethylene may accelerate the loss of GA and, in this way, it may increase the level of ABA. These works may suggest that several hormone action in senescence are finally mediated by ABA. But others have reported that ABA levels increase after others senescence processes have been initiated (Samet and Sinclair, 1980). Many of these works could be understood by assuming a homeostatic regulation of hormone levels in plants. A change in that homeostatic equilibrium leading to a hormonal disbalance in which the promotors of senescence are at hight level can produce an autocatalytic acceleration of senescence. It is possible that the different hormones have independent effects in senescence as described for the comparative effects of cytokinins and gibberellins and of ABA in leaf senescence of *Rumex* (Manos and Goldthwaite, 1975b). On the other hand, the different hormonal levels are interrelated. For example, a decrease in cytokinins favours senescence. Moreover, for a determinate homeostatic equilibrium of cytokinin decrease produces a decrease in free GAs and an increase in ABA which result in the acceleration of senescence. The transition from a hormonal equilibrium retarding

senescence to another equilibrium stimulating of senescence, may be triggered by different genes, including a marked change in the supply or in the synthesis of an individual hormone. It may exist different equilibrium states of hormonal balance. In the neighbour of an equilibrium, homeostatic mechanisms nullify mall oscilations in hormone levels. Either due to a different external circumstances or to an unusual changes in one hormone, a new equilibrium is reached, and again homeostatic mechanisms adjust the level of other hormones to the new equilibrium state.

Although it can not be excluded that a hormonal change be the initial event of senescence, hormones can also independently affect to the different processes associated with senescence. Thus one can find that the different processes of senescence have different hormone specificity. In this line, it has been found that chlorophyll loss in detached barley leaves (Sabater *et al.*, 1981) and *Helianthus annuus* (Purohit, 1982a). is retarded by the different cytokinins. However, in the same system, phosphate loss is retarded by natural cytokinins only when they are applied together with GA_3.

Cytokinins are the most studied hormones in relation with the mechanism of hormone action in senescence. As indicated earlier that cytokinins can affect to senescence through very different mechanisms. The problem is also complex because cytokinins can affect to the level of other hormones. Whether action of cytokinins is direct or an indirect effect due to their effect on the level of other hormones. On the other hand, cytokinins action can not be studied independently of other agents such as light, which also affect senescence. Thus, in the following although we will mainly discuss the mechanism of cytokinins action in retarding senescence, we incidently will mention the related mechanisms of other agents that affect senescence. The biochemical mechanism of a symptom of senescence is poorly known. Thus, the research of the mechanism of hormone action in senescence is parallel with a research of the enzymatic (and in general biochemical) mechanism responsible for each symptom of senescence.

Lipids and membranes

X-ray diffraction studies show structural changes of chloroplast and microsomal membranes during leaf senescence (McKersie and Thompson, 1978; Chian, 1981). As the tissue senesces, the membrane lipids progre-

ssively become crystalline. The structural change seems to be associated with a 4-fold increase in the sterol to phospholipids ratio, but it seems to be independent of the change in the ratio of the different fatty acids components of phospholipids. It is possible that the structural change could increase the permeability of the membranes and so contribute to the loss of compartmentation which probably occurs in senescence.

Senescent mesophyll cells usually accumulate lipid granules in chloroplasts (Cohen *et al.*, 1979; Wilhelm and Wilhelmová, 1981), which probably derives from thylakoid breakdown. In cytoplasm of senescent mesophyll cells also accumulates lipid grain or oleosomes (Parker and Murphy, 1981). It has been suggested (Dhindsa *et al.*, 1981) that free-radical induced peroxidative reactions could modify the membrane lipids and tryiglycerides. Lipid peroxides may descompose to malonyl-dialdehyde which attacks various cellular components (Wilhelm and Wilhelmová, 1981). Lipid peroxides probably also transform into other lipid derivatives which can not be metabolized determinating structural modifiaction in membranes. Lipid peroxides may also be accumulated as lipid granules.

Both malonyldialdehyde (Chia *et al.*, 1981) and lipid peroxides (Dhindsa *et al.*, 1981) increase in senescence. In attached tobacco leaves, the increase in lipid peroxides is parallel with the decrease of chlorophyll and protein. Dhindsa *et al.* (1981) attribute the increase of lipid peroxides to a previous increase in the concentration of the radical superoxide (O_2) due to a diminution of the enzymes superoxide dismutase and catalase which destroy O_2. According to this interpretation, lipid peroxidation is a consequenee of the general decrease of proteins (among them superoxide dismutase and catalase) which occurs in senescence. Leshem *et al.* (1981) suggest that superoxide radical level may be more directly controllèd by cytokinins. They show in acellular assays that kinetin can act as a radical scavenger and also kinetin can indirectly inhibit to an enzyme forming radical superoxide such as xanthine oxidase. Although these results can explain some of the effects of cytokinins retarding senescence, the same authors found that benzoiladenine, a structural analog of cytokinins which does not affect radical production, has some effect in retarding senescence. This implies that at least not all of the effects of cytokinins retarding senescence are mediated by their possible effets on free radicals diminution. A deteriorative effect of free radicals has been claimed (Borg, 1970) during animal aging, but

their significance during senescence of leaves is not clear. In chloroplasts, secondary reactions mostly associated with the photolysis of water can produce high amount of superoxide radicals. Several reactions, among them that catalyzed by superoxide dismutase, destroy that radicals (Foyer and Hall, 1980). The chloroplats are among the mesophyll orgnelles more sensible to senescence, but this is quicker in the dark when very low superoxide radicals amount can be formed associated with photosynthetic structures. In fact, isolated chloroplasts in the dark are more stable than in senescent detached leaves in the dark (Choe and Thimann, 1974). However, when isolated chloroplasts are at light, at least their chlorophylls are less stable than in detached leaves in light (Choe and Thimann, 1975). The mechanisms of chloroplasts deterioration are probably different in each of the two circumstances. At light free radical production may be a factor of senescence but probably in the dark free radicals are not important. The relative importance of free radical production during senescence under natural conditions remain to be determined.

The change in the properties of the membranes during senescence may be important if they facilitate the withdraw of the components of the cellular compartment. However it is difficult to find a role in senescence for the accumulation of pilid granules.

Other lipid change associated with senescence is the early decrease of the characteristic lipids of chloroplat membranes : sulfolipids and galactolipids (Noodén and Leopold, 1978). Here arises a question that is this symptom a primary factor for controlling other posterior symptoms ? Related to the decrease of galactolipids, Dalgarn *et al.* (1979) found a decrease in galactosyl transferase of chloroplast envelopes during senescence of soybean cotyledons, but hormonal studies on these aspects are still not available.

During soybean cotyledon senescence the percentage of linolenic acid decreases (Wetterau *et al.*, 1979). It is important to note that this acid greatly increases during chloroplat biogenesis. One of the most rapid effects of zeatin (2 h) has been described for its stimulation of the increase of linolenic acid in the leaves of *Coleus blumei* and *Impatiens sultani.* Rapid effects of kinetin have also been described for the synthesis of lipids by mesophyll protoplasts (Kull and Ultes, 1980). The possible relation of these last results with senescence is not clear. On the one hand, zeatin was applied at very high concentrations (100

μg/ml), on the other hand the results may reveal a general effects of cytokinins are not necessary implicated in senescence. Its importance would depend of the membrane lipids turnover during senescence and in mature leaf. However the results show the importance of cytokinin action on membrane composition. It is possible that appropriate membrane integrity and composition depend on a continuous supply of cytokinins to the leaf. A diminution in the level of cytokinins at the beginning of senescence may determinate membrane changes. The relation of these chages in linolenic or in other fatty acids with the membrane changes in senescence is still unclear. A possibility is that a deviation of acetate use from the synthesis of fatty acids to the synthesis of steroids may trigger some of the X-ray diffraction changes observed in membranes.

Respiration

Mitochondria is one of the most stable organelles during leaf senescence (Rhodes, 1980). It remains active until late senescence when it declines (Hardwick, 1968). During the senescence of detached leaves a low (0.74 whereas in normal leaves is 1.0) respiratory quotient is detected which probably indicates the combustion of amino acids produced from proteolysis. Sometimes a climateric respiratory rise is detected during the senescence of both intact (Hardwick, 1968) and detached leaves (Tetley and Thimann, 1974) which is correlated with a rise in proteinase levels (Kannagara and Woolhouse, 1967). Inhibition of respiration retards senescence (Rhodés, 1980). The respiration, or the ATP produced in oxidative phosphorylation, is used for some biosynthesis processes probably required in senescence (e. g., the biosynthesis of hydrolytic enzymes). But this would be only a passive role of respiration in senescence in the same way that respiration is also required for the maintenance of young or not senescing leaves. In fact, from experiments with the uncoupler dinitrophenol, Tetley and Thimann (1974) concluded that respiration rate increases (when present) during senescence is mostly due to uncoupling. However, more recently, Malik and Thimann (1980) have found that the level of ATP increases during senescence and that kinetin prevent that increase just as the increase in respiration rate. However, light (which delays senescence) also produce an increase is the levels of ATP. The results on ATP levels point to a dual role of ATP. ATP retards senescence in light by stimulating the biosynthetic processes in general. In the dark, ATP accelerates senescence by providing energy for the biosynthesis of only degradative enzymes and of other possible

proteins required for senescence. At light, the main source of ATP is the photophosphorylation. In the dark, ATP must be produced during respriration (Thimann, 1980). If this model is correct, the question is : What determinates that the biosynthetic processes at light are mainly directed against senescence but in the dark the results of biosynthesis promote senescence?. The most plausible possibility is that at light genes which retard senescence are expresed (e. g., structural genes for protein present in normal functional mesophyll cells). However, in the dark, genes for degradative enzymes and other possible protein implicated in senescence symptoms are expresed. Independently of these genetic controls in relation with the role of respiration in senescence, the question is if respiration rate increases (when present) are a consequence of other senescence processes or are directly affected by the hormonal changes occurring during senescence. In excised leaves of *Rumex* Goldthwaite (1974) found that gibberellins and cytokinins inhibit respiratory metabolism and senescence in the dark. However, ABA accelerates senescence and increases the rate of respiration. The effects of GA_3 and ABA on respiration are detected as soon as 3 h after hormone treatment. This suggests that hormone effects on respiratory metabolism are direct. Sucrose which stimulates respiration does not however inhibits chlorophyll or protein breakdown. Thus, increased respiration is not sufficient for senescence. Probably respiration in senescnce is higher due to the low GA and cytokinins levels relative to the ABA levels. But here again, it seems that although similarly responding to hormones, respiration and other senescence phenomena (as protein and chlorophyll breakdown) are controlled by hormones through different mechanisms.

A direct effect of cytokinins inhibiting respiration has been claimed. In mitochondria prepeared from six plant species Miller (1980) found an inhibition of respiration by benzylaminopurine. The cytokinin seems to inhibit at a level between NADH dehydrogenase and cytochrome b. The relevance of these results is still unclear, as zeatin has no effect and the concentration used of benzylaminopurine is too high (0.5 mM).

Protein level and nutrients transport

It was advanced that a characteristic of leaf senescence is due to the degradation of proteins. The amino acids produced migrate to young or to reproductive structures. Both, protein degradation and nutrients export by leaves have been intensively studied in relation to the mecha-

nism of hormone action in senescence and, in general, in relation to the control of senescence.

Protein degradation determinates, or at least is parallel, with a diminution of the photosynthetic capacity of the leaf and with the ability of the leaf to export carbohydrates. Carbohydrates are also required as energy source and as carbon precursors for biosynthesis in reproductive structures. One can think that an adjustment of the nitrogen and carbohydrate demands of developing reproductive structures may control leaf senescence in monocarpic plants, but the problem may be difficult for an experimental examination. In fact, frequently reproductive structures can carry out considerable photosynthesis. Bazzaz *et al.* (1979) have estimated that in a number of plants the probable contribution of photosynthesis of reproductive structures to their total carbon demands are in the range of 50%. Of course this contribution varies along the reproductive structure and with the senescence of the photosynthetic apparatus. In relation with the hormonal effect on carbohydrate export by leaves, there are only few works, although it may be considered that in general cytokinins, as stimulators of anabolic processes (Kull and Hollwarth, 1974), favour the synthesis of starch in the leaves. At the beginning of senescence in *Lolium temulentum* leaves, Lloyd (1980) found a rapid loss of insoluble leaf material (probably including polysasccharides previously marked with [^{14}C]-sucrose or $^{14}CO_2$. As soon as 6 h after the induction of senescence, an increase of proteolysis is detected in oat leaves (Shibaok and Thimann, 1970) and polysaccharide breakdown soon follows.

While studying grain filling in different oat varieties, Welch *et al.* (1980) found that the relative low non-structural carbohydrate content of the high grain protein varieties is apparent only at the end of the period of grain filling. However, grain protein content differences among varieties are apparent during all the period of grain filling. The authors concluded that the high protein varieties have more efficient mechanisms for the mobilization of nitrogen and its translocation to the grain. They attribute the low carbohydrate contents of the high grain protein varieties to an increased level of respiration associated with the greater mobilization and translocation of nitrogen. However, a low photosynthesis can not be excluded in the protein rich varieties at the end of the period of grain filling.

A typical symptom of senescence of attached leaves in natural condition is the decrease of free amino acid in leaves (Story and Beevers, 1978; Kao, 1980b; Kang and Titus, 1980). The decrease is usually interpreted as due to the rapid transport of the products of proteolysis from the senescent leaves to young or to reproductive structures. In contrast, in detached leaves free amino acids accumulate during senescence (Kao, 1980a; Mishra, 1965; Thomas, 1978) which is interpreted as due to the absence of sink structures for the amino acids in this system. These changes in amino acids levels are inhibited by retardants of senescence, among them cytokinins. In attached leaves of peas (Storey and Beevers, 1978) the decrease of free amino acids levels is parallel with chlorophyll decrease and slightly preceeds protein decrease. It is not clear if this is a general phenomenon, but its knowledge would be of interest in relation with the mechanism of senescence. In contrast with the senescence in natural conditions, if senescence is induced by exposure of attached leaves to dark, free amino acids increases at the beginning of senescence (Wittenbach, 1978; Lloyd, 1980) in parallel with a decrease of soluble protein. Again, zeatin retards the rise in free amino acids and the decrease in soluble protein (Wittenbach, 1978). The rise in proteolytic activity (which is also retarded by zeatin) frequently occurs after the rise in free amino acids (Goldthwaite, 1974; Wittenbach, 1978). The relation of this dark induced senescence with the nature is uncertain. But it must be noted that usually senescence of detached leaves is carried out in the dark, which means that the increase of free amino acids in the senescence of detached leaves may not be due to the absence of sink structures. One possibility is that in dark-induced senescence in attached leaves, there is little sink activity of the yonug structures. Indeed, Mor and Halevy (1980a) found that sink activity of young shoot is promoted by light. However this light-promoted activity of young structures seems to be mediated through phytochrome (Mor and Halevy, 1980b) but in *Lolium* (Lloyd, 1980) senescence is also induced by CO_2 starvation at light. This senescence in the absence of CO_2, although not as quick as the dark-induced senescence, is also accompained by an increase of free amino acids and a decrease of protein in the leaf. Today, the reason of the decrease of amino acids in attached leaves and its increase in detached leaves is not clear. The hypothesis of the presence of sinks in assays with attached leaves is, at least, too simple. The possibility of an effect of the light favouring an export of amino acids through a direct effect in the leaves can not be excluded. Years ago, Chibnall (1939) described increased in free amino acids in attached leaves in night and fall again in the day.

It is well known that cytokinins and other stimulating hormones promotes the sink activity of the treated areas (Mothes and Engelbrecht, 1961; Penot and Beraud, 1978), This effect of cytokinins seems independent of the stimulation of the biosynthetic processes by cytokinins (Thimann, 1980). Penot and Beraud (1978) also found that the sink promoting activity is also independent of antisenescent hormone aċtivity. The hypothesis that the transpiration stream pulls substances to transpirating structures is attarctive but it lacks of experimental basis for senescing leaves. Moreover, that hypothesis is in contrast with the fact that senescence of attached leaves at light (with open stomes) produces a decrease of amino acids, but senescence in the dark produces an increase in amino acids. Penot (1978) proposed that hormones activate nutrient loading in the treated areas thus originating a down stream movement of nutrients.

Amino acids can reduce the retarding effects of cytokinins on senescence which may complicate the regulation of the senescence mechanisms.

Summarizing, with the available evidence it may be stated that a hormonal balance in leaves with a low concentration of stimulating hormones and with a high concentration of inhibitory hormones favours the senescence processes and inhibits the sink activity (at least the sink activity for amino acids). Studies on the effect of chemical and physical agents (for example the response to light) suggest that hormones affect to the senescence and to the sink activity of the leaves through different mechanisms. Under natural conditions, both the senescence and the low sink activity of the leaves occurs simultaneously and determinate the mobilization of the leaf components to young or to reproductive structures. However in many experimental conditions it can affect by a different way to senescence and to sink activity. It is significant that in monocarpic senescence of rice, Biswas and Choudhuri (1980) found a clear phosphate transport from leaves to developing grains before the appearance of senescence symptoms in the leaves. The senescence must affect to the sink status and similarly the sink status must affect to senescence. The mechanism and the extension of these reciprocal effect are of interest for an understanding of the control of senescence.

Classical experiments in the laboratory of Noodén (1980) suggested that the hypothetical senescence signal moves little and downward from pods to leaves in soybeans. Together with this interpretation, the possibi-

lity exists that leaf also requires to be conditioned to respond to senescence signals. The senescence-conditioned state may be induced in leaves by the same photoperiodic treatment which induces flowering. Wittenbach has recently (1982) reported that although depodding retards leaf senescence (as measured by chlorophyll or protein loss) in soybeans, ribulose bisphosphate carboxylase and photosynthesis strongly declined in leaves of depodded plants. Two polypeptides of 25-30000 daltons and one polypeptide of 75000 daltons appear (probably by synthesis *de novo*) in leaves of depodded plants. The leaves and the whole depodded plant seem to be transformed in a sink of carbohydrate and other materials (Wittenbach, 1982). Possibly the leaves of depodded plants may be considered as senescent leaves in a plant without the strong sink activity of the pods. The normal course of senescence may be look as the coincidence in time of degeneration symptoms in leaves and the filling of reproductive structures. In this regard it may be of interest to remember the results of Proebsting *et al.* (1978) discussed previously. On genetic lines of peas, which demonstrated the independence of the genetic determinants for flowering and for senescence, although both, flowering and senescence, may be under photoperiodic control. We have previously discussed the evidences for the control of the senescence by phytochrome. The molecular weights of the proteins level increase in depodded soybeans are striking similar to those of the polypeptides synthesized by chloroplast of senescent barley leaves (Garcia *et al.*, 1983). Senescence (as measured by chlorophyll loss) is a accelerated by ABA in normal soybean plants, but not in depodded plants (Noodén, 1980). ABA has been proposed (Kao, 1980a) to mediate the manifestation of senescence symptoms in soybeans. One can speculate that after induction of a senescence-conditioned state in leaves by some mechanism similar to that of the induction of flowering, reproductive structures, in an action mediated by ABA, withdraw nutrient from leaves, thus producing the typical senescence symptoms.

The senescence-conditioned state may be similar to the early senescence symptoms which appear in detached leaves. Cytokinins seem to affect negatively to both the senescence-conditioned state and the loss of nutrient by the leaves. Phytochrome may control the induction of the senescence-conditioned state. Ethylene would mediate the induction of the senescence-conditioned state in detached leaves (Garcia *et al.*, 1983) and in monocarpic senescence. A competition among leaves for root

cytokinins (Kao, 1980a) would cause senescence of basal leaves during the non-reproductive stages.

Chloroplasts are the main source of the released amino acids by senescent leaf (Morita, 1980) and precisely the abundant Fraction–I protein or Ribulose bisphosphate carboxylase is the main source of amino acids (Peoples *et al.*, 1980; Wittenbach, 1978; Wittenbach, 1979), at least during the first stage of senescence. The sequential degradation of the different proteins will be dalt in next pages. Now, we will mostly consider the role of the proteolytic enzymes although a decrease in the rate of protein synthesis may also contribute to the loss of proteins by the leaf.

Increases in proteinase levels have been described during senescence (Martin and Thimann, 1972; Drivdahl and Thimann, 1978) and cytokinins usually retard that increase (Frith and Dalling, 1980). As also cycloheximide inhibits the increse of proteinase activities, probably cytokinins act, directly or indirectly, by inhibiting the synthesis of proteinases in the cytoplasm. However, some problems remain in the control of proteinase levels in senescent leaves. Some problems in the interpretation of the results may raise because frequently proteinaes activity has not been assayed with its natural substrates in the leaf. By using ribulose bisphosphate carboxylase as substrate of proteinase, Peoples *et al.* (1980) found a biphasic increase in proteolytic activity which is parallel with a decrease in the content of protein in leaf during senescence. The proteinase assayed is of the acid type with an optimal pH of 4.8. However we have yet mentioned results (Wittenbach, 1978) indicating a decrease of leaf protein before the increase of proteinàse levels measured with casein as substrate. At the stage of the two days which precceds the raise of proteinase activity in the dark-induced senescence, the senescence process is reversible. Acid proteinase is localized in vacuoles (Heck *et al.*, 1981) and seems to be absent in chloroplasts. Probably the initial protein decay in senescence, before the increase in proteinase activity, is catalyzed by the proteinase usually present in vacuoles in a process which probably implicates organelle recognisation mechanisms (here between chloroplasts and vacuole). Further progress of senescence would be dependent of increases in proteinase levels. The increase in proteinase during dark-induced senescence (Wittenbach, 1978) in wheat mainly occurs after a two days period and coincides with the beginning of the irreversible stages of senescence. There are considerable evidences indi-

cating that the increases of proteinase are inhibited by cytokinins, but little is known on possible effects of cytokinins on the availability of chloroplast protein to the lytic enzymes of the vacuoles. One possibility is that also low cytokinin levels determinate the loss of membrane permeability thus making accesible the lytic enzymes to their substrates. However, the high sensibility of the chloroplast protein to senescence points to a specific interaction of chloroplasts with the lytic compartments at least during the initial stages of senescence.

Amides are the main forms of nitrogen transported in plants (Bollard, 1960; Zimmerman, 1960). On the other hand detached leaves accumulate high amount of the amides glutamine and asparagine (Chibnal 1939; Yemm, 1950). Thus, amino acids reconversions are probably another aspect of senescence. Changes in the levels of same enzymes of nitrogen metabolism have been reported in relation with probably amino acids interconversion during leaf senescence (Cuello and Sabater, 1982; Kang and Titus, 1980b; Simpson and Dalling, 1981, Storey and Beevers, 1978; Thomas, 1978). Usually, the associtated enzyme level changes are inhibited by cytokinins which not necessary means that these cytokinins actions are direct. One of the most characteristic changes in these enzymes is the continuous increase of glutamate dehydrogenase during senescence which has been related to the formation of NH_3 to be used in glutamine synthetase reaction. Glutamine synthetase does not seem to increase during senescence, in fact its actity declines. However, glutamine synthetase levels during senescence seem to be sufficient for glutamine formation. In attached wheat flag leaf Simpson and Dalling (1981) have reported that glutamate, aspartate, serine, alanine and glycine are the main amino acids exported at the beginning of senescence. Only during late senescence glutamine become the main amino acid exported. Again the possibility of two phases in the course of senescence is revealed in attached leaves.

On the basis of its senesitivity to protein synthesis inhibitors (Cuello and Sabater, 1982), glutamate dehydrogenase increase during senescence seems to be controlled through different mechanisms that other enzyme changes such as that of transaminases.

Nucleic acid and protein synthesis

The nucleus and the mitochondria are the most stable subcellular organelles during senescence. Reversion of senescence has been described (Beevers, 1967) in both attached and detached leaves which implies that

the cellular hereditary material retain all of its potentilities until advances stages of senescence. Moreover, in many cases in which senescence reversion has not been achieved, the hereditary material may be intact until the end of senescence. Here, probably, that fail to reverse senescence may be due to a scarce knowledge of the appropriate hormonal and nutritional treatments for reversion. With appropriate techniques, sequential changes in the state of the DNA has been found (Dhillon and Miksche, 1981) during senescence which should affect to a differential control of genetic expression. But there are powerfull reasons to think that an irreversible loss or deterioration of DNA is not the first cause of plant senescence.

A typical symptom of senescence is the decrease of the content of RNA which is retarded by cytokinins (Beevers, 1976). In barley leaf Srivastava (1968) found a chromatin-associated ribonuclease which level increases during senescence. The ribonuclease increase is accelerated by ABA and reversed by kinetin. *De novo* ribonuclease synthesis has been demonstrated in morning glory (Baumgartner *et al.*, 1975) and *Rhoeo* (Sacher and Davids, 1974). In senescent pod of bean, however, Sacher and Leo (1977) found an activation of ribonuclease activity. On the other hand, a decrease in RNA polymerase activity has also been found during senescence (Schneider, 1980). This decrease is retarded by cytokinin treatment. Probably the decrease in RNA during senescence is due to both an increased degradation and a decreased synthesis of RNA. However, the loss of RNA may be more than a symptom of senescence. The central question is if the loss of RNA shows some selectivity or it is only an aspect of an indiscriminated cellular deterioration. Related to the RNA decay is the question of the protein synthesis activity during senescence. In plant cell we must to consider the RNA and the protein synthesis activity in three compartment, cytoplasm, mitochondria and chloroplast. The genetic expression in mitochondria is usually considered to have only minor importance in senescence. Indeed, in mesophyll cells, the main contributions to protein synthesis are that of chloroplasts and cytoplasm. Thus, the change in RNA and protein synthesis activity during senescence have been mostly studied in chloroplast and cytoplasm. The results on protein synthesis activity during senescence are conflictive. It has been reported a decline, a maintenance or an increase in the rate of amino acids incorporation during senescence (Beevers, 1976; Thomas and Stoddart, 1980). The origin of these disparing results may be multiple. A change in the permeability of the tissue to the exogenous

supplied labeled amino acids, a dilution of the radioactive label with the endogenous increased free amino acids in detached leaves or a masking of the label incorporation due to the presence of increased proteinase activities, make difficult measure of the genuine protein synthesis activity in senescent leaves. The report on polyribosome level during senescence are also conflictive (Thomas and Stoddart, 1980). In general a decay of all of the species of RNA (tRNA, rRNA and mRNA) is usually found during senescence. However, the effect of protein synthesis inhibitors retarding senescence (Thomas and Stoddart, 1980) frequently indicate that some mRNA are translated in active ribosomes in a process which is required for senescence. The mRNAs coding the hydrolytic enzymes, which leavels increase during senescence, are probably translated during senescence. The effect of the inhibitors of protein synthesis in retarding senescence may de due to their action on the synthesis of the hydrolytic enzymes, but effect on the synthesis of other unidentified senescent proteins can not be ruled out.

Cycloheximide, which inhibits protein synthesis in cytoplasm, has been frequently described as a senescence retardant (Thomas, 1974; Martin and Thimann, 1972; Peterson and Huffaker, 1975), which delays chlorophyll and protein loss and the increase of proteolytic enzymes. Other inhibitor of protein synthesis in cytoplasm : 2-(4-methyl-2, 6-dinitroanilino) -4-methylpropionamide (MDMP) also delay leaf senescence (Thomas, 1976). Thomas and Stoddart (1980) concluded that senescence processes are dependent on protein synthesis in cytoplasm. Sometimes the problem may be more complex. Cycloheximide accelerate senescence in tobacco leaves (Takegami, 1975). Pjon (1981) reported that cycloheximide inhibits the loss of chlorophyll, and in a lesser extent the loss of protein, in detached maize leaves, but it does not affect to the loss of chlorophyll in detached hydrangea leaves. It is possible that the effect of protein synthesis inhibitors depend on the plant material (plant species and physiological state) and of the conditions of incubation. In detached leaves of *Lolium* incubated under alternate light and dark periods, Thomas (1974) found that cycloheximide inhibits senescence but chloramphenicol has no definitive effect which suggests that chloroplasts have only a passive role in senescence (Thomas and Stoddart, 1980). However, in detached barley leaves incubated in the dark, both cycloheximide and chloramphenicol prevent the high levels of chlorophyllase associated with senescence (Sabater and Rodriguez, 1978). In this regard, it may be the significant finding that chloramphe-

nicol retards several senescence symptoms (among them chlorophyll loss) in detached barley leaves incubated in the dark, but chloramphenicol accelerates the same senescence symptoms in detached barley leaves incubated at light (Cuello and Sabater, 1992).

The effects of the inhibitors of protein synthesis may be difficult to interpretate (Thomas and Stoddart, 1980). Sometimes, the effect of a protein synthesis inhibitor in senescence may not be directly related to its action on protein synthesis. Thus, an inhibition of protein synthesis may determinate a rise in the level of ATP. Of course, some protein synthesis occurs along senescence (Choe and Thimann, 1975. One possibility is that at light, photophosporylation provides sufficient ATP for the biosynthesis of the material normally present in the leaf, in this way light partially masks the degradative processes of senescence. Protein may be among the material synthesized at light. Alternatively, in the dark, both cytoplasm and chloroplast specifically synthesize proteins which favours the degradative processes. Kinetin has effects very similar to that of chloramphenicol in the senescence of detached barley leaves in the dark (Cuello and Sabater, 1982), which suggests a possible effect of kinetin inhibiting the synthesis in chloroplasts of proteins which accelerate senescence. In fact, a small but significant effect of kinetin retarding protein breakdown has been found in senescenct isolated chloroplasts (Choe and Thimann, 1975).

The inhibitors of the synthesis of RNA, actinonycin-D and rifampicin do not affect to the course of senescence (Thomas and Stoddart, 1977) which suggests that no specific genes of senescence are transcribed. However a lack of incorporation of the inhibitor of RNA synthesis or an inactivation can not been ruled out. In RNA-DNA hybridization competition studies, Srivastava (1972) does dot found a significant change in RNA during senescence of barley leaves. But, here again the hybridization competition assays, probably with high amount of rRNA, can not detect small amounts of possible specific mRNA synthesized during senescence. One possibility is that the transcription programme in senescent leaf is not significantly different to that of mature leaf. The probable qualitative differences in the protein synthesized by mature and senecent leaves may be due to either a specific translation of some mRNA or to a specific increased degradation of some mRNA during senescence.

During the senescence of detached barley leaves, Legocka and Szweykowska (1981) found that the rRNA of chloroplast ribosomes shows a higher stability than the rRNA of cytoplasm ribosomes. However, in *Xanthium* and tobacco (Beevers, 1976; Takegami, 1975) chloroplast rRNA is more labile than cytoplasm rRNA. Dyer and Osborne (1971) found a greater stability of tRNA than of other RNA fractions during senescence. Almost all reported that cytokinins retard the RNA decay and the increase of RNAse activity. At the same time cytokinins stimulate RNA synthesis. Changes in specific mRNA during senescence have been deduced from changes in their protein products in heterologeus systems (Watanabe and Imaŝeki, 1982). It seems probably that some mRNA may be translated during senescence in chloroplast ribosomes, in cytoplasm ribosomes or in both. Ribosomes were still observed in intact yellowish green leaves (Naito *et al.*, 1981) and polysomes increase after cytokinin treatment. The role of cytoplasmic protein synthesis may be clear in *de novo* synthesis of hydrolytic enzymes. However we have previously mentioned that the increase in the level of these enzymes is a late process in senescence. More complicated is the possible role of protein synthesis in chloroplasts during senescence.

With chloroplasts isolated from senescent tocacco leaves, Vonshak and Richmond (1975) found a decrease in the rate of protein synthesis during senescence. However, in detached barley leaves (Garcia *et al.*, 1982; Cuello *et al.*, 1980) it has been found a transitory (10 to 25 hours after detachment) increase in the activity of protein synthesis by isolated chloroplasts in spite of a decrease in the amount of rRNA of chloroplasts. Kinetin prevents the decrease of rRNA of barley chloroplasts and produces high increase in the activity of protein synthesis. The reasons for the differences in the evolution of protein synthesis activity in chloroplasts between tobacco and barley are not clear, but before we have mentioned that the rRNA of chloroplasts is less stable in tobacco than in barley when compared with the rRNA of cytoplasm. The transitory increase in protein synthesis activity in chloroplast of senescent barley leaves may be in contrast with the decrease of rRNA. However, Simpson *et al.* (1980) found in barley a ribonuclease which attacks rRNA in intact ribosomes. Despite scission of rRNA in multiple sites, the RNAse-treated ribosomes remain active in protein synthesis.

The transitory increase during senescence of protein synthesis by isolated chloroplasts of barley mainly produces (G arcia *et al.*, 1983) two or

three polypaptides (25,000 and 60–75,000 daltons) may be important in senescence. Kinetin produces a bulk of proteins of molecular weight ranging from 10,000 to 60,000 daltons. Some modified bases of RNA, particularly of tRNA, have the same structure that cytokinins. However, cytokinins do not incorporate in these tRNA, modification of the bases occurs after synthesis of a precursor chain with the usual bases. Cytokinin action on protein synthesis does not seem to be mediated by its incorporation in tRNA. The effect of cytokinins may be more probably related to a direct binding to ribosomes (Chung *et al.*, 1979) or to other protein receptor, although little work has been made in this way in senescence. It seems reasonable that the kinetin-stimulated synthesis of protein in chloroplasts produces different proteins than the senescence dependent protein synthesis. Cytokinins have a general stimulatory effect on protein synthesis in leaves (Guern and Péaud-Lenoel, 1981), and probably by stimulating the synthesis of protein of chloroplast biogenesis, cytokinins inhibit the synthesis of senescence protein. The importance of chloroplast and of plastid rRNA on the inhibitory effect of cytokinins in senescence has been suggested before (Dyer and Osborne, 1971), It is possible that the transitory increase in protein synthesis in chloroplasts of senescent leaves, although related with senescence, may be also dependent of the detachment treatment. Davies and Schuster (1981) have found an increased protein synthesis activity at the neighbour of a wound. One possibility is that the wound stimulus is an important factor in the induction of the accelerated senescence detected in detached leaves. The increased, and probably specific, protein synthesis induced by the wound moving signal may play a role in the mechanism of senescence in detached leaves.

We mentioned some contrasting effect of polyamines in senescence. Polyamines increase the fidelity of the process of nucleic acid and protein synthesis (Abraham and Pihl, 1981). Thus, it is possible that, associated with senescence, occurs a decreased fidelity of protein synthesis. Polyamines also inhibits synthesis and ethylene production (Apelbaum *et al.*, 1982; Fahrer *et al.*, 1982) and protease activity (Shin *et al.*, 1982).

Other biochemical changes

Many other biochemical changes have been described to occur during senescence. Frequently, their significance in senescence is unknown, but at least the study of the mechanism of their appearance may be of interest for an understanding of the senescence process.

Together with an increase in glutamate dehydrogenase, RNAses, proteinases and chloropoyllase, it has been described increases of phenolase (Meyer and Biehl, 1980) and invertase (Pollock and Lloyd, 1978) activities. More interesting is the parallel changes in acetyl-cholinesterase and acetylcholine in senescing cotyledons of *Phaseolus* (Lees *et al.*, 1978).

Chloroplast in Senescence

The most prominent aspect of leaf senescence is the deterioration of chloroplast structure and function. One can hardly see fortuituous that light and cytokinins, which delay senescence, are powerfull activators of plastid biogenesis (Feierabend and de Boer, 1978; for references see : Guern and Péaud-Lenoel, 1981). In mature leaf, the processes of synthesis and degradation of chloroplast components are apparently in equilibrium. The individual rates of synthesis and degradation processes are difficult to determinate in mature cells but they, reasonably, must be slower than the biosynthetic processes at the biogenetic stage of young leaf. In senescent leaf, the degradative processes seem to be activated in respect to mature leaf, but the contribution of a decreased rate of synthetic processes to senescence symptoms can not be neglected from the discussion in the prceeding pages. We have cited many reports on the inhibition by cytokinins and light of the hydrolytic enzyme increases accompaning senescence. Thus, cytokinins and light, which stimulates biosynthetic processes during plastid biogenesis, inhibit the deteriorative processes during the degradation of chloroplasts in senescent leaves. During plastid biogenesis, cytokinins and light act both on nuclear and chloroplast genoma (see referencs in Guern and Péaud-Lenoel, 1981). One can ask if during senescence the protective effects of cytokinins and light are only due to effects in nucleus and cytoplasm (Thomas and Stoddart, 1980) or cytokinins and light have also some direct action in chloroplasts. There are some evidences for a role in senescence of the experssion of specific genes of chloroplasts. Because of their main importance, here we discuss with some details the processes of deterioration of chloroplasts during senescence.

The different chloroplast activities are not lost simultaneously during senescence. In detached barley leaves in the dark Biswall and Mohanty (1978) found that the processes of the dark reactions of photosynthesis are more sensitive to degradation during senescence than electron transport and photophosphorylation, specially cyclic photophosphorylation

and electron transport. In this line, Morita (1980) found that in leaf senescence during the vegetative stage of rice, the stroma was the main responsible for the loss of leaf nitrogen. However during reproductive stages, nitrogen was released from lamellar and stromal fraction at almost the same rate which suggests that different mechanisms occur in the two types of senescence. Ribulose bisphosphate carboxylase, the majoritary protein of chloroplast, is the most studied enzyme of the dark stages of photosynthesis during senescence (Peoples *et al.*, 1980; Wittenbach, 1978) and probably it is one of the most quickly degraded soluble proteins. An acid proteinase seems to be responsible for the degradation of chloroplast proteins. That proteinase is localized in the central vacuole (Heck *et al.*, 1981) which poses the problem of the factors responsible for vacuole and chloroplast interaction which allow the deterioration of chloroplasts. We have previously mentioned that frequently, the increase in proteinase during senescence is preceeded by a protein degradation. Probably one of the earlier consequences of the initiating event of senescence is the interaction between vacuole and chloroplast. A possibility is an initial disruption in the chloroplast envelope. In this regard, Tomomatsu and Asahi (1981), have found a chloroplast-damaging factor in mung bean leaves.

In relation with thylakoid changes, we have previously discussed the deterioration of thylakoid structure during senescence and the aparition in chloroplast of lipid granules derives from thylakoid lipids. From electron microscopic studies, Wrischer (1978) deduced the formation of intrathylakoid protein crystals in senescent detached spinach leaves. It must be remembered that also thylakoid lipids from crystalline structures during senescence. Bricker and Newman (1980) distinguish two groups of thylakoid polypeptides according to their stability during senescenecr. Interesting, the same authors found that a thylakoid polypeptide of 62600 daltons increases during senescence and decreases during regreening in soybean cotyledons.

The relation of thylakoid disassembly with chlorophyll degradation is not clear. Thomas and Stoddart (1975) described a mutant of *Festuca pratensis* which retains chlorophyll during senescence. This 'non-yellowing' mutant loss stroma proteins and a fraction of thylakoid proteins at the same rate than the normal 'yellowing' strain. But some thylakoid proteins are most stable in the 'non-yellowing' mutant than in the yellowing strain (Thomas, 1977). These more stable proteins

probably protect chlorophyll against enzyme attack. The existence of this mutant seems more compatible with an enzymatic degradation of chlorophyll than with a non enzymatic degradation (Simpson *et al.*, 1976). Thomas and Stoddart conclude that chlorophyll degradation is a secondary consequence of thylakoid protein degradation. However, there is no reason to think that thylakoid proteins are more easily accesible to proteinases than chlorophyll to enzyme degradation. Chlorophyll may be hydrolyzed by chlorophyllase, an enzyme usually assayed in hydro-organic mixtures (Holden, 1966) and so, chlorophyllase may be appropriate to act at the membrane level. The frequent earlier loss of protein than of chlorophyll has been argued as probing that chlorophyll is a secondary process. However, sometimes, chlorophyll is lost earlier than protein (Biswas and Choudhuri, 1980). In senescence at light, chlorophyll may be photodestroyed (Brow *et al.*, 1980; Choe and Thimann, 1974). However during senescence in the dark increased chlorophyll degradation (in the dark there is no chlorophyll synthesis) may be enzyme catalyzed. Several enzymes can destroy chlorophyll *in vitro* (Holden, 1966). Chlorophyllase is a candidate for the first step in the degradation of chlorophyll during senescence. Thermodynamic reasons suggest that chlorophyllase acts *in vivo* in the direction of the hydrolysis of chlorophyll. During fruit maturation (Looney and Patterson, 1967; Sacher, 1973) there is a direct correlation between chlorophyllase levels and chlorophyll loss. Similar results has been found during the senescence of barley, oat and sunflower (Purohit and Chandra, 1980; Sabater and Rodriguez, 1978; Purohit, 1982). Phillips *et al.* (1969), however, did not find such a correlation during senescence of radish leaves. With the available data it is difficult to explain these differences. But up to date chlorophyllase remains the main candidate for a possible enzyme degradation of chlorophyll. The effect of cytokinins and protein synthesis inhibitors on chlorophyllase leavels also suggest the role of chlorophyllase in chlorophyll degradation. The degradation of chlorophyllides, the products of chlorophyllase action on chlorophylls, may be catalyzed by peroxidase (Matile, 1980).

More than a cause-effect relations chain among the different senescence symptoms, these frequently appears as unrelated and as if they are controlled through different mechanisms. Accordingly, different physical and chemical factors can induce senescence symptoms in leaves. Leaf senescence is not the only but one of the several different processes which occur during the reproductive phase of monocarpic plants. Other of these

processes as seeds development, nutrient transport, sink and source changes, etc.... may be under different control mechanism than senescence, but they are not independent. Their appropriate coupling allows the best adaptative adventages for the plant species (seed production, seed viability, quick seed development under stress conditions, etc...). Thus, it is possible that, for example, seed development and leaf senescence simultaneity arosed in evolution as an adaptive adventage resulting from two previously unrelated processes. The actual simultaneity results in a series of interferences of one process on the other. Hormones seem to mediate most of the relations among the different processes which occur during the reproductive stage, but actually the mechanisms of hormone level changes are not clear. The ontogeny of the leaf can determinate a change in hormone metabolism and/or a change in hormone import and export which favours the senescence of the leaf. But also, hormone export by other organs may led to leaf senescence.

The mechanism of hormone action in senescence are not still clear. The nature of hormone interaction is almost a black box. Related to cytokinins, actions at the membrane and genetic levels appear as the most probable mechanism in senescence. The possible relation between these two mechanisms is still unclear. Today, there is no evidence for possible intracelluar messenger between cytokinins and their genetic or membrane action in senescence.

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Hormonal Regulation of Ion Transport in Plants

J. L. Karmoker

Introduction

Plant hormones and inorganic nutrients share a common physiological function *i. e.*, both of these growth factors influence the growth and development of plants. The former regulates the internal control mechanism of plant growth while the latter controls growth by providing the mineral requirements for a range of functions which include maintanence of the osmotic potential of cells and tissues as constituents of organic compounds and as cofactor in important biological reactions. Any interaction between phytohormones and inorganic ion transport may change the ionic balance in plants and consequently growth may be affected. Since the 1950's, a number of reports have accumulated in the literature concerning the effect of plant hormones on the uptake and transport of ions in a wide variety of excised plant tissues (Van Steveninck, 1976a). Table 1 summarises effects of phytohormones on ion transport recorded to date and most of these relate to cation (K^+, Na^+ or H^+) transport. This suggests a possible role in cation exchange (e. g., K^+ or Na^+ for H^+).

Reports on the effect of exogenous plant hormones on ion transport in excised plant tissues (Table 1) provide strong evidence that endogenous plant growth substances play a role in the regulation of ion transport also in whole plants. Plant hormones were found to influence physiological phenomena which govern water and ionic balance in plant cells. This was evident from kinins and abscisic acid-induced control of stomatal guard cell movement and their effects on water and ion transport of decapitated roots and of storage tissues (Van Steveninck, 1976a).

However, the mechanism of hormone-regulated ion transport is poorly understood. The recent trend of research in this area is the study of its mechanistic aspects. In this regard, the questions are whether plant hormone exert their effects diractly on ion pump (e. g., H^+, K^+ and Cl^- pumps) or less directly by affecting the membrane permeability, the ion

Table-1. Effect of plant hormones on ion transport processes.

Hormones	Effective concentration (M)	Tissue	Ion transport process affected	Reference
IAA	5.7×10^{-6}	Pea epicotyl segments	Increase in Rb absorption	Higinbotham *et al.* (1953)
α-NAA	10^{-5}	Intact wheat plants	Decrease in cation uptake in the order of K^+, Na^+, Mg^{2+}, Ca^{2+},	Swenson and Burstrom (1960)
IAA	2×10^{-5} to 10^{-4}	Sunflower hypocotyl segments	Promoted K^+ and Rb^+ uptake	Ilan and Reinhold (1963)
IAA	10^{-6} to 10^{-4}	*Avena* coleoptile	Increase in Cl^- uptake	Rubinstein and Light (1973)
IAA	10^{-5}	Callus culture cells of *Petroselinum sativum*	Decrease in Cl^- uptake	Bentrup *et al.* (1973)
IAA	10^{-9} to 10^{-5}M	Suspension cultured of parsley	Increase in influx of ^{86}Rb, ^{22}Na and ^{36}Cl after an initial inhibition	Pfruener and Bentrup (1978)
B A	1.3×10^{-4}	Bean leaf slices	Inhibition of Na^+ uptake in expanding leaves	Jacoby and Dagan (1970)
Kinetin	4.6×10^{-5}	Sunflower cotyledons	Increase in K^+ uptake	Ilan *et al.* (1971)
B A/Kinetin	7×10^{-5}	Beet root discs	Decrease of K^+ and Na^+ uptake in aged tissue	Van Steveninck (1972b)
Kinetin	1×10^{-6}M	Isolated Maize roots	Inhibition of K^+ and Cl^- transport into the xylem	Collins and Karrigan (1973, 1974)
BA and Kinetin	5×10^{-8} and 5×10^{-6}M	Intact barley plant	Decrease in K^+ export in guttation fluid from hydathodes	Dieffenbach *et al.* (1980)

ABA	3.8×10^{-5}	Beet root	Increase in K^+, Na^+ and uptake in aged tissue	Van Steveninck (1972a)
ABA	$0.4–1.9 \times 10^{-5}$	Excised barley and maize roots	Inhibition of K^+ and Cl^- transport into the xylem	Carm and Pitman (1972)
ABA	10^{-2}M	Excised barley	Decrease in ^{86}Rb transport into the xylem	Pitman and Wellfare (1978)
ABA	5×10^{-7} to 10^{-6}	Excised bean root systems	7– to 8–fold in K^+ and 19–to 20– fold increase in Cl^- transport into the xylem	Karmokar and Van Steveninck (1978)
ABA	10^{-6} to 10^{-4}	Intact barley plant	Increased K^+ transport in guttation fluid from hydathodes	Dieffenbach *et al.* (1980)

carriers and other physiological processes which are involved in ion transport phenomenon.

The present review provides a general discussion of the effect of phytohormones on ion transport in excised tissues and in intact plants and the reconciliation of results with excised tissues to that in whole plants. Finally, an attempt will be made to explain the possible mechanism of hormonal regulation of ion transport in plants. This will be followed by a conclusion giving a birds'eye view of the problem and prospects for future work.

Effects of Plant Hormones on Proton Pump and K^+/H^+ Antiport

The striking resemblance between the stimulatory effect of acid buffer solution (Bonner, 1934) and that of auxin (Pohl, 1948) on the growth rate of *Avena* coleoptile indicated that growth by cell elongation depends on an output of protons supported by a process of cation exchange. Recent work has demonstrated that auxins like IAA, α-NAA and 2, 4-D mimick the effect of high concentration of protons. For example, Ilan and Shapira (1976) has shown that auxin-induced growth of hypocotyl segments of *Helianthus annuus* grown in 20 mM Na_2SO_4 was accompa-

nied by an auxin-induced pH drop in the medium. Similarly, Kholdebarin and Oetrli (1977) has shown that 3 ppm IAA reduced the pH of the external medium bething the barley coleoptile tissues. Pavlenko (1978) showed that 1×10^{-4}M IAA caused secretion of H^+ from isolated protoplast of tobacco. More recently, Evans *et al.* (1980) found that IAA and L-napththalene acetic acid increased the pH of the bathing medium. Similarly the synthetic auxin 2, 4-D also initiated H^+ excretion, but 3, 5-D which lacks growth promoting capacity did not increase H^+ extrusion (Rayle and Johnson, 1973). On the other hand, ABA at a concentration of 5×10^{-5}M completely inhibited auxin-induced H^+ extrusion while ABA at a relatively low concentration (5×10^{-7}M) caused a partial inhibition. It was concluded that the auxin-induced extension of coleptiles is a secondary effect resulting from an auxin-induced increase in excretion of H^+ ions (Rayle 1973). However, in a concurrent review on this topic, Evans (1974) did not favour this conclusion and this opinion was strengthened by the work Ilan (1973) who recorded that sunflower hypocotyls an auxin-induced pH drop in an external solution which was dependent on the presence of K^+. However, in an NH_4-containing solution, IAA caused an initial drop in pH which rose some hours later. Ilan (1973) proposed that an IAA-induced stimulation of K^+ influx preceeded the proton efflux thus maintaining that IAA-induced promotion of proton efflux is of secondary nature.

Hager *et al.* (1971) found that auxin-starved sunflower hypocotyls grown in buffer of pH 4 showed a growth rate which was equivalent to auxin-induced growth and no growth occurred in buffer of pH 6 or higher. The acid-induced growth of sunflower hypocotyls placed in buffer of pH 4 did not depend on aerobic conditions but a auxin-stimulated growth was found to have a strict requirement of aerobic conditions. However, nucleotides like ATP or GTP could substitute for the latter requirement (Hager *et al.*, 1971). The inhibitory effect of metabolic uncouplers on auxin-induced H^+ extrusion and its dependence on respiratory energy led to the proposition that IAA affects an active proton pump. This view was confirmed by the works of Cleland (1973) who found that auxin caused a rapid induction of H^+ excretion from *Avena* coleoptile sections with the epidermis removed in order to facilitate the escape of H^+. Uncouplers of respiration (CCCP and KCN) and an inhibitor of protein synthesis (CHM) were found to inhibit H^+ efflux (Cleland 1973). In suspension cultured bean or cycamore cells, auxin-induced H^+– ion efflux required

the presence of Ca^{2+} in the medium (Fisher and Albershem, 1973). More recently Cocucci and Dalla Rosa (1980 a 1980b) found that canavanine, cycloheximide and cordycepin (3 – deoxyadenosine, which depresses uridine incorporation into RNA) strongly inhibited H^+ extrusion and K^+ uptake in maize coleoptiles.

Auxin-induced promotion of K^+ uptake by *Mnium* leaves was associated with a drop in pH of the external solution (Luttge *et al.*, 1972). The cation exchange theory of ion transport proposed that the transport of protons out of the cell and into the cell wall is compensated by a flow of monovalent cations like K^+ into the cell (Smith and Raven, 1976). In addition to cation exchange, anions in the solution may be transported into the cell periphery to balance the charge of protons excreted into the cell wall compartment. Theory of K^+ for H^+ exchange was supported by Haschke and Luttge (1973) with work on *Avena* coleoptiles which demonstrated that IAA-regulated K^+ for H^+ exchange showed a 1 : 1 stoicheometry. On the contrary, Dejaegere and Neirnckx (1978) reported that although H^+ efflux was correlated with the uptake of monovalent cations in barley seedlings, the process did not show 1:1 stoichiometry. Using lipophilic cations in the medium in place of K^+, Bellando *et al.* (1970) suggested that the nature of K^+ for H^+ exchange is electrical rather than chemical. Fusicoccin (FC) increased K^+ uptake with a concomitant stimulation of H^+ extrusion in pea internode sections (Marrè *et al.*, 1974c) and in excised barley roots (Pitman *et al.*, 1975a, 1975b). Gabella and Pilet (1979) has shown that H^+ extrusion from maize root segments was proportional to the concentration of FC used. Moreover, FC-induced H^+ extrusion was also enhanced in presence of K^+ at concentrations higher than 2×10^{-4}M when chloride was the accompanying anion. This effect was not significant when slowly penetrating SO_4^{-2} or benzenesulfonate were the accompanying anions (Lado *et al.*, 1976a). This result was supported by Lado *et al.* (1976b) who found that in presence of K^+ (1–10 mM KCl) and Rb^+, fusicoccin treated pea internode segments showed a marked increase in H^+ extrusion and K^+ uptake. However, Na^+ or other monovalent cations had little or no effect. Marre (1977) showed that FC–induced H^+ extrusion and K^+ transport showed a 1:1 stoichiometry but in dwarf maize coleoptiles more H^+ than K^+ was transported (Nelles, 1978).

Cleland (1976) found that the FC stimulation of H^+ excretion and K^+ uptake took place within 90 seconds of application while the IAA stimu-

lated H^+ extrusion and R^+ uptake occurred after a long lag period. Also in the later case, more H^+ than K^+ was transported. It was suggested that IAA did not affect K^+/H^- exchange directly and that the mechanism of H^+ extrusion and cell elongation brought about by these two hormones differs. However, both phenomena are affected by metabolic inhibitors and external pH.

Mentze *et al.* (1977) found that removal of the cuticular barrier prevented auxin-induced H^+ extrusion and cell elongatian in *Heliathus* hypocotyl segments. On the contrary, peeling off cuticular barriers did not affect the FC-induced increase in H^+ efflux and growth. This shows that the auxin effect is tissue specific *i. e.*, the epidermis and closely associated cells may control auxin-induced H^+ excretion. This result support the acid growth theory of auxin action.

Recently, Karmoker (1981) found that ABA had an effect opposite to that of IAA and FC on H^+ extrusion and K^+/Rb^+ uptake as indicated

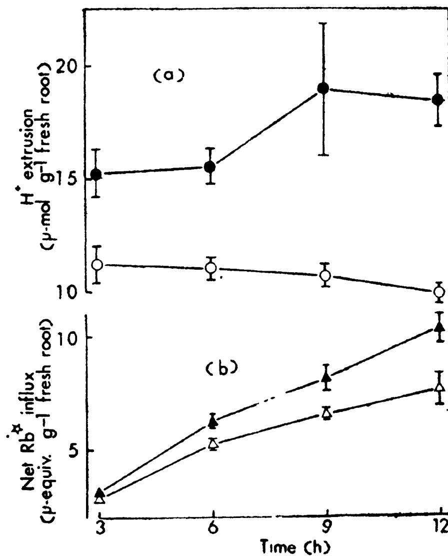

Fig. 6. 1. (a) The effect of ABA on H^+ extrusion from the root system of intact bean seedlings. (b) The effect of ABA on net ^{86}Rb influx intact bean seedlings placed in 0.5 mM KCl+ 0.1 mM $CaSO_4$. Solid symbols : Control ; open symbols : 1×10^{-6}M ABA. (○) H^+ efflux; (△) ^{86}Rb transport. Each point represents the means of four replicates (12 plants). The bars represent $\pm$ standard error (Karmoker and Van Steveninck, 1979a).

by ABA-induced inhibition of both H^+ extrusion and ^{86}Rb transport in intact bean root systems (Fig. 6.1).

Effects of Plant Hormones on Ion Transport in Excised Plant Tissues

Excised roots have long been used in ion transport research (Van Steveninck 1975, 1976b, 1978) because it is a simple and reproducible tool to study ion uptake without any interference from metabolic and physiological functions operating in the whole plant.

Auxins

A growing number of reports regarding the effect of auxin on ion transport has offerred explanations in terms of cation exchange theory of ion transport. Thus, IAA was reported to stimulate Rb^+ influx into pea epicotyl segments, slices of rutabaga *(Brassica napobrassica)* and potato tuber tissue (Higinbotham *et al.*, 1953) and K^+ uptake by sunflower hypocotyl segments (Ilan, 1973) and *Mnium* leaves (Luttage *et al.*, 1972). IAA caused a stimulations of K^+ or Rb^+ uptake in sunflower hypocotyl segments with an concomitant inhibition of NH_4^+ uptake. It was suggested that K^+, Rb^+ and NH_4^+ compete for a same carrier site (Ilan and Reinhold, 1963). Sucrose (0.05M) nullified the stimulatory effect of IAA on K^+ uptake in presence of NH_4^+ but this could be reversed if NH_4^+ in the medium was replaced by Na^+ (Ilan and Reinhold, 1964). In another instance, $\alpha-$NAA treatment strongly stimulated ^{36}Cl uptake by coleoptile cells within 15 min of application over a wide range of external chloride concentrations. Such a stimulation did not occur at 0° C or in the presence of CCCP indicating that it is an energy linked process (Rubinstein, 1973; Rubinstein and Light, 1973). On the other hand, IAA was found to have no effect on ^{86}Rb and ^{36}Cl transport into excised maize roots (Weigal, 1969) and beet root slices (Van Steveninck, 1974) while it inhibited ^{36}Cl transport in root callus cells of *Petroselinum sativum* within 30 min of treatment, and had no effect on $^{86}Rb/K^+$ influx (Bentrup *et al.*, 1973). On the contrary, (Stout *et al.*, 1978) reported an IAA-induced stimulation of ^{86}Rb uptake in *Avena* coleoptile sections within a lag period of 15 to 20 min. In contrast, FC caused a rapid stimulation of ^{86}Rb uptake with a lag period of only one minute.

Further stimulation of K^+ uptake by IAA was observed in pea internode segments (Lado *et al.*, 1976), in bean cuttings at 4 h of application during the first stage of the development of adventitious roots (Yakushina *et al.*, 1977), in hypocotyl sections, in homogenate extracts and a microsomal fraction of kidney bean (Yakushkina *et al.*, 1979) and in barley

coleoptile segments placed in tris-buffer solution (Kholdebarin, 1981), IAA increased K^+, Na^+ uptake in pea internode segments and at low concentrations it favoured K^+ over Na^+ (Lado *et al.*, 1976). Further more, IAA and 2, 4-D increased the influx of $^{86}Rb/K^+$, ^{22}Na and ^{36}Cl in suspension cultured cells of parsley after an initial reduction of apparent influx of these ions within 30 min of application (Pfruener and Bentrup, 1978).

In contrast, auxins were also found to inhibit ion uptake in excised plant tissue. For example, Hourmant and Penot (1978a) found that IAA inhibited the increase in the rate of phosphate absorption. Recently, Poder *et al.* (1981) showed that the increase in the rate of phosphate uptake in aged potato tuber discs was partially prevented by addition of IAA at a final concentration of 50 μM in the medium. It also inhibited the incorporation of ^{32}P in different fractions of phospholipid without any changes in total phospholipid. The data supported the hypothesis that IAA specifically affects the development of the uptake mechanism during the aging period. Similarly, 2, 4-D at a concentration of 0.01mM in the incubation medium with a low pH caused an inhibition of K^+, NH_4 and NO_3^- uptake in rice roots. Lowering the pH caused a rapid entrance of 2, 4-D into the root and consequently an inhibition of ion uptake (Zsoldus and Haunold (1982), Castro and Oliviera (1982) also found that IAA decreased the Na,Ca and Mg content in the stem of soybeans. Ilan and Shapira (1979) observed that IAA inhibited the release of K^+ from hypocotyl segments of sunflower bathed in 2mM sodium phosphate buffer. However, this effect was counteracted by the addition of 20mM Na_2SO_4 to the medium. Recently, Buckhout *et al.* (1981) found that IAA promoted the release of calcium from membrane pellets of soybean hypocotyls at auxin concentrations ranging from 1 nM to 1 μM. In this case, the calcium release is accompanied by a decrease in calcium binding sites in the membrane. However, 2, 4-D did not promote Ca^{2+} release but promoted in some cases, Ca^{2+} association with the membrane. IAA also released Mn^{2+} from the membrane pellet in a manner similar to that of Ca^{2+}.

Gibberellins

Gibberellins did not receive much attention as far as their effects on ion transport is concerned. Much earlier, Van Steveninck (1961) found that GA_3 at a relatively high concentration (10^{-4}M) increased the leakage of K^+ and Na^+ from freshly sliced beet root discs. This increase in

K^+, Na^+ leakage was attributed to an GA_3-induced increasc membrane permeability. GA_3 ($10^{-8}$to 10^{-5}M) reduced the time required for the onset of Na^+ uptake capacity from 22 to 8 h but had no effect on this phenomenon with respect to K^+ uptake. Ten years later, Eastwood and Laidman (1971) found that gibberellins from germinating embryos initiated the release of K^+, Mg^{2+}, Ca^{2+} and inorganic phosphate which were retained in the cells by the action of a cytokinin like hormone from the endosperm. This induction process was counteracted by ABA.

Recent reports shows that gibberellins stimulate or inhibit ion transport in plants depending on the concentration of hormone used and the type of tissue and ions in question. For example, in a dwarf maize mutant, GA_3 increased the ion content of chloroplast and vacuole but had no effect on the cytoplasmic ion content (Newmann and Janossy, 1977). Kannan (1978) found that GA_3 increased the rate of ^{86}Rb transport from the middle to the base of corn leaves. Similarly, GA_3 at a concentration of 10^{-4}M stimulated both K^+ and water transport in excised cucumber cotyledons (Ezekiel *et al.*, 1978). On the other hand, the increase in phosphate uptake during aging of potato tuber discs was decreased by GA_3 (Hourmant and Penot, 1978b). In another instance, Bartolome and coworkers (1981) found that application of GA_3 together with kinetin is essential for delaying the loss of phosphate from senescing leaf segments of barley.

Abscisic Acid

Action of ABA on Stomatal Guard Cell Mechanism

ABA caused a rapid closure of stomata (Mittelheuser and Van Steveninck, 1969, 1971; Cummins *et al.*, 1971; Kriedemann *et al.*, 1972) while other reports showed that the stomatal closure depends on cations, particularly K^+ transport into guard cells (see Raschake 1979; Purohit, 1983). Stomatal closure by ABA appeared to be due to its direct action on guard cells rather than an effect on water potential throughout the leaf (Horton, 1971). More specifically, ABA was found to inhibit K^+ transport into stomatal guard cell of *Commelina communis* leaves (Mansfield and Jones, 1971). This resûlted in a drop in osmotic potential of guard cells with a little or no effect on osmotic potential of subsidiary cell. Thus, a difference in turgor pressure between guard cells and subsidiary cells was established and consequently stomatal closure occurred. This view was supported by the discovery of a shuttle of K^+ and Cl^- between guard cells and subsidiary cells resulting in a difference in turgor pressure

in these cells (Raschke and Fellows, 1971, Fischer, 1972). Horton and Moran (1972) also reported that in leaves of *Vicia faba* ABA treatment caused a closure of stomata with a concomitant inhibition of K^+ influx into the guard cells. Recently, Weyers and Hillmann (1980) found that when stomata were floated in KCl or RbCl-containing buffer solution, the uptake of ^{86}Rb increased linearly with the degree of opening of stomata. However, ABA decreased ^{86}Rb uptake into the gurad cell region within 80 min of incubation and at the same time caused closure of stomata. Efflux of tracer from ^{86}Rb-loaded epidermal tissue increased following the treatment of ABA. In another instance, Itai and Meidner (1978a, b) found that ABA decreased the accumulation of neutral red in the guard cell while it increased the rate of accumulation and final concentration of neutral red in the adjacent epidermal cells. However, there exists a controversy as to whether the shuttle of ions between guard cells and accessory cells occurs by means of symplastic or apoplastic transport. Hsiao (1976) believed that in maize, symplastic transport of ions is unlikely because of the absence of plasmodesmata between guard cells and the surrounding cells. This view was supported by Willmer and Sexton (1979) who found that the plasmodesmatal connections which are present between guard cells and epidermal cells in developing epidermal tissue of *Phaseolus vulgaris* are ultimately lost in fully differentiated tissue. Furthermore, this movement of ions is considered to be passive *i. e.*, down a activity gradient. However, other reports have shown that K^+ accumulation in the guard cells involve an active transport *i. e.* against an electrochemical gradient (Penny and Bowling, 1974).

It appears that the effect of ABA on K^+ fluxes into the guard cells might result from ion exchange but the need for accompanying ions should also be considered in maintaining ionic balance and pH control (Smith and Raven, 1979). In maize, for example, the K^+ shuttle between guard and subsidiary cells was dependent on Cl^- which acts as an accompanying ion (Raschke and Fellows, 1971) while organic acid anions may play a role during K^+ for H^+ exchange in *Vicia faba* (Pallas and Wright, 1973; Raschke and Humble, 1973). Allaway (1973) showed that malate is the principle anion accmulating in guard cells of *Vicia faba*. Raschke (1975) showed that stomatal closure was related to high proton and malate content. The recent work of MacRobbie (1980) with isolated and intact guard cells of *Commelina communis* showed that changes in K^+ content were not sufficient to fully explain the recorded changes in stomatal

aperture which indicated that osmotic adjustment of stomatal guard cells was likely to be dependent on additional solutes such as halides.

Effects of ABA on Water Transport and Hydraulic Conductivity and its Relation to Ion Transport

Abscisic acid appears to play an important role in the regulation of water and the ionic balance in plants (Van Steveninck, 1983). The best known effect of ABA is the induction of rapid closure of stomata (Mittelheuser and Van Steveninck, 1969, 1971; Cummins *et al.*, 1971 and Kriedemann *et al.*, 1972) which in turn reduces the rate of transpiration (Little and Eidt, 1968; Mizrahi *et al.*,1970). However, experiment with excised roots/root systems have shown that ABA may cause a dramatic increase of water transport in decapitated tomato plants (Tal and Imber, 1971), excised sunflower roots (Glinka, 1973) and excised maize roots (Collins and Kerrigan, 1973, 1974). This increase in volume flow was attributed to an increase in hydrulic conductivity of root cell membranes (Glinka and Reinhold, 1971, 1972; Glinka, 1973). However, Cram and Pitman (1972) finding that ABA inhibited both water and ion transport through the cut end of barley roots claimed that the decrease in water transport was primarily due to an inhibition of ion transport and that hydraulic conductivity (L_p) remained unaltered by ABA treatment. This view was supported by Pitman and Wellfare (1978) who found that

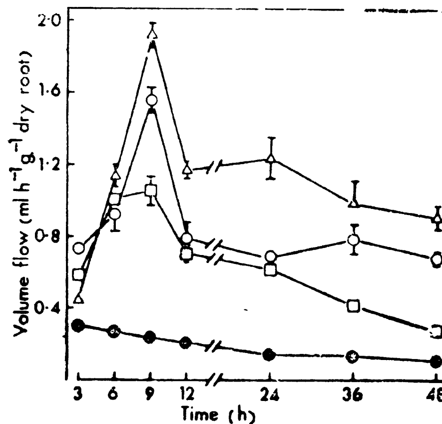

Fig. 6.2. The effect of different concentrations of ABA on volume flow in excised root systems *Phaseolus vulgaris* cv Redland Pioneer bathed in 0.5mM KCl+0.1mM $CaSO_4$. ● Control; ○ 5x10⁻⁷M ABA; △ 10⁻⁶M ABA; □ 10⁻⁵M ABA. Each point represents the mean of six replicates; the bars represent ± standard error. (Karmoker and Van Steveninck, 1978).

ABA did not affect L_p (excpet for a brief initial increase) while ion transport was inhibited. Glinka (1977) showed that when decapitated sunflower root systems were placed in tritiated water (THO), ABA increased the rate of appearance of radioactivity in the exudate within 1h of application. The specific activity of THO in the exudate was unaffected by an imposed hydrostatic pressure gradient.

Karmoker and Van Steveninck (1978) found that in excised bean root systems, ABA (10^{-6} to 10^{-5}M) caused a 6- to 8- fold increase in the rate of volume flow (J_v). The increase in J_v was apparent within 3h of treatment and was sustained over a period of 48h (Fig. 6.2). It was suggested that the maintenance of volume flow over a period in excess of 48h could not be ascribed to a transient change in hydraulic conductivity and also the increase in volume flow was shown to be dependent on the stimulation of solute flow from the external solution into the xylem. Recently, this work was supported by Fiscus (1981) who found that ABA caused an initial increase in J_v matched by ion transport in bean *(Phaseolus vulgaris)* root systems. Then, this was followed by a long term ABA-induced increase in solute transport caupled with a decrease in hydraulic conductivity. The interdependence of transport of water on that of ion was also evident from the fact that ABA-stimulation of both water and ion transport was nullified by carbonyl cyanide-m-

Table-2. Interaction of CCCP and ABA on ^{42}K transport in excised bean root systems bathed in 0.5mM KCl + 0.1mM $CaSO_4$ over a period of 3h. (Karmokar and Van Steveninck, 1978).

Treatment	Rate of ^{42}K transport as % of control	Rate of volume flow as % of control
Control	100	100
10^{-6} M	479	255
10^{-6} M ABA + 5μM CCCP	75	99
10^{-6} M ABA + 10μM CCCP	71	96

chlorophenyl hydrazone (CCCP) (Table-2, Karmokar and Van Steveninck, 1978). On the contrary Erlendsson and coworkers (1978) have reported that ABA did not affect water transport but decreased the transport of ^{86}Rb and ^{32}P into the xylem of excised sunflower roots within 30 to 70 min while Behl and Jeschke (1979) reported a severe inhibition of both water and ion transport in excised barley roots. Similar inhibitory effects of ABA were also found in suflower hypocotyl segments (Dorffling, 1973) and in barley roots (Carm and Pitman, 1972;

Pitman and Carm, 1973; Pitman *et al.*, 1974a and b). Generally the recorded changes in water and ion transport support the theory that exudation in excised root systems result from a difference between the osmotic potential of the external solution and that of xylem sap (Anderson, *et al.*, 1970).

Glinka (1980) found that the discontinuation of external K^+ supply to sunflower roots resulted in a rapid decrease in the rate of volume flow and K^+ transport. Under this condition, addition of ABA restored the rate of water transport, presumably by increasing the hydraulic conductivity. Glinka (1980) suggested that ABA may cause a release of K^+ from vacuoles which would facilitate its role in subsequent transport to the xylem. This view is also supported by further work of MacRobbie (1983) who has suggested that the ABA-induced K^+ release from the vacuole may be an active process. However, Markhart II *et al.* (1979) showed that 5×10^{-6}M and 2×10^{-4}M ABA caused a decrease in hydraulic conductivity of soybean roots.

Effects of ABA on Ion Transport in Excised Tissue

Reports on the effect of ABA on ion transport in excised tissues are contradictory. For example, ABA increased net uptake of K^+, Na^+ and Cl^- in beat root discs (Van Steveninck, 1972a) while it inhibited K^+ uptake by leaf slices of *Vicia faba* but did not affect K^+ transport in leaf discs of mature nonexpanding leaves (Horton and Bruce, 1972). The latter was supported by the work of Reed and Bonner (1974) who found that ABA caused a decreased in K^+ and Cl^- uptake by *Avena* coleoptile sections. ABA caused in increase of K^+ flux into the xylem of isolated maize roots (Collins and Kerrigan, 1973, 1974) while it inhibited K^+, ^{86}Rb and Cl^- transport into the xylem of excised barley and maize roots (Cram and Pitman, 1962; Pitman and Cram. 1973; Pitman *et al.*, 1974a, 1974b and Dieffenback *et al.*, 1980).

Kannan (1978) observed that 25 ppm, ABA increased the rate of transport of ^{86}Rb from the middle to the base of young corn leaves. Pandey and Kannan (1976) found that in bean plants ABA (10 μg/ml) increased the movement of ^{86}Rb and ^{59}Fe from the primary leaves (site of application) to the roots. Similarly, ABA increased the transport of Fe^{2+} supplied to the roots to the trifoliate leaves. On the other hand, Cocucci and Cocucci (1977) showed that ABA-induced inhibition of seed germination is accompanied by an inhibition of K^+ uptake which is reversed by fusicoccin and to some extent by GA_3.

The alternatives of stimulatory or inhibitory effects may depend on the concentration of hormone used. For example, low concentration of ABA ranging from 0.01 to 0.1 ppm increased Ca^{2+} content in *Lemna gibba*. However, higher concentration of ABA (1 to 10 ppm) decreased Ca^{2+} as well as the K^+ and P content of fronds but also caused an increase in N content. The uptake of K^+ and P was also related to change in dry weight of the fronds. Corresponding reduction of P to Fe and Fe to Ca ratios were also observed in *Lemna gibba* (Dekock *et al.*, 1978).

Migliaccio and Rossi (1977) have found that ABA, at a concentration of 10^{-5}M increased the transport of anions like chloride and sulfate into the xylem of excised roots at 22°C over a period of 24h. The stimulatory effect was apparent within 1h of treatment. On the contrary, Hemberg (1978) found that ABA inhibited P uptake in discs of potato pith while Palmer (1981) has shown that ABA increased NO_3^- accumulation and nitrate reductase activity in fresh potato tuber slices incubated in 0.1 to 5mM KNO_3.

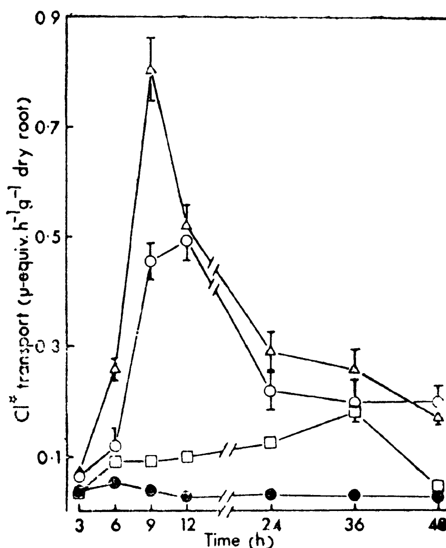

Fig. 6.3. The effect of different concentrations of ABA on ^{36}Cl transport in excised root systems of *Phaseolus vulgaris* cv. Redland Pioneer bathed in 0.5mM KCl + 0.1mM $CaSO_4$. ● : Control; ○ 5×10^{-7}M ABA; △ 10^{-6} M ABA; □ 10^{-5}M ABA. Each point represents the means of six replicates; the bars represent ± standard error (Karmoker and Van Steveninck, 1978).

In a demonstration of ABA effeets on ion transport, Karmoker and

Van Steveninck (1978) reported that in bean root systems ABA caused a 19 - to 20 - fold increase in ^{36}Cl flux (Fig. 6.3) and a 7- to 8-fold increase in ^{42}K flux into the xylem. The effect was quite prolonged *i. e.* the stimulatory effect was sustained over a period of 48h of treatment (Fig. 6.4). This finding was supported by Fiscus (1981) who

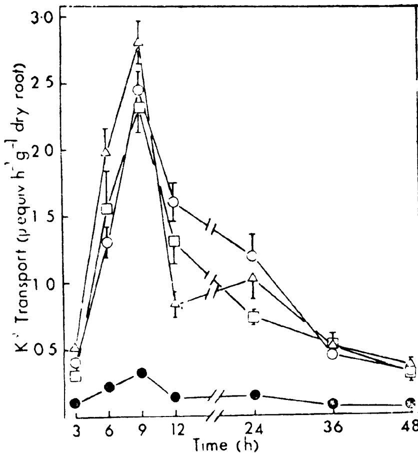

Fig. 6.4. The effect of different concentrations of ABA on ^{42}K transport in excised root systems of *Phaseolus vulgaris* cv. Redland Pioneer bathed in 0.5mM KCl + 0.1mM $CaSO_4$. ● Control; ○ 5× 10^{-7}M ABA; △ 10^{-6}M ABA; □ 10^{-5}M ABA. Each point represents the mean of six replicates; the bars represent ± standard error. (Karmoker and Van Steveninck, 1978).

found that ABA caused a long term stimulation of ion flux in bean root systems. Moreover, Karmoker and Van Steveninck (1978) also fonnd that ABA-induced stimulation of ^{42}K transport into the xylem occurred in root systems placed in a widely different concentration of KCl (e. g., 0.5mM to 100mM. Most interesting point is that a reversal of the relevant electrochemical gradient of either K^+ or Cl^- is likely to occur along the wide rang of KCl concentration and as a result, a reversal of the effect on exudate flow should take place. The fact that ABA increased both K^+ transport and volume flow over a wide range of concentrations led to the suggestion that ABA may affect energised process of ion transport (Karmoker and Van Steveninck, 1978). ABA caused similar but less pronounced effects on ^{24}Na transport *i.e.* 5×10^{-7}M and 10^{-6}M ABA caused a 2 – fold stimulation of ^{24}Na transport into the xylem after 24h of application. Moreover, 10^{-6}M ABA caused approximately 2 – fold increase in vacuolar accumulation of ^{36}Cl and ^{24} Na respectively into the vacuoles of root cells but had no effect on ^{42}K transport into these root cells (Karmoker and Van Steveninck, 1978). These results are supported by Glinka's (1980) observations that ABA increases ion flux into the xylem and volume flow in sunflower roots. He suggested that ABA increased the release of ions from the vacuole to the cytoplasm (ψ_{vc})

and then from the cytoplasm to the xylem (ψ_{cx}). In contrast, Behl and Jeschke (1979) observed that ABA caused 93–98% inhibition of K^+ and Na^+ transport in excised barley roots which are placed in 0.2mM external solution of the respective ions with a 2–3h period of treatment. Withdrawal of ABA from the solution caused a reversal of ABA effects. However, ABA caused a permanent inhibition of K^+ transport when sulfate and chloride were the accompanying anions. ABA increased the vacuolar accumulation of K^+ when NO_3^- was the accompanying anion. This increase was ascribed to a decrease in K^+ accumulation by the roots before they approached saturation. With respect to Na^+, both vacuolar accumulation and uptake by roots was increased by ABA. Latter on, Behl and Jeschke (1981) found that ABA significantly increased tonoplast transport (ψ_{cv}) of K^+ and Na^+ in excised roots of barley. Thus, a drastic reduction of K^+ and Na^+ transport into the xylem occurred. They suggested that this latter effect of ABA was responsible for the observed decrease of K^+ influx (ψ_{oc}) at the plasmalemma.

Kinins

Action of Kinins on Water Transport and Hydraulic Conductivity

Kinins play an important role in regulating salt and water balance in plants. Kinetin was reported to cause stomatal opening and an increase in the rate of transpiration in barley leaves (Livne and Vaadia 1965), but a decrease in volume flow (J_v) in isolated maize roots (Collins and Kerrigan 1973, 1974) and excised tomato root systems (Tal and Imber, 1971). The decrease in water transport was attributed to an increased hydraulic resistance of the root system (Tal and Imber 1971).

Karmoker (1982) found that kinetin (10^{-6}M to 5×10^{-6}M) caused 34 to 47% inhibition of volume flow over a time span from 12 to 48h of application while a higher concentration of kinetin in the medium (10^{-5}M) caused a progressive inhibition of J_v from 3h to 48h of treatment. Recently, Dieffenbach and coworkers (1980) reported that benzyladenine (BA) (5×10^{-8}M to 10^{-6}M) and kinetin (5×10^{-6}M) progressively decreased volume flow with a concomitant decrease in hydraulic conductivity. Kinetin-induced inhibition of volume flow was also accompanied by a decrease in ion transport (Collins and Kerrigan 1973, 1974, Glinka 1980, Karmoker 1982).

Effects on Ion Transport

It is apparent from the previous discussion that kinins have effects on volume flow and hydraulic conductivity opposite to that of ABA. This observation to possible effects of kinins on ion transport. In expanding primary leaves of intact bean plants, BA inhibited the rate of Na^+ absorption (Jacoby and Dagan, 1970). BA also inhibited Rb^+ and molybdate uptake by slices of potato (Hourmant and Penot, 1973a, 1973b) and other reports on experiments with excised plant tissues have shown that kinetin decreased the transport of K^+, Ca^{2+} and Cl^- into the xylem of isolated single maize roots (Collins and Kerrigan, 1973, 1974). Kinetin increased the transport of K^+ and Rb^+ in excised sunflower cotyledons (Ilan *et al.*, 1971) and appeared to affect K^+/Na^+ selectivity (Ilan *et al.*, 1971, and Ilan, 1981). Ilan (1971) found that kinetin increased K^+ uptake while it decreased Na^+ uptake into leaf discs of *Helianthus annuus*.

The rate of exudation from isolated single roots of honey locust (*Gleditsia triacanthus*) increased following the removal of root tips. This stimulatory effect was nullified by 5×10^{-6}M kinetin with a subsequent inhibition of exudation coupled with a decrease in ^{86}Rb transport (Hong and Sucoff, 1976). Glinka (1980) found that kinetin inhibited the transport of ions into the xylem of sunflower roots.

BA (10μg/ml) stimulated the migration of Rb^+ and Fe^{2+} from leaves (sits of application) to the roots of bean. Similarly, kinetin increased the transport of Fe^{2+} from roots to the primary and trifoliate leaves when it was supplied to the roots (Pandey and Kannan, 1976). In excised maize roots, kinetin inhibited the translocation of chloride and sulfate after 6h of application but had no effect on chloride efflux (Migliaccio and Rossi,1977). On the other hand, BA stimulated the uptake of water and K^+ in excised cucumber cotyledons (Ezekiel *et al.*, 1978). This work was supported by Prasad *et al.* (1978) who found that BA stimulated the uptake of both K^+ and Na^+ but the stimulation of K^+ uptake was larger than that of Na^+ when the excised cucumber cotyledons were bathed in equimolecular concentrations of KCl and NaCl.

Dieffenbach *et al.* (1980) found that kinetin decreased J_V and ^{42}K flux into the xylem of barley roots. Similarly Karmoker (1982) found that in bean root systems, kinetin (10^{-6} to 10^{-5}M) inhibited ^{42}K transport into the xylem by 55% after an initial promotion which ranged from 45

to 76% at 3 to 12h of treatment. In aged beet root tissue, kinetin and BA were found to prevent the net accumulation of K^+, Na^+ and Cl^- (Van Steveninck 1972b). This was supported by Hourmant and Penot (1978a, and 1978b) who found that BA, furfuryl aminopurine (FAP) and methyl aminopurine partially prevented the development of phosphate uptake capacity in ageing discs of potato tuber which normally shows a large increase in phosphate absorption in aerated liquid medium. Hourmant and Penot (1978a) found that the degree of inhibition depends on the time of ageing and also on the concentration of BA. It was apparent from this work that the pattern of inhibitory effect of kinetin of ion uptake capacities was very similar to that of cycloheximide and actinomycin – D (Van Steveninck and Van Steveninck, 1972).

Effects of Plant Hormones on Ion Transport in Whole Plants: Reconciliation of Results obtained with Excised Tissue versus Whole Plants

Earlier consideration of effects of phytohormones on ion transport in excised tissue poses the question whether they also apply to whole plants. Reports on the effect of plant hormone on ion transport in whole plant are relatively rare. Quite early Swenson and Burström (1960) showed that α–NAA over a range of concentrations of $10\times$ to 3×10^{-5}M decreased cation uptake in intact wheat plants. The degree of inhibition was in the order of $K^+ > Na^+ > Mg^{2+} > Ca^{2+}$. Most recently, Castro and Oliviera (1982) showed that IAA decreased Na, Ca, and Mg content in the stem of intact soybean plants. Thus, the inhibition of ion transport by auxin in intact plants (Swenson and Burström 1960; Castro and Oliviera, 1982) is opposite to the stimulatory effects of auxins observed in excised plant tissue (see Table 1).

Pitman *et al.* (1974a) found that gibberellic acid (GA_3) had no effect on transport of ions from root to shoot in intact barley seedlings. However, GA_3 did stimulate K^+ uptake in NaCl—treated bean plants. Further more, it increased P and Ca^{2+} content in metabolically active organs and decreased the accumulation of Na^+ (Stark and Kozinska, 1980). GA_3 also increased the uptake of Ca^{2+} in seedlings of *Pisum sativum* cv. Progress and to lesser extent that of Mg^{2+} and K^+. On the other hand, it decreased the uptake of N, P in cultivars Alaska, Progress and Dark Skin Perfection (Garcia and Guardiola, 1981). They suggested that stimulation or inhibition of ion uptake by GA_3 is influenced by the size and geometry of the root system, the sink strength of the shoot, the

variety of the plant and ions in question. Moreover, Castro and Oliviera (1982) showed that GA_3 increased the K^+ content of leaves and stem and the S content of the stem in soybean. However, it reduced the Ca^{2+} and Mg^{2+} content in the stem. In fact, the stimulatory effect of GA_3 on ion transport in whole plants agrees well with the GA_3– induced increase in ^{86}Rb translocation in isolated corn leaves (Kannan, 1978) and K^+ transport in excised cucumber cotyledons (Ezekiel *et al.*, 1978).

Abutalybov and coworkers (1975) showed that some analogues of kinins slightly inhibited and other had no effect on K^+ and phosphorus transport in intact pumpkin plants depending on the concentration of kinins used. Karmoker (1981) found that kinetin inhibited the uptake and transport of ^{22}Na, ^{36}Cl and ^{42}K in intact bean seedlings in a manner similar to that in excised root systems (Karmoker 1982). Kinetin and BA also decreased the transport of K^+ in the guttation fluid of intact barley seedlings (Dieffenbach *et al.*, 1980).

Cram and Pitman (1972) found that ABA inhibited K^+ and Cl^- transport into the xylem of excised barley roots while it caused an approximately 49% inhibition of ^{36}Cl transport into the shoot of intact barley seedlings yet, they recorded a 19% increase of ^{36}Cl accumulation in the roots. This result was supported by Shanner *et al.* (1975) who found an inhibition of K^+ uptake both in excised maize roots and intact seedlings. In sharp contrast to these results, ABA caused a dramatic increase in ^{36}Cl, ^{42}K and ^{24}Na transport into the xylem of excised bean root systems. It also increased the accumulation of ^{36}Cl and ^{24}Na in the roots but not that of ^{42}K (Karmoker and Van Steveninck, 1978). However, in whole bean seedlings, ABA inhibited the long distance transport from the root to the shoot of ^{36}Cl, total chloride, ^{22}Na and total Na^+ after a brief initial promotion. However, long distance transport of ^{42}K and total K^+ was consistently inhibited by ABA. Accumulation of Na^+ and Cl^- increased in intact roots while that of K^+ was decreased by ABA (Karmoker and Van Steveninck, 1979a). Similarly, in intact barley seedlings, ABA decreased K^+ transport in the guttation fluid from passive hydathodes after an initial promotion during first 2h of application (Dieffenbach *et al.*, 1980). Thus, the increase in the accumulation of Na^+ and Cl^- in roots and transient increase in transport of these ions to the shoot by ABA is to some extent consistent with its stimulatory effects on excised root systems. The greatest discrepancies between whole plants and excised roots were observed with

respect to K^+ transport into the xylem in excised bean root systems while it caused a substantial inhibition of ^{42}K transport in intact bean seedlings (Karmoker and Van Steveninck, 1978). Discrepancies are to be expected because the two systems are completely different from each other.

Based on the possible ABA effects on two key processes, a simple model is proposed which may represent the mechanism of ABA-mediated ion transport in intact plants as compared to that of excised root systems

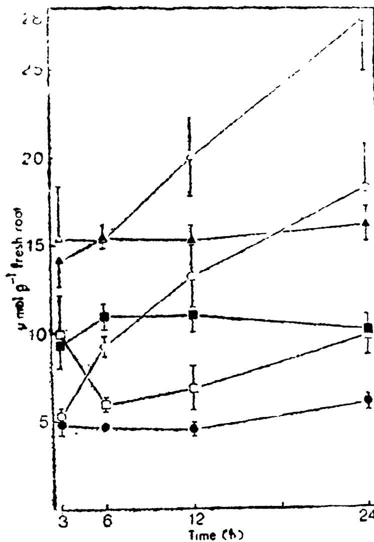

Fig. 6.5. The effect of ABA on sugar content root systems of intact been seedlings placed in 0.5mM KCl+0.1mM $CaSO_4$. Solid symbols, Control; open symbols. $1x10^{-6}$M ABA. ● ○ reducing sugar ▲ △ total sugar; ■ □ nonreducing sugar. Each value represents mean of six replicates; the bars represent ± Standard errors of the mean. (Karmoker and Van Steveninck, 1978).

(Fig. 6.5). These two processes which operate in intact seedlings are (a) co-transport of sugar and ions in the phloem and (b) a negative feed back system and it may be assumed that are removed in excised root systems. Karmoker and Van Steveninck (1979b) have shown that ABA may stimulate the transport of sugar from the shoot to the root. This transport may be associated with an increase in transport of ions in the phloem because co-transport of sugar and inorganic ions has been reported (Menzel and Haider, 1977; Hutchings 1978a, 1978b). Hence, an increased removal of ions from the plant tops via the phloem may create a demand which in turn, will stimulate the removal of ions from the xylem vessels thus creating a rapid circulation of ions from the shoot to the root in the phloem and from the root to the shoot in the xylem (Fig. 6.5). A possible K^+ recirculation of this kind in plants has already been suggested (Ben Zioni *et al.*, 1971; Armstrong and Kirby, 1979).

Further more the uptake and long distance transport of ions in ABA-treated seedlings may be controlled through a negative feed back system. Negative feed back is defined as flow of information "input" from "output" (concentration of substances, osmotic pressure etc.)to controlled transport processes. Thus, an increase in "output" value will cause a decrease in the rate of controlled processes and vice versa (Cram, 1976). In ABA-treated intact seedlings the ionic concentration in the xylem may act as an "output" where ψ_{oc} and ψ_{cx} are the controlled transport processes. An ABA-induced decrease in transpiration may lower the removal of ions from the xylem. Consequently, the concentration of inorganic ions in the xylem vessel will rise. In order to maintain a constant "output" value, "input" will be signelled to the ion pumps (controlled processes). The function of these pump which may operate at the xylem parenchyma or endodermis (ψ_{cx}) and plasmalemma (ψ_{oc}) may be affected accordingly (Fig. 6.6). Thus, this model provides an adequate explanation for ABA-induced inhibition of K^+ and ^{86}Rb influx and long distance transport of Na^+, Cl^- and K^+ in hole plants and stimulation when control process have been removed by decapitation.

It was estimated that the net influx of chloride and sodium in intact seedlings either increased or remained constant under the influence of ABA while at the same time fluxes of these ions into root vacuole were increased (Karmoker and Van Steveninck, 1979a). It is suggested that the ABA-induced increase in influx of chloride and sodium and the concomitant decrease of their long distance transport (ψ_{cx}) will lead to an increase in the ionic concentration of the cytoplasm. This increase in ionic concentration of the cytoplasm may trigger or stimulate the flux of sodium and chloride into the vacuole (ψ_{cv}), and hence, the cytoplasmic concentration of these ions may be maintained at a 'reference level'. However, this suggested feed back system may not operate in excised root system having "open" vessels (Fig. 6.6) and thus ABA may continue to cause an increase in ion transport (Karmoker and Van Steveninck, 1977).

Action of Fusicoccin on Ion Transport

Fusicoccin (FC), a nonspecific phytotoxin produced by *Fusicoccin anygdali* Del. is characterised as a diterpene glucoside with a complex ring system (Ballio *et al.*, 1968; Chain *et al.*, 1971). The principal physiological effects of fusicoccin are an increase of cell permeability, stimulation of respiration (Van Steveninck 1976a; Penot *et al.*, 1981), and H^+ extrusion in a manner similar to that caused by IAA.

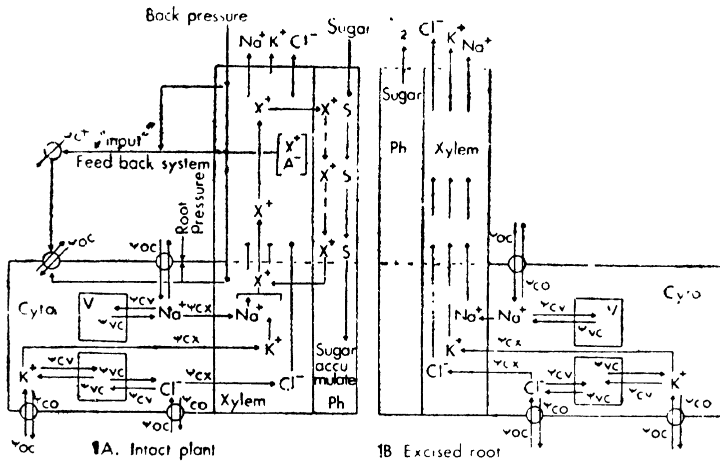

Fig. 6.6. A diagramatic representation of the effect of ABA on ion transport in roots and the mechanism involved (Karmoker, 1981). X^+ = Cations (e. g. K^+, Na^+), S = Sugar, [X^+/A^-] = Concentration of ions (cations and anions), Ph. = Phloem, Cyto. = Cytoplasm, V = Vacuole, ψ_{oc} = Flux of ions from the outside solution into cytoplasm, ψ_{co} = Flux of ions from cytoplasm into the outside solution, ψ_{cv} = Flux of ions from cytoplasm into vacuole, ψ_{vc} = Flux of ions from vacuole into the cytoplasm.

The stimulatory effect of fusicoccin on H^+ extrusion was discussed earlier. This phenomenon is normally associated with a stimulation of monovalent cation transport particularly of K^+ and Na^+ but other cations and anions may also be affected (Marre, 1979). FC increased the uptake of K^+ into pea internode sections, excised maize roots, detached squash cotyledons (Marre *et al.*, 1974a, 1974c), excised barley roots (Pitman *et al.*, 1975a, 1975b), *Avena* coleoptile sections (Cleland, 1976b); apical root segments (Lado *et al.*, 1976a), pea stem segments (Lado *et al.*, 1976b) and dawrf maize coleoptiles (Nelles, 1978). Furthermore, Cocucci and Co-workers (1976) found that in excised root apices of maize FC stimulated the uptake of ^{42}K, ^{22}Na and ^{134}Cs uptake in the order of $K^+/Rb^+ > Na^+ > Cs^+$.

In *Avena* coleoptile sections FC caused a rapid stimulation of ^{86}Rb uptake which was apparent within 1 min of application (Stout *et al.*, 1978). Cocucci and Cocucci (1977) observed that FC reversed the ABA-induced inhibition of development of K^+ uptake capacity in germinating raddish seeds. Aldasore and co-workers (1981) also found that FC counteracted the ABA-induced inhibition of K^+ uptake in chick pea seeds, but Marco

and co-workers (1977) showed that FC did not affect passive transport of water and solute in excised living tissue and artificial phospholipid membranes. Kholdebarin (1981) recorded that FC stimulated Cl^- uptake in barley coleoptile segments placed in Tris-buffer in a manner similar to that of IAA. It was suggested that FC and IAA may involve a common mechanism of action.

Fusicoccin also increased the uptake of diverse monovalent and divalent anions like Cl^-, NO_3^{2-} HPO_3^{2-} and SO_4^{2-}, benzenslfonate (reviewed by Marre, 1979).

The red light component of the phytochrome (red, far-red perception) system, filipin B, and polylysine inhibited, promoted or otherwise had no effect on K^+ and Na^+ transport in plants (reviewed by Van Steveninck 1976a).

Ophiobolin B (OPH B), a sesterpene metabolite of *Heliminthosporium oryzae* has a basic structure similar to FC. But, OPH B and FC have opposing effects that is, OPH B nullified FC-promoted H^+ extrusion and K^+ uptake leading to an inhibition of the process (Luciana *et al.*, 1979). In another instance, Tipton and co-workers (1977) found that ophiobolin-A increased the leakage of electrolytes and glucose from roots of maize seedlings.

Ethrel (2-chloroethane phosphonic acid) at a concentration of 20 ppm progressively inhibited the absorption and transport of Rb^+ in wheat seedlings grown in low salt (0. 2mM $CaCl_2$) or high salt (dilute nutrient) conditions. Pretreatment of the root with 20 ppm ethrel for 48h also resulted in a prominent inhibition of Rb^+ transport in low salt roots and to a lesser degree in high salt roots (Kannan, 1977). It was suggested that ethrel may mimick the effect of respiratory inhibitors on ion transport. However, Kannan (1978) recorded that 25 ppm ethrel did not affect the transport of ^{86}Rb from the middle to the base of excised corn leaves. Ethrel (50, 100 or 200 ppm) supplied to the root of bean seedlings decreased the incorporartion of ^{32}P but foliage treatment of ethrel increased the incorporation of ^{32}P into RNA-P, DNA-P, phospholipid-P and phosphoprotein (Karivaratharaju and Thangaraj, 1978). Ferulic acid (0.5 and 1 mM) inhibited ^{32}P uptake by roots of intact soybean seedlings but had no effect on the onward transport of this element to the shoot(McCure *et al.*, 1978).

CCC (2-chloroethyl) trimethyl-ammonium chloride) at a concentration of 2000 ppm increased of K^+ content in the stem and Mg^{2+} content in leaves of soybean (Castro and Oliverira, 1982).

Possible Mechanism of Hormonal Regulation of Ion Transport in Plants

Physico-chemical phenomena which participate in the regulation of ion transport into plant cells are membrane permeability, transmembrane electropotential, ion pumps (e.g., ATPase pump) and ion carriers. Reports on the effect of plant hormone on the above phenomena are scattered over a wide range of plant species. In this section an attempt will be made to co-ordinate this in formation into a composite picture of possible mechanisms of hormone-directed ion transport.

Effects of plant hormones on transmembrane potential

The electrical potential difference between the interior of the cell and external medium acts as a driving force on ions across a membrane. Thus, any change in transmembrane potential difference (or P.D.) will affect ion flux across the membrane.

In many instances, plant hormones were found to change membrane potential. For example, IAA caused a hyperpolarization in dwarf maize coleoptiles in a few minutes (Nelles, 1977). However, it caused no change in the membrane potential of young or old *Mnium* leaf cells (Luttge *et al.*, 1972). In maize root segments, FC caused a rapid hyperpolarization (20-50mV) depending on the external salt concentration (Cocucci *et al.*, 1976). For example, 5×10^{-5}M FC caused a maximum hyperpolarization within six minutes. Fusicoccin-induced hyperpolarization of membrane potential is an enegry-linked process as shown by the rapid reduction of normal and FC-induced membrane potential by low temperature (6°C) and by (p-trifluoromethoxy)-carbonyl cyanide-phenyl-hydrazone (FCCP), an uncoupler of phosphorylation. Fusicoccin-induced hyperpolarization of membrane potential was associated with a stimulation of monovalent cation uptake in maize root segments. This work was supported by Cleland and co-workers (1977) who found that in oat coleoptile, FC caused a hyperpolarization which reached a value of-49 mV and -75 mV within 20s at external K^+ concentrations of 1 mM and 0.1 mM. Nelles (1978) also found that FC caused a hyperpolarization in dwarf corn coleoptile cells within 6-8 minutes. He considered rapid hyperpolarizarion of membrane potential as evidence of the electrogenic nature of H^+ extrusion.

Kinetin (4.6×10^{-8}M) induced a hyperpolarization of 10 to 20 mV of membrane potential in root hairs of intact *Trianea bogotensis* after a short term depolarization for 10 minutes. Removal of the meristematic root tip reduced the K^+ concentration in the root hair and this effect was neutralized by kinetin (Abutalybov *et al.*, 1980).

Kasamo (1981) reported that ABA increased passive K^+ efflux which was accompanied by a depolarization of membrane potential. For example, 2×10^{-4}M ABA decreased the membrane potential from -205 to -170 mV. This was related to K^+ motive force ($\Delta \mu K/F$) responsible for the net passive transport of K^+.

Hyperpolarization generally represent an increase in the driving force for cation transport along the electrochemical gradient (Marre, 1979). This phenomenon may explain the FC - and IAA - induced promotion of transport of K^+, Rb^+ and other monovalent cations. This view is further supported by the fact that K^+ and other monovalent cations are required in the medium in order to maintain FC-induced H^+ extrusion (Marre, 1977).

Effects of hormones on permeability characteristics of membranes

It is apparent from the previous presentation that plant hormones affect the energy-dependent transport of protons and ions in higher plant tissues. It is also likely that such an effect of plant hormones on ion transport phenomena may, to some extent, result from their effects on physico-chemical characteristics of the membrane. Changes in the structure of membrane due to alteration of its lipoprotein composition may change its permeability charactersitics.

James and Bracker (1976) found evidence of IAA-induced change in the ultrastructure of the isolated and *in situ* plasmamembranes. They observed that plasmamembrane were 10-15% thinner in 1 μM IAA than in the control after 20 minutes of exposure. However, calcium had opposite effects *i. e.* membranes treated with 0.5 mM $CaCl_2$ for 20 minutes were 15 to 20% thicker than the control. IAA had a reverse effect on membrane thickness. Furthermore, if a membrane was subjected to a series of alternating treatments of IAA and calcium then the membrane would show the characteristics of the last treatment. This effect of IAA on membrane thickness was specific and temperature dependent. Moreover, Helgerson and co-workers (1976) found that 1 μM IAA increased the microviscosity of the hydrocarbon region of isolated

membranes of soybean hypocotyls as shown by a 25% increase in fluorescence polarization of the probe N-phenyl-1-naphthylamine. This increase in stimulation was temperature dependent and maximum polarization occurred in a temperature range of 12 - 22°C. In addition, Weigl (1969) showed that IAA had a specific affinity for lecithin which facilitated its incorporation in the membrane structure. It was suggested that incorporation of IAA could change membrane permeability to ions.

Marco and co-workers (1977) found that FC does not affect passive transport of urea, thiourea and the influx or efflux of water in artificial phospholipid membrane and its electric conductance. Hence, he concluded that FC-induced stimulation of ion transport is dependent on the interaction of hormone with some specific protein component of the membrane and not on FC-induced changes of its lipid component. Doharmann and co-workers (1977) showed that FC is bound specifically to a protein component of a plasmalemma-enriched membrane preparation.

GA_3 increased the permeability of model phospholipid membrane systems (Wood and Paleg, 1972, 1974). However, Paleg and co-workers (1973) while studying the permeability characteristics of liposomes came to the conclusion that in GA_3 -induced regulation of membrane transport may involve a direct effect on the physical properties of the phospholipid component of natural membranes. This hypothesis was supported by the detection of a GA_3 -lecithin complex by means of nuclear magnetic resonance studies (Wood *et al.*, 1974). Earlier, Luttge and co-workers (1978) suggested that the GA_3- induced increase in electrolyte transport from the root to shoots of pea seedlings within 4h was an indication of a direct effect of GA_3 on membrane permeability. This is because they considered that a period of 4h was too short for a transcription process to occur. This view was opposed by Evins and Varner (1971) who observed that GA_3 (1 μM) caused a 4- to 8- fold increase in the incorporation of ^{14}C-choline into the insoluble fraction of barley aleurone cells within 4h of application which could be reversed by 2.5 ABA within 2h of application.

ABA was found to effect lipid metabolism in a vide range of plant material. ABA increased the palimatic acid (saturated fatty acid) content of saponifiable lipid with a concomitant decrease in unsturated fatty acids such as linolenic and linoleic acid (Khul and Unger, 1974). These changes in fatty acid content of membrane may affect its diffusional permeability constant (P_d) (Trauble, 1971). ABA also affected the phospho-

lipid metabolism in wheat aleurone tissue of imbibed seed (Varty and Laidman, 1976). Moreover, when germination was suppressed by ABA treatment in imbibed dormant embryos of wild oat, a change in phosphotidyl serine coccured, but this effect was not apparent in imbibed nondormant embryos (Cuming and Osborne, 1978). In a recent view Vansteveninck and Van Steveninck (1983) quoted work which showed that environmental stresses like water stress, low temperature stress and salt stress affected both lipid composition and ABA accumulation in plants. The most relevant example in that water stress treatment on bean and barley plants caused an increase in ABA and fatty acid and fatty acids such as decnnaic acid and undecanoic acids in leaves when compared to controls (Willmer *et al.*, 1978).

Using synthetic lipid membranes analogous to those with hydrophilic pores (ionophores), Lee and Collins (1979) found that ABA induced fluctuations in conductance of the lipid membrane. This result supports the view that ABA may directly affect membrane permeability by being incorporated into the structure of the latter. This is in contrast to plant hormones such as GA_3, IAA, ABA and C_2H_4 which had relatively small effects on the conductance of liposome preparations (Parups and miller, 1978).

Effects of Hormones on Ion Pumps and Carrier Mechanism

Ion pumps and ion carriers are two important modes of ion transport in plants which involve expenditure of metabolic energy. However, one important difference between ion pumps and ion carrier is that the former is directly linked to a metabolic process whereas the latter may not be. Ion pumps may be either neutral (e.g. cation change phenomena or co-transport of cations and anions) or electrogenic *i. e.*, transport of a net electric charge across the membrane (Pool, 1978). Ion selectivity is determined by the nature, direction and specificity of ion pumps. Ion carriers are involved in the transport of ions throuh the hydrophilic interior of membranes which would otherwise be less permeable to certain specific ions (Van Steveninck and Van Steveninck, 1983).

It is apparent fron cation exchange phenomenon that influx of potassium is intimately linked to proton extrusion. The latter is specifically associated with a K^+ dependent membrane bound ATPase activity (Marre, 1979). Similarly, the activity of a chloride pump in the salt gland of *Limoniun* is related to specific chloride-stimulated ATPase (Hill and Hill, 1973). Plasmalemma ATPases were found to be involved in K^+ trans-

port in plant cells. This is supported by the fact that inhibitors of ATPase activity like N, N-dicyclohexylcarbodiimide (DCCD), diethylstilbestrol (DES) and actylguanidine led to the inhibition of FC-promoted K^+/H^+ exchange and K^+ uptake (review by Marre, 1979).

Yakushkina and co-workers (1979) have shown that IAA increased K^+ uptake wiht a concomitant increase of ATPase activity in the homogenate extract and the microsomal fraction of kidney bean hypocotyl sections. ATPase activity of the microsomal fraction was Na^+, K^+ and Mg^{2+} dependent. Recently, Scherer (1981) found that 10^{-6}M IAA caused a 50% stimulation of membrane bound ATPase activity in the membrane fraction abtained from pumpkin hypocotyl in the presence of 10^{-4}M phenylacetic acid (PAA). However, in the absence of phenyl acetic acid, IAA also promoted ATPase activity but only when a higher concentration of IAA (10^{-4}M) was used. PAA alone had little or no effect. 2, 4-D, an auxin analogue also stimulated ATPase activity in presence of 10^{-4}M PAA. 2, 3-D and 3, 5-D, inactive stereoisomers of 2, 4-D, did not have any effect on ATPase activity (Scherer, 1981). However, IAA had no effect on ATP content in aged potato tuber disc (Podder *et al.*, 1981).

10^{-5} to 10^{-3}M kinetin caused an increase in adenylate kinase activity in pea seed extract leads to an increase in ATP synthesis (Perl, 1981). In bean cotyledous, ABA inhibited GA_3 – or C – AMP – induced synthesis of Na^+, K^+ stimulated ATPase (Maslowski *et al.*, 1974). Inhibitiors of protein sŷnthesis cycloheximide (CHM) and actinomycin – D had effects on ATPase similar to that of ABA. ABA-induced inhibition of ATPase activity agreès well with ABA-induced inhibition of ion transport in plants. ABA (10^{-5} to 10^{-6}M) stimulated Mg^{2+} activated ATPase activity in epidermal strips of tabacco leaves in light and darkness. The ATPase activity was completely inhibited by CCCP and DCCD (Kasamo, 1979).

Kinetin is known to promote the synthesis of protein (Mothes *et al.*, 1961, Gunning and Barkley, 1963). The decrease in ion transport ellicited by inhibitors of protein synthesis led to the speculation that proteins might act as carriers of ion transport across the plasmamembrane and in transport from the symplasm in to the xylem (Lauchli *et al.*, 1974 and Schaefer *et al.*, 1975). Kinetin-induced stimulation of protein synthesis may explain kinetin-mediated stimulation of ion transport in terms of a carrier concept. However, inhibitory effects of kinetin on ion transport cannot be explained in the light of a carrier concept unless the

effect of kinetin on the synthesis of specific proteins is opposite to that of general protein synthesis. Another example of the divergent effects of kinetin on ion transport and protein syathesis is shown in the work of Waisel and co-workers (1965). They found that kinetin and other analogues of kinins increased the incorporation of ^{14}C – leucine into protein fractions of excised barley roots but did effect th Rb^+ uptake.

Effects of Hormones on Metabolic Processes Involved in Ion Transport

Transport of ions across membranes against a concentration gradient was found to be dependent on metabolic processes like respiration, protein synthesis and photosynthesis. In this section, the effect of plant hormones on different metabolic processes will be examined in order to establish a relation between hormone-induced changes in ion transport and metabolic processes.

On many occasions, it has been shown that the energy requirement of ion transport may be lead to a stimulation of respiration (salt respiration) (Mitchell, 1966, Robertson, 1968). Yakushkina (1977) showed that IAA increased K^+ uptake with a concomitant increase in respiration in bean cuttings. However recently, Podder *et al.* (1981) found that IAA did not affect respiration although it showed a promotion of ion uptake.

In an interesting demonstration of the relationship between hormone-induced changes in respiration and ion transport. Hourmant and Penot (1978) showed that analogues of kinetin like benzylaminopurine (BAP), methylaminopurine (MAP) and furfuryl aminopurine (FAP) partially inhibited stimulation of respiration and phosphate uptake in aged potato tuber discs. ABA, IAA and GA_3 had a similar action with respect to phosphate transport but had opposite effects on respiration. Thus, it was suggested that hormone-induced inhibition of phosphate uptake were most related to changes in respiration. However, Palmer (1966) reported that kinetin inhibits phosphate uptake with a concomitant inhibition of respiration and invertase activity.

Hemberg (1978) found that ABA increased oxygen uptake by potato pith discs. Similarly, Hourmant and Penot (1979) showed that ABA caused a slight stimulation of respiration without any effect on ATP production. On the other hand, ABA partially prevented the stimulation of phosphate uptake in aged potato tuber discs. Recently, Karmoker (1981) found that ABA caused 3- and 2.3 fold increase in oxygen uptake by the roots of intact bean seedlings from 0 to 1.5 and 1.5 – 3h

of treatment respectively. ABA also caused a 37% increase in oxygen uptake in excised root systems of 0-2h and 2-4h of treatment respectively. The ABA-induced increase in respiration may be related to its stimulatory effect on ion transport in excised root systems (Fig. 6.3 and 6.4) and initial stimulation of ion uptake in intact bean seedlings (Karmokar and Van Steveninck, 1979a). However, the stimulatory effect of ABA on respiration does not appear to be a general phenomenon as it was shown not to affect respiration in isolated barley aleurone layers (Chrispeels and Varner, 1966) and in excised cotyledons of *Phaseolus vulgaris* (Yomo, 1971).

Earlier, the effect of kinetin on protein synthesis was discussed in relation to carrier mediated ion transport. To this can be added that inhibitors of protein synthesis like canavanine and CHM severely inhibited IAA-induced and to a lesser extent FC-induced stimulation of H^+ extrusion and K^+ uptake (Cocucci and Dalla Rosa, 1980a). These authors suggested that maintenance of normal protein synthesis is essential for hormone-induced stimulation of growth and ion transport. Similarly, Cordycepin (an inhibitor of uridine incorporation into RNA) prevented IAA and FC-induced stimulation of H^+ and K^+ uptake together with cell elongation in maize coleoptiles (Cocucci and Dalla Rosa, 1980b).

Recently, Karmoker (1981) has shown that ABA slightly decreased soluble protein levels in roots of intact seedlings at 6h after treatment but caused a slight increase 24h after treatment. However, insoluble and total protein levels remained unchanged by ABA application except for a slight increase at 0-3h. Thus, the inhibitory effect of ABA on protein synthesis does not seem to bear any relationship with ABA–induced stimulation of ion transport in bean (Fig. 6.3 and 6.4).

Finally, in order to establish a relationship between the effect of plant hormones on ion transport and there effects on protein synthesis, it would be more useful to carry out a detailed study on the effect of plant hormones on the synthesis of protein(s) which are specifically involved in ion transport. This should be done with emphasis on translation and trancription processes rather than on the total rate of protein synthesis (Glasziou, 1969).

An interesting relationship between the effect of ABA on sugar level and ion transport was observed in bean seedlings (Karmoker and Van Steve-

ninck, 1979b). They found that 10^{-6}M ABA caused a 3-fold increase in the reducing sugar content and about 86% increase in the total sugar content of root tissue of intact bean seedlings after 12-24h of transport (Fig. 6.5). But ABA did not have any effect on sugar level of excised bean root systems (Karmoker and Van Steveninck, 1979b) which indicated that ABA increase the transport of sugar from the shoot to the root. This increase in sugar level in roots of intact bean seedlings (Fig. 6.5) may be closely related to ABA-induced inhibition of ion transport in intact seedlings (Karmoker and Van Steveninck, 1979a). Other reports have shown that plant hormones may be involved in the transport of metabolites in plants (reviewed by Patrick, 1976). Pitman *et al.* (1971) suggested that a relationship may exist between sugar level and ion transport in barley roots.

Role of Endogenous Hormone Levels in Ion Transport

Cline (1976) emphasized that the importance of quantitative estimation of the amount of hormones in tissue while studying their effects on cell metabolism. The study of exogenous hormone treatment on ion transport in excised tissues and whole plants showed that the stimulatory or inhibitory response of these treatments was related to the concentration of hormones used (Abutalybov *et al.*, 1975; Van Steveninck 1976a). In that respect, hormonal action on ion transport may depend on changes in the endogenous hormonal level and balance which may result from such treatments. Therefore, information regarding the uptake and transport of exogenously supplied hormone in excised tissue and whole plants with the subsequently induced changes in endogenous levels will be essential in understanding the mechanism of hormonal action on ion transport.

Karmoker (1981) found that the exogenous supply of ABA caused a dramatic increase in endogenous ABA level in roots of bean. It was shown that ABA levels in excised roots and roots of intact bean seedlings increased by 31-fold and 23 fold respectively within 3h of application. Such massive increases in ABA level should cause a major change in the balance of ABA with other hormones. Furthermore, the ABA concentration gradient in whole plants was reversed (*i. e.*, the ABA content in the root became higher than that in the leaf (Karmoker, 1981). Such a reversal of concentration gradient of endogenous ABA may explain the inhibitory effect on ion transport in intact plants (Karmoker and Van Steveninck, 1979a). However, the dramatic increase in ABA contents did not seem to have any adverse effect on uptake and transport of ions in excised root systems (Karmoker and Van Steveninck, 1978).

In conclusion, the rapid action of FC and IAA on ATPase activity and on K^+/H^+ antiport indicate that these hormones may have a direct effect on ion pumps. In other instances, plant hormones required a relatively long lag period before there effect on ion transport became apparent. This long lag period suggests that hormones may affect ion transport through the modification of molecular constituents of the membrane. The indirect aspects of hormone effects on ion transport may also be apparent through effects on membrance potential and metabolic processes which are linked to ion transport and also via aspects of hormonal balance.

Acknowledgements

The author thanks Professor R.F.M. Van Steveninck, School of Agriculture, La Trabe University and Dr. Margaret E. Van Steveninck, School of Botany, La Trabe University, Victoria, Australia for taking keen interest while writing up the article, going through the final draft of the manuscript, making critical comments and valuable suggestions.

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Growth Regulating Activity of Penicillin in Higher Plants

S.S Purohit and S. Mukherji

Introduction

The first among the modern antibacterial antibiotics and still the most well known is penicillin. It is produced by an Ascomycetous saprophytic fungus *Penicillium (P. notatum* and *P. chrysogenum)*. It was first isolated by Sir Alexander Fleming, Professor of Bacteriology, St. Mary's Hospital, London in 1929, but the extensive use of penicillin as a therapeutic agent against some bacterial infections dates from 1940 when Professor H. W. Florey and his associates working at Oxford University prepared penicillin in a concentrated and stable form. Penicillin shows antibacterial action towards most of the Gram-positive and a few Gram-negative bacteria. It acts by blocking some stages in the biosynthesis of bacterial cell wall mucopeptide (Gale, 1962; Strominger, 1962; Salton, 1964). Such compound might act selectively against bacteria and display little toxicity to mammalian cells (Newton, 1965). As compared to the volume of literature about penicillin action available on bacteria, its role in higher plant metabolism is, on the contrary, little elucidated (Brian, 1957; Anderson, 1968; Anderson and Wood, 1969; Enomoto and Saito, 1972; Kado, 1976).

The regulatory role of penicillin as a plant hormone on various plant physiological processes has recently been investigated in India by the authors. Among the various responses produced by penicillin are induction of α-amylase and ribonuclease synthesis (Biswas and Mukherji, 1979, 1982; Mukherji and Biswas, 1979 a), synthesis and retention of chloroplast pigments (Biswas and Mukherji, 1978; Mukherji and Biswas, 1979 b, 1981; Purohit and Chandra, 1983 a), effects on leaf morphology, stem circumference and internode length (Purohit and Purohit, 1983). formation of endogenous gibberellin and cytokinins (Mukherji and Wareing, 1983), enhancement of rooting of cuttings in *Phaseolus areues* (Bhattacharya *et al.*, 1983) and in *Helianthus annuus* seedlings (Purohit and Bhargawa, 1983; Purohit, 1984), regulation of growth and embryogenesis in cultured carrot tissue (Biswas *et al.*, 1983), inhibition of abscission (Purohit, unpublished). At the molecular level, penicillin effect is mediated through its influence on DNA, RNA and protein synthesis (Mukherji and Biswas, 1983). In view of these properties of penicillin,

it points to the validity of assuming penicillin actions being of hormonal nature. The present review accounts for most of the experimental work done with penicillin on higher plants and reports on its biochemical, morphological and physiological aspects.

Chemistry and Biosynthesis

It is a fascinating question in the field of antibiotics that why these substances are produced by microorganisms. These substances are either natural wastes or byproducts of their metabolism without any significant role. They are produced in detectable concentrations in certain micro-ecological conditions of the soil. The naturally occurring penicillins are the compounds of the monobasic acid-penicillanic acid (6-amino penicillanic acid). All penicillins have common basic nucleus, β-lactam ring which is synthesized through L-cysteine and D-valine (though valine enters the biosynthetic pathway as L-valine). The work carried out in Arnstein's laboratory has conclusively demonstrated that intact cysteine molecule is incorporated into the penicillin molecule. The cysteine in turn is shown to be derived from inorganic sulphate and acetate or formate. During condensation of L-cysteine and D-valine, another amino acid (L-α-amino adipic acid) also participates in biosynthesis. The initial reaction of penicillin biosynthesis is probably a condensation of L-cysteine and L-α-aminoadipic acid. α-Aminoadipyl cysteine condenses with valine and form penicillin.

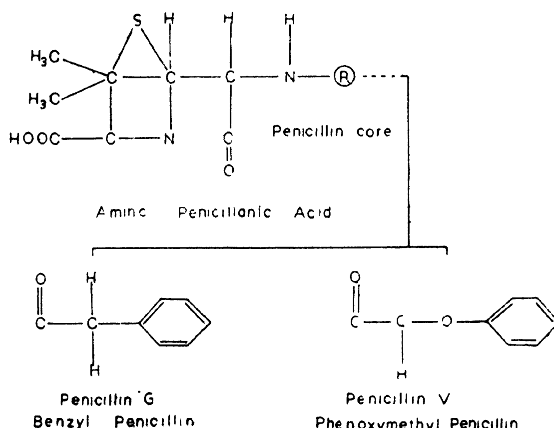

Fig. 7 1. Chemical Structure of Penicillin G and Penicillin V.

New biosynthetic penicillins could be made by feeding different precursors to *P. chrysogenum*. Penicillins can be hydrolyzed by the enzyme

'penicillin acylase' which is widely prevalent among several microbial species. 6-amino penicillainc acid is the product of hydrolysis of penicillin and the same may be used as an intermediate for the preparation of new penicillins by chemical acylation (see Jayaraman and Rangaswami, 1969). Natural penicillins can be prepared as salts of sodium, potassium, procaine and other bases. They are unstable to heat, strong acids, alkalies, cysteine and penicillinase enzyme. The crystalline sodium and potassium salts are freely soluble in water, ethyl alcohol, ether esters and dioxane but slightly soluble in chloroform and benzene. The pure crystalline forms of penicillins are colourless.

Induction of α-Amylase and Ribonuclease Synthesis

Penicillin induces the synthesis of α-amylase, ribonuclease and increases total soluble protein content in embryoless rice endosperms (half seed). Biswas and Mukherji (1979) have observed these effects in the presence and abscence of GA_3. Penicillin (500 parts 10^{-6} concentration) maximally stimulates the synthesis of α-amylase. They have further characterized penicillin-induced α-amylase production using different compounds in the medium. The production was prevented almost completely by actimomycin-D (inhibitor of DNA-dependent RNA synthesis) cycloheximide (inhibitor of protein synthesis) FudR (inhibitor of DNA synthesis), CCC (inhibitor of gibberellin biosynthesis) and 2,4-DNP (uncopuler of oxidative phosphorylation). Data obtained by them are presented in Table 1.

Table 1 : Effects of different compounds on the induction of amylase formation in rice endosperm by penicillin.

Treatment	Maltose released (μg/5 half-seed/5 min)
Water (Control)	230
Penicillin, 100 parts 10^{-6}	590
+ actinomycin-D	250
+ FudR	250
+ CCC	250
+ CCC	210
+ DNP	190
+ Glucose	600

After Biswas and Mukherji (1979).

Presence of glucose in the medium was also found ineffective during penicillin-induced α-amylase synthesis. However, Kessler and Kaplan's results (1972) indicate that the glucose acts as an inhibitor of gibberellin induced α-amylase synthesis.

In conformity with increased production of α-amylase by penicillin, total protein content of the enzyme extract was also increased (Table 2). In this respect penicillin exhibits similar behaviour as observed with GA_3 (Filner *et al.*, 1969) which suggests that the expression of penicillin action needs the synthesis of RNA and protein.

Table 2 : Effects of penicillin with or without GA_3 on the incorporation of labeled amino acids into cellular proteins of rice half-seeds. Each flask contained 30 half-seeds in 2 ml with 10 μmol acetate buffer (pH 4.8), 80 μmol $CaCl_2$ and μCl lable.

Treatment	ct min $^{-1}$/30 half seeds
Water (Control)	1030
GA_3, 10^{-4}M	3273
Penicillin	
100 parts 10^{-6}	2321
500 parts 10^{-6}	2970
1000 parts 10^{-6}	1690
Penicillin 100 parts 10^{-6} + GA_3	2740
Penicillin 500 parts 10^{-6} + GA_3	3387
Penicillin 500 parts 10^{-6} + GA_3	3297

After Biswas and Mukherji (1979)

The inhibition by CCC of α-amylase formation induced by penicillin may be due to control exerted by penicillin over gibberellin synthesis, which is a prerequisite response for α-amylase synthesis via an induction of gibberellin biosynthesis. This speculation was confirmed by measuring the time course of α-amylase synthesis in different concentrations of penicillin with or without GA_3 (10^{-4}M) in rice embryoless half seeds. The studies reveal that penicillin promotes both gibberellin and α-amylase synthesis.

Recently, Biswas and Mukherji (1982) compared the activities of penicillin-induced α-amylase activity with cAMP-induced activity in rice half seeds. When penicillin was applied alone, it markedly stimulated the formation of α-amylase and 500 ppm was found maximally effective. Cyclic AMP, however, excelled over penicillin in inducing α-amylase formation in as much as the concentrations of penicillin required for an indentical amount of α–amylase formation and was more than 10 times that required by cyclic AMP; more than 40% higher activity on the average was obtained than observed with penicillin alone.

Previous data indicate that the increased production of α-amylase by cyclic AMP is limited to increased synthesis rather than both synthesis and secretion typical of GA response (Galsky and Lipponcott, 1969; Barton *et al.*, 1973). Mukherji and his coworkers have proved that

penicillin-caused α-amylase production is characterized by both synthesis and secretion similar to GA but contrary to cAMP-induced response (Biswas and Mukherji, 1982).

A characteristic gibberellin-like activity in the extracts from penicillin-treated endosperms became detectable after 12h from the addition of penicillin to the incubation medium. The gibberellin-like activity is located on paper chromatograms at the Rf typical of GA_3 and its formation is blocked by CCC-an inhibitor of GA biosynthesis.

The time course study of the levels of different constituents and incorporation of ^{14}C thymine and ^{14}C uracil into the half seed suggests that penicillin probably induces DNA and RNA synthesis in the first place, which results in gibberellin biogenesis, and this in turn stimulates the synthesis of both α-amylase and RNase. As a result, endosperm reserves become mobilized rapidly to support embryo growth leading to considerable increase in fresh and dry weights, root and shoot lengths, respiration rate and IAA-oxidase activity. Typical gibberellin responses, viz., second leaf sheath elongation of Taichung Native-1 (a drawf rice cultivar) and reversal of abscisic acid-induced inhibition of α-amylase can also be achieved by penicillin (Biswas and Mukherji, 1982; Mukherji and Biswas, 1983).

Synthesis and Retention of Chloroplast Pigments

Penicillin enhances chloroplast pigments formation in intact rices eedlings (Biswas and Mukherji, 1978), *Helianthus annuus* (Purohit and Chandra, 1983), *Glycine max* (Purohit and Purohit, 1983), *Chrysanthemum* (Purohit, 1984) and mungbean cotyledons greening in presence of light (Mukherji and Biswas, 1979 b). Chlorophyll contents of leaf discs of *Euphorbia pulcherrima* incubated with penicillin are maintained at much higher level and the increment is almost linear as a function of increasing penicillin concentrations (Mukherji and Biswas, 1981). In this respect, penicillin probably mimics cytokinin action through the maintenance of higher nucleic acid and protein levels, and it serves to stimulate the synthesis of these compounds. Joint application of penicillin and cytokinin was found synergistic and enhanced chlorophyll synthesis in *Phaseolus aureus* cotyledons (Table 3).

Penicillin acts as an antisenescence compound by preventing chlorophyll destruction. This action of penicillin is due to its inhibitory action on a chlorophyll degrading enzyme- chlorophyllase. Mukherji and Biswas (1981) found that penicillin treatment of leaf discs caused a maintenance of chlorophyll by lowering of chlorophyllase levels.

During monocarpic senescence in *Helianthus annuus*, the developing fruits cause leaf chlorosis and increase chlorophyllase activity in leaves. Foliar application of penicillin (50-250 ppm) prevented fruit-induced chlorophyll loss (Table 4 and Plate 7.1 [4]).

Table 3. Chlorophyll, carotene and xanthophyll, formation in excised mungbean cotyledons induced by a 48 h period after 72 h germination in the dark. Chlorophyll content expressed as mg l^{-1}. Carotene and xanthophyll contents expressed as absorbancy $\times 10^2$ at 425 and 450 nm respectively.

Treatment	Time embryonic axis attached during dark germination								
	24 h			48 h			72 h		
	Chlo	Caro	Xan	Chlo	Caro	Xan	Chlo	Caro	Xan
Control	2.89	5	20	2.54	2	19	2.06	1	17
Penicillin (250 ppm)	3.73	6	22	2.99	3	21	2.34	1	20
Benzyladenin (10^{-3} M)	4.98	7	32	3.81	5	25	2.91	2	22
Penicillin + benzyladenin	6.81	10	46	6.09	7	40	4.97	5	34

Chlo : Chlorophylls, Caro : Carotene, Xan : Xanthophylls

Table 4. Effect of foliar spray of penicillin on chlorophyll (Chl) content [g kg^{-1} (fr. m.)] and chlorophyllase (Chlase) activity [units] in *Helianthus annuus* plants with (HF) or without (O) heads or with heads without fruits (H).

Plant age [d]	HF Penicillin [g m^{-3}]				O Penicillin [g m^{-3}]				H Penicillin [g m^{-3}]			
	0	50	100	250	0	50	100	250	0	50	100	250
50 Chl	12.7	13.7	14.4	14.7	13.6	15.2	15.8	16.0	12.8	14.5	14.9	15.3
Chlase	5.33	4.43	4.02	3.91	4.49	3.15	3.16	3.52	4.33	3.98	3.82	3.66
60 Chl	6.26	8.91	9.20	9.53	12.5	14.0	14.6	14.4	11.6	14.2	14.8	15.0
Chlase	6.52	6.33	6.00	5.92	4.92	4.27	4.05	3.94	4.83	4.11	3.80	3.72
70 Chl	5.30	6.75	7.22	7.40	10.7	11.9	12.4	12.3	8.42	10.0	10.7	0.9
Chlase	9.33	7.90	7.43	7.22	5.90	5.12	4.80	4.90	6.10	5.81	5.52	5.30
80 Chl	2.66	3.60	4.05	4.34	7.62	8.80	9.25	9.42	5.72	6.90	7.40	7.82
Chlase	11.9	11.5	11.2	10.7	6.83	6.35	6.10	5.91	6.90	7.37	7.07	6.84

After Purohit and Chandra (1983).

In addition to, halting leaf yellowing and shedding of leaves, this treatment also inhibits chlorophyllase activity (Purohit and Chandra, 1983a). Aboveground biomass and fresh mass per 100 fruits of *Helianthus annuus* also increased in the plants treated with penicillin.

It has been shown that retardation of senescence by cytokinin is associated with the lowering of both protease (Anderson and Rowan, 1965)

and RNase (Srivastava and Ware, 1965). It is, therefore, reasonable to predict that dreakdown of these important macromolecules is well prevented by penicillin as possible means of retardation of catabolism with concomitant rise in chlorophyll and carotenoids.

Penicillin effect and well known kinetin effect (Richmond and Lang, 1957; Purohit, 1982; Purohit and Chandra, 1983a) on leaf discs are similar in nature in that both are effective in maintaining chlorophyll. This action is mediated through their effects on chlorophyllase activity (Purohit, 1982). As compared to kinetin, the degree of response to penicillin is lower and a little lower effect than kinetin is obtained at fairly high concentrations. When the leaf material is placed in penicillin plus kinetin solutions, no significant interaction could be found between them. It may be assumed that penicillin apparently replaces kinetin in maintaining chlorophyll and that penicillin may be involved in the metabolism and availability of cytokinins; and the action of kinetin and penicillin may be identical in this system.

Effects on Leaf Morphology, Stem Circumference and Internode Length

Penicillin (400 mg/l) is found effective in causing difference in leaf morphology of *Glycine max* (Plate 7.1 [3]) and increasing leaf area of *Chrysanthemum* (Plate 7.1 [1 & 2]). The leaf area of *Chrysanthemum* increases considerably from 28 cm^2 (control) to 44 cm^2 (penicillin 400 mg/l). Results presented in Fig. 7.3 reveal that expansion of leaf lamina is associated with increase in chlorophyll level. The fresh and dry weights of penicillin leaf discs also increase with increasing concentrations (Fig. 7.2). The stem circumference and internode length also increase with increasing concentrations of penicillin. Such promotion may be due to cell elongation. A reduction in internode length in untreated plants may be due to reduced leaf growth. Watmore and Garison (1966) have shown that internode elongation is directly related to supra adjacent leaf development. Therefore, it might be possible that penicillin application might have increased the levels of growth and nutritional factors (hormone, assimilates and minerals) besides increasing leaf area together with chlorophyll biosynthesis and its retention.

Levels of Endogenous Gibberellins and Cytokinins

Role of penicillin in increasing GA-like activity in endosperm and seedlings has also been recorded. Biswas and Mukherji (1979) measured gibberellin-like activity in extracts from penicillin-treated endosperms

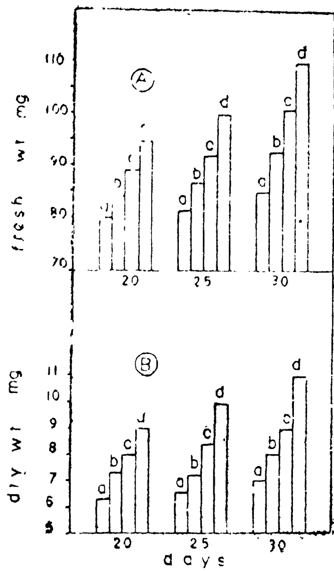

Fig. 7.2

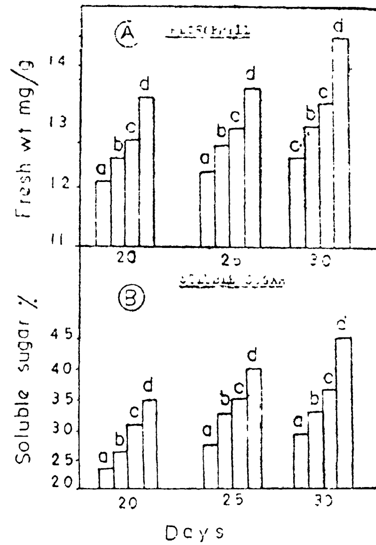

Fig. 7.3

Fig. 7.2 Effect of foliar spray of penicillin (mg/l) on *Chrysanthemum.* a : cantrol, b : 100 mg/l, c : 200 mg/l, d : 400 mg/l. A : fresh weight per 15 leaf discs; B : dry weight per 15 leaf discs. Fig. 7.3 : Effect of foliar spray of penicillin (mg/l) a, b, c, d. as in Fig. 7.2 on *Chrysanthemum.* A : chlorophyll content. B : soluble sugars.

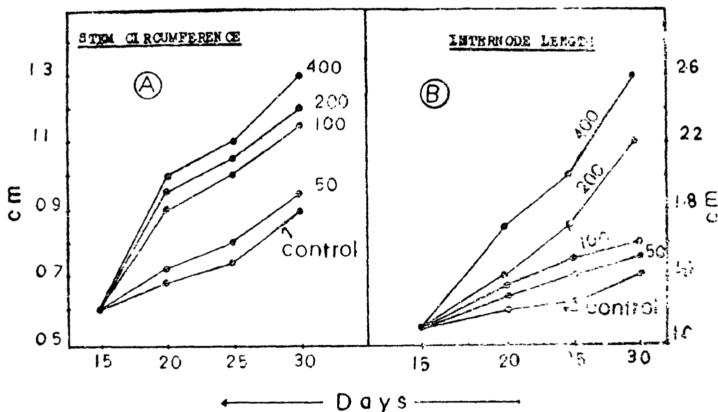

Fig. 7.4 : Effect of foliar spray of penicillin (mg/l) a, b, c, d as in Fig. 7.2 on *Chrysanthemum.* A : stem circumference, B : internonde length.

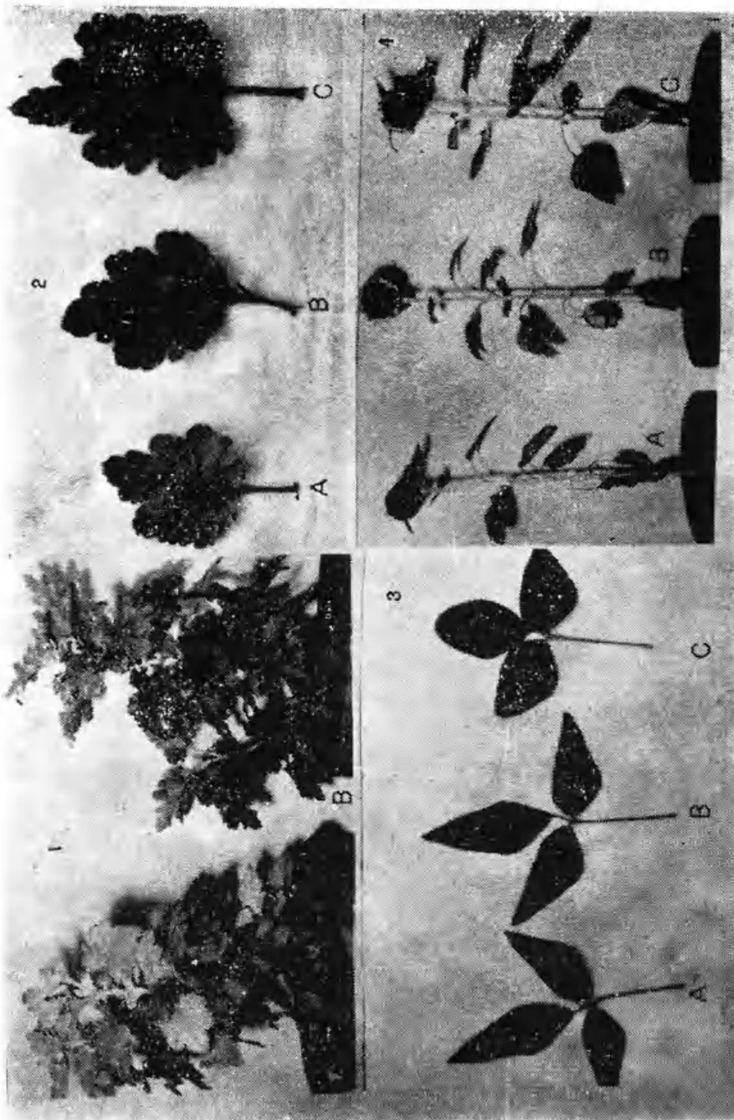

Plate 7.1 (1) Effect of foliar spray of penicillin on *Chrysanthemum* plants. A: control ; B: pencillin 400 mg/l. Note the greenness of the leaves, internode elongation and leaf size. The treated leaves were dark green while the control leaves show yellowing. (2) A: control : B: penicillin 200 mg/l : C: penicillin 400 mg/l. Photograph was taken after 15 spray-treatments for alternate days. (3) Effect on *Glycine max* leaves. A: control ; B: penicillin 200 mg/l; C: penicillin 400 mg/l. (4) Effect of foliar spray of penicillin on *Helianthus annuus* during monocarpic senescence. A: control ; B: penicillin 200 mg/l, C: penicillin 400 mg/l.

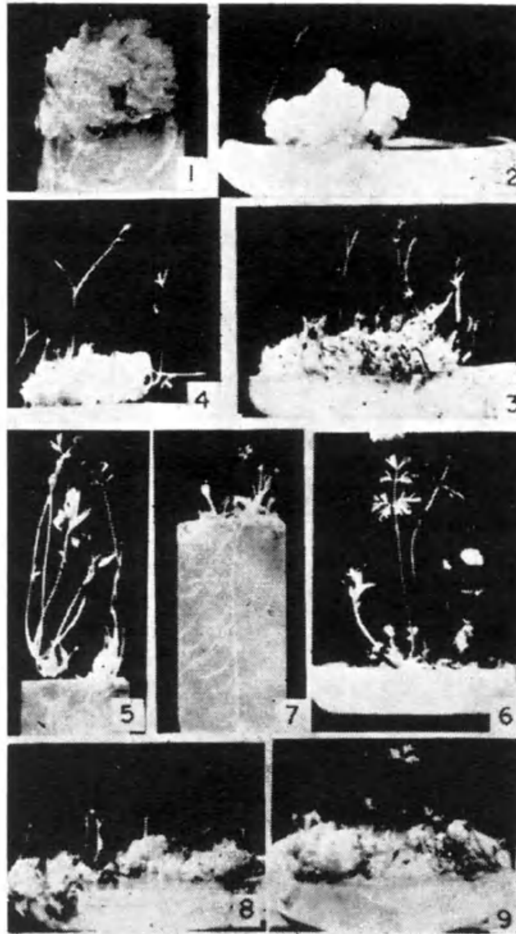

Plate 7.2 (i) 28 days old carrot callus in B5 medium+2,4-D (0.1 ppm) subculture from 4 month old callus raised from hypocotyl segments (ii) 28 days old callus with plantletes in B5+ penicillin 100 ppm medium. (iii) 28 days old callus with plantlets in B5+ 200 ppm penicillin medium. (iv) 28 days old callus with plantlets in B5+ penicillin 500 ppm medium. (v) 56 days old plantlets in B5+ penicillin 200 ppm medium showing nature of shoot and leaf (vi) as (v). (vii) 56 days old seedlings with highly developed roots and less developed shoot and leaves in B5+ penicillin 200 ppm. (viii) callus with small regenerated plantlets in B5+BA (0.1 ppm) medium after 14 days subculturing (ix) plantlets in B5 + medium alone after 14 days of subculturing showing normal regeneration of plantlets.

and found that between 12 and 16 h gibberellin-like activity rose to 10 folds. They further observed a clear gap of about 8 h between the appearance of GA-like activity and α-amylase synthesis, and regarded the latter process as a consequence of the former. They also added CCC in 16 h treatment-medium during which gibberellin like activity was inhabited.

The gibberellin like activity was located out on paper chromatograms at Rf typical for GA_3 and was confirmed by chemical identification. Recently, increase in gibberellin like compounds, as determined by lettuce hypocotyl bioassay and cytokinin like substances as determined by amaranthin betacyanin assay have been reported to occur in mungbean seedlings in response to penicillin (Mukherji and Wareing, 1983).

Effect on DNA, RNA and Protein Synthesis

Penicillin induces DNA and synthesis in rice endosperm (Biswas and Mukherji, 1979) and sunflower (Purohit, 1983a). A linear increase in DNA content upto 16 h and RNA content upto 12 h is recorded. From the time course of increase in DNA and RNA levels, Biswas and Mukherji (1979) concluded that these events precede gibberellin biosynthesis and are related to the ability of the half seeds to synthesize proteins. Penicillin increases nitrogen level from 0.82% (control) to 1.29% (penicillin 400 mg/l) in *Chrysanthemum* leaves which consequently increases protein contents (Purohit, 1984).

Penicillin induces incorporation of ^{14}C-labelled amino acids into cellular proteins of rice half seeds, which became more pronounced in presence of GA_3. This further suggests that effects of penicillin and GA_3 are at least partly independent of each other. An incorporation of radioactive amino acids into protein, and promotion of α-amylase formation on one hand, and inhibition of α-amylase formation by inhibitors of protein synthesis on the other lead one to conclude that penicillin promotes *de novo* synthesis of protein in this system. As suggested earlier that probably synthesis of RNA, a minor one, is necessary for α-amylase induction. It is possible that penicillin has certain preferential action on the synthesis of a particular species of RNA inducing messanger RNAs or any specific protein for which they code.

Promotion of Root Growth and Development

Penicillin initiates rooting of cuttings when applied singly. The interaction studies show that the increase in the number of roots per cutting

in presence of IAA and penicillin is simply additive. From the present Investigation, it thus appears that treatment with penicillin may be utilized with success for the vegetative propogation of cuttings (Bhattacharya *et al.*, 1983). Similarly, Purohit and Bhargawa (1983) and Purohit (1984) observed that aqueous solutions (50, 100 and 200 mg/l) of penicillin exert promotive action on root growth in *Helianthus annuus* seedlings. Penicillin increases tryptophan, protein, DNA and RNA contents in primary and lateral roots (Purohit, 1984). Further root promoting property of penicillin was tested on Dikegulac-sodium-which inhibits root growth. This growth inhibitor causes inhibition of root elongation above 100 mg/l in *Helianthus annuus* (Purohit, 1980). It also inhibits tryptophan, protein, DNA and RNA synthesis (see Purohit and Chandra, 1983 b). When Dikegulac-sodium-treated (250 and 500 mg/l) seedlings of *H. annuus* were transferred to the penicillin solutions, new laterals developed after 36-40 h of treatment. Penicillin did not cause further development of primary root while lateral roots developed well (Purohit and Bhargawa, 1983).

Regulation of Growth and Embryogenesis in Cultured Carrot Callus Tissue

Penicillin promotes growth and embryogenesis in cultured carrot callus tissue. The plantlets formed are somewhat abnormal in appearance with respect to shoot/root ratio and morphology of shoot and root. Penicillin fails to overcome the inhibitory effects of 2,4-D on plantlets regeneration, whereas its joint application with benzyladenine is more beneficial regarding the number of regenerated plantlets than either of the agents applied alone (Plate 7.2). Penicillin either alone or when applied jointly with 2, 4-D or benzyladenine increases the fresh weights of tissues. Although penicillin has little effect on DNA and protein when applied alone, it shows slight stimulating effect on RNA level. When combined with benzyladenine, the levels of these constituents are increased in most of the cases measuring more than those obtained in single application. The activities of peroxidase and IAA-oxidase are inhibited by penicillin whereas IAA synthesis activity have been found associated with enhanced growth and embryogenesis in response to penicillin treatments. This suggests that these enzymes are responsible for maintaining an optimum auxin balance which controls growth and differentiation.

Probable Mode of Action

Penicillin causes rapid seedling growth either directly by stimulating cell division and cell elongation or by some indirect manner. The direct effect would seem to be that penicillin alters the S-H contents within the cell by donating this group because penicillin is a derivative of cystein- a sulphur amino acid. As a result, the ratio of S-H and S-S is altered which has a great significance both in cell division and cell elongation. It has also been shown that increase in S-H content through decreased number of S-S bridge should increase the extensibility of the wall in the longitudinal direction and that S-S/S-H quotient shifts during different phases of mitosis (Svensson, 1971).

Another interpretation may be that penicillin donates S-H group for the production of various active enzymes responsible for the synthesis of nucleic acids, proteins, hormones and several other metobolites which ultimately stimulate growth and development.

During early stages of rice seed germination, the auxin concentration in the endosperm is supraoptimal for growth, as elimination of fractions of endosperms increases the seedling growth in comparision to full endosperms (Sircar *et al.*, 1955). Mukherji and his co-workers have demonstrated increase in IAA-oxidase activity after penicillin treatment which helps to destroy the supraoptimal auxin concentration within the seed and thus presumably brings the IAA level to an optimum which is conducive to growth. Data on seedling growth caused by an interaction of penicillin with IAA also support this indirect effect of penicillin. While only IAA at certain concentrations inhibits root and shoot elongation; penicillin helps to overcome this inhibition by joint application with IAA (Mukherji and Biswas, 1983).

Within 10 to 15 minutes after auxin application to an isolated stem or a coleoptile segement there is a dramatic increase in growth rate. Penicillin also exerts more or less similar action on root growth and its elongation. The major effect of auxin leading to increased elongation rates is the enhancement of cell wall loosening. The 'acid growth hypothesis of auxin' suggests that H^+ ions are released from cytoplasm into the cell wall upon auxin treatment thus making it acidic which may be held responsible for auxin-induced wall loosening by breaking acid-labile bonds. An aqueous solution of penicillin probably helps in loosening of cell wall in a similar manner and thus promotes growth. Therefore, the action of penicillin seems to be similar with auxin and is

possibly based on 'acid growth hypothesis'. Work is in progress to resolve the precise mechanism of action of penicillin.

Conclusion

Since 1950 there has been a spectacular advancement in the technology of chemical regulation of plant growth and development. From the foregoing results it appears that penicillin behaves as a potent plant growth stimulant. Range of plant responses points to the possibility of penicillin action being a conceptual breakthrough in phytohormone research. Since the range of bioregulators that can be exploited for increasing production through more efficient plant performance will certainly continue to expand, the results obtained so far with penicillin will definitely brighten the hope for the agricultural application of this antibiotic pertaining to hormone-penicillin link-up.

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Physiology and Biochemistry of Ethylene

K. Shimokawa

Introduction

Atmospheric gases make existence possible through respiration and photosynthesis in animals and plants. Among these gases are oxygen and carbon dioxide abundantly present in the earth. Nitrogen is required for preserving nitrogen fixing microorganisms. The recent energy crisis has aroused interest in presence and utilization of methane and hydrogen as energy sources. In contrast to abundant gases, ethylene is the simplest unsaturated hydrocarbon compound and is a major product of the petroleum industry. In Japan, about four million tons of ethylene were produced in 1979 alone. It is of interest that this gas aets as a plant hormone.

The introduction of. gas chromatography as an analytic technique in 1959 has enhanced the progress of research on ethylene. However, the major discoveries in regard to ethylene biology were made before the use of gas chromatography had come about. A review published in 1962 cited many references indicating plant sources of ethylene and the various effects of ethylene in plant growth and development. Harvey (1928) showed ethylene to be a ripener of fruits and vegetables. Hanson (1939) and Kidd and West (1942) suggest that ethylene functions as a ripening hormone. The necessity of oxygen for ethylene production is a known fact. These findings were given complete confirmation through gas chromatography. Actually, there could be no significant progress in the research on ethylene biosynthesis without gas chromatography and radioisotope technology,

Outline of Ethylene Research

The discovery of the properties of ethylene preceeds that of other plant hormones. Progress in ethylene research has been slow and stepwise, because of the difficulty in making various physical and chemical determination of this compound. This is one of the reasons that workers have avoided ethylene research.

Burg and Thimann (1959) reviwed the study of ethylene biology with the coming of new analytical techniques such as gas chromatography and radioisotope tracer. They completed the first report on the biosynthesis of ethylene in 1960. It is little known that the metabolism of ^{14}C-ethylene was reported prior to the publication of Burg and Thimann (Hall *et al.*, 1959). Since then, interest in ethylene research has spread throughout the world.

Prompted by reports of research on ethylene, the author was prompted to undertake the study of the biosynthesis of ethylene at the radiobiochemistry laboratory of Kyoto University. First an attempt was made to develop a radioisotope tracer technique for $^{14}C_2H_4$ (Shimokawa and Kasai, 1965).

Abeles and Rubinstein (1964) and Morgan and Hall (1962) demonstrated that auxin increased ethylene production. This was found 29 year later after the original discovery (Zimmerman and Wilcoxon, 1935) of auxin-induced ethylene production. This rediscovery should prompt a reevaluation of the significance of ethylene research. The interest of many plant physiologists and horticulturists has been aroused on biosynthetic mechanism and the role of ethylene in the effecting auxin. Abeles and his collaborates (1971) studied the abscission of cotton, bean and *Coleus* explants and daveloped an aging-ethylene theory on the basis of data on the explants of *Phaseolus vulgaris* L. An attempt was made to systematize the abscission as phytogerontology.

It is strange that the useful application of the data of endogeneous plant growth substances has been much slower than expected. The degreening of various citrus fruits with ethylene is widely done on a commercial scale through out the world. In Japan (Kitagawa, 1975, Shimokawa and Tominaga, 1972) the practical use of ethylene in improving the quality of *Citrus unshiu* fruits has been establish. Cohen (1979) developed a degreening technique in Israel. Shimokawa and his coworkers investigated ethylene-induced degreening (chlorophyll degradation) from morphological (Shimokawa *et al.*, 1978a) and biochemical stand points (Shimokawa *et al.*, 1978b). Recently, Shimokawa (1981, 1982) purified ethylene induced chlorophyllase from ethylene-treated fruits of *Citrus unshiu.* This research has the way to further development of the degreening tecnique.

Generally, the commercial application of ethylene is difficult since it is a

gas. The discovery of ethephon- an ethylene releasing compound has facilitated the practical use of ethylene. Now, many attempts are being made to find commercial applications for ethephon as a control for crop growth. But sofar, there has been no significant success in this regard.

Although methionine is certainly converted to ethylene in plants, a more direct precursor has not been found in the last fifteen years. Shimokawa and Kasai (1970c) showed the presence of an enzyme which catalyzes the formation of ethylene from acrylic acid in presence of Mg^{2+} and thiamine pyrophosphate. This is only report dealing with the characterization of an ethylene forming enzyme. However, no biosynthetic pathway has found general acceptance.

Table 1. Historical summary of ethylene research

Age	Running title of work
1901	Discovery of strnage phenomenon induced by a gas (Neljubow, 1901)
1934	Identification of the gas as ethylene (Gane, 1934)
1932-1935	Investigation of ethylene physiology (e. g. Crocker *et al.*, 1932)
1940-1954	Involvement of ethylene in fruit ripening (e.g. Kidd and West, 1945)
1950	Ethylene formation of infiltrated plants (Williamson, 1950)
1959	Metabolic fate of ethylene-^{14}C (Hall *et al.*, 1959)
1959	Gas chromatography of ethylene (Meigh, 1959)
1959	Beginning of research of ethylene biosynthesis (Burg and Thimann, 1959)
1962	Fruit ripening hormone (Burg and Burg, 1962)
1964	Auxin induced ethylene formation (Abeles and Rubinstein, 1964)
1965	Discovery of methionine as a precursor (Lieberman *et al.*, 1965)
	Liquid scintillation counting method of ethylene-^{14}C (Shimokawa and Kasai, 1965)
1966	Ethylene-induced abscission (Abeles, 1966)
1967	CO_2 - a competitive inhibitor of ethylene action (Burg and Burg, 1967)
1968	Discovery of ethephon-an ethylene releasing compound (e.g. Cooke and Randall, 1968)
	Phytogerontology (a concept of abscission mechanism) (Abeles, 1968)
1970	Preparation of an enzyme catalyzed ethylene formation from acrylate (Shimokawa and Kasai, 1970)
1971	Inhibition of ethylene formation with enol ether amino acid analogs (Owens *et al.*, 1971)
	Autoinhibition of ethylene formation (Vendrell *et al.*, 1971)
1977	Establishment of ethylene biosynthesis from methionine (Adams and Yang, 1977)
1979	Discovery of ACC as a precursor (Lurssen *et al.*, 1979, Adams and Yang, 1979, Konze and Kende, 1979)

Lieberman, one of the leaders in ethylene research found that ethylene formation was inhibited by enol ether amino acid analogs (Lieberman *et al.*, 1975). This indicates that pyridoxal phosphate may be included as a cofactor in ethylene formation from methionine. Yang and his cow-orkers (1977) postulated a biosynthetic pathway of ethylene via S-adeno-syl methionine from methionine. This hypothetical biosynthetic pathway includes pyridoxal phosphate-S-adenosyl methionine complex as an inte-rmediate. Recently, 1-aminocyclopropane-1-carboxylic acid (ACC) as a precursor of ethylene was discovered independently by Lurssen *et al.* (1979) and Adams *et al.* (1979). Their research is different in regard to the iedntification of the amino acid as a more direct precursor than methionine. Adams *et al.* (1979) identified 1-aminocyclopropane-1-cor-boxylic acid-^{14}C as a metobolite in apple tissues administered methio-nine-U-^{14}C and proposed ACC as a useful precursor. On the other hand, Lurssen *et al.* (1979) found a high yield from ACC during a survey of ethylene forming activity to find ethylene-releasing compounds. They postulated a biosynthetic pathway from the non-protein amino acid. Table 1 presents a historical summary of ethylene research.

Analysis of Ethylene

In early research (Crocker and Zimmerman, 1932), biological respon-ses were used to determine ethylene concentration. The reliability of this method was questioned in regard to its specificity and sensitivity. Manometric methods were used to enhance specificity and sensitivity by concentrating ethylene in mercuric perchlorate solutions (Young *et al.*, 1952). But even this method was not sufficient to analyze ethylene produced from plant sources. However, gas chromatagraph equipped with a hydrogen flame ionization determining small quantities of ethyl-ene (levels as low as 0. 1μl/liter). The determination of ethylene by gas chromatography is very simple, because no extraction is required prior to analysis. This simplicity has assisted the progress of ethylene research, but has also caused confusion in regard to the operative conditions. One uses alumina for column packing, and the other charcol. Recently, Porapack Q has been used. Also, the temperature and length of the column are different from each other.

To compare data obtained by numerous investigators and further the development of ethylene research, there must be standard operative conditions. Fig. 8.1 shows chromatogram of ethylene and other gases obtained under optimum conditions. The retention time of ethylene is short when alumina and high polymers such as Porapack are used.

When alumina is used as a column packing, alumina treated with tailing reducers is necessary. Retention time of ethylene for active carbon is long. When active carbon is used, the length of the column is short. High polymer beads are superior for qualitative uniformity and wide scope of application.

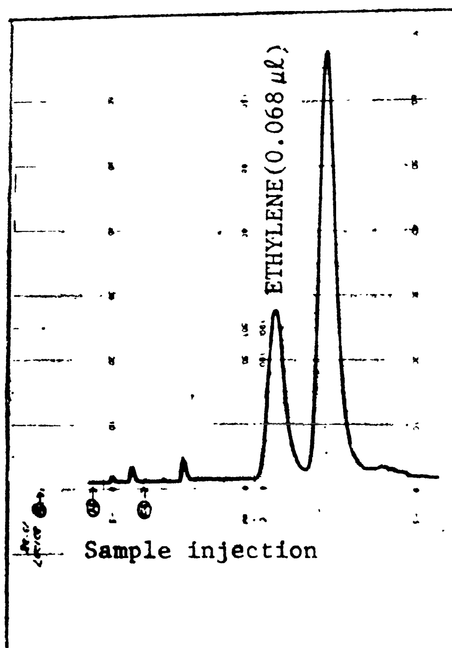

Fig. 8.1. Gas chromatogram of ethylene. Ethylene determinations were made with Shimadzu gas chromatograph 3A using a flame ionization detector. The analytical column (50 cm long and 0.3 cm in diameter) was packed with active carbon (60-80 mesh). The oven was maintained at 100° C. The retention time of ethylene was six minutes. By this method, we are able to measure concentrations of ethylene as low as 0.001 μl/ml.

Except for certain climacteric fruits, ethylene production in most of the biological tissues is low, ranging from 0.5 to nl/g hour (Leopold 1972). Therefore, several methods have been developed to increase ethylene concentration. The first technique used for measuring ethylene produced from biological materials is the ethylene-mercury complex formation. Released ethylene is measured manometrically (Young *et al.*, 1952). But, this method requires harsh conditions and the ethylene released is only 80% (DeGreef *et al.*, 1976). They developed a new and very

sensitive method using a Porapak S precolumn cooled at -95°C as a trapping unit. By this method, concentration of ethylene as low as 0.01 ppb could be measured. For calibration, diluted ethylene (in N_2 or in artificial air) is used. Ethylene of 52.4 ppm has been used at this laboratory. The rate of ethylene production by plant tissues has been estimated by measurements of the atmosphere surrounding plant tissues in sealed are aerated containers. These techniques are not completely adequate because of the ethylene concentration gradient between the fruit interior and the atmosphere. One factor (2ppm/μl/kg/h) was estimated to determine the ethylene concentration gradient across a fruit skin and the rate of ethylene production. This indicates that an apparatus for ethylene extraction from plant tissue requires a sufficient estimation of the total ethylene. Blanpied (1971) has described a method for this purpose, which requires less than 1 h, from sampling to analysis. When a continuous flow system containing trace amounts of ethylene requires, the most common methods for this are premixing the desired concentration and metering very small amounts of ethylene into a measured air stream. Barmore and Wheaton (1978) describe a diluting and dispensing system for providing concentrations of ethylene as low as 0.1 ppm. This apparatus will facilitate the investigation of physiological effects of ethylene.

Ethylene as a Plant Hormone

It is a fact that ethylene is a plant hormone. About 15 years ago, it was unclear as to whether ethylene was a plant hormone or a metabolite having unusual effacts on growth. But, ethylene was recognized in 1968 by plant physiologists as a true growth regulator or plant hormone. This is evident by the fact that Pratt and Goeschl (1969) reviewed ethylene biology in 1968 and applied the term "plant hormone" to ethylene. The rediscovery of auxin-induced ethylene formation was important for this recognition.

The terms plant growth substance or plant hormone are defined as follows: A plant growth snbstance (or plant hormone) is an organic substance produced within a plant and, at low concentrations, promotes, inhibites or qualitatively modifies growth, usually at a site other than its place of origin. Its effects do not depend on its calorific value or content of essential elements. Indeed, ethylene is an organic compound consisting of carbons and hydrogens, and is produced within a plant. Ethylene promotes epinasty, prolification, root formation and absci-

ssion and inhibits growth and anthocyanin formation of morning glory seedlings (Plate 8.1). Ethylene freely moves about within a plant or sometimes among plants without definite direction in the manner of the polar transport of IAA. Also, one can not specify the place of origin. Responce forward ethylene is everywhere evident in plant organs. But, exogeneous ethylene exposed to stem or roots causes stem prolification in the upper parts of hypocotyl of morning glory seedlings (Plate 8.2). This suggest that exogeneous ethylene or its stimuli reach the responding part and modify plant organs quantitatively or qualitatively. Therefore, ethylene satisfied the difinition of plant hormone.

Ten years before the discovery of the growth promotion of IAA, ethylene-hastening effects on degreening of lemon fruit were observed. Ethylene was identified as a plant metabolite in 1934 and 10 years later, IAA was found to be in plants. This, however, may be unfavourable to further research on ethylene. This is because the interest of plant physiologists was initially focused on IAA. Unfortunately, the leaders of plant physiologists at that time did not take up the problem of ethylene in their articles, since young research personal had no interest in ethylene biology. Ethylene research was started again by Burg and Thimann, a leader of plant hormone research. Consequently, ethylene has been firmly established as a plant hormone.

Ethylene as a Ripening Hormone

There were two schools as to the role of ethylene in fruit maturation : ethylene as a ripening hormone (Hansen 1942, Kidd and West 1945), ethylene as a by-product of fruit ripening (Biale 1960a). Porritt (1951) reviewed an article in support of the concept that ethylene is a ripening hormone. Biale and his collaborators (1954, 1960b) reported several articles oppose to this concluding that ethylene is a by-product of fruit maturation. But, Biale *et al.* (1954) took into account the following excerpt from the article presented in the by-product theory, "in the absence or information correlating the internal ethylene content with the rate of ethylene production, one can advance the argument that small quantities sufficient to induce ripening are produced prior to the rise of respiration, but measurable amounts are detected only after the onset of the climacteric". Burg and Burg have challenged this assertion (Burg and Burg 1962). Their results are summarized as follows : Internal ethylene content and the rate of ethylene production are determined for a variety of fruits including several previously been described as devoid

of ethylene; e. g., the mango, pineapple, orange and lemon. The application of ethylene hastens the ripening of mangoes even after the climacteric has began. This effect is related to the log of the gas concentration. Direct measurements indicate that accumulation of a stimulatory concentration of ethylene precedes the onset of the climacteric in both mangoes and bananas.

McGlasson *et al.* (1975) raised the question as to whether or not ethylene is a true ripening hormone. They examined the role of propylene in respiration and ethylene production of tomoto fruits of *rin*- a nonripening mutant. Although the application of propylene to normal tomato advanced repening, but no concomitant increase in ethylene production could be found. Propylene stimulated respiration in the immature fruits of *rin*, but there was no change in endogeneous ethylene production. It was concluded that the onset of ripening in no rmal tomato fruit is not controlled by endogeneous ethylene, and increased ethylene production is probably an integral part of the ripening process. This is the first work calling into question the fruit ripening hormone theory of ethylene.

The Kinds of Stress Produced Ethylene

One characteristic of ethylene is that it is produced from plants subjected to stress, such as disease, radiation, mechanical wounding and chemicals. Stress ethylene or wound ethylene formation occupies an important part of ethylene biology. The induction mechanisms of stress ethylene formation and the role of the gas in these stresses has been studied. This study involves important problems of a practical nature.

Disease

Since the discovery of ethylene production by leaves of *Physalis floridiana* infected with potato virus (Williamson, 1950), similar observations have been on the difference in host-parasite relationships (Pegg, 1976; Pegg and Cronshaw, 1976). As in the case of fruits treated with ethylene, in infected plant organs, the rate of respiration increased with that of ethylene production. This may be referred to as a "pseudo" climacteric rise- a sudden increase in respiration before death.

It is of interest that, in tomato plants infected with *Verticillium albatrum*, ethylene production is recognized in susceptible plants but not in those resistant fungi. Although this suggest a direct role of ethylene as a phytotoxin. Pegg *et al.*, (1976) concluded that ethylene production results from the infection but not a cause of the infection. Actu-

ally, stress ethylene represses the formation of tulipaline-an antifungal compounds in plants infected by *Fusarium oxysporum* f. *tulipei* (Beijesbergen and Bergman, 1973). It may be assumed that pathogen enhances cellulase and/or pectinase activity through the action of ethylene produced by pathogen, causing tissue disorganization in host plants. Pathogen, however, is known to stimulate peroxidase activity but not cellulase activity. The stimulated peroxidase may participate in the reconstruction of disordered tissues, for instance through, peroxidase-enhanced lignin biosynthesis (Hall and Sexton, 1972).

The biochemistry of ethylene formation in wounded tissues has been studied in a number of plants. Sakai *et al.* (1970) compared the formation of ethylene from various substrates in freshly cut and black rot infected sweet potato tissue. Their data show clear differences in normal and diseased tissue. Hyodo (1977) observed an increase in ethylene production in albedo tissue excised from satsuma mandarin. The ethylene production was accompanied by the conversion of methionine for a period of 30 h after cutting. Neither ethylene production nor the conversion of methionine to ethylene was obsereved in intact fruit. As in this case, there are differences between stressed and intact plant materials when methionine is used as a substrate. However, recent work shows that ACC is also a useful precursor of ethylene in wounded plant tissues and that ethylene formation is based on the rapid enhancement of the activity of ACC synthase,- a key enzyme of ethylene biosynthesis (Boller and Kende, 1980).

Physical stress

Since the discovery of mechanically-stimulated changes in growth by Darwin (quoted from Jaffe 1973), many investigators have observed this phenomenon. In particular, Jaffe (1973) observed a significant retardation in internode elongation by rubbing (a gentle mechanical stimulus), and termed this morphogenetic response as thigmomorphogenesis. Furthermore, Jaffe suggested the participation of ethylene in thigmomorphogenesis (for details see review by Jaffe in this volume).

Independent of Jaffe's work, Goeschl *et al.* (1966) happened to observed an unexpected increase in ethylene evolution from epicotyl pressed with a container stopper. They investigated in detail this strange ethylene production and concluded that the production of ethylene by pea epicotyls increases by nonwounding physical stress, and that ethylene acts as an endogeneous growth regulator.

Matsukawa and Kashiwagi (1971) observed thigmomorphogenesis in *Lilium longiflorum*, induced by stroking with a brush. Hiraki and Ota (1975) showed that this is caused by ethylene produced after stress. They conclude with Jaffe that ethylene may play an important role in thigmomorphogenesis. The ethylene-induced thigmomorphogenesis is interesting from a practical standpoint because it makes possible growth regulation and developments without any requirement for growth retardants.

Autocatalytic ethylene formation (ethylene stress)

A characteristic of ethylene formation is that its formation is autocatalytically regulated by ethylene itself. This characteristics has been documented from time to time. However, the first characterization of autocatalytic ethylene formation was done by Vendrell and McGlasson (1971) using banana pulp slices. Further evidence was obtained using propylene as an ethylene analogue (McMurchie *et al.*, 1972). Propylene suppressed ethylene formation and delayed the peak of ethylene production in intact green bananas. Zeroni and Galil (1976) showed that ethylene formation of fig fruits in jar stopped when the concentration of ethylene reached a certain level and was renewed when removed ethylene by aeration. Exogeneous ethylene also repressed immediately and completely ethylene formation of figs.

Autoinhibition of ethylene production was more directly evidenced with the use of methionin-^{14}C (Vendrell and MeGlasson, 1971). Recent work has shown that autoinhibition of ethylene production in *Citrus* flavedo discs results from a suppression of ACC formation through repression of the synthesis of ACC synthase and inhibition of its activity (Riov and Yang, 1982). A series of these works suggests the direction of regulation of ethylene action in practical situaions.

Auxin-induced ethylene formation

Zimmerman and Wilcoxon (1935) first noted the stimulation of ethylene when tomato plants treated with IAA produced a gas that caused an epinastic response in plants. Later, Morgan and Hall (1962) presented evidence that this gas produced is ethylene. Abeles and Rubinstein (1964) concluded that ethylene evolution is closely allied with auxin levels and that the acceleration of abscission by NAA may be due to ethylene produced following auxin applications. Burg and Burg (1966) also observed IAA-induced ethylene formation in etiolated pea stem sections. Based on these findings, it is beyond doubt that auxin induces ethylene formation.

Biochemical studies on auxin-induced ethylene formation have continued to be carried out. Abeles (1966) has shown that auxin-induced ethylene production by etiolated bean hypocotyl section is inhibited by actinomycin-D and to a lesser extent by puromycin. Also, ethylene production by etiolated pea seedlings induced by auxin and kinetin is inhibited by cycloheximide and actinomycin-D (Lieberman and Kunishi, 1975). Sakai and Imaseki (1971) have shown that auxin-induced ethylene production of hypocotyl segments of etiolated mung bean seedlings is inhibited by cycloheximide but not by chloramphenicol, and also, that an ethylene producing system is a state of rapid turn over. These studies suggest the involvement of RNA and protein synthesis in the induction of ethylene production.

Sakai and Imaseki (1973) purified a protein which reversely inhibits auxin-induced ethylene synthesis from the hypocotyls of etiolated mungbean seedlings. This inhibitor does neither degrades nor binds IAA, and has no peroxidase activity. An attempt has been made to clarify the unique properties of this ptoteinous inhibitor; it has been found that the inhibition is completely reversible and attaches to the cell surface of the plasma membrane.

A biosynthetic pathway of auxin induced ethylene production is similar to endogeneous and wound ethylene production. Methionine is also an effective precursor in these productions (Sakai and Imaseki, 1971). Recently, ACC has been postulated as an intermediate from methionine in auxin-induced ethylene production (Yoshii and Imaseki, 1981).

Ethylene formation in soils

Since the appearance of a brief report of K.A. Smith *et al.* (1969), some soil scientists have also been interested in the formation and role of ethylene in soils. Smith and co-workers (1971) reported that ethylene is produced in anaerobic soils by microbial activity, and can affect the growth of crop plants in waterlogged soils. A. Smith and Cook, (1974) in studying ethylene as a cause of soil fungistasis developed the concept of the O_2-C_2H_4 cycle, a self-regulating microbial cycle in soil. This cycle has great practical potential for agriculture because its successful manipulation may lead to regulation of organic matter turn over in soil, better availability of essential plant nutrients, and development of effective biological control of soil borne plant pathogens (Smith, 1976). The decomposition of ethylene by a group of aerobic microorganisms and the effects of ethylene on plant growth in waterlogged soils were also

examined (Cornforth, 1975). However, no information related to decomposed product was obtained. Soil conditions are important for improving agriculture efficiency. Ethylene may be important as a gas capable of causing injury to plant roots. Crossett and Campbell (1975), using a solution culture, examined the shoot and root growth of barley plants after long-term exposure of the roots to ethylene. Both root and shoot dry weights were reduced slightly by ethylene treatment. Seminal root extension was greatly inhibited while lateral root growth was stimulated.

Although research in this field is slow because of the complexity of soil as an experimental system, it goes without saying that crop cultivation is of great practical importance.

Biosynthesis of Ethylene

Prehistoric age of ethylene biosynthesis

Following the discovery of ethylene production, the first report on ethylene biosynthesis appeared in 1954. Fergus (1954) found that ethylene was produced from mannose, mannitol, and citric acid in Citrus mold *(Penicillium digitatum)*. Phan-Chon-Ton (1960) found that glycerol and alanine increased the production of ethylene. Whether these substrates are utilized as precursors of ethylene by molds or not is doubtful on the basis of current data, at least on the results of radioisotope experiments. But, articles dealing with early research on ethylene are very important. Lieberman (1961), Spencer (1959) and Hall (1951) independently undertook a biochemical approach to the ethylene biosynthesis of plant materials. However, a significant ethylene forming system was not obtained and the results of his research have been wontonly disputed since it was not specifically directed foward ethylene.

Technological Innovation of ethylene biosynthesis

As mentioned in the introduction, ethylene physiology has progressed with the advance of gas chromatography. In particular, the radioisotope tracer method is indispensable for promoting the research of ethylene biosynthesis. The first application of gas chromatography to the measurement of biologically produced ethylene was reported by Burg and Stolwijk (1959). Their detector was a high sensitivity katharometer capable of detecting less than 10^{-9} moles of ethylene. Meigh (1959) used a gas chromatographic system provided with flame ionization as a detector. This system can detect less than 10^{-11} moles of ethylene.

The gas chromatographic system was still novel even in 1982 except for the addition of Porapak Q as new column packing.

The first application of radioisotope tracer method to the study of ethylene biosynthesis was done by Burg and Thimann (1959). But, their method of radioactivity assay was characterized by low sensitivity because of the Geiger Muller counter. Shimokawa and Kasai (1965) introduced an assay method to the study of ethylene biosynthesis using a scintillation counter, the most sensitive apparatus for radioactivity assay. This method has been used in other work for determinating of ethylene-^{14}C produced from various labelled compounds.

The non-enzymatic approach of ethylene formation

Research on ethylene biosynthesis encountered trouble from the start of the difficulty in obtaining significant ethylene forming cell free systems. This difficulty has rendered in effective orthodox approaches found useful in other fields of biosynthetic research. However, attempts have been made to develop systems to overcome this difficult and approximate an *in vivo* system. An article concerning lipid peroxidation damage by free radicals suggests the methionine is a possible precursor of ethylene. A pioneering work in ethylene formation *in vitro* finally emerged. Lieberman and Mapson (1964) first described the formation of ethylene from peroxided linolenate and methionine in a non-enzymatic model system consisting of Cu^{++} and ascorbate. Methionine was converted to ethylene more effectively than linolenate in a Cu^{++}-H_2O_2-ascorbate system.

Yang *et al.* (1967) developed a non-enzymatic photochemical system which produces ethylene from carbon 3 and 4 of methionine in the presence of flavin mononucleotide and light. The FMN-light system is much more efficient than the Cu^{++}-H_2O_2-ascorbate system. Shimokawa and Kasai (1967) reported the formation of ethylene from ethyl moiety of methionine in a system similar of Yang *et al.* It appears that radicals generated in these reaction provide the driving force which degrades the methionine molecule.

Beauchamp and Fridovich (1970) obtained evidence for the free radical nature of these ethylene-forming reactions. Methionine was converted to ethylene in a reaction system consisting of xanthine oxidase and xanthine to generate superoxide radicals and hydrogen peroxide, which

react to produce the hydroxy radical. However, recent work (Bors *et al.*, 1976, Pryor and Tang, 1978) questions the participation of ·OH redical in the ethylene-forming system.

Mapson *et al.* (1967) demonstrated the conversion of methional and the α-keto analog of methionine, KMB, to ethylene by cell-free extracts of cauliflower florets. This system may reflect ethylene formation *in vivo*. Mapson's system is similar to the model system developed by Yang (1967), in which horseradish peroxidase, sulfite, phenols, and manganese, or catalytic amounts of hydrogen peroxide, convert methional or KMB to ethylene. It is assumed that the cell-free extracts of cauliflower florets provide peroxidase and its cofactor system.

Following disorganization such as wounding, disease, cell free preparation and senescence, peroxidase, cofactors and substrates discharged from tissues or cells are in contact with each other. It is difficult to determine whether such ethylene production has physiological significance or is related to a natural *in vivo* system.

Knowledge from studies on these model systems has contributed to the further elucidation of ethylene biosynthesis. In particular, the requirement for oxygen in ethylene forming systems and the involvment of free radicals are two important facts.

Establishment of methionine as a precursor of ethylene

Methionine as an effective precursor of model systems was converted to ethylene with high conversion efficiency in apple tissues (Lieberman *et al.*, 1966), pea stem segments and bananas (Burg and Clagett, 1967). Labeled ethylene was produced by tissues only when carbons 3 and 4 of methionine were labeled. Labeled carbon 1 form CO_2, C-2 form formic acid, and the CH_3 S was retained in the tissues. Admas and Yang (1977) proposed a hypothetical mechanism of ethylene production from methionine via S-adenosylmethionine and ACC.

Appearance of ACC as a new precursor

It has been shown that carbon atmos 3 and 4 of metnionine are converted to ethylene in a model system (Lieberman *et al.*, 1965) and in plants (Lieberman *et al.*, 1965). Fourteen years following this discovery, ACC was found to be a more direct and immediate precursor by three independent research groups. Lurssen *et al.* (1979) found that ACC enhances

ethylene formation in soybean leaves. Konze and Kende (1979) reported the properties of a cell-free system that catalyzes the conversion of ACC, and concluded that the ACC-dependent ethylene formation may result from a chemical reaction between ACC and a product from a reaction between an enzyme and a heat-stable co-substarate. Adams and Yang (1979) found ACC in the metabolites of methionine and proposed that ACC is oxidised to ethylene *in vivo.*

Theoretical end products other than ethylene from methionine should differ depending on the mechanisms postulated by Adams and Yang (1979) and by Lurssen *et al.* (1979). For either case, the discovery of an approach to the purification and characterization of enzymes in the ethylene-synthesizing system is being looked forward to.

Biochemical Action of Ethylene

The important biochemical actions of ethylene are summarized in Table 2. These actions are divided into five main categories: DNA, RNA and protein biosynthesis, mitochondrial swelling, pigment metabolism, enzyme induction and modification of endoplasmic reticulum. The pigment metabolisms result from the ethylene-enhancement of related enzymes. The modification of endoplasmic reticulum indicates a change in protein synthesis or transportation of proteins. Eventually ethylene action results from DNA, RNA and protein synthesis on efficient utilization.

A knowledge of molecular biology has facilitated the determination of the action mechanisms of plant hormones such as ethylene, auxin and gibberellins. However, the rapid response to the plant hormones as in the case of IAA, if it actually occurs, can not be explained by the molecular biological concept involving enzyme biosynthesis. The molecular biological concept of ethylene-induced abscission was put forth on the basis of studies carried out from 1966–1968.

Little is known about the mode of action of ethylene, except for abscission. An increase in the rate of respiration may be possible due to ethyllene as has been described elsewhere (Shimokawa, 1983).

Molecular Requirement of Ethylene Action

Ethylene, simplest unsaturated hydrocarbon, regulates growth development and senescence to a considerable extent. Burg and Burg (1967) challenged the idea that this was due to the molecular structure of ethylene and discovered that biological activities requiring an unsaturated bond to be

Table 2. Biochemical action of ethylene

DNA, RNA, Protein Synthesis	
DNA synthesis (inhibition)	Apelbaum and Burg, 1972
RNA synthesis	Marei and Romani, 1971
	Hulme *et al.*, 1971
Protein synthesis	Frenkel *et al.*, 1968
	Brady *et al.*, 1970
	Hulme *et al.*, 1971
Protein loss	Abeles and Holm, 1967
Enzyme	
Cellulase	Hall, 1964
	Horton and Osborne, 1967
	Abeles, 1969
	Ridge, 1969
	Abeles and Leather, 1971
	Rasmussen, 1973
	Greenberg *et al*,, 1975
Glucanase	Abeles *et al.*, 1971
Pectinase	Rasmussen, 1973
Polygalacturonase	Greenbe'rg *et al.*, 1975
α- amylase	Herrero add Hall, 1960
Catalase	Herrero and Hall, 1960
Chitinase	Abeles *et al.*, 1971
Phenylalanine ammonia lyase	Riov *et al.*, 1969
	Hyodo and Yang, 1971
Malic enzyme	Hulme *et al.*, 1963
Peroxidase	Ridge and Osborne, 1970
Leakage of peroxidase	Ridge and Osborne, 1970
Release of α- amylase	Jones, 1968
Stabilization of enzyme activity	Fuchs and Gertman, 1973
Ribosome	
Modification of ribosomes	Valdovinos *et al.*, 1971
	Sargent and Osborne, 1975
	Freytag, *et al.*, 1977
Mitochondria	
ATPase stimulation	Olsen and Spencer, 1968
	Malphotra and Spencer, 1974
Swelling	Lyons and Pratt, 1964
Volume change	Turkova *et al.*, 1965
Inducement of alternative path of respiration	Solomos and Laties, 1974
Chloroplast (Chromoplast)	
Chlorophyll synthesis	Frenkel *et al.*, 1968
Chlorophyll degradation	Shimokawa *et al.*, 1978,
	Purohit, 1982
Degreening	Barmore and Wheaton, 1976
Carotenoid synthesis	Kang *et al.*, 1972
Pigment	
Formation of anthocyanin	Kropfitsch, 1951
	Hall, 1952
	Craker *et al.*, 1971

adjacent to a terminal carbon atom is related to molecular size, and that activity decreases with substitution. Furthermore, they observed that CO_2, a close structural analogue, is a competitive inhibitor of ethylene action. Although the application of a Linewever-Burk plot for enzyme kinetics is still questionable to some extent, it is recognized that the effects of CO_2 are the opposite of those of ethylene. The molecular requirements for abscission are in agreement with Burg's results (Abeles and Gahagan, 1968). It was not possible to adopt Lineweaver-Burk plots in the abscission of a bean petiole explant since abscission cannot be expressed as a rate phenomennon (Abeles and Gahagan, 1968). However, the idea that CO_2 is a competitive inhibitor in the abscission arises from the results of early research.

Dollwet and Seeman (1975) found that propylene competes with ethyleneinduced reduction in the epicotyl of an etiolated garden pea. They conclude that ethylene become loosely attatched to a site.

Mechanism of Ethylene Action

Ethylene biologists have also been interested in this action mechanism. But so far, there has not been developed a mechanism concept capable of explaining the wide scope of action such as ACC as a common precursor of ethylene biosynthesis under various conditions.

Ribosomes as a target

Shimokawa *et al.* (1969) found that ethylene-^{14}C was fixed into high molecular immobile molecules in the leaves of morning glory plants. It was shown that ethylene has a high affinity for ribosome RNA and some affinity for ribosomal protein (Shimokawa and Kasai, 1968). They consider that the incorporation of ethylene into high molecules may induce a conformational change. This is the first evidence suggesting that a primary action site of ethylene may be ribosomes. In support of this, Freytag *et al.* (1977) published their research data. Ribosomes in ethylene-treated cotton and sugarbeet radicles were larger than ribosomes in control and nitrogen treated cells. The ribosomes in ethylene-treated plants were swollen and less dense. The swollen ribosomes observed in electron micrographs of ethylene–treated materials underwent conformational changes. as was indicated by significant differences in sedimentation patterns. The ribosomes from ethylene-treated material were less dense than those from the control material.

In experiments on the ethylene-enhanced chlorophyll degradation of satsuma mandarin, lamellar membranes of chloroplasts in ethylene-treated

fruits were swollen and less dense (Shimokawa *et al.*, 1976). These conformational changes in plant cell organelles are perhaps important to ethylene action.

Abscission

The analysis of ethylene-enhanced abscission is possible using simple system constructed of explants prepared from plant seedlings. The action mechanism of plant hormones was studied in relation to the molecular biology involved. Studies on abscission resulting from the action of ethylene seem to indicate that the participation of an ethylene-enhanced enzyme activity is the action mechanism involved. Generally it is accepted that ethylene hastens abscission and auxin delays it. Also, abscisic acid (ABA) enhances the abscission of some plants as a third abscission regulator. *In vivo*, these plant hormones regulate abscission through complicated interrelationships with each other. In particular, the ethylene and auxin balance in the separation zone is very important. The ability of ethylene against abscission increases with a decrease in auxin concentration in the separation zone. This is a phytogerontological effect of ethylene, recently postulated by Abeles *et al.* (1971).

Results obtained by Abeles and his collaborates are summerized as follows: Ethylene-enhanced abscission depends on the synthesis of RNA and protein. The addition of actinomycin-D results in the loss of this ability by inhibiting RNA synthesis. Cycloheximide inhibits abscission through the inhibition of protein synthesis. Horton and Osborne (1967) reported an ethylene-induced enzyme to be cellulase. The cellulase synthesis was inhibited by IAA, cytokinins, actinomycin-D, cycloheximide, and CO_2. These observations agree with earlier work which show that these substances inhibit abscission and with histochemical observation (Halland and Sexton, 1974). An investigation of the effects of ethylene on the fine structure of abscission cells showed the development of a rough endoplasmic reticulum (Valdovinos *et al.*, 1971). This undoubtadly reflects an increase in protein synthesis and translocation. An increasingly long profile of rough and smooth endoplasmic reticulum appears in sections of epidermal and cortical cells from the hook region of intact etiolated seedlings of *Pisum sativum* L. (Sargent and Osborne, 1975). The stimulation of rough endoplasmic reticulum formation and modification of its profile following hormone treatment occurs in both plant and animal tissues and lead to the enhancement of protein synthesis and secretion.

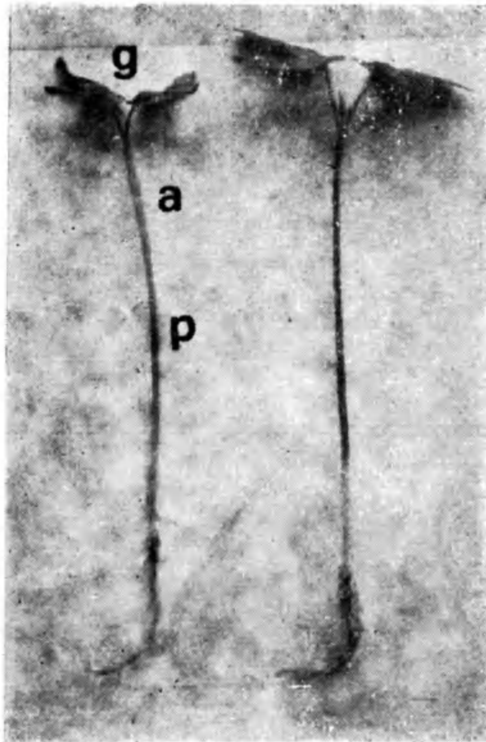

Plate 8.1 Effect of ethylene on morning glory seedling. a. Proliferation, p. Inhibition of anthocyanin formation, g. Growth inhibition.

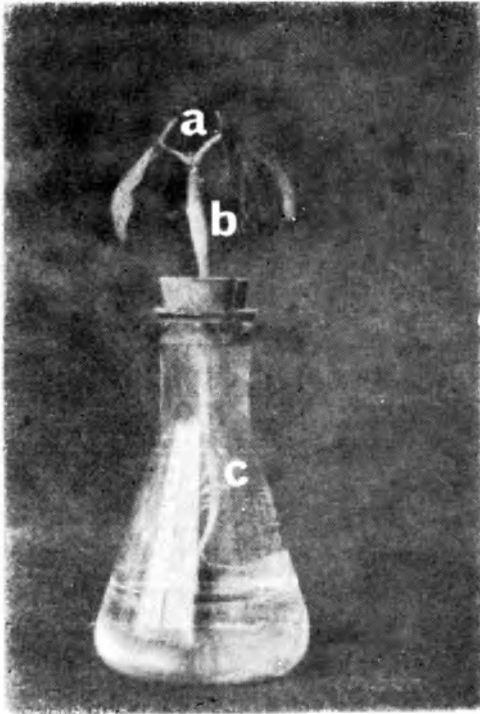

Plate 8.2 Effect of ethylene on morning glory seedling. a. Epinasty, b. Proliferation, c. Ethylene-induced root formation.

Finally, attention should be directed to the work of Koehler and Lewis (1979). These authors observed an increase in the buoyant density of a plasma membrane due to the ethylene treatment of bean abscission zones. This increase in denisty was observed by following the shift of cellulase and KCl-stimulated ATP activity. It was concluded that such a change is consistent with the loss of lipids from the senescents cells of the abscission zone. Changes in density of cell organelles may possibly be a common phenomenon due to ethylene treatment.

Chlorophyll degradation

Ethylene degreening of *Citrus* fruits is a common commercial practice in USA, Japan, Israel, Australia to insure product quality in the market. Practical degreening methods are generally complete, but the role of ethylene is still not certain.

Degreening does not occur during exposure to ethylene in the peel of satsuma mandarin fruits, but does by exposing the ethylene-treated fruits to air. Chlorophyllase activity is low during exposure to ethylene and during the first 6 h of the subsequent exposure to air. These observations indicate that a lag phase is a prerequisite for the activation of chlorophyllase and also that oxygen is required for ethylene-enhanced chlorophyll degradation and chlorophyllase activity. The constructing effeets of these two inhibitors of protein synthesis indicate that *de novo* synthesis of cytoplasmic chlorophyllase is essential for ethylene-enhanced chlorophyll degradation.

Changes in chloroplast structure during degreening of *Citrus* fruits treated with ethylene were examined by light and electron microscopy (Shimokawa *et al.*, 1978). The number of chloroplast decreased with ethylene treatment and tree-ripening. Reduction in chloroplast size was a characteristic feature of ethylene-treated fruits lost grana and lamellar systems. This disintegration of the membranes within the chloroplasts is expressed by the word "melt". In the chloroplasts of ethylene-treated fruit, double-layered structures were degraded and the membrane layers become separated from each other. These observations are quite similar to the case of the ribosomes of the ethylene-treated separation zone (Freytag *et al.*, 1977).

Ethylene-enhanced chlorophyllase was purified and the properties investigated (for details see Shimokawa, 1983).

Fate of ethylene *in vivo*

Early works on the fate of ethylene were carried out by Buhler *et al.* (1957) and Behmer (1958). They attempted to find out if ethylene could be metabolized further and obtain information on ethylene precursors. This is an unusual approach in the study of biosynthesis. They concluded that ethylene was a terminal product in fruits and could not be metabolized further. Contrary to these results, Hall *et al.* (1961) found that cotton and *Coleus* plants incorporated ethylene-^{14}C into a variety of substances. However, they could not obtain information on ethylene biosynthesis and ethylene action. It should be noted that these studies on ethylene-^{14}C metabolism appeared before the orthodox study of Burg and Thimann (1959). This indicates the difficulty encountered in the study of ethylene biosynthesis.

Shimokawa and Kaŝai (1968) reported on the incorporation of ethylene -^{14}C in morning glory seedlings. ^{14}C of the ethylene-^{14}C was found in the ribosomal protein and ribosome RNA. If the incorporation of ethylene is of physiological significance, it is very interesting as a primary site of ethylene action. However, this research used ethylene regenerated from its mercuric perchlorate complex to facilitate obtaining known amounts of ethylene. No adequate precautions were taken with regard to $^{14}C_2H_4$ purity or microbial contamination. But, recent work has excluded the regeneration method to avoid the effects of impurities generated during storge. One of Bayer's works clearly shows that ethylene is metabolized to CO_2. He showed that this conversion to $^{14}CO_2$ is possibly linked to ethylene action and that it represents the means of reducing the endogeneous ethylene level. Contrary to this, Sisler and Wylie (1978) assume that the binding of ethylene to its attachment site is weak and is readily displaced. No significant results have yet been obtained from the experiments using $^{14}C_2H_4$.

Bayer (1972) and Abeles *et al.* (1972) attempted to gain an understanding of the ethylene receptor site and its activation and carried out experiments dealing with the use of deutrated ethylene (C_2D_4, *cis*-$C_2D_2H_4$, *trans*-$C_2D_2H_2$). Deutrated ethylene was used on the basis of theoretical reasoning: Deuteration increases the stability of silver ion-olefin complexes. If ethylene binds to a metal-containing receptor site as proposed by Burg and Burg (1967), C_2D_4 might possibly be slightly more biologically active than C_2H_4. If C_2D_4 is less active, this would warrant consideration of a mechanism of receptor site activation. No apparent

differences were observed in the biological activity af C_2D_4 and C_2H_4 using the pea stem straight growth assay and the pea root elongation assay. There was no detectable exchange between the D atoms and H atoms of the tissues, and no conversion of *cis* and *trans*-$C_2D_2H_2$. These results suggest that the mechanism of ethylene action does not involve an intermediate ethylene complex resulting in hydrogen exchange or *cis-trans* isomerization during a possible activation of the receptor sites.

Conclusion

Twenty years ago, the decision was made by the author to undertake a study on the control of plant aging under the direction of Prof. Z. Kasai of Kyoto University. Attention was directed to the yellowing of leaves, especially tobacco leaves because of their practical importance. According to an article of Hall *et al.* (1961), Genevois in 1954 expressed the opinion that carotenoids may arise from ethylene. Since some 65 per cent of ether-soluble radioactivity was present in the carotenoid fraction, it may be assumed that ethylene has reacted with β-carotene or served as a precursor in its formation. This sentence prompted the author to gather literature on ethylene. Literature on ethylene measurement available at that particular time was scarce. Therefore, research was started on the methodology of measurements of ethylene and ethylene $-^{14}C$ (Shimokawa and Kasai,1965).

This review has raised and attempted to answer the following question: (i) Is there an enzyme that may be termed ethylene synthase ? (ii) Is there a metabolic interrelationship between methionine (or ACC) as a dominant ethylene precursor and other substrates such as acrylic acid or linolenate, as a minor precursor ? (iii) Why is it that a cell free system for research of ethylene biosynthesis cannot be established ? (iv) In what respects is the action of ethylene significant ?

It is hoped that this review will stimulate interest in various areas of research on ethylene.

Acknowledgement

I am very grateful to Mrs. T. Kinoshita for her assistance in preparation of the manuscipt.

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Plant Responses to Ethylene and Ethylene Releasing Compounds

Klaus Lurssen

Introduction

Long before it was established that plant metabolism and growth is regulated and influenced by plant hormones, the action of the plant hormone ethylene on plants was observed and ethylene was recognized as the inducing agent. The first reports on ethylene action date back to 1858, describing plant responses to illuminating gas. At the end of the 19th century a pineapple farmer on the Azores, experimenting with fumes in the greenhouse to kill insects, discovered an earlier flowering of the pineapple plants after this treatment. In the following years the method to induce flowering of pineapple in the greenhouse by fumes became common on the Azores. This was probably the first application of a "plant growth regulator" in agriculture. In a report of 1901, the "triple response" of etiolated pea seedlings (growth inhibition, thickening of the subapical region, horizontal nutation) was observed to occur in the presence of illuminating gas and ethylene was identified as the inducing agent. In 1912 the induction of fruit ripening by ethylene was recognized. The role of ethylene in flower induction of pineapple was established in 1932 (Rodriguez, 1932). In 1934 the production of high amounts of ethylene by ripening fruit was described, indicating that ethylene is a natural substance produced by plants- the first basis for the later arising opinion that ethylene is a plant hormone (for references see Zimmer, 1968; Burg, 1973).

Mode of Action of Ethylene

Plant hormones induce many different plant responses on the metabolic level as well as on the level of cell organelles and finally on that of whole plants. It is unknown whether plant hormones have just one or more primary sites of action. Depending on the availability of receptor sites, on the compartmentation within the plant cells, on the metabolic and physiological status of the plant and plant cells, and on the environmental conditions causing a certain status one may well assume that one

primary site of action can lead to many different responses. So far the primary reaction is known for none of the plant hormone for certain. However, the dose-r esponse relations of ethylene and the competitive inhibition of most ethylene effects by carbondioxide make a single site of primary action of ethylene probable (Abeles 1973).

It is generally accepted today, that certain ethylene induced plant responses, as for example fruit ripening or abscission, are preceded by an ethylene stimulated increase of protein synthesis. It was also shown, that ethylene affects the synthesis of the different RNA species (Holm, O' Brin, Key and Cherry, 1969; Hulme, Rhodes and Wooltoroton, 1971a; Marei and Romani, 1971). Lately the ethylene action on the protein synthesis machinery in general and on the expression of specific gene messages was confirmed (Christoffersen and Laties, 1982). The common problem of all experiments mentioned above is the long time elapsed between ethylene treatment and analysis of the events measured. Thus gene expression as a primary action of ethylene seems possible but is not proved.

A different approach to study primary reactions of plant hormones is the investigtion of their binding to receptors in the cells. The interpretation of such expriments is difficult, because of the low amounts of receptor that can be expected in the tissue and of unspecific binding of the hormones. Interpretation of data has become even more doubtful, since Murphy (1979) showed, that naphthaleneacetic acid binds to bovine serum albumin in exactly the way, one would expect for a receptor site. Although the studies of ethylene binding sites are of extreme importance for illucidation of the primary reaction, one should be very coutious in interpreting the results.

A calculation of the amount of ethylene receptors in plant tissue by Abeles (1973) revealed 10 mg per metric ton or approximately 500 attachment sites per cell, assuming the receptor has a molecular weight of 100 000. Based on kinetic studies of ethylene binding and displacement in tobacco leaves, Sisler (1979) estimated 4000 binding sites per cell.

Concerning the nature of the binding site, Burg and Burg (1967) proposed a metal atom at the binding site centre. Experiments with deuterated ethylene revealed no exchange of deuterium (Abeles *et al*, 1972: Beyer, 1972). It can be concluded from these experiments, that ethylene action does not involve a type of chemical binding by which hydrogen respecti vely deuterium can be exchanged or *cis-trans*-isomerisation can occur.

A biphasic release of ethylene from plant tissue was observed, possibly indicating a compartmentation of ethylene in the cell (Sisler 1979). Similar conclusions were drawn by Jerie, Shaari and Hall (1977). In the latter study the meaning of the ethylene binding site is discussed alternatively as the primary site of action or as a mechanism for controlling the internal ethylene concentration.

After establishing binding of ethylene as a general phenomenon in plant tissue there were several attempts to isolate and characterize the binding site. Sisler (1980) partially purified an ethylene binding component of high molecular weight with the properties of a lipophilic protein, resembling a membrane protein. Hall's group isolated a cell free system from *Phaseolus* cotyledons with an apparent dissociation constant for ethylene of 0.64 nM, which is well in the range of half maximal saturation of ethylene responses in physiological systems. Also a variation of the number of binding sites during ontogeny was found, which is an additional support for the assumption, that indeed a relevant binding site for ethylene was isolated (Bengochea *et al.*, 1980a). Furthermore, structural analogues of ethylene behave in this system in a similar way as they do in physiological processes in plants (Bengochea *et al.*, 1980b). Characterisation of sedimentation properties and identification of marker enzymes in the preparations suggest three different binding sites, possibly on protein body membranes, on smooth and on rough endoplasmic reticulum (Evans *et al.*, 1982a) These results were confirmed with high resolution autoradiography (Evans *et al.*, 1982b).

Another approach to identify the primary reaction of ethylene turned out to be the study of ethylene metabolism in plants. Since 1957 there were several reports on this topic, some of which show incorporation or metabolism to carbon dioxide of small amounts of the applied ethylene. The main problem of interpreting these results is the fact, that impurities of the radio-labelled ethylene and microbial contaminations may have obscured the analysis. Other workers did not find indications for ethylene turnover. Abeles concluded in his book in 1973, that "it seems reasonable to assume that the incorporation measured represents an artifact due to impurities in the ethylene used" (Abeles, 1973, for further references and detailed discussion of these earlier results also see in this reference).

In a series of investigations with highly purified radio-labelled ethylene and special precautions against microbial contaminations and other

analytical errors Beyer demonstrated, that ethylene is indeed incorporated into the tissue and metabolised to carbon dioxide. The metabolised fraction of the total natural occuring ethylene in the cell can be estimated as only a few per cent. This low rate of turnover does not seem to be sufficient to be regarded as an effective system for ethylene detoxification. Furthermore, as ethylene diffuses rapidly out of the plant tissue (Abeles, 1973), detoxification seems not necessary. It was therefore speculated, that a relation between ethylene action and ethylene metabolism might exist (Beyer, 1975a; Beyer 1975b; Beyer, 1977).

In flowers of *Ipomoea tricolor* a correlation between ethylene-induced senescence (Kende and Baumgartner, 1974), sensitivity to ethylene, and capacity to metabolise ethylene to carbon dioxide was shown (Beyer and Sundin, 1978). The oxidation rate of naturally occurring ethylene in this system was estimated to be less than 0.2%. Further investigations into the relationships between ethylene action and ethylene metabolism in etiolated pea seedlings (Beyer, 1979a) and in abscission zones of cotton leaves (Beyer, 1979b) indicate a close correlation between ethylene action and ethylene metabolism. Ethylene incorporation into plant tissue and ethylene oxidation to carbon dioxide can be inhibited independently by silver ions and by carbon dioxide, while the inhibition of either of these metabolic pathways coincides with the inhibition of the physiological action of ethylene. These findings suggest, that ethylene action and ethylene metabolism are related and that ethylene metabolism is possibly involved in the first step of ethylene action. The two independent pathways of ethylene metabolism could " acount for the wide divergence of ethylene responses that can occur in different tissues depending on their physiogical state" (Beyer 1979b).

Recent investigations into the metabolic fate of incorporated ethylene revealed an oxidation to ethylene oxide in *Vicia faba* cotyledons (Jerie and Hall, 1978) and in cell free preparations obtained from this source (Dodds *et al.*, 1979; Dodds *et al.* , 1980). The oxidation to ethylene oxide seems to be peculiar for this tissue and ethylene oxide might be a precursor of the identified end product, ethylene glycol (Blomstrom and Beyer, 1980). It is an open question whether, respectively how, ethylene metabolism is related to ethylene binding located at the endomembrane system.

Effects of Ethylene on Plant Metabolism

The primary reaction of ethylene must lead to influences upon the metabolism of plants, which then will cause the different physiological plant

responses. As stated in the preceding chapter, there is today no definite knowledge about the primary reaction of ethylene. Nothing at all is known about the reactions following the primary reaction. On the other hand, many different, sometimes even opposite effects on plant metabolism are reported in the literature. Opposite effects to the same metabolic reaction sequence indicate, that this regulating effect is far away from primary or secondary reactions (Lurssen, 1981). In Table 1 the effects of ethylene on plant metabolism are summarized. Effects upon gene expression are not included, as they were discussed already in the preceding chapter (see review paper by K. Shimokawa in this volume).

Table 1. Effects of ethylene on plant metabolism

Influence upon enzyme activity and protein synthesis	
Enhancement of amylase synthesis	Jacobson, 1973
Enhancement of aspartate aminotransferase activity	Sankhla and Huber, 1974
Enhancement of alanine aminotransferase activity	Sankhla and Huber, 1974
Enhancement of alcohol dehydrogenase activity	Hulme *et al.*, 1971b
Enhancement of cellulase activity	Goren and Huberman, 1976
	Huberman and Goren, 1979
Enhancement of cellulase synthesis	Lewis and Varner, 1970
	Abeles and Leather, 1971
Enhancement of cell wall-bound peroxidase activity	Ridge and Osborne, 1971
Enhancement of chlorophyllase activity	Shimokawa *et al.*, 1978;
	Purohit, 1982
Enhancement of glutamate dehydrogenase activity	Sankhla and Huber, 1974
Enhancement of malic enzyme activity	Hulme *et al.*, 1971b
Enhancement of PEP carboxylase	Tupy and Primot, 1976
Enhancement of peroxidase activity	Stahmann *et al.*, 1966
	Imaseki *et al*, 1968
	Imaseki, 1970
	Ridge and Osborne, 1971
	Morgan and Fowler, 1972
Enhancement of phenylalanine amonia lyase activity	Imaseki *et al.*, 1968
	Hyodo and Yang, 1971
	Rhodes and Wooltorton, 1971
Enhancement of polygalacturonase activity	Sawamura *et al.*, 1978
	Huberman and Goren, 1979.
Enhancement of polyphenoloxidase activity	Stahmann *et al*, 1966
Enhancement of polyribosome formation	Christoffersen and Laties, 1982
Enhancement of pyruvate decarboxylase activity	Hulme *et al.*, 1971b
Enhancement of xylanase activity m-RNA formation	Eastwell and Spencer, 1982
	Christoffersen and Laties, 1982
Reduction of phenylalanine ammonia lyase activity	Buhler *et al.*, 1978

Table 1 (Continue)

Reduction of Pryuvatdecorboxylase activity	Tupy and Primot, 1976
Stimulation of protein synthesis	Brady *et al.*, 1970 Marei and Romani 1971
Stimulation of ribonucleic acid synthesis	Holm *et al.*, 1970 Marei and Romani, 1971
Stimulation of ribosome synthesis	Marei and Romani, 1971
Influence upon concentrations of substances in plants	
Decreased Auxin content	Lieberman and Knegt, 1977 Riov and Goren, 1979
Decreased sucrose content	Tupy and Primot, 1976
Increased Chlorogenic acid content	Imaseki *et al.*, 1968
Increased gibberellin content	Dimalla and van Staden, 1977
Increased pH of *Hevea* latex	Tupy, 1980
Increased phenolic compounds content	Rhodes and wooltorton, 1973
Increased water soluble pectin content	Sawamura *et al.*, 1978
Phytochrome	Stone and Pratt, 1978
Influence upon metabolic pathways	
Enhancement of auxin conjugation	Ernest and Valdovinos, 1971 Riov and Goren 1979
Enhancement of auxin decarboxylation	Beyer and Morgan, 1970
Enhancement of anthocyanin synthesis	Craker *et al.*, 1971
Enhancement of Chlorophyll synthesis	Alscher and Castelfranco, 1972 Buhler *et al.*, 1978b
Enhancement of lignin biosynthesis	Rhodes and wooltorton, 1973
Enhancement of respiration	Biale, 1960 (see this reference also for older literature) Eaks, 1970 Marei and Crane, 1971 Rhodes and wooltorton, 1971 Hulme *et al.*, 1971b Reid and pratt, 1972
Inhibition of anthocyanin synthesis	Craker *et al*, 1971 Kang and Burg, 1973; Buhler *et al.*, 1978a
Inhibition of auxin formation	Ernest and Valdovinos 1971
Inhibition of carotenoid synthesis	Kang and Burg, 1973
Inhibition of phospholipid synthesis	Irvine and Osborne 1973
Inhibition of carbon dioxide fixation	Kays and Pallas, 1980; Pallas and Kays, 1982
Inhibition of phytochrome destruction	Stone and Partt, 1978
Influence upon membranes, transport and secretion	
Decrease in membrane permeability	Naik *et al.*, 1980

Table 1 (Continued)

Enhancement of amylase secretion	Jones, 1968 Ho *et vl.*, 1982 Eastwell and Spencer, 1982
Enhancement of calcium secretion	Eastwell and Spencer, 1982
Inhibition of auxin transport	Morgan and Gausman, 1966 Beyer and Morgan, 1970 Ernest and Valdovinos, 1971 Beyer and Morgan, 1971 Beyer, 1973 Riov and Goren, 1979
Inhibition of proton excretion	Craker *et al.*, 1978
Stimulation of cellulase secretion	Abeles and Leather, 1971
Interactions with plant hormones or phytochrome	
Abscisic acid	Jacobson, 1973
Auxin	Kang and Burg' 1973
Gibberellins	Jacobson, 1973 Ho, *et al*, 1982 Eastwell and Spencer, 1982
Phytochrome	Kang and Burg, 1972a Kang and Burg, 1973 Buhler *et al.*, 1978a

Involvement of Ethylene in Plant Growth

There are many different influences of ethylene upon plant growth reported in the literature (Table 2). Most effects have been known already for a long time (for older literature see Abeles, 1973; Zimmer, 1968; Burg, 1973). Only recently there were several examples of a closer understanding of physiological plant responses in relation to influences of ethylene upon metabolism. Such relations could be established for abscission processes and enhancement of cellulase and polygalacturonase activity in abscission zones, for abscission and influence upon auxin concentration, for ripening (degreening) of citrus and stimulation of chlorophyllase activity, and for growth inhibition and modification of cell shape and influence upon auxin concentration. Further investigations into the field of relations between metabolic processes and physiological actions are of extreme value for the understanding of the growth of plants.

Another field of special interest is the interaction between plants and plant pathogens. After infection of plants by certain viruses and fungi an enhanced ethylene production is observed. Further studies are needed to learn about possible defense mechanisms on the biochemical level of

plants against these pathogens and about the role of ethylene in inducing such mechanisms.

Table 2. Involvement of ethylene in plant growth

Abscission : promotion	Hallaway and Osborne, 1969
	Addicott, 1970
	Jackson and Osborne, 1970
	Lewis and Varner, 1970
	Abeles *et al.*, 1971
	Abeles and Leather, 1971
	Beyer and Morgan, 1971
	Morgan and Durham, 1972
	Beyer, 1973
	Lipe and Morgan, 1973
	Poovaiah and Rasmussen, 1973
	Osborne, 1974
	Wright and Osborne, 1974
	Hanisch ten Cate *et al.*, 1975
	Osborne and Sargent, 1976
	Wheaton *et al.*, 1977
	Goren *et al.*, 1977
	Riov and Goren, 1979
no effect :	Dorffling *et al.*, 1978
Cell structure : modification	
Cell size	Apelbaum and Burg, 1971
Cell wall	Eisinger and Burg, 1972
	Apelbaum and Burg, 1972
	Sargent *et al.*, 1974
	Freytag *et al*, 1977
	Geballe and Galston, 1981 a, b
Chloroplasts	Shimokawa *et al.*, 1978
Endoplasmic reticulum	Valdovinos *et al.*, 1971
	Freytag *et al.*, 1977
Microfibrils	Apelbaum and Burg, 1971
	Steen and Chadwick, 1981
Microtubuli	Steen and Chadwick, 1981
Mitochondria	Makimoto and Asahi, 1981
Plasma membrane	Koehler and Lewis, 1979
Ribosomes	Freytag *et al.*, 1977
Female flower induction	McMurray and Miller, 1968
	Iwahori *et al.*, 1969
	Splittstoesser, 1970
	Iwahori *et ai.*, 1970
	Rudich *et al.*, 1972

Flower induction	Rodriguez, 1932 Zimmer, 1968 Cooke and Randall, 1968 Py et Guyot, 1970 Teisson, 1979
Fruit development	Marei and Crane, 1971
Germination: inhibition	Taylorson, 1979 Suzuki and Taylorson, 1981 Jackson *et al.*, 1981
promotion	Toole *et al.*, 1964 Ketring and Morgan, 1969 Ketring and Morgan, 1970 Egley and Dale, 1970 Ketring and Morgan, 1971 Takayanagi and Harrington, 1971 Burdett and Vidaver, 1971 Ketring and Morgan, 1972 Eplee and Langston, 1976 Taylorson, 1979
Growth : inhibition	Radin and Loomis, 1969 Chadwick and Burg, 1970 Turgeon and Webb, 1971 Apelbaum and Burg, 1972 Sargent *et al.*, 1974
Growth : promotion	Ku *et al.*, 1970 Musgrave *et al.*, 1972 Suge *et al.*, 1971 Suge, 1972 Takahashi, 1973 Suge, 1974 Craker *et al.*, 1978
Involvement in differentiation of ferns	Brooks, 1973 Elmore and Whittier, 1973
Involvement in stress phenomena	
Mechanical stress	Goeschl *et al.*, 1966 Pickard, 1971 Turgeon and Webb, 1971 Hiraki and Ota, 1975 Mitchell, 1977
Water deficit	McMichael *et al.*, 1972 Hall, *et al.*, 1977

	Apelbaum and Yang, 1981
Water logged soil	Jackson and Campbell, 1975
	Jackson, 1976
	Jackson and Campbell, 1976
	Hall *et al.*, 1977
	Jackson *et al.*, 1978
Involvement in tropisms	
Gravitropism	Chadwick and Burg, 1970
	Jackson, 1979
	Wheeler and Salisbury, 1981
	Bucher and Pilet, 1981
Phototropism	Humphrey, 1980
Thigmotropism	Bangerth, 1974
Latex flow Stimulation	Abraham *et al.*, 1968
Other morphogenetic effects	
Adventitious root formation	
	Drew *et al.*, 1981
	Jackson *et al*,, 1981
Aerenchyma formation	Drew *et al.*, 1981
	Konings, 1982
Callus formation	Goren *et al.*, 1979
Epinasty: induction	Abeles, 1973
	Jackson, 1976
	Amrhein and Schneebeck, 1980
no influence	Reid *et al.*, 1981
Plumular hook formation	Kang and Burg, 1972b
Tillering	Harrison and Kaufman, 1981
Pollen sterility	Rowell and Miller, 1971
	Bennett and Hughes, 1972
Response to plant diseases: ethylene evolution	
	Hislop *et al.*, 1973
	Montalbini and Elstner, 1977
	Paradies *et al.*, 1980
	Wien and Roesingh, 1980
	de Laat *et al.*, 1981
	Walther *et al.*, 1981
	de Laat and van Loon, 1982
	Esqerre-Tugaye *et al.*, 1982
Ripening: no direct influence	
	Reid *et al.*, 1973
Promotion	Burg and Burg 1965
	Burg and Burg, 1969
	Hulme *et al*, 1969
	Hale *et al.*, 1970
	Ben-Yehoshua *et al.*, 1970
	Brady *et al.*, 1970
	Zeroni *et al.*, 1972
	Lieberman *et al.*, 1975

	Cohen, 1978 Shimokawa *et al.*, 1978 Bufler und Bangerth, 1981
Senescence : promotion	Mayak and Halevy, 1982 Purohit, 1982 Kende and Baumgartner, 1974 Nichols and Ho, 1975 Aharoni and Lieberman, 1979
Stomatal closure no effect of ethylene :	Pallas and Kays, 1982 Pallaghy and Raschke, 1972
Wound ethylene formation	Elstner and Konze, 1976 Saltveit and Dilley, 1978 Peiser and Yang, 1979 Boller and Kende, 1980 Yu and Yang, 1980 Wang and Adams, 1980 Konze and Kwiatkowsky, 1981 Hyodo and Nishiono, 1981 Field, 1981

Ethylene Related Plant Growth Regulators

Some of the reactions of plants to ethylene are not only of theoretical interest but could be useful in agriculture and horticulture. Ethylene as a gas can only be applied in practice under certain circumstances in more or less closed systems like glasshouses or incubation chambers, limiting the usefulness of the hormone itself to very few applications. Beginning in the nineteensixties there were several efforts in the agrochemicals industry to develop liquid or solid ethylene releasing compounds for easier handling. These compounds degrade in presence of water, evolving ethylene (Draber, 1977). The development of such compounds still is the only example of a rational design of plant growth regulators or pesticides with a new mode of action.

2-chloroethylphosphonic acid (CEPA, Ethephon) is the best known compound of this group. It releases ethylene above pH 4 in aqueous solutions but is relatively stable below pH 4. The possibility of stabilisation under certain conditions is of course important for a commercial plant growth regulator, as such a compound is stored for some time before use. The degradation of 2-chloroethylphosphonic acid to ethy-

lene is a base catalysed elimination reaction (Fig. 9.1) (Cooke and Randall 1968; Sterry, 1969).

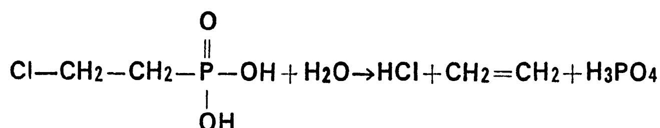


Fig. 9.1 Degradation of 2-chloroethylphosphonic acid (Ethephon) to ethylene.

Several esters and other derivatives of 2-chloroethylphosphonic acid are described in the patent literature (see Draber, 1977). Most probably all these compounds are converted to 2-chloroethylphosphonic acid in the cell. Theoretically an improved performance could be achieved by these derivatives by a better penetration into the cell or by a different time course of ethylene evolution (Lurssen, 1982). However, these derivatives generally were not superior to ethephon itself.

2-chloroethyl-tris-(2-methoxyethoxy)-silan (Etacelasil) belongs to the group of 2-chlorosilanes (see Draber 1977), also releasing ethylene in aqueous solutions. The chemistry of ethylene evolution is compatable to that of ethephon (Fig. 9.2).

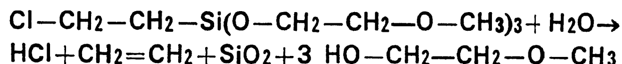


Fig. 9.2 Degradation of 2-chloroethyl-tris-(2-methoxyethoxy) silan (Etacelasil) to ethylene.

A third group of ethylene releasing compounds are the 2-haloethylsulfinic acids and derivatives (Draber, 1977; Lurssen, 1982). These compounds also have a similar mechanism of ethylene release as compared with ethephon (Fig. 9.3.).

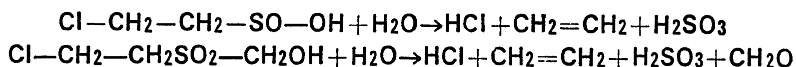


Fig. 9.3 Degradation of 2-chloroethylsulfinic acid (upper formula) and (2-chloroethyl) sulfonyl-methanol (lower formula) to ethylene.

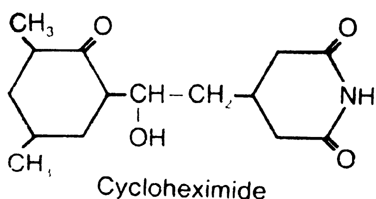
The growth regulator 2-hydroxyethylhydrazine (Fig. 9.4) causes typical ethylene reactions in plants (Gowing and Leeper, 1956; Palmer *et al.*,

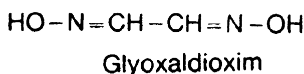
Fig. 9.4 2-Hydroxyethylhydrazine is possibly converted to ethylene in the plant.

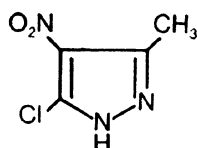
1967). However, the mode of action of rhis compound is not clear. The compound does not release ethylene spontaneously in aqueous solution (Block and Young, 1971). In more complex systems an ethy-

lene generation can be measured and it could be assumed, that in plant tissue a conversion to ethylene can occur (Palmer *et al.*, 1967; Dollwet and Kumamoto, 1972).

Three chemically unrelated growth regulators should be mentioned: cycloheximide (trade name Act-Aid), glyoxal–dioxim (trade name Pik-Off), and 5-chloro-3-methyl-4-nitro-1H-pyrazol (trade name Release) Fig. (9.5). These compounds are useful only for a very limited field of application, namely citrus fruit abscission. They do not generate ethy-







5-Chlor-3-methyl-4-nitro-1H-pyrazol

Fig. 9.5 Citrus abscission agents which slightly injure the fruit causing wound ethylene production.

lene themselves but obviously slightly injure the fruit, causing wound ethylene production, which induces abscission layers (Cooper and Henry, 1971; Devies *et al.*, 1976). Cycloheximide applied to the tissue directly does not enhance ethylene production, but inhibits it by inhibiting protein synthesis (Hyodo, 1978).

1-aminocyclopropane-1-carboxylic acid (ACC) (Fig. 9.6), known since 1957 as a constituent of ripe fruits (Burroughs, 1957; Vähätalo and Virtanen, 1957), is an intermediate of ethylene biosynthesis (Adams and Yang, 1979; Lurssen *et al.*, 1979). ACC and its derivatives enhance ethylene biosynthesis and induce typical ethylene reactions in plants

(Schröder and Lurssen, 1978; Lurssen, 1981; Lavee and Martin, 1981; Lurssen, 1982).

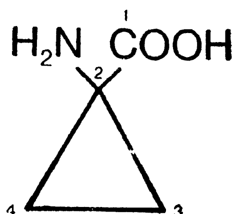


Fig. 9.6 1-Aminocyclopropane-1-carboxylic acid (ACC), the precursor of ethylene in ethylene biosynthesis.

Chemical analogs of ethylene are far less active in biological systems. Propylen causes similar reations, but 100 times the amount is needed compared with ethylene. Acetylene gives similar reactions only at about 3000 times the amount of ethylene (Burg and Burg, 1969). Other hydrocarbones are even less active.

Application of Ethylene Related Plant Growth Regulators in Agriculture and Horticulture

The application of ethylene as a plant growth regulator dates back to the last century (see Introduction). The gas itself however is not easy to handle. The only practical application until recently is the ripening of banana fruit in special gas tide chambers: green banana fruit, imported from the producing countries, are stored in climatised chambers at 12 °C, keeping the fruits unripe. Ripening is induced by rising the temperature to at least 14.5°C and simultaneously applying ethylene gas. The introduction of Ethephon (Cooke and Randall, 1968; Sterry, 1969) opened up a number of different application in practice. Furthermore, many investigations into the actions of ethylene on plant growth and metabolism were done using Ethephon as a convenient source and surely this compound stimulated basic research on actions of ethylene. On the other hand, a detailed knowledge about reactions of plants following ethylene treatment facilitates the development of a commercial ethylene plant growth regulator.

Some of the ethylene actions listed in Table 2 are of special interest from the viewpoint of modification of plant growth and development in agriculture and horticulture. These aspects of ethylene action are promotion of abscission, female flower induction, flower induction, latex flow

stimulation, promotion of germination, growth inhibition, enhancement of ripening and induction of pollen sterility.

Stimulation of Ripening

There are several aspects regarding the usefulness of stimulation of ripening. The most interesting consequence is the possibility of a once-over mechanical or manual harvest in crops that produce ripe fruits over a longer time period and therefore normally must be harvested several times. A second aspect is the possibility to speed up the time of cultivation of a crop, especially in greenhouses were follow up crops are planted immediately. A further advantage of stimulating ripening is the possibility to plan harvest and marketing times of fruits. In some crops it may also be of interest, to harvest and market fruits generally earlier in the year.

Ethephon is used for stimulation of tomato fruit ripening especially in California on processing tomatoes. The fruit quality is not negatively affected compared with normally ripened tomatoes. The application of the growth regulator about 8 days before harvest with 20% colored fruits in the field allows a once-over mechanical harvest without loosing a substantial amount of unripe fruits (Sims, 1969; Iwahori and Lyons, 1969). The use on fresh market tomatoes is also possible (Sims and Kasmire, 1972; Rabinowitch *et al.*, 1970). Tomato fruit ripening cannot be enhanced by ACC sufficiently. However, a derivetive of ACC, N-Formyl-ACC, is very effective in stimulation of tomato fruit ripening (Lurssen, 1982).

Ethephon enhances ripening also in other vegetables and fruits (Sterry, 1969) like peppers (Sims *et al.*, 1970), fig (Crane *et al.*, 1970; Gerdts and Obenauf, 1972), melon (Rabinowitch *et al.*, 1970; Tyler *et al.*, 1970), peach (Martin *et al.*, 1969), blueberry (Dekazos, 1978), papaya (Shanmugavelu *et al.*, 1976), apple (Sterry, 1969; McBride and Faragher, 1978) and grape (Jensen *et al.*, 1975; Chakrawar and Rane, 1977) when applied shortly before harvest. In apple and grape an earlier time of application during fruit development also enhances the ripening process (Hale *et al.*, 1970). However the ripening of some fruits- possibly the non-climactric ones- cannot be enhanced by ethylene or Ethephon. No influence on ripening was reported for strawberry (Nestler, 1978) and in citrus only a coloration of the peel is achieved without affecting internal ripening (Fishler and Monselise, 1971). In pineapple Ethephon is also used shortly before harvest to enhance ripening. Here the compound also

causes mainly a eoloration of the fruit, the maturity is hardly affected (Poignant, 1971; Crochon *et al.*, 1981). The use of Ethephon to ripen coffee berries is complicated by the fruiting behaviour of coffee with fruits of different developmental stages being simultaneously on the tree. When Ethephon is applied to early during fruit ripening a loss of quality due to an apparent enhanced ripening of the fruit without ripening of the bean is observed. Furthermore, problems can arise with ethylene-induced leaf drop (Browning and Cannell, 1970; Sondahl *et al.*, 1974; Oyebade, 1976; Opile and Browning, 1977; Clowes, 1977; Opile, 1978).

Recently the usefulness of Ethephon a ball opening agent in cotton could be demonstrated, The application allows a once-over harvest and increases total yield (Cothren, 1981).

Apart from ripening of fruits an enhanced ripening (senescence) of vegetative plant parts can also be of interest. Positive results have been reported in sugarcane. The harvested material has a higher sugar content and higher juice purity when Ethephon is applied (Anonymous, 1976). An enhanced leaf maturity is desirable in tobacco to reduce the harvest frequencies. With Ethephon it is possible to reduce the number of harvest from about 6 to 3. Furthermore the curing time after harvest is reduced (Long *et al.*, 1974; Domir and Foy, 1976).

Stimulation of latex flow in *Hevea* trees

Tapping of *Hevea* trees to collect latex from latex vessels in the bark is done by cutting a spiral into the bark. At the lower end of this spiral the runs down into a cup or a plastic bag. Depending on the conditions of the tree and on the season in the year tapping has to be done every few days. The work needs highly trained and reliable people and rubber plantations in the producing areas sometimes have difficulties to find appropriate workers. The application of stimulants of latex flow has several beneficial aspects: Firstly, the number of tappings can be reduced, as latex flow continues for a longer time if stimulants are used. Thus the tapping can be done more effectively. Secondly, the length of the spiral can be reduced from one full spiral down to a quarter of a spiral without reducing the yield. By reducing the length of the cut, a worker can tap more trees in the same time. Furthermore the bark of the tree is saved and the trees can possibly be used for a longer time. Thirdly, by applying stimulants it is possible to increase the total yield of a tree up to approximately 50% without affecting the trees livetime, as long as

the nutrient status is well balanced. In earlier trials with higher amounts of stimulants much higher yield increases up to a few hunderd percent could be achieved, but after a few years of tapping the trees gave only very low yields, nullifying the earlier effects. Today the application of high amounts of stimulant is recomended only on old trees for the last tappings with special application methods (Primot et Tupy, 1976).

Abraham *et al.* (1968) proposed ethylene as the active principle for stimulants of latex flow. Experiments were done with many compounds including ethylene gas absorbed on charcoal (Pakianathan, 1971). Ethephon is the most active compound for this purpose. Special techniques have been developed for its application in combination with tapping methods (Abraham, 1970; Abraham *et al.*, 1973). Today it is commercially applied in most of the *Hevea* plantations.

ACC and its derivatives are not effective in latex flow stimulation (Lurssen, K., unpublished work). A reason could be a lack of the enzyme converting ACC to ethylene in the bark.

Flower Induction in Bromeliaceae

Individual plants of Bromeliaceae normally donot flower simultaneously. This causes problems with commercially grown plants in horticulture (ornamental plant production) as well as in agriculture (pineapple production). In ornamental plant production several Bromeliaceae like *Vriesea*, *Aechmaea*, *Guzmannia*, *Neoregelia*, *Tillandsia* etc. are important flowering plants. The ornamental plant market is a seasonal market and flowering pod plants must be produced accordingly. The discovery, that ethylene stimulates flower induction in Bromeliaceae (Rodriguez, 1932) opened up the possibility to time production of ornamental plants and to plan the production of pineapple some month in advance. The possibility to induce flowering in more than 95% of the pineapple plants in ihe field facilitates greatly the cultivation of the crop.

Not only ethylene and ethylene releasing compounds (Cooke and Randall, 1968), but also acetylen and 2-hydroxyethylhydrazine (Zimmer, 1968) are suitable stimulants. In the main pineapple producing areas Ethephon is widely used today (Guyot et Py, 1970 a; Guyot et Py, 1970 b; Py et Guyot, 1970; Teisson, 1979).

Abscission

Abscission processes are induced by ethylene. Abscission zones form at predetermined cell layers (Table 2). Metabolic and physiological

processes leading to abscission involve induction of polysaccharide hydrolysing enzymes (Table 1) and interference with auxin transport (Morgan and Durham, 1972). Possibly a decrease in auxin concentration caused by ethylene also plays a role in abscission. Generally induction of abscission processes is helpful in agriculture and horticulture for several aspects (Cooper *et al.*, 1968).

Induction of ripe fruit abscission is of practical interest to facilitate mechanical harvest of fruits grown on trees. The use of shaking machines for mechanical fruit harvest may lead to damages of the trees depending on the forces to be applied: young roots may be damaged and twigs may fall down if the necessary shaking forces are high. Induction of fruit loosening by chemicals allows the shaking forces to be diminished. However, a general problem arises if fruit loosening chemicals are applied: abscission is not induced selectively in fruits but also in leaves. Fortunately mainly older leaves, which would abscise within a short time period, are affected. The dependence of leaves abscission and age of the plants could be demonstrated for example in roses: maximum defoliation following treatment with ethylene growth regulators occured only later in the growing season (Lurssen, 1982). The problem of leaves abscission varies from species to species, and even cultivars may react differently. Simultaneously with fruit abscission an ethylene treatment shortly before harvest often also enhances the colour development and ripening of the fruits (see above).

Another aspect of abscission is that of young fruit to prevent biennial cropping and to increase fruit size and quality. The problem of biennial cropping is probably best known in apple. In one year blooming and fruit set is extremely high, while in the following year the fruit set is low. Thinning the young fruits in the first year by about 20 to 40% results in bigger fruits of higher quality. Of course the total amount of fruits is reduced in the first year. In the second year generally a normal fruit set will occur in response to last years treatment. The total yield and quality of fruits is improved over the years by thinning. Similar problems also occur in other crops like peach, coffee, grape and some plum varieties.

A special aspect in the ornamental tree sector is the prevention of fruit formation to avoid nuisance created by ripe fruits fallen down to the ground. These fruits might cause people to slip and they stain concrete

and other surfaces. Young fruit abscission can prevent such problems (Furuta *et al.*, 1970).

Defoliation of plants is also useful in some cases. Early defoliation of decideous nursery stock is of interest. The induction of earlier leaf abscission allows earlier sale and transplanting of the plants and earlier storage of the plants for the winter season. An important aspects is the defoliation as a harvest aid procedure. This is professionally done before mechanical harvest of cotton to prevent a coloration of the fiber by leaf pigments. However, the commercially applied products do not seem to act via ethylene. Relatively high amounts of ethylene plant growth regulators are needed to defoliate cotton. Defoliation of grape is discussed as a harvest aid procedure firstly for mechanical harvesting for a better separation of grapes and leave material, and secondly as a harvest aid procedure to speed up mechanical as well as hand harvesting. A further aspect of early defoliation of fruit trees is the possibility to prune the trees a few weeks earlier in the season. In *Hevea brasiliensis* a simultaneous defoliation of the trees is of interest. Normally old leaves and young leaves would occur at the same time in a plantation. To avoid infection of the highly susceptible young leaves with several diseases from the old leaves still on the tree a suitable defoliant is needed to induce an artificial 'autumn leaf fall'.

In Table 3. the several purposes of induction of abscission processes in agricultural and horticultural practice are summarized.

Table 3. Induction of abscission in agriculture and horticulture

Crop plant and purpose of abscission	Chemical*)	Reference
fruit abscission (harvest aid) :		
apple	Ethephon	Schumacher *et al.*, 1969
cherry	Ethephon	Schumacher und Fankhauser, 1969
	Ethephon	Liebster, 1970
	Ethephon	Stosser, 1970
	Ethephon	Al-Jaru and Stosser, 1973
	Ethephon	Stosser, 1974
citrus	Ethephon	Wardowski and Wilson, 1970
	Cycloheximide	
	Cycloheximide	Cooper, 1971
	Cycloheximide	Cooper and Henry, 1971
	Cycloheximide	Cooper and Wilson, 1971
	Ethephon	Ismail, 1971

Table 3 (continued)

	Cycloheximide	
	Cycloheximide	Wardowsky and Wilson, 1971
	Cycloheximide	Wilson, 1971
	Cycloheximide	Davies *et al.*, 1976
	Release Pik-Off	
	Cycloheximide	Holm and Wilson, 1976
	Release Pik-Off	
	Cycloheximide	Holm and Wilson, 1976
	Release	
	Release	Wheaton *et al.*, 1977
coffee	Ethephon	Browning and Cannell, 1970
	Ethephon	Oyebade, 1976
grape	Ethephon	Eynard, 1975
	Ethephon	Mortensen, 1980
olive	Ethephon	Hartmann *et al.*, 1968
	Etacetasil	Hartmann *et al.*, 1975
	Ethephon	
	Cycloheximid	
	Ethephon	Forlani *et al.*, 1976
	Etacelasil	
	Ethephon	Ben-Tal and Lavee, 1976
	Etacelasil	
plum	Ethephon	Schumacher und Fankhauser, 1969
	Ethephon	Al-Jaru and Stosser,
	Cycloheximide	1973
Syrian plum	Ethephon Cycloheximide	Al-Jaru anu Stosser, 1973
fruit thinning :		
apple	Ethephon	Knight, 1978
		Link und Bavendorf, 1978
Coffee	Ethephon	Adenikinju, 1975
grape	Ethephon	Weaver and Pool, 1971
peach	Ethephon	Blake *et al.*, 1969
plum	Ethephon	Schumacher und Fankhauser, 1972
leaf abscission :		
nursery stock, early autumn	Ethephon	Knight, 1979
leaf fall		Insley and Boswell, 1980
fruit trees, early autumn	Ethephon	Gerdts *et al.*, 1977
leaf fall for early pruning		
grape, harvest aid	Ethephon	Fader *et al.*, 1976

*Only ethylene-active compounds mentioned.

Stimulation of germination :

Weed control is sometimes difficult, if weeds do not germinate before or shortly after application of herbicides. Induction of germination in these weeds could solve this problem. Germination of a number of plant spicis is enhanced by ethylene (Table-2). So there are some efforts to apply ethylene releasing compounds in combination or prior to application of herbicides to increase the effectiveness of the herbicide treatment (Steward, 1969; Egley and Dale, 1970; Chancellor, *et al.*, 1971). Even the application of ethylene as a gas seems promising (Eplee and Langston, 1976).

Induction of female flowers :

Cucumber and melon normally produc male flowers earlier than female flowers. Ethylene stimulates the early production of female flowers. Often male flower formation is completely inhibited following ethylene treatment (Table-2). Treatment of plants with Ethephon has the same effect (McMurray and Miller, 1968; Iwahori *et al.*,1969). Female flower induction in Cucurbitacae is of commercial interest to achieve an earlier harvest and a yield increase (Tompkins and Shulteis, 1970: Tompkins and Smay, 1971; Cantliffé and Woods, 1978).

Prevention of Lodging in Cereals

In Western Europe cereal production is highly intensified. Herbicides, fungicides and high amounts of fertilizer are commonly used to reach the high production level. Under such conditions cereals tend to lodge when rainfalls and storms occur later in the growing season. Mechanical harvest of lodging cereals is very time consuming. Furthermore, the quality of the crop is lower because of prolonged ripening. Under wet weather conditions the drying of the grains is severely affected. Especially in rye germination of grains on the culm may occur.

In wheat the problem of lodging is solved by the use of chlorocholinchloride, a gibberellin antagonist. Apart from a growth inhibition a thickening of the culm is achieved. However, this compound does not prevent lodging in barley and is not very effective in other cereals. The ethylene-induced modification of cell size and shape (Table 2) offers the possibility to inhibit the growth of plants in sensitive developmental phases. A stabilisation of the culm by thicker cell walls according to the observed modification of cell walls (Table 2) may also occur. Under field conditions ethylene growth regulators cause a growth inhibition in

cereals especially when they are applied at a late developmental stage after the appearance of the second node. In barley Ethephon alone or in combination with gibberellin antagonists can be applied succesfully, however, the efficacy is not as good as that of chlorocholinchloride in wheat (Karchi, 1969; Wunsche, 1972; Wunsche, 1977). In rye lodging prevention with Ethephon is also possible (Raafat und Kuhn, 1975; Kuhn und Linser, 1977; Wunsche, 1977; Kuhn *et al.*, 1980).

ACC, the precursor of ethylene in plant metabolism, inhibits the growth of cereals only at very high concentrations. Amazingly N-Formyl-ACC and similar derivatives of ACC are much more effective and prevent lodging at relatively low concentrations (Lurssen, K., unpublished work).

Growth Inhibition

The growth inhibiting properties of ethylene in many plant species are moderate compared with those of plant growth inhibitors interfering with gibberellic acid biosynthesis. In water-plants even a stimulation of growth observed by ethylene (Table 2). The use of ethylene growth regulators in cereal production to prevent lodging is based on growth inhibition (see above). Some investigations show the usefulness of Ethephon in other crops for growth inhibition. In grape vigorous vegetative growth interferes with reproductive growth, berry size, coloration and ripening of the fruits. Therefore special pruning systems have been developed for grape production. Ethephon may be useful in inhibiting vegetative growth of grape vines resulting in higher yields and better quality (Weaver and Pool, 1969; Hartmair und Hepp, 1974; Leave *et al.*, 1977). Similar effects may be achieved in apple (Sandke, 1978). The problem of alternate fruit bearing in this crop was discussed already in connection with fruit thinning. Control of vegetative growth may also overcome this phenomenon.

Male Sterility

In plant breeding of cereals the production of hybrid seed is of great interest to improve quality and to increase yields. Ethylene applied at a certain time of pollen development causes male sterility (Table 2). Reports on the use of Ethephon to induce male sterility in wheat seem promising, however, the effect may not be sufficient for commercial application (Bennett and Hughes 1972; Hughes *et al.*, 1974; Dotlacil and Apltauerova, 1978).

Other Effects of Ethylene Growth Regulators

Apart from the above mentioned effects of ethylene plant growth regulators, a lot of studies on other possible uses of Ethephon in agriculture and horticulture were performed : tuber size distribution and yield of potato may be positively influenced (Marlowe *et al.*, 1968; Bodlaender, 1972). Sweetpotato plants are reduced in vegetative growth and yield of tubers is increased (Tompkins and Bowers, 1970; Shanmugam and Srinivasan, 1973). Production of gladiolus corms is increased and fungicide treatment is more effective after Ethephon application (Halevy *et al.*, 1970; Simchon *et al.*, 1972). Shoot development of roses is stimulated. Almond bloom can be delayed to overcome the problem of early and later blooming varieties, which normally could not fertilize each other. A fertilization between varieties may increase yields (Browne *et al.*, 1978). An earlier walnut harvest can be achieved with Ethephon treatment. The kernels are mature but lighter in color and thus of higher quality (Olson *et al.*, 1977; Sibbett *et al.*, 1978). In cranberry coloration of the fruits can be improved by Ethephon. Yields are not affected in this crop (Devlin and Demoranville, 1970). Also in sugarbeet (Kuhn *et al.*, 1977) and strawberry (Sachs and Iszak, 1974) treatment with Ethephon to enhance yield was not successful.

Factors Influencing the Performance of Ethylene Plant Growth Regulators

Ethylene is the active principle of all ethylene releasing compounds or intermediates in ethylene biosynthesis. However, the individual compounds markedly differ in their action upon plants. What are the reasons for those differences in performance ?

Theoretically differences in the total amount of ethylene generated, in the duration of ethylene generation, in the rate of ethylene generation and in the penetration of the compounds into the plant cells could cause different plant reactions. Furthermore, the mode of ethylene generation-non-enzymatically, like ethephon, or enzymatically, like ACC and derivatives-may be of importance for the responses of plants, as the activity of the ACC converting enzyme may change during the development of plants or from plant organ to plant organ.

Regarding the time course of ethylene evolution, Ethephon shows a long lasting effect over a few days while Etacelasil and sulfinic acid derivatives degrade almost completely within the first 24 hours. ACC

shows an intermediate behaviour, here the time course of ethylene generation may vary, depending on the plant tissue used (Ben-Tal and Lavee, 1976; Lurssen, 1982).

The duration of ethylene generation should play a major role in long-lasting processes like ripening of fruits. Tomato fruits for example respond to ethylene treatment only after they have reached a certain stage of development. Application of ethylene before this stage does not result in enhanced ripening. A tomato plant simultaneously bears fruits of different ripening stages, including fruits which are still growing and will not easily respond to ethylene treatment. So at a certain day only a part of the fruits will react to ethylene with a stimulation of ripening. Therefore it is essential to generate ethylene for a period of several days to achieve an optimal response of most of the fruits. Growth inhibition of cereals may also be regarded as a process requiring ethylene development for a longer time. As far as data are available, these considerations are in agreement with the observed actions of ethylene plant growth regulators: Ethephon, releasing ethylene for a longer time period, stimulates ripening, while sulfinic acid derivatives, releasing ethylene only for a day, are more or less ineffective. According to other field results, abscission processes and flower induction in Bromeliaceae seem to require only a short ethylene pulse: Ethephon is active, sulfinic acid derivatives are sometimes even more active (Lurssen, 1982 and unpublished results).

N-formyl-ACC causes only an extremely low stimulation of ethylene production in plant material. However, the stimulation of fruit ripening and the growth inhibition in cereals caused by this compound is much more pronounced than that caused by ACC, which enhances the measurable ethylene evolution much more and for at least as long as N-formyl-ACC. It seems, that ethylene generated from N-formyl-ACC is much more effective than that generated from ACC. Of course there are normal dose-response curves for ACC, ethylene generated from ACC and plant responses on the one hand and for N-formyl-ACC, ethylene generated from this compound and plant responses on the other hand. Similar results were observed with excised plant parts (Konze, personal communication, Lurssen, 1982 and unpublished results). Isotopic dilution experiments showed, that N-formyl-ACC definitely is converted to ACC and ethylene in plant tissue (Konze, personal communication). Taking into account also the data of the other ethylene plant growth regulators, the total amount of ethylene generated from the compounds seems to be of minor importance (Lurssen, 1982).

The discrepancies between the amount of ethylene measured after application of ACC and N-formyl-ACC and the observed plant responses, which are typical "ethylene responses", are not easy to explain. They led us to the hypothesis, that applied ACC may be converted to ethylene in at least two plant compartments: one which is nearby the ethylene receptor, and another one clearly seprated from the site of ethylene action, in which most of the applied ACC is converted to ethylene. Ethylene diffussion to the receptor site must be limited. N-formyl-ACC, due to its different physico-chemical properties, easily penetrates into the compartment of the ethylene receptor. It is converted there to ACC and finally to ethylene, which then easily occupies the receptor sites. The total conversion of N-formyl-ACC to ethylene can be very low, because the ethylene generated from N-formyl-ACC near the receptor site is more effective than that generated from ACC in other compartments (Konze and Lurssen, in preparation). Obviously ACC can be stored in plant tissue, as it was isolated from ripe fruit in relatively high amounts (Burroughs, 1957; Vähätalo and Virtanen, 1957). Furthermore, more than half of the amount of ACC applied to plants is converted to malonyl-ACC (Amrhein *et al.*, 1981). Taking into account these facts, a rather complicated system of compartmentation of ACC must be assumed (Fig. 9.7).

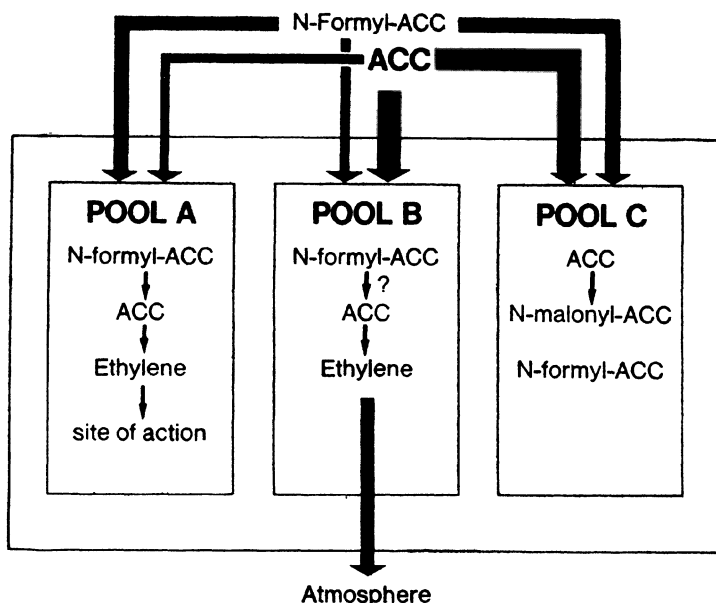


Fig. 9.7 Proposed scheme for the compartmentation of ACC and N-formyl-ACC in relation to metabolism and action.

The hypothesis, that only a very small parts of the ethylene generated or measured in natural processes is needed for plant responses, fits well with the observations and measurements of Beyer (see contribution of Shimokawa, K. in this volume). If ethylene metabolism is an essential process in ethylene action, here again only a small part of the ethylene produced by plants or fed to plants is converted to its metabolities initiating the first step in ethylene action.

Outlook

Today ethylene is the plant hormone, which is best understood with regard of biosynthesis, regulation of biosynthesis (see the contribution of Shimokawa in this volume), effects upon plant metabolism and plant development, and there are promising approaches to the elucidation of the primary reaction. Further studies to confirm the nature of the primary reaction are certainly needed. A very important aspect of hormone physiology, the question of the role of sensitivity of the plants to a hormone during plant growth (Trewavas, 1981; Trewavas, 1982) could possibly be answered first for ethylene due to the relatively good knowledge of the other aspects of this hormone.

The ethylene plant growth regulators known today are extremely effective. It is not likely, that new compounds with the same mode of action-ethylene release- would be more effective. As the market size for such products is limited compared with that of pesticides, new industrial developments in this field can hardly be expected (Lurssen, 1981 b). However, an understanding of the regulation of plant sensitivity to a plant hormone, could open up new classes of growth regulators, which influence sensitivity and not hormone concentration. Such products might be far more active than the plant growth regulators known today.

Acknowledgement

I wish to express my thanks to Dr. J. Konze for critically discussing this review and help in preparing the manuscript.

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Ethylene and Other Plant Hormones in Thigmomorphogenesis and Tendril Thigmonasty

Mordecai J. Jaffe

Introduction

Although botanists have long been aware that plants will respond to experimental stimuli such as light, gravity, temperature or chemicals, most observers have felt that excepting certain extraordinary cases (e. g., Sensitive *Mimosa*, Venus' Flytrap), vascular plants neither sense nor respond to mechanical perturbation (MP). If the plant systems mentioned above are the only kind that respond to mechanical caues, we might choose to say that reaction to touch does not represent a general kind of response to environmental MP by ordinary growing plants. It can now be demonstrated that the growth of almost all of the species that have been tested is markedly retarded, and the stem diameter increased, by very slight rubbing or bending of their stems (Fig. 10.1). I have called this phenomenon Thigmomorphogenesis (Jaffe, 1973). In nature, we may assume that wind is the environmental factor most responsible for this phenomenon, and indeed, this supposition has been supported in the laboratory (Jaffe, 1976a) and in the field (Biro, *et al.*, 1980).

Plants possessing the vining habit also exhibit thigmomorphogenesis. But, in addition, many of them also display the ability to undergo thigmonastic reactions. This is especially true of tendril bearers (Fig. 10.1). This review will describe what is currently known about the mediation of these two phenomena by the phytohormones, with special emphasis placed on the role of ethylene.

Thigmomorphogenesis

Only recently has a direct effort been made to elucidate thigmomorphogenesis, so much of the literature on the topic is anecdotal. Nevertheless, in searching the literature, one is able to find a number of papers that report thigmomorphogenetic responses in a wide variety of vascular plants. Over fifty species from over 20 families all have been reported to respond to some form of mechanical stimulation.

The primary thigmomorphogenetic response is one of growth retardations coupled with increased radial growth (Fig. 10.1) (Jaffe, 1973). In most of the species tested, shaking, bending rubbing or blowing wind on the plant produces a retardation of elongation. The ubiquity of this response is significant. It should be stressed that thigmomorphogenesis is as common a phenomenon as other, much more widely studied plant growth responses, such as gravitropism or photomorphogenesis. Currently, some interesting observations have been made concerning the roles of the phytohormones in mediating this phenomenon. They will be reviewed in the next several sections.

Role of Ethylene in Thigmomorphogenesis

Physiological systems

The first time that ethylene was implicated in thigmomorphogenesis was in a report by Goeschal, Rappaport, and Pratt (1966). They found that when etiolated peas were mechanically perturbed by growth through glass beads or by the pressure of a foam neoprene stopper, the elongation of the internodes decreased, their diameter increased, and there were greater than control levels of ethylene produced by the tissue. Rubbing tomato plants induces epinasty in the leaves near the site of perturbation (Jaffe, 1973) and mechanically perturbing poinsettia plants does the same thing (Saltveit *et al.*, 1979). Epinasty is a phenomenon that is normally associated with ethylene production, and the latter authors found increased ethylene evolution in mechanically perturbed plants. Furthermore, those cultivars which experienced the most epinasty, also produced the most ethylene. For example, Eckespoint C-1 Red cultivar displayed hardly any epinasty four hours after the perturbation and perturbation caused only 1.3 times as much ethylene to be produced than the controls. On the other hand, the cultivar Annette Hegg Hot Pink, had severe epinasty by that time, and the perturbed samples. produced 5.2 times as much ethylene as the unperturbed controls. In a further study of this phenomenon (Saltvelt and Larson, 1981), the respective inhibitors of ACC biosynthesis and ethylene biosynthesis, 10mM AVG and 3mM silver ions, caused a reduction of epinasty of 68% and 44% respectively.

Ethylene has been shown to promote femaleness in members of the cucurbitaceae. Accordingly, Takahashi and Suge (1980) tested the effect of stem rubbing as a mechanical perturbation of several types of cucumber cultivars. They found that rubbing increased the number of pistillate

flowers in monoecious flowers, a response that might be expected if ethylene were produced (Table 1). Perturbed plants that were also treated with silver nitrate, however, did not produce an increased number of pistillate flowers. While these findings do not directly demonstrate that ethylene produced as a result of mechanical peturbation caused female sex expression, they are certainly not inconsistant with such an interpretation.

Table 1. The effects of MP and the anti-ethylene agent, $AgNO_3$, on growth and feminization of monoecious (M) or gynoecious (G) cucumber plants. Modified from Takashi and Suge (1980).

Cultivar	treatment	Growth rate (cm/Day)	No. of pistillate nodes up to 15 nodes
Mastsunomidori (M)	control	5.5	1.8
	MP	3.7	3.9
	MP + $AgNO_3$	4.4	0.7
Higan-Fushinari (G)	control	3.8	13.0
	MP	1.2	13.2
	$AgNO_3$	3.5	0.2

One of the most well know effects of ethylene on plant growth is an increase of stem diameter, often coupled with a gnarled, knobley appearance of the stem (Jaffe, 1976 ; Jaffe and Biro 1979). Jaffe and Biro (1979). demonstrated that such an effect due to either MP or exogenous ethephon is prominent in bean stems, in addition to thedecrease in elongation. In a subsequent study Biro and Jaffe (1983), they showed that the ethylene inhibitors AVG and $CoCl_2$ both blocked the MP-induced thickening reaction (Table 2). The anatomical basis for the thickening reaction in beans has been determined (Jaffe and Biro, 1979). Both exogenous ethylene and MP were shown to induce thickening and distortion of the epidermal cells, as well as enlargement of the cuticular ridges. In a subsequent report, Biro *et al.*, (1980) demonstrated that exogenous ethylene was able to mimic the anatomical effects caused by MP. These includedan increase in radial cell divisions in the pith and secondary xylem and an increase in cell size of the treated cortical cells. Thus it may be that ethylene mediates the thickening response of thigmomorphogenesis in beans by causing these

anatomical changes, since it also mimics the thickening effect on supertending, non-MP'd internodes when it is applied to the first internode only (Erner, Biro and Jaffe, 1980).

Table 2. The effects of pro- and anti-ethylene agents on MP-induced bean stem thickening and elongation.

Additive	% of Control			
	Elongation		Thickening	
	C	MP	C	MP
none (control)	100	48	100	143
IAA (10 μm)	64	77	63	—
ACC (5mM)	56	51	165	161
Hypobaria	93	71	—	—
AVG (0.5 mM)	91	44	100	104
$CoCl_2$ (0.5 mM)	88	47	108	118

Ethylene also has been shown to mimic the effects of MP on woody plants. When ethephon is exogenously applied to the stems of loblolly pine seedlings (*Pinus taeda*), their elongation is retarded, and the stem thickening is increased (Telewski and Jaffe, unpublished) (Table 3). The tthickening reaction to both MP and exogenous ethephon is due to an increase in tracheid number and cross-sectional area, and in the volume of the resin ducts.

Table 3. The effects of MP and exogenous ethephon (E) on loblolly pine hypocotyls

Half-sibling	Elongation			Thickening		
	C	MP	E	C	MP	E
8—61	100	72	90	100	97	110
8—27	100	70	87	100	110	128

Interaction with other Physiological Systems

Ethylene can be shown to mediate the interaction of other physiological reactions with thigmomorphogenesis. Usually, when plants are placed in the horizontal position, gravitropism causes the stems to bend upward. Jaffe and Biro (1979) have shown that a prior MP retards the development of gravitropic curvature. This effect can be mimiced by the exogenous application of ethephon (Jaffe, 1979a). MP also inhibits photo-

tropism in beans, and removal of native ethylene by a partial vacuum, restores the normal rate of both gravitropism and phototropism (Jaffe and Biro, 1979).

Plants growing in nature are normally mechanically perturbed by the wind. These plants usually are more resistant to rupture injury by subsequently greater wind than are sheltered plants (Jacobs, 1954; Somervile, 1981). This is, perhaps, to be expected. However, several studies have also shown that, at least in some instances, such plants are more resistant to other stresses, as well. For example, Jaffe and Biro (1979) have shown that bean plants exposed to drought stress or freezing stress, are better able to recover if they were previously mechanically perturbed. Similarly, Suge (1980) demonstrated that mechanically perturbed beans were more resistant to drought stress injury than were controls.

When tomato plants are subjected to drought stress, and then re-irrigated, the stem pith parenchyma undergoes autolysis, and the resulting stem becomes a hollow tube (Aloni and Pressman, 1981). This syndrome is known as "pithiness". We have used video image processing to quantify the amount of pithiness in this and other plants. If the internodes of the plants are pretreated with MP, they become resistant to drought induced pithiness (Table 4) (Pressman *et al.*, 1983). Furthermore, pretreatment with ethephon can substitute for MP in inducing hardiness (Table 4). Since rubbed plants produce ethylene and AVG blocks MP-induced hardening, it may be concluded that the MP-induced ethylene formation mediates the hardening mechanism.

Table 4. The effects of MP on stem elongation, ethylene evolution and pithiness and of ppm ethephon (E) on pithiness in Alcobaca tomatoes.

	C	MP	E
Stem elongation after 6 days of MP or 2 days E (CM)	8.5 ± 0.8	4.3 ± 0.4	—
Peak ethylene production (pm/g fr. wt./h)	45	57	—
Pithy internodes (%)	95 ± 3	6 ± 2	18 ± 1

Ethylene Production

Mechanical perturbation of many tissues induces the production of the gaseous plant hormone, ethylene. Rubbing tomato stems induces epinasty in the leaves (Jaffe, 1973) and rubbing bean internodes causes

them to markedly increase in diameter and elongate less (Jaffe, 1976a). Both of these responses are known to be caused by ethylene. Therefore, it is not surprising that various workers have shown that mechanical perturbation induces ethylene production in plants as diverse as lily, bean, white and loblolly pine, apple and peach. For example, Goeschal *et al.* (1966) demonstrated that when dark grown peas push up through glass beads, they produce greater than control levels of ethylene. Similarly, Jaffe and Biro (1979) have shown a peak of stimulated ethylene production to occur about 90-180 minutes after flexing or rubbing bean stems (Fig. 10.2). The ethylene production is related to the long term slowing

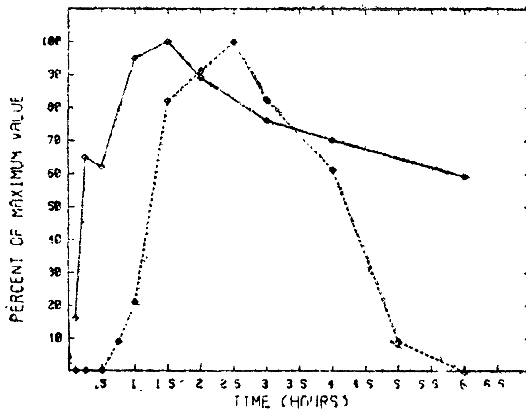

Fig. 10.2. The time course of MP-induced ACC (□—□) and ethylene (□---□) production in the bean first internode.

of growth, but not to the immediate responses (during the first hour), which seem to be membrane related (Jaffe, 1976). Hypobaric conditions significantly nullify growth retardation due to mechanical perturbation, and ethylene is the only phytohormone which, when applied exogenously in the form of ethephon, precisely mimics all the morphological effects of mechanical perturbation (Jaffe and Biro, 1979).

However, we have several lines of evidence that support the view that the "pulse" production of ethylene directly mediates thigmomorphogenetic stem thickening, but only indirectly affects the decrease in elongation. When inhibitors of different steps of the biosynthetic pathway of ethylene biosynthesis (Biro and Jaffe, 1983) are applied to the plant before

MP, AVG and Co^{++} ions block the thickening reaction, but not the decrease in elongation (Table 2). Further, we have looked at the effect of MP on both thickening and ethylene production in young plants of different genetic lines of loblolly pine and tomato. Pine half sibling 8-27, which thickens when given MP, shows a large amount of MP-induced ethylene evolution, whereas 8-61, a half sibling which does not show thickening, displays much less of an increase in ethylene production. Similarly, 3 tomato cultivars that exhibit no MP-induced thickening, also show no increase in ethylene production, while a cultivar which does thicken, also shows an MP induced increase in ethylene production.

By a series of experiments which measured MP-induced ethylene production in both 15 mm long stem segments and in 5 mm long segments, the effect of wound ethylene at the cut ends was accounted for. There was still appreciable non-wound ethylene produced due to MP (Huberman and Jaffe, unpublished). This, together with the observation that MP induces twice as much ethylene in MP treated stem segments as in controls which only have wound ethylene evolution, shows that the MP-induced ethylene represents an increase in "endogenous" ethylene (Abeles, 1973). MP-induced ethylene seems to follow the "methionine-to-5-adénysyl methionine-to-ACC-to-ethylene pathway (Adams and Yang 1979). Since we have been able to measure an increase in ACC due to MP, which begins immediately and peaks about the time that ethylene production is beginning (Fig 10.2). Furthermore, the MP signal is transmitted both up and down the plant, since both ACC and ethylene are produced above and below the region that is rubbed). It is interesting, however, that ethylene production is not found in the petioles when the stem is rubbed. Thus it seems that some active principal is induced to translocate up or down the stem from the mechanically perturbed site. This principal then mediates thigmomorphogenesis in other parts of the plant (Erner, *et al.* 1980).

The Endomembrane System

Irvine and Osborne (1973) found that both exogenous ethylene and Mechanical perturbation decreased the rate of incorporation of ($1\text{-}^{14}C$) glycerol into the phospholipids of etiolated pea stems. Since phospholipids are an important component of the endomembrane system, Erner and Jaffe (1983) have studied the effect of both MP and exogenous ethylene on the endomembrane system of cells of the first and second internodes of bean plants. MP or exogenous ethylene increases the level of endomembrane-associated proteins, but decreases specific activities of

the enzymes succinic acid cytochrome c oxidase (a mitochondrial marker), KCN-resistant NADPH cytochorme c reductase (a marker for endoplasmic reticulum), and latent inosine diphosphates (a dictyosome marker). Both MP and exogenous ethylene cause marked decreases in endomembrane-associated phosphatidyl choline and increases in phosphatidyl ethanolamine and phosphatidyl inositol, when compared to controls. When membrane-associated free fatty acids were examined, lauric acid (16:1) was less in controls, but as the chain length increased and as unsatruration increased, the fatty acid titers decreased after treatment with ethylene or mechanical perturbation. This was particularly striking in the case of linolenic acid (18:3), which decreased about 60% in the MP and ethylene treatments. MP or exogenous ethylene had no effect on the sterol content of the extracted endomembranes. It is as yet too early to be able to interpret these data, but it is interesting to note that when plant tissues are challenged with linolenic acid or its peroxide, ethylene synthesis is induced (Mapson, 1969). These experiments indicate that one of the ways that ethylene mediates thigmomorphogenesis is by inducing profound changes in the protein and lipid components of the endomembrane system.

Other Hormones in Thigmomorphogenesis

The role of gibberellins

Quite the reverse of ethylene, gibberellins seemd to oppose thigmomorphogenesis instead of being involved in its induction. Suge (1978) has shown that chromatographed and bioassayed bean extracts have much less gibberellin-like activity after MP than in controls (Table 5). Further, Jaffe and Biro (1979) have demonstrated that physiological levels of exogenous gibberellic acid reverse the elongation decrease of thigmomorphogenesis (but not the thickening response) due to MP. Thus, it seems that an MP-induced decrease in stem gibberellins may be involved in the decrease in stem elongation which occurs during thigmomorphogenesis.

The role of abscisic acid

Both Jaffe and Biro (1979) and Suge (1980) have shown that prior MP protects bean plants from injury due to drought stress followed by dehydration (Table 5). Further, Pressman *et al.* (1983) have shown that MP-induced thigmomorphogenesis or exogenous ethephon prevent drought induced pithiness from occuring in tomato stems. These experiments suggest that the phytohormone abscisic acid (ABA), which is induced by drought conditions (Moore, 1979), might play a role in thig-

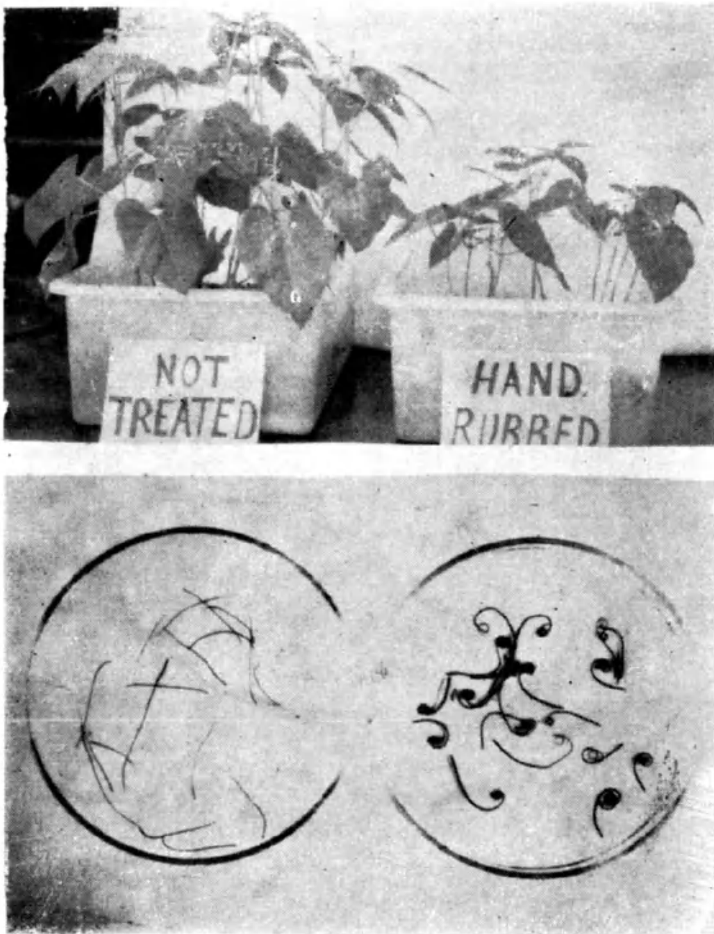

Plate 1. The effects of MP (rubbing) on beans (Top) and on pea tendrils (Bottom). In each case, the controls are on the left and the MP plants are on the right.

momorphogenesis. Accordingly, exogenous ethephon was applied to bean stems with or without MP. In the former case, 1 μM ABA reduced elongation to the same extent as did MP but had no significant effect on the stem diameter (Jaffe and Biro 1979, Erner and Jaffe, 1982). If ABA is part of the thigmomorphogenetic mechanism, it might be expected that its titer should increase in MP plants. Jeong and Ota (1980) rubbed two varieties of rice plants for 30 sec per day for 30 days. They found that the two cultivars displayed 22% and 30% decreases in elongation and 98% and 52% increases in ABA titer, respectively. In another study, Erner and Jaffe (1982), bean plants given MP once daily for 10 days were found to have up to 68% more ABA than controls. In this latter study, it was also demonstrated that as low a concentration of exogenoues ABA as 10 nM was able to significantly decrese control stem elongation. However, even a concentration as high as 0.1 mM caused only a slight additional decrease in elongation when ABA was applied to MP plants.

Table 5. The effects of MP or ethephon (E) on auxin, gibberellin and abscisic acid in several species.

Plant	Treatment	% of Control		
		Auxin	Gibberellin	Abscisic Acid
Phaseolus vulgaris	C	100*	650**	100*
	MP	556	100	313
	E	296	—	—
Bryonia diocia	C	100***	—	—
	MP	12	—	—
Oryza sativa	C	—	—	100****
	MP	—	—	198

* Erner & Jaffe (1882) ; **Suge (1978) ; ***Boyer (1967) ; ****Jeong & Ota (1980).

These experiments suggest that ABA may be involved in the thigmomorphogenetic syndrome. However, from the reported results, it seems likely that ABA plays no role in the thickening reaction, but only in the MP induced decrease in elongation.

The role of auxin

The growth hormone auxin, also seems to be involved in thigmomorphogenesis. Mitchell (1977) have demonstrated that mechanical perturbation reverses auxin promoted elongation in soybeans and peas. Boyer (1967) has shown that auxin is not present in the lower internodes of MP plants of *Bryonia dioica*, a wild cucumber, although it is in the controls (Table 5).

In an attempt to explain this phenomenon, Boyer *et al.* (1979) have shown that MP induces changes in both soluble and ionically bound cell wall basic peroxidases,with the appearance of an additional peroxidase. These observations suggest that in *Bryonia,* auxin catabolism is hastened by mechanically stimulated peroxidase. In addition, Boyer, Chappelle and Gaspar (1979) pretreated plants with Lithium and showed that it prevents boths thigmomorphogenesis and the appearance of the specific cathodic isoperoxidase induced by rubbing, thus lending further credence to the possibility that the peroxidase-auxin system is involved in thigmomorphogenesis in *Bryonia.*

However, we have evidence that the mechanism may be different in beans. We have been able to show by bioassay that auxin-like substances accumulate in internodes that have been mechanically perturbed or treated with exogenous ethephon (Erner and Jaffe, 1982) and that a high concentration of exogenous auxin causes growth retardation in bean internodes (Biro and Jaffe, 1979 ; Earner and Jaffe, 1982) (Table 5). We interpret this to indicate that ethylene production induced by MP blocks basipetal auxin transport (since MP has been reported to do this in both peas (Mitchell, 1977) and beans (Erner and Jaffe 1981), causing auxin to accumulate in the internode to such an extent that it induces further ethylene production. However, this scheme presupposes that thigmomorphogenetic ethylene does not cause peroxidase activity in bean as it does in *Bryonia.* Just such a difference can be found in the literature. Thus, whereas Ivanoff (1932) found that exogenous ethylene induced increased peroxidase in cucumber, Gahagan *et al.* (1968) were able to show that it had no effect on peroxidase in beans. It seems possible then, that in *Bryonia* thigmomorphogenetic ethylene increases peroxidase activity which reduces the auxin titer in the tissue to a level low enough not to support normal elongation ; whereas in beans the ethylene has no effect on the peroxidase (Gahagan, Holm and Abeles, 1968), but causes auxin to accumulate to such high levels that growth is also inhibited.

A Model of the Involvement of Phytohormones

On the basis of the available evidence, I have constructed a first approximation of the possible way in which phytohormones may help to mediate thigmomorphogenesis in bean internodes. It should be stressed that this is only a working model, and that some of it is still speculative. However, it is useful, since it helps to place this aspect of the syndrome in the perspective necessary for further analysis. According to the

model, mechanical perturbation causes membrane changes which induce a burst of ethylene production. The ethylene in turn may block basipetal auxin transport and the endogenous auxin level may increase in the stimulated internodes to such a high level that it may induce further ethylene production via a kind of feedback loop. In the case of the latter, an increased ABA titer could be, at least in part, responsible for the ovserved retardation of elongation. At the same time, gibberellin induced elongation would be severly reduced, as the gibberellin titer drops. The integrated action of the hormones may in turn be responsible for profound changes in the balance of the lipids and proteins of the endomembranes. These changes are almost surely related causally to the anatomical and morphological changes which constitute thigmomorphogenesis.

Thigmonasty in Tendrils

Tendrils are long thin organs, often modified from leaf primordia which respond thigmonastically when their ventral (i. e. underside) is rubbed. This response enables them to wrap around a support which they encounter during circumnutation (Jaffe 1972, 1979). In peas, as well as in many other species, the ventral side contracts following MP, and as the tendril bends around the support, the dorsal side then begins to grow faster than the ventral side. Thus, the tendril grows round the support.

Table 6. The effect of exogenous phytohormones on the coiling of pea tendrils with or without MP.

	Contact coiling (Degrees/Hours)	
	— Hormone	+ Hormone
GA_3 (10^{-6}M)+MP*	650°/20h	480°/20h
IAA (10^{-6}M)—MP**	26°/3h	315°/3h
IAA (10^{-6}M)+MP*	860°/20h	1100°/20h
Ethephon (5g/L) — MP***	18°/2h	264°/2h
Ethephon (5g/L) + MP***	264°/2h	252°/2h
ABA (10^{-8}M) — MP****	35°/1h	78°/1h
ABA (10^{-8}M) + MP****	155°/1h	152°/1h
Kinetin (10^{-6}M) — MP****	21°/1h	30°/1h
Kinetin (10^{-6}M) + MP****	153°/1h	120°/1h

*Jaffe & Galston (1966) ; **Jaffe (1975) ; ***Jaffe (1970) ; ****Jaffe (unpublished).

Reinhold (1967) noted that the symmetric application of ethylene induced a slight coiling of tendrils of *Marah fabaceus* L. Jaffe (1970) applied ethephon assymetrically by painting it onto the ventral or dorsal surfaces of pea tendrils. The ventral ethylene treatment mimiced the reaction to ventral MP. In the same study, Jaffe also showed that rubbed tendrils produced three times as much ethylene as did resting tendrils. This suggested that ethylene may mediate contact coiling. Support for this hypothesis came from the work of Bangerth (1974). Using cucumber tendrils, he found that auxin-stimulated ethylene production in the ventral side was several times greater than in the dorsal side. Since auxin can move basipetally in the tendril (Jaffe, 1975), it may be that MP sensitizes the ventral side so that translocating auxin induces ethylene production; and that the localized ethylene production is, in turn, responsible for the curvature.

The role of auxin in tendril coiling is, to some extent, clear. Exogenous auxin both enhances MP-induced coiling (Jaffe and Galston 1966) and is capable of inducing coiling (Reinhold 1967, Jaffe 1975). In the latter case, if the tip of a tendril is dipped into an auxin solution, the tendril coils, but only after a long lag period. In addition, exogenous auxin abolishes the normal, MP-induced ventral contraction. These findings strongly suggest that auxin is not involved as a growth stimulator in the early stages of contact coiling. However, it is likely that auxin is involved in two other ways. Auxin induced coiling is much slower in the early stages than MP-induced coiling. However, after about 1.5 hours, the auxin treated tendrils continue to coil, whereas the rubbed plants do not. Thus, auxin may be involved in the sustained asymmetrical growth around a support, although not in the early rapid contraction phase. Conversely, auxin may also play a role in the very early events of contact coiling since only a few seconds of dipping the tip into auxin suffice to induce coiling, suggesting that very few auxin molecules are needed to start the response (Jaffe, 1975). Only the active auxins IAA, NAA or 2, 4-D cause this effect, whereas 2,6-D and TIBA inhibit IAA-induced coiling (Jaffe, 1975). In the same study, Jaffe (1975) also showed that ^{14}C-IAA was translocated basipetally, an observation confirmed by Junker (1976), both noting no change in the amount of translocated label after either mechanically induced or auxin-induced coiling. Junker (1977) also failed to detect any change in the dorsiventral ratio of labeled IAA (43:57) following MP.

An interesting observation has been made concerning the tendril tip which is sensitive to auxin. Hertel, *et al.* (1972) have shown that auxin

can be specifically bound to a putative receptor which can be recovered in a membrane-rich fraction from various plants. They also showed that this binding exhibits the same kinetic properties as does auxin action in these plants. Jaffe (1975) was subsequently able to demonstrate that specific auxin binding activity could be extracted from the tips but not the remainder of uncoiled tendrils, and that extracts of coiled tendrils no longer exhibited this specific binding property. Thus it may be that upon MP, auxin may act as a trigger by being released at the tip, thence becoming bound to specific receptor molecule (Jaffe, 1975).

In summary, auxin may act as an immediate trigger at the distal end of the tendril, responding to MP. The ventral side being more sensitive to auxin-induced ethylene production, more ethylene may be produce on the ventral side, causing ventral contraction. Finally, the continued basipetal translocation of auxin down the tendril, may be involved in the dorsi-ventral asymmetrical growth which occurs later on, and asqures that the tendril is well attached to the support.

Acknowledgement

This work was supported by grants from the National Science Foundation (USA) and the National Aeronautics and Space Administration (USA).

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Hormonal Regulation of the Gravitropic Response in Pulvini of Grass Shoots

Peter B. Kaufman and P. Dayanandan

Introduction

The gravitropic responses in plants can be visualized most easily in terms of three distinct phases: gravity perception, transduction, and cellular response. The second one, transduction, involves the development of the hormonal asymmetry which has been experimentally verified for both roots and shoots that have been gravistimulated. How this hormone asymmetry is established is still not clear for most root and shoot systems. For the graviperceptive "organs" of shoots of grasses that we call pulvini (swollen bases of leaf sheaths or swollen regions near the bases, but above the intercalary meristems of internodes), the transduction process is especially an enigma since lateral movement of both auxin (Bridges and Wilkins, 1973) and gibberellin (Kaufman, Pharis, Noma and Rood, 1982) does not occur in gravistimulated leaf sheath pulvini.

This review represents a summary of previous (Sachs, 1887; Arslan and Bennet-Clark, 1960; Maeda, 1958; Bridges and Wilkins, 1973; Wright and Osborne, 1977; Dayanandan *et al.*, 1976) and contemporary investigations (see references) that shed light on the transduction process, and thus, how we think hormones regulate, the gravitropic curvature response in grass shoots.

The Grass Pulvinus and Its Response to Gravistimulation

Location of Pulvini in Grass Shoot Systems

In grass shoots, we encounter swollen regions at the bases of leaf sheaths and near the bases of internodes near the intercalary meristem regions. Such pulvini are mistakenly called nodes, which, in fact, occur the pulvini at sites where leaves are attached to the stems. To dispel any confusion that may exist regarding nodes and pulvini, refer to Fig. 11.1 that illustrates leaf-sheath and internodal pulvini.

The gravity-sensitive leaf-sheath pulvini (Fig. 11.1A) are found in almost all members of the subfamily, Festucoideae (Pooideae), such wheat, oats, barley, and rye. In contrast, most members of the subfamilies, Panicoideae, Eragrostoideae, Bambusoideae and Arundinoideae, possess a swollen, gravity-sensitive region just above the base of the internode (Fig. 11.1B) in addition to the more or less developed leaf-sheath pulvinus. Examples of this category include sorgum, maize, sugarcane, millets, bamboos, and *Panicum* species. Further discussion of the structure of leaf-sheath and internodal pulvini, and their occurrence in different grass taxa is found in papers by Brown, Prat and Mobley (1959), Brown, Harris, and Graham (1959), and Dayanandan *et al.* (1977).

Paucity of Lignin and Biogenic Silica in the Grass Pulvinus

Using the lignin-specific stains, pararosaliline hydrochloride and phloroglucinol-HCl, one can demonstrate that the gravisensitive leaf-sheath and internodal pulvini of grasses are lignin-poor as compared with leaf--sheath or internodal tissues above and below the pulvini (Fig. see also, Dayanandan *et al.*, 1977).

This is due to the fact that the vascular bundle-associated collenchyma present in leaf-sheath pulvini (ca. 40% of the pulvinus tissue in leaf-sheaths) is non-lignified, whereas the fibers that form caps associated with the same vascular bundles in leaf-sheath above or the internode below are highly lignified. The same applies to internodal pulvini. The only tissue elements that are lignified in the pulvini are xylem tracheids and vessel elements.

Using an energy-dispersive X-ray analyzer coupled to the scanning electron microscope (SEM), one can produce X-ray maps for silicon in surface layers of pulvini that one photographs with the SEM. Our results show that in essentially all grasses we have surveyed (e. g., *Festuca, Polypogon, Agropyron, Panicum, Oryza, Avena, Hordeum, Leersia, Echinochloa*) the gravisensitive leaf-sheath and internodal pulvini are silica-poor as compared with relatively high amounts of silica present in leaf-sheath and internodal tissue above and below the pulvini (Fig. 11.2). The only cells we have found to be silicified in the pulvini are isolated, well-separated trichomes and silica cells that are present in the epidermal systems of many leaf-sheath pulvini (Figs. 17, 18 in Dayanandan *et al.*, 1977).

The above results show that gravisensitive leaf-sheath and internodal pulvini can specifically respond to gravistimulation because their component cells are relatively unslicified and are lignin-poor.

Growth Response of Gravistimulated Pulvini in *Avena* and *Hordeum*

The grass pulvinus, when experimentally gravistimulated, typically starts to curve upward within 20 to 30 minutes and continues upward for 48 to 60 hours at a rate of about 1.5° per hour. This is shown for tip-held and base-held *Avena* shoot portions in the time-lapse photographs in Fig. 11.3. Typical kinetics of curvature, base on angular recording transducer traces, are shown for tip-and base-held oat shoot portions in Fig. 11.4. Fig. 11.5 is a similar kinetic analysis that compares the negative gravitropic response in pulvini in *Hordeum* and *Avena*.

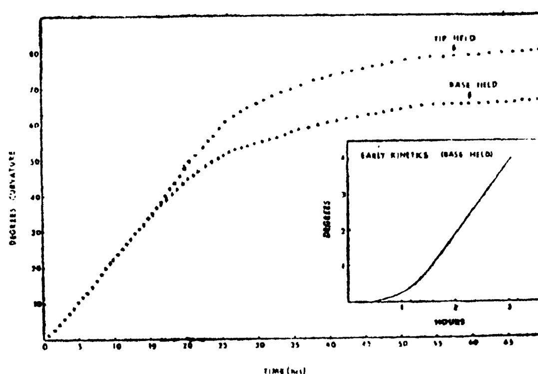


Fig. 11.4. Kinetics for gravitropic curvature in pulvinus of tip-held and base-held /oat (*Avena*) shoots, based on tracings from an angular recording transducer. Inset shows early kinetics for curvature response in the base-held shoot. Curvature upward is initiated within 30 minutes.

Not all grass shoot pulvini respond to gravistimulation. They must be fully developed to respond to gravity. Thus, young pulvini below the shoot apex within the bases of still elongating leaves will not respond. Further, older pulvini do not respond because they become silicified and lignified. Those "competent" to respond to gravistimulation are typically 2 to 3 pulvini in most Festucoid grasses, and as many as 6 to 8 in Panicoid grasses such as *Zea mays*, bamboos, *Phragmites* and *Sorghum*.

The upward negative gravitropic growth response of grass shoot is, very important in agriculture, as witnessed by cereal grain shoot that become are lodged (prostrated) due to the action of wind and/or rain. Grain yields can be greatly reduced as a result of lodging in grain crops such as rye, millets, wheat, barley, oats, and rice. The prostration (lodging)

does not occur in dwarf cultivars and is not so likely to occur in those with high silica contents in their shoots.

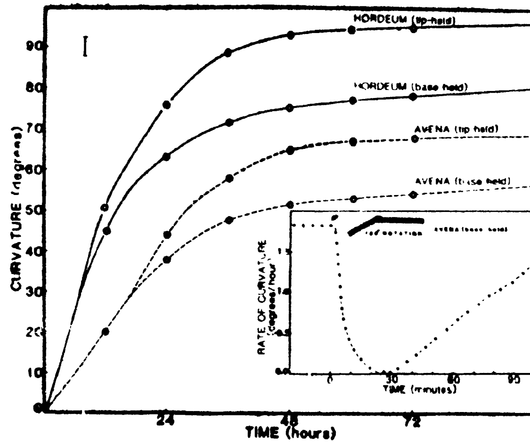


Fig. 11.6. Time-course of bending in barley and oats. Segments are held either by their bases or tips. The insert shows the response of a segment turned 180 degrees during bending. Growth stops within 5 minutes followed by new curvature in the opposite direction.

Cellular Basis of the Gravitropic Curvature Response in the Grass Pulvinus

In all of the grass shoot pulvini that we have examined, it is clear that the primary basis for the asymmetric growth that elevates the shoot upward is one of the differential cell elongations. The pulvinus consists of mature cells which, in our hands, can only be stimulated to undergo cell division by wounding or by growing pulvinus tissue under sterile conditions with auxin (e. g., 1mg/l 2, 4-D in Murashige/Skoog medium) present.

The cell asymmetry in elongation response occures because there is zero cell elongation at the top of a gravistimulated pulvinus increasing amounts of cell elongation occuring as one of the progresses downward from top to bottom, and maximal cell elongation at the base. In other words it is not just the cells in the bottom half that responds, but all pulvinus cells respond except the uppermost ones in the epidermis, which actually become compressed. The cell elongation responses of epidermal cells,

collenchyma cells, and parenchyma cells in a gravistimulated pulvinus are illustrated in Fig. 11.6 and 11.7.

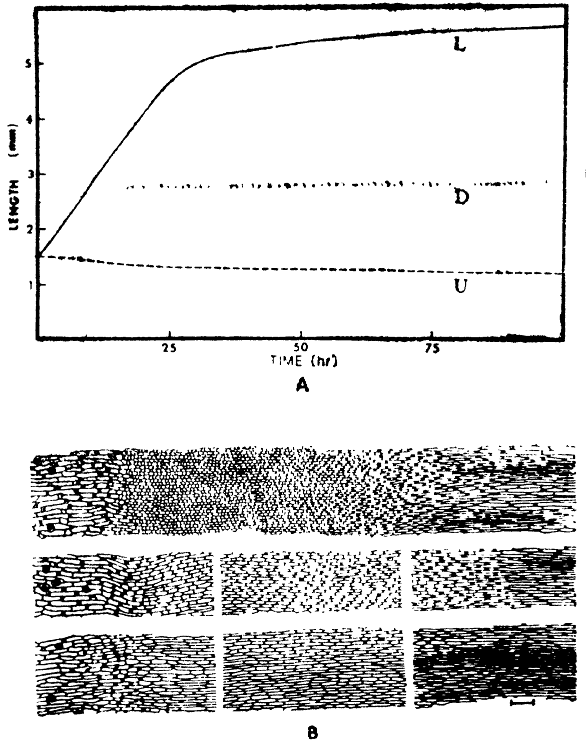


Fig. 11.6 A shows the length of the upper (U) and lower (L) regions of the same pulvinus during gravitropic bending. Some constriction is seen in the lower region, while the upper region elongates considerably. Diameter (D) of the pulvinus show a small inc^ease after 12 hours of gravistimulation. Tracings made of epidermal cells peeled off from a bent pulvini are shown in Fig. 11.6 B. Such measurements demonstrated the precise control of cell elongation seen in grass pulvini. Cells in the mid region respond half as much as the lower region.

The gradation in cellular response in gravistimulated pulvini can be visualized in longisection and transection of the pulvinus, as shown in Fig. 11.8 and 11.9. The cellular response can also be expressed mathematically according to the equation $S=0.5-r\cos\theta$ as shown in Fig. 11.9, where S represents a gravity-sensitivity profile that indicates the predicted response of average growth of a cell in a gravistimulated pulvinus (Dayanandan *et al.*, 1981).

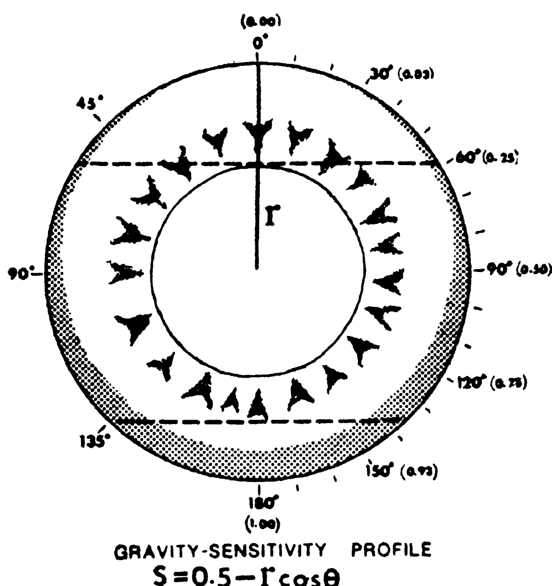


Fig. 11.9 is a diagrammatic representation of a cross section of a pulvinus. The upper and lower surfaces are here indicated by the 0° and 180° positions respectively. The responses of the cells in the periphery are shown by values in brackets The upper surface has no response while the lower surface has a maximum of 1 whereas the mid-point (90° position) has a value of 0.5. The shaded area along the inner periphery is a diagrammatic representation of the relative responses of the cells in the periphery. The dashed lines are two represcntive isobars indicating identical elongation of cells inside the pulvinus.

Based on the trigonometric relationships that exists between the different positions shown in Fig. 11.2 we have derived an equation that describes the sensitivity of every cell in the organ to gravitropic stimulus. The sensitivity $S = 0.5 - r \cos \theta$ where r is the radius and theta is in degrees. When r itself is 0.5 the equation describes the sensitivity of the epidermal layer. When r is 0.25 the equation describes the sensitivity of cells lying halfway from the centre of the circle. Thus, by varying the value of r the sensitivity of any cell can be determined.

The inner circle of wedge-shaped stipled areas indicate the location of the statenchyma. If statenchyma is involved in the release of IAA as is often assumed, it is difficult to explaie the growth response of cells well above the level of statcchyma. (from Dayanandan *et al.*, 1981).

Preception of Gravity by Starch Statoliths in the Grass Pulvinus

Starch statoliths are located aside of each vascular bundle in the grass leaf-sheath pulvinus (Fig. 11.10) near the centre of the pulvinus. These statoliths are easily visualized by standing them with I_2KI, a starch-speci-

fic stain that colors starch grains blue-black. On gravistimulation, they fall to the bottom of the statenchyma cells within ten minutes. It is interesting that the starch statoliths carry with them the tonoplast membrane (starch statolith or amyloplast, that is surrounded by a double membrane, which in turn in surrounded by an unit membrane, the tonoplast) (Fig. 11.11). Thus, any enzymes associated with the starch statoliths (as we have shown for esterase- an important enzyme which releases IAA from its inositol ester conjugate) may play a role in releasing hormones, such as GAs or IAA from their conjugates. GA conjugates are stored in vacuoles and IAA conjugates (e. g., inositol ester of IAA or peptidyl IAA) may be associated with the plasma membrane. Thus, a key part of the gravity perception process is the fall, under gravistimulation, of the starch-statoliths to the plasma membrane. But, how gravity perception leads to transduction has been a mystery up till now. It may be that starch statoliths that have fallen act as vehicles to bring enzymes to the substrate compartments, resulting in release of free, active hormones from presumbly inactive conjugates. This idea is particularlty attractive for the grass pulvinus since neither

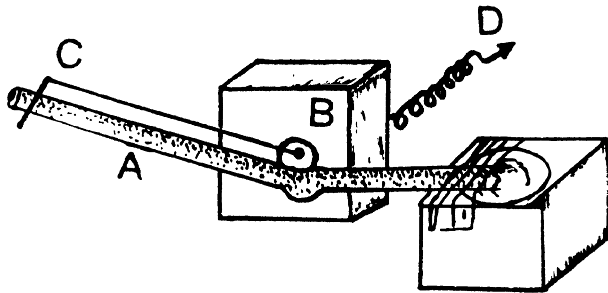


Fig. 11.12 Diagram of angular position transducer auxonometer for the measurement of curvature in grass pulvinus. One end of segment (A) is held firmly and is supplied with sucrose solution. The pulvinus proper is positioned to be at the same spot where the axis of the arm of the transducer (B) is located. End of the transducer arm is bent and placed over the distal end of the segment (C). Signal from the transducer is fed to an amplifier and recorder (D) for display. The entire set-up is placed inside an incubator. A curvature response of 0.01° can be recorded as a full-scale displacement of recorder pen to 24 cm.

IAA nor GA is transported basipetally in gravistimulated pulvini (Bridges and Wilkins, 1973 ; Kaufman, Bandurski, *et al.*, 1979 ; Pharis and Kaufman *et al.*, 1982).

Gravitropic Response in Grass Pulvini Treated with Different Hornones

Auxin and its Conjugates

Exogenous IAA does not stimulate gravitropic curvature. The segments are grown in the dark at 30°C. for periods up to 48 hours O. 1M sucrose + hormone. Curvature is measured manually with a protractor or electronically with a Metripak angular transducer. To localize IAA application, we also apply it directly to the lower side of the pulvinus with lanolin. Fig. 11.13 illustrates the kinetics of upward curvation for IAA--treated segments as compared with sucrose control segments.

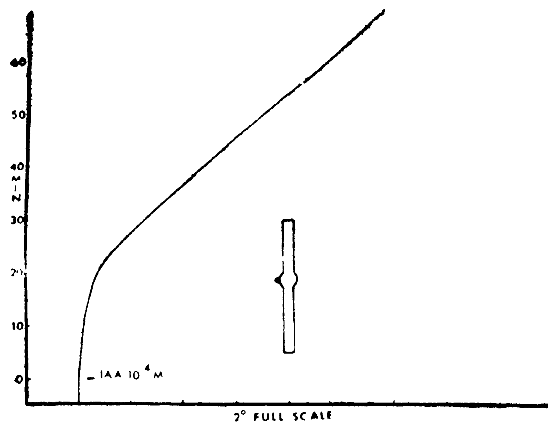


Fig. 11.13 Angular recording transducer tracing showing the kinetics of early stages of binding response induced in up right pulvinus by the unilateral application of IAA. Elongation of cells on the side application that leads to bending is apparent within 10 minutes of application.

Fig. 11.14 indicates the elongation growth response for upright barley pulvini treated all around with IAA, inositol ester conjugate of IAA, and the growth regulator brassin. Interestingly, the auxin conjugate does not by itself promote elongation of the pulvini, but in the presenee of brassin brings about maximal elongation response. We interpret this to mean that brassin is possibly promoting the release of IAA from its conjugte. Brassin alone promotes elongation of the pulvini. Although this response is gréater than that induced by IAA alone, the effect of brassin is slower, involving 5 to 8 hrs before response is noticed. Response to IAA can be detected as early as 10 min aftar application.

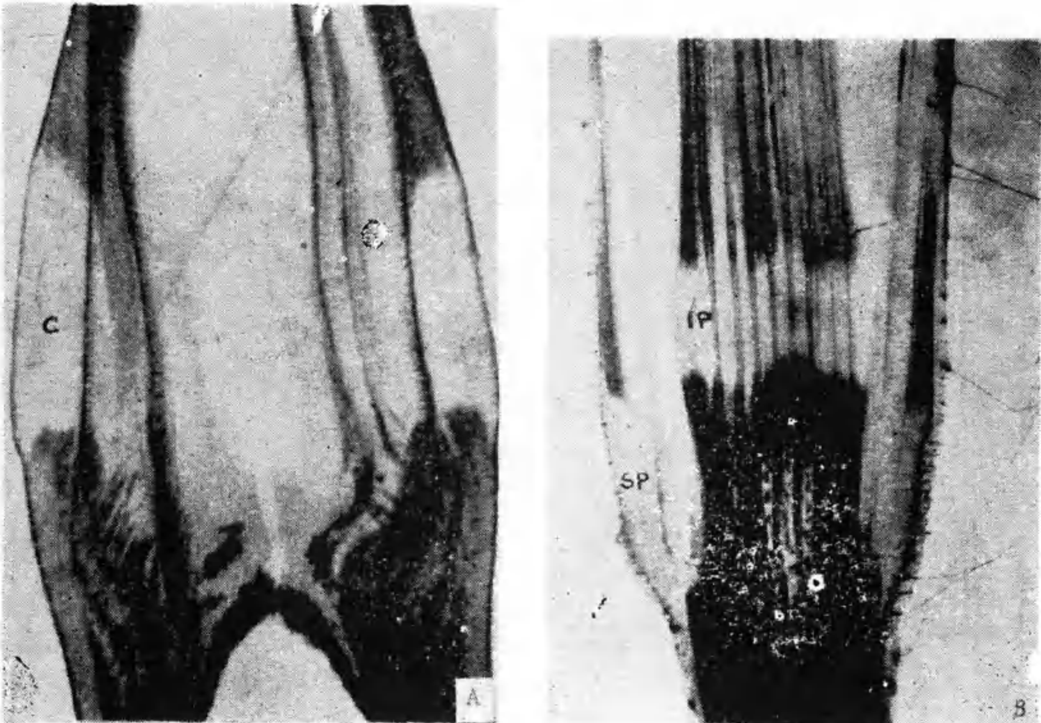
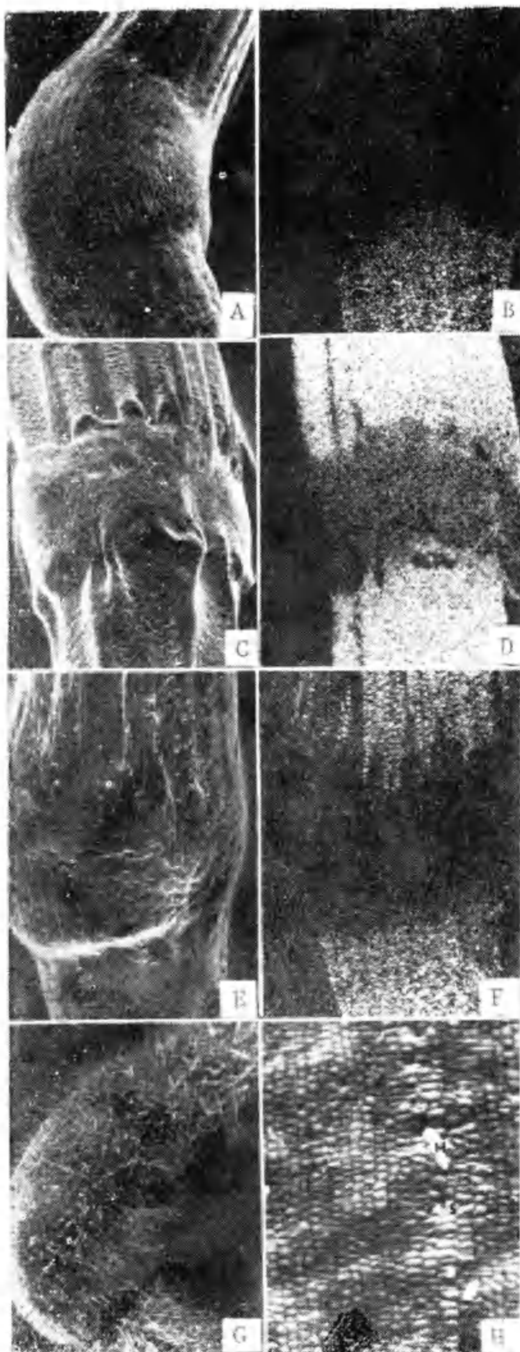


Fig. 11.1 (A) Longitudinal section of *Hordeum distichon* treated with pararosaniline-HCl for lignin and counter-stained with fast green. The collenchymatous (C) bundle caps are free from lignin except for the xylem. The internode in this festucoid grass does not possess a pulvinus. × 23. (B) A longitudinal section (not quite median of a shoot of *Panicum capillare* treated with pararosaniline-HCl and fast green. Leaf-sheath pulvinus (SP) and internodal pulvinus (IP) are free from lignin except in the vascular tissue. × 11. Both from Dayanandan *et al.* (1977).

Fig. 11.2 Scanning electron microscope (SEM) views and X-ray maps for silicon distribution in leafsheath pulvini of selected grasses A,B SEM view and Si X-ray map of *Festuca rubra*. The shoot had undergone natural gravitropic stimulation. The regions above and below the pulvinus are highly silicified. The pulvinus itself shows no more than background distribution × of Si. 27. C.D. *Oryza minuta*. The leaf sheath pulvinus has numerous silica cells which accumulate Si. Yet, there is less silica here than in the sheath above and in the internode and below the pulvinus. × 27. E, F. Pulvinus and regions immediately above and below it in *Panicum* sp The pulvinus is devoid of epidermal idioblasts (e g., trichomes, silica cells). Again, the pulvinus has very little silica. × 42 G Pulvinus of *Polypogon monspeliensis* which is covered with numerous epidermal trichomes. The hairs and the rest of the pulvinus accumulate very little silica. × 23. H. Magnified view of the leaf-sheath pulvinus in *Zea mays* Stomatal apparatuses (S) and bicellular trichomes (H: hairs) are seen among the short epidermal cells over the pulvinus × 88. (from Dayanandna *et al.*, 1977).



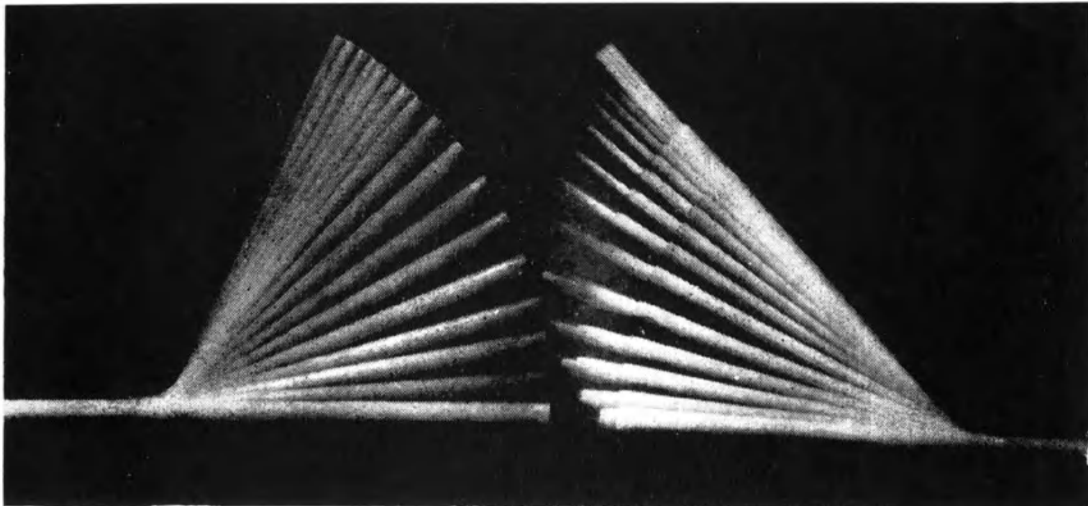


Fig. 11.3 Gravitropic response in two oats segments photographed every three hour for 48 hours. The segment on the right was held in normal position, with the basal stem portion held firmly to permit the apical leaf sheath portion to curve upwards. On the left, the apical leaf sheath portion was held down permitting the internodal stem to curve up

Fig 11.7 Growth response due to geotropic stimulation in *Avena sativa* A. Longitudinal section through the hollow internode and leaf sheath pulvinus of a shoot that had bent during 2 days of geotropic stimulation. The lower side of the pulvinus has elongated while the upper side has contracted. The inner epidermis of the sheath is emphasized by the lines drawn over them. C, collenchymatous bundle cap.×10 B. Collenchymatous cells isolated from a control leaf sheath pulvinus (viewed between crossed polarizers). The cells are uniformly birefringent and the walls uniformly thick. ×98. C. Isolated collenchymatous cells from a leaf sheath pulvinus that had grown in response to 24 h geotropic stimulation. As seen between crossed polarizers, regions that have stretched appear thin and almost isotropic whereas the unstretched regions remain as thick and birefringent as in control cells. × 98. D. Isolated collenchymatous cell from a control leaf sheath pulvinus compared with one derived from a shoot that has been geotropically stimulated for 2 days. Photographed between crossed. polarizers × 98. (Figures Band are the same as in Dayanandan *et al.*, 1976. (from Dayanandan *et al*,1977).

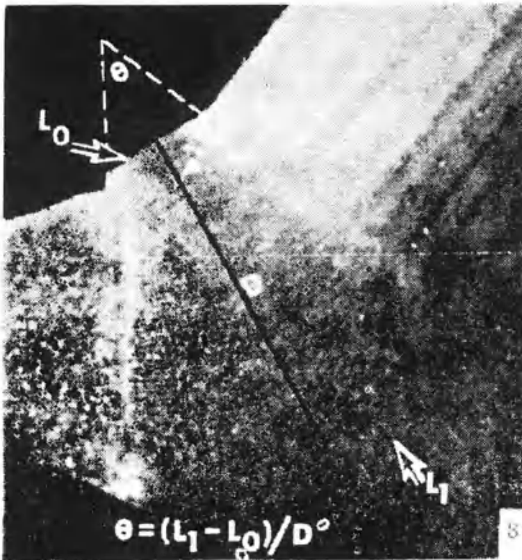


Fig. 11.8 is a photograph of an oats segment that has curved to 52°. Growth response is confined to the pulvinus. As seen in this photograph, the response of the pulvinus is linear with no growth on the upper surface to maximum growth on the lower surface. Since a cylindrical organ is being viewed from one side, the half-cricumferential distance between the upper and the lower sides represents the pulvinus. L_0 represents the initial length of the pulvinus which has not changed on the upper surface. L_1 is the maximal length seen on the lower side. When two lines are drawn along the boundaries of the pulvinus and allowed to meet on the upper side, and angle Theta is generated. The value of this angle is the same as the angle of curvature of the segment. The equation given in figure make it clear why it is that the magnitude of growth responses in different cells has not been easy to analyze. The difference in length between the initial and final sizes is a function of the initial length for any given angle and diameter. (Dayanadan *et al.*, 1981).

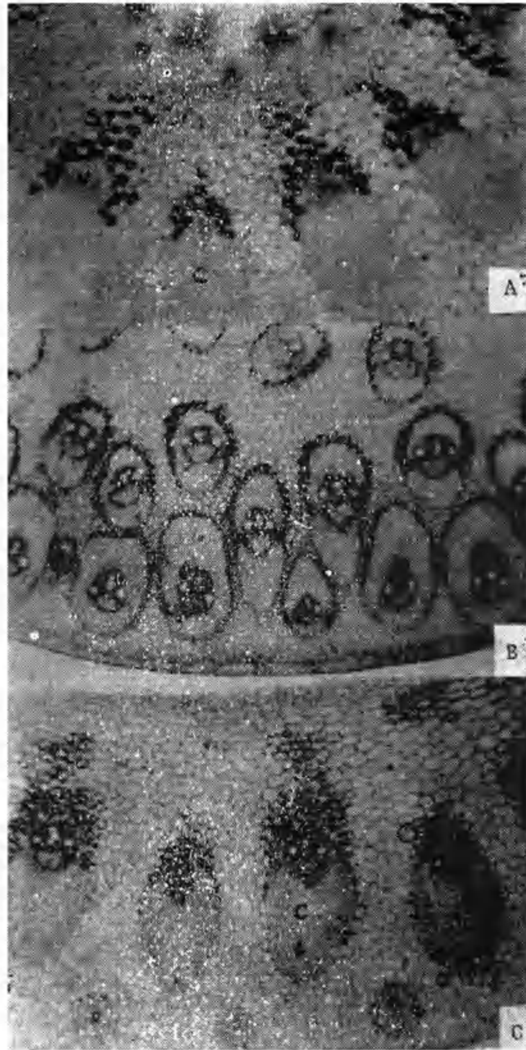


Fig. 11.10 Anatomical features of leaf sheath and internodal pulvini of selected grasses as seen in cross section. All preparations are from the lower halves of pulvini that have been geotropically stimulated for 1 h. C. collenchymatous bundle cap: V, vascular tissue: S. Statocytes. (A). Leaf sheath pulvinus surrounding the internode in *Avena sativa.* The statoliths have sedimented towards the gravitational field due to geotropic stimulation. A few statocytes are also seen in the internode. Free-hand section stained with I_2 KI. × 100. (B). Internodal pulvinus of *Zea mays.* Vascular bundles are completely surrounded by collenchymatous cells, which in turn, are partially or compltely surrounded by 1-2 layers of statocytes. Collenchymatous cells are also found in the hypodermis. Free-hand section stained with I_2 KI. × 50. (C) Vascular bundles, collenchymatous cells, and statocytes of a leaf sheath pulvinus of *Zea mays.* × 36.

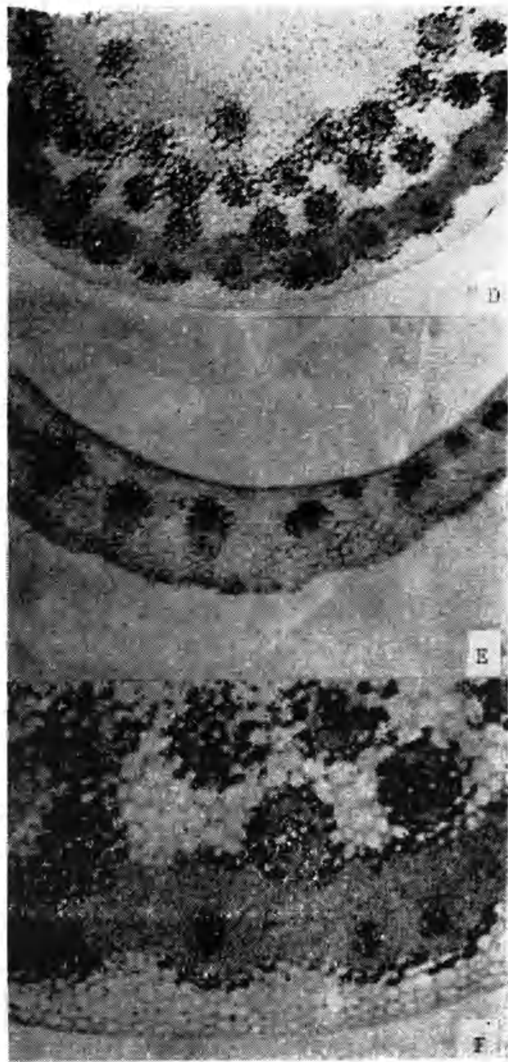
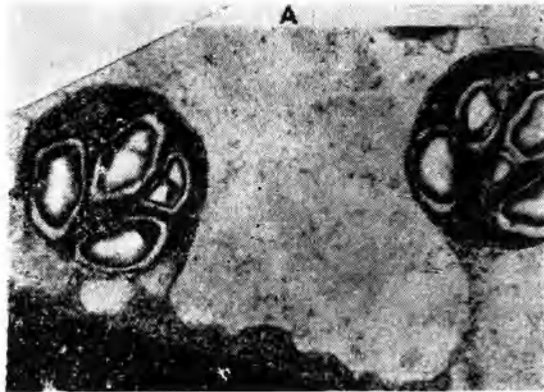


Fig. 11.10 (D). Free-hand section of an internodal pulvinus of *Panicum capillare* stained with I, KI. 28. (E) leaf sheath pulvinus of *Panicum capillare*. Outer epidermis of the pulvinus has epidermal hairs. × 21. (F) An enlarged view of a portion of the pulvinus in Fig. E. Statocytes of 1-2 layers surround the collenchymatous bundle cap. Outermost bundle caps have coalesced to form a continuous band of collenchymatous cells. There is no hypodermal collenchymatous tissue in this plant. × 58.



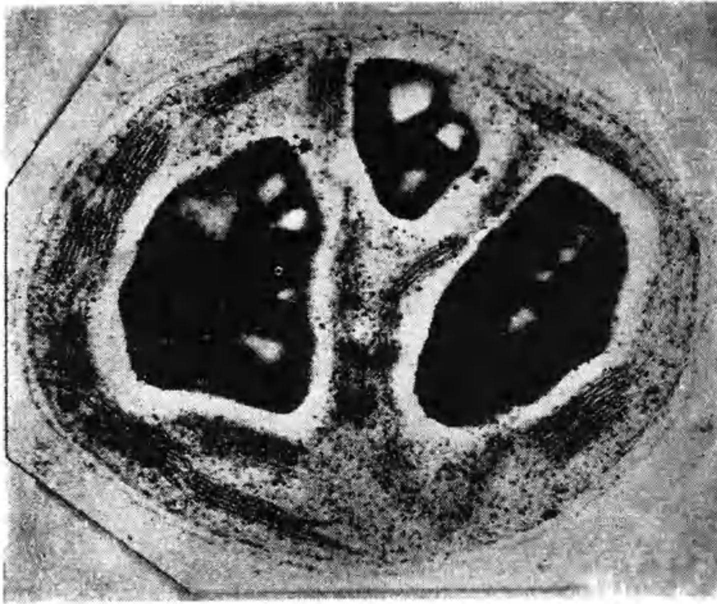


Fig. 11.11 When statoliths sediment during gravistimulation, they seem to be enveloped by the tonoplast membrane In Fig. (A) two statoliths are seen partially surrounded by tonoplast membrane. Dense Cytoplasm is seen between the tonoplast and plasmamembranes, At some levels of sectioning, it is possible to see the tonoplast seen entirely surrounding the statolith. (B): There is space, about 4 to 5 times that of the double membrane thickness, between the statolith and tonoplast membrane. Such association between statoliths and tonoplast can be observed within an hour after gravistimulation. We believe that such interaction could bring about changes normally associated with graviperception.

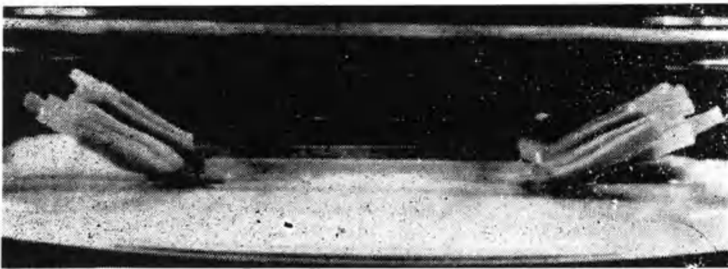


Fig. 11.16 Illustration of *Avena* gravitropism bioassay set-up for testing effects of exogenously supplied hormones on gravitropic curvature. Segments include the leaf sheath pulvinus, the internode below it, and the leaf sheath and enclosed basal portion of internode above it. The internode portions are placed under the four microscope slide with the pulvini just outside the stackes. Sheath and enclosed internode portions extend out beyond the pulvini. Under the segments is a dise of filter paper which is saturated with 6 ml, O. 1M sucrose. The entire set-up is enclosed in a standard sized Petri dish (90 mm diameter).

Effects of Exogenously Supplied GAs

In another excised stem segment system, (Fig. 11.16), adapted for use as a bioassay system for analyzing long-term gravitropic curveture responses of the grass pulvinus to exogenously supplied hormones and other regulatory substances, we find that GAs (30 μm + .01M sucrose), as well as GA_4 and GA_7, accelerate the upward curvature response of pulvini, as compared with control pulvini of excised stem segments maintained in sucrose (0. 1M) alone. The amount of curvature is from 15-20° greater in the presence of GAs after 20 hours than in control segments. But with GAs, by 48 hours, the curvature response is $<$ control curvature because of GA-promoted stem elongation out of the sheath portion beyond the pulvinus, which imposes a significant weight factor that results in a slowing down in the rate of pulvinus curvature.

Ethylene

Ethylene, when applied exogenously, does not stimulate gravitropic curvature significantly. Its role as a regulatory hormone in the process of gravitropic curvature of grass pulvini is thus doubtful, especially in light of data we shall present later on in endogenous $CH_2{=}CH_2$ during gravitropic curvature.

Changes in Endogenous Hormones During Gravistimulation

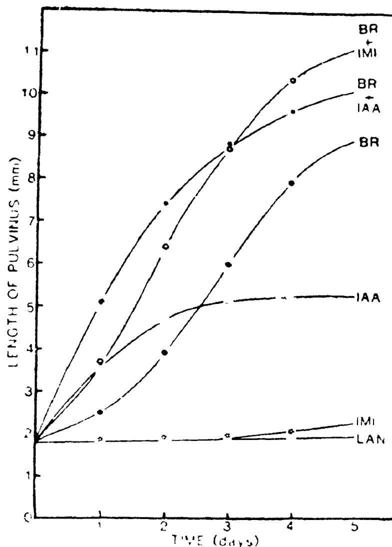


Fig. 11.14 Time- course change in length of pulvini of *Hordeum* (barley) treated with brassin (BR), indole-3-acetic acid (IAA), BR + IAA, lanolin (LAN =control), and myo-inositol ester of (IMA) . About 1μg of each reagent was applied in lanolin around intact pulvini of excised stem segment.

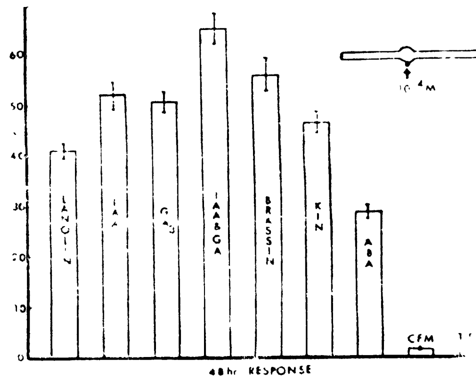


Fig. 11.15 Effects of exogenous, unilateral applications of different growth regulators on gravitropic curvature of pulvini after 48 hours in the dark at 30°C. IAA = indole-acetic acid, GA_3 = gibberellic acid, Brassin = brassinosteroid (kindly provided by Dr. Werner Meudt of the USDA al Beltsville, Maryland). KIN = kinetin (6-furfurylamino purine). ABA = abscisic acid (mixed *cis* and *trans* isomers), CFM = the morphactin (chloroflurenol), TIBA = 2, 3, 5-triiodobenzoic acid. Twenty segments were used for each treatment. They were incubated in the dark at 30°C. The internode ends were placed in 0.1m sucrose-saturated towels between two glass plates

Table 1. Negative gravitropic curvature response and net internodal extension*/ in *Avena* stem segments**/ in the presence and absence of GA_3, GA_4/A_7, or IAA.

Growth Response	Treatments ***/			
	Sucrose	Sucrose + GA_3	Sucrose + GA_4/A_7	Sucrose + IAA
Mean Negative Geotropic Curvature (degrees)	28°	34°	42°	44°
Mean Net Internodal Extension (mm)	1.9	14.6	15.7	1.8

*/ After 20 hours of incubation in the dark at 30°C. **/ These are excised stem segments including the basal intercalary meristem, the surrounding sheath base portion, the sheath pulvinus, and the node. 24 segments were used for each treatment. ***/ Sucrose = 0.1 M, GA_3, GA_4/A_7 = 30 μM, and IAA = $^{-5}$M.

Native IAA

Using 10 gm of fresh wt. of oat pulvins tissue and the double isotope dilution technique of Bandurski, Schultz and Cohen (1980), we analyzed the levels of free IAA in vertical as compared with gravistimulated oat

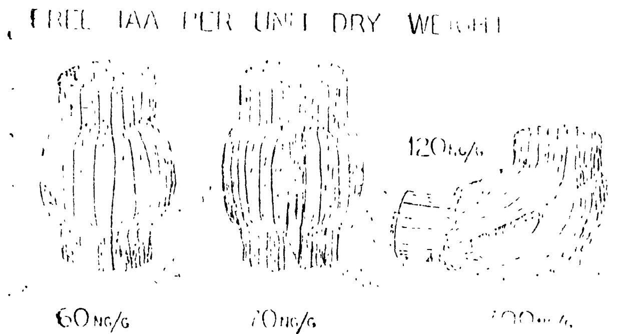


Fig. 11.17 Amount of free IAA present unit dry weight in *Avena* pulvini which were left upright and harvest intact (left), left upright, but divided into left and "right" halves (middle), and gravistimulated to 30° (ca. 24 hrs.), then divided into upper and lower halves.

leaf-sheath pulvini. Fig. 11.17 indicates the results. Intact upright pulvini in halves of upright pulvini have 67 and 70 ng. free IAA present/g dry wt., whereas in the pulvini gravistimulated to 36°, top halves had 130 ng. free IAA per gm dry wt. and bottom halves had 349 ng. free IAA per gm. dry wt. This represent an increase of about seven times in amount of free IAA in the pulvini as a result of gravistimulation and a 1:2.5 ratio of free IAA in top halves as compared with bottom halves. The conjugates of IAA (insoitol ester of IAA and peptidyl IAA) were not analyzed but should be analyzed because of the possibility that the above increase in free IAA that occurs in gravistimulated pulvini, and the 1:2:5 top/bottom asymmetry that develops, arises as a result of release of free IAA from its conjugates. This is especially attractive in light of the fact that ^{14}C-IAA does *not* move in a lateral fashion in gravistimulated oat or wheat pulvini (Kaufman, Bandurski *et al.*, 1979; Bridges and Wilkins, 1976). Alternatively, the increase in free IAA in gravistimulated pulvini, and the asymmetry could be due to enhanced synthesis of IAA under these conditions.

Native GAs

The primary active, polar GAs *Avena* pulvini area GA_3, GA_4 and GA_7.

Glucosyl ester conjugates of these GAs are also present. When the pulvinus is gravistimulated to 30°, it shows marked changes in the distribution of free, polar GAs and GA conjugates, as compared with those in vertical, control pulvini (Fig 11.18 and 11.19). Free GA_3, GA_4 and GA_7 increase significantly (29-fold) in gravistimulated pulvini with these active, free GAs being two times greater in amounts in the lower halves than in the upper halves, and inactive glucosyl ester conjugates of these GAs being 1.8 times greater in upper halves than in lower halves.

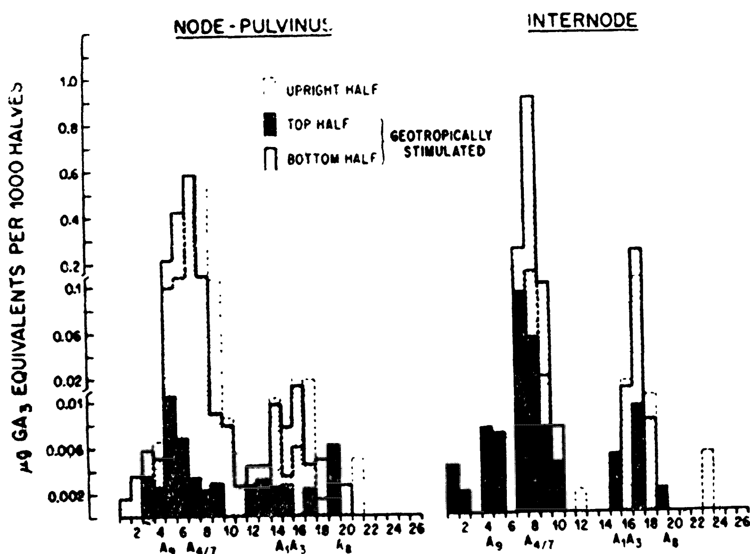


Fig. 11.18 Elution pattern of standard GAs (shown below the fraction number) and GA-like substances present in *Avena* plant parts, as assayed on dwarf rice, 'Tan--ginbozu' in serial dilution, for each of 26 fraction from a gradient-eluted SiO_2 partition column. (From Pharis *et al.*, 1981).

Ethylene

In collaboration with Devid Reid and Richard Pharis, we have found that upon gravistimulation, ethylene evolution, increase drammatically in *Avena* leaf-sheath pulvini within 6 hours in gravistimulated, intact oat plants. This is also confirmed by work of Osborne and Wright (1979) with gravistimulated *Echinochloa colonum*. Further, in view of the fact the exogenous ethylene does not appear to stimulate gravitropic curvature in *Avena* pulvini, it is probable that ethylene production may be the *result* of gravitropic curvature and early establishment of auxin

asymmetry (see Bandurski *et al.*, 1983) in gravistimulated grass shoots (within 15 minutes in *Zea mays*).

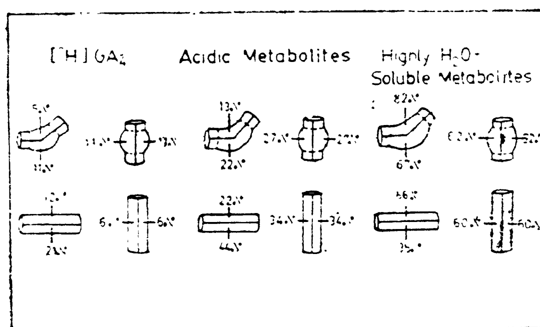


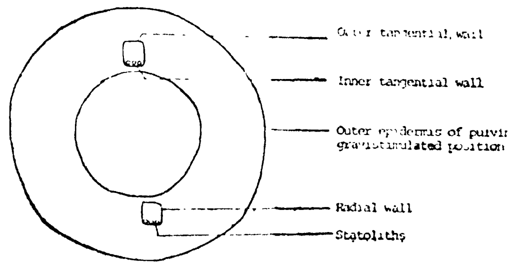
Fig.11.19 Diagrammatic representation of percentage distribution of ^3H- dpm in node -pulvinus and internode halves after SiO_2 partition column chromatography of 20 upright or 20 geostimulated plants (30° curvature) incubated in 10×10^6 dpm of [^3H] GA_4 (1.4 Ci/mmol) for 24 h prior to geostimulation (t_0) of 20 plants, and 44.5 h after t_0. The 80% methanolic extract was chromatographed without further purification and the highly H_2O- soluble matabolites (probably glucosides and/or glucosyl esters) were eluted from the column with absolute methanol after the completion of the hexane to ethyl acetate gradient. Actual dpm persent were 945 (nodepulvini) and 4918 (internodes for left plus right halves, and 3774 (nodepulvini) and 3938 (internodes) for upper plus lower halves. For plant parts from geostimulated shoots the sum of all bottom halves for each plant part=100%, as does the sum of top halves. Left halves were within 8% of right halves for node-pulvini, but the 4918 dpm value for internodes may be anomalous since the left halves contained 1226 dpm, right halves 3692 dpm. Hence, since there is no reason to believe that left halves should not equal right halves, the sum of left + right ÷ 2 is shown for verical plant part. Lower halves of geostimulated plant parts contained 1927& 2027 dpm and upper halves 1847 and 1811 dpm for each of node-pulvini and internodes, respectively (From Pharis *et al.*, 1981).

An Overall Model for Hormonol Regulation of the Gravitropic Response in Grass Pulvini

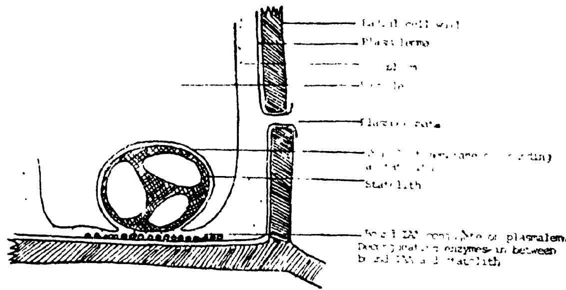
Fig. 11.20 represents a working model to explain how gravitropic response occurs in grass shoot pulvini in light of current experimental evidence, as we have presented in this chapter.

The main points of this physiological/biochemical model are as follows :

(1) The outer tangential wall (OTW) has associated with it the plasmalemma where there is more bound auxin present than in the plasmalemma associated with the inner tangential wall (ITW).



C. S. of gravistimulated pulvinus.



Interaction between statolith, tonoplast and plasmalema.

Figs. 11.20A and 11.20B are diagrams, respectively, of the location of statocytes in the grass pulvinus, relationship between the statoliths and the inner tangential (ITW) and outer tangential (OTW) as well as radial walls, and of a partial view of statocyte cell in which statolith descended to the bottom of the cell and how hormonal transduction could occur as a result.

(2) The statolith-tonoplast interaction leads to localized release of activation of IAA deconjugating enzyme (s).

(3) Release of these enzymes can be due to mere contact between the tonoplast and statolith or due to tension that must develop (due to stretching) in the tonoplast membrane. Such deformation in the tonoplast membrane can also release the GAs that are stored in the vacuole as conjugates.

(4) Free IAA can have limited transport through the lateral walls (it is not transported basipetally in the grass pulvinus (Bridges and Wilkins, 1973; Kaufman *et al.*, 1978) once it is released from its conjugates at the plasmalemma at the OTW. This would allow IAA to promote cell lengthening in cells located in positions on either side of the statocytes at the sides of the pulvinus.

(5) Both free, active GAs and IAA, once "liberated" from their inactive conjugates. could act on the cell walls directly or indirectly by stimulating the synthesis or by activating cell wall-loosening enzymes which would plasticize the cell wall; they could also stimulate cell wall synthesizing enzymes either by activating them or stimulating their synthesis (as with the cell wall hydrolases). The geometry of the pulvinus, with morphological and/or biochemical assymmetry in the radial vs. tangential walls and OTW's vs. ITW's, would then account for the differential release of active hormones as one progresses from top to bottom of the pulvinus.

(6) Transport of IAA is radial (from statenchyma outwards) and not lateral as proposed in classical models.

Implication for the Future

Basic Studies on Gravitropic Responses in Cereal Grains and Their Agricultural Significance

Hormonal regulation of negative gravitropic curvature in grass pulvini is just beginning to be understood in a most rudimentary way. For the future, we need to focus precisely on how gravity perception is translated into the transduction process, that is, the primary events that occur between the fall of starch statoliths and the establishment of hormone asymmetry. Such ideas as release of IAA and of GAs from their respective conjugates in an asymmetrical fashion and the occurrance of radial (lateral hormone transport must be proved or disproved in this system. The mechanism of action of free IAA and of free GAs on cell wall-loosening must be investigated in order to elucidate the asymmetric cell response component of gravitropic curvature. Finally, the process of lignification and silicification that occurs after a pulvinus has responded to gravity, or which occurs in older, upright pulvini, preventing them from responding to gravistimulation, should be examined. Such studies are of importance in agriculture so that we can develop cereals and other grasses which do not lodge or which will recover from lodging. Hormones and other chemicals are being used at present to prevent lodging; e.g., CCC (an antigibberellin) is used to dwarf wheat or as silica slags are applied to rice paddies to stiffen the straw through enhanced silicification.

Space Biology and Growth of Grasses in Near Null Gravity of Outer Space

Already, the U.S. National Space and Aeronautics Administration, Western Europe Space Biology Programs, and the USSR Space Biology

Programs have given a tremendous impetus to exploration of the nature of gravitational responses of plants at 1 g on earth, but at the same, have spurred efforts to study plant growth and development under conditions of near null gravity that prevail in outer space.

The grasses we plan to grow include wheat *(Triticum)* and rice (*Oryza sativa*) in the U.S. Space Shuttle, Space Lab., and the Space Station between 1985 and 1993. These plants will be grown in fully-automated culture regimes and controlled environments. Shoots of cer... grasses will be "directed" upward in their growth by means of uniderectionally oriented light sources, space planting, and physical constraint. Hormones may be applied exogenously and unilaterally to the shoots if they become lodged because of vibrations. Monosilicic acid [Si $(OH)_4$ n H_2O] will be supplied in solutions applied under aeroponic conditions to the roots of cereal grasses (which are known to accumulate high amounts of silicate and store it irreversibly as biogenic silica) so as to enhance silicification in the shoots, hence strengthening the latter. Semi-dwarf plants will also be used because of their lodging resistance and higher silica contents. Such plants will be analyzed for their contents of lignin and silicon; protein, starch, and total carbohydrates; and levels of native auxin and gibberellins and their conjugates. Growth habits will be monitored by means of photographs and "real-time bio-growth monitors" under computer control. The future prospects for carrying out this work are exciting. The plants eventually will be used for food, CO_2 consumption, provision of O_2 and water, and processing of wastes.

Acknowledgements

We are thankful to Dr. Il Song and Dr. Dennis Inman for help in various ways. This work is supported by Grant NAGW-34 from the National Aeronautics and Space Administration (NASA) Space Biology Programm (USA).

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Hormonal Regulation of Root Formation

K. Gurumurti, B. B. Gupta and Adarsh Kumar

Introduction

With the growing trend towards more and more of plantation forestry, introduction of fast growing trees and the genetic improvement of forest tree species, it is of practical significance to develop fast, convenient and economical method of raising the planting stock. Reproduction of forest tree species by shoot cuttings is very useful for the multiplication of species and for developing clones. Clone is of considerable importance in practical forestry because it offers advantage of uniformity of growth and development and immediate availabilty of superior individual for large scale afforestation and seed orchard work, it also eliminates differences in genetic constitution between trees. In comparison to other methods, planting of shoot cuttings is very convenient, as it may save time and labour usually involved in seed collection, lot of nursery work and several years of weeding. The early flowering of shoot cuttings further saves time and given an easy approach to the tree breeder for genetical work at considerably lower height. Stem or shoot portion are generally very good material for rooting purposes, because they usually have undifferentiated tissues which may permit initiation of root primordia and they also have preformed buds.

There is wide spread agreement on the need for handling forests to produce selected species of trees with improved form, good quality and fast growth, as well as resistant to insects and diseases. The growth of trees is affected by internally produced growth substances in addition to fodd, minerals and water. Among the best known of these growth regulating substances are the auxins which cause elongation of shoot cells. They play a regulatory role in several aspects of growth such as shoot elongation, diameter growth, root growth, wound healing formation of galls and tumors, fruit development and abscission of leaves and fruits. Rooting can be induced by treatment with natural and synthetic auxins. However, rooting is influenced by a variety of factors, including age of parent tree, season when cuttings are taken and the part of tree sampled.

There are numerous reports on the rootability of shoot cuttings, e.g., Yin and Liu, 1948; Fielding, 1954; Ohmasa, 1956; Nienstaedt *et al.*, 1958; Schrieber, 1963; Thulin and Faulds, 1968; McKnight, 1970; Hill and Libby, 1970. Poplars (*Populus* spp.) for example are being established extensively in suitable localities in Indiä through shoot cuttings (Anon, 1979). Similarly, various studies aimed at improving rooting techniques with forest tree species have involved the physiology of rooting, with particular reference to auxins and nutritional relationships (Snow, 1941; Mirov, 1944; Thimann and Behnke 1947; Yim, 1962; Matthews *et al.*, 1960; Fielding 1964; Libby, 1964; Bhatnagar *et al.*, 1968; Bhatnagar and Joshi, 1978; Nanda *et al.*, 1968; Nanda, 1970).

Factors Affecting Rooting Response

An intensive study on the physiological factors controlling rooting response of branch cuttings (Nanda, 1975) provided considerable basic information. It was found that environmental factors like season, light, temperature and humidity have paramount role to play on root initiation. The other factors like injuvenility of the mother tree, size of cuttings, presence of leaves and vegetative buds and the change in the content of carbohydrates, protein and nitrogen have also greatly influnced the rooting of branch cuttings.

Detailed investigation made on the rooting behaviour of branch cuttings of hundreds of species revealed that while some plants rooted without the application of auxins and other failed to root even with application of auxins (Nanda *et al.*, 1968, 1970; Nanda, 1970). These studies further revealed that the rooting behaviour of different species belonging to the same family was similar. It was further observed that the rooting potential of branch cuttings varied with season (Bhatnagar, 1973; Bhatnagar and Joshi, 1978). It was generally observed that the seasonal dormancy in rooting concided with winter dormancy (Nanda, 1970; Nanda and Anand, 1970, Nanda *et al.*, 1970) whereas the spurt in rooting concided with the period of high cambial activity (Nanda, 1970; Nanda and Anand, 1970). In certain cases like *Ficus infectoria*, it was observed that schlerenchymatous tissues surrounding the vascular tissue prevented the emergence of root initial which could be broken by slitting the cuttings (Nanda, 1970; Nanda *et al.*, 1970). During enhancement of rooting in response to externally applied auxins to branch cutting in a number of tree species is well known. It is also known that the effectiveness of externally applied

auxins is not always the same in all the seasons (Nanda, 1975; Nanda and Sethi, 1979). Thus, it has been reported that treatment of the cuttings with auxins inhibited rooting during seasons when untreated cuttings root profusely. In certain season when the control cuttings do not root or root only negligibly, the external application of auxins enhanced number of roots formed (Nanda *et al.*, 1968). This has been ascribed to the fact that during period of active growth the endogenous level of auxin was high due to high meristamatic activity (Nanda, 1970; Nanda and Anand, 1970; Adarsh *et al.*, 1969). In species, *Dalbergia sissoo*, (Nanda *et al.*, 1969; Nanda, 1970; Nanda *et al.*, 1970; Nanda and Anand, 1970), it was observed that untreated cuttings rooted well during May to June while auxin application inhibited or reduced the number of roots. In certain species like *Ficus infectoria* and *Hibiscus rosa-sinensis* it was observed that even during active growth period external application of auxin enhanced the root formation of branch cuttings suggesting probably the inadequecy of auxin produced within the system (Nanda, 1975). It was further observed that in certain species like *Alnus nitida* and *Dalbergia sissoo* which rooted only in May, the treatment of cuttnigs with auxin even during August enhanced the root formation (Nanda *et al.*, 1967, 1968, Nanda. 1970). Such widening of rooting behaviour as a result of the externally applied auxins has been reported for a number of species. Another important factor was the response of branch cuttings to nature and concentration of applied auxin which is well illustrated in work of Nanda *et al.*, 1967; 1969; Nanda, 1970. It is, thus, clear from the foregoing that there exist distinct correlation between the cambium activity, auxin production and root initiation in branch cuttings. Evidences presented in the literature show that leaves and vegetative buds on the cuttings exert considerable influence in the root initiation. This close relationship was confirmed by Wareing (1951) Larson (1962), Wort (1962), Haissig (1970a,b) observed that root primordia formation in Brittle Willow was governed by auxins. Gibberellins have been reported to inhibit rooting in many plants. However, Nanda *et al.* (1967) reported that GA_3 promoted rooting in certain species. Though Libbert (1956) and Hess (1962) reported higher rooting response to applied kinetins on leafy cuttings but it has not been found to play and shows direct role in root initiation.

Kawase (1964) demonstrated the seasonal response in rooting of branch cuttings which was latter on shown to be a function of cambial activity and auxin production in many woody species. Nanda *et al.* (1968) have

also found seasonal variation in rooting response in the shoot cutting of some tree species. However, within limits, ability to form root is related to the physiological conditions of the plant and the climate rather than the time of the year. Rooting of conifers usually is poor if shoot cuttings are taken in late spring or early summer while shoots are still growing. The best season for rooting differs with the plant species. Tarasenko and Stefan (1960) and Vieitez and Pena (1968) reported high rooting of the stem cuttings taken form the mother plant during the active period of growth. Similarly Hartmann and Loreti (1965) showed that leafy cutting of olive tree rooted profusely in June–August but poorly in November-January. Seasonal rooting response is also influenced by the nature of stem cuttings. Soft wood cuttings of many trees and shrubs rooted better in June while the older one did well in December (Sonnenfeld, 1960). Wareing and Smith (1963) found that actively growing soft wood cuttings of *Populus* rooted best in June-July but the dormant hardwood ones in autumn. Deolle and Mitchell (1964) reported that spring was the best season for rooting stem cuttings of many plants and Nichloson (1965) considered that for rooting stem cuttings of bamboo and cotton wood the best month were February-March. Marygina (1966) reported that stem cuttings of *Picea abies* rooted best when these were taken from the mother plant in early spring before the bud break or in mid summer after the cessation of extension growth. An annual rhythm in rooting response of stem cuttings has been reported by many workers (Bhatnagar *et al.*, 1963; Bhatnagar, 1973; Bhatnagar and Joshi, 1978). Thus Lanphear and Mehl (1961) observed that rooting of stem cuttings of Juniper was the lowest during the active period of vegetative growth and the highest during the period of dormancy. Hartmann and Loretoi (1965) observed that stem cuttings of *Olea europea* developed adventitious roots and a fast rate during winter months. Vieitez and Pena (1968) reported a similar seasonal rhythm in the rooting response of stem cuttings of *Salix atrocinera.* The seasonal changes in rooting response of stem cuttings have been ascribed to either changes in food forming substances (Klein, 1953) or to physiological status of the cuttings or to changes in the endogenous content of growth substances (Nanda and Jain, 1971). Hartmann and Loreti (1965) attributed changes in rooting to differences in the production of photosynthates. The photosynthetic output of leaves would be less during winter when days are short and the intensity of light and temperature is lower than during summer. There is a decrease in the production of metabolites necessary for the initiation and developments of roots in winter. Zabielski (1965)

attributed high rooting in autumn to accumulation of nutrients. Table 1 summarises the results achieved in rooting branch cuttings for some of the important forest tree species, which have been generally classified as obstinate to root by other workers (Nanda *et al.*, 1968, 1969, 1970; Nanda, 1970). The studies also revealed that many forest plants have a narrow period for eliciting maximum rooting response.

Table 1. Successful rooting of difficult to root forest tree species of economic importance* at Plant Physiology Branch, Forest Research Instt., Dehra Dun.

Species	Hormone and dose	Month for optimal rooting
Bombax ceiba	IBA 100 ppm	March-April
Bombax insigne	IBA 100 ppm	March
Dalbergia sissoo	IAA, IBA 100 ppm	August-September
Eucalyptus camaldulensis	IBA 100 ppm	September
Eucalyptus tereticornis	IAA, IBA, NAA 100 ppm	August, September
Ficus elastica	IAA, IBA 100 ppm	March
Ficus krishneii	IAA, IBA 100 ppm	March
Gmelina arborea	IAA, IBA, NAA 100 ppm	March. July, August
Grewia oppositifolia	IBA 100 ppm	March
Ginkgo biloba	IBA 100 ppm	February, March
Leucaena leucocephala	IBA 200 ppm	February, August
Platanus orientalis	IAA, IBA, NAA 100 ppm	March, July
Pinus caribaea	IBA, NAA 200 ppm	July, August
Pinus roxburghii	IBA 50 ppm	July
Populus gamblei	IAA 200 ppm	November
Tectona grandis	IBA 100 ppm	March
Toona ciliata	IBA 100 ppm	March

* See also Plates 11.1 & 11.2.

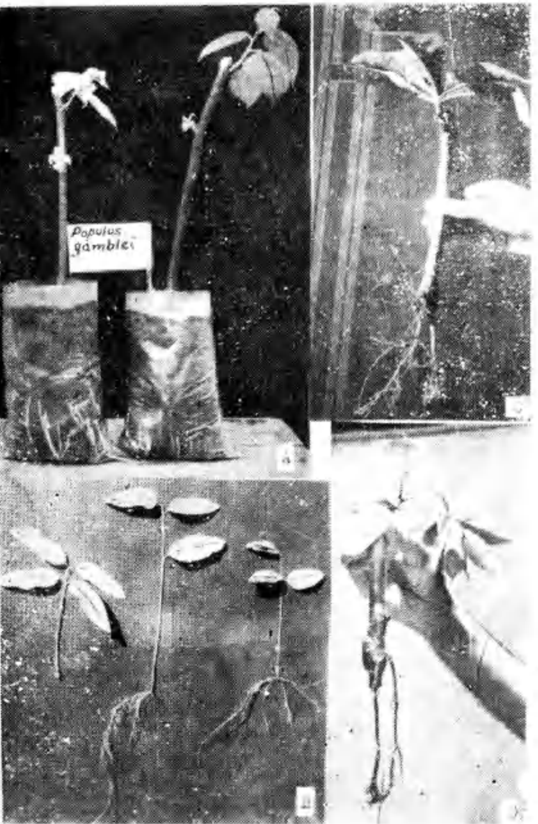
Though the seasonal response in rooting of cutting is basically the function of auxin produced as well as the result of cambial activity, the availability of carbon nutrition also plays an important role in initiation and development of roots. Sachs (1882) stated that leaves are essential for root formation as they supply certain root forming substances whereas Went (1938) suggested that leaves supply nutrients. It was later shown by Pearse (1943) that leaves supply carbohydrate and other root promoting substances. Despite of the fact that both auxin and nutrition are present in branch cuttings many a times they fail to root. A careful analysis of both auxin and nutrition level as well as the rooting response in *Populus nigra* suggested a probable existance of certain

balance between certain auxins and nutrition (Nanda, 1970). Studies were, therefore, designed to understand the nature of morphophysiological factors which control the formation of adventitious root and the mechanism involved in cellular and subcellular levels. Nanda and coworkers developed two systems, etiolated segment of *Populus nigra* (Nanda and Jain, 1971) and etiolated mungbean hypocotyl cuttings (Gurumurti and Nanda, 1974). The experiments were designed in such a way that experimental material was totally devoid of auxin and nutrition hence it was possible to control the supply of both externally in desired quantities to study their effect alone or in combination. The results obtained by them clearly showed that a proper balance between auxin and nutrition is absolutely essential for optimum root formation (Table 2 and 3). Based on these results it was also possible to explain the rooting behaviour of different species in various seasons (Nanda *et al.*, 1968). A model concept was presented for the initiation and development of roots in stem cutting of *Populus robusta*, which is presented in Fig. 12.1 (Nanda and sethi, 1979).

Mechanism of Adventitious Root Formation

Table 2. Effect of varying concentration of glucose with or without addition of auxins on the number of segments rooted and number of roots per rooted segment on etiolated stem segment of *Populus nigra* in light (Data from Nanda & Sethi, 1979).

Parameter	Glucose %	Concentration of auxin mg/l				
		IAA			IBA	
		0	0.5	0.1	0.5	0.1
Rooted	0	0	0	0	0	0
Segments	0.01	2	5	5	10	9
(Out of 10)	0.1	5	7	6	10	10
	0.5	8	10	10	9	10
	1.0	6	10	10	9	10
	5.0	0	0	3	10	10
	10.0	0	0	0	0	5
Roots per	0	—	—	—	—	—
rooted	0.01	1.5	1.4	1.0	5.3	3.4
segment	0.1	1 6	2.0	1.6	7.7	9.4
	0.5	2.3	3.1	4.1	10.1	11.7
	1.0	1.3	3.6	4.3	11.0	14.7
	5.0	—	—	1.3	8.1	11.9
	10.0	—	—	—	—	1.8



←Plate 11.1 Rooting of shoot cuttings: A. *Populus gamblei*; B. *Tectona grandis* C. *Eucalyptus tereticornis*; D. *Bombax ceiba*. (Left)

Plate 11.2 Rooting of shoot cuttings: A. *Pinus roxbrughii*; B. *Pinus caribaea*; C. *Leucaena leucocephala*. (Top)

Table 3. Rooting response of hypocotyl cuttings of *Phaseolus mungo* after 7days (Data from Gurumurti & Nanda, 1974).

Treatment	Segments rooted	No. of roots per rooted segment	Time of microscopic root initiation (hr.)
Watar (control)	4	8.4±0 4	96
IAA 5 mg/l	10	7.8±0.6	48
Sucrose 1%	10	28.7±1.2	48
IAA 5 mg/l + Sucrose 1%	10	46.3±1.2	48
Cyclohaximide 1 mg/l	0	—	—
IAA 5 mg/l + Sucrose 1% + Cycloheximide 1 mg/l	8	8.0±0.5	96

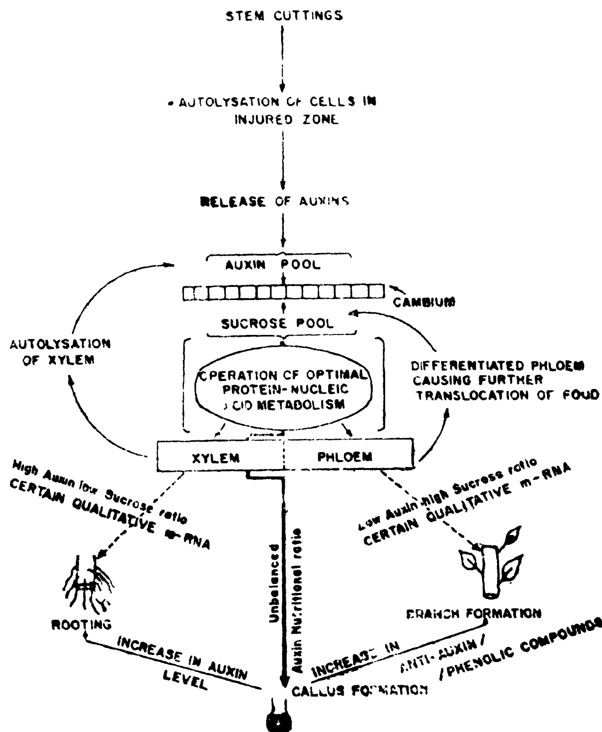


Fig. 12.1 Scheme to show the operation of potential mechanism for callus formation.

Nanda *et al.* (1974) carried out detailed investigation with a view to understand the adventitious root formation in etiolated hypocotyl cutting with excised apex and cotyledons made from mungbean seedling raised in dark. It was observed that invertase registered steep increase prior to microscopic root initiation in five day old cuttings cultured in IAA sucrose or IAA+sucrose which suggested either fresh synthesis of invertase or enhancement in activity as prerequisite for root initiation, chloromaphenicol, cyclohexamide, actinomycine-D, which inhibit protein synthesis either directly or indirectly or inhibited rooting. In fact, inhibitors of DNA synthesis and RNA synthesis, DNA-dependent RNA synthesis, protein synthesis singly or in combination of auxin or nutrition inhibited rooting (Nanda *et al.*, 1971; Jain and Nanda, 1972; Nanda *et al.*, 1973a Nanda *et al.*, 1973b, Nanda and Bhattacharya, 1973; Nanda *et al.*, 1974; Dhaliwal *et al.*, 1974; Gurumurti and Nanda, 1974). Gurumurti and Nanda (1974) showed that certain peroxidase isoenzymes were associated with initiation of root while other with their development but maleic hydrazide (mitotic inhibitor) applied initially suppressed rooting and also prevented the formation of isoenzyme associated with root initiation. The initiated roots continued to develop but the initiation of new root primordia was arrested by transfer of cutting to maleic hydrazide cultured in IAA+ sucrose during first 48 hrs. One of the isoenzymes associated with root initiation also disappeared within 12 h of transfer to maleic hydrazide (Chibbar *et al.*, 1980). These results suggest that IAA induced the formation of certain isoenzyme associated with rooting. Endo (1968), Yoneda and Endo (1970) have shown that some of peroxidase isoenzyme may act as IAA oxidases. The induction of certain peroxidase isoenzyme in media containing IAA (Gurumurti and Nanda, 1974) lends credence to the theory proposed by Endo (1968) and Yoneda and Endo (1970). Nanda *et al.* (1973a, 1974) have shown that new isoenzyme of peroxidase, NAD dependent dehydrogenase, amylyse and catalase appeared during rooting. The association of some isoenzyme with morphogenetic phenomenon has been reported by some other workers as well. Kinetic studies of enzymic oxidation of IAA in homogenates prepared from *Ipomea fistulosa* was carried out in detail by Chibbar *et al.* (1974). These studies revealed that reaction kinetics change with change in pH, the maximum activity was observed at pH 3.6. They further observed that at certain concentration of IAA, the rate of IAA oxidation increased with sucrose in homogenate concentration (Nanda *et al.*, 1975). on the basis of results obtained they suggested that IAA oxidase is allosteric in nature with two sites for binding IAA Site I, having high affinity with low cata-

lytic activity and Site II having low affinity but hlgh catalytic activity The role of IAA oxidase in plant system is also very controversial. Which some workers ascribe the role of detoxification of IAA to IAA oxidation (Galston and Davis, 1969), others consider that it produces oxidation product that cause physiological responses, characteristic of auxin (Meudt, 1971, Tuli and Mohyed 1969; Ockerse and Waber, 1970). In order to test the hypothesis that the oxidation product of IAA causes the auxin affect, Gurumurti *et. al.*, (1974) devised experiments and come to the conclusion that IAA effects are mediated through its oxidation produce formed by IAA oxidase. The complete suppression of rooting of mungbean hypocotyl cutting by even as low as 100 μg of specific protein anceous inhibitor of IAA oxidase (Chibbar *et al.*, 1974) supported the postulate that enzymatic oxidation of IAA is essential for positive rooting rseponse. Based on the observation that IAA oxidase exhibits allosteric behaviour with Side I representing the oxidation Site and the other peroxidase Site (Nanda *et al.*, 1975), it was suggested that Site I is concerned in the production of active IAA oxidation products while Site II acts to detoxify the excessive IAA if and when present in the system. Earlier Tuli and Mohyed (1969) had shown that methylene oxyindole, a product of oxidation of IAA was more effective in stimulating the elongation of stem segment in peas and beans. However Sabater *et el.* (1976) disagreed with this suggestion and showed IAA oxidase enzyme primarily function for degradation of the hormone itself Nevertheless, results obtained by Gurumurti *et al.* (1974) had conclusively shown that sodium metabisulphite (enhances the formation of oxidation product of IAA by IAA oxidase in the medium containing IAA + sucrose greatly enhanced the adventious root formation in mungbean hypocotyl. Further a diphasic concentration response was also observed in this system. It is, thus clear that IAA acts through its oxidation products and IAA oxidase has a dual function in plant system.

The involvement of nucleic acid in protein in auxin induced rooting has been clearly demonstrated by various workers (Nanda *et al.*, 1973a, Nanda *et al.*, 1973b, 1974; Dhaliwal *et al.*, 1974; Bhattacharya *et al.*, 1974). The diagrammatic mechanism of adventious root formation is presented in Fig. 12.2 (Nanda and Sethi, 1979). In fact, it was shown the some new RNAs that were induced in etiolated stem segment of *Populus nigra* cultured in glucose+IAA were supressed by actinomycin-D, suggest involvement in root initiation. These RNAs had low molecular weight therefore may be either mRNA and tRNA type (Nanda *et al.*,

1973c; Bhattacharya *et al.*, 1974). It, thus, appears that IAA or its oxidation products probably act as trigger at transcriptional level and nutrition as source of carbon to regulate the translation in synthesis of

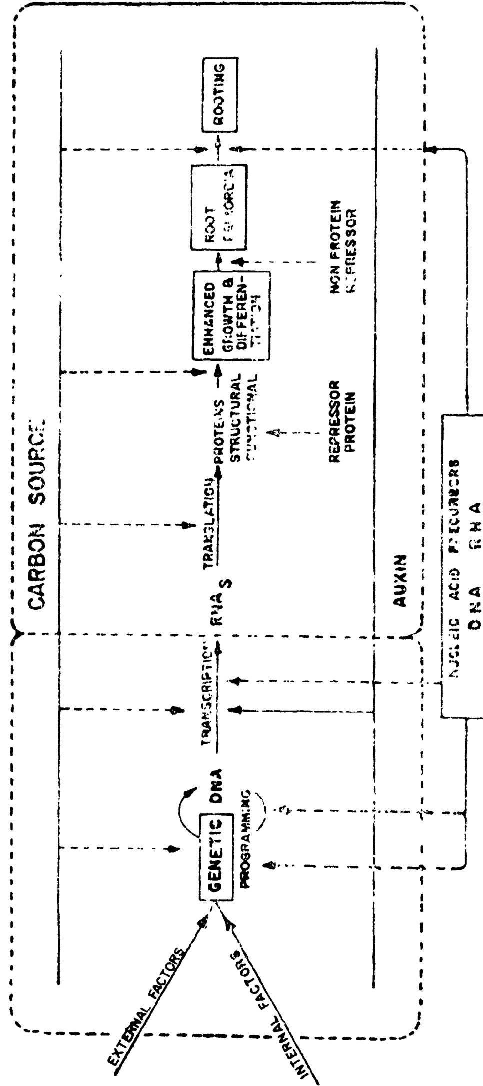


Fig. 12.2 Proposed mechanism of adventitious root formation.

proteins that are required for differentiation of cambial activity into root primordia and their development. In this context the scheme of root/shoot initiation presented in Fig. 12.1 assumes considerable significance.

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Plant Hormones in Relation to Air Pollution Injury

H. S. Srivastava

Introduction

Phytotoxic effects of air pollutants are well known. In experiments with relatively higher concentration of pollutants, reduction in plant growth and productivity and visible injuries such as chlorosis and necrosis have been frequently observed. However, in the ambient atmosphere, air pollutants exist at low concentrations and at these concentration they may not induce any visible damage, although they may interefere with several metabolic or enzymic activities of the plants (Srivastava, 1978). Since hormones play an important role in growth and differentiation of plants and they also modify metabolic or enzymic activities, very often the effects of air pollutants on plants are interpreted in terms of changes in hormonal metabolism. In addition, applicalion of exogenous hormones modifies the effects of air pollutants on plants by interferring with the normal growth and metabolism. The present review relates to these interactions of air pollutants and hormones.

Air Pollution and Hormonal Metabolism

The most extensively studied class of plant hormones in relation to air pollutants is the auxins. Both, *in vitro* and *in vivo* studies have been conducted. When bubbled through the solution of indole acetic (IAA), oxidants peroxyacetyl nitrate (PAN) and ozone inactivate it (Ordin and Propst, 1962). This inactivation is possibly due to the oxidation of IAA into 5-oxindolic and other similar compounds (Hall *et al.*, 1971). Sulfite, a possible product of cell sap, also destroys IAA in *in vitro* conditions, in the presence of Mg^{2+} and oxygen (Yang and Saleh, 1973). It has been suggested that sulfite undergoes oxidation to sulfate and during this oxidation several free radicals are formed, which are able to oxidise biologically important molecules such as NADH, NADPH, tryptophan and indoles. Air pollutants interfere with auxin metabolism in *in vivo* condition also. In 1952, Hull *et al.* demonstrated the destructive effects of air pollutants on IAA by employing the hormone bioassay technique. In their experiments with *Avena* coleoptiles, the curvature due to exogenou-

sly supplied auxin was significantly reduced in the presence of synthetic some, a mixture of ozone and hexene-1 or gasoline. Exposure to PAN also inhibits the metabolism of exogenously supplied labelled (2-C^{14}) IAA to tabacco leaves both in light and dark.

Ethylene is a hormone as well as an air pollutant. Often it is associated with the ripening of fruits and senescence. When supplied exogenously, it induces abscission of plant parts at 0.01 to 1.0 ppm level. In addition it causes leaf yellowing, abnormality and early drop of fruits, abnormal inflorescence and epinasty in several plants (Ota, 1974; Ota *et al.*, 1976). Since very often the visible effects of air pollutants on abscission add senescence are similar to those of ethylene; a correlation between pollutant injury and ethylene production has been often looked far. Such a correlation has been observed in by Cracker (1971), Tingey *et al.* (1976) and Bressan *et al.* 1978,a,b). Production of ethylene during exposure to ozone, as observed by other workers as well (Uno and Miyake, 1972) is rather short lived, lasting for about 48 h or so (Abeles and Abeles, 1972) Occasional epinastic movements in tomato leaves exposed to 10 to 34 ppm ozone, has also been inferred due to production of ethylene (Hodgson *et al.*, 1973). Exposure to sulfur dioxide also increases the production of ethylene by plant tissues. In a study with alfalfa plants, the production of ethylene by plants exposed to 0.7 ppm SO_2 was about 10 times higher than the control plants (Peiser and Yang, 1979). In chinese cabbage, wheat and barley, the damage due to SO_2 is always associated with the evolution of ethylene and in most cases, the effect of SO_2 can be stimulated by the exogenous application of ethylene (Baba and Sakai, 1974). Evolution of ethylene by spruce twigs chronically injured by SO_2 has been demonstrated by Guttenberger *et al.* (1979).

The sensitivity of plants to air pollutants is sometimes related to the amount of ethylene produced. In a study with 4 varieties of pea with differing sensitivity to ozone damage, the exposure to ozone increased ethylene production in each variety (Dizak and Ormrod, 1982). Generally the average increase in sensitive cultivars was more than in resistant ones, although the correlation between the injury and ethylene production was not very close.

Possible mechanism of ethylene production during SO_2 exposure may be examined in the light of some known facts about sulfur metabolism. In kidney bean, spraying leaves with sulfur dust increases ethylene production at two stages of plant growth, one just before full bloom and other

preceeding fruit set (Recalde-Manrique and Diaz-Miguel 1981). It has been proposed that sulfurdust is oxidised partially to SO_2 in the air (Turel 1950), and SO_2 in turn is absorbed and metabolised by the leaves to sulfur containing amino acids such as cysteine and methionine. Significant increase in total soluble amino acids and in serine and homoserine (which are intermediates in the biosynthesis of methionine) during exposure to SO_2 have been reported in several plants (Jager, 1974, 1976). The evolution of ethylene is perhaps related to the build up of methionine pool, which is precursor for ethylene biosynthesis. Perhaps the acceleration of senescence of mature maize leaf segments by H_2S (Nagar and Srivastava, 1979) could be explained on similar lines. Exposure to H_2S increases sulfur containing amino acids in the leaves of maize seedlings (Srivastava and Kumar, unpublished) which may in turn induce the generation of ethylene and consequently the senescence. The possible metabolic route for induced synthesis of ethylene during exposure to sulfur containing air pollutants is shown in Fig. 13.1.

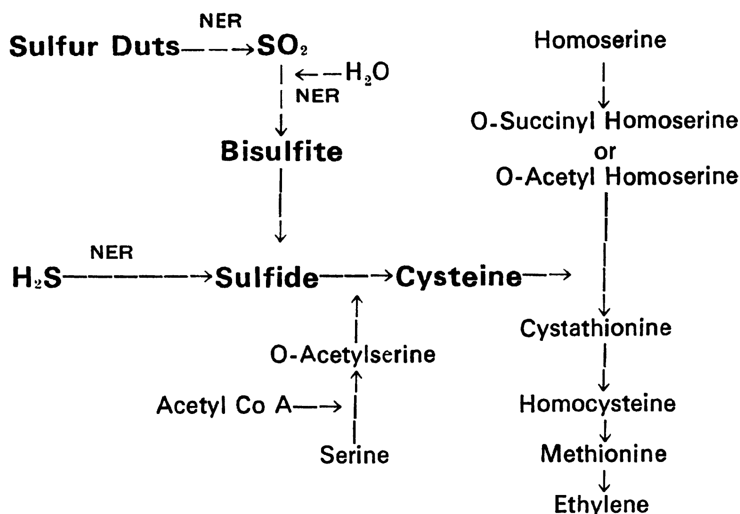

Fig. 13.1 Possible metabolic route of the iuduced synthesis of ethylene during exposure to sulfur containing air pollutants. NER=Non Enzymatic Reactions.

Ethylene affects the metabolism of other hormones as well. It inhibits the transport of IAA in petiole and stem tissues of several plants (Morgan and Gaussman, 1966; Morgan *et al.*, 1968). Exposure to ethylene lowers endogenous IAA level in some species (Ernest and Valdovinus, 1971, Lieberman and Knegt, 1977). This decrease may be due to decreased

synthesis of auxin (Ernest and Valdovinus, 1971), increased decorboxylation (Beyer and Morgan, 1969; Minato and Okazawn, 1978) or by increased conjugation of IAA with the amides (Riov *et al.*, 1982). Increase in IAA oxidase (involved in the oxidative decorboxylation of IAA) by ethylene has been reported by Hall and Morgan (1964) and Morgan *et al.* (1968).

Amelioration from Air Pollution Injury by Plant Hormones

Several endogenous and environmental factors are known to influence the phytotoxic effect of air pollutants on plants. Some of these factors reduce or completely elliminate the potential injury to plants. Possible use of fungicides, antioxidants and growth regulators for protecting horticultural crops from air pollutants has been reviewed by Ormrod and Adedipe (1974). Protection of injury by plant hormones has also been reported in several cases. Although the possible mechanism of protection by hormoes has not been investigated in most cases, the alternate possibilities may be as follows: (1) Inhibition of the uptake of air pollutant (2) Protection of cellular and sub-cellular organisation (3) Stimulation of general metabolism. These possibilities are explained with examples as follows:

It is generally believed that stomata must be open for air pollutant injury to occur ; since they are major portals of air pollutant entry. Therefore, factors affecting the stomatal opening may regulate injury by the air pollutant. Reduction of ozone phytotoxicity by abscisic acid (ABA) has been reported in bean leaves (Fletcher *et al.*, 1972). Since abscisic acid is known to induce stomatal closure (Addicott and Lyon 1969, Walton 1980), this reduction in phytotoxicity is believed to be due to decreased uptake of ozone by the leaves. Sensitivity of radish plants to SO_2 also seems to involve ABA levels controlling the stomatal movements. Kondo and Srigahara (1978) observed that the leaves of sensitive radish cultivar sprayed with 10^{-}[illegible] M ABA became resistant to SO_2 and showed only a little chlorosis even when they were exposed to 2.0 ppm SO_2 for one day in light. Regulation of the effect of hydrogen sulfide (H_2S) on plants by abscisic acid has been shown in maize also (Kumar and Srivastava, unpublished). When maize seedlings are enclosed in a chamber containing 50 ppm H_2S for 2h, the activity of nitrate reductase increases considerably (over no H_2S). However, if the leaves are painted with 1 ppm ABA prior to H_2S exposure, the increase in enzyme activity is reduced. Almost similar effect is observed when the plants are pretreated in dark.

The protecting effects of cytokinins appears to be due to their protecting effects on cellular compartments. Exogenous application of kinetin and benzyladenine reduces ozone injury to some extent in leaves and isolated chloroplasts from bean (Runeckles and Resh, 1975; Tomlinson and Rich, 1973), although neither treatment is able to provide complete proctection. It has been suggested that the reduction in ozone injury is due to maintenance of integrity of chlorolastic membranes by cytokinins. Apparently, this suggestion is based on two types of facts: (i) Ozone causes damage to membranes by oxidising the lipid component of the membrane (Rich, 1964; Pauls and Thompson, 1981) and (ii) cytokinins alter lipid bilayer portion of the membranes (Stillwell and Hester, 1983) and protect the organisation of chloroplastic membranes. Consistent with this suggestion, chemicals such as 2-4-dichloro-6-phenyl phenoxyethyl-diethylamine (DPDA) and 2-diethylaminoethyl-2, 2-diphenylvalerate (SKF 525-A), which are known inhibitors of mixed function oxidase enzyme involved in the oxidation of lipid component of the membrane), are known to protect tobacco leaves from ozone injury (Koiwai and Kisaki, 1973). Alternatively, toxic effects of ozone on membrane are believed to be mediated via free radicals, formed due to reaction of ozone in water (Pauls and Thompson, 1982). Cytokinins are able to scavange these free radicals and therefore protect membrane integrity against ozone. By way of mechanism, it has been suggested that alpha carbon of the amine bond in cytokinin donates a hydrogen thereby providing a partner for the unpaired electron in free radicals. Sodium benzoate, a compound which is often used as a hydroxyl radical scavanger also decreases the severity of ozone damage to chloroplastic membranes (Pauls and Thompson, 1982). This possibility neeeds to be tested with other pollutants as well.

In many cases, the protecting effects of hormones may not be related to a specific biochemical effect of the hormone. A variety of anti-oxidants or related to a specific biochemical effect of the hormone, a variety of anti-oxidants or reducing substances such as ascorbic acid or IAA protect cucumber seedlings against acute ozone damage (Siegel, 1962). The inhibition of root and leaf growth in radish is overcome by the application of benzyladenine. The leaf weight of plants treated with gibberellic acid and IAA does not decrease due to ozone exposure (Adedipe and Ormrod, 1962). The authors in these cases have suggested that protection from ozone induced growth reduction by hormones was due to long term hormone mediated metabolic response of the plants. These studies

where a direct correlation between hormone induced growth and metabolism and reduction in air pollution injury could be demonstrated, should be extended to other plants and other air pollutants as well. Whatever the mechanism may be, use of hormones and related compounds to protect plants against air pollutants show some promise at least in localised incidences of air pollution. However, an overall assessment could be made only when the cost of such an application and appropriate methodology has been worked out.

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Hormonal Regulation of Plant Growth and Development : Problems and Perspectives

Some of the important problems and prespectives of hormonal regulation of plant growth and development are presented for further research.

Molecular Basis of Hormone Action

Hormonal control of physiological processes can be understood only when we are in a position to describe each fact of hormone action in molecular terms. Plant hormones differ from animal hormones in several respects, the most important of which is that they are relatively non-specific and several target organs may be effected by one plant hormone; plant systems also differ from the animal systems in that they have the cell wall in addition to the cell membrane. The rigidity of the cell wall which restricts the expansional needs of the elongating and the dividing cell, has to be reduced. Consequently the primary action of hormones has to be concerned in some way with the loosening of the cell wall. The acid growth theory involving a "proton pump" explains to a certain extent the process and the mechanism of cell wall loosening as an early event being a consequence of the interaction of auxin and probably gibberellin also, with some primary site(s) in the cell, but it is not adequate enough to meet all the requirements and does not answer satisfactorily several questioned raised. The primary site of auxin or gibberellin action also has to be located unequivocally, this is also true for other hormones.

The large variety of responses which the hormones evoke clearly indicate a multiplicity of the sites of action of each hormone. Parameters other than extension growth have received very little attention and there are also only one or two organs- the internode and coleoptile sections- - which have been the test systems; leaf, fruit and other rapidly growing tissues have received much less attention. Our knowledge regarding molecular mechanism of hormone effects on ion uptake, mobilization of organic metabolites, protoplasmic streaming, photosynthesis and various other metabolic processes, differentiation of tissue components and

organs and the control of senescence is fragmentary. Some of these, for example, hormone effects on protoplasmic streaming (even though it is not directly related to growth) are fantastically rapid, no less spectacular than auxin effect on growth of coleoptile or internode cells, while others e. g., differentiation may be slow and time consuming but they are all equally important and the nature of the primary reactions in these processes may well be different. For substance of growth as also of many of these processes continued nucleic acid and protein synthesis may be essential and for some of them interaction with the genome may well be the primary event.

It is doubtful whether any physiological effect is concerned exclusively with one hormone, and there is considerable evidence to suggest that the net effect is due to more than one regulatory substance. The biogensis, transport and degradation of several hormones appear to be interrelated and the need of a sequential activation of reactive sites (whatever be their nature) is being recognized. Variation in the competence and availability of the sites to initiate or sustain the reactions must also be taken into consideration. The polyamines, triterpenoids, phytosterols and steroids and various cellular metabolites have been found to modify hormone action in several cases and the influence of these substances on the mode of action of the hormones in the cellular miliev have also to be explained at the molecular level. If receptor proteins are essential for hormone action, we have to visualize the existence of a large numbet of such proteins and hormone receptor complexes have to compete with the free hormones as also other cellular metabolites and ions, which are known to interact with chromation or other sites. Very little information is available regarding the concentration of the regulatory substances in the microenvironment of the genome the cell membranous structures or the space between the cell wall and the cell membrane. Methods have to be devised for estimation of nano- or picograin quantities of such subtances within the cell.

We thus, have a long way to go, but the progress in this field is very rapid, new subtle analytical methods are being developed and it seems plant endocrinology will soon receive the recognization it deserves.

Hormonal Regulation of Flowering

Many factors such as physical, chemical, genetic and physiological, affect development process of plants, probably mediated by plant hormonese. Therefor, it is quite conceivable that several plant hormones and the interactions among them play very important roles in a series

of morphogenetic events which occur during floral initiation and development. A great number of experimental results showed various flower-inducing and inhibiting actions of phytohormones in different plant species, but sometimes the data seem to be inconsistent or even contradictory, making interpretation of results rather difficult. Also, current state of the studies in this field indicate that none of the known plant hormones is really the flower-inducing substance in a strict sense.

Among many unsolved questions concerning the physiology of flowering, the followings must be treated and answered with priority: (i) Characterization of flower inducing and inhibiting principles which seem exist at least in some of photoperiodic and cold requiring plants (ii) The localization and type of cells which are responsible for the perception of photoperiodism. (iii) Roles play by roots in relation to flower differentiation. (iv) Time measuring mechanism (s) of plants.

Hormonal Control of Enzyme Secretion

Numerous enzymes can be detected as exocellular in the culture media of the plants, organs, tissues or cells from which they derived but in most cases there are no indications for a discrimination between a passive leakage and a controlled active secretory process. The few available examples of well studied secretory processes, let appear that the hormonal control of secretion of a precise enzyme is generally not an isolated mechanism but part of a series of integrated phenomenons. For instance, the hormones (auxin, ethylene) which control abscission, - where among other enzymes cellulase is known to be secreted, - may play three roles at least in this process : a phytogerontological effect which is responsible for aging of plant tissue, an enhancement of cellulase biosynthesis and finally a promotion of enzyme secretion. There is no other indication whether a hormone-controlled enzyme synthesis or activation is a prerquisite for an active secretion. More advanced studies of the relationship between the endogenous hormones and potential second hormonal messengers such as c-AMP, Ca^{2+}- calmodulin, polyamines involved in secretory processes will be necessary. The change observed as consequence of an hormonal treatment may result either from a "hormonal stress" or from a secondary effect following the external addition of a growth regulator. This addition can also produce quite different results, depending upon the physiological state of tissues and the environmental conditions. This is to say that the problem of the hormonal control of enzyme secretion has just began to be opened in plants.

Hormonol Control of Gravitropic Curvature in Gravistimulated Grass Pulvini

The chief problems that remain unresolved in connection with negative gravitropic curvature in gravistimulated grass pulvini include following: (1) How is the transduction signal perceived by starch statoliths ? (2) In what way do starch statoliths act as agents for "information transfer" during the perception phase of the gravitropic responses ? (3) What is the nature of the events that occur during and after starch statoliths reach the bottoms of the statocytes up to the time that the transduction process is initiated ? (4) What are the kinetics for the establishment of asymmetry in levels of free IAA and its conjugates, and how is this asymmetry established (differential release from conjugates, or differential, or both) ? (5) What are the kinetics for the establishment of asymmetry in levels of free GAs and their conjugates, and how is this asymmetry established (differential release from conjugates, or differential synthesis, or both) ? (6) What is the physiological biochemical basis for the differential cell elongation process that underlies the asymmetric growth in gravistimulated pulvini ? (a) Are wall-loosening enzymes activated or synthesized ? (b) Are wall-synthesis enzymes activated or synthesized ? (3) What new m-RNA's are synthesized, and if so, what new proteins are made during gravitropic curvature ? (d) Since m-RNA, protein, and cellulose synthesis are necessary for gravitropic curvature to occur, do hormones, such as GAs and/or IAA regulate these processes, and if so during transcription or translation ?

We are currently on the verge of working out some of the roles that starch statoliths play in the gravity perception, the kinetics for establishment of free GA/GA- conjugate and free IAA/IAA- conjugate asymmetry during transduction, and the nature of the cell wall-loosening/synthesis processes that occur during gravitropic in curvature in cereal grass pulvini. In addition, the cereal grass pulvinus system will soon be employed to study how we can regulate shoot growth of cereal grasses in space under conditions of near null gravity. Thus, we can predict that in the next decade we shall know great deal more about the basic mech–anism underlying the gravitropic curvature eesponse in cereal grass shoot and how it can be regulated both on earth for agricultural applications and in space for the production of the atmosphere in space vehicles with oxygen and water, and recycling of wastes. We are confident that botanists, physiologists, horticulturists, agronomists, space biologists, and other interested researchers will find this volume as source book for further research.