UV-B Radiation: From Environmental Stressor to Regulator of Plant Growth

UV-B Radiation: From Environmental Stressor to Regulator of Plant Growth

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WILEY Blackwell

This edition first published 2017 © 2017 by John Wiley & Sons Ltd

Registered Office
John Wiley & Sons, Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK

Editorial Offices
9600 Garsington Road, Oxford, OX4 2DQ, UK
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111 River Street, Hoboken, NJ 07030-5774, USA

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Library of Congress Cataloging-in-Publication data has been applied for.

ISBN: 9781119143604

A catalogue record for this book is available from the British Library.

Wiley also publishes its books in a variety of electronic formats. Some content that appears in print may not be available in electronic books.

Cover design: Wiley
Cover image: Courtesy of Vijay Pratap Singh, previously published in Journal of Photochemistry & Photobiology, B: Biology 153 (2015) 334–343 © Elsevier.

Set in 10/12pt Warnock by SPi Global, Pondicherry, India

10 9 8 7 6 5 4 3 2 1

Raja Ramanuj Pratap Singhdev (1901–1954)
Founder of Education System in Korea State, India

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Preface

In the course of acquiring knowledge about UV-B research in plant systems from the past up to the present day, we have found a considerable gap between the availability of books and emerging areas of research. This book has been written to bridge the gap between researches being conducted from the past up to today, and the direction these researches might take in the future with respect to UV-B.

The title itself indicates that this book has mapped UV-B research from past up to recent times. It is a book of theoretical knowledge, and the compilation has been done on the basis of practical work done by the researchers and scientists. We have briefed out the historical backgrounds of UV-B namely, how it reaches the earth's surface, its action spectra and its interaction with living systems, using the research work conducted by researchers in the past, to recent studies that show how research in UV-B has taken a U-turn with the discovery of UVR8.

A good book is one that includes knowledge for all readers, including students, and of course we are indebted to the many authors who have contributed to it. This book includes chapters which cover several aspects of UV-B, starting from the basics of UV-B research and going on to the present date, and a brief outline has been provided below.

The first chapter gives an overview of the ozone layer and the reasons for its depletion and UV-B reaching the earth's surface, and it also offers a brief introduction to action spectra and biologically effective irradiance. In later sections, the authors also discuss the impact of UV-B on plants by analysing the researches performed in the past.

The second chapter gives a brief historical background for the effect of ambient UV-B on plants, with special reference to accumulation of secondary metabolites, such as phenolic compounds, alkaloids and terpenoids. The authors have also discussed recent studies regarding phenolics under ecologically relevant UV-B radiation, and changes in the content of secondary metabolites, with reference to species variation, changes in the UV-B : UV-A : PAR ratio, UV-B doses and UV-B spectral quality.

In the next few chapters, authors discuss risk arising due to the interaction of UV-B with the components of plants, and biological effects arising due to absorption of UV radiation, whether from UV-A or UV-B, by important biomolecules like nucleic acids, lipids and proteins. They also examine the impact on the phytochrome system and photosynthetic machinery. In addition, the authors also discuss the effects of UV-B radiation in terms of oxidative stress, and the responses generated by plants to combat from the stress arising due to UV-B induced toxicity, which includes accumulation of sun-screen molecules. These chapters basically focus on the past researches that have been performed with UV-B. With technology and research advancement,

the introduction of photomorphogenic responses came into existence, which compelled researchers to gain a deeper insight into this phenomenon, and this curiosity for innovation led to the discovery of UVR8.

In later chapters, authors have very well documented the history of photomorphogenic responses and how UVR8 was discovered – and all the regulators, whether positive or negative, involved with this component. In the last chapter, the authors discuss the mechanism of regulatory action by UVR8 and its integration with other pathways.

In concluding, it is a pleasure to express our thanks to all the authors for contributing chapters that have helped us in giving a clear picture of the changing scenario of research in UV-B. We hope that this book will be of special value to environmentalists, researchers and students seeking knowledge on UV-B, which has not yet been assimilated in textbooks.

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1

An Introduction to UV-B Research in Plant Science

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1.1 The Historical Background

About 3.8×10^9 years ago, during the early evolutionary phase, the young earth was receiving a very high amount of UV radiation and it is estimated that, at that time, the sun was behaving like young T-Tauristars and was emitting 10,000 times greater UV than today (Canuto *et al.*, 1982). Then, the radiance of the sun became lower than it is in the present day, thereby resulting in temperatures below freezing. On the other hand, due to high atmospheric carbon dioxide (CO_2) level, which was 100–1000 times greater than that of present values, liquid water did occur and absorbed infrared (IR) radiation, and this shaped an obvious greenhouse effect (Canuto *et al.*, 1982). Due to the photosynthesis of photosynthetic bacteria, cyanobacteria and eukaryotic algae, oxygen (O_2) was released for the first time into the environment, which led to an increase of atmospheric O_2 and a simultaneous decrease of atmospheric CO_2.

About 2.7×10^9 years ago, due to the absence of oxygenic photosynthesis, oxygen was absent from the atmosphere. About 2.7×10^9 years ago, with the deposition of iron oxide (Fe_2O_3) in Red Beds, aerobic terrestrial weathering occurred and, at that time, O_2 was approximately about 0.001% of the present level (Rozema *et al.*, 1997). In proportion with gradual atmospheric O_2 increase, the accumulation of stratospheric ozone might have been slow. Alternatively, about 3.5×10^8 years ago, due to a sheer rise in atmospheric oxygen, it might have reached close to the present levels of 21% (Kubitzki, 1987; Stafford, 1991). Nevertheless, terrestrial plant life was made possible by the development of the stratospheric ozone (O_3) layer, which absorbs solar UV-C completely and a part of UV-B radiation, thereby reducing the damaging solar UV flux on the earth's surface (Caldwell, 1997).

Before focusing on the various aspects of UV-B radiation, we should firstly understand the electromagnetic spectrum. The electromagnetic spectrum consists of ultraviolet

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Table 1.1 Regions of the electromagnetic spectrum together with colours, modified from Iqbal (1983) and Eichler *et al*. (1993).

Wavelength (nm)	Frequency (THz)	Colour
50 000–10^6	6–0.3	far IR
3000–50 000	100–6	mid IR
770–3000	390–100	near IR
622–770	482–390	red
597–622	502–482	Orange
577–597	520–502	yellow
492–577	610–520	Green
455–492	660–610	blue
390–455	770–660	violet
315–400	950–750	UV-A
280–315	1070–950	UV-B
100–280	3000–1070	UV-C

(UV) and visible (VIS) radiations (i.e. also PAR). The wavelength ranges of UV and visible radiation are listed in Table 1.1. Solar radiations, with a longer wavelength, are called infrared (IR) radiations. The spectral range between 200 and 400 nm, which borders on the visible range, is called UV radiation, and is divided into three categories: UV-C (100–280 nm), UV-B (280–315 nm) and UV-A (315–400 nm). The shorter wavelengths of UV get filtered out by stratospheric O_3, and less than 7% of the sun's radiation range between 280 and 400 nm (UV-A and UV-B) reaches the Earth's surface.

The level of UV-B radiation over temperate regions is lower than it is in tropical latitudes, due to higher atmospheric UV-B absorption, primarily caused by changes in solar angle and the thickness of the ozone layer. Therefore, the intensity of UV-B radiation is relatively low in the polar regions and high in the tropical areas. Over 35 years ago, it was warned that man-made compounds (e.g. CFCs, HCFCs, halons, carbon tetrachloride, etc.) cause the breakdown of large amounts of O_3 in the stratosphere (Velders *et al.*, 2007) thereby increasing the level of UV-B reaching the Earth's surface. Increase in the UV-B radiation has been estimated since the 1980s (UNEP, 2002), and projections like the Kyoto protocol estimate that, even after the implementation of these protocols, returning to pre-1980 levels will be possible by 2050–2075 (UNEP, 2002).

1.2 Biologically Effective Irradiance

The term 'biologically effective irradiance' means the effectiveness of different wavelengths in obtaining a number of photobiological outcomes when biological species are irradiated with ultraviolet radiations (UVR). The UV-B, UV-A and photosynthetically active radiations (PAR; 400–700 nm) have a significant biological impact on organisms (Vincent and Roy, 1993; Ivanov *et al.*, 2000). Ultraviolet irradiation results into a

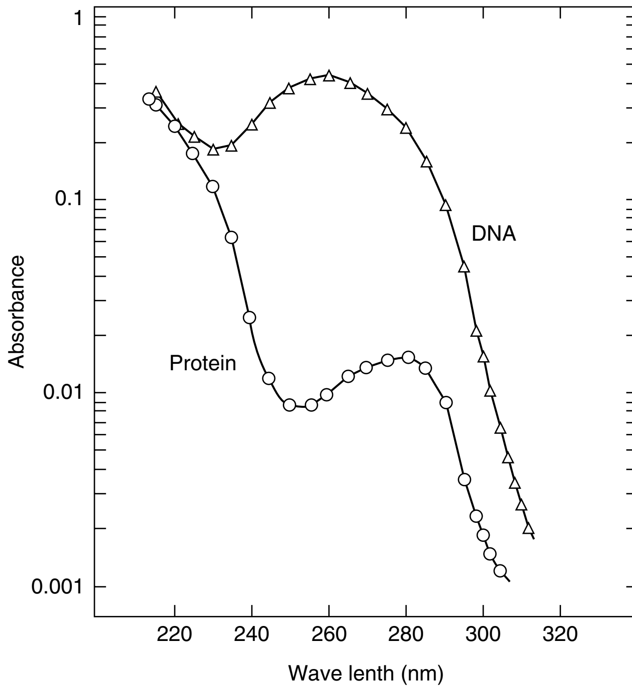

Figure 1.1 Absorption spectra of protein and DNA at equal concentrations (adapted from Harm, 1980).

number of biological effects that are initiated by photochemical absorption by biologically significant molecules. Among these molecules, the most important are nucleic acids, which absorb the majority of ultraviolet photons, and also proteins, which do so to a much lesser extent (Harm, 1980).

Nucleic acids (a necessary part of DNA) are nucleotide bases that have absorbing centres (i.e. chromophores). In DNA, the absorption spectra of purine (adenine and guanine) and pyrimidine derivatives (thymine and cytosine), are slightly different, but an absorption maximum between 260–265 nm, with a fast reduction in the absorption at longer wavelengths, is common (Figure 1.1). In contrast with nucleic acids solutions of equal concentration, the absorbance of proteins is lower. Proteins with absorption maxima of about 280 nm most strongly absorb in the UV-B and UV-C regions (Figure 1.1). The other biologically significant molecules that absorb UVR are caratenoids, porphyrins, quinones and steroids.

1.3 UV-B-induced Effects in Plants

In the past few decades, a lot of studies have been made on the role of UV-B radiation. Due to the fact that sunlight necessity for their survival, plants are inevitably exposed to solar UV-B radiation reaching the earth's surface. From the point of view of ozone depletion, this UV-B radiation should be considered as an environmental stressor for photosynthetic organisms (Caldwell *et al.*, 2007). However, according to the evolutionary point of view, this assumption is questionable.

Although UV-B radiation comprises only a small part of the electromagnetic spectrum, the UV-B reaching on earth's surface is capable of producing several responses at molecular, cellular and whole-organism level in plants (Jenkins, 2009). UV-B radiation is readily absorbed by nucleic acids, lipids and proteins, thereby leading to their photo-oxidation and resulting in promotional changes on multiple biological processes, either by regulating or damaging (Tian and Yu, 2009). In spite of the multiplicity of UV-B targets in plants, it appears that the main action target of UV-B is photosynthetic apparatus, leading to the impairment of the photosynthetic function (Lidon *et al.*, 2012). If we talk about the negative impact of UV-B, it inhibits chlorophyll biosynthesis, inactivates light harvesting complex II (LHCII), photosystem II (PSII) reaction centres functioning, as well as electron flux (Lidon *et al.*, 2012).

The photosynthetic pathway responding to UV-B may depend on various factors, including UV-B dosage, growth stage and conditions, and flow rate, and also the interaction with other environmental stresses (e.g., cold, high light, drought, temperature, heavy metals, etc.) (Jenkins, 2009). The thylakoid membrane and oxygen evolving complex (OEC) are highly sensitive to UV-B (Lidon *et al.*, 2012). Since the Mn cluster of OEC is the most labile element of the electron transport chain, UV-B absorption by the redox components or protein matrix may lead to conformational changes, as well as inactivation of the Mn cluster. The D1 and D2 are the main proteins of PSII reaction centres and the degradation and synthesis of D1 protein is in equilibrium under normal condition in light, however, its degradation rate becomes faster under UV-B exposure thereby, equilibrium gets disturbed (Savitch *et al.*, 2001; Lidon *et al.*, 2012). In the OEC coupled to PSII, during light-driven photosynthetic electron transport, tri-molecular oxygen is produced continuously, which can be converted in the sequential reduction to superoxide radical ($O^{2\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\bullet}OH$) (Apel and Hirt, 2004). Furthermore, PSI and cytochrome b6/f complex are less affected by UV-B radiation in comparison to PSII (Lidon *et al.*, 2012).

Stomatal movement is an important regulatory process that limits the rate of photosynthesis. In *Vicia faba*, high UV-B radiation stimulates either stomatal opening or closing, depending on the metabolic rate (Jansen and van-den-Noort, 2000). However, the stimulated reduction of stomatal conductance can be responsible for CO_2 limitation, as reported in many plants (Zhao *et al.*, 2003; Lidon and Ramalho, 2011), but the reduction in the stomatal conductance has a lesser extent than that of net photosynthetic rate. Additionally, UV-B radiation strongly affects the activity as well as content of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) in plants (Correia *et al.*, 1998; Savitch *et al.*, 2001). Besides this, the intermediate stage of the Calvin cycle (i.e. sedoheptulose 1,7-bisphosphatase), as well as the regeneration of RuBP, was found to be decreased upon exposure to UV-B radiation (Allen *et al.*, 1998).

UV-B radiation has long been perceived as a stressor. Many studies have shown that it impedes photosynthetic activities, damages DNA, proteins and membranes, and impedes plant growth. Oxidative stress has been flagged as a pioneer factor in such UV-B stress responses (Lidon *et al.*, 2012). However, DNA damage, membrane degradation products, and ROS also play a role in mediating UV-B protection, and have done so since the origin of the first plants. Cyanobacteria first evolved on the earth at a time when UV-B levels were at their highest and no ozone layer existed. Under such high UV-B radiation during the early evolution of photosynthetic organisms, they might have coevolved their genetic machinery along with the ambient UV-B level, which might have also helped the

transition to terrestrial life (Rozema *et al.*, 1997). Therefore, it can be assumed that plants' metabolic machinery must have all the compulsory elements for normal coexistence with present UV-B levels, so the solar UV-B radiation reaching the earth should not be considered to be an environmental stressor. Actually, the current ambient UV-B radiation level should be considered as a signal factor which is capable of inducing the expression of genes related to the normal growth and development of plants (Jenkins, 2009).

A conceptual U-turn has been taken place, and UV-B is rarely considered as a damaging factor. There is overpowering evidence that UV-B is an environmental regulator that controls gene expression, cellular and metabolic activities, and also the growth and development (Jenkins, 2009). Under low UV-B fluence rate, the regulatory role of UV-B can be observed, and these effects are mediated by the UV-B-specific UV Resistance Locus 8 (UVR8) photoreceptor, which has opened the door to elucidate the UV-B signalling pathways in plants (Christie *et al.*, 2012; Wu *et al.*, 2012; Singh et al., 2012; Srivastava *et al.*, 2014).

The UVR8 photoreceptor exists as a homodimer that undergoes immediate monomerization following UV-B exposure, and the process is dependent on an intrinsic tryptophan residue (Rizzini *et al.*, 2011). Upon exposure to UV-B, UVR8 accumulates rapidly, and interacts with Constitutively Photomorphogenic 1 (COP1) to initiate the molecular signalling pathway that leads to gene expression changes. UVR8 monomer is redimerized by the action of RUP1 and RUP2, which interrupts the UVR8-COP1 interaction, thereby inactivating the signalling pathway and regenerating the UVR8 homodimer again, ready for UV-B perception. This signalling leads to UVR8 dependent responses, such as UV-B-induced photomorphogenic responses, and also the accumulation of UV-B-absorbing flavonols (Tilbrook *et al.*, 2013). Elongated Hypocotyl 5 (HY5) acts as a downstream effector, and is regulated by the negative feedback pathway.

Favory *et al.* (2009) hypothesized that during UVR8 interaction with COP1, COP1 might have been taken out from phytochrome (red light receptor) and cryptochrome (blue/UV-A light receptor) under UV-B exposure, and this fact was supported by the phenotype of the *COP1* overexpressing line of UVR8. Conversely, Oravecz *et al.* (2006) and Favory *et al.* (2009) have noted that COP1 was excluded by the nucleus upon exposure to visible light, while UV-B exposure results in nuclear accumulation and stabilization of COP1. In addition, being a repressor of photomorphogenesis, COP1 is dependent on SPA protein, which is not a part of the regulatory action by COP1 (Laubinger *et al.*, 2004; Oravecz *et al.*, 2006). Interestingly, SPA and Repressor of Photomorphogenesis (RUP) genes show similarity in their phylogeny while interacting with COP1 (Gruber *et al.*, 2010; Fittinghoff *et al.*, 2006). All these similarities suggest towards the evolution of complex photoreceptor UVR8 from the other photoreceptors, and the role of UVR8 as a signalling molecule.

1.4 Conclusion and Future Perspectives

Over recent years, significant progress has been made in identifying the molecular players, their early mechanisms and signalling pathway in UV-B perception in plants, but there is more we have to do. Several questions remain to be uncovered, regarding the photochemistry, signal transduction and regulatory mechanisms of UVR8, that need to be addressed and, of course, this will open a new horizon in the field of UV-B perception and signalling. Questions that remain to be traced out include: the primary

responses of UVR8 after UV-B perception; whether functioning at the chromatin level exists; sites of UVR8 functioning in the cell; crosstalk of UVR8 pathway with COP1 and visible light photoreceptors along with their signalling; whether UVR8 has evolved from other photoreceptors as a need of environmental changes and is now towards the degrading or evolutionary phase.

Now the stage is set to tackle these questions. No doubt, the answers will pave a new direction and a deep understanding of plant UV-B responses. Of course, the future of UV-B signalling will be more realistic after the preparation of a detailed molecular map of various signalling molecules regarding UV-B.

Acknowledgements

The University Grants Commission, New Delhi is greatly acknowledged for financial support.

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2

Stimulation of Various Phenolics in Plants Under Ambient UV-B Radiation

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2.1 Introduction

Under natural conditions, plants are constantly exposed to dynamic changes of solar radiation, which mainly consists of infrared (IR, >700 nm), photosynthetically active radiation (PAR, 400–700 nm) and minor portion of ultraviolet (UV) radiation (UV-B, 290–315 nm and UV-A, 315–400 nm). Besides being the primary source of energy in photosynthesis, sunlight is an important signal which regulates plant growth and development. In addition to light quantity, plants are able to monitor the quality, periodicity and direction of light (reviewed in Caldwell *et al.*, 2007; Jiao *et al.*, 2007). Plants perceive light signals through several protein photoreceptors: five phytochromes (PHY A-E), which are sensitive to red and far red light (600–750 nm), and two cryptochromes (CRY1 and CRY2), two phototropins (PHOT1 and PHOT2) and zeitlupe proteins (ZTLs) for blue and UV-A radiation (315–500 nm), while UV-B radiation is sensed by UV Resistant Locus 8 (UVR8) (reviewed in Jiao *et al.*, 2007; Heijde and Ulm, 2012).

During the period from the 1970s to 1990s, investigations on UV-B effects on organisms were in the centre of attention, due to alarming depletion of stratospheric ozone layer and increased UV-B radiation reaching the Earth's surface. However, the results of numerous studies that explored the impact of high UV-B radiation on plants were often contradictory. In following years, this was explained by different unrealistic UV-B : UV-A : PAR ratios, high UV-B doses applied, different spectral distribution in the UV-B region, as well as simultaneous effects of other environmental stressors (drought, high temperature, nutrient deprivation), and previous plant exposure to UV-B radiation (plant history). Inconsistent reports on UV-B effects on photosynthesis and stomata conductance were a result of different UV-B doses applied, species-specific, and even genotype-specific responses, but also plant history and overall plant metabolism.

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In the light of these findings, during the last decade, research on UV-B radiation effects on biological systems has advanced towards more controlled conditions aiming to imitate ambient solar radiation. Using sun simulators with realistic balance of UV-B, UV-A and PAR, is a very good solution to achieve realistic and reproducible experimental conditions (Döhring *et al.*, 1996; Aphalo *et al.*, 2012). Contrary to previous widely accepted beliefs, in the last several years it has been demonstrated that UV-B radiation, at low and ecologically relevant doses, presents an important regulator of plant growth and development (Jenkins, 2009; Hideg *et al.*, 2013). Plants grown in the open field, exposed to natural UV-B doses, have higher nutritional and pharmacological value than plants grown in polytunnels and glasshouses, which are non-transparent to UV radiation (Jansen *et al.*, 2008; Behn *et al.*, 2010). Moreover, it has been shown that UV-B radiation improves plant adaptive capacity to drought, high temperatures, pathogen and insect attack, and nutrient deficiency conditions (Schmidt *et al.*, 2000; Caputo *et al.*, 2006). These findings have a strong impact on the agricultural, pharmaceutical and food industries.

A hallmark of UV-B response in plants is accumulation of secondary metabolites, such as phenolic compounds (particularly flavonoids and phenylpropanoids), alkaloids and terpenoids. Phenolics are the most abundant secondary metabolites in plants, and 20% of carbon fixed in photosynthesis is directed to their biosynthesis (Hernández and Van Breusegem, 2010). Phenolic compounds in plants are involved in many processes, from growth and development, to flowering, reproduction and seed dispersion, defence against pathogens, plant–insect interactions and protection against numerous abiotic stresses (Gould and Lister, 2005; Sedlarević *et al.*, 2016). The most well-studied mechanism of UV-B induction of phenolic metabolism is certainly the UVR8 pathway, which will be discussed in detail in this chapter. However, regarding UV-B and sunlight exposure in general, antioxidative *vs.* UV-B-absorbing (screening) functions of phenolics remain debatable (Agati *et al.*, 2013). Genes encoding UVR8-like proteins are highly conserved, and have been identified in a large number of plants, algae and mosses, suggesting the importance of this pathway for the adaptation of autotrophic organisms to sunlight (Tilbrook *et al.*, 2013).

In this chapter, we have provided overview of publications reporting phenolics induction by supplementary UV-B radiation in the last decade. Plant response depends on UV-B fluence rate and spectrum. Therefore, it is important to standardize UV-B exposure experimental designs to adequately compare the responses of phenolic metabolism obtained in different studies. In order to interpret morphological and physiological changes in plants, phenolics function and distribution on the cellular, tissue and plant level should be understood. Moreover, recent findings on relationship between photosynthesis and storage molecules, such as starch, and stimulated flavonoid biosynthesis under UV-B radiation are considered.

2.2 UV-B Radiation

Ultraviolet radiation (UVR) covers solar radiation wavelength range between 200 and 400 nm. It is classified in three spectral regions: UV-A (315–400 nm), UV-B (280–315 nm), and UV-C (200–280 nm). Atmospheric oxygen and ozone completely absorb UV-C, as well as the largest amount of UV-B radiation <290 nm and only about 3% of UV-A (Seckmeyer *et al.*, 2008). Therefore, UVR contributes only about 6% (UV-A) and less

than 0.5% (UV-B) of total solar radiation on the Earth's surface (Frohnmeyer and Staiger, 2003; Favory *et al.*, 2009). In spite of these small percentages, UV-B radiation is highly energetic and is biologically active towards macromolecules (e.g. DNA, RNA, proteins), and it can initiate photochemical reactions and reactive oxygen species (ROS: hydroxyl radical, singlet oxygen, superoxide radical and hydrogen peroxide) generation, even at low fluence rates (Hideg and Vass, 1996; Jansen *et al.*, 1998; Hideg *et al.*, 2002; Brosché and Strid, 2003; Hideg *et al.*, 2013).

Seasonal and diurnal dynamics in UVR are influenced by weather conditions (cloud cover), solar zenith angle and amount of aerosols and pollutants dispersed in the atmosphere (Jenkins, 2009; Aphalo *et al.*, 2012). UV-B increases with elevation or decreasing latitude, and reaches the highest levels on high mountains in equatorial regions. Since the 1980s, the stratospheric ozone layer has decreased by 3–6%, thus allowing up to 14% increase of UV-B radiation reaching the Earth's surface (Herman, 2010; Kataria *et al*,. 2014). Such reductions in ozone layer are observed annually every spring in the Southern Hemisphere. In the Northern Hemisphere, changes in ozone amounts are less pronounced and are also less predictable, but UV-B levels have still increased significantly in the last 30 years (Herman, 2010).

As a consequence of ozone depletion, UV levels have increased in high and middle altitudes (Seckmeyer *et al.*, 2008). The figure below (Figure 2.1) shows the UV index, the effective UV irradiance (one unit is 25 mW m^{-2}) reaching the Earth's surface, based on erythema action spectrum. This spectrum is based on the susceptibility of Caucasian skin to sunburn (erythema), and is valid for clear sky at local noon. The highest UV index is in lower latitudes, especially at high mountains. Increased UV index can be seen in the higher latitudes of the Northern Hemisphere in Greenland.

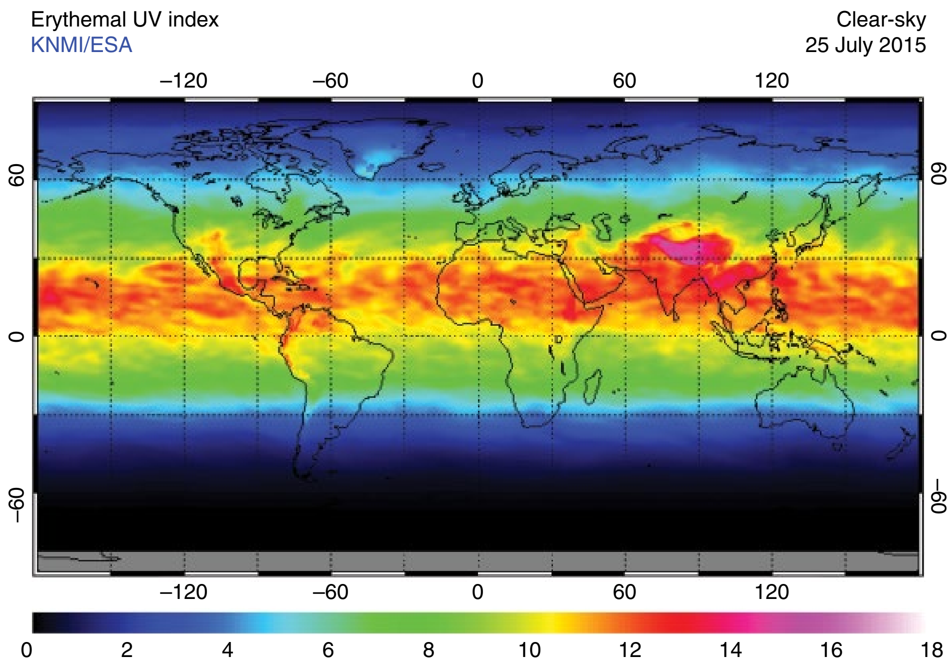

Figure 2.1 Global erythemal UV index in 2015 (http://www.temis.nl/uvradiation/world_uvi.html).

It is important to note that seasonal variations in UV-B irradiance are higher than PAR variations, resulting in variations in the UV-B : PAR ratio. These variations are sensed by plants, and may influence the intensity of plant responses to light changing environment (Grant, 1997). Moreover, it is considered that temporal and spatial changes in UVR in the past have influenced the diversity and speciation of plants (Willis *et al.*, 2009).

There are two basic approaches to investigate UV-B effects on plants: exposure to supplementary UV-B radiation and UV-B filtration. UV-B filtration is used to attenuate or exclude all, or part of the solar UV-B radiation, while, at the same time, allowing UV-A and PAR to remain unchanged. Filters such as cellulose diacetate, polythene or polytetrafluoroethylene (PTFE) are UV-B and UV-A transparent, while polyester film (e.g. Mylar, Melinex, Autostat) attenuates UV-B with small effects on UV-A (e.g. Wargent *et al.*, 2009; Comont *et al.*, 2012). Theatrical 'gels' (e.g. Rosco E+, #226, Westilighting, Finland) are suitable for complete attenuation of both UV-B and UV-A (Kotilainen *et al.*, 2009; Aphalo *et al.*, 2012).

When investigating UVR effects on plants in the field, one should consider the influences of both direct and diffuse UVR. Moreover, it is important to consider that plants do not respond to all wavelengths equally. Wavelength spectra which initiate a response in plant photo-receptors are defined as response and action spectra (for more details see Aphalo *et al.*, 2012). The action spectrum is used to show the effectiveness of radiation of different wavelengths (and different fluences) in inducing a given size of response, and is used as biological spectral weighting function (BSWFs). BSWF is needed for calculating biologically effective UV doses (UV-B_{BE}, Caldwell, 1971; Kotilainen *et al.*, 2011).

The most commonly used BSWF for investigating photobiological plant response to UV is Generalized Plant Action spectrum (GEN), where daily biologically effective UV-B dose has been calculated by Green *et al.* (1974), according to the measurements of Caldwell (1971), normalized at 300 nm. This action spectrum is not based on plant growth responses, and predicts no action in the UV-A spectral region (Kotilainen *et al.*, 2009). The second, more recent, is Plant Growth spectrum (PG, proposed by Flint and Caldwell, 2003), which was originally used for monitoring growth responses in oats at 275, 297, 302, 313 and 366 nm, in the absence of any visible radiation. For example, Comont *et al.* (2012) studied the effects of latitudinal variation in ambient UV-B radiation on *Lolium perenne* biomass production. Daily biologically effective UV-B doses of 2.3, 3.2, 4.1, 5.0 and 5.7 kJ m^{-2}, simulating 70, 60, 50, 40 and 30 °N latitudes, respectively, were determined using a UV software radiation model, and UV-B irradiation was weighted with Caldwell generalized plant damage action spectrum (Caldwell *et al.*, 1986). In addition, erythema action spectrum is widely used for quantifying UV-B effects on plants (Webb *et al.*, 2011). In the following text, all biologically effective UV-B doses were calculated using GEN, unless otherwise stated.

2.3 Phenolics

Phenolic compounds are a widespread class of secondary metabolites, with diverse functions in plant growth and development, as well as in plant interactions with the environment (Gould and Lister, 2005; Lattanzio *et al.*, 2006; Michalak *et al.*, 2006;

Agati and Tattini, 2010). In this chapter, we briefly present an overview of phenolics structure, biosynthesis, distribution and their functional significance.

2.3.1 Chemistry of Phenolic Compounds

Phenolic compounds consist of an aromatic ring (C_6) bearing one or more –OH group(s) (polyphenols), including functional derivatives (esters, methyl ethers, glycosides, etc.). Based on their chemical structure, natural phenolic compounds can be classified as: hydroxybenzoic acids (HBA, C_6–C_1), hydroxycinnamic acids (HCA, C_6–C_3), coumarins (C_6–C_3), flavonoids (C_6–C_3–C_6), proanthocyanidins [(C_6–C_3–C_6)$_n$], stilbenes (C_6–C_2–C_6), lignans (C_6–C_3–C_3–C_6) and lignins [(C_6–C_3)$_n$] – see Figure 2.2. Based on the degree of oxidation and saturation present in the C_3 element (C ring), flavonoids are further divided into the following groups: flavones, flavon-3-ols, flavanones, flavanols, chalcones and anthocyanidins (Antolovich *et al.*, 2000; Marais *et al.*, 2006).

Most flavonoids and hydroxycinnamic acids are glycosylated in the plant cells, usually with two or three sugar moieties, thus they might be considered as an important storage of mono- and disaccharides (Winkel, 2006). The sugar moiety may be acylated by hydroxycinnamic acids (caffeic, ferulic, *p*-coumaric or sinapic acids) and by aliphatic acids (malonic or acetic acids) (Pereira *et al.*, 2009). Glycosylation provides better water solubility, but it also protects reactive –OH groups from autooxidation during flavonoid transport in plant cell (Hernández *et al.*, 2009).

2.3.2 Biosynthesis and Subcellular Localization of Phenolics

Biosynthesis of phenolic compounds is the best described biosynthesis pathway of secondary metabolites. Extensive reviews and books address the characterization of the phenolics biosynthetic pathway and enzymes involved in detail (Winkel, 2004, 2006; Tzin and Galili, 2010; Martens *et al.*, 2010; Vogt, 2010; Petrussa *et al.*, 2013), and will not be discussed herein. Instead, we have focused on the cellular location of phenolic biosynthesis, which is an important clue to understand their function in the plant cells.

Starting point of phenolic biosynthesis is the shikimate pathway, a critical link between primary and secondary metabolism. This pathway directs carbon from glycolysis (in the form of phosphoenolpyruvate, PEP) and from the reductive pentose phosphate pathway (in the form of erythrose 4-phosphate, E4P), towards synthesis of aromatic compounds. All enzymes of the shikimate pathway are located in plastids (Tzin and Galili, 2010). The shikimate pathway is linked to linear electron transfer, since the first enzyme, 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase (DAHPS), which catalyzes ligation of PEP and E4P, requires the presence of the reduced form of thioredoxin. Phenylalanine and tyrosine derived from chorismate (the final product in the shikimate pathway) are the precursors for all phenolic compounds (Vogt, 2010). The first step of phenylpropanoid biosynthesis from phenylalanine is catalyzed by phenylalanine-ammonia lyase (PAL), whose expression is regulated by different biotic and abiotic stressors, as well as by conditions that demand increased cell wall lignification (Sewalt *et al.*, 1997; Huang *et al.*, 2010).

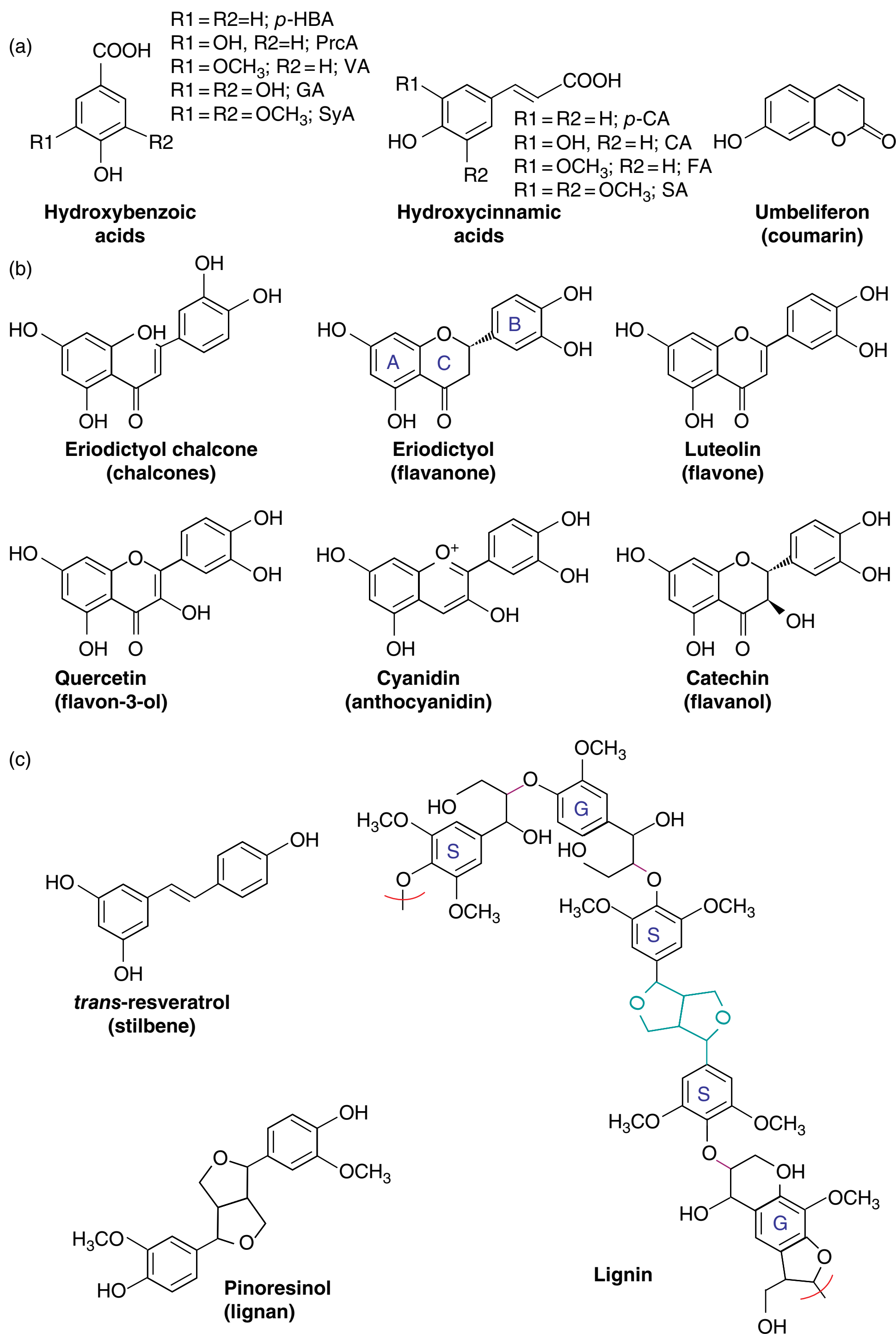

Figure 2.2 Chemical structures of the main sub-classes of phenolic compounds.
a) Common hydroxybenzoic and hydroxycinnamic acids, and umbeliferon. *p*-HBA, *p*-hydroxybenzoic acid; PrcA, protocatechuic acid, VA, vanillic acid; GA, gallic acid; SyA, syringic acid; *p*-CA, *p*-coumaric acid; CA, caffeic acid; FA, ferulic acid; SA, sinapic acid.
b) Representatives of flavonoids with labelled A, B and C rings in the eriodictyol structure.
c) Representatives of stilbenes, lignans and presentation of a part of lignin polymer from poplar. Adapted from Vanholme *et al*. (2010). G: guaiacyl unit; S: syringyl unit.

The large diversity of phenylpropanoids and flavonoids is a result of the activity of various enzymes: hydroxylases and oxygenases of the Cyt P450 superfamily, ligases, oxidoreductases, and superfamilies of *O*-methyl, acetyl- and glycosyl-transferases, which are organized in multienzyme complexes (Saslowsky and Winkel-Shirley, 2001; Martens *et al.*, 2010; Winkel, 2004). This multienzyme complex, also known as flavonoid metabolon, is associated to the cytoplasmic surface of the endoplasmic reticulum (Petrussa *et al.*, 2013), although particular enzymes, such as chalcone synthase (CHS), chalcone isomerase (CHI) and anthocyanidin synthase (ANS) are detected in the chloroplasts, vacuoles, nuclei and cytosol (Saslowsky *et al.*, 2005; Tian *et al.*, 2008; Wang *et al.*, 2011).

Upon biosynthesis, phenolic compounds are transported to the vacuole or apoplast. The transfer is enabled after conjugation with glutathione (by glutathione-S-transferase, GST), esterification with malonate, after glycosylation through membrane transporters, via vesicles from endoplasmic reticulum, chloroplast or Golgi apparatus (Kitamura, 2006; Zhao *et al.*, 2015). Flavonoids and anthocyanins are transferred into the vacuole through specific transporters on the tonoplast, such as proton dependent transporters, ATP-binding cassette (ABC) transporters, multidrug resistance-associated proteins (MRPs; preferentially glutathione-flavonoid complexes), and multidrug and toxic compound extrusion proteins (MATE, preferentially glycosides) (Agati *et al.*, 2012; Petrussa *et al.*, 2013).

2.3.3 Functions of Phenolic Compounds Depend on Their Localization

Phenolic compounds as secondary metabolites are considered as non-essential for plants; however, they provide various advantages for interaction of plants with the environment, and also for plant growth. Flavonoids are important for protection and adaptation to abiotic and biotic stressors, such as exposure to UVR and high intensity of white light, wounding, pathogen infection, chilling, ozone, pollution, nutrient deficiency (Gould and Lister, 2005; Lattanzio *et al.*, 2006; Morina *et al.*, 2008; Agati *et al.*, 2013; Vidović *et al.*, 2015a).

Phenolics accumulated in the vacuole are involved in plant defence mechanisms against herbivores, insects and phytopathogens, since they possess antimicrobial, antifungal and repellent properties (Nagy *et al.*, 2004; Gould and Lister, 2005; Lattanzio *et al.*, 2006). Anthocyanins, as pigments in flowers and fruits, have a role in attracting pollinators and in seed dispersal. Phenolic compounds are involved in signalling mechanisms between plants and beneficial microorganisms, such as stimulation of *Rhizobium* bacteria for nitrogen fixation in legumes (Taylor and Grotewold, 2005; Cooper, 2007). Furthermore, in specific plant-insect interaction, the signalling role of phenolics in gall induction has been proposed (Sedlarević *et al.*, 2016). Flavonoids are also involved in the regulation of cellular processes such as hormone signalling, transcriptional regulation, and cell-to-cell communication (Rice-Evans *et al.*, 1996; Gould and Lister, 2005; Agati *et al.*, 2013). Phenolic compounds are necessary for promotion of pollen tube growth and the resorption of mineral nutrients from senescing leaves (Taylor and Grotewold, 2005). In addition, flavonoids are the basis for allelopathic interactions with other plant species.

The structural role of phenolic compounds is based on formation of lignin, a polymer associated with the secondary cell wall in plants (Dixon and Paiva, 1995). Lignin is

formed by oxidative coupling of hydroxycinnamoyl alcohol monomers, catalyzed by class III peroxidases (Vanholme *et al.*, 2010). Covalent cross-linking of lignin with polysaccharide polymers, esterified with hydroxycinnamic acids and with proteins, reinforces the cell wall, making it resistant against mechanical and enzymatic actions (McLusky *et al.*, 1999; Lattanzio *et al.*, 2006; Stewart *et al.*, 2009; Agati *et al.*, 2012).

Phenylpropanoid and flavonoid glycosides and derivatives predominately accumulate in the vacuoles and cell walls of epidermal and guard cells (Schmelzer *et al.*, 1988; Cerović *et al.*, 2002; Gould *et al.*, 2002; Ferreres *et al.*, 2011). In addition, *p*-coumaric and *p*-hydroxybenzoic acids, chalconaringenin and naringenin were dissolved in the epicuticular wax of tomato, and their composition changed during ripening (España *et al.*, 2014). In soybean leaves, flavon-3-ols and hydroxycinnamic acids have been detected in the upper epidermal cells, while only flavon-3-ols were detected in guard cells of the lower epidermis (Gitz and Liu-Gitz, 2003). Quercetin and kaempferol derivatives have been detected in the cell wall of leaf epidermal cells in Scots pine, and in the cell wall of epidermal cells in *Eustoma grandiflorum* flower petals (Strack *et al.*, 1988; Markham *et al.*, 2000). Furthermore, isoflavonoids released in the apoplast and produced phytoalexins are crucial components in root–microbe interactions (Hassan and Mathesius, 2012).

Due to their strong absorption in UV spectral range, flavon-3-ols (maximal absorption in the 250–285 nm range is from the A ring, and in the 320–385 nm range from the B ring) and hydroxycinnamic acids (maximal absorption in the 310–330 nm range) and their preferential accumulation in the epidermis, they act as a shield against UVR with no effect on PAR transmission to mesophyll cells (Bilger *et al.*, 2001; Cerović *et al.*, 2002; Morales *et al.*, 2010; Agati *et al.*, 2011a). On the other hand, anthocyanins have absorption maxima in 260–280 nm and 500–550 nm intervals, allowing cyanic leaves to absorb the green range of PAR proportionally to the logarithm of their concentration (Neill and Gould, 2000). As a consequence, cyanic leaves have lower quantum efficiency of photosystem II (PSII) (Neill and Gould, 2003; Hughes *et al.*, 2005). However, by absorbing green light, anthocyanins protect photosynthetic apparatus from excess of visible light (excitation pressure) and potential photooxidative stress. The photoprotective role of anthocyanins has been demonstrated in several plant species: *Lactuca sativa* cv. Dark Lolo Roso (Neill and Gould, 2003), *Pseudowintera colorata* (Gould *et al.*, 2002), *Quintinia serrata* and *Elatostema rugosum* (Neill *et al.*, 2002a,b). In the case of evergreen mountain species *Galax urceolata*, under low temperatures and high solar radiation, accumulation of anthocyanins was enhanced in the cells of outer mesophyll layer, where they could protect photosynthetic apparatus under high excitation pressure on PSII (Hughes *et al.*, 2005).

Another very important role of phenolic compounds is their antioxidative function (Rice-Evans *et al.*, 1996; Chen and Ahn, 1998; Gould *et al.*, 2002; Agati *et al.*, 2007; Hernández *et al.*, 2009; Agati and Tatini, 2010). The hydroxyl group of phenolic compounds is a good electron and proton donor, and it is capable to react with ROS and reactive nitrogen species (RNS), forming more stable radicals (Pereira *et al.*, 2009; Morina *et al.*, 2015). Stabilization of these radicals is based on delocalization of π-electrons, intramolecular hydrogen bonding or condensation with other radicals (Croft, 1998; Procházková *et al.*, 2011). Rice-Evans and co-workers (1996, 1997) reported four times higher antioxidative activity of flavonoids and anthocyanidins with *ortho*-dihydroxyl substitution in the B ring than other phenolics. This structural property enables electron delocalization, while the 2, 3-double bond, in conjugation with a 4-oxo

function in the C ring, provides electron delocalization from the B ring. The glycosylation of flavonoids reduces their total antioxidant activity *in vitro*, as shown for quercetin 4′-*O*-glucoside and quercetin-3-*O*-rutinoside compared to quercetin (Morina *et al.*, 2015). In addition, *ortho*-dihydroxy B-ring-substituted phenolic compounds have high metal chelating activity (particularly for aluminium, iron and copper) (Chen and Ahn, 1998), preventing ROS generation via Fenton or Haber-Weiss reactions. However, flavonoids can act as pro-oxidants by reduction of Cu^{2+} and Fe^{3+}, enabling them to participate in the Fenton reaction (Cao *et al.*, 1997; Procházková *et al.*, 2011).

Accumulation of flavonoids has been reported not only in epidermal cells, but also in mesophyll cells, in vacuoles and chloroplasts (Neill and Gould, 2000; Agati *et al.*, 2002; Ferreres *et al.*, 2011; Bidel *et al.*, 2015). Additionally, flavonoids, particularly *ortho*-dihydroxy B-ring-substituted ones, have been detected in the chloroplast's envelope and in the nucleus of mesophyll cells of several species (Gould *et al.*, 2002; Polster *et al.*, 2006; Agati *et al.*, 2007). In a recent study, Guidi *et al.* (2016) hypothesized that accumulation of quercetin and luteolin derivatives in the chloroplasts of mesophyll cells of *Ligustrum vulgare* may compensate for UV-B-induced inhibition of xanthophyll cycle. They suggested that these *ortho*-dihydroxylated flavonoids protect thylakoids and photosynthetic machinery from UVR-induced photo-oxidative damage by direct ROS scavenging and prevention of lipid peroxidation.

Besides their potential to directly react and scavenge ROS and RNS, flavonoids and hydroxycinnamic acids are endogenous substrates for vacuolar and apoplastic class III peroxidases, and are involved in H_2O_2 scavenging, together with ascorbate (Takahama and Oniki, 1997; Takahama, 2004; Ferreres *et al.*, 2011). Similarly, cyanidin serves as an electron donor for class III peroxidases in H_2O_2 scavenging, followed by re-reduction with ascorbate in vacuoles (Yamasaki *et al.*, 1997). Gould *et al.* (2002) observed that red mesophyll cells of *P. colorata*, enriched with anthocyanins, flavon-3-ols, and dihydroflavonols and hydroxycinnamic acids, were more efficient in H_2O_2 scavenging than green cells. Moreover, UV-B radiation systemically induced accumulation of cyanidin glycosides with significant role in antioxidative defence, in both abaxial and adaxial epidermis of *Plectranthus coleoides* leaves (Vidović *et al.*, 2015b).

Unfavourable environmental conditions might inhibit antioxidative enzymes in plants but, at the same time, phenylpropanoids and flavonoids may become key antioxidative defence components (Veljović-Jovanović *et al.*, 2006, 2008; Fini *et al.*, 2011). For example, during the critical first few hours of rehydration of the resurrection plant *Ramonda serbica* (Serbian phoenix flower), cellular enzymatic antioxidant systems were unable to scavenge ROS, due to a decrease in superoxide dismutase, ascorbate peroxidase and class III peroxidases activities (Veljović-Jovanović *et al.*, 2006). Instead, a transient increase in phenolics (mostly hydroxycinnamates) could have prevented lipid peroxidation during that period (Quartacci *et al.*, 2002; Veljović-Jovanović *et al.*, 2008).

In the nucleus, accumulated anthocyanins make complexes with DNA, leading to reduced oxidative DNA damage (Sarma and Sharma, 1999). Additionally, they can protect DNA from oxidative damage by direct ROS scavenging (Glei and Pool-Zobel, 2005). It has been proposed that glycosylation/deglycosylation changes can influence association of flavon-3-ols with histones, changing the histones/DNA interactions and altering gene expression (Polster *et al.*, 2006).

Briefly, depending on their structure and localization at organ and cellular level, phenolic compounds may have various physiological roles. They can act as a shield against

solar radiation (sun screeners, e.g. in the epidermal cells), or they can act as antioxidants (e.g. in the chloroplasts and vacuoles of mesophyll cells).

2.4 UV-B Radiation Stimulates Phenolic Induction

A hallmark of UV-B-induced changes in plant metabolism is the induction of phenylpropanoid and flavonoid pathways (Brown *et al.*, 2005; Heijde and Ulm, 2012). In the following text, we address recent findings on mechanisms of phenolics induction and classification of inducible phenolics. Important interactions of UV-B radiation with other environmental factors, reflecting natural conditions for plants, are also discussed.

2.4.1 Mechanisms of UV-B Perception

UV-B radiation activates at least two independent signalling pathways that regulate the expression of different sets of genes, depending on its fluence rate (Brosché and Strid, 2003; Frohnmeyer and Staiger, 2003; Ulm *et al.*, 2004; Brown and Jenkins, 2008; González Besteiro *et al.*, 2011). One is UV-B stress response, and the second one is crucial for UV-B acclimation response.

High fluence rates and short wavelengths of UV-B radiation might induce ROS accumulation, which is involved in non-specific UV-B signalling pathway (A-H-Mackerness *et al.*, 2000; Kilian *et al.*, 2007; Jenkins, 2009). This pathway activates expression of genes characteristic for defence, wounding or general stress responses (e.g. stimulation of jasmonic acid and ethylene). The evidence for the involvement of ROS in transmitting UV-B signals relies on several observations. Firstly, it was demonstrated that UV-B radiation might provoke generation of different kinds of ROS in the leaves (Hideg and Vass, 1996; Hideg *et al.*, 2002). Moreover, several genes involved in antioxidative protection (e.g. glutathione and pyridoxine metabolism) were upregulated by UV-B radiation (Brosché *et al.*, 2002; Ulm *et al.*, 2004; Brown *et al.*, 2005; Hectors *et al.*, 2007; Favory *et al.*, 2009). Kalbina and Strid (2006) showed that NADPH oxidase is involved in fine tuning of the *CHS* expression levels after exposure of *Arabidopsis thaliana* to higher UV-B doses. Finally, it is known that ROS might play important roles in redox signalling in plant cells (reviewed in Neill *et al.*, 2002c; Foyer and Noctor, 2009; Suzuki *et al.*, 2012).

It was revealed that two mitogen-activated protein kinases (MAPK), MPK3 and MPK6, were activated in response to high fluence rates of UV-B radiation in *A. thaliana* (González Besteiro *et al.*, 2011). Furthermore, they showed that particular MAPK phosphatase (MKP1) had a specific role in UV-B stress response by inhibition of MPK3/MPK6 activities. According to Brosché and Strid (2003) UV-B acclimation response, triggered by low UV-B doses, is perceived by an unknown UV-B receptor, which activates at least two signalling pathways – one that induces the expression of pathogenesis-related (PR) proteins and accumulation of salicylic acid, and a second one that upregulates the expression of *CHS* (involving calcium/calmodulin pathway and protein phosphorylation). In the years following, more extensive research on UV-B radiation effects on plant growth and development was done. It was revealed that even short exposure to very low UV-B irradiances (1/40 of the fluence rate of UV-B in full sunlight) regulates plant metabolism by the induction of genes involved in phenolic biosynthesis

and in photomorphogenic responses (Brown *et al.*, 2005; Favory *et al.*, 2009; Heijde and Ulm, 2012; Paul *et al.*, 2012). A UV-B receptor, which directly senses UV-B radiation, UVR8, was originally identified during screening for Arabidopsis mutants hypersensitive to UV-B (Kliebenstein *et al.*, 2002).

UVR8 is responsible for signal transduction that results in the reprogramming of expression of more than 100 genes (Favory *et al.*, 2009). In the absence of UV-B radiation, UVR8 protein exists as a homodimer, maintained by cation-π interactions between positively charged and aromatic amino acids (arginine and lysine residues with tryptophan and tyrosine residues) and charge-stabilized hydrogen bonds at the dimer interface (Rizzini *et al.*, 2011; Christie *et al.*, 2012; Wu *et al.*, 2012; Heilmann *et al.*, 2016). Exposure to UV-B radiation induces excitation of Trp^{285} and Trp^{233} indole rings, which results in the disintegration of cation-π interactions and UVR8 monomerization (Ulm and Jenkins, 2015). UV-B-mediated UVR8 monomers accumulate in the nucleus and interact with the protein COP1 (Constitutively Photomorphogenic 1) (Kaiserli and Jenkins, 2007; Cloix *et al.*, 2012; Yin *et al.*, 2015). During the night, COP1, an E3 ubiquitin ligase, targets photomorphogenesis–promoting transcription factors (such as Elongated Hypocotyl 5, HY5; HY5 Homolog, HYH; Long after Far-Red, LFR 1; and Long Hypocotyl in Far-Red, HFR1) for degradation by proteasomes (Jiao *et al.*, 2007). For effective repression of photomorphogenesis, it is required that COP1 and SPA (Suppressor of Phy A) form complexes with other components of ubiquitin-proteasome system (Chen *et al.*, 2010). Recent investigations have proposed that, under UV-B radiation, UVR8 monomers inhibit association of COP1-SPA complexes with ubiquitin-proteasome apparatus and, thus, enable gene transcription mediated by HY5 and HYH (Huang *et al.*, 2013).

Blue light and UV-A radiation inhibit COP1 via cryptochromes, disabling it to alter transcriptional factors like HY5 (Yi and Deng, 2005). Therefore, COP1 is a negative regulator of the visible light response, and an important positive regulator of responses to low UV-B irradiances, coordinating the HY5-dependent and the HYH-dependent pathways in signalling transduction. UVR8/COP1 pathway is crucial for UV-B acclimation response, leading to accumulation of HY5/HYH transcriptional factors in the nucleus, which are, in combination with MYB and PFG transcription factors, responsible for upregulation of genes encoding enzymes involved in UV tolerance (e.g. key enzymes of flavonoid biosynthesis pathway, including CHS, CHI and flavonol synthase, FLS) (Ulm *et al.*, 2004; Brown and Jenkins, 2008; Singh *et al.*, 2014). In addition, the UVR8 signalling pathway activates genes involved in antioxidative defence, mostly related to glutathione metabolism (such as glutathione reductase, glutathione peroxidases, peroxiredoxins, glutaredoxins and GST), which is also involved in flavonoid transport in the cell (Brosché *et al.*, 2002; Brown *et al.*, 2005).

Moreover, in Arabidopsis, HY5 and FHY3 (Far-red elongated Hypocotyl 3) positively regulate induction of COP1 transcripts (Huang *et al.*, 2012). The UVR8/COP1/HY5 pathway activates the expression of two proteins, RUP1 and RUP2 (Repressor of UV-B Photomorphogenesis 1 and 2), providing a negative feedback regulation of this pathway (Gruber *et al.*, 2010). Both RUP1 and RUP2 disrupt UVR8/COP1 complexes and promote UVR8 redimerization following UV-B exposure, balancing UV-B-specific responses and ensuring normal plant growth (Heijde and Ulm, 2013; Yin *et al.*, 2015). Two other transcription factors, STO/BBX24 (Salt Tolerance) and RCD1 (Radical-induced Cell Death1), are also proposed to have a repressing role in this pathway (Jiang *et al.*, 2012).

The potential interplay of UV-B stress response with the UVR8-mediated pathway is still not defined. González Besteiro *et al.* (2011) proposed that UV-B-induced activation of MKP signalling was not regulated by UVR8-dependant pathway; however, both were needed to achieve full UV-B tolerance. Previously, Brown and Jenkins (2008) showed that both high and low UV-B fluence rates could activate UVR8. These findings imply that UV-B perception and signalling mechanisms are not simple and distinctive. This was originally demonstrated by Ulm and co-workers (2004), who highlighted the presence and interaction of at least two UV-B perception and signalling pathways; one is activated by the longer wavelengths of UV-B radiation, while the second is activated by the shorter wavelengths of the UV-B spectrum. Furthermore, some gene clusters are positively regulated by the shorter, and negatively regulated by the longer UV-B wavelengths.

2.4.2 UV-B-Induced Accumulation of Phenolic Compounds

Upregulation of the phenylpropanoid and flavonoid pathway is considered as the most frequently observed response to UV-B radiation in most plant species. An overview of recent publications reporting the accumulation of phenolics in different plant species and organs (leaf, fruit, and root) is given in Table 2.1. In order to compare the effects of UV-B radiation under different treatment conditions, UV-B exposure is presented as UV-B_{BE} normalized according to GEN and PG (see Section 2.2).

Table 2.1 lists publications regarding induction of phenolics in different plant species under supplemental UV-B radiation in growth and sun simulator chambers, or in greenhouses. Different techniques were used to describe changes in phenolics content: (i) *in vivo*, measured by fluorimeters: Multiplex Research or Dualex 4, FORCE-A (Orsay, France, see Cerović *et al.*, 2012); (ii) spectrophotometrically for content of total phenolics and flavonoids or UV-B absorbing compounds (e.g. Singleton *et al.*, 1999); and (iii) thin layer and high pressure liquid chromatography for determination of specific compounds (e.g. Vidović *et al.*, 2015a).

At first glance, changes in phenolics profiles in response to UV-B are species-specific; however, the most common response is induction of flavonoid glycosides and anthocyanins, followed by hydroxybenzoic and hydroxycinnamic acids. It is interesting to note the occurrence of phenolic glycosides, which are highly energy-demanding compounds; energy sources required for their biosynthesis will be discussed in Section 2.6.1. Furthermore, the differential structure-specific response of flavonol glycosides and hydroxycinnamic acid derivatives to different UV-B radiation doses is presented (e.g. Lavola *et al.*, 2003; Neugart *et al.*, 2012). The most frequently detected flavonoids are quercetin and kaempferol glycosides, and these compounds were detected in both leaves and fruits exposed to UV-B radiation. Noteworthy, Table 2.1 shows that a broad range of UV-B doses, UV-B : PAR ratios and durations of exposure were used in experiments. However, in some publications, there is not enough information for readers to repeat the experiment under the same conditions.

In our study we obtained similar results on UV-B impact on phenolics metabolism in specific plant species (e.g. resurrection endemic plant *R. serbica*, variegated plants *P. coleoides* and *Pelargonium zonale*, and native Mediterranean herbs, *Ocimum basilicum, Salvia officinalis* and *Eruca sativa*).

Table 2.1 An overview of recent publications (since 2000) reporting the accumulation of phenolics induced by UV-B radiation in different plant species and organs (leaf, callus, fruit). Estimated biologically effective UV doses (UV-B_{BE}, kJ m^{-2} day^{-1}), PAR (μmol m^{-2} s^{-1}) and experiment duration are presented where available. The experiments were performed in the temperature range 20 ± 5 °C, unless otherwise indicated.

Plant species	Phenolic compound	UV-B_{BE}, kJ m^{-2} d^{-1}	PAR, μmol m^{-2} s^{-1}	Exposure duration, days	Reference
leaves					
Arabidopsis thaliana Columbia ecotype	quercetin derivatives	[a] 0.54	1310; 14 h day^{-1}	14	Götz *et al.*, 2010
Arabidopsis thaliana Columbia ecotype	quercetin derivatives	[a] 0.47	540; 14 h day^{-1}	14	Götz *et al.*, 2010
Arabidopsis thaliana Columbia ecotype	quercetin glycosides total anthocyanin concentration was not affected	[a] 0.5 W m^{-2} 12 h per day	730; 14 h day^{-1}	14	Gruber *et al.*, 2010
Artemisia annua	total flavonoid content	[a] 4.2	100; 14 h day^{-1}	14	Rai *et al.*, 2011
Betula pendula	chlorogenic acid, quercetin-3-*O*-galactoside, quercetin-3-*O*-rhamnoside, kaempferol-3-*O*-rhamnoside	[a] 7.3–8.5	280–350; 16 h day^{-1}	10	Tegelberg *et al.*, 2004
Betula pendula	chlorogenic acid, myricetin-3-*O*-galactoside, myricetin-3-*O*-rhamnoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-rhamnoside, kaempferol-3-*O*-rhamnoside, condensed tannins	[a] 2.47 [a] 1.31	18.23 MJ m^{-2} day^{-1}	47	Kotilainen *et al.*, 2009
Betula pubescens ssp. czerepanovii	myricetin 3-*O*-glucoside, myricetin 3-*O*-glucoside + glucuronide	[d] 2.75–5.23	200/50 (d/n)	several weeks	Anttila *et al.*, 2010

(*Continued*)

Table 2.1 (Continued)

Plant species	Phenolic compound	UV-B_{BE}, kJ m^{-2} d^{-1}	PAR, μmol m^{-2} s^{-1}	Exposure duration, days	Reference
Brassica napus	quercetin-7-*O*-glucosides, kaempferol-7-*O*-glucosides, kaempferol-3-*O*-*p*-coumaroyldiglucoside-7-*O*-glucoside	[a] 1	250; 14 h day^{-1}	6	Wilson *et al.*, 2001
Brassica campestris L. ssp. *chinensis* var. communis	caffeoylmalate (9 °C), coumaroylmalate (9 °C), feruloylmalate (9 °C), kaempferol-3-*O*-diglucoside-7-*O*-glucoside (9 °C and 22 °C), kaempferol-3-*O*-coumaroyldiglucoside-7-*O*-glucoside/isorhamnetin-diglycoside (9 °C and 22 °C)	[e] 0.35 mW m^{-2} 16 h day^{-1}	100; 16 h day^{-1}	7	Harbaum-Piayda *et al.*, 2010
Brassica oleracea var. sabellica	kaempferol-3-*O*-feruloyl-sophoroside	[a] 0.22	72	1	Neugart *et al.*, 2012
Brassica oleracea var. sabellica	kaempferol-3-*O*-caffeoyl-sophoroside-7-*O*-glucoside, kaempferol-3-*O*-feruloyl-sophoroside-7-*O*-glucoside, caffeoylquinic acid, disinapoyl-gentiobiose, sinapoyl-feruloyl-gentiobiose	[a] 0.44	72	1	Neugart *et al.*, 2012
Brassica oleracea var. sabellica	caffeoylquinic acid, kaempferol-3-*O*-sinapoyl-sophoroside, kaempferol-3-*O*-caffeoyl-sophoroside-7-*O*-glucoside, kaempferol-3-*O*-feruloyl-sophoroside-7-*O*-glucoside, kaempferol-3-*O*-disinapoyl-triglucoside-7-*O*-glucoside	[a] 0.66	72	1	Neugart *et al.*, 2012
Brassica oleracea var. sabellica	kaempferol-3-*O*-feruloyl-sophoroside, kaempferol-3-*O*-hydroxyferuloyl-sophoroside, kaempferol-3-*O*-sinapoyl-sophoroside, quercetin-3-*O*-disinapoyl-triglucoside-7-*O*-glucoside	[a] 0.88	72	1	Neugart *et al.*, 2012
Centella asiatica	flavonoids (FLAV index, measured by Multiplex Research, FORCE-A, Orsay, France)	[e] 0.3 W m^{-2}	455 and 835; 12 h day^{-1}	35	Müller *et al.*, 2013

Centella asiatica	flavonoids (FLAV index, measured by Dualex 4, FORCE-A, Orsay, France), kaempferol-3-*O*-β-D-glucuronopyranoside, quercetin-3-*O*-β-D-glucuronopyranoside	[e]2.16 [e]15.3	655–2150	different durations	Bidel *et al*., 2015
Hordeum vulgare var. Barke	flavonols (FLAV index, measured by Dualex 4, FORCE-A, Orsay, France)	[a] average 3.9 ± 0.8	average 3.7 ± 1.1 $MJ m^{-2} day^{-1}$	7	Klem *et al*., 2012
Hydrocotyle leucocephala	quercetin derivatives and total quercetin	[a] 2.4	516 and 906; $12 h day^{-1}$	28	Müller *et al*., 2015 12/22 °C
Kalanchoe pinnata	total flavonoid content, quercetin 3-*O*-α-L-arabinopyranosyl (1 → 2) α-L-rhamnopyranoside, quercitrin	[a] 4.86 – 18.36	200–400; $11.5 h day^{-1}$	5 and 10	dos Santos Nascimento *et al*., 2015 32 ± 4 °C
Lactuca sativa cv. Expedition	chlorogenic acid, quercetin-3-*O*-glucuronide, luteolin-7-glucuronide, quercetin-3-(6"-malonyl-glucoside)	[a] 10	750; $14 h day^{-1}$	6	Wargent *et al*., 2015
Lolium perenne cv. AberDart	total hydroxycinnamic acids (mostly chlorogenic acid), total flavonoids	[a] 4.1 [a] 5.0 [a] 5.7	minimum 200; $8 h day^{-1}$	35	Comont *et al*., 2013
Melissa officinalis	rosmarinic acid, melitric acid, caffeic acid	[e]1 [e] 2.5	360 and 306, $10 h day^{-1}$	112	Manukyan, 2013
Mesembryanthemum crystallinum	mesembryanthin (flavon-3-ol), ferulic glucoside	[a] 28.0	1520 $10 h day^{-1}$	5	Ibdah *et al*., 2002 12/22 °C
Nasturtium officinale young leaves	quercetin and kaempferol glycosides	*ca*. $0.1 W m^{-2} nm^{-1}$	*ca*. $1 W m^{-2} nm^{-1}$	2	Reifenrath and Müller, 2007 13/33 °C

(*Continued*)

Table 2.1 (Continued)

Plant species	Phenolic compound	UV-B_{BE}, kJ m^{-2} d^{-1}	PAR, μmol m^{-2} s^{-1}	Exposure duration, days	Reference
Nepeta cataria var. citriodora	rosmarinic acid, caffeic acid, *p*-coumaric acids (only under 1 kJ m^{-2} d^{-1} UV-B_{BE}	[e] (i) 1 [e] (ii) 2.5	(i) 360 (ii) 306, 10 h day^{-1}	112	Manukyan, 2013
Ocimum basilicum	gallic acid, cinnamic acid, ferulic acid, catechin, quercetin, rutin, kaempferol, luteolin	[e] 2.30 W m^{-2} [e] 3.60 W m^{-2}	min. 52 max. 1480	6,8 h	Ghasemzadeh *et al.*, 2016 average 30 ± 2 °C
Oryza sativa cvs. Cypress, Cheniere, Cocodrie, CLXL729	total phenolics	[b] 16	800	85–105	Mohammed and Tarpley, 2011 22/31 °C
Pelargonium zonale green leaf sectors	flavonoids not affected	[a] 7.0	1350; 12 h day^{-1}	10	Vidović *et al.*, 2015c
Pelargonium zonale green leaf sectors	*p*-hydroxybenzoic acid, protocatechuic acid, gallic acid, epicatechin	[a] 7.0	395; 12 h day^{-1}	10	Vidović *et al.*, 2015c
Populus tremula	cyanidin and cyanidin glucoside, quercetin-3-*O*-rhamnoside	[a] 46.8	max 1400; 16 h day^{-1}	5, 9 and 13	Kaling *et al.*, 2015 17/27 °C
Pinus sylvestris	dicoumaroyl-astragalin, dicoumaroyl-isorhemnetin, astragalin, trifolin, isoquercitrin, isomyricitrin, isorhamnetin-3-*O*-glucoside, myricetin-3-*O*-galactoside, hyperin	[a] 1.57 [a] 2.61 [a] 4.46 [a] 7.84 [a] 13.07	max 700; 20 h day^{-1}	60	Lavola *et al.*, 2003

Plectranthus coleoides green leaf sectors	apigenin glycosides, cyanidin glycosides, caffeic acid, hydroxybenzoic acids	[a] 7.0	1350; 12 h day^{-1}	10	Vidović *et al.*, 2015b
Plectranthus coleoides green leaf sectors	apigenin glycosides, cyanidin glycosides,	[a] 7.0	395 12 h day^{-1}	10	Vidović *et al.*, 2015b
Rosmarinus officinalis	caffeic, rosmarinic acid, carnosic, vanillic acid, naringin, cirsimaritin, carnosol, hispidulin	[a] 5.4 and 31	n.a.	14	Luis *et al.*, 2007
Salvia officinalis	rosmarinic acid, caffeic acid, meltric acid	[e] (i) 1 [e] (ii) 2.5	(i) 360 (ii) 306, 10 h day^{-1}	112	Manukyan, 2013
Sinapis alba young and old leaves	quercetin glycosides, kaempferol glycosides, hydroxycinnamic acids (old leaves)	ca. 0.1 $W\,m^{-2}\,nm^{-1}$	ca. 1 $W\,m^{-2}\,nm^{-1}$	2	Reifenrath and Müller, 2007 13/33 °C
Solanum tuberosum cv. Désirée	unknown flavonoid compounds	[a] 7.83	450; 11 h day^{-1}	8	Santos *et al.*, 2004
Trifolium repens	quercetin glycosides, kaempferol glycosides	[a] 13.3	425; 12 h day^{-1}	12	Hofmann *et al.*, 2003
Vigna acontifolia	total phenolics, total flavonoids	[e] 9.8, 11, 12.2, 13.4, 15.8	minimum 550; 13 h day^{-1}	2	Dwivedi *et al.*, 2015 28 ± 2 °C
Vigna mungo	total phenolics, total flavonoids	[e] 11, 12.2, 13.4, 15.8	minimum 550; 13 h day^{-1}	2	Dwivedi *et al.*, 2015 28 ± 2 °C

(*Continued*)

Table 2.1 (Continued)

Plant species	Phenolic compound	UV-B_{BE}, kJ m^{-2} d^{-1}	PAR, µmol m^{-2} s^{-1}	Exposure duration, days	Reference
Vitis vinifera cv. Tempranillo (at veraison stage)	quercetin-3-*O*-glucoside, kaempferol-3-*O*-glucoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucopyranoside	[c] 16.4 [c] 19.2	7.4; 8.4 MJ m^{-2} day^{-1}	93	Del-Castillo-Alonso *et al.*, 2016
Vitis vinifera cv. Chardonnay, young leaves	UV-B absorbing compounds, total anthocyanins, total phenolics	[c] 8.04	50; 16 h day^{-1}	4	Majer and Hideg, 2012b
callus					
Passiflora quadrangularis	apigenin-*C*-glucosides (vitexin, isovitexin), orientin	[e] 12.6	25; 16 h day^{-1}	2	Antognoni *et al.*, 2007
Passiflora quadrangularis	apigenin-*C*-glucosides (vitexin, isovitexin), luteolin-*C*-glucosides (orientin, isoorientin)	[e] 25.3	25; 16 h day^{-1}	2	Antognoni *et al.*, 2007
fruits					
Vitis vinifera cv. Tempranillo-grape berries (at harvest stage)	resveratrol, resveratrol-3-*O*-glucoside, epicatechin, procyanidin B1, myricetin-3-*O*-glucoside, kaempferol-3-*O*-glucoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucuronide quercetin-3-*O*-glucopyranoside, isorhamnetin-3-*O*-glucoside	[c] 16.4 [c] 19.2	7.4; 8.4 MJ m^{-2} day^{-1}	93	Del-Castillo-Alonso *et al.*, 2016

fruits (postharvest UV-B exposure)					
Citrus limon, cv. Limoneira 8A, flavedo peel tissue	total phenolics	[a] 22	none	2 and 3 min	Interdonato *et al.*, 2011
Brassica oleracea var. italica flower buds	quercetin, kaempferol, isorhamnetin	[e] 0.22–0.25 W m^{-2}	19	3	Rybarczyk-Plonska *et al.*, 2015 at 10 °C after 4 days of pre-storage at 4 °C
Ribes nigrum cv. Titania	total flavonols, total anthocyanins, hydroxybenzoic acids	[a] 0.27 [a] 0.41	n.a.	60, 90 and 120 min	Huyskens-Keil *et al.*, 2007
Ribes nigrum cv. Titania	total flavonols, total anthocyanins, hydroxybenzoic acids, hydroxycinnamic acids	[a] 0.54	n.a.	60, 90 and 120 min	Huyskens-Keil *et al.*, 2007
Malus domestica cv. Aroma peel	chlorogenic acid, epicatechin, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-rhamnoside, cyanidin-3-*O*-galactoside	[a] 0.2 W m^{-2} 12 h day^{-1}	25–30	10	Hagen *et al.*, 2007

Biologically effective UV doses were calculated with different biological spectral weighting functions (BSWFs):
[a] according to Caldwell (1971), as formulated by Green *et al.* (1974);
[b] according to Madronich *et al.* (1998);
[c] according to Flint and Caldwell, 2003;
[d] according to Björn, 1990;
[e] not available (n.a).
Units of UV-B_{BE} are presented as kJ m^{-2} d^{-1}, unless stated otherwise.

Table 2.2 Changes in the content of phenolic compounds (determined as aglycones) in *Ramonda serbica* leaves exposed to moderate UV-B_{BE} for five days.

Phenolic compounds	Control	UV-B
hydroxybenzoic acids		
protocatechuic acid	15.3 ± 2.3	51.6 ± 5.8 *
p-hydroxybenzoic acid	21.0 ± 1.0	32.3 ± 4.4 *
syringic acid	12.3 ± 1.2	40.7 ± 5.2 *
hydroxycinnamic acids		
chlorogenic acid	0.52 ± 0.04	0.71 ± 0.05 *
caffeic acid	4.45 ± 0.55	4.76 ± 0.20
ferulic acid	0.89 ± 0.08	0.97 ± 0.13
flavanols		
catechin	55.4 ± 7.2	68.4 ± 4.2
flavones		
luteolin	0.68 ± 0.08	0.56 ± 0.05
apigenin	8.70 ± 0.77	9.39 ± 1.17
anthocyanidins		
delphinidin	8.9 ± 1.2	51.3 ± 9.0 *
cyanidin	12.9 ± 1.9	76.6 ± 9.4 *
peonidin	1.4 ± 0.2	5.3 ± 0.9 *
petunidin	1.5 ± 0.2	7.4 ± 1.5 *

Values are shown in μmol gFW^{-1} ± SE, and in nmol gFW^{-1} ± SE, for anthocyanidins ($n = 4$). Significant differences between control and UV-B exposed plants, according to Mann-Whitney U-test, are indicated (*$P < 0.05$).

In *R. serbica* leaves, derivatives of hydroxybenzoic acids and anthocyanins were significantly enhanced (in total, 2.6 and 5.7 folds, respectively) after UV-B radiation exposure (2.0–2.5 kJ m^{-2} day^{-1} UV-B_{BE} combined with 210 μmol m^{-2} s^{-1} PAR for five days), whereas apigenin and luteolin glycosides and most of hydroxycinnamic acid derivatives were unaffected (Table 2.2).

Similarly, in our study with variegated *P. coleoides* plants, we described the stimulating effect of moderate UV-B doses (7 kJ m^{-2} day^{-1} UV-B_{BE} for ten days) on the accumulation of apigenin and cyanidin glycosides, particularly in white leaf tissue (Vidović *et al.*, 2015b). The first visible signs of anthocyanin accumulation were observed in the leaves of *P. coleoides* plants after four days of exposure to UV-B radiation, and were more noticeable under higher PAR (1350 μmol m^{-2} s^{-1}), compared to lower PAR (395 μmol m^{-2} s^{-1}).

Leaf tissue type (source/sink) was an important factor in UV-B-provoked phenolic induction in another variegated species, *P. zonale* (Vidović *et al.*, 2015c). The same UV-B irradiance (under the same conditions as in *P. coleoides* study) induced significant accumulation of different phenolic subclasses: *p*-coumaric acid, kaempferol and quercetin glycosides only in white leaf tissue. We observed similar dynamics of flavonoid

induction by UV-B radiation (UV-B: 22.5 kJ m^{-2} d^{-1} UV-B_{BE} GEN, UV-A: 0.60 MJ m^{-2} d^{-1}, PAR: 13.1 MJ m^{-2} d^{-1}) in the leaves of several species from the *Lamiaceae* family previously grown in a non-UV-transparent glasshouse. Accumulation of epidermal flavonoids was determined using Dualex4 (Cerović *et al.*, 2012). Significant increase in quercetin, or apigenin and luteolin, as well as hydroxycinnamic acids' accumulation was observed 30 hours after exposure to ambient light conditions, compared with greenhouse conditions in *O. basilicum* var. Americanum, *O. basilicum* var. Genovese, *S. officinalis* and *E. sativa* while, in *O. basilicum* var. Purpurescens, significant response was observed after three days (unpublished results). Among the analysed species, *O. basilicum* var. Purpurescens was the only one containing anthocyanins, while the content of flavonoids was similar. The delay in flavonoid induction in *O. basilicum* var. Purpurescens may be explained by constitutive protection against UVR by anthocyanins, similarly as less pronounced UV-B effect on green tissue, which had constitutively higher phenolics concentration than white one, in *P. coleoides* and *P. zonale* (Vidović *et al.*, 2015b, 2015c). Hofmann *et al.* (2000) reported population-specific differences in response to UV-B radiation in *Trifolium repens*. Populations with low constitutive flavonoid content (quercetin and kaempferol glycosides), responded to UV-B radiation (13.3 kJ m^{-2} d^{-1}) by more than twofold induction. On the contrary, *T. repens* populations with a higher constitutive content of flavonoids responded to UV-B radiation in a lower extent.

2.4.3 Interactive Effects of UV-B with UV-A Radiation and PAR on Phenolics Accumulation

The final effect of UV-B radiation on plants (detrimental or beneficial) depends not only on the biologically effective dose applied and/or the spectral quality (Ibdah *et al.*, 2002; Antognoni *et al.*, 2007; Huyskens-Keil *et al.*, 2007; Neugart *et al.*, 2012), but also on UV-B interactions with other environmental stimuli, such as background PAR intensity and UV-A radiation (Jenkins *et al.*, 2001; Caldwell *et al.*, 2007; Götz *et al.*, 2010; Majer and Hideg, 2012a; Behn *et al.*, 2010; Hideg *et al.*, 2013; Vidović *et al.*, 2015b, 2015c), temperature (Hughes *et al.*, 2005) and water supply (Nogués *et al.*, 1998; Hofmann *et al.*, 2003; Arróniz-Crespo *et al.*, 2011; Doupis *et al.*, 2016).

Under natural light conditions, UV-A, blue light and high intensity of white light can upregulate *CHS* via the previously mentioned COP1/HY5 signalling pathway, and can subsequently induce accumulation of flavonoids (Neil and Gould, 2003; Jenkins *et al.*, 2001; Ibdah *et al.*, 2002; Brown *et al.*, 2005; Page *et al.*, 2012; Heyneke *et al.*, 2013; Grifoni *et al.*, 2016). Therefore, acclimative responses to UV-B radiation and high PAR intensity may overlap, imposing cross-tolerance (Behn *et al.*, 2010; Majer and Hideg, 2012a; Vidović *et al.*, 2015b, 2015c). High PAR- induced hydroxycinnamates and flavon-3-ols in epidermis may attenuate UVR effects (Searles *et al.*, 2001; Kotilanen *et al.*, 2009; Götz *et al.*, 2010; Vidović *et al.*, 2015c). It is also known that blue light induces an increase of photolyases, enzymes involved in the repair of cyclobutane pyrimidine dimers of DNA, which are induced by UVR (reviewed in Sinha and Hader, 2002; Britt, 2004; Ballare *et al.*, 2011).

In fully sun-exposed leaves (combined effect of PAR and UVR) of *Phyllostachys aureosulcata* Aureocaulis and *Tilia platyphyllos*, we observed significantly higher accumulation of flavonoids compared with shaded ones (Figure 2.3). Increased flavonoids

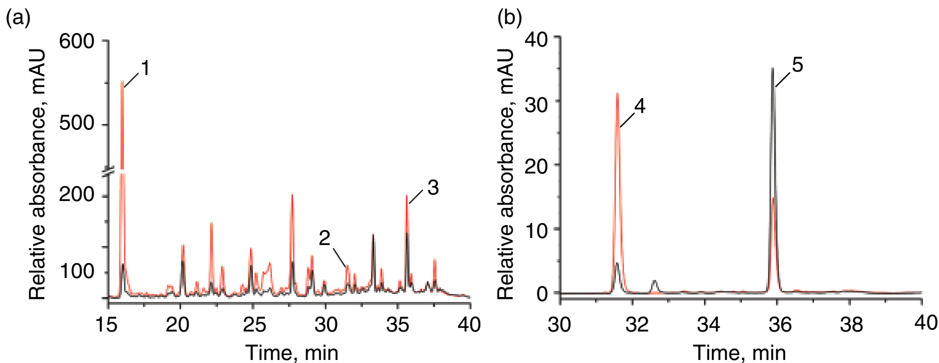

Figure 2.3 HPLC chromatograms recorded at 340 nm, showing sunlight-induced accumulation of flavonoids in bamboo (a) and linden (b) leaf hydrolyzed methanol extracts.
1, homoorientin; 2, luteolin; 3, tricin; 4, quercetin; 5, kaempferol.
Grey—sunlight; black—shade.

were those with *ortho*-dihydroxyl substitution on the B-ring: luteolin-*C*-glycosides and derivatives (homoorientin, tricin) in bamboo leaves, and quercetin and myricetin glycosides in linden leaves. Similar stimulation of flavonoids with higher antioxidative potential in sun-exposed linden leaves was observed by Majer *et al.* (2014).

Increase in the quercetin-to-kaempferol ratio in response to sunlight, or UV-B radiation only, has already been reported for a number of species. Reifenrath and Müller (2007) observed preferential accumulation of quercetin over kaempferol in response to UVR in *Sinapis alba* and *Nasturtium officinale*, and the same trend was observed in *Vaccinium myrthillus* (Jaakola *et al.*, 2004) and *Glycine max* (Winter and Rostas, 2008) and *Centella asiatica* (Bidel *et al.*, 2015). In addition, high PAR induced higher quercetin-to-kaempferol ratio in green leaf tissue of *P. zonale* (Vidović *et al.*, 2015c). Similarly, the luteolin-to-apigenin ratio increased under sunlight in both *L. vulgare* and *Phillyrea latifolia* (Tattini *et al.*, 2005; Guidi *et al.*, 2016). Higher accumulation of *ortho*-dihydroxylated flavonoids by different sunlight components emphasizes their antioxidative, rather than screening, function. This should be related to localization of flavonoids in different leaf tissues (epidermis and mesophyll layers, as mentioned above).

2.4.4 Interactive Effects of UV-B Radiation with other Environmental Factors on Phenolics Accumulation

Flavonoids are crucial components of developmental processes in plants and, as mentioned above, they are often regarded as hallmarks of acclimative metabolism to abiotic and biotic stress, exerting regulative and protective properties (Chalker-Scott, 1999; Petrussa *et al.*, 2013). In order to understand the complex interactions of environmental factors on phenolics metabolism, a number of studies have addressed the combined effects of UV-B radiation and other factors, namely CO_2 levels, temperature, and drought. Recent data have shown that plant responses to a single factor are different compared with responses to combinations of other stressors. When *Vigna unguiculata* genotypes were exposed to UV-B radiation in combination with elevated temperatures and increased CO_2 levels, significant interaction of all three factors on phenolics content was observed (Singh *et al.*,

2010). Although UV-B radiation increased phenolics content alone, UV-B + CO_2 induced further increase of their content, while UV-B + CO_2 + T induced phenolics to a lower extent. Another study by Lavola and co-authors (2000) showed differential effects of CO_2 and UV-B radiation on secondary metabolism in *Betula pendula* seedlings. While UV-B stimulated biosynthesis of phenolic acids and flavonoid glycosides, elevated CO_2 favoured accumulation of condensed tannins. As a result, elevated CO_2 could ameliorate the effects of UV-B radiation through regulation of carbon allocation between phenolic metabolites.

Additive effects of ambient light and low temperatures regarding flavonoids accumulation have been observed in several species. In *G. urceolata* leaves and detached grape berries the highest accumulation of anthocyanins was observed under lower temperature combined with higher light treatment (Hughes *et al.*, 2005; Azuma *et al.*, 2012).

Plants with high anthocyanin content tend to be tolerant to drought, as reviewed by Chalker-Scott (1999) and Gitz and Liu-Gitz (2003). Since the main strategy for drought tolerance is based on water loss limitation, the accumulation of phenolic compounds in the leaf epidermal cells and guard cells might be helpful for maintenance of stomatal functioning. Indeed, Nogués *et al.* (1998) showed that drought stress was alleviated in *Pisum sativum* grown under 32 kJ m^{-2} d^{-1} UV-B_{BE} (biological weighting function of Caldwell, 1971) for 24 days. In this study, flavonoids and anthocyanins were significantly accumulated in epidermis as a result of synergistic effect of both drought and UV-B radiation. Similar synergistic effects of these two factors were obtained in *V. unguiculata* (Balakumar *et al.*, 1993) and *T. repens* (Hofmann *et al.*, 2003). Moreover, exclusive distribution of flavon-3-ols in the abaxial guard cells in soybean (Gitz and Liu-Gitz, 2003) might offer protection to chloroplasts and photosynthetic apparatus from oxidative stress induced by drought.

Besides cross-tolerance to abiotic stress, UV-B radiation can reduce the effects of biotic stress on plants. Inhibition of insect herbivory under UV-B radiation can be achieved through induction of signalling pathways similar to those induced by wounding (reviewed by Caldwell *et al.*, 2007). Izaguirre *et al.* (2007) observed overlapping in accumulation of UV-B- absorbing and anti-herbivore phenolic compounds in *Nicotiana attenuata* and *N. longiflora* leaves. Similar findings were reported for UV-B effects on broccoli sprouts (Mewis *et al.*, 2012). Accumulation of phenolics under UV-B radiation contributes to plant defence – for example, against aphides in broccoli (Kuhlmann and Mullert, 2010), and diamondback moth in *A. thaliana* (Caputo *et al.*, 2006). In addition, UV-B radiation can mediate pathogen development directly, or by changing the biochemical profile of plant hosts (reviewed by Raviv and Antignus, 2004).

It is important to note that interactions between UV-B radiation and other environmental factors are complex and variable, due to the plant species, extent and severity of stress, etc. Further research should focus on untangling and understanding these interactions, which are significant from the global climate change and agricultural perspectives.

2.5 UV-B-Induced Photomorphological Responses

UV-B radiation is an environmental signal that induces morphological changes in plants, such as decreased leaf size and total leaf area, increased leaf thickness, leaf curling, inhibition of hypocotyl elongation and auxiliary root and shoot branching

(recently reviewed by Yokawa *et al.* (2014) and Robson *et al.* (2015)). All these responses help plants to reduce surface area exposed to UV-B radiation, and minimize eventual damaging effects induced by other, associated environmental factors (Jansen, 2002).

2.5.1 Connection Between UV-B-Induced Morphological Responses and Phenolics

UV-B effects on morphological characteristics are highly variable between species, and may be transient (where the plant recovers and reaches full growth) or persistent (leading to growth reduction). In acclimated *A. thaliana* plants, UV radiation provoked a decrease in the rosette diameter and inflorescence height and increased the number of flowering stems, which can be considered as redistribution, not as suppression of growth (Hectors *et al.*, 2007). The recent review by Robson and co-authors tackled the complex relationship between morphological changes and phenolics induction under UV-B radiation. Most of the previous reports address the interaction between phenolics and metabolism of auxin (Peer *et al.*, 2004; Taylor and Grotewold, 2005; Besseau *et al.*, 2007; Hectors *et al.*, 2007; Ringli *et al.*, 2008; Yin *et al.*, 2014). It has been shown that flavonoids can alter auxin homeostasis by downregulation of auxin efflux carriers (Peer *et al.*, 2004) and enhancement of auxin catabolism. On the other hand, recent study of Hectors and co-workers (2012) has shown that auxin may also affect the profile and accumulation of flavonoid glycosides under UV-B exposure, and this was observed using Arabidopsis mutants with altered auxin synthesis (*nit3*) or auxin influx (*arx4-1*).

In addition, the involvement of phenolics in cell wall thickening and lignification in response to ambient UV-B radiation, should not be neglected. Only a few studies have addressed the importance of cell wall-bound phenolics in responses to UV-B radiation in plants and mosses. Tolerance to higher UV-B radiation of *Ceratodon purpureus* compared to other two Antarctic mosses in the field conditions was attributed to accumulation of cell wall-bound phenolics (Clarke and Robinson, 2008). Ruhland and Day (2000) have observed a tendency for increased accumulation of cell wall-bound ferulic acid in *Deschampsia antarctica* under ambient and 'near ambient' (87% of ambient) UV-B radiation in a field experiment. Accumulation of ferulic acid in *D. antarctica* following UV-B exposure may be responsible for reduction of its leaf elongation, due to crosslinking with other cell wall components, such as pectin and extensine (Wang *et al.*, 2013). Accordingly, short term exposure to UV-B irradiance of 7.5 $W\,m^{-2}$, four hours per day for three days, increased cell wall thickness of epidermal cells, lignin accumulation and the activity of class III peroxidases in cotyledons of quinoa seedlings (Hilal *et al.*, 2004).

UV-B radiation stimulated accumulation of tannins in the epidermal cells and cell wall-bound phenolics in *Pinus taeda* during needle development (Laakso *et al.*, 2000). Stimulation of sinapyl and coniferyl alcohol dehydrogenases, enzymes linked to lignin biosynthesis, was observed in cucumber cotyledons, following continuous exposure to UV-B radiation ($0.57 \pm 0.16\ W\,m^{-2}$) to for several days. In addition, activity of peroxidases which polymerize monolignols was significantly increased in epidermal cells surrounding trichomes (Yamasaki *et al.*, 2010). Similarly, Jansen *et al.* (2001) observed significantly increased lignification in UV-tolerant duckweed mutant *mTR*, as well as increased peroxidase activity following 24 hours of exposure to 4.4 $W\,m^{-2}$ UV-B radiation.

An increased number of trichomes is also considered as acclimative response of plants to UV-B radiation. Twenty-day exposure of Arabidopsis trichome mutants to moderate UV-B

radiation (3.4 kJ m^{-2}) showed that mutants with increased trichome density were more tolerant than *wt* (wild type), while transcript levels of GLABRA3 (GL3), the transcription factor responsible for trichome initiation, increased (Yan *et al.*, 2012). Particularly, it has been reported that trichomes present hot spots of flavonoid accumulation in olive leaves (Liakopoulos *et al.*, 2001), while trichomes in two oak species storaged UV-B absorbing compounds (Liakoura *et al.*, 2003).

2.5.2 Effect of UV-B Radiation on Root Morphology in Relation to Phenolics

Numerous reports have shown the UV-B effects on aboveground (aerial) plant organs; however, effects of UV-B radiation on root development are poorly understood. Recent studies have shown that roots respond to UV-B radiation (Yokawa and Baluška, 2015). This is not surprising, considering that all photoreceptors found in Arabidopsis were expressed both in aerial parts and in roots (Briggs, 2014). Moreover, in addition to expression of UVR8 receptor (Rizzini *et al.*, 2011), specific UV-B sensing proteins, Root UVB Sensitive 1 (RUS1) and Root UVB Sensitive 2 (RUS2) have been detected in Arabidopsis (Tong *et al.*, 2008; Leasure *et al.*, 2009) and rice root seedlings (Yu *et al.*, 2016). In RUS1 deficient mutant (*rus1*) primary roots had reduced growth and were hypersensitive to very low UV-B fluence rates, $< 0.1\,\mu mol\,m^{-2}\,s^{-1}$ (Tong *et al.*, 2008). Elevated UV-B doses reduced root biomass in *Glycine max* cv. Heidou (Feng *et al.*, 2003). Moreover, the ecologically relevant UV-B doses, simulating 70, 60, 50, 40 and 30 °N latitudes decreased (proportionally with the applied doses) root biomass and length in *Lolium perenne* (Comont *et al.*, 2013).

Flavonoids can be synthesized in roots, and accumulated in epidermal and cortex cells of the root elongation zone (Saslowsky and Winkel-Shirley, 2001). Here, they are involved in the regulation of root branching, stress adaptation and gravitropism (Buer *et al.*, 2013; Agati *et al.*, 2011b). Fasano *et al.* (2014) have shown that over-expression of UVR8 receptor in 35S-UVR8 transgenic Arabidopsis lines reduced growth development, due to inhibition of cell expansion. In addition, the 35S-UVR8 phenotype had decreased primary root growth and lateral root density compared with control plants. Inhibition of root growth and lateral branching indicated alterations in auxin metabolism. Indeed, under low UV-B radiation, the concentration of root flavonoids was higher in 35S-UVR8 transgenic plants compared with control ones, while a tendency of auxin decrease was also observed. However, down regulation of UVR8 in *uvr8-6* Arabidopsis mutants had no effect on the content of root flavonoids, suggesting that lack of UVR8 expression did not influence flavonoid content in roots. Overall, flavonoid accumulation mediated by UV-B radiation and UVR8/COP1 pathway may affect auxin homeostasis, and induce changes in root morphology observed in 35S-UVR8 lines. Meng (2015), revealed that root growth and anthocyanin accumulation in Arabiopsis under light were mediated by COP1 and AN3, a transcription coactivator.

2.6 Photosynthesis Under UV-B Radiation

Reports on UV-B effects on gas exchange parameters are not consistent. A large number of studies have described deleterious effects of UV-B radiation on photosynthetic activity, usually as a consequence of UV-B-supplemented treatments with unrealistic UV-B, UV-A and PAR spectra and intensities (Kakani *et al.*, 2003; Lu *et al.*, 2009; Kotilainen *et al.*, 2011;

Ranjbarfordoei *et al.*, 2011; Lidon *et al.*, 2012). Negative effects of UV-B radiation on photosynthetic machinery refers to disruption of thylakoid membranes, damage of the photosystems (PSII more sensitive than PSI), decrease in CO_2 assimilation and stomata closure (Jansen *et al.*, 1998; Kataria *et al.*, 2014 and references therein). In environments where UV-B radiation is naturally high (e.g. high altitudes, polar regions), UV-B exclusion studies have shown that UV-B radiation can decrease photosynthesis (Ruhland *et al.*, 2005; Albert *et al.*, 2011; Berli *et al.*, 2013; Gitz *et al.*, 2013). On the other hand, the influence of lower, ecologically relevant fluence rates of UV-B radiation on photosynthetic activity is minimal or negligible (Searles *et al.*, 2001; Valkama *et al.*, 2003; Ballaré *et al.*, 2011; Hideg *et al.*, 2013; Comont *et al.*, 2013; Müller *et al.*, 2013; Vidović *et al.*, 2015c).

The positive influence of ambient UV-B radiation on photosynthetic rate is rarely documented. It has been reported that low UV-B doses up-regulate proteins important for maintenance and protection of photosynthesis (Favory *et al.*, 2009; Davey *et al.*, 2012). For example, the UVR8/COP1/HY5(HYH) signalling pathway leads to the expression of *SIG5* (which encodes sigma factor of plastidic RNA polymerase, involved in D2 protein biosynthesis) and induction of ELIP1 (Early-Light Inducible Protein 1), which may interact with D1 protein of PSI (Singh *et al.*, 2014). In addition, ELIP1 is induced in chloroplasts during maturation, protecting photosynthetic apparatus from photooxidative stress (Rossini *et al.*, 2006).

Musil and Wand (1994) reported stimulation of net CO_2 assimilation rates and growth in winter ephemeral *Dimorphotheca pluvialis*, under low, ambient doses of UV-B radiation. In our study with variegated *P. coleoides* exposed to 7.0 kJ m^{-2} d^{-1} UV-B_{BE}, combined with 48.8 and 14.2 mol m^{-2} d^{-1} of PAR, rapid stimulation of CO_2 assimilation, already after four hours was observed, and remained higher until the end of the experiment (Vidović *et al.*, 2015b). Furthermore, after nine days of treatment under high PAR with UV-B supplementation, increased influx of electrons in alternative electron pathways was detected, compared with only high PAR. These results suggested that stimulation of photosynthesis was related to an enhanced requirement for building blocks for biosynthesis of apigenin and cyanidin glycosides.

Results related to UV-B effects on stomatal conductance are controversial, due to various UV-B: UV-A: PAR ratios, and different times of exposure and metabolic state of the plant (Jansen and van der Noort, 2000), as well as previous plant exposure to UV-B radiation (Surabhi *et al.*, 2009; Klem *et al.*, 2012; Gitz *et al.*, 2013). For example, during summer months in Finland, enhanced stomatal conductance was observed in birch leaves (Kostina *et al.*, 2001) while, in two poplar populations exposed to 12.4 kJ m^{-2} d^{-1} UV-B_{BE} during summer in central China, stomatal conductance was decreased (Lu *et al.*, 2009). The presence of environmental stressors, such as drought and low nutrient conditions in combination with UV-B treatments, has a detrimental influence on CO_2 assimilation rate and stomatal conductance (Musil and Wand, 1994; Nogués *et al.*, 1998; Lu *et al.*, 2009; Arróniz-Crespo *et al.*, 2011; Doupis *et al.*, 2016).

2.6.1 Interplay of Phenolics and Photosynthesis Under UV-B Radiation

Over 20% carbon derived from the Kalvin-Benson cycle is introduced to the shikimate pathway (Jensen, 1986), which points to the significance of phenolic compounds in plant metabolism. Inspite of that, the exact relationship between photosynthesis and phenolic metabolism is insufficiently investigated (Fritz *et al.*, 2006; Hernández and Breusegem, 2010).

In contrast to the results obtained with variegated *P. coleoides* discussed above, in another variegated species (*P. zonale* cv. Frank Headley), identical UV-B irradiances, under the same experimental conditions, did not influence photosynthetic machinery (Vidović *et al.*, 2015c). Interestingly, UV-B radiation induced carbon allocation from source-green to sink-white leaf tissue, decreasing trehalose concentration and, therefore, mediating regulation of starch degradation. The involvement of trehalose-6-phosphate in carbon allocation to sink tissues (e.g. root), as well as in regulation of starch degradation, has been observed previously (Ramon *et al.*, 2007; Smeekens *et al.*, 2010). Therefore, phenylpropanoid and flavonoid accumulation under UV-B radiation is closely related to, and regulated by photosynthesis.

In two mentioned variegated species, under identical conditions, UV-B differentially affected photosynthesis (Figure 2.4). Biosynthesis of phenylpropanoids and flavonoids, especially flavonoid glycosides, which are carbon rich compounds, consumes ATP, NADPH and photoassimilates (triosophosphates). Stimulation of carbon assimilation and preferential directioning of electrons into this pathway enables enhanced flavonoid production in both leaf tissues of *P. coleoides* under UV-B radiation (Figure 2.4a). On the other hand, in the leaves of *P. zonale*, UV-B radiation stimulated starch degradation and sugar transport from source to sink leaf tissue, providing the building blocks for biosynthesis of *p*-coumaric acid, kaempferol and quercetin glycosides, which were induced in white tissue (Figure 2.4b).

Biosynthesis of phenylpropanoids and flavonoid glycosides can be regarded as an energy escape valve for photosynthetic electron transport (PET) under unfavourable conditions (Grace and Logan, 2000; Hernández and Van Breusegem, 2010). In addition, another link between the components of PET, the redox state of plastoquinone (PQ) pools and flavonoid biosynthesis was proposed. The role of over-reduced PQ pool in retrograde signalling transduction (from chloroplasts to nucleus) has been confirmed (for review see Pogson *et al.*, 2008; Suzuki *et al.*, 2012; Szechyńska-Hebda and Karpiński, 2013). In a recent study, Akhtar *et al.* (2010) showed that low UV doses provoked over-reduction of the PQ pool, followed by accumulation of apigenin and luteolin glycosides in *Lemna gibba*. The mechanism of flavonoid biosynthesis stimulation in *L. gibba* was not dependent on ROS. Furthermore, the authors proposed that UV-B might inactivate Rubisco activity inducing the over-reduction of PQ pool. Clearly, this can not be related to the *P. coleoides* case.

Some authors highlighted the importance of ROS and components of the antioxidative metabolism in promoting flavonoid and anthocyanin accumulation. Ascorbate is a cofactor for four 2-oxoglutarate-dependent oxygenases involved in flavon-3-ol and anthocyanidin biosynthesis (Turnbull *et al.*, 2004; Martens *et al.*, 2010; Tzin and Galili, 2010). Page and co-authors (2012) demonstrated that Arabidopsis mutants: *vtc1*, *vtc2* and *vtc3*, with at least 20% lower ascorbate content in the leaves after exposure to high PAR (550–650 μmol m^{-2} s^{-1}), had decreased concentration of cyanidin glycosides, while no change in kaempferol glycosides was observed. The transcript levels of all enzymes involved in anthocyanidin biosynthesis, except cinnamate-4-hydroxylase, were increased in *wt* Arabidopsis, while in mutants they were not affected.

Additional investigation with Arabidopsis mutants expressing only 7% of normal catalase activity showed reduced anthocyanin accumulation, which implicates regulatory role of H_2O_2 in activation of this pathway (Vanderauwera *et al.*, 2005). On the other hand, double Arabidopsis mutants deficient in cytosolic and thylakoid

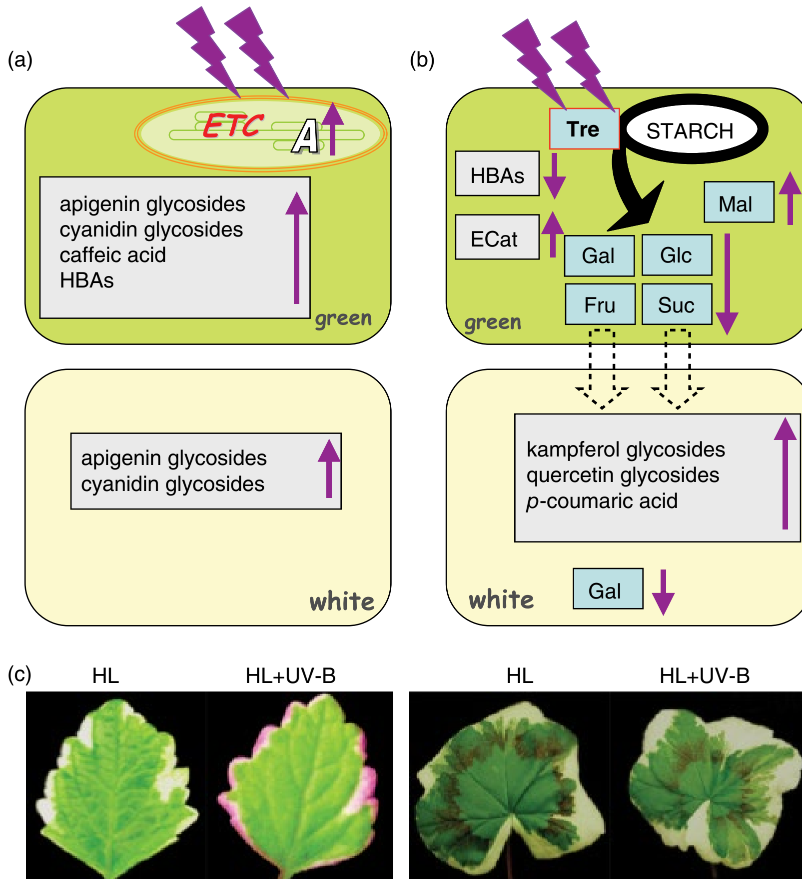

Figure 2.4 Schematic overview of the link between photosynthesis, sugar content and phenolic induction under ecologically relevant UV-B doses, in green and white leaf sectors of variegated (a) *P. coleoides* and (b) *P. zonale*. Arrow directions indicate increased or reduced concentration of specific metabolite. Dotted arrows represent transport between source and sink leaf tissue. (c) Photographs of representative leaves of *P. coleoides* (left) and *P. zonale* (right) plants after exposure to: $UV\text{-}B_{BE}$: 7.0 kJ m^{-2} day^{-1} combined with 48.8 mol m^{-2} day^{-1} of PAR (HL). Tre, trehalose; Glc, glucose; Fru, fructose; Suc, sucrose; Gal, galactose; HBAs, hydroxybenzoic acids; ECat, epicatechin; *A*, CO_2 assimilation rate; ETC, linear electron transport chain. For detailed results, see Vidović *et al.* (2015b, 2015c).

ascorbate peroxidases (and consequently increased H_2O_2 concentration) had enhanced accumulation of anthocyanins during high PAR exposure (Miller *et al.*, 2007).

Taken together, the role of H_2O_2 or altered redox homeostasis in the cell in the regulation of anthocyanin biosynthesis is rather complex. It is obvious that flavonoid biosynthesis is tightly connected with photosynthetic processes. This is also supported by the induction of 19 genes involved in flavonoid biosynthesis after exposing Arabidopsis plants to conditions which also increase CO_2 assimilation rate (such as high PAR, low temperatures, nitrogen and phosphate deficiency) whereas, during the night, these genes were suppressed (Vanderauwera *et al.*, 2005). Moreover, the species-specific, as well as source-sink dependence of UV-B effect on photosynthesis and associated processes, and induction of flavonoids has to be considered. The targets and the mechanisms of this regulation remain to be revealed.

2.7 UV-B Radiation Induces Phenolics Accumulation in Fruits

Increasing evidence of beneficial effects of phenolics for human health have drawn attention to food sources, such as fruits and vegetables, and means of improving their nutritional value. Phenolics have proved to be effective protectors against cardiovascular diseases and cancer, along with antiallergenic and antiseptic properties (reviewed by Schreiner and Huyskens-Keil, 2006; Del Rio *et al.*, 2013; Zhang and Tsao, 2016), mostly attributed to their antioxidative function (Tsuda, 2012; Carocho and Ferreira, 2013; Croft, 2016). Several factors influence the phenolics composition of foods, such as genotype, environmental conditions, storage and preparation (Giuntini *et al.*, 2008; Huyskens-Keil *et al.*, 2007; Bian *et al.*, 2014; Schreiner *et al.*, 2014).

UV radiation is considered to be stimulative for the accumulation of secondary metabolites, especially flavonoids, terpenoids and vitamins in fruits, thus increasing their nutritional, organoleptic and pharmacological value (Jansen *et al.*, 2008; Becatti *et al.*, 2009; Avena-Bustillos *et al.*, 2012; Schreiner *et al.*, 2014). However, in the glasshouses and polytunnels widely used in agriculture, most of UVR is excluded. In order to produce fruit and vegetables with a high content of bioactive compounds (i.e. phenolics), a number of experiments with manipulation of light quality and quantity, both PAR and UV-B radiation, have been performed, most of them on fruits and vegetables such as tomatoes, lettuce, peppers, berries and apples (Kolb *et al.*, 2003; Luthria *et al.*, 2006; Berli *et al.*, 2008; Wargent *et al.*, 2011; Del-Castillo-Alonso *et al.*, 2016).

Tomato (*Solanum lycopersicum*) is a high-value crop commonly grown in polytunnels. In our study, two varieties of tomato (Big Beef and Marathon) were planted at two locations in central Serbia, using three commercially available plastic covering materials with different UV transmission properties. We showed different responses regarding flavonoid accumulation in the tomato peel and skin when plants were grown in the open field (under maximal daily PAR of 1850 $\mu mol\,m^{-2}\,s^{-1}$, UV-A irradiance of 0.5 $W\,m^{-2}$ and UV-B irradiance of 1.8 $mW\,m^{-2}$), compared with polytunnels (Milić *et al.*, 2014). All three polytunnels transmitted about 50% PAR and 25% UV-A, and differed in UV-B transmittance. In Big Beef cultivar, quercetin accumulated to the highest extent in the peel of fruits from the open field, and its content was correlated with UV-B transmittance in two polytunnels (7.4% and 0.2%), while kaempferol content did not change regarding UV-B irradiances. No differences in quercetin concentration were observed in the Big Beef flesh. In Marathon fruit peels, quercetin contents were similar between open field and polytunnel with 38% UV-B transmittance, while the opposite was observed in the flesh (higher quercetin content in the flesh was in polytunnel-grown tomatoes, compared with full sunlight ones) (Milić *et al.*, 2014).

In another study by Giuntini *et al.* (2008), UV-B radiation strongly stimulated accumulation of flavonoids in two tomato lines, but at different ripening stages. The total amount of flavonoids, expressed as a sum of naringenin, quercetin, rutin, and quercetin 3-*O*-pentosylrutinoside, increased in the peel of tomatoes exposed to UV-B radiation at mature green and ripening red stages (DRW 5981 line), and at the mature green and turning stages in Esperanza line. Low content of flavonoids in UV-shielded fruits was correlated with diminished and delayed transcript levels of *CHS*. Furthermore, in two other studies, the total concentration of hydroxycinnamic acids in fully ripe tomato fruits was higher under ambient UV-B than in UV-B shielded fruits (Luthria *et al.*, 2006; Calvenzani *et al.*, 2015).

Similarly, in sun-exposed skin of Braeburn apples, accumulation of both anthocyanins and flavonoids (mostly quercetin glycosides) was increased compared with shielded ones (Solovchenko *et al.*, 2003), while Merzlyak *et al.* (2002) also reported accumulation of flavonoids (mainly rutin) and anthocyanins in full sunlight-exposed peel of cv. Zhigulevskoye apples. UV-B effects on another commercially attractive crop, grapes, were investigated by several research groups. Grape berry skin exposed to full sunlight and UV-B radiation had a higher content of anthocyanins and resveratrol, compared with filtered UV-B radiation (Berli *et al.*, 2008). Six-day exposure of white grape cultivar to natural UVR led to significant stimulation of flavonol biosynthesis (Kolb *et al.*, 2003), and both flavonols and anthocyanins increased in red grape berries exposed to UV-B radiation under controlled conditions (Martínez-Lüscher *et al.*, 2014). Moreover, in the leaves of two different grapevine genotypes (cvs. Romeiko and Soultanina) exposed to above-ambient UV-B doses, the amount of UV-B absorbing compounds was increased (Doupis *et al.*, 2016). In another study by Josuttis *et al.* (2010), although total phenolic content was similar in strawberries grown under UV-blocking film and in the open field, the phenolic profiles were different. The contents of quercetin-3-*O*-glucuronide, kaempferol-3-*O*-glucoside and cyanidin-3-*O*-glucoside were higher in fruits in the open field.

However, it should be noted that, besides reports listed above, a number of reports showed that UV-B radiation did not stimulate phenolics accumulation (Mewis *et al.*, 2012; Ordidge *et al.*, 2010; Schreiner *et al.*, 2014; Solovchenko *et al.*, 2003; Giuntini *et al.*, 2008). These discrepancies were attributed to genotypic differences, constitutive phenolics pool, and/or induction of metabolites other than phenolics (e.g. glucosinolates).

2.8 Conclusion and Future Perspectives

A literature survey on UV-B effects on plants revealed numerous inadequacies in studying relevant UV-B influence on plant metabolism. Very high UV-B doses used, unrealistic UV-B : UV-A : PAR ratios and non-standard units impede comparison of experimental outputs and reproducibility. Fortunately, in the latest reports, the authors highlighted the use of appropriate UV-B lamps and exclusion filters in experimental design, and standardized calculations of biologically UV weighting functions. Plant history and ecogeographic origin are also carefully considered, while details about growth conditions (PAR, UV-A, temperature, nutrient supplementation) ensure comparison of results with other studies and their relevance.

Naturally, UV-B radiation is associated with PAR and UV-A radiation, and should not be taken as a single, isolated factor. In addition, UV-B radiation is frequently interlaced with drought and high temperatures and can induce cross-tolerance. For example, decrease and thickening of leaf surface area, specifically induced as a morphogenetic response through UVR8 signalling pathway, reduces water loss and damage from visible light excess. Accordingly, UV-B-induced growth reduction should not be considered as a yield loss in agriculture, due to significant accumulation of secondary metabolites, which increase plant resistance to herbivores and pathogens. Moreover, secondary metabolites, such as flavonoids, have multiple beneficial effects for human health.

Great efforts have been made to discover the molecular basis of UV-B perception and signalling mechanisms in plants. However, it is still questionable how UVR8 activation is dependent on the quality and quantity of UV-B radiation. Additional complications

arise from overlapping and interaction of the UV-B signalling pathway with signalling pathways activated by other components of solar radiation (UV-A radiation and PAR). Although several new regulators of the UVR8 pathway were recently discovered, there are still components which should be characterized. Moreover, information on other pathways involved in UV-B response is scarce. For example, the role of ROS and antioxidative enzymes (especially glutathione-related), induced even by very low UV-B doses, are poorly considered.

Although phenolics accumulation is considered as general plant response to solar radiation, other factors, such as cold, metal excess and nutrient deficiency, also induce increase of their content. There is growing evidence that photosynthesis is involved in phenolics biosynthesis through redox changes in the electron transport chain. Additionally, it has been suggested that carbon-rich flavonoid glycosides act as energy escape valve. Furthermore, the general effect of UV-B radiation on photosynthetic electron transport, CO_2 assimilation and associated processes has to be elucidated.

Briefly, further research should contribute to understand the rapid changes in UV response and its dependence on specific UV-B dose and spectral characteristics. Investigating the link between primary and secondary metabolism mediated by UV-B radiation presents a challenge for future studies. The use of various plant species, and studying metabolites and their glycosylation profiles, should be included, too. Finally, the presented data lead to the conclusion that production of high quality plants for nutrition and pharmaceutical industry in protected cultivation can be achieved by using UV-B transmitting covering materials and UV-B supplementation.

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3

UV-B Radiation: A Reassessment of its Impact on Plants and Crops

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3.1 Introduction

Global climate change will lead to changes in agricultural ecosystems, and will influence plant yield and crop productivity. The impacts on agricultural crops will differ across regions, depending on the local climate and the adaptive potential of locally cultivated species. Climate change increases exposure to UV-B radiation mainly outside the tropical zone. Therefore, photoautotrophic organisms (plants and crops) have to constantly adapt to changing environmental conditions.

UV-B radiation reaches the biosphere with solar energy, the major source of energy for plant growth and development (Heisler *et al.,* 2003; Lidon *et al.,* 2012; Piri *et al.,* 2011). The levels of atmospheric CO_2 are also related to UV-B radiation reaching the surface of the Earth. UV-B can reduce the rate of CO_2 assimilation up to three-fold, thus disrupting photosynthetic processes in exposed plants (Bornman and Teramura, 1993).

Schlesinger (2000) demonstrated that the conversion of natural vegetation to agricultural land is a major source of CO_2, not only due to losses of plant biomass, but also due to increased decomposition of soil organic matter resulting from physical disturbance, and energy costs associated with various agricultural practices (fertilization and irrigation). The use of high-yielding plant varieties, fertilizers, irrigation, residue management and reduced tillage can minimize losses and increase CO_2 uptake in managed areas (Blume *et al.,* 1998). An increase in the photosynthetic efficiency of plants in the absence of solar UV components could enhance rapid fixation of atmospheric carbon dioxide, and reduce global warming. UV-B radiation significantly influences morphological, physiological and biochemical processes in agricultural crops, plants species and ecosystems. Those impacts should be taken into account in climate change assessments.

UV-B Radiation: From Environmental Stressor to Regulator of Plant Growth, First Edition. Edited by Vijay Pratap Singh, Samiksha Singh, Sheo Mohan Prasad and Parul Parihar.
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3.2 Plant Production

The impacts of global climate change on agricultural crops and plants are being researched extensively around the world. Ultraviolet-B (UV-B) radiation generally exerts a negative impact on most agricultural crops and plants. It has a more damaging influence on plants that are particularly sensitive to this environmental stressor. The effect of UV-B radiation should be analyzed comprehensively, in view of other aspects of climate change (Heisler *et al.,* 2003).

The changes induced by UV-B radiation in plant morphology and the nutritional value of vegetables, herbaceous and ornamental plants influence their appeal for consumers. Intensified UV-B radiation influences many agricultural crops, and can affect food supply (Piri *et al.,* 2011; Żuk-Gołaszewska, 2003). Adverse changes in the biometric and physiological parameters of oat plants (*Avena sativa* L.) have been observed under exposure to UV-B radiation ($UV\text{-}B_{BE} = 4\,kJ\,m^{-2}\,d^{-1}$, 15 days) (Skórska and Lewandowski, 2003).

Sosa-Flores *et al.* (2014) evaluated the effect of UV-B irradiation time on the growth and morphology of melon plants growth from irradiated seeds. Radiation contributed to an increase in plant height, stem diameter, number of leaves, leaf area, fresh weight and dry weight. Seeds exposed to UV-B radiation for 15 minutes ($99\,mJ\,cm^{-2}$) produced plants whose fresh weight and dry weight were 24.87% and 32.42% higher, respectively, in comparison with control. Radiation also decreased the concentrations of biological elements in leaves (P, Ca and Na). In a study by Shaukat *et al.* (2015), *Vigna mungo* (L.) Hepper plants grown from seeds, irradiated for 10, 20, 30 and 40 minutes, were characterized by lower fresh weight of radicles and seedling shoots in comparison with control plants. UV-B radiation also increased the content of total soluble phenols.

UV-B radiation reduces plant height and leaf area, increases leaf thickness and affects plant growth and development (Gerhardt *et al.*, 2005; Vyšniauskienė and Rančelienė, 2014). Irradiated soybean plants were characterized by a 50% reduction in the weight of aboveground parts (Szwarc and Skórska, 2007). In turn, when cotton plants (*Gossypium hirsutum* L.) were exposed to 9.5% higher UV-B radiation during the growing season, their height, leaf area and total biomass were reduced by 14%, 29% and 34%, respectively, in comparison with plants grown under natural conditions. In plants exposed to local allograft irradiation (LAI), the greatest reduction in morphological parameters was observed in the flowering and boll-filling stages. The Net Assimilation Rate (NAR) and the Relative Growth Rate (RGR) decreased, subject to the UV-B dose, and were determined at 42% and 35%, respectively, at the highest level of irradiation. Exposure to UV-B also lowered fibre quality, and led to a 72% decrease in economic yield and a 58% drop in profitability (Gao *et al.*, 2003).

Similar results were reported by Kataria *et al.* (2012) in *Gossypium hirsutum* L., by Sullivan (1997) in agricultural crops (*Glicinehispida* L. and *Oryza sativa* L.), by Yuan *et al.* (1999) in *Triticum aestivum* L., and by Tapia *et. al.* (2010) in varieties of *Cucumis sativus* L. Wheat has been found to be less sensitive to UV-B radiation than other agricultural crops. In a study by Szwarc and Skórska (2007), UV-B radiation decreased net photosynthetic rates, chlorophyll content, and the height and dry matter content of shoots.

The height of greenhouse-grown bush bean plants (*Phaseolus vulgaris* L.) decreased up to 31.8% under exposure to high levels of UV-B radiation. Irradiation reduced the fresh weight, dry weight and leaf area of bush bean plants, and delayed flowering by

one day. Growth regulators inhibiting gibberellin biosynthesis also delayed flower induction under enhanced UV-B (Saile-Mark and Tevini, 1997).

According to Biever *et al.* (2014), inhibition of hypocotyl growth in etiolated *Arabidopsis* seedlings under exposure to UV-B radiation, a photomorphogenic response, resulted from the absorption of UV-B by DNA, which can arrest the cell cycle. Exposure to UV-B radiation under field conditions enhanced the photosynthetic rate, PS II efficiency and, consequently, increased biomass accumulation and crop yield (Kataria *et al.,* 2014). Photosynthetic carbon reduction is also sensitive to UV-B radiation, which has a direct effect on the activity and content of the Rubisco enzyme.

Gerhardt *et al.* (2005) suggested that, after absorption by a UV-B chromophore, reactive oxygen species are generated by photosensitization, which ultimately leads to cotyledon curling in the analyzed agricultural crops. Plant DNA, plant proteins and membranes are highly sensitive to UV-B radiation, and have to be protected for normal growth and development (Jansen *et al.,* 1998). Despite the above, Yao *et al.,* (2014) demonstrated that amylose, amylopectin and total starch content of wheat grain was not affected by increased exposure to UV-B.

Kakani *et al.,* (2003) described characteristic visual symptoms on leaves exposed to UV-B radiation, including chlorotic or necrotic patches. The leaf anatomy was altered due to changes in the thickness of epidermal, palisade and mesophyll layers. Chlorophyll concentrations decreased by 10–70%, whereas the content of UV-B-absorbing compounds increased (10–300%) in many crops. The observed 3–90% decrease in photosynthetic rate, which was particularly pronounced under exposure to higher UV-B doses, resulted from both direct (effect on the photosystem) and indirect effects (decrease in pigments and leaf area). The decrease in chlorophyll pigments and photosynthesis resulted in lower biomass and yield of most agricultural crops. Genotypes of crop species exhibited variability in leaf wax layer thickness, loss of chlorophyll and increase in phenolics as mechanisms of tolerance to enhanced UV-B radiation resulting in changes in biomass and level yield.

Balouchi *et al.* (2009) found adverse changes in photosynthetic pigments and in physiological and biochemical parameters of durum wheat plants exposed to UV-B, UV-A and UV-C radiation. The content of carotenoids, anthocyanins, flavonoids and proline decreased significantly when UV wavelength was increased, relative to control.

UV-B radiation has shaped evolutionary processes since the beginnings of life on Earth. UV-B was not always a destructive element, and species had to adapt to new light conditions when they colonized new areas (Heisler *et al.,* 2003). Therefore, UV-B radiation also exerts positive effects on agricultural crops and plants. In plants such as tomatoes and tobacco, UV-B stimulates pathogenesis-related (PR) protein synthesis, which can directly promote resistance to pathogens (Barka *et al.,* 2000; Fujibe *et al.,* 2000; Charles *et al.,* 2009). In dune grassland plants, increased hormone levels lowers susceptibility to fungal infections (Staaij *et al.,* 2001).

In a study by Brzozowska *et al.* (2014), exposure to dispersed solar radiation during germination increased the content of L-ascorbic acid and polyphenols in red clover seeds, and it enhanced the antioxidant activity and improved the sensory properties of germs. Kumari *et al.* (2009) reported that exposure to lower levels of UV-B radiation ($1.8\,kJ\,m^{-2}\,d^{-1}$ higher than ambient conditions) increased the net photosynthetic rate, stomatal conductance and water-use efficiency (WUE) in the sweet flag. This considerably increased productivity, in particular the production of rhizome biomass.

3.3 Plant Protection Against UV-B

UV-B radiation is an important stress factor for crops and plants. Many agricultural crops and plants have many defence mechanisms that limit the harmful effects of UV-B radiation. Plants of the class *Monocotyledones* are generally more resistant to UV-B radiation due to the vertical arrangement of leaves, which protects the base of the leaf sheath and the apical meristem. Crops exposed to UV-B were characterized by a smaller leaf area (Caldwell *et al.,* 1994). Reduction of leaf area was also observed in other species of agricultural crops, including *Zea mays* L., *Helianthus annuus* L. (Saile-Mark and Tevini, 1997), *Beta vulgaris* L. (Panagopoulus *et al.,* 1990) and *Avena sativa* L. (Skórska and Lewandowski, 2003). The observed effects constitute a defence mechanism, because reduced leaf area decreases exposure to harmful radiation.

Plants exposed to UV-B also rapidly synthesize radiation-absorbing compounds, mostly flavonoids. Their specific location in the epidermal layer (mainly in leaves) protects internal cell layers by attenuating the impinging UV-B radiation at the epidermis (Braun and Tevini,1993; Tevini *et al.,* 1991). Plants generally rely on two mechanisms to alleviate the harmful effects of UV-B light (Caldwell *et al.,* 1994; Jordan, 1996; Piri *et al.,* 2011; Szwarc and Skórska, 2007). In a study by Szwarc and Skórska (2007), the defence mechanisms developed by irradiated plants involved increased activity of antioxidant enzymes (peroxidase and catalase) and higher content of flavonoids as the protective compounds. In *Arabidopsis* plants, the accumulation of UV-absorbing pigments suggests that protection against UV-B radiation is determined genetically (Li *et al.,* 1993; Lois and Buchanan, 1994).

Other authors demonstrated that exposure to UV-B can increase the content of UV-B absorbing pigments (flavonoids) in rice (Ziska and Teramura, 1992; Dai *et al.,* 1992) and barley (Liu *et al.,* 1995). Similar results were reported by Ravindran *et. al.* (2010). Antioxidant enzymes and UV-B absorbing compounds protect *Indigofera tinctoria* seedlings against oxidative damage.

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4

Interaction of UV-B with the Terrestrial Ecosystem

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4.1 Introduction

The solar energy of most interest to environmental chemistry is the UV component of sunlight. Below 290 nm, solar radiation is significantly absorbed by a thin stratospheric ozone layer that surrounds the Earth at an altitude of about 30–40 km above the earth surface, while above 400 nm, it does not provide enough energy to break most chemical bonds (Bonzongo and Donkor, 2003). UV radiation can reach the earth surface in both UV-A (315–400 nm) and UV-B (280–315 nm) ranges, while solar light UV-C (100–280 nm) is absorbed by ozone in the stratosphere. During the course of evolution, the first photosynthetic organisms were cyanobacteria, which released oxygen into the environment that led to the development of several cereal communities, until a climax community developed by the process of succession, and a stable terrestrial ecosystem established. This was possible due to the formation of the ozone layer which protects the earth from incoming UV rays, thus acting as a filter and, hence, protecting the ecosystems (Rozema *et al.*, 2005).

Terrestrial ecosystems include agricultural lands, agro ecosystems, and less intensively managed lands such as forests, grasslands, savannahs, deserts, tundra, and so on. However, in recent times, due to rapid industrialization, several toxic gases have been released from industries, including CH_4, SO_2, CO_2, CFC, NO_2, etc. The main effect of these gases has been to destroy the stratospheric ozone, leading to significantly elevated UV irradiances in the northern mid and high latitudes (United Nations Environment Program, 2012). The thickness of ozone has also been reduced as a result of ozone breakdown by chlorine released from emitted chlorofluorocarbons in the Antarctic and Arctic regions (Paul, 2001; Paul and Gwynn-Jones, 2003).

Accordingly, surface UV-B radiation has increased by about 5% (WMO, 2003). High doses of UV-B radiation cause plant stress, leading to reduced biomass accumulation, DNA damage, photosynthetic impairment and lipid peroxidation (Jansen *et al.*, 1998; Ballaré *et al.*, 2011). Elevated solar UV-B radiation may exert effects on

UV-B Radiation: From Environmental Stressor to Regulator of Plant Growth, First Edition. Edited by Vijay Pratap Singh, Samiksha Singh, Sheo Mohan Prasad and Parul Parihar.
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terrestrial ecosystems through actions on plants and microbes, as well as on some animals. Ecosystem attributes that could potentially be affected by increased solar UV-B radiation include plant biomass production, seed production, plant consumption by herbivores including insects, disease incidence of plants and animals, population fluctuations of plants and animals, and changes in species composition and mineral nutrient cycling (Zepp *et al.*, 1998). At the ecosystem level, the effects are less well understood than at the molecular and organism levels. Here, we describe major recent breakthroughs in the understanding of the enhancement effect of UV-B on terrestrial ecosystems.

4.2 Growth and Development

In any ecosystem, plants are primary producers, and they are directly influenced by solar radiation (UV-B). In recent times, it is estimated that about 6–14% UV-B increased over pre-1980 levels, and the current level is 2–12 kJ m^{-2} day^{-1} on the Earth's surface, which may increase in future years. Plants are sensitive to UV-B radiation, and this sensitivity can be species-specific. It is now generally accepted that the development of phenolic polymer metabolism, as well as flavonoids and lignin, are partly induced by UV-B, and has played a major role in the evolution of land plants. It is reported that elevated UV-B radiation causes a slight decrease in plant height growth (3–10%) and total leaf area (6–13%), above ground biomass (15–16%), while the concentrations of UV-B-absorbing compounds like DNA, protein and lipid are often enhanced (Searles *et al.*, 2001).

Growth and biomass accumulation of woody deciduous species are not expected to be strongly affected by UV-B radiation, because the leaves, with possible injuries, shed every year. Two species of *Betula* (*B. pendula* and *B. resinfera*), when exposed to UV-B supplemental levels (5–6 kJ m^{-2} day^{-1}) for up to 2.5 months, did not indicate any change, which suggested that all these birch or *Betula* populations were capable of protecting themselves against UV-B radiation (de la Rosa *et al.*, 2003).

Temperature also plays a crucial role along with UV-B which, at high temperatures, has a positive effect on plant growth but reduces the concentrations of phenolic compounds (Zvereva and Kozlov, 2006; Lavola *et al.*, 2013). Thus, it is noted that UV-B and temperature might affect plant growth and phenolic concentrations in opposite directions (Randriamanana *et al.*, 2015). The leaf perceives the light, but outdoor leaf curling occurs due to elevated UV-B levels. Due to this, leaf size has been found to be consistently decreased (Newsham *et al.*, 1999; Keiller and Holmes, 2001), although the final leaf size was not affected by irradiation (Kostina *et al.*, 2001). Radiation also causes thickening of leaves, by which the capacity of UV-B inside photosynthetic cell layers decreases due to different anatomic changes – i.e., there is conscious development of more spongy parenchyma and intercellular space, and density of glandular trichomes increases (Kostina *et al.*, 2001). Under elevated levels of UV-B, stimulation of height and reduction in diameter has been observed (Tegelberg *et al.*, 2001). This UV-B sensitivity is associated with the accumulation of soluble sugars, mainly glucose, in the stems, suggesting disturbances in carbon utilization (Tegelberg *et al.*, 2002).

4.3 Secondary Metabolites

Every plant produces a diverse array of compounds which have no direct role in growth and development. Secondary metabolites are mainly three types – terpenes, phenolics and alkaloids. Among these, phenolics are most affected by UV-B. The two key enzymes PAL (Phenylalanine ammonia lyases) and CS (chalcone synthase) are involved in the biosynthetic pathway of phenolics. These compounds are produced from aromatic amino acids (phenylalanine), via the phenylpropanoid pathway, where several steps are affected by UV-B.

Various derivatives of phenolics are present as cinnamic acids, chlorogenic acids, glycosylated flavonoids and aglycons and high amounts of salicylates and polymeric tannins (Laitinen *et al.*, 2002). The functions of these compounds are involved in defence processes, to attract pollinators, and are used in medication and to activate other pathways (e.g. to activate the jasmonic acid pathway (Lavola *et al.*, 1997; Schmid *et al.*, 2001)). The concentration of these secondary metabolites (mainly flavonols and flavones of flavonoides) are increased excessively, because these compounds absorb harmful UV-B radiation, act as antioxidant and minimize the generation of ROS. Hence, it is stated that UV-B increases the level of secondary metabolites.

4.4 Susceptibility to Herbivorous Insects

Herbivorous insects play a peculiar role in ecosystem functioning at a particular tropic level, but the nature of insects is ramified to different tropic levels, due to elevated UV-B radiation. Plants have the ability to protect themselves from herbivores by primary and secondary compounds and, if the characteristics of these compounds change, it also changes the behaviour of insects, although the effect is extremely low.

UV-B affects herbivores either directly or indirectly. Direct effects include changing their behaviour or physiological processes (Buck and Callaghan, 1999; Antignus *et al.*, 2001; Veteli *et al.*, 2003), while indirect effects include affecting the host plant quality, predators, parasitoids and pathogens (Roberts, 2001; Veteli, 2003). Some insects have the capacity to absorb UV, so increasing UV-B radiation may make plants more apparent to their herbivores, resulting in higher numbers of insect observed on plants under UV-B stress. Changes in the phytochemistry of the plant can affect the choice of host plant, amount of feeding, and the performance of herbivores. Flavonoids alter the feeding behaviour of the insect but inhibit the growth of the insect. Insects that feeds on leaves exposed with UV-B show altered patterns of growth, survivorship and feeding (McCloud and Berenbaum, 1999; Lindroth *et al.*, 2000).

4.5 Plant Sexual Reproduction

With regard to their response to UV-B, the vegetative characteristics of plants have been most studied, but plants reproduction capacity has also been regulated in recent literature. In plants, flowers occupy the topmost position and are continuously exposed to solar radiation, so therefore they develop some strategies to tolerate elevated UV-B radiation.

UV-B can affect sexual reproduction by affecting flowers' colour development, phenology, size or number, pollination success and seed numbers (Llorens *et al.*, 2005). Petal and fruit colour are most affected by UV-B, due to accumulation of UV-B absorbing pigments such as flavonoids, anthocyanins (Hennayake *et al.*, 2006).

Apple pre bud-treatment that blocked UV-B exposure led to decrease anthocyanin production and the flowers developed pink petals instead of red petals (Dong *et al.*, 1998). As a consequence, floral temperature and the capacity of the flowers to attract pollinators or nectar thieves was changed, ultimately affecting plant reproductive success. Fruit colour plays a significant role in dispersing the seeds when animals eat fruits, and it also is an indicator of toxicity of unripe fruits. Apart from impacts on coloration, flower size and number are also affected that ultimately affects pollination, because pollinators prefer large flowers, and pollinate the plants having maximum flowers. Flower size decreases, due to changes in petal numbers and bract area (Essenberg, 2012; Barbir *et al.*, 2014; Kakani *et al.*, 2003). The length of sepal also decreases when exposed to UV-B radiation, as observed in *Brassica napus* (Qaderi and Reid, 2005).

Interaction between plants and pollinators are of crucial importance for natural communities and for agriculture, and this relationship depends on plant and pollinator species, as well as time and speed of wind (Kwak and Jennersten, 1986; McCall and Primack, 1992). It is noticed that when there is high UV-B radiation, high numbers of pollen grains are released (Munoz-Rodríguez *et al.*, 2011). Insect vision has the capability to detect UV, and there are UV-reflectant patterns and nectar guides on many flowers (Peter and Johnson, 2008). Most pollinator insect eyes have photoreceptor cells which are sensitive to UV, blue, and green wavelengths, but their sensitivity varies among different orders (Briscoe and Chittka, 2001). For instance, Hymenoptera show sensitivity around 340 nm (i.e. UV-A region), the green receptors around 535 nm and the blue receptors around 430 nm (Peitsch *et al.*, 1992), while thrips prefer low UV-B environments that perceive UV-B radiation (Mazza *et al.*, 2010).

Interestingly, it is noticed that honeybees and nectar thieves avoid visiting flowers when UV-B is present at high levels (Stephanou *et al.*, 2000) and, consequently, pollination success decreases. To tolerate this mechanism, plants increase the size of nectaries, by which honeybees and nectar thieves stay longer (Stephanou *et al.*, 2000). Thus, pollination success increases, both in terms of number of seeds per fruit and total seed mass per plant (Manetas and Petropoulou, 2000). In conclusion, the most susceptible stage to suffer UV-B stress is pollen, during the anther dehiscence and pollen tube penetration into the stigma. In annual species, increases in UV-B radiation tend to delay (or not affect) the onset of flowering, which can also be interpreted as a regulatory response to stress.

4.6 Genomic Level

The DNA of all creatures is subjected to damage by environmental and chemical agents, including ultraviolet (UV) light, ionizing radiation and chemical mutagens. Under high UV-B radiation, plants alter their morphological, physiological and molecular responses (Casati and Walbot, 2003; Zu *et al.*, 2010), as well suffer from damage to macromolecules such as DNA, RNA and proteins. This results in a reduced net photosynthesis rate, and modification of the activities of some antioxidant enzymes (Ries *et al.*, 2000; Feng *et al.*,

2003; Agrawal *et al*., 2009). However, the plants cope with this damage and develop several strategies to repair DNA damage, because DNA damage causes deleterious mutations in plants. For example, a locus named UV Resistance Locus 8 (UVR8), identified in *Arabidopsis thaliana*, activates morphological changes, antioxidant mechanisms, photorepair and accumulation of UV-B photoprotective compounds (Rizzini *et al*., 2011; Heijde and Ulm, 2012).

The main target of UV-B is phenolics production, and several key steps in the formation of these compounds are up- and downregulated by some transcription factors either reduced or induced by solar radiation. MYB is a important transcription factor that regulates the genes for enzyme PAL, CHS and ANS (Davies and Schwinn, 2003), and MYB is also involved in the regulation of the metabolism of phenylpropanoid compounds, such as flavonoids in red apples and grape vines subjected to sunlight (Takos *et al*., 2006; Matus *et al*., 2009). Hydroxycinnamic acids (HCAs) are a derivative of phenylalanine widely distributed in plants consumed as beverages, and are health-protectants due to their capacity to scavenge free radicals, which prevents DNA and lipid peroxidation by reactive oxygen species (El-Seedi *et al*., 2012). Under low UV-B radiation, two R2R3-MYB genes have been shown to be the negative regulators of the HCA biosynthesis. AtMYB4, from *Arabidopsis*, is a repressor of cinnamate 4-hydroxylase and reduces the synthesis of sinapate esters but, in the presence of UV-B light, AtMYB4 is repressed, thus resulting in an increase of sinapate esters production in leaves (Jin *et al*., 2000).

The duration of UV-B exposure or fluence rate is regulating factor. High fluence rate stimulate the expression of genes involved in the perception and signalling of stress, wound and defence responses (Stratmann, 2003). Tomato varieties having hp-1 mutant are characterized by exaggerated photo responsiveness and increased fruit pigmentation, due to more HCA synthesis at low UV-B (Calvenzani *et al*, 2015).

UV-B damages the DNA by changing its nucleotide sequences, or by breaking strands, or by a deamination process. Therefore, to cope with DNA damage, plants develop DNA repair techniques, in which several proteins are involved and act as a single unit. DNA repair is carried out by several processes, including photoreactivation, mismatch repair (either direct or indirect), base excision repair, nucleotide excision repair and homologous recombination. Among these, homologous recombination, mediated by RecA-mediated DNA repair machinery, is the most well characterized DNA damage repair system (Rowan *et al*., 2010). In *Arabidopsis*, five putative RecA homologues were identified in the genome and predicted to be localized in mitochondria and chloroplasts (Peng *et al*., 2012; Shedge *et al*., 2007). DNA-damage repair/tolerance 100 (DRT100) is one of the plant RecA proteins, and localizes in chloroplasts (Rowan *et al*., 2010).

4.7 Conclusion

From this chapter, it is clear that enhanced UV-B radiation causes deleterious effects on the ecosystem due to ozone depletion. Firstly, it affects growth and development, with a slight decrease in plant height (3–10%), total leaf area (6–13%) and above ground biomass (15–16%) as well as increases leaf thickening and reduces photosynthetic cells, so that stems start accumulating soluble sugars (mainly glucose). DNA damage is the ultimate effect of UV-B radiation, as it removes or breaks nucleotide sequences, resulting in reduced net photosynthetic rate. It also reduces sexual reproduction, by

changing the number of petals and sepals, as well as flower size and numbers, and it changes the fruit colour, ultimately reducing the seed dispersion of plants.

In order to cope with the damage due to excessive UV-B radiation, plants, at the biochemical level, synthesize more and more solar UV-B-absorbing compounds, including several phenols, flavones and flavonoids, other photoprotective agents such as carotenoids and proteins, and some antioxidants system as well. The changes for adaptation at morphological level include an increase in the amount of nectaries and the diameter of nectar-producing glands, by which insects stay longer to pollinate plants. Thus, the damaging effects of UV-B affect the plants at morphological, biochemical and genetic level.

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5

A Review on Responses of Plants to UV-B Radiation Related Stress

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5.1 Introduction

The sun radiates energy in a wide range of wavelengths, and the non-ionizing part of the electromagnetic spectrum is ultraviolet radiation. Further, UV radiation is divided into three ranges: UV-A (315–400 nm), UV-B (280–315 nm) and UV-C (100–280 nm). Among these, the UV-C region (100–280 nm) is completely absorbed by the stratospheric oxygen/ozone and is not received at the earth surface. The UV-A region (315–400 nm) is not much attenuated by ozone (O_3), and more than 70% is received at the earth's surface. The UV-B region (280–315 nm) is largely absorbed by ozone, and about 20% reaches the earth's surface.

Although UV-B is a minor component of sunlight, its potential for causing biological damage has become a point of concern, due to its high energy. However, over the last few decades, UV-B in the biosphere has increased, due to substantial reduction of the stratospheric ozone layer in the upper atmosphere (McKenzie *et al.*, 2011). Transmission of UV-B is mainly controlled by ozone, and ozone is depleted by gases such as CFCs (CFC-11, CFC-12, and CFC-113), with a high potential to deplete ozone and a half-life ranging from 50 to 150 years (Dentener *et al.*, 2001; Kakani *et al.*, 2003b).

However, some astronomical parameters, such as solar zenith angle, as well as physical characteristics of the earth's surface, like altitude, albedo and meteorological conditions, also affects the transmission of UV-B (Madronich *et al.*, 1998; Porfirio *et al.*, 2012). Catalytic destruction of ozone molecules by atomic halogens in the stratosphere can result in a decrease of UV radiation absorption, and each 1% reduction in ozone results in an increase of 1.3–1.8% in UV-B radiation reaching the biosphere (Caldwell and Flint, 1994; McKenzie *et al.*, 2003). Though the Montreal Protocol is working, recovery of O_3 layer is not expected before 2070 (Caldwell *et al.*, 2007).

UV-B Radiation: From Environmental Stressor to Regulator of Plant Growth, First Edition. Edited by Vijay Pratap Singh, Samiksha Singh, Sheo Mohan Prasad and Parul Parihar.
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All living organisms of the biosphere are exposed to UV-B at intensities that vary with the solar angle and the thickness of the stratospheric ozone layer. The amount of increase of UV-B is dependent mainly on latitude, with the greatest increases in Arctic and Antarctic regions (Zlatev *et al.*, 2012). Global terrestrial UV-B radiation levels have been found in the range of 0 and 12 kJ m^{-2} day^{-1} near the equator, while mid-latitudes receives higher doses of UV-B (McKenzie *et al.*, 2011). Since 1980, there has been an increase of 6–14% in the level of UV-B radiation (Forster *et al.*, 2011). Studies on ozone predictions, based on GISS, UKMO and DLR, indicate that increases in the annual Northern Hemispheric UV doses are predicted to be 14% between 2010–20 and 2% in 2040–50. In the Southern Hemisphere, 40% enhancement is expected during 2010–20, and 27 % during 2040–50 (Taalas *et al.*, 2000).

Life evolved from unicellular forms under the sea to multicellular forms on Earth's surface, this could have happened due to the formation of an ozone layer that reduced ultraviolet-B (UV-B) radiation received on the Earth surface by about 10,000 times (Canuto *et al.*, 1983; Rozema *et al.*, 1997). However, current rate of changes in the atmosphere, due to anthropogenic activities, can threaten life on the Earth's surface. Though UV-B radiation comprises only a small portion of the electromagnetic spectrum (only < 0.5%)it has pleiotropic effects on both plants and animals because of many biologically important macromolecules and cellular components, such as nucleic acids, proteins, lipids and quinones which can absorb UV-B radiation directly (Jordan 1996; Caldwell *et al.*, 2003; Heisler *et al.*, 2003).

Variation in UV-B radiation affects various tropic level interactions and processing of biogeochemical cycles (Ballaré *et al.*, 2011). UV-B induces plant photomorphogenic development and several regulatory effects on plant morphology, development, physiology and biochemical composition (Jansen *et al.*, 1998; Brosché and Strid, 2003; Caldwell *et al.*, 2003; Frohnmeyer and Staiger, 2003; Kataria and Guruprasad, 2012a, 2012b; Huang *et al.*, 2013). In mammalian systems, UV-B radiation hampers molecular processes such as transcription and replication, which results in reduction of RNA synthesis, arrest of cell cycle progression and apoptosis (Sancar *et al.*, 2004). The effects of ambient UV-B radiation on plants in respect to plant growth and morphological structure, biochemical metabolism, plant genetic material, UV-B-absorbing compounds and protection against UV-B are reviewed in this chapter.

5.2 Morphological and Yield Response to UV-B

Plants, being sessile organisms, are unavoidably exposed to different type of 'stress' at every stage of their life cycle, which can lead to disruption of metabolic processes at the molecular, cellular, organism or even at ecosystem level (Bijlsma and Loeschcke, 2005). The fluence of UV-B is getting higher at the earth's surface as the stratospheric ozone decreases (Ormrod and Hale, 1995). The effects of UV-B on diverse species of plants have been reported in the literature, and it is evident that different responses are observed at different UV-B fluence rates (Brosché and Strid, 2003; Frohnmeyer and Staiger, 2003).

Numerous physiological and metabolic responses in animals and plants are mainly controlled by the wavelength of UV, fluence rates and duration of exposure to a particular wavelength. Ultraviolet-B radiation is an energetic intrinsic component of sunlight

and, at high fluence rates or ambient condition, it can affect various physiological and metabolic processes (Jordan 1996; Pal *et al.*, 1997; Mazza *et al.*, 1999; Pal *et al.*, 2006; Guruprasad *et al.*, 2007; Moussa and Khodary, 2008). In plants, UV-B exerts physiological damage to the photosynthetic apparatus (Musil *et al.*, 2002; Correia *et al.*, 2005), damage to DNA (Schmitz and Weissenbock, 2003), has effects on membranes (An *et al.*, 2000) and causes morphological damage, such as stunting of plants and leaf discoloration, as well as reducing vegetative biomass and grain yield (Kakani *et al.*, 2003a; Yao *et al.*, 2008).

Variability in crop exists in responses to increased UV-B radiation, possibly due to differences in the sensitivity of plant species (Teramura, 1983), and the sensitivity may be within and between plant species (Hidema and Kumagai, 2006) and also depends on developmental stage and experimental conditions (Al-Oudat *et al.*, 1998). Extensive studies on different crops, including wheat, maize, cucumber, rice, soybean and others, have shown a tremendous interspecific variability in plants in response to UV-B radiation, although the reason behind these variations are yet to be fully explored (Musil *et al.*, 2002; Li *et al.*, 2002; Zuk-Golaszweska *et al.*, 2003; Kataria and Guruprasad, 2012a, 2012b).

The effects of UV radiation are higher in tropical climates, as the plants are exposed to sunlight for longer duration. Studies on tropical crops such as *Vigna mungo*, *V. radiata*, and *Glycine max* (Mazza *et al.*, 2000; Amudha *et al.*, 2005; Guruprasad *et al.*, 2007), *Triticum aestivum* (Kataria and Guruprasad, 2014), *Amaranthus tricolor* varieties (Kataria and Guruprasad, 2014) and *Oryza sativa* (Teramura *et al.*, 1991) have shown retarded growth, reduced leaf area expansion and yield on exposure to ambient UV-B. The most likely reason for reduction in the growth is direct damage to DNA (Giordano *et al.*, 2004).

UV-B radiation also affects the reproductive or floral morphology of crop plants and, in turn, results in the lowering of yield. It was found that cotton flowers produced on plants exposed to UV-B treatments were smaller due to reduced petal and bract size, and had reduced anther number (Kakani *et al.*, 2003a, 2003b). As cotton floral morphology is sensitive to enhanced UV-B radiation, pollination, then boll formation and development and, finally, the lint yield, could also be affected.

Evidence from *in vitro* experiments shows that pollen germination and tube growth was inhibited by exposure to enhanced UV-B (Torabinejad *et al.*, 1998; Conner and Neumeier, 2002; Koti *et al.*, 2004). A study with 34 plant species showed that UV-B radiation reduced pollen germination and, more severely, the pollen tube growth. The authors reported that pollen tube lengths of crop species (corn, rye and tobacco) were reduced by 10–25%, depending on crop species, which would severely limit fertilization and the yield-forming capability of these crops.

In addition, UV-B radiation may also affect nitrogen (N) cycling of ecosystems, by decreasing mobilization of N into the microbial biomass, the decomposition rate of litter, and the N concentration of plants (Moody *et al.*, 2001; Johnson and Lapadat, 2002). In plants, UV-B radiation caused a decrease in the nitrate reductase activity of maize (*Zea mays L.)* (Quaggiotti *et al.*, 2004) and barley (Ghisi *et al.*, 2002). However, in sun hemp (*Crotalaria juncea*), the opposite result was reported (Saralabai *et al.*, 1989; Rail 1998). Ghisi *et al.* (2002) observed significant reductions in the activities of nitrate reductase and glutamine synthetase in barley, not only in the UV-B-receiving leaves, but also in the root system. Reduction in nitrogen concentration on exposure to

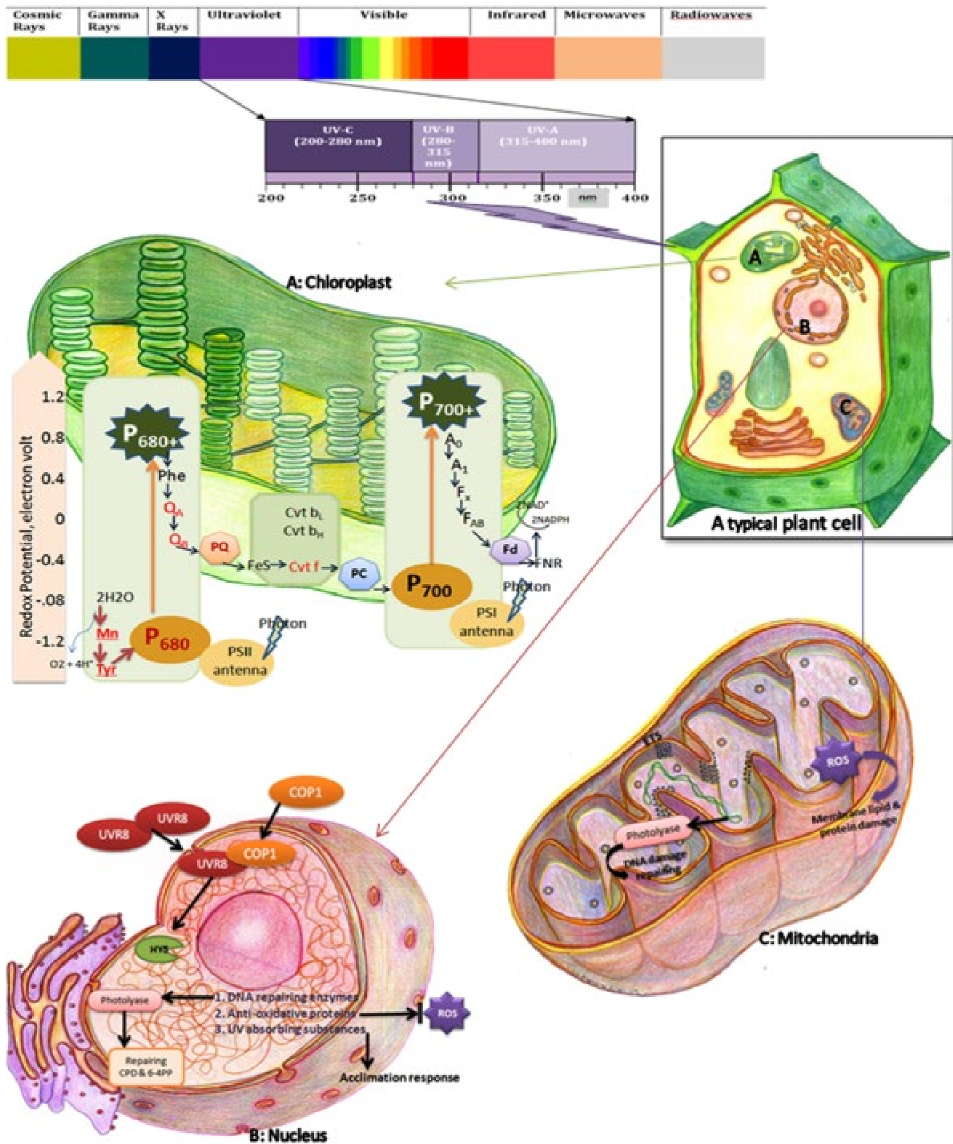

Figure 5.1 Plants' response to ambient UV-B radiation at cellular and molecular level (schematic presentation), emphasising the effect of ambient UV-B radiation on major cell organelles like Chloroplast (A), Nucleus (B), and Mitochondria (C).
(A) Chloroplast: UV-B radiation causes reduction in photosynthesis due to loss of thylakoid membrane integrity, chlorophyll pigments and downregulation of genes associated with photosynthesis. UV-B targets several components of Z scheme in chloroplast (text in red). Abbreviations: Mn- manganese complex containing four Mn atoms, bound to Photosystem II (PSII) reaction centre; Tyr- tyrosine in PSII; O_2- oxygen; H^+- protons; P680 (Primary electron donor)- PSII reaction centre in chlorophyll (Chl), on receiving a photon of light, P680 gets excited to P680*; Phe- pheophytin molecule, primary electron acceptor of PSII; Q_{A-} plastoquinone molecule; Q_B- loosely bound plastoquinone molecule to PSII; FeS- Rieske iron sulphur protein; Cyt.f- Cytochrome f; Cytb6(L & H) for Cytochrome b6 (low & high Energy); PC- copper protein plastocyanin; P700- primary electron donor of PSI excited to P700* by absorbing energy; Ao- chlorophyll molecule and primary electron acceptor of PSI; A1- phylloquinone

supplemental UV-B was also observed in the roots of *Glycine max* and *Phaseolus vulgaris*, indicating suppressive action of UV-B on nitrogen fixation (Singh, 1995). In a UV-B supplementation experiment, decrease in root length was found in *Acorus calamus*, and also root biomass in soybean (Feng et *al.*, 2003), *Trigonella foenumgarecum* (Sharma and Guruprasad, 2012), *Calamagrostis purpurea* (Jones and Johanson, 2006) and *Lolium perenne* (Comont *et al.*, 2013).

5.3 Targets of UV-B in the Carbon Fixation Cycle

UV-B radiation is one stress which has become a serious point of concern for plant biologists (Shanker, 2006), as plants require sunlight for photosynthesis and, during photosynthesis, they are continuously exposed to extreme variations in the level of solar radiation, including UV radiations. Hence, it is important to understand how plants protect and increase their tolerance against the potentially damaging effects of UV-B (Figure 5.1; Brown *et al.*, 2005; Kakani *et al.*, 2003b).

In plants, photosynthesis is the only physico-chemical process through which carbon can be fixed in the form of organic compounds by using light energy. In the photosynthetic process, sunlight trapped by the chloroplast passes through a set of complex protein molecules, arranged on a highly organized membrane, and a series of energy-transducing reactions converts it in to ATP, NADPH and organic matter. Exposure to UV-B radiation causes inactivation of light-harvesting complex II, and alters gene expression for synthesis of PSII reaction centre proteins. Also, the Mn cluster of the water oxidation complex is the most important primary target of UV-B stress, while D1 and D2 proteins, quinone molecules and cytochrome b6/f are the subsequent targets of UV-B (Kataria *et al.*, 2014).

Exposure to UV-B results in the inhibition of photosynthesis in pea (Nogues and Baker 1995), cotton (Zhao *et al.*, 2004) and oilseed rape (Allen *et al.*, 1997). Solar UV radiations are known to reduce photosynthetic efficiency by impairing some important processes of photochemical reactions in thylakoid membranes, enzymatic processes in the Calvin cycle and stomatal limitations to CO_2 diffusion (Allen *et al.*, 1998; Keiller *et al.*, 2003) of the plants by causing structural and functional changes in the chloroplast. Besides these, many genes associated with photosynthetic proteins (32 kDa PSII

Figure 5.1 (*continued*) (Vitamin K) molecule; FX, FA, and FB- three separate iron sulphur centres; FD- ferredoxin; FNR- Ferredoxin-NADP-oxido-Reductase(FNR); NAD^+-Nicotinamide adenine dinucleotide; NADPH is the reduced form of $NADP^+$.
(B) Nucleus: Plants sense UV-B through UVR8 photoreceptor that activates a UVR8-dependent photo-morphogenesis, signalling leads to interaction with the E3 ubiquitin ligase COP1 and stabilization of the bZIP transcription factor HY5 that transmits the UV-B signal resulting in changes in gene expression, which further leads to encoding of proteins helps in UV protection by (1) increasing level of DNA repair enzymes (e.g. photolyases) can act on CPDs and 6-4 PPs lesions (2) Increased anti-oxidative proteins (antioxidants) can act as ROS scavengers and (3) Increased level of UV-absorbing sunscreens gives acclimation response Abbreviations: HY5-Elongated hypocotyl 5; UVR8- UV resistant locus 8; COP1-constitutive photomorphogenic 1; ROS- Reactive oxygen species.
(C) Mitochondria: In the presence of UV-B, mitochondrial DNA (mtDNA) becomes photoactivated, and UV-induced polymerase (DNA repair enzyme CPD photolyase) helps in repairing of DNA; also, UV radiation causes release of ROS from the electron transport chain, resulting in membrane lipid and protein oxidation. Abbreviations: ROS-Reactive oxygen species; Cyclobutane pyrimidine dimer (CPD)].

protein, Lhcb, Rubisco, etc) are downregulated (Allen *et al.*, 1998; Nogues and Baker, 1995; Takeuchi *et al.*, 2002). UV-B causes dilation of thylakoid membranes and disintegration of the envelope around chloroplasts (He *et al.*, 1994), inhibition of photosynthetic enzymes (Teramura and Sullivan, 1994), reduction of electron transport rate (Pfündel *et al.*, 1991), and damage to PSII (Caldwell, 1981), photosynthetic pigment concentrations and leaf anatomy (Teramura, 1983; Feng *et al.*, 2003).

Anatomical response towards UV-B in plant leaves have also been reported, such as increase in leaf thickness due to UV-B (Bornman and Vogelman, 1991; Nagel *et al.*, 1998), trichomes on the abaxial leaf surface (Barnes *et al.*,1996), reduction in number and diameter of xylem tubes, decreased stomatal frequency and distorted leaf area (Lingakumar and Kulandaivelu 1993). UV-B exposure results in reduction in net carbon assimilation capacity (photosynthesis) which, in turn, affects biomass allocation and growth (Musil, 1996). Increase in yield is administered by the rate of net CO_2 assimilation, by available light energy, conversion efficiency of intercepted light into biomass, and the proportion of biomass partitioned into grain (Russell *et al.*, 1989; Long *et al.*, 2006; Murchie *et al.*, 2009).

Many researchers have reported reduction in biomass accumulation due to UV-B exposure in several trees (Searles *et al.*, 1995; Liu *et al.*, 2005) and crop species (Kakani *et al.*, 2003a) as the rate of biomass production is directly proportional to the rate of photosynthesis, leaf area index and light intercepting efficiency. It has been reported in *Amaranthus tricolor* varieties that the efficiency of PSII (F_v/F_m), rate of photosynthesis and stomatal conductance are significantly enhanced, along with a remarkable increase in carbonic anhydrase, PEP carboxylase and total soluble proteins (Kataria and Guruprasad, 2014).

5.4 Photoreceptors and Signalling Pathway in Response to UV-B Radiation

Plants try to acclimatize according to variations in the UV-B fluence rate by several defence responses, including morphological changes, accumulation of effective UV-screening compounds, production of increased amounts of antioxidants and stimulation of DNA repair, as well as other regulatory adjustments. Perception of specific light is beneficial for optimization of photon capture for photosynthesis and other responses also regulated by light, including de-etiolation, phototropism, shade-avoidance, stomatal opening and the intracellular distribution of chloroplasts in response to weak or strong light intensity. Moreover, reproductive achievements, including germination and flowering, are also affected by perception of light by the plant (Sullivan and Deng, 2003; Kami *et al.*, 2010; Arsovski *et al.*, 2012).

Photomorphogenic response in plants is regulated by several photoreceptors associated with signal transduction pathways, that create a link between environmental stimuli and physiological responses (Figure 5.1; Jiao *et al.*, 2007). With the introduction of molecular genetics, the functions of different photoreceptors in photomorphogenesis have been established; the phytochrome photoreceptors detect principally red and far-red light, whereas the cryptochromes, phototropins and zeitlupe family proteins detect UV-A and blue light (Christie, 2007; Li and Yang, 2007; Franklin and Quail, 2010). For example, in Arabidopsis (*Arabidopsis thaliana*), more than 13 photoreceptors take part

in photomorphogenic response, including five red/far-red perceiving phytochromes (phyA-E), two phototropins (phot1 and phot2), two cryptochromes (cry1 and cry2) and three members of the Zeitlupe family (ZTL, FKF1 and LKP2) to perceive blue light, and recently characterized UV-B photoreceptor UVR8 (Kami *et al.*, 2010; Heijde and Ulm, 2012).

Low levels of UV-B light educe photomorphogenic responses in plants (Frohnmeyer and Staiger, 2003; Ulm and Nagy, 2005; Jenkins, 2009; Jiang *et al.*, 2012; Heijde and Ulm, 2012). Significant achievements have been made in the last few years in the identification and functioning of additional components involved in this UV-B-specific signalling pathway. It has been shown that UV-B-specific UV Resistance Locus 8 (UVR8) and the multifunctional E3 ubiquitin ligase Constitutively Photomorphogenic 1 (COP1) are main regulators of the UV-B response, and their interaction in the nucleus helps in acclimation and protection in the natural environment against UV-B radiation. This was further confirmed by using uvr8-null mutants, and it was found that these mutants are deficient in UV-B-induced photomorphogenesis and hypersensitive to UV-B stress, whereas overexpression of UVR8 results in enhanced UV-B photomorphogenesis, acclimation and tolerance to UV-B stress (Favory *et al.*, 2009).

Studies on *Arabidopsis thaliana* have established that the protein encoded by *UV Resistant Locus 8* (*UVR8*) controls the expression of numerous genes involved in acclimation, as well as protection against UV-B radiation. Studies have indicated that the proteins Repressor of Photomorphogenesis1 (RUP1) and RUP2 interact with UVR8, and are negative regulators of the UVR8 pathway (Cloix *et al.*,2012). The genes regulated by UVR8 include genes involved in the biosynthesis of flavonoids (protective phenolic sunscreens), the gene encoding a cyclobutane pyrimidine dimer (CPD) photolyase (UVR2, which is essential for repair of UV-B-induced DNA damage), and genes connected with protection against oxidative stress and photooxidative damage.

Plants showing resistance and protection against UV-B are highly efficient in the synthesis and accumulation of phenol compounds, especially flavonoids and hydroxycinnamic acids. It has been demonstrated that uvr8 mutants exposed to supplementary UV-B under controlled environment conditions accumulate smaller amounts of phenolics than their respective wild types (Kliebenstein *et al.*, 2002; Brown *et al.*, 2005; Demkura and Ballare, 2012).

Regulation of genes involved in flavonoid metabolism are mediated by activation of the bZIP transcription factors Elongated Hypocotyl 5 (HY5) (Ulm *et al.*, 2004; Brown *et al.*, 2005) and HY5 Homolog (HYH) (Brown and Jenkins, 2008). Structurally, UVR8 is a symmetric homodimer of seven-bladed β-propeller, without any external cofactor as the chromophore; arginine (Arg) residues like Arg 286 and Arg 338 stabilize the homodimeric interface by making elaborate intramolecular cation–π interactions with surrounding Trp 285 and Trp 233, tryptophan amino acids that collectively serve as the ultraviolet-B chromophore (Wu *et al.*, 2012).

Exposure to UV-B radiation destabilizes the intramolecular cation–π interactions, causing disruption of the critical intermolecular hydrogen bonds mediated by Arg 286 and Arg 338, and subsequently results in dissociation of the UVR8 homodimer. The UVR8 monomer then associates with COP1, ultimately resulting in the activation of ultraviolet-B-responsive genes, which starts a cascade for signalling pathway for ultraviolet protection (Wu *et al.*, 2012). However, underlying mechanism by which UVR8 senses ultraviolet-B is still unknown. Comprehensive studies on uvr8 mutant

Arabidopsis plants reveals that the UVR8 photoreceptor is essential in order to maintain photosynthetic efficiency as, in mutants, both F_v/F_m and PSII decreased, showing greater susceptibility to photoinhibition than wild types exposed to elevated UV-B (Davey *et al.*, 2012).

5.5 Acclimatization and Protection in Response to UV-B

A low fluence rate of UV-B upregulates the expression of range of genes involved in UV-B protection, such as synthesis of flavonoids compound (Brosché and Strid, 2003; Casati and Walbot, 2003; Frohnmeyer and Staiger, 2003; Ulm *et al.*, 2004). However, at high fluence rates, UV-B causes damage to DNA, lipids and proteins by generating reactive oxygen species (Kliebenstein *et al.*, 2002). Exposure to UV-B provokes two effective systems in response. The first is the production of secondary metabolites that effectively absorb UV-B, and the second is antioxidant defence systems (Jenkins and Brown, 2007). The biosynthesis of secondary metabolites plays a major role in protecting plants from UV-B damage. These include phenylpropanoids such as cinnamoyl esters, flavones, flavonols and anthocyanins esterified with cinnamic acids after irradiation with UV-B (Wellmann, 1983).

In addition to phenylpropanoids, other important products of the shikimic acid pathway, such as furanocoumarins, and polyketides and terpenoids such as canabinoids, also accumulate under increased UV-B radiation. Flavonoids usually absorb the light in the region of 280–320 nm and, consequently, are able to act as a UV filter (Singh *et al.*, 2012; Schaller *et al.*, 2013), thus protecting the photosynthetic tissues from damage (Treutter, 2005). On the other hand, UV-absorbing substances (UAS) act as sunscreens, preventing it from penetrating into the leaf mesophyll cells (Landry *et al.*, 1995; Bieza and Lois, 2001).

UV-absorbing substances are produced and deposited in leaf epidermal cells or hairs (Manetas, 2003). However, the effects of UAS are species-specific, as either increases (Poulson *et al.*, 2006) or no change (Cechin *et al.*, 2007) in their concentration has been reported. Besides UAS, carotenoids have antioxidant properties that act against UV-B radiation. Some of the genes identified so far as being regulated by UV-B encode proteins involved in the biosynthesis of protective pigments, DNA repair and antioxidative enzymes, genes regulating photosynthesis, cell cycle genes and stress genes (Brosché and Strid, 2003).

5.6 Oxidative Stress and Antioxidant System in Response to UV-B

During metabolic processes, ROS are generated routinely in chloroplasts, mitochondria and peroxisomes. In chloroplasts, $O_2^{\bullet-}$ and H_2O_2 are mainly produced by the electron acceptor of photosystem I, whereas singlet oxygen is generated by the transfer of an electron from an excited chlorophyll molecule to molecular oxygen (Asada and Takahashi, 1987; Hernandez *et al.*, 1995). The generation of ROS was also reported in the electron transport chain of chloroplast and mitochondria during abiotic stress condition (Zhang *et al.*, 1990; Nawkar *et al.*, 2013). At high fluence UV radiation, ROS

generated from chloroplasts and mitochondria causes membrane lipid and protein oxidation, mitochondrial transmembrane potential (MTP). Loss from mitochondria results in cytochrome C release and activation of caspases which, in turn, causes DNA laddering (Nawkar *et al.*, 2013).

When plant cells are exposed to UV-B then, like other environmental stresses, this activates cell signalling pathways (Knight and Knight, 2001; Zhu, 2001, 2002) and cellular responses, such as production of stress proteins, upregulation of antioxidants and accumulation of compatible solutes (Vierling and Kimpel, 1992; Cushman and Bohnert, 2000). To counteract the effect of oxidative damage caused by UV-B, ROS scavenging systems are involved in plants (Bowler *et al.*, 1992). Various enzymatic and non-enzymatic scavenging system become activated in the oxidative stress condition. Superoxide dismutase (SOD), catalase (CAT) and Halliwell/Asada pathway enzymes (Foyer *et al.*, 1994) are involved in the enzymatic scavenging system, whereas the non-enzymatic scavenging system includes low molecular mass antioxidants such as ascorbate (ASA), glutathione (GSH), carotenoids (Car), proline and compounds such as phenols (Asada, 1999).

Antioxidant enzymes or the contents of antioxidants change in response to UV-B oxidative stress (Zlatev *et al.*, 2012). UV-B irradiation enhances the level of superoxide dismutase (SOD), ascorbate peroxidases (APX) and glutathione reductase (GR), as reported in cyanobacterium (Prasad and Zeeshan 2005), wheat (Sharma *et al.*, 1998), cucumber (Tekchandani and Guruprasad, 1998) and Arabidopsis (Rao *et al.*, 1996). Through extensive studies, it was reported that the tolerance of seedlings to UV-B is due to the enhancement of SOD activity and other antioxidative enzymes in *Cassia auriculata* (Agarwal, 2007), potato (Santos *et al.*, 2004), pea (Mackerness *et al.*, 1999), cucumber (Kondo and Kawashima 2000), and in *Plectonema boryanum* (Prasad and Zeeshan, 2005).

The isoenzymes expression pattern also differs in different stress conditions. For example, Santos *et al.* (2004) have found different SOD isoenzymes on exposure of UV-B. Other antioxidant enzymes, like CAT and peroxidases (POX) activity in *Cassia* species (Agarwal and Pandey, 2003), cucumber (Krizek *et al.*, 1993; Jain *et al.*, 2004), sugar beet (Panagopoulos *et al.*, 1990), potato (Santos *et al.*, 2004), sunflower (Costa *et al.*, 2002; Yannarelli *et al.*, 2006); soybean (Xu *et al.*, 2008) and *Acorus calamus* (Kumari *et al.*, 2010) were found enhanced on irradiating with UV-B. However, enhancement in the non-enzymatic antioxidants on UV-B exposure was also observed in pepper plants (Mahdavian *et al.*, 2008), *Cassia auriculata* (Agarwal, 2007) and *Acorus calamus* (Kumari *et al.*, 2010).

5.7 DNA Damage and Repair Mechanism

UV-B can affect the growth and development process in plants, either directly by damaging DNA which can cause inheritable mutations, or indirectly by affecting various physiological functions (Ormrod and Hale, 1995; Lidon, 2012), or by changes in membrane and protein denaturation. These damaging effects of UV-B consequently result in diminished plant growth and productivity. To afford protection against UV radiation, a range of defence mechanisms and DNA damage control strategies get switched on in response. High fluence or longer duration of exposure to UV-B causes damage to DNA

and the activity of the photolyase enzyme(s), and the products formed as a result of damage to DNA are involved in repair mechanism (McLennan, 1987; Pang and Hays, 1991; Quaite *et al.*, 1992; Stapleton, 1992; Taylor *et al.*, 1997).

UV radiation induces pyrimidine dimers on exposure, which are categorized into two major classes: the pyrimidine [6-4] pyrimidone photoproduct (6-4 product), and the cyclobutane pyrimidine dimer (CPD) (Jenkins, 2009; Xu and Sullivan, 2010). On counteracting against UV radiation, some genes related to defence are upregulated, although other important genes involved in photobiological process, such as photosynthetic genes, are downregulated (Jordan *et al.*, 1992; Baker *et al.*, 1997; Britt, 1997). UV radiation shows detrimental effects on absorption by nucleic acids and proteins, which can result in photodamage and conformational changes and can later disturb essential metabolic functions such as transcription, DNA replication, and translation (Buma *et al.*, 1995; Lao and Glazer, 1996; Buma *et al.*, 2003).

In a greenhouse study, a native herb from southern Patagonia, *Gunnera magellanica*, was exposed to a gradient of UV-B from zero to moderate fluxes. The results inferred that, with increase in UV-B radiation, leaf expansion decreases and the CPD density increases, due to increase in DNA damage (Giordano *et al.*, 2004). Moreover, studies on rice supported that the reason behind the detrimental growth on exposure to UV-B is DNA damage in the form of CPDs. Also, results showed slight variation in photolyase activity involved in the repairing process, which provides difference in the tolerance power (Teranishi *et al.*, 2004).

In plant cells, total CPDs account for approximately 75% of these lesions, while the remainder are (6–4) photoproducts (Mitchell and Nairn, 1989). DNA damage caused by the UV-B irradiation can be mutagenic, or lethal, by hampering replication and transcription (Brash *et al.*, 1987, 1991). Since the DNA and RNA polymerase cannot read photoproducts, plants possess special repairing mechanisms to deal with UV-B-induced CPD in nuclear DNA (ncDNA), such as photoreactivation (photorepair) and nucleotide excision repair (dark repair), helping plant cell survival (Britt and May, 2003).

Photoreactivation is a light-dependent enzymatic process using UV-A and blue light to monomerize pyrimidine dimers. Photolyase binds to the generated photoproducts, and uses light energy to initiate electron transfer, breaking the chemical bonds of the cyclobutane ring and restoring integrity of the bases (Frohnmeyer and Staiger, 2003). CPD photolyase is induced by UV-B, whereas 6-4 PP photolyase protein is constitutively expressed (Waterworth *et al.*, 2002). It has been demonstrated that *Arabidopsis* contains photolyases with substrate specificity for either CPDs or 6-4 PPs, respectively (Hoffman *et al.*, 1996; Ahmad *et al.*, 1997).

In nucleotide excision repair (NER; dark repair), *de novo* DNA synthesis occurs to replace dimers, and the undamaged complementary strand is employed as the template (Britt, 1996). This repairing mechanism employs some critical proteins, such as CUL4-based E3 ligase with the core subunits CUL4, RBX1, and DDB1. DDB1 can interact with either CSA (Cockayne Syndrome Factor A); in *Arabidopsis thaliana* the protein is called ATCSA-1 (Biedermann and Hellmann, 2010) or DDB2. CSA and DDB2 both act as key players in damaged DNA recognition and initiating NER, whereas DDB2 binds directly to damaged DNA and is involved in genome wide control of damaged DNA. CSA does this in concert with CSB (Cockayne Syndrome Factor B; in Arabidopsis called CHR8) and RNA polymerase II, and is only involved in repair of genes actively transcribed.

In addition to the nuclear genome, plant cells of higher plants contain organelles having additional genomes, one in chloroplasts and another in mitochondria. The genome of these organelles is involved in encoding proteins important for photosynthesis and respiration, respectively. UV-B irradiation also affects DNAs and induces the formation of CPDs (Chen *et al.*, 1996). Thus, chloroplasts and mitochondria might undergo repairing of photoproducts through a pathway that efficiently removes the DNA lesions before replication and transcription. This repairing is yet to be fully explored.

In the presence of UV-B, photoactivation was noticed in the chloroplast DNA (cpDNA) and mitochondrial DNA (mtDNA) of *Zea mays* (maize) leaves (Stapleton *et al.*, 1997), and UV-induced polymerase-blocking lesions in the cpDNA of Glycine max (soybean) cells (Cannon *et al.*, 1995). In rice, it was found that single DNA repair enzyme CPD photolyase has 'triple targeted' in rice cells, functioning in nuclei, chloroplasts and mitochondria (Takahashi *et al.*, 2011).

5.8 Exclusion of UV Components: Experimental Approach to Study the Effect on Plants

Earlier scientists used growth chambers or greenhouses, in which plants were exposed to unnaturally high UV-B from lamps with low UV-A and photosynthetically active radiation (PAR, 400–700 nm), to study the effect of UV-B on terrestrial plants. Then, in the early 1990s, it was accepted that these indoor experiments are not very realistic in understanding the field responses towards ambient UV radiation, as the plants were exposed to unnaturally high ratios of UV-B/UV-A and UV-B/PAR, (Caldwell and Flint, 1994; Caldwell and Flint, 1997; Krizek and Mirecki, 2004). Some scientific groups adopt the supplementation (high UV-B provided) approach to correlate the effect of enhanced levels of UV-B on plant physiology and development, by using lamp banks of fluorescent lights to supplement ambient levels of UV-B. Another most reliable approach is the field experiment by using plastic filters with different UV-B transmission properties to filter natural sunlight (Rousseaux *et al.*, 2004), which excludes UV-B from the total solar spectrum, and helps in understanding the impact of natural UV-B on photosynthesis and photomorphogenesis of plants.

Exclusion studies on soybean, cotton, wheat and *Amaranthus* have shown that UV-B has inhibitory effect on growth and expansion of leaves (Kataria *et al.*, 2013). Moreover, an enhancement in the net rate of photosynthesis after the exclusion of UV has been reported in populous (Schumaker *et al.*, 1997), maize and mung bean (Pal *et al.*, 1997), wheat and pea (Pal *et al.*, 2006) and sorghum (Kataria and Guruprasad, 2012b), which indicates the detrimental effect of UV on plants. The contributory components of the photosynthesis process, like amount of Chl, was shown to increase in the leaves of *Cyamopsis, Amaranthus, Sorghum,* cotton and wheat by excluding UV radiation (Amudha *et al.*, 2005; Kataria *et al.*, 2013). Also, in the exclusion experiment, it was reported that flowering increased in the crop growing under a Mylar sheet that filters UV-B radiation (Caldwell *et al.*, 1968). Shine and Guruprasad (2012) demonstrated that generation of ROS ($O_2^{\bullet-}$ and $^{\bullet}OH$) was less in the plants raised under UV exclusion filters then in the plants exposed to ambient UV radiation.

At the biochemical level, UV exclusion influences both carbon (Kataria *et al.*, 2013, 2014; Kataria and Guruprasad, 2014) and nitrogen metabolism (Sharma and Guruprasad,

2012; Baroniya *et al.*, 2014) in a beneficial manner, resulting in an increase in the yield and biomass of plants. Through UV exclusion technique, it has been demonstrated that the activity of nitrogenase enzyme is enhanced in *Trigonella*. Besides enhancing nitrogenase activity, exclusion of UV also enhances the leghemoglobin and heme-chrome, along with the accessory protein required for efficient fixation of nitrogen (Sharma and Guruprasad, 2012). Other studies have shown that enhancement in the growth of leaves and net rate of photosynthesis in the leaves of plant under UV-excluded condition contributed to the increase of yield in crop plants (Kataria *et al.*, 2013; Kataria and Guruprasad, 2014).

5.9 Conclusion and Future Perspectives

Increasing ultraviolet radiation (UVR) has become one of the most important issue affecting terrestrial ecosystems. Anthropogenic activities are causing an increase in ultraviolet-B (280-315 nm) radiation at the Earth's surface. Cellular components, such as nucleic acids, proteins, lipids and quinones, can absorb UV-B radiation directly in plants, which has various deleterious effects at the morphological, physiological and metabolic level. This, in turn, alters plant growth, reduces yield, and causes damage to photosystem II (PSII) and decrease in chlorophyll content. It harshly inhibits photosynthesis in various plant species, increases oxidative stress, and damages DNA. However, plants have developed certain repairing mechanisms to cope with the UV-induced DNA damage to nucleus, chlorophyll and mitochondria. These damages apparently cause reduction in biomass and yield.

Enhanced UV-B radiation also has critical effect on plant growth and reproductive development. Plants' success of establishment in the field depends upon the ability to efficiently capture and use sunlight, along with counteracting various stress factors like UV. Response to enhanced UV-B radiation varies distinctly within and between species. In plants, both antioxidant enzymes like superoxide dismutase (SOD), ascorbate acid peroxidase (APX), glutathione reductase (GR) and peroxidase (POD), and non-enzymatic antioxidants like ascorbate (AsA), glutathione (GSH), carotenoids, tocopherols, and the phenolics-dependent antioxidant defence system act to neutralize oxidative stress. Secondary metabolites like flavonoids and phenolic compounds help in protecting photosynthetic tissues in leaves from detrimental effects of UV-B.

Enhanced UV-B causes photomorphogenic as well as genetic changes in plants. Signals received are regulated by two main proteins encoded by UV Resistance Locus 8 (UVR8) and Constitutively Photomorphogenic 1 (COP1), which act as photoreceptors through signal transduction pathways. However, the molecular basis of perception and signal transduction in response to UV-B to give relevance to present and future scenario of climatic change globally is yet to be understood.

Also in the future, ambient UV-B radiation and its interactions with other environmental factors may cause significant economic loss. Exclusion studies will provide meaningful information on the field response of plants towards enhanced UV-B radiation, and can ultimately be used to develop crop models to combat the predicted level of UV-B in the near future. Another important future prospect can be the revelation of the significant changes at molecular level due to UV damage. It will be highly important to explore the connection of these signalling events with the adaptation and acclimation

processes occurring under ultraviolet exposure. In agriculture, increasing knowledge of UV-B protective mechanisms employed by the plant may potentially lead to industrial applications.

Acknowledgement

We wish to convey our sincere thanks to Mrs. Swagata Chatterjee for drawing the figures for this chapter.

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6

Oxidative Stress and Antioxidative Defence System in Plants in Response to UV-B Stress

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6.1 Introduction

Plants often face the challenge of different environmental conditions, which include stressors such as drought, salinity, pesticides, low temperature, mineral nutrient deficiency, metal toxicity and UV irradiation, all of which exert adverse effects on plant growth and development (Foyer *et al.*, 1994; Gupta *et al.*, 2011). Due to anthropogenic pollutants, UV-B is one of the main environmental constraints, which can increase with thinning and depletion of the O_3 layer (McKenzie *et al.*, 2011). As a result of ozone loss, UV-B flux at the surface of the earth inevitably produces negative impacts on organisms (Coohill, 1991).

The potential harmful impact of increased UV-B intensity on ecological and biological systems has attracted global attention (Caldwell *et al.*, 2007; Ballaré *et al.*, 2011). UV-B radiation is currently near its maximum levels, and is expected to revert to the pre-1980s (WMO, 2003). However, many factors, including rising concentrations of greenhouse gases, could delay this return (Newman *et al.*, 2001). The intensity of UV-B radiation reaching the biosphere is dependent on solar zenith angle, the thickness of the ozone layer clouds, and aerosols. In the tropics, due to the small solar zenith angle and thin stratospheric ozone layer, terrestrial plants encounter much higher levels of UV-B radiation than at higher latitudes (Caldwell *et al.*, 1989; Madronich *et al.*, 1995). Ambient UV-B irradiance at low latitudes is also high, due to the high solar angle and a relatively low stratospheric ozone amount. India lies in a low ozone belt and receives more UV-B radiation, compared with temperate higher latitudes (Mitra, 1991). Sahoo *et al.* (2005) observed a significant decline in the total ozone column (TOC) at numerous stations in northern India.

Enhanced UV-B radiation produces deleterious effects on physiological and morphological traits of plants, and thus poses a severe threat to the existence and survival of organisms (Frohnmeyer and Staiger, 2003; Prasad *et al.*, 2005; Klem *et al.*, 2012). Many studies have reported effects of strong UV-B radiation on the growth and physiological properties of crops, such as leaf area, plant biomass, photosynthesis, UV absorption

UV-B Radiation: From Environmental Stressor to Regulator of Plant Growth, First Edition. Edited by Vijay Pratap Singh, Samiksha Singh, Sheo Mohan Prasad and Parul Parihar.
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substances, antioxidant systems, endogenous hormone regulation and yield (Hidema and Kumagai, 2006; Lizana *et al.*, 2009; Surabhi *et al.*, 2009; Kataria *et al.*, 2013).

Since higher plants are immobile, they cannot escape from environmental stresses, so they cannot avoid exposure to damaging UV-B radiation (Boldt and Scandalios, 1997). The susceptibility to elevated and ambient UV-B irradiation is dictated by a complex interplay between protection, repair and other factors that may lead to highly variable UV-B susceptibility among the species. Some plant species are tolerant, or even show stimulation when exposed to UV-B radiation, while some are highly susceptible (Xiong *et al.*, 1995, 1996). Differences in UV-B sensitivity between cultivars of the same species have been investigated in rice (*Oryza sativa* L.) (Kumagai *et al.*, 2001; Wu *et al.*, 2011), wheat (*Triticum aestivum* L.) (Pinto *et al.*, 2000; Kataria and Guruprasad, 2012a), sorghum (*Sorghum bicolor*) (Kataria and Guruprasad, 2012b) and cucumber (*Cucumis sativus* L.) (Tapia *et al.*, 2010). The UV effect on plants occurs within the regulatory systems controlling the causing factor of the plant's response to stress (Wu *et al.*, 2011).

When a plant absorbs UV-B radiation, leaf photosynthetic apparatus is heavily damaged. Pigment degradation (chlorophylls and carotenoids) and thylakoid disruption occurs (Strid and Porra, 1992), and this process mainly affects photosystem II, thus reducing chlorophyll b content. Carotenoids are less affected by UV-B treatment than chlorophylls (Sharma *et al.* 1998; Barsig and Malz, 2000). Under UV-B stress, energetic and metabolic resources are diverted towards the synthesis of scavenger compounds, among which are phenols and, especially, flavonoids. The latter are localized in the uppermost parts of the leaf mesophyll as protective compounds, which reduce disruption of the photosynthetic apparatus. Direct evidence of the role of phenolic accumulation in conferring UV tolerance has been obtained in *Arabidopsis thaliana* (Sheahan, 1996).

Apart from increasing phenolic compound content, other adaptive responses have been observed, such as an increase of free radical scavenging capacity, due to the fact that UV-B can generate reactive oxygen species at various sites of respiratory and photosynthetic electron transport (Arora *et al.*, 2002; Stratmann, 2003), as well as during various biochemical reactions in cellular systems. These reactive oxygen species (ROS) are highly deleterious for cell structures and functions (Halliwell and Gutteridge, 1984; Hideg and Vass, 1996; Foyer *et al.*, 1997). UV-B radiation promotes ROS formation and exerts oxidative stress to the plant (Yannarelli *et al.*, 2006a). The ROS include not only free radicals such as superoxide ($O_2^{\bullet-}$) and hydroxyl radicals ($^{\bullet}OH$), but also hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$). These ROS can cause oxidative damage to membrane lipids, nucleic acids and proteins (Figure 6.1).

The ability of higher plants to scavenge the toxic effects of reactive oxygen seems to be a very important determinant of their tolerance to UV-B stress. Antioxidants are the first line of defence against free radical damage. They are critical for maintaining the optimum health of plant cells. In order to prevent the harmful effects caused by UV-B stresses, organisms develop radical quenchers and antioxidants, which provide protection by scavenging harmful radical or oxygen species (Middleton and Teramura, 1993; Prasad and Zeeshan, 2005).

To keep this damage to a minimum, plants possess enzymatic and nonenzymatic antioxidative defence systems. Among the latter are ascorbic acid (ASC), tocopherols, carotenoids and flavonoids (UV-absorbing substances). Enzymes involved in the defence system include catalase (CAT; EC1.11.1.6), superoxide dismutase (SOD; EC1.15.1.1) ascorbate peroxidase (APX; EC1.11.1.11), peroxidase (POD; EC1.11.1.7),

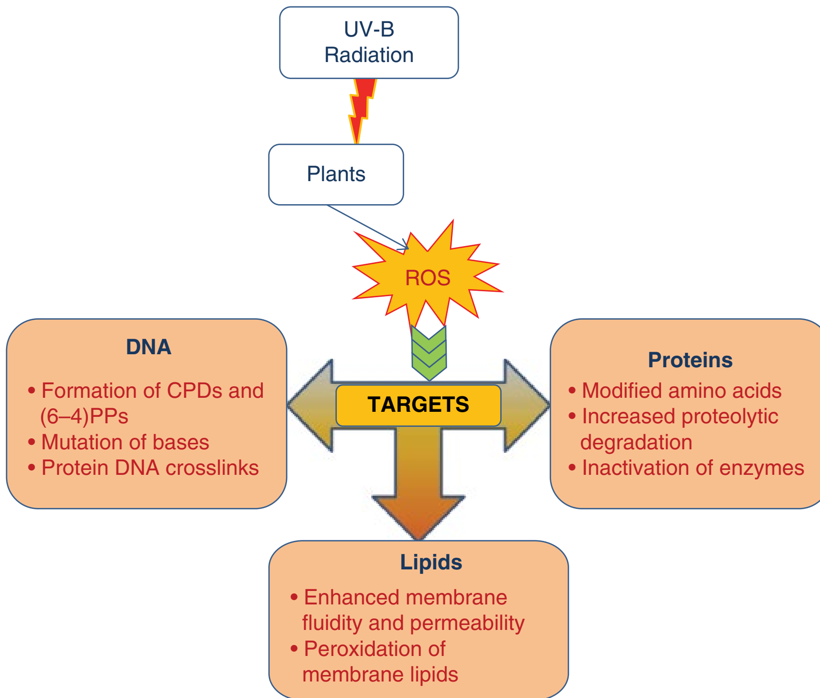

Figure 6.1 Targets of ROS generated by UV-B stress.

glutathione reductase (GR; EC1.6.4.2), dehydroascorbate reductase (DHAR; EC1.8.5.1) and monodehydroascorbate reductase (MDHAR; EC1.6.5.4) (Noctor and Foyer, 1998).

Plants use antioxidant enzymes to remove ROS in cells directly or indirectly, thus ensuring normal metabolic reactions, which is a major mechanism to ameliorate the toxic effect of ROS generated by UV-B stress. The key factor for UV-B tolerance may be considered as UV-B-absorbing pigments, regulation of active oxygen species levels and activity of antioxidants, and the effective repair mechanism for PSII, one of the important components of photosynthetic electron transport chain (Xiong *et al.*, 1995; Mackerness *et al.*, 2001). In the present chapter, we discuss the oxidative stress and antioxidative defence system in plants in response to UV-B (280-315 nm) stress.

6.2 Plant Protection Against UV Radiation

In response to the damaging effects of UV radiation, plants have developed defence mechanisms against UV-B, while allowing photosynthetically active radiation (PAR) to penetrate through the outer cell layers to support photosynthesis in the mesophyll and palisade tissues (Cen and Bornman, 1993). This may involve the rapid biosynthesis of protective pigments that absorb the damaging UV radiation (Jordan, 1996). There is also a series of enzymes, called photolyases, that are capable of repairing critical molecules such as DNA (Cen and Bornman, 1993). UV-B has a high energy level, and is readily absorbed by a number of important macromolecules in plants, including nucleic

acids, proteins, lipids, and phytohormones (Rozema *et al.* 1997). Jansen *et al.* (1998) demonstrated that DNA, proteins and the photosynthetic apparatus of plants are primary potential targets of UV-B radiation. Therefore, metabolic processes of plants can be influenced through damage to their DNA by UV-B radiation (Wang *et al.*, 2012). Surabhi *et al.* (2009) demonstrated that UV-B radiation impairs amino acid residues and damages unsaturated fatty acids in plant cell membranes.

Plants are protected against the penetration of UV-B into internal tissues by accumulating phenolic compounds to absorb the excess UV-B radiation (Rozema *et al.*, 1997; Soheila and Mackerness, 2000). The most common classes of phenolic compound are the flavonoids, produced by the phenylpropanoid pathway. Flavonoids are water-soluble flavone and flavonol glycosides or their derivatives – particularly kaempferol and quercetin – and are located in vacuoles (Rice-Evans *et al.*, 1997). Flavonoids are produced primarily in the epidermal layers of the leaves, and they absorb UV-B radiation effectively while transmitting PAR to the chloroplasts (Jordan, 1996). In addition to their role as sunscreens, flavonoids are also known to have an antioxidant function, and can help dissipate UV-B radiation within the leaf (Hofmann *et al.*, 2000).

Foliar concentrations of UV-B-absorbing compounds commonly increase in response to UV-B exposure. In the meta-analysis, Searles *et al.* (2001) found that increase in foliar concentrations of UV-B-absorbing compounds were the most consistent response to UV-B supplements. Several enzymes involved in UV-B absorbing compound synthesis, such as phenylalanine ammonia lyase, chalcone synthase and chalcone isomerases, are stimulated by UV-B (Fujibe *et al.*, 2004; Ryan *et al.*, 2002). Increases in UV-B absorbing compounds appeared to protect DNA in *Arabidopsis* (Fujibe *et al.*, 2004) and maize (Stapleton and Walbot, 1994), and to reduce the sensitivity of PSII to UV-B in grape (Kolb *et al.*, 2001), *Arabidopsis* (Fujibe *et al.*, 2004; Rao and Ormrod, 1995), and rye (Tevini *et al.*, 1991).

Soybean cultivars with higher constitutive concentrations of these compounds experience less DNA damage and biomass reduction upon UV-B exposure (Mazza *et al.*, 2000). Many studies have looked at UV-B sensitivity in various mutants, to test if UV-B-absorbing compounds can provide protection against UV-B radiation. Mutants with genetic blocks in phenolic synthesis have been shown to exhibit increased sensitivity to UV-B in comparison with the wild type of that species (Booij-James *et al.*, 2000; Landry *et al.*, 1995; Rao and Ormrod, 1995). Also, other mutants with elevated accumulation of UV-B absorbing compounds display a remarkable tolerance to UV-B (Bieza and Lois, 2001; Jin *et al.*, 2000). Hence, it is well established that UV-B-absorbing compounds do afford the plant protection against UV-B radiation. Higher concentrations of UV-B-absorbing compounds are usually inferred to reduce epidermal transmittance and to provide selective sunscreen protection to targets in the mesophyll. This is because these compounds absorb effectively in the UV-B region, show little absorption in the visible region and are located predominantly in the vacuoles of epidermal cells (Markstädter *et al.*, 2001; Robberecht and Caldwell, 1983).

In higher plants, UV-B-absorbing compounds include a large number of phenylpropanoids, with flavonoids and hydroxycinnamic acids likely to be most important in terms of UV-B sunscreen (Cockell and Knowland, 1999). In *Arabidopsis*, the hydroxycinnamic acids are the most abundant phenylpropanoid and provide the most protection (Booij-James *et al.*, 2000; Landry *et al.*, 1995) while, in maize and barley, flavonoids are the most abundant and provide the most effective protection (Reuber *et al.*, 1996;

Stapleton and Walbot, 1994). Anthocyanins and flavonoids act not only as UV filters, but also as active oxygen scavengers (Peng *et al.*, 2003; Gould *et al.*, 2002). Also, UV-B-induced flavonoid can affect auxin polar transport and catabolism, which has been linked to UV-B tolerance (Jansen, 2002; Jansen *et al.*, 2001).

6.3 UV-B and ROS

Generation of reactive oxygen species (ROS) is one major process for UV-B radiation to cause damage to the plants. The term 'reactive oxygen species' (ROS) is generic, embracing not only free radicals such as superoxide ($O_2^{\bullet -}$) and hydroxyl radicals ($^{\bullet}OH$), but also hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$). While it is generally assumed that the hydroxyl radical and singlet oxygen are so reactive that their production must be minimized, superoxide and peroxide are synthesized at very high rates, even under optimal conditions. A regulated balance between ROS production and destruction is required if metabolic efficiency and function are to be maintained in both optimal and stress conditions.

There is a wide array of sources of ROS in plants. The electron transport chains of the chloroplast and mitochondria are two important sources of ROS. In the chloroplast, environmental stress may limit CO_2 fixation and reduce the $NADP^+$ regeneration by the Calvin cycle. In this case, the photosynthetic electron transport chain is over-reduced, which leads to the formation of superoxide radicals and singlet oxygen (Asada, 1999; Foyer *et al.*, 1994).

Chloroplast is very sensitive to UV-B radiation. Excessive radiation may lead to over-saturation of the photosynthetic light reactions, which eventually can cause photoinhibitory damage to the photosynthetic apparatus (Powles, 1984; Aro *et al.*, 1993). ROS is harmful to plant cells affecting plant growth and development and physiochemical reactions (Abd El-Baky *et al.*, 2004; Mahmood *et al.*, 2012). Oxidized, endogenous target molecules can also be used as ROS reporter molecules. For example, accumulation of malondialdehyde (MDA) (Hideg *et al.*, 2003; Lidon *et al.*, 2011) or of DNA thymine dimers (Schmitz-Hoerner and Weissenbock, 2003), products of ROS-mediated oxidation of polyunsaturated membrane lipids and of DNA, respectively, imply the presence of ROS. MDA has been reported in the leaves of rice cultivars treated with UV-B (Dai *et al.*, 1997). Increasing indirect evidence suggests that ROS are involved in the damage caused by UV- B radiation (Yao and Liu, 2007; Kalbina and Strid, 2006; Wang *et al.*, 2006; Prasad *et al.*, 2005) and lipid peroxidation (Yao and Liu, 2007; Yannarelli *et al.*, 2006a; Prasad *et al.*, 2005; Yang *et al.*, 2005) in plants.

Direct evidence for the formation of superoxide radicals was observed in isolated cucumber cotyledons exposed to UV-B radiation by ESR spectroscopy (Jain *et al.*, 2004a). Shine and Guruprasad (2012) also found that the amount of $O_2^{\bullet -}$ and $^{\bullet}OH$, radicals and the radical scavenging activity were significantly higher in soybean leaves exposed to ambient UV radiation by ESR spectroscopy. Enhanced production of ROS in plant tissues exposed to supplemental level of UV-B (sUV-B) has detrimental effects on enzyme activities and gene expression, which ultimately leads to cellular damage and programmed cell death (Mackerness *et al.*, 1998).

Plants subjected to UV-B radiation accumulate H_2O_2 in leaves, and intracellular MDA and other harmful products are also induced (Wang *et al.*, 2010; Li *et al.*, 2012). Previous

reports showed that UV-B radiation results in fast regeneration of oxidative oxygen, over-production of H_2O_2, extensive oxidation of membrane lipids, and higher MDA content in leaves of rice plants (Dai *et al.*, 1997; Fedina *et al.*, 2010; Mohammed and Tarpley, 2010). UV-B irradiation activated over-production of oxidative products (H_2O_2 and MDA) which, in turn, induced stronger antioxidant enzyme activity and higher total antioxidant capacity to remove those harmful products (He *et al.*, 2014).

Although it is not known how plants irradiated with UV-B generate ROS, it is thought that NADPH oxidase may be involved in the generation (Rao *et al.*, 1996). Reactive oxygen species increase in response to UV-B, and are an important component in the regulation of both upregulated and downregulated genes. The nature and origin of the ROS involved in the early part of UV-B-induced signalling pathways have been investigated in *Arabidopsis thaliana* (Mackerness *et al.*, 2001). The increase in PR-1 transcript and decrease in Lhcb transcript in response to UV-B exposure was shown to be mediated through pathways involving hydrogen peroxide (H_2O_2) derived from $O_2^{\bullet-}$. In contrast, the upregulation of PDF1.2 transcript was mediated through a pathway involving $O_2^{\bullet-}$ directly.

The origins of the ROS were also shown to be distinct, and to involve NADPH oxidase and peroxidase(s) (Mackerness *et al.*, 2001). Mackerness *et al.* (2001) provided evidence to show that UV-B exposure induced NADPH oxidase and cell wall peroxidases-mediated ROS synthesis in the leaves of *Arabidopsis*, suggesting that there are multiple sources of H_2O_2 production in response to UV-B radiation. It may be possible that the plants recognize the UV-B radiation through mechanisms identical to those involved in pathogen infection.

In addition, increased H_2O_2 levels are detected simultaneously with the inhibition of photosynthesis by UV-B irradiation (Fujibe *et al.*, 2004). This suggests that the UV-B-induced oxidative bursts of H_2O_2 are associated with the damage and degradation of PSII. It is widely accepted that UV-B damages the donor side of PSII by inactivating the Mn cluster of water oxidation (Messinger, 2004). Hydroxyl radicals are the dominating reactive oxygen induced by UV-B radiation in the thylakoids (Hideg and Vass, 1996). Production of highly damaging $^{\bullet}OH$ radicals in the heart of the Mn cluster by UV-B has been suggested as one of the possible mechanism of UV-B-induced damage (Szilard *et al.*, 2002). H_2O_2 reacts with $O_2^{\bullet-}$ via the Fenton reaction to produce $^{\bullet}OH$, which is the most reactive ROS and may be responsible for higher lipid peroxidation in UV-B exposed leaves (Takshak and Agrawal, 2014). Direct damage to the key enzymes involved in photosynthesis and respiratory pathways may also promote ROS formation (Jordan, 1996).

6.4 UV-B and Antioxidant Enzymes

It is well known that plants respond to detrimental solar UV-B irradiation via several repair and defence mechanisms that allow them to tolerate, counteract or avoid its effects (Larkum and Wood, 1993). Efficient antioxidant defence systems, including anti-oxidants and enzymes, have developed in plants to counteract the toxicity of ROS. Bowler *et al.* (1992) have concluded that UV-B produces $O_2^{\bullet-}$, $^{\bullet}OH$ and H_2O_2. Superoxide is rapidly converted to H_2O_2 by the action of SOD, or reduced by ascorbate (Noctor and Foyer, 1998). Dismutation of $O_2^{\bullet-}$ leads to the formation of oxygen and hydrogen

peroxide (H_2O_2), and the latter can react with $O_2^{\bullet-}$ to create the highly reactive hydroxyl radical ($^{\bullet}OH$) via the Haber-Weiss cycle (Bowler *et al.*, 1992).

H_2O_2 is known to diffuse across biological membranes and cause cellular damage. H_2O_2 is effectively scavenged by CAT in peroxisomes, while this task is performed by ascorbate-glutathione cycle in cytosol and chloroplasts (Miller *et al.*, 2010). H_2O_2 production was increased by high level of UV-B in experiments conducted indoors (Kalbina and Strid, 2006; Wang *et al.*, 2006; Prasad *et al.*, 2005). An increase in the H_2O_2 content was found in the leaves of UV-B treated plants (Rybus-Zając, 2005; Kubiś and Rybus-Zając, 2008). An increase in the SOD activity is one of the reasons for higher production of H_2O_2 in plants (Figure 6.2). H_2O_2 is scavenged by catalase, various peroxidases, and enzymes of ascorbate-glutathione cycle (Figure 6.2).

In plant cells, the most important reducing substrate for H_2O_2 reduction is ascorbate (Mehlhorn *et al.*, 1996; Nakano and Asada, 1987). Ascorbate peroxidase uses two molecules of ascorbate to reduce H_2O_2 to water, with the generation of two molecules of monodehydroascorbate (MDHA). MDHA can be reduced to ascorbate, catalyzed by MDHAR, and ascorbate can also be non-enzymatically regenerated from MDHA. Dehydroascorbate (DHA) is always produced during the rapid disproportionation of the MDHA radical, and DHA is reduced to ascorbate by the action of DHAR, using GSH as the reducing substrate and generating glutathione disulphide (GSSG), which is reduced to GSH by GR (Figure 6.2). The removal of H_2O_2 through this series of reactions is known as the ascorbate-glutathione cycle (Noctor and Foyer, 1998). It is generally assumed that this cycle is mainly responsible for the scavenging of ROS, especially in the chloroplast.

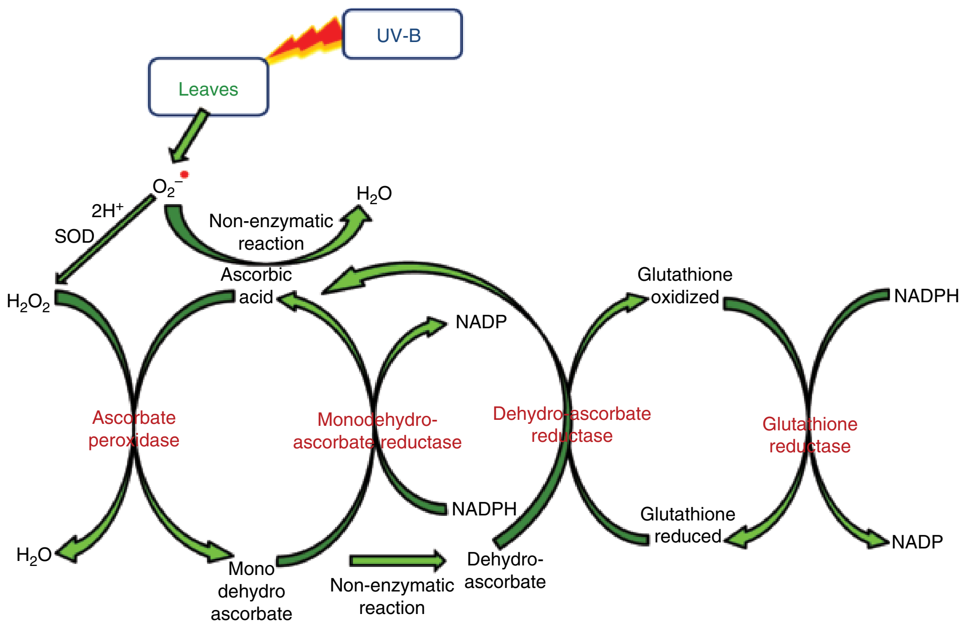

Figure 6.2 UV-B-induced ROS generation followed by Asada-Halliwell pathway of oxyradicals scavenging and involvement of various antioxidant enzymes.

SOD removes $O_2^{\bullet-}$ by catalyzing its dismutation to H_2O_2 and O_2 (Agarwal, 2007; Gill and Tuteja, 2010). Many responses of SOD to UV-B exposure have been reported, revealing no uniform responses. For instance, in indoor experiments, SOD activity was increased by UV-B radiation in pea and wheat (Alexieva *et al.*, 2001), *Arabidopsis* (Rao and Ormrod, 1995), soybean (Prasad *et al.*, 2005), poplar (Ren *et al.*, 2006), and rice (Dai *et al.*, 1997), but was not affected in buckwheat (Jovanovic *et al.*, 2006) and soybean (Malanga *et al.*, 1999), and was decreased in sunflower cotyledon (Costa *et al.*, 2002). Also, SOD expression was not affected by UV-B radiation in *Nicotiana plumbaginifolia* (Willekens *et al.*, 1994), but was decreased in *Pisum sativum* (Strid, 1993).

In a field study, supplemental UV-B increased SOD activity in wheat and bean (Agrawal and Rathore, 2007) and *Picea asperata* (Yao and Liu, 2007) but had no effects in barley (Mazza *et al.*, 1999), and caused different responses among soybean cultivars (Yanqun *et al.*, 2003). In other experiments, the effects of artificial UV-B on SOD activity were found to vary with temperature (Takeuchi *et al.*, 1996), duration of the treatment (Dai *et al.*, 1997), leaf age and PAR source, even under the same level of PAR (Krizek *et al.*, 1993).

Catalase can convert H_2O_2 to water and oxygen, but it is found predominantly in the peroxisomes, where it functions chiefly to remove the H_2O_2 formed during photorespiration and oxidation of fatty acids. An alternative mode of H_2O_2 destruction is via peroxidase, which is found throughout the cell (Jimenez *et al.*, 1997; Yamaguchi *et al.*, 1995), and has a much higher affinity for H_2O_2 than CAT. Peroxidase (POD) enzymes are heme proteins that also function in H_2O_2 scavenging, and are often found in multiple molecular forms. POD transduces extracellular signals into redox signals that eventually stimulate the intracellular Ca^{2+} signalling required for induction of defence responses (Kawano, 2003).

PODs are enzymes that catalyze the H_2O_2-dependent oxidation of a wide variety of substrates, mainly phenolics (Dunford, 1986). PODs are involved in numerous physiological roles in plant tissues, including lignin biosynthesis, indole-3-acetic acid degradation, wound healing and pathogen defence (Kawano, 2003; Bestwick *et al.*, 1998; Sato *et al.*, 1993). POD decomposes H_2O_2 by oxidation of co-substrates (Gaspar *et al.*, 1991).

UV-B radiation increased POD activity in several plant species, including wheat and mung bean (Agrawal and Rathore, 2007), peanut (Tang *et al.* 2010), cucumber (Tekchandani and Guruprasad, 1998; Hagh *et al.*, 2012), *Hibiscus rosa-sinensis* (Panagopoulos *et al.*, 1989) and *Beta vulgaris* (Panagopoulos *et al.*, 1990). CAT directly dismutates H_2O_2 into H_2O and O_2, and is an important ROS-scavenging enzyme during stress conditions (Garg and Manchanda, 2009; Gill and Tuteja, 2010). CAT activity was reported to increase under UV-B (Balakumar *et al.*, 1997; Hagh *et al.*, 2012; Kumari *et al.*, 2010). Decreased CAT activity could also be due to the destruction of peroxisomes, due to high lipid peroxidation under UV-B stress (Ravindran *et al.*, 2010).

In cucumber, the expression levels of genes coding for CAT and POD were enhanced by UV-B treatments. A different response of catalase to UV-B has been reported in *Nicotiana plumbaginifolia* (Willekens *et al.*, 1994) and *Zea mays* (Boldt and Scandalios, 1997), due to the presence of several isoforms and to the different metabolic functions. It has already been demonstrated that UV-B treatment can trigger upregulation of antioxidant enzymes (Chen *et al.*, 2003). Studies conducted by Yannarelli *et al.* (2006a) in soybean showed an increase of CAT activity under the lower UV-B doses, demonstrating that this enzyme is upregulated to safeguard normal cellular functions and survival.

Cantarello *et al.* (2005) have found that UV-B radiation dramatically stimulates gene expression and enzyme activity of POD in cucumber, and have suggested the enhancement in the activity of POD may either be due to *de novo* synthesis of the enzyme, related to the rather high amount of POD transcripts accumulated, or suppression of the activity of a natural enzyme inhibitor caused by UV-B radiation. A regulatory role for a peroxidase inhibitor in the UV-B induced enhancement of peroxidase activity in cucumber cotyledons has been demonstrated by Tekchandani and Guruprasad (1998).

APX plays an important role in scavenging H_2O_2 produced by SOD, and is required to maintain the redox state of cells under stress (Asada, 1992). An increased APX activity by UV-B has been also observed in cucumber (Jain *et al.*, 2004a,b; Kataria *et al.* 2007), kidney bean (Singh, 2011), cotton (Dehariya *et al.*, 2011) and sunflower (Hagh *et al.*, 2012). However, a decline in the APX activity in the treated plants might probably be due to APX degradation or repression of *APX* gene expression under prolonged UV-B exposure (Casati *et al.*, 2002). Experiments conducted in chambers (Yannarelli *et al.*, 2006a; Rao *et al.*, 1996; Takeuchi *et al.*, 1996; Landry *et al.*, 1995), or even in the field (Mazza *et al.*, 1999), suggest that APX has an important role in the control of endogenous H_2O_2 content. An increase in ascorbate peroxidase activity has also been observed in response to supplemental UV-B radiation in cucumber cotyledons (Takeuchi *et al.*, 1996), rice and cucumber mature leaves (Kim *et al.*, 1996).

GR is an important enzyme of the ascorbate glutathione cycle (Rao and Reddy, 2008). An increase in GR activity by UV-B has been found in the earlier reports of Xu *et al.* (2008) and Cakirlar *et al.* (2011). Dertinger *et al.* (2003) reported that the GR activity was maximal in the youngest leaves, and was reduced during leaf development in tobacco. High GR activity maintains the pool of glutathione in the reduced state, allowing GSH to be used by DHAR to reduce dehydroascorbate to the reduced form of ascorbate (Noctor and Foyer, 1998). Increased GR activity was detected one hour after resveratrol treatment or UV-C treatment in peanut seedling leaves (Figure 6.7B), which have thus enhanced their tolerance to oxidative stress (Noctor and Foyer, 1998).

Changes in GR activity has been reported in other species during UV treatment (Mahdavian *et al.*, 2008), but multiple forms of GR may be expressed differentially in response to various stresses, and total GR activity changes may be less significant than changes in individual isoenzymes (Edwards *et al.*, 1990). Each of the antioxidant enzymes comprises a family of isoforms, often with different characteristics. UV-B exposure could induce different enzyme isoforms, such as POD (Yannarelli *et al.*, 2006b; Kataria *et al.*, 2007), CAT (Willekens *et al.*, 1994), SOD (Rao *et al.*, 1996) or APX (Yannarelli *et al.*, 2006b). Studies on the effects of UV-B on the enzymatic antioxidants, at both the activity level (Agrawal and Rathore, 2007; Yao and Liu, 2007; Yannarelli *et al.*, 2006b) and the mRNA level (Willekens *et al.*, 1994; Zinser *et al.*, 2007), have yielded inconsistent results.

6.5 UV-B and Antioxidant

There is also evidence indicating that UV-B radiation has an impact on non-enzymatic antioxidants, such as ASA (Giordano *et al.*, 2004; Jain *et al.*,2004a, 2004b; Kataria *et al.*, 2007), GSH (Kalbin *et al.*, 1997; Galatro *et al.*, 2001) and α-tochopherol (Carletti *et al.*, 2003; Jain *et al.*, 2004a, 2004b). α-Tocopherol is a principal biochemical antioxidant

defence molecule against lipid peroxidation, with a capacity to scavenge $O_2^{\bullet-}$, $^{\bullet}OH$ and $^1O_2$ (Fukuzawa *et al.*, 1985). Besides being an active *in vitro* chain-breaking antioxidant, the long-chain phytol tail on α-tocopherol allows the compound to partition into lipophilic membranes of cells and organelles, where it exerts its antioxidant activity in the prevention of oxidative damage (Burto *et al.*, 1983).

The α-tocopherol present in the thylakoid membrane protects the structure and function of photosynthetic membranes by scavenging active O_2 species and peroxyl radicals produced as a result of stress (Hess, 1993). In addition, exogenous application of α-tocopherol increases membrane stability under elevated UV-B (Pelle *et al.*, 1990). The tocopheroxyl radicals formed during the conversion of oxyradicals to hydroperoxides are reduced back to tocopherol by the ascorbic acid–glutathione cycle. Thus, ascorbic acid and α-tocopherol can act synergistically in the reduction of free radicals formed during any of the stress conditions (Leung *et al.*, 1981).

Ascorbic acid is a water-soluble antioxidant, and it can rapidly react *in vivo* with $O_2^{\bullet-}$ and $^{\bullet}OH$ to produce water (Nishikimi and Yagi, 1976). Since antioxidants like α-tocopherol and ascorbic acid are effective quenchers of oxyradicals, plants often respond to oxidative stress by enhancing the biosynthesis of antioxidants. An increase in the endogenous level of ascorbic acid after UV-B exposure has been reported in *Arabidopsis thaliana* (Rao and Ormond, 1995), wheat (Sharma *et al.*, 1998), cucumber cotyledons (Jain *et al.*, 2004a, 2004b) and *Vigna* species (Dwivedi *et al.*, 2015), to alleviate the detrimental effects of UV-B induced oxyradicals. In contrast to ASA, α-tocopherol level did not enhance in the cucumber cotyledons by UV-B. Instead, α-tocopherol content was decreased by UV-B exposure, indicating its utilization in quenching of oxyradicals (Jain *et al.*, 2004a, 2004b).

Increase in the AsA pool in response to UV-B exposure have also been observed (Galatro *et al.*, 2001; Takeuchi *et al.*, 1996; Rao and Ormrod, 1995). However, in maize seedlings, UV-B exposure had no effect on the AsA content (Carletti *et al.*, 2003). Under field conditions, long-term exposure to enhanced levels of UV-B did not change the AsA content (Taulavuori *et al.*, 1998). Baroniya *et al.* (2013) found that AsA content was decreased, while the DHA content was increased by solar UV-B, resulting in a decreased ratio of AsA/DHA. These UV-B effects on AsA are consistent with the results in wheat and bean (Agrawal and Rathore, 2007; Prasad *et al.*, 2005), and this could be explained by the increase of APX activity under UV-B exposure. Higher APX activity consumes more AsA and produces more DHA.

The glutathione pool was also slightly affected by solar UV-B exposure in soybean (Xu *et al.*, 2008), where only GSSG content was decreased by UV-B radiation. Increased thiol content by UV-B radiation has been reported in several studies (Galatro *et al.*, 2001; Kalbin *et al.*, 1997; Dai *et al.*, 1997; Rao and Ormord, 1995), but all these increases were found under high levels of UV-B. In the field, long-term exposure to enhanced UV-B radiation did not affect the thiol content in *Vaccinium myrtillus* (Taulavuori *et al.*, 1998), but increased it in wheat and bean (Agrawal and Rathore, 2007).

6.6 UV-B and Signalling

Plants show elevated levels of ROS due to disruption of metabolic activities and increased activity of membrane-localized NADPH-oxidase, in response to UV radiation (Hideg and Vass, 1996; Kalbina and Strid, 2006). In addition to being a

cell-damaging agent, ROS have been described as secondary signalling molecules (Mittler *et al.*, 2011). ROS-mediated signalling, a complex mechanism that depends on the nature of the individual ROS species, produced the balance between ROS-producing enzymes and the oxidation-reduction states of various antioxidants (De Tullio, 2010). Recently, the role of ROS as a trigger of UV-induced cell death processes in plants has been studied. ROS acts as a signalling molecule leading to the opening of the permeability transition pore (PTP) in the mitochondrial membrane, which leads to the release of cytochrome c and the generation of more ROS, causing a feedback loop that amplifies the original PCD-inducing stress signal (Reape and McCabe, 2008).

UVR8-mediated acclimatization is important for the survival of the plant against oxidative stress caused by UV- B radiation. Plants have a UV-B-specific signalling pathway that requires UV Resistance Locus 8 (UVR8), which has been recently reviewed in detail (Tilbrook *et al.*, 2013). Brown and Jenkins (2008) showed that UVR8 is a UV-B-specific signalling component that regulates UV-protective responses in *Arabidopsis*. UVR8-dependent signalling for UV-protective responses is triggered by UV-B below 1.0 μmol m^{-2} s^{-1} and, in most cases, as low as 0.1 μmol m^{-2} s^{-1}.

Recently, Wu *et al.* (2012) showed UVR8 as a photoreceptor for UV-B in *Arabidopsis* and, upon UV-B radiation, UVR8 undergoes an immediate switch from homodimer to monomer, hence triggers a signalling pathway for protection. Dimers of UVR8 function as a UV-B photoreceptor (Rizzini *et al.*, 2011), and the elegant crystallographic and spectroscopic studies of Christie *et al.* (2012) and Wu *et al.* (2012) demonstrated that the absorption of UV-B by specific tryptophan residues in UVR8 causes dissociation of the UVR8 dimer *in vitro*. Subsequent studies showed that the UVR8 monomer is necessary for interaction with Constitutively Photomorphogenic 1 (COP1) and downstream transduction though Elongated Hypocotyl 5 (HY5) (O'Hara and Jenkins, 2012).

Differential behaviour of UV-B was demonstrated by the type of response of plants to UV-B, which is dependent substantially on its fluence rates (Frohnmeyer and Staiger, 2003; Brown *et al.*, 2005). High fluence rate (UV-BH) of UV-B produces ROS, and may cause damage to DNA, proteins and lipids, while low fluence rate (UV-BL) may produce a protective response against other stress types (Frohnmeyer and Staiger, 2003). Low-fluence UV radiation activates UVR8-dependent photomorphogenesis. For example: increased level of UV-absorbing sunscreens gives acclimation response; increased antioxidative proteins can act as ROS scavengers; increased level of DNA repair enzymes can act on CPDs and 6-6 PPs lesions, and may result in cell cycle arrest and overall growth inhibition (Nawkar *et al.*, 2013). The authors suggested that UV defence responses are mediated by the UVR8-COP1-HY5 pathway, which increases sunscreen pigments and ROS scavenging activity upon ambient levels of UV exposure.

UV-B radiation induces DNA damage, resulting in cell cycle arrest, and may lead to cell death. The use of higher dosages of UV-B, or UV-C radiation which is physiologically irrelevant, can induce programmed cell death (PCD) in plants under laboratory conditions. The stress pathway activated under high fluence UV-B radiation is independent of UVR8 signalling (Nawkar *et al.*, 2013). UV overexposure induces an oxidative burst and subsequently disrupts the function of the vital organelles, chloroplast and mitochondria. Loss of mitochondrial transmembrane potential (MTP) causes the release of cytochrome c and, in turn, activates the metacaspase cascade in plants. Moreover, how the UV photoreceptor is involved in UV mediated cell death is not yet clear.

In order to answer the questions raised above, it will be important to study the effect of UV signalling components of the cell death pathways under conditions of high fluence UV radiation.

6.7 Conclusion and Future Perspectives

In conclusion, though sunlight is obligatory for photosynthesis and survival of plants, it also represents one of the major threats to their genomic integrity. Sunlight contains energy-rich UV-A (315–400 nm), UV-B (280–320 nm) and UV-C (100–280 nm) light. While UV-C is filtered out in the stratosphere, UV-B and UV-A can reach the earth's surface. UV-B is a key environmental signal that regulates diverse responses in plants. UV-B promotes UV protection and plant survival in sunlight, and influences metabolism, development and plant defence.

Reactive oxygen species are involved in UV-B induced responses in plants, both as signalling and damaging agents. Plants have developed complex antioxidant defence systems, involving several antioxidant enzymes, such as superoxide dismutase, ascorbic acid peroxidase, glutathione reductase and peroxidase, as well as antioxidants like ascorbate, glutathione, tocopherols and phenolics to scavenge excess ROS produced under UV-B stress. Effectively upregulating these enzymes is a key factor in conferring tolerance to UV-B. Reactive oxygen species produced by UV-B stress cause oxidative damage to membrane lipids, nucleic acids and proteins. This leads to reduction in photosynthetic pigments and proteins, which imposes limitations on photosynthesis, due to reduced photosynthetic efficiency of PSII and reduced activity of Rubisco under UV-B stress, which ultimately results in reduced yield of crop plants.

The role of UV light in signal transduction events in cells of photosynthetic organisms is an emerging field of UV research concerns. It will be highly important to explore the connection of these signalling events with the adaptation and acclimation processes occurring under ultraviolet exposure. In agriculture, our increasing knowledge of UV-B protective mechanisms employed by the plant may potentially lead to industrial applications. UVR8-mediated UV-B signalling may be exploited to alleviate the detrimental effects or to harness the desirable effects of UV-B exposure to improve plant productivity and quality overall. For example, changes in plant secondary metabolism in response to UV-B should be considered in terms of nutritional value. Also, with a clearer understanding of the interplay between UV-B, phytohormones and responses to other environmental signals, UVR8 UV-B signalling may prove a means to manipulate plant growth and/or plant tolerance to abiotic and biotic stress.

The molecular mechanisms behind UV-B responses are poorly understood. The biochemical changes, paralleled by strong modulation of gene expression of antioxidant enzyme after exposure to increase in the terrestrial solar UV-B radiation, will have to be evaluated in future. Further studies are needed in order to better understand the molecular basis of the UV-B responses and the impacts of solar UV-B on the ROS metabolism, along with other environmental factors on the proteome and ROS metabolism. Future progress in genomics, metabolomics, and proteomics will help in clear understanding of the biochemical networks involved in cellular responses to oxidative stress caused by UV-B. Improved understanding of these will be helpful in producing plants with inbuilt capability of improved levels of tolerance to ROS, using a biotechnological approach.

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7

Major Influence on Phytochrome and Photosynthetic Machinery Under UV-B Exposure

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7.1 Introduction

Sunlight is the primary energy source for photosynthesis, and it also serves as an environmental signal which regulates the growth and development of the plant. The electromagnetic spectrum constitutes different bands, based upon the wavelength. The electromagnetic radiation emitted from the sun has 7% of radiation in the UV range (200–400 nm) which contains UV-A (315–400 nm), UV-B (280–315 nm) and UV-C (100–280 nm). According to the theory of quantum mechanics, shorter wavelengths have higher energy and have detrimental effects on biological macromolecules.

The earth is surrounded by a stratospheric ozone layer that totally filters out UV-C, and absorbs a large proportion of the UV-B irradiation (Li *et al.*, 2013). As a result, the UV-B radiation is minimised to approximately 0.5% of the total amount of solar radiation, which has the highest energy in the daytime and also has a substantial effect on the biosphere (Caldwell *et al.*, 2003; Jenkins, 2009; Heijde and Ulm, 2012). However, UV-B radiation on the earth's surface has been stimulated due to changes in the depth of the ozone layer, either due to anthropogenic, atmospheric pollutants or natural factors (latitude, altitude, season), which may cause severe changes to biological systems (Madronich *et al.*, 1998).

In general, UV-B can induce two types of responses in plant: positive or photomorphogenic responses; and negative or stress responses. These kinds of responses depend mainly on fluence rate of UV-B. Low fluence rates of UV-B positively affect the plant by promoting photomorphogenic responses, such as inhibition of hypocotyl growth, cotyledon expansion, biosynthesis of anthocyanins and flavonoids, and stomatal opening (Kim *et al.*, 1998; Ulm and Nagy, 2005; Jenkins, 2009). In contrast, high fluence rates of UV-B negatively affect the plant by generating stress, which includes DNA damage, production of reactive oxygen species (ROS) and altered physiological processes, namely photosynthesis (Ulm and Nagy, 2005; Jenkins, 2009; Heijde and Ulm, 2012) (Figure 7.1). Therefore, the purpose of this chapter is to summarize our present knowledge of UV-B-mediated

UV-B Radiation: From Environmental Stressor to Regulator of Plant Growth, First Edition. Edited by Vijay Pratap Singh, Samiksha Singh, Sheo Mohan Prasad and Parul Parihar.
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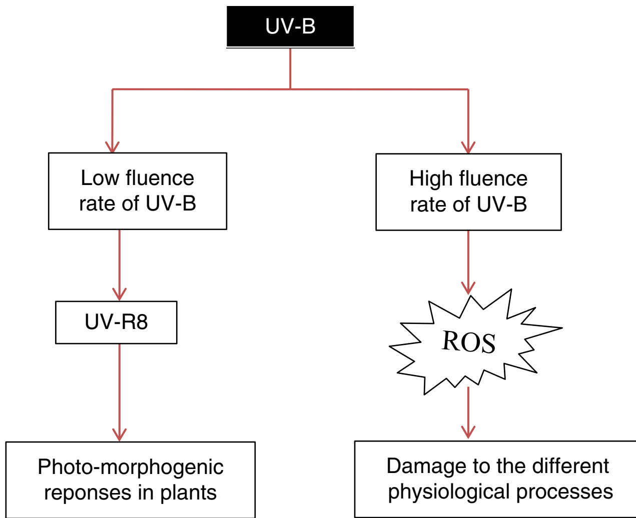

Figure 7.1 UV-B-mediated dose-dependent response of higher plants.

responses on the phytochrome system and photosynthetic processes in plants. We include the phytochrome system and its relation with UV-B, photomophogenic responses of UV-B, and the effect of UV-B on photosynthetic activity of plants.

7.2 Photomorphogenesis in Higher Plants

Photosynthetic organisms show several adaptation to regulate the light to optimize their performance and this phenomenon known as photomorphogenesis. These organisms have developed the ability to adjust in response to light and the phenomenon known as photomorphogenesis (Shinkle et *al.*, 2004). Light has a wide range of effects on the development of plants. The most prominent effects of light are observed when a germinating seedling is exposed to light. The developmental changes (pigment synthesis, leaf growth promotion, stem radical expansion, lateral root development) characteristic of photomorphogenesis, shown by de-etiolated seedlings, are induced by light. Characteristically, plants are responsive to the blue, red and far-red regions of the electromagnetic spectrum, through the action of several different photosensory systems (Figure 7.2). In this section, we have summarized the photomorphogenic responses mediated through phytochrome system and UV-B radiation.

7.2.1 Phytochrome System and its Interaction with UV-B

The development of plants needs a signal transduction pathway which includes photosignals from the environment. The photo-signals are regulated by an important class of chromoproteins and phytochromes. The phytochrome family consists of five members (*phyA*, *phyB*, *phyC*, *phyD*, *phyE*), and was the first class of plant photoreceptors to be identified. Phytochromes absorb light principally within the red and far-red region of the electromagnetic spectrum, and mediate a wide range of light-regulated developmental processes in plants (Franklin *et al.*, 2005).

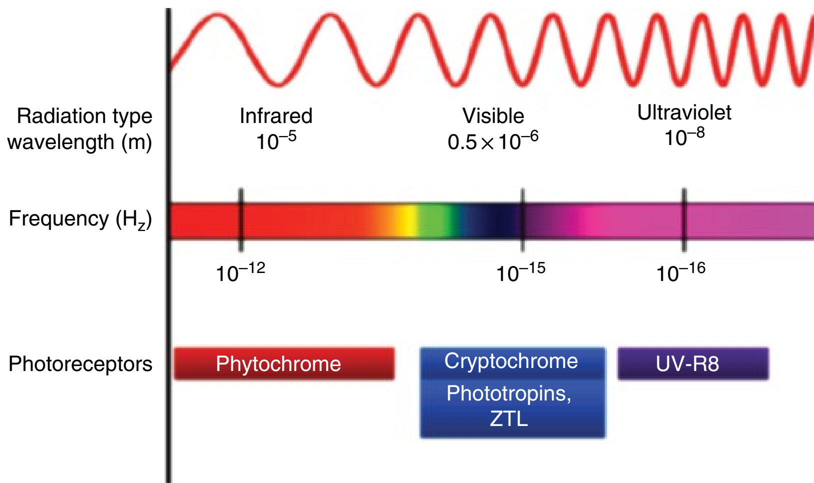

Figure 7.2 Photoreceptors-mediated signalling in higher plants.

Phytochrome protein structure is characterised by an N-terminal photosensory domain that non-covalently associates with the tetrapyrrole chromophore, phytochromobilin, and a histidine-like kinase at the C-terminus (Jiao *et al.*, 2007). Depending on the red/far-red light content, phytochromes exist in two isoforms – Pr or Pfr. Pr primarily absorbs red light, which results in photoconversion to its biologically active Pfr form (Franklin *et al.*, 2005). Upon illumination, both *phyA* and *phyB*, in the form of activated homo-dimers, translocate from the cytoplasm into the nucleus, where they form 'speckles' (Yamaguchi *et al.*, 1999; Gil *et al.*, 2000; Hisada *et al.*, 2000). The phytochrome affects all phases of plant development, such as seed germination, vegetative growth, reproduction and senescence.

Photo-transformation between the Pr inactive and Pfr active forms is efficiently achieved by red light (around 660 nm), but it is also driven by many other wavelengths, such as UV (300 nm) at a less efficient rate (Shinomura *et al.*, 1996). Under wavelengths other than red light, low quantities of Pfr accumulate, which allows plants to use phytochrome signalling pathways in response to particular light conditions such as very low irradiance, and therefore to best adapt to their environment (Shinomura *et al.*, 1996). *PhyA* is the only phytochrome able to produce such a very low fluence response (VLFR) (Casal, 2013), due to its highest sensitivity (Shinomura *et al.*, 1996). Kim *et al.* (1998) have also shown that low-fluence UV-B-induced hypocotyl inhibition response is mediated by more than one class of phytochromes, needing only a small amount of active photoreceptor for WT (wild type) response.

7.2.2 Photomorphogenic Responses of UV-B

One of the first stages in plant development involves photomorphogenesis, and the generation of photosynthetic apparatus, alterations in plant morphology and gene expression, in order to maximise light utilization for energy production. Photomorphogenesis in seedlings is largely controlled by red/far-red-absorbing phytochromes (*phyA–E*) and by blue/UV-A-absorbing cryptochromes (Batschauer, 1999; Quail, 2002). UV-B radiation

generally affects the biological tissues negatively, but a low fluence rate of UV-B mediates various physiological responses. Interestingly, a low fluence rate of UV-B also stimulates photomorphogenesis in etiolated seedlings, because the inhibition of hypocotyl elongation and opening of the apical hook are mediated independently of phytochromes and cryptochromes, and exhibit a UV-B fluence response relationship (Ballaré *et al.*, 1991, 1995; Kim *et al.*, 1998; Suesslin and Frohnmeyer, 2003).

Beggs *et al.* (1986) reported UV-B-mediated increase in the biosynthesis of flavonoids in Parsley (*Petroselinum crispum*) plants in isogenic cell cultures. Results suggest that phytochromes and cryptochromes are modulating the UV-B response, but are not sufficient to stimulate increased flavonoid levels without UV-B. This response pattern is not confined to parsley, but was also described for defined developmental stages of other plant species (Batschauer *et al.*, 1996; Wade *et al.*, 2001), as well as in cell cultures (Beggs *et al.*, 1986).

The UV-B specific pathway that regulates developmental processes appears to operate independent of the DNA damage pathway, according to action spectra comparisons (Kucera *et al.*, 2003). UV-B-specific acclimation responses are primarily induced by changes in gene expression, which lead to an increase in the activity of photoprotective enzymes, accumulation of UV-absorbing secondary metabolites and stimulation of DNA damage repair. However, photomorphogenic UV-B signalling is not independent of other light signalling pathways; UV-B and *phyB* interact to regulate cotyledon opening (Boccalandro *et al.*, 2001), and UV-B-induced chalcone synthase (CHS) expression is negatively regulated by *phyB* and synergistically enhanced by UV-A and blue light detected by photoreceptor(s) (Wade *et al.*, 2001).

As discussed above, responses to a low fluence rate of UV-B can be defined as photomorphogenic in character. The most extensively studied examples are the suppression of hypocotyl extension by low fluence rates of UV-B (Ballaré *et al.*, 1991, 1995; Kim *et al.* 1998; Boccalandro *et al.*, 2004; Suesslin and Frohnmeyer 2003; Shinkle *et al.*, 2004) and the UV-B induction of genes involved in flavonoid biosynthesis, such as CHS (Jenkins *et al.* 1997, 2001; Frohnmeyer and Staiger, 2003). Together, the results from a number of studies show that these UV-B responses are not mediated by DNA damage signalling, stress/wound/defence signalling, or the known photoreceptors, but instead involve distinct photomorphogenic signalling processes.

The threshold UV-B doses that initiate photomorphogenic responses are much lower than those that cause detectable DNA damage or induce stress/defence/wound gene expression. Less than 0.1 $\mu mol\, m^{-2}\, s^{-1}$ UV-B (approximately 1/40 of the fluence rate of UV-B in sunlight) is sufficient both to suppress hypocotyl extension (Kim *et al.*, 1998; Britt, 2004) and to induce CHS expression in *Arabidopsis* (Brown and Jenkins, 2008). Several genes associated with stress pathways require at least an order of magnitude higher fluence rate for UV-B induction (Brosché and Strid, 2003; Brown and Jenkins, 2008). Moreover, less than five minutes' exposure to UV-B increases CHS transcript abundance in *Arabidopsis* (Jenkins *et al.*, 2001), and second illumination is reportedly sufficient to stimulate transcription from the CHS promoter in parsley cells (Frohnmeyer *et al.*, 1999).

The photomorphogenic induction of gene expression does not correlate with CPD formation (Frohnmeyer *et al.*, 1999; Kalbin *et al.*, 2001). Moreover, mutants defective in DNA repair, which would be expected to show increased levels of responses mediated by DNA damage signalling, do not show altered suppression of hypocotyl

extension, promotion of cotyledon opening, or induction of several genes by low-fluence UV-B (Allan and Fluhr, 1997; Kim *et al.*, 1998; Ulm *et al.*, 2004). Furthermore, co-illumination with light that would repair DNA damage by photoreactivation does not reduce the UV-B induction of CHS, but actually enhances it through synergistic relations (Fuglevand *et al.*, 1996; Wade *et al.*, 2001). The very brief, low-fluence UV-B treatments that are sufficient to induce genes such as CHS are very unlikely to cause detectable accumulation of ROS or signalling molecules such as ethylene, salicylic acid (SA), and jasmonic acid (JA). It is therefore very unlikely that these molecules mediate photomorphogenic UV-B signalling.

Consistent with this hypothesis, the UV-B stimulation of defence gene expression is reduced in the JA and ethylene signalling mutants *jar1* and *etr1* (A-H-Mackerness *et al.*, 1999). Similarly, the UV-B induction of defence genes is inhibited by antioxidants (Green and Fluhr, 1995; Surplus *et al.*, 1998), these compounds do not impair the UV-B induction of CHS in *Arabidopsis* cells (Jenkins *et al.*, 2001). CHS expression shows little or no stimulation by ROS in either *Arabidopsis* cells (Jenkins *et al.*, 2001) or plants (Desikan *et al.*, 2001; Gadjev *et al.*, 2006), and hydrogen peroxide accumulation actually reduces the level of CHS expression (Vanderauwera *et al.*, 2005). Nevertheless, it has been reported that the UV-B induction of CHS is reduced in the *Arabidopsis atrbohdf* double mutant, leading to the suggestion that NADPH oxidase quantitatively affects the response (Kalbina and Strid, 2006).

Taken together, the above studies indicate that the photomorphogenic UV-B induction of CHS does not require either ROS or wound/defence signalling molecules. Further research has demonstrated that photomorphogenic UV-B responses are not mediated by the known photoreceptors. Although phytochromes, cryptochromes, and phototropins are able to absorb UV-B and, therefore, have the potential to mediate UV-B responses, various mutants lacking these photoreceptors retain low fluence UV-B induction of CHS and a number of other genes (Wade *et al.*, 2001; Brosché and Strid, 2003; Brown and Jenkins, 2008; Ulm, 2006). Similarly, the suppression of hypocotyl extension by UV-B is present in mutants that lack phytochromes and cryptochromes (Ballaré *et al.* 1991; Boccalandro *et al.*, 2001; Suesslin and Frohnmeyer, 2003).

7.2.3 UV-B Signal Transduction (UVR8)

The existence of UV-B receptors has been questioned for decades, although the effect of UV-B on anthocyanin biosynthesis has long been known (Arthur, 1936). Studies with mutants devoid of these photoreceptors now demonstrate that UV-B radiation independently affects the hypocotyl elongation response (Kim *et al.*, 1998; Suesslin and Frohnmeyer, 2003). Because some UV-B responses, such as chalcone synthase (CHS) expression, can be modulated by blue or red light, there is evidence that a complex web exists between phytochrome, cryptochrome and UV-B-signalling chains in cell cultures (Ohl *et al.*, 1989) and plants (Boccalandro *et al.*, 2001; Wade *et al.*, 2001). Photomorphogenic UV-B signalling is mediated by the UV-B-specific component UV Resistance Locus8 (UVR8). Both UVR8 and Constitutively Photomorphogenic1 (COP1) are required for UV-B induced signal transduction of the Elongated Hypocotyl5 (HY5) transcription factor. This regulates target genes involved in photomorphogenic responses of UV-B, which also include UV protection (Jenkins, 2009; Rizzini *et al.*, 2011; Wu *et al.*, 2012; see Figure 7.3).

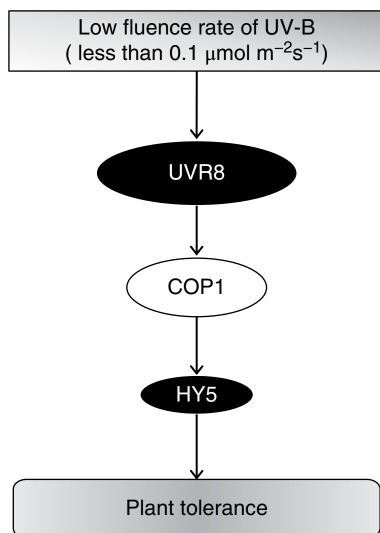

Figure 7.3 Schematic representation for signalling of low fluence rate of UV-B through UVR8. COP1, Constitutively Photomorphogenic 1; HY5, Elongated Hypocotyl 5; UV-B, ultraviolet-B radiation; UVR8, UV Resistance Locus 8 (Jenkins, 2009).

7.3 Effect of UV-B Exposure on Photosynthetic Machinery

Photosynthesis is a crucial step performed by all the autotrophic plants, and it is responsible for providing food to the organism. Photosynthetic apparatus (i.e. chloroplast) contains several components to perform the photosynthesis process, such as photosynthetic pigments, photosystems, the electron transport system, and the CO_2 reduction pathway. When these sites are affected by environmental stress, then it leads to reduction in photosynthetic yield of green plants.

Among different stress factors, UV-B stress affects the photosynthetic apparatus both directly and indirectly. The activity of PSII and PSI, Rubisco and ATP synthase activity, CO_2 fixation and oxygen evolution and total chlorophyll content are directly affected by UV-B stress (Lidon *et al.*, 2012). Indirect impacts include induction of stomatal closure, and changes in leaf thickness or anatomical structure and canopy structure, which indirectly impairs the rate of photosynthesis. To mitigate these adverse effects of UV-B stress, plants possess various defence mechanisms. Plants can protect themselves against UV-B radiation by increasing length of epidermal cells, by producing a waxy cuticle, and by accumulating UV-B absorbing compounds such as phenylpropanoids in the epidermal layer (Hollosy, 2002). These direct and indirect adverse effects and, protective defence mechanisms are discussed in the following sub-sections.

7.3.1 Direct Effects of UV- B on Photosynthetic Machinery

7.3.1.1 Effects of UV-B Stress on Components Involved in Light Reaction

The light-harvesting complex of PSII (LCH II) shows adverse effects against UV-B stress. In the presence of UV-B rays, there is a reduction in chlorophyll a/b binding proteins, due to reduction in the transcriptional level of cab genes (Vass *et al.*, 2005). Reductions in photosynthetic pigments lead to loss of photosynthetic yield (Jordan *et al.*, 1994). Marwood and Greenberg (1996) have suggested that UV radiation is mainly

responsible for the destruction of Chl *a*, more so than Chl *b*. Along with these pigments, significant reduction of carotenoids has also been noticed in barley under UV-B stress (Cicek *et al.*, 2012).

As carotenoids play an important role in the protection of chlorophyll, reduction in carotenoids could have serious impact on Chl pigments (Agrawal and Rathore, 2007; Mishra *et al.*, 2003). In the case of blue/green algae, phycobilisomes participate in light harvesting, and are profoundly affected by UV radiation. Due to destruction of the phycobiliproteins in the presence of UV-B rays, the energy transfer towards the photosynthetic reaction centres is also impaired (Sinha *et al.*, 1996). Restoration of phycobilisomes can be done, but it requires the development of new cells, via cell division (Vass *et al.*, 2001).

The thylakoid membrane, made up of these pigment-protein complexes, also shows the deleterious effects of UV-B radiation. UV-B rays result in leakage of the membrane, which increases ion permeability (Vass *et al.*, 2005). Gupta *et al.* (2008) have observed that there was distortion in the thylakoid membrane of *Spirulina platensis* under UV-B stress. UV-B radiations damaged the ultrastructure and photosynthetic light-harvesting complex of cyanobacteria by affecting the conformation of thylakoid membrane proteins. These modified proteins possibly originated from the UV-B-induced cross-linking of the thylakoid proteins. The thylakoid membrane is the main target of UV-B radiation, so it leads to reduction in its functioning, and alterations in the membrane organization (Petroluleas, 2002). Along with this, UV-B radiations also result in dilation of thylakoid membranes, and rupture of the chloroplast double membrane, which ultimately changes membrane permeability.

The photosynthetic pigments (Chl *a* and *b*) are important components of thylakoid membranes. They play a role as a light receptor, and help in the absorption, transmission and transformation of light during photosynthesis. With significant changes in their amount, there was significant decrease in the photosynthetic rate of plants. As discussed above, Zhang and Chen (2013) have also reported that chlorophyll *a*/*b* is lower in UV-B treated rice plants than that of the control. This indicates that degradation under UV-B stress is higher for Chl *a* than for Chl *b*. Chlorophyll *b* is present in the antenna system of two main optical components (particularly in PSI), whereas chlorophyll *a* exists in the core complex of PSI and PSII. This suggests more susceptibility of the core complex towards enhanced UV-B, compared with the peripheral antenna complex.

7.3.1.2 Effect of UV-B Stress on Photosystems and Cytochrome b6/f Complex

Photosystems are functional and structural units of the photosynthetic system. They have several functions, such as maintaining the primary photochemistry of photosynthesis and helping in the absorption of light and electron transfer. They are localised in the thylakoid membranes of chloroplastin plants, algae and cyanobacteria. There are two types of photosystems; PSI and II.

PSI is slightly affected by a high intensity of UV-B radiation. Several studies have showed minor or insignificant effect on PSI, compared with PSII (Turcsanyi and Vass, 2000). In the presence of a high intensity of UV-B radiation, there was destruction in the amount of oxidized reaction centre chlorophyll (P700) of PSI, which decreases the amplitude of absorption change at 700 nm. A significant downregulation of genes that encode PSI protein subunits in UV-B-exposed cells of the cyanobacterium *Synechocystis*

6803 were observed with the help of DNA microarray experiments (Huang *et al.*, 2002). A corresponding decreases in PSI activity has not been reported, and the possible targets within PSI and damage to its protein structure have not been studied in detail.

Between the two photosystems, PSII is the major component of photosynthetic apparatus, so it can be called the heart of photosynthesis. At this site, conversion of solar energy into chemical energy takes place. PSI and PSII are connected by the electron transport system, which is also affected by UV-B. The photosynthetic electron transport chain is made up of different components – namely, PSII, the Cyt b6/f complex, PSI, and the free electron carriers plastoquinone and plastocyanin. Electron transportation occurs on light stimulation at PSII and PSI, which are also linked with oxidation of water at the other end of the chain. Thereafter, electron flow occurs within the electron transport chain and results in the creation of the proton pump into the thylakoid lumen, which is utilized by the enzyme ATP synthase in order to synthesize ATP. On the other end of the chain, reducing power is generated, which helps in the CO_2 assimilation with the help of ATP.

All these components of electron transport chain become inactivated under UV-B stress, which leads to destruction of the water-oxidizing manganese (Mn) cluster of PSII. UV-B stress also affects electron acceptors and donors, such as quinine and tyrosine, and the reaction centres of the D1 and D2 protein present in PSII. As D1 and D2 are made up of polypeptides, they are the sites most susceptible to UV-B. UV-B also results in the generation of reactive oxygen species, which consequently decreases the rate of oxygen evolution and variable fluorescence.

Cytochrome b6/f complex acts as a mediator between the two photosystems. It helps in the oxidation of plastoquinol at PSII, and reduction of plastocyanin at PSI. Among different sites, it was found to be the site least affected by UV-B stress (Strid *et al.* 1990). This resistance is provided by quinone binding sites, through oxidation and reduction at the quinol site (Hope, 1993). The resistance of quinone under UV-B stress suggest its role in the attenuation of UV-B-induced damage in the photosynthetic apparatus.

Figure 7.4 shows the different centres of electron transport chain between PSII and PSI susceptible to UV-B damage. Wang *et al.* (2010) have also supported this fact by exposing the plant *Wolffia arrhiza* to UV-B radiation for 12 hours. They observed significant inhibition in CO_2 assimilation rate and the chlorophyll *a*, chlorophyll *b* (Chl *a*, Chl *b*) and carotenoids content. Other parameters, such quantum yield of primary photochemistry (Φ_{Po}), electron transport (Φ_{Eo}) and efficiency per trapped excitation (Ψ_o), were also decreased under high UV-B radiations. Along with this, the amount of active PSII reaction centres per excited cross section (RC/CS), and the total number of active reaction centres per absorption (RC/ABS), also showed significant changes. Thus, overall, it was concluded that the rate of photosynthesis decreased under high irradiance of UV-B, due to inactivation of reaction centres in the electron transport chain (Wang *et al.*, 2010).

Chlorophyll fluorescence can be used to assess changes in photosynthetic rate of higher plants grown under stress condition (Rajagopal *et al.* 2000). This study depicted the physiological status of plants by observing the process of absorption, transmission and conversion of light energy under different environmental conditions. F_v/F_m is the maximum quantum yield of PSII photochemistry, affecting the quantum yield when all reaction centres are open at PSII. In the presence of high doses of UV-B, chlorophyll fluorescence parameters change significantly, including: the quantum yield of primary

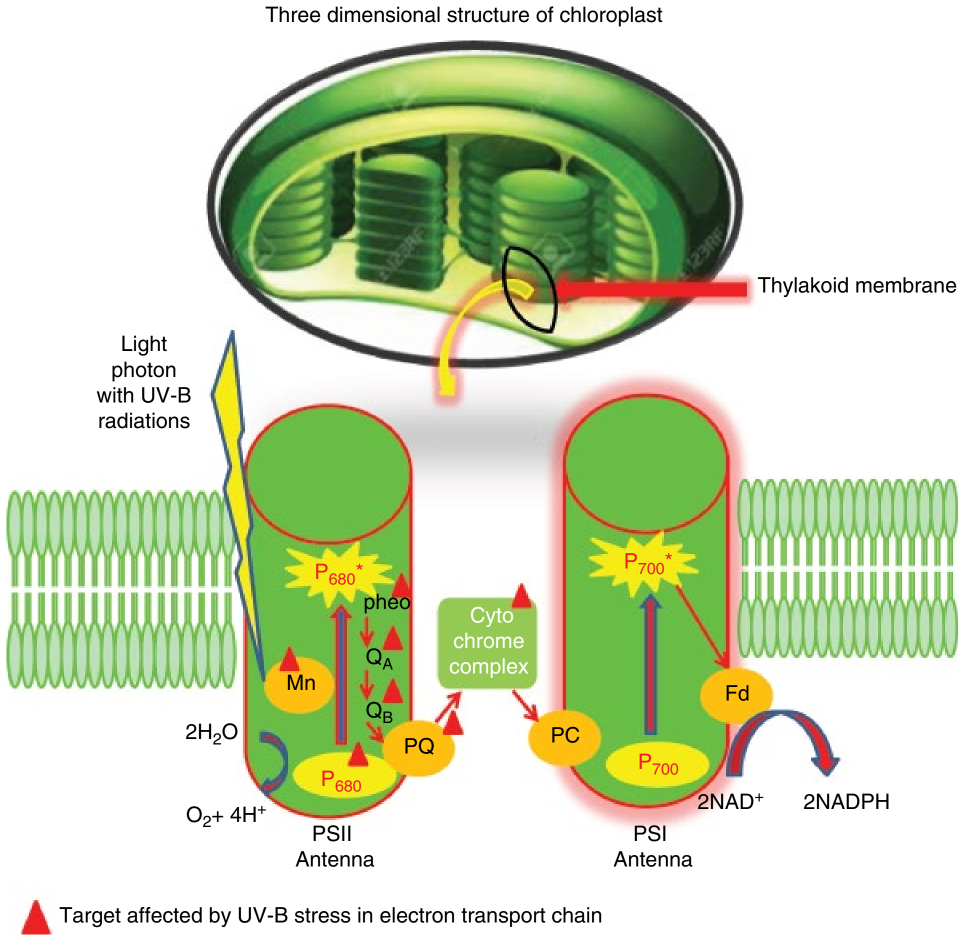

Figure 7.4 Different centres of electron transport chain susceptible to UV-B damage (Kataria *et al*., 2014)

photochemistry (ϕP_0); yield of electron transport per trapped excitation (Ψ_0); quantum yield of electron transport (ϕE_0); performance index of PSII (PI_{ABS}); the energy fluxes for absorption of photon per active RC (ABS/RC); trapped energy flux per active RC (TR_0/RC); electron transport flux per active RC (ET_0/RC); and energy dissipation flux per active RC (DI_0/RC). It consequently results in a decrease in the potential photosynthetic rate, disruption of photosynthetic electron transport and reduction in thylakoids proton gradient (Zhang and Chen, 2013). Thus, chlorophyll fluorescence parameters can be used as the internal probe of photosynthesis.

UV-B exposure of *Spirulina platensis* results in alterations in fluorescence emission in the pigment-protein complexes of thylakoids. Photoinhibition of effective quantum yield ($\Delta F/Fm'$) has been found to be decreased in 30% *of Gelidium latifolium* under UV-B exposure (Gomez and Figueroa, 1998). Ranjbarfordoei *et al*., (2011) have observed effect of UV-B stress on *Prunus dulcis* plant by estimating the range of chlorophyll (Chl) fluorescence parameters (FPs), Chl contents and photosynthetic gas-exchange. In the

presence of UV-B stress, they found an increase in F_0, which indicates the impairment of the light harvesting complex of PSII due to its adverse effects on photosystem II (PSII) activity. Due to a reduction in the rate of quencher QA, there was significant reduction in Fv, which indicates a decrease in PSII quantum yield that affect variable fluorescence (Fv, F_v/F_m, and F_0/F_m).

7.3.2 Indirect Effect of UV-B Stress on Components Involved in Dark Reaction

7.3.2.1 Impact on Regulation of Stomata and Rubisco Enzyme

Stomatal regulation is one of the important steps during the photosynthesis process, as its movement is responsible for influx of CO_2 influx and loss of water. The opening and closing of stomata is dependent upon several environmental factors. UV-B radiations also showed their adverse effect on stomatal movements (Eisinger *et al.*, 2003; He *et al.*, 2013). It was found that in *Vicia faba* stomatal opening and closing is induced by UV-B radiations (Jansen and Noort, 2000).

The adverse impact of UV-B radiation has been studied in woody perennial plants. (*Fraxinus excelsior*, *Betula pendul*, *Quercus robur* and *Acer pseudoplatanus*, *Tilia cordata*). They were exposed to UV-B radiation for five years, in field conditions. After the fifth year of UV-B exposure, reductions in photosynthesis, transpiration, water use efficiencies, stomatal density, stomatal conductance and carboxylation efficiency were noticed (Keiller and Holmes, 2001). Eisinger *et al.*, (2003) have also reported that in Arabidopsis (*Arabidopsis thaliana*), stomatal opening is stimulated by lower doses of UV-B, whereas stomatal closure is induced by higher irradiance of UV-B.

Tossi *et al.*, (2014) have found that, in the presence of a high fluence rate of UV-B, stomata become closed in abaxial epidermal strips of the *Arabidopsis* ecotype *Landsberg erecta*. The rate of CO_2 assimilation is reduced by UV-B rays through induction of stomatal conductance (Jansen and Noort, 2000; Lu *et al.*, 2009; Reddy *et al.*, 2013). The guard cells of stomata are directly affected by high UV-B irradiances that affect the process of stomatal opening (Nogués *et al.*, 1999). In the presence of UV-B radiation, the aperture of a guard cell is unable to readjust its opening and closing by affecting solute fluxes, leading to stomatal movement.

Nogués *et al.* (1999) have observed that UV-B radiation doses result in a substantial decrease of stomatal conductance. Compared with the abaxial side, the adaxial side of stomata is more sensitive towards UV-B treatments. This demonstrates the direct effect of UV-B on the guard cells. Adaxial guard cells receive much higher UV-B irradiation than the mesophyll cells and abaxial guard cells, due to presence of UV-B-absorbing pigments such as flavonoids on leaves, particularly in the epidermal layer. Gitz *et al.* (2013) conducted an experiment to show the effect of ambient levels of UV-B radiation on stomatal development and its density and, consequently on water-use efficiency (WUE). For this, soybean [*Glycine max* (L.) Merr.] isolines with variation in distribution of stomata and flavonoid content were grown in the field. The accumulation of UV-screening phenolic pigments was higher in isolines exposed to solar UV-B. UV-B exposure resulted in reduction in stomatal density and conductance in all isolines.

Along with stomatal regulation, other enzymatic activities are also affected by UV-B radiations. Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco, EC 4.1.1.39), is the most abundant leaf protein in plants, and it is also susceptible to UV-B damage (Yu *et al.*, 2013). In the Calvin cycle, this enzyme helps in CO_2 incorporation to

synthesise sugar. The aromatic amino acids in the Rubisco protein absorb UV-B and, due to this, it can be an excellent tool for investigating protein damage in the presence of UV-B rays. The amino acid tryptophans (Trp), which is present in each of eight large subunits (LSU, 53 kDa) and eight small subunits (SSU, 14 kDa) of the Rubisco holoenzyme, is sensitive to UV-B rays.

Researchers have reported that Rubisco activity is inhibited by UV-B radiation (Takeuchi *et al.*, 2002; Fedina *et al.*, 2010).The modification of the peptide chain and protein degradation under UV-B stress lead to inactivation of Rubisco activity (Takeuchi *et al.*, 2002; Bouchard *et al.*, 2008). Under UV-B stress, reduction in Rubisco activity is correlated with reduction in the mRNA level of Rubisco subunits. The larger subunit gets divided into two polypeptides by ROS in chloroplasts under UV-B illumination. Along with this, there is also expression of senescence-associated genes (SAGs), namely SAG12, under UV-B stress in *Arabidopsis* sp. It helps in encoding an enzyme, cysteine protease, which is responsible for enhancing Rubisco degradation (John *et al.* 2001).

Other enzymes, such as RuBP and sedoheptulose 1,7-bisphosphatase, also showed degradation in their activities by UV-B radiation (Savitch *et al.*, 2001). A reduction in photosynthetic rate in *Brassica napus* under UV-B radiation of 200 mol m^{-2} s^{-1} PAR has been observed. This reduction was due to a decrease in the capacity of sucrose biosynthesis, limitation of triose-P consumption, and a decrease in the regeneration rate of RuBP. Along with this, inhibition of PS II photochemistry and reduction in ATP supply were also observed.

7.3.3 UV-B induced ROS Production in Plants

UV-B rays are also responsible for generating reactive oxygen species (ROS) in plant systems. In photosynthetic apparatus, photosystem I and II (PSI and PSII), and the electron transport chain (ETC) in chloroplasts are the major sites for the production of ROS (Gill and Tuteja, 2010). The ROS are responsible for increasing activity of antioxidative enzymes, and production of oxidative membrane damage products (Jansen *et al.*, 2008). There is a significant increase in the thiobarbituric acid (TBARS) reacting substance which impairs cell defence systems, it is a good indicator of UV-B induced damage (Li *et al.*, 2010; Hassan *et al.*, 2013).

Excess of UV-B rays lead to the inhibition of photosynthesis as well as the formation of ROS. The singlet oxygen produced from photsensitization plays a crucial role in damaging the D1 protein. Hydroxyl radicals ($^{\bullet}$OH) are produced as the dominating reactive oxygen in the thylakoids membrane under UV-B stress (Szilard *et al.*, 2002). The Mn cluster is also affected by $^{\bullet}$OH radicals in the presence of high irradiance of UV-B. The production of superoxide radicals disturbs the balance between the light phase and dark phase, which might be due to a reduction in of ribulose-1,5- bisphosphate carboxylase/oxygenase (Rubisco) activity (Bischof *et al.*, 2000).

7.3.4 Protective Adaptation

In response to UV-B stress, plants possess various defence mechanisms to protect photosynthetic machinery. These include: increased length of epidermal cells; production of a waxy cuticle; accumulation of UV-B absorbing compounds, particularly phenylpropanoids, in the epidermal layer; and activation of different scavenging systems of various active oxygen species (Hideg *et al.*, 2003). Blue/green algae can mitigate the

damaging effects of UV-B rays by accumulating enzymes and xanthophylls to nullify the toxic effects of highly reactive oxidants produced under UV-B stress. Along with this, some UV-absorbing compounds are also synthesized, and water-soluble oligosaccharide-mycosporine amino acids (OS-MAA) are accumulated to prevent UV photodamage (Ivanov *et al.*, 2000).

Photosynthetic components show difference in sensitivity, depending upon the efficiency of their repair processes in the presence of UV-B stress. It was reported that in cyanobacterium *Syenechocystis* 6803, the restoration of PSII activity can be done by *de novo* synthesis of the damaged D1 and D2 protein subunits (Sass *et al.*, 1997). In another study, with *Synechocystis* sp. 6803, it was found that three psbA genes (called psbA1, psbA2 and psbA3) were responsible for restoration of PSII. Under UV-B stress, psbA3 is expressed significantly (Máté *et al.*, 1998), and the protein synthesized with this helps in the restoration of damaged PSII complex.

There is also another mechanism to obtain protection against the detrimental UV effects in *Synechoccus* sp. 7942. This shows two type of D1 protein, D1:1 and D1:2, which are encoded by psbAI and psbAII, AIII, respectively. Exchanging these two types of protein with each other can mitigate the damaging effect of UV-B irradiation (Campbell *et al.*, 1998). Under UV-B stress, the D2 subunit encodes psbD1, and psbD2 shows some changes. In this situation, the expression level of psbD2 is significantly enhanced in *Synechocystis* sp.6803, and provides protection against UV-B rays by increasing the synthesis of D2 protein (Viczián *et al.*, 2000).

Many phenylpropanoid compounds are effective attenuators of sunlight and are, therefore, considered to serve important functions in protecting photosynthetic organs faced with a superabundance of radiant energy (Agati and Tattini, 2010). Of all the phenylpropanoids, the anthocyanins are unusual, as they absorb quanta in the green region of the solar spectrum, possibly protecting chloroplasts from the effects of absorbing supernumerary photons (Kytridis and Manetas, 2006; Lev-Yadun and Gould, 2007; Gould *et al.*, 2010). There is, indeed, good experimental evidence that anthocyanins mitigate photoinhibitory and photooxidative damage across a range of species, the pigments acting not only as light attenuators, but also as quenchers of reactive oxygen species (ROS) (e.g. Neill and Gould, 2003; Gould, 2004; Kytridis and Manetas, 2006).

Nevertheless, the putative involvement of anthocyanins in plants is challenged against an excess of light, a condition transiently experienced by leaves on a daily, as well as on a seasonal basis (Li *et al.*, 2009). However, anthocyanins, when acylated with coumaroyl or sinapoyl moieties (Andersen *et al.*, 2010), are effective attenuators of ultraviolet light, not only of green solar wavelengths. This is important, as UV attenuation by phenylpropanoids may have a tremendous impact on leaf/whole-plant architecture and, in turn, on light-induced adjustments in the physiology and biochemistry of leaves/plants (Potters *et al.*, 2007; Pollastri and Tattini, 2011).

There are antioxidant defence systems in plants to scavenge excess ROS produced under UV-B stress. They contain several enzymes and metabolites (Jansen *et al.*, 2008). The enzymatic antioxidants include: superoxide dismutase (SOD; EC 1.15.1.1); catalase (CAT;EC 1.11.1.6); peroxidase (POD; EC 1.11.1.7); ascorbic acid peroxidase (APX; EC 1.11.1.11); glutathione reductase (GR; EC 1.6.4.2); dehydroascorbate reductase (DHAR; EC 1.8.5.1); monodehydroascorbate reductase (MDHAR; EC 1.6.5.4), and nonenzymatic antioxidant systems: reduced glutathione (GSH); ascorbic acid (AsA); α-tocopherol; and carotenoids, etc.

Under UV-B stress, transcription of key enzymes of the antioxidative enzyme system, such as APX, SOD, POD, GR and CAT is increased (Foyer and Noctor, 2009; Garg and Manchanda, 2009). UV-B stress leads to the production of ROS such as $O_2^{\bullet -}$, H_2O_2, and enhanced the lipid peroxidation and electrolyte leakage (Prasad *et al.*, 2005), which are scavenged by different antioxidants. SOD plays an important role in the detoxification of highly reactive super oxides radicals, produced by over-reduction of election transport chains in the thylakoid membranes (Foyer and Noctor, 2009). H_2O_2 is another ROS, detoxified through the action of CAT, peroxidases and low-molecular-weight antioxidants (Garg and Manchanda, 2009).

Other non-enzymatic antioxidants, such as ascorbate, α-tocopherol and reduced glutathione, are also responsible for decreasing the level of oxidative stress under UV-B stress (Kataria *et al.*, 2007). The oxidation state of ascorbate is very important for its functioning in the signalling process. A change in its oxidation state results in a decrease in plant growth and development. With the help of enzymes MDHAR and DHAR, the regeneration of oxidized ascorbate is achieved. Thus, upregulation of these enzymatic and non-enzymatic antioxidants play a crucial role in conferring resistance towards UV-B stress (Selvakumar, 2008).

Dobrikova and Apostolova (2015) have reported that quercetin (a compound of natural flavonoids), which is a dihydroxy β-ring substituted flavonoid and present in the chloroplast envelope membrane, presumably in the outer envelope membrane, is very important for the defence of photosynthetic apparatus against UV-B damage. It provides protection by increasing the production of antioxidant and by absorbing the UV-B. In addition, under alkaline conditions, it increases the fluidity of the membrane and the transfer of energy from PSII to PSI. The authors concluded that quercetin results in modification of the thylakoid membranes' structure and the Mn cluster, in order to decrease the adverse effect of UV-B rays.

7.4 Conclusion and Future Perspectives

Ultraviolet-B (UV-B) radiation is a small component of sunlight, but it can induce two types of responses on the basis of its fluence rate. A low fluence rate of UV-B induces photomorphogeneic responses alone, or by interacting with the phyrochrome system. The photomorphogenic responses of UV-B are mediated through a special signal transduction pathway which has a photoreceptor, UVR8. In contrast to a low fluence rate, a high fluence rate of UV-B can generate ROS, which inhibits the physiological processes (i.e. photosynthesis rate). A high fluence rate of UV-B can damage the photosynthesis apparatus and decline the photosynthetic activity, and it also causes CO_2 fixation to decline, by inhibiting the activity of Calvin cycle enzymes, and affects the growth and development of the plant.

Little is known about the interactions between UV-B and other photomorphogenic responses regulated by blue and red light signalling. UV-B is a very variable environmental signal, and fluctuations in its fluence rate will probably modulate the levels of ROS. ROS levels are expected to change rapidly through alterations in the balance of production and scavenging. These pathways are likely to function in plants at different levels of UV-B, although relatively little information is available on this point.

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8

UV-B Radiation-Induced Damage of Photosynthetic Apparatus of Green Leaves: Protective Strategies *vis-a-vis* Visible and/or UV-A Light

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8.1 Introduction

Solar electromagnetic radiation with relevance to photosynthesis of green leaves comprises the visible region (400 nm–700 nm) and ultraviolet (UV; 100–400 nm) radiation. The UV spectrum on the Earth's surface, comprising 5% of the solar spectrum, includes UV-A (315–400 nm) and UV-B (280–315 nm) radiation, while the UV-C (100–280 nm) component is filtered out completely (McKenzie *et al.*, 1999). These two UV bands have several adverse effects on plants in general, and the photosynthetic apparatus (PSA) of green leaves in particular. With the depletion of stratospheric ozone, the level of UV-B radiation is increasing on the Earth's surface. In this chapter an attempt has been made to summarize the progress on systematic analyses and rational approaches of plant responses to UV-B stress.

8.2 UV-B Effects on the Photosynthetic Apparatus of Leaves

UV-B radiation affects several physiological processes of green leaves, including photosynthesis. The radiation downregulates photosynthesis by inactivating photosystem (PS) II at multiple sites (Turcasanyi and Vass, 2000; Gaberscik *et al.*, 2002; Vaas *et al.*, 2005), while its effect on PSI is negligible (Teramura and Ziska, 1996; Biswal *et al.*, 1997). The overall damaging effects of UV-B radiation include downregulation of photosynthetic genes, inactivation of the PSII reaction centre (RC), decrease in the levels of Chl and Cars, loss in thylakoid integrity, alteration in chloroplast ultrastructure and reduction in the activity of Rubisco (Melis *et al.*, 1992; Friso *et al.*, 1994a, 1994b; Strid *et al.*, 1994; Teramura and Sullivan, 1994; Teramura and Ziska, 1996; Greenberg *et al.*, 1989, 1996; Jansen *et al.*, 1996, 1998; Vass, 1997; Kulandaivelu and Lingakumar, 2000; Vaas *et al.*, 2005; Lidon, 2012; Zlater *et al.*, 2012). Figure 8.1 depicts the sites of damage of PSA in response to UV-B radiation.

UV-B Radiation: From Environmental Stressor to Regulator of Plant Growth, First Edition. Edited by Vijay Pratap Singh, Samiksha Singh, Sheo Mohan Prasad and Parul Parihar.
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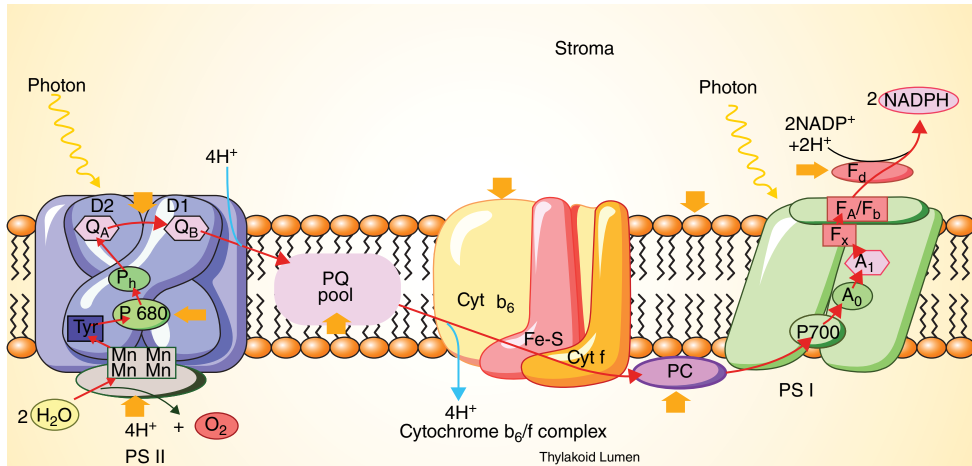


Figure 8.1 A schematic representation showing different sites of damage in the PSA of green leaves, as induced by UV-B radiation. The scheme depicts the arrangement of major protein complexes of PSII, PS I, cyt b6/f complex, oxygen-evolving complex and components of electron transport chain within the thylakoid membrane. The sites of damage are indicated by the thick arrows. Modified after http://en.wikipedia.org/wiki/thylakoidmembrane.

Although UV-B-mediated damage of 47 and 43 kD pigment-protein complexes associated with PSII (Gupta *et al.*, 2008), Q_B binding site and electron transport chain between PSII and PSI (Bornman, 1989; Jordan, 1996) are significant, the inactivation of RC (Bornman, 1989; Jordan, 1996) and oxygen evolving complex (OEC) (Vaas, 1997) are vital for the decline in efficiency of PSII. The inactivation of all these components leads to a drastic loss in O_2 evolution (Renger *et al.*, 1986, 1989; Barbato *et al.*, 1995; Segui *et al.*, 2000).

The precise nature of inactivation of OEC and RCII has not yet been understood, nevertheless, the tetra-nuclear Mn complex of OEC has been identified as the primary site of inactivation (Hideg *et al.*, 1993; Vaas, 1997). The sensitivity of OEC to UV-B radiation is significantly higher in the S_3 and S_2 states of Mn-complex than in the S_1 and S_0 states (Szilárd *et al.*, 2007). The redox components at both the donor and the acceptor sides of PSII are also affected by the radiation. The acceptor side of PSII is affected, either due to direct damage to plastoquinone (PQ) molecules (Bornman and Teramura, 1993), or due to modification of quinone binding sites (Renger *et al.*, 1989).

The radiation, besides inducing degradation of D1 and D2 proteins (Vaas *et al.*, 2005), downregulates the D1 and D2 proteins' turnover (Jordan, 1996). The damage to D1 and D2 proteins is mediated through ROS and semiquinone radicals, which are induced in response to UV-B radiation (Brosché and Strid, 2003; Zvezdanovic *et al.*, 2013). Joshi *et al.* (2011) have opined that the accumulation of Q_A^- alters the redox poising between Q_A and Q_B of the photosynthetic electron transport chain, leading to a loss in the redox homeostasis. They have linked the generation of excitation pressure and loss in redox homeostasis to the degradation of D1 and D2 proteins of the PSII reaction centre, as observed by Friso *et al.* (1994a, 1994b). The losses in the balance between energy source-sink relation and redox homeostasis lead to an increase in the production of ROS, finally culminating in disruption of thylakoid membrane and pigment loss.

8.3 UV-A Effects on Photosynthetic Apparatus of Leaves (Damage and Promotion)

Although UV-A radiation has certain beneficial effects on some physiological processes (Shiozaki *et al.*, 1999; Helsper *et al.*, 2003; Krizek, 2004), it is known to inflict damage on plants' photosynthesis (Biswal *et al.*, 2011; Joshi *et al.*, 2013b). Under different environmental and growth conditions, the damaging effects on PSA become significant, and even alarming (Joshi *et al.*, 1997; Turcasanyi and Vaas, 2000; Vaas *et al.*, 2002; Nayak *et al.*, 2003). The radiation is known to induce a loss in photosynthetic pigments, Car-to-Chl energy transfer efficiency, thylakoid membrane integrity and photosynthetic efficiency (Joshi *et al.*, 1994; Biswal *et al.*, 2006; Ivanova *et al.*, 2008). The electron transfer chain, oxygen-evolving system (OES) and Q_B binding site of RCII are also damaged by the radiation (Joshi *et al.*, 1997; Turcasanyi and Vaas, 2000; Nayak *et al.*, 2003). However, damages of PSA in response to UV-A exposure depend on the intensity of the radiation and the duration of exposure (Unal *et al.*, 2009). The sensitivity of the apparatus to the radiation also depends on the status of the leaf and environmental conditions.

Conversely, UV-A radiation effectively promotes the growth of cotyledons, photosynthetic pigment synthesis, anthocyanin formation and flavonoid synthesis, and inhibits hypocotyls elongation in certain plants (Mohr and Schopfer, 1995). The gene expression for RCII proteins is activated by UV-A radiation (Christopher and Mullet, 1994). Additionally, a blue/UV-A light induced nuclear-encoded protein ELIP is known to prevent the degradation of PSA under light stress (Adamska *et al.*, 1992). These non-damaging effects have been suggested to be controlled by a blue/UV-A photomorphogenic photoreceptor (Mohr and Schopfer, 1995).

8.4 UV-A-Mediated Modulation of UV-B-Induced Damage

UV-A radiation, when it accompanies UV-B (UV-A + UV-B), moderately reverses the negative changes of PSA caused by UV-B radiation (Joshi *et al.*, 2007; Lud *et al.*, 2002). UV-A + UV-B treatment partially restores the photochemical potential (F_v/F_m) from UV-B-induced decline, suggesting that accompanying UV-A helps in developing a protective pathway against UV-B-induced impairments. Bernal *et al.* (2013) have observed an increase in Car content and Chl*a*/*b* ratio in Mediterranean plants grown under UV-A + UV-B radiation, compared with plants grown without UV-A. UV-A exposure helps in acclimatization to UV-B radiation through accumulation of flavonoids, increase in stomatal conductance and reduction in the functional size of PSII (Joshi *et al.*, 2013a). UV-A-specific chloroplast movement, a photomorphogenic response which varies the excitation energy distribution between two PS, is known to protect PSA from UV-B-induced damage (Davis and Hangarter, 2012).

Additionally, plants have an inbuilt defence mechanism, consisting of both enzymatic and non-enzymatic processes, to tackle the problems of oxyfree radicals. The processes include the activity of super oxide dismutase, catalase, ascorbate peroxidase, glutathione reductase, monodehydroascorbate reductase, dehydroascorbate reductase, guaiacol peroxidase, and glutathione-S-transferase (enzymatic), and defence mechanisms

involving, reduced ascorbate, reduced glutathione, α-tocopherol, β-carotene, carotenoids and the xanthophyll cycle (non-enzymatic). These mechanisms are reported to be upregulated in response to UV-B exposure. The flavonoids also save the chloroplasts from oxidative damage by prohibiting oxygen-promoted redox reactions.

Photo-reactivation involves the process of activating the enzyme photolyase with the help of longer wavelength electromagnetic radiation, to repair the damaged DNA (Pang and Hays, 1991). The PSII repair mechanism, on the other hand, depends on an enhancement in turnover of D1 and D2 proteins. These newly synthesized proteins replace the damaged ones. This process is activated by blue/UV-A light (Christopher and Mullet, 1994). The degree of DNA damage as induced by UV-B radiation is also effectively diminished (Lud *et al.*, 2002). Plants possess an efficient PSII repair mechanism pertaining to both DNA repair and *de novo* synthesis of UV-B-sensitive PSII reaction centre proteins (Bornman, 1989; Vass, 1997).

8.5 PAR-Mediated Balancing of UV-B-Induced Damage

Findings of Kolb *et al.* (2001) and Xiong and Day (2001) suggest that UV-B-induced inactivation of PSII is temporary, and the damage is alleviated in course of time under natural conditions. Recovery from UV-B-induced structural and functional damage of PSII in the presence of visible light has been reported by Bergo *et al.* (2003). The degree of damage of PSA is also lessened when UV-B radiation is accompanied by visible light (Pradhan *et al.*, 2006). Higher plants usually demonstrate their potential to balance the damage caused by UV-B radiation in the presence of PAR through protective, repair and acclimation mechanisms (Jansen *et al.*, 1998). They develop a protective mechanism by evoking DNA repair to counteract the damaging effects (Frohnmeyer and Staiger, 2003).

Plants in high UV-B regions appear to be well protected against UV-B damage, due to accumulation of UV-B absorbing phenolic compounds in the outer tissues to screen the radiation (Mazza *et al.*, 2000; Kolb *et al.*, 2001; Rozema *et al.*, 2002; Storch *et al.*, 2008). They engage Cars in negotiating UV-B induced oxidative stress for dissipation of unused quanta and scavenging ROS (White and Jahnke, 2002; Gartia *et al.*, 2003).

8.6 Photosynthetic Adaptation and Acclimation to UV-B Radiation

Plants, being sessile, do not have any means of evading the harmful effects of UV-B radiation, and have evolved mechanisms to counter the damaging effects. While the question of how plants perceive the signal for development is still haunting, there is plenty of literature documenting UV-B signal perception and photomorphogenic responses to confer adaptation and acclimation (Beggs and Wellman, 1994; Barnes *et al.*, 1996). Radiation at a higher intensity is known to trigger photomorphogenic responses through a putative UV-B photoreceptor in inducing developmental processes (Kim *et al.*, 1998). The photoreceptor interacts synergistically with other two-photomorphogenic photoreceptors, namely phytochrome and blue/UV-A photoreceptor (Boccalandro *et al.*, 2001; Krizek, 2004). Therefore, UV-B induced damage is likely to be

altered in response to light absorbed by phytochrome and blue/UV-A photoreceptor, which are known to modulate the structure and function of PSA.

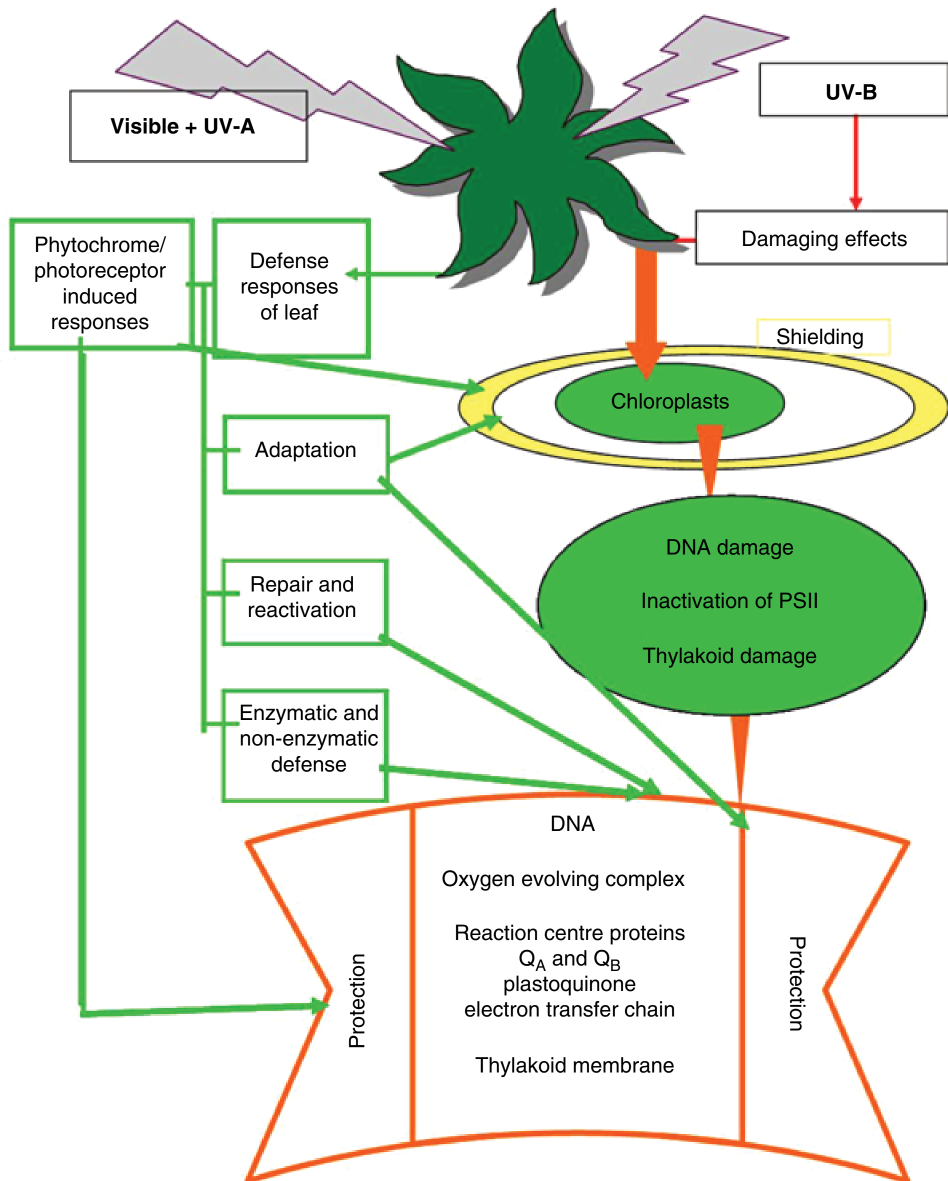
In tandem with other photomorphogenic photoreceptors, UV-B radiation elicits responses such as induction of phenylalanine ammonia lyase, chalcone synthase and enzymes involved in the formation of UV-B absorbing compounds (Fuglevand *et al.*, 1996; Wade *et al.*, 2001). There are many reports showing co-action of UV-B photoreceptor with these photoreceptors (Beggs and Wellman, 1994). The level of accumulation of epidermal flavonoids and anthocyanin is known to increase due to UV-B irradiation, and is further increased in response to light absorbed by phytochrome and blue/UV-A photoreceptors (Mohr and Schopfer, 1995). These compounds reduce the transmittance of UV-B radiation, and shield the photosynthetic apparatus from damage (Storch *et al.*, 2008). However, the process limits photosynthesis, as the transmittance of PAR is also diminished.

Tossi *et al.* (2009), on the other hand, have observed that UV-B radiation induces an increase in ABA production, which can trigger higher NO synthesis and emission by plants. This could lead to a rise in NO concentration in the troposphere, which may help in counterbalancing the deleterious effects of UV-B radiation. Even a school of thought regarding the development of cross-protection against other stresses in UV-B exposed plants has taken ground. The works demonstrating development of protective mechanism against cold (Chalker-Scott and Scott, 2004) and tolerance against high light and drought stresses (Poulson *et al.*, 2002) in plants grown under elevated UV-B environment are noteworthy. Involvement of UV-B-induced photomophogenic responses in providing such tolerance to other stresses could be a plausible explanation (Gitz and Liu-Gitz, 2003). The modes of inactivation induced by UV-B radiation, and the responses of plants to meet the challenges, are depicted in Scheme 8.1.

8.7 Corroboration with Sensible Approach

The description of UV-B-induced damage above is the result of studies conducted under unrealistic intensities of UV-A, UV-B and PAR in laboratory conditions (Middleton and Teramura, 1994; Fiscus and Broker, 1995; Krizek, 2004). The idea that the deleterious effects of UV-B on a plant's photosynthesis are plausibly exaggerated, due to unrealistic proportions of the various components of solar spectrum used in the growth chamber (Booij-James *et al.*, 2000; Alexieva *et al.*, 2001; Krizek, 2004), is gaining momentum. Experiments based on exclusion of UV-A and/or UV-B from solar radiation have been conducted, for better understanding of UV-B-induced damage and adaptation under ambient levels of these radiations (Krizek and Mirecki, 2004; Guidi *et al.*, 2011; Baroniya *et al.*, 2013).

The exclusion of solar UV-A and UV-B radiation is known to enhance vegetative growth, total biomass accumulation and yield, compared with those in plants grown under ambient UV. The activities of superoxide dismutase, guaiacol peroxidase, ascorbate peroxidase, and glutathione reductase and the levels of ascorbic acid are significantly decreased, while the level of α-tocopherol is increased after the exclusion of UV-B and UV-A (Guidi *et al.*, 2011; Baroniya *et al.*, 2013). Ambient levels of UV-A along with UV-B accumulate significantly high amount of active oxygen species, which are responsible for damage of PSA. The removal of UV components from solar spectrum leads to an eventual increase in photosynthesis (Láposi *et al.*, 2002; Rousseaux *et al.*, 2004; Kataria *et al.*, 2014).



Scheme 8.1 A model schematizing the defence mechanisms exhibited through visible/UV-A light by green leaves to counter UV-B-induced damages. [For color representation in this figure legend, please refer to the online version of this book.] Inactivation of PSA occurs due to damage to DNA, PS II (OEC, reaction centre II proteins, Q_A, Q_B electron acceptors, electron transport between PSII) and PSI and thylakoid disorganization. The red arrow indicates the damaging path of PSA. Green leaves respond to UV-B assault in two distinctly different modes:

(I) Educing defence responses, comprising of: (a) adaptation (through promotion of anthocyanin and flavonoid synthesis that filter out UV-B radiation and activation of gene expression for D1 & D2 reaction centre protein); (b) repair and reactivation (activating the enzyme photolyase to repair the damaged DNA and diminishing the degree of DNA damage); (c) enzymatic (activity of super oxide dismutase, catalase ascorbate peroxidase, glutathione reductase, monodehydroascorbate reductase, dehydroascorbate reductase, guaiacol peroxidase, and glutathione-S-transferase) and non-enzymatic (ascorbic acid, reduced ascorbate, reduced glutathione, α-tocopherol, β-carotene, carotenoids and xanthophyll cycle) defence;

(II) Photomorphogenic responses involving phytochrome and/or blue/UV-A photoreceptor, which act at the level of development and protection, and overlap with the mechanism of adaptational responses. The green arrow indicates the mechanism involved in protecting different sites of damage.

8.8 Conclusion

Perusal of the relevant literature suggests that UV-B radiation inflicts damage to PSA of green leaves in at least two pathways:

1) The radiation brings about structural and functional changes to PS II by inducing damage at the molecular level, leading to a decline in photosynthetic efficiency.
2) UV-B radiation, besides inactivating the molecular defence mechanisms, induces a rapid loss in photochemical potential of thylakoid membrane, in spite of relative pigment stability, leading to a photoinhibitory condition that furthers the damage of PSII.

Damage of PSA in either way upsets the energy source-sink relation and the equilibrium in redox homeostasis between Q_A and Q_B, resulting in an enhancement in the level of ROS metabolism, which leads to thylakoid membrane lipid peroxidation and PSA damage. These changes transmit necessary signals for cellular readjustment, to restore photostasis of photosynthesis and redox homeostasis, by evoking different defence mechanisms for its protection, repair and adaptation. Moreover, green leaves elicit photomorphogenic responses to alleviate the UV-B-induced damage by engaging PAR and/or UV-A light.

Acknowledgements

I am indebted to Prof UC Biswal and Prof B Biswal for their kind help throughout my research career. I would like to thank Dr MK Pradhan and Dr S Gartia for their help in preparing the MS. Financial assistance (MRP F.35-161/2008(SR) Dt 18 Feb, 2010) from UGC, New Delhi is gratefully acknowledged.

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9

Ultraviolet Radiation Targets in the Cellular System: Current Status and Future Directions

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9.1 Introduction

The sun is the major source of energy to photosynthetic organisms. The energy reaching the earth's surface is in the form of radiation. There are basically two quantities characterising radiation, frequency (f) and wavelength (λ; lambda) which are defined by the relation:

$$f = c/\lambda$$

where c is the velocity of light ($3 \times 10^8\,m\,s^{-1}$).

The energy of a single photon is determined by the wavelength (λ) of the photon, as described by the relation:

$$\left(\text{photon energy}\right) = hf = hc/\lambda$$

where h = 1.24 eV nm.

However, apart from constituting an important requisite for life on earth, organisms are inevitably exposed to UV radiation originating from the sun. The global UV (total solar UV reaching the earth) is divided into two components: direct and diffuse. The spectral and amount of solar UV irradiance depends on several factors, such as wavelength of UV, solar angle, source spectrum, ozone thickness, absorption and scattering by molecule, and altitude above sea level.

UV radiation has been divided traditionally into three regions on the basis of wavelength:

- UV-C (100–280 nm), not relevant under natural conditions, but which has the most harmful effect;

UV-B Radiation: From Environmental Stressor to Regulator of Plant Growth, First Edition. Edited by Vijay Pratap Singh, Samiksha Singh, Sheo Mohan Prasad and Parul Parihar.
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- UV-B (280–315 nm), which represents only 1.5% of total spectrum, but has a high potential for disrupting membrane integrity and function of biological macromolecules (such as DNA, proteins and lipids), and inhibition of photosynthetic apparatus, ultimately affecting growth and productivity of plants (Mathur and Jajoo, 2015);
- UV-A (315–400 nm), which constitutes about 6.3% of incoming radiation, but is less effective.

The intensity of damage caused by UV reaching the earth's surface solely depends on the shielding afforded by the atmosphere and ozone is the most important factor affecting how solar UV undergoes absorption. The ozone layer filters out most of the detrimental radiation shorter than 280 nm, but its absorption coefficient decreases for wavelengths longer than 280 nm, and reaches zero at 330 nm (Robberecht, 1989). Thus, terrestrial organisms are exposed to significant radiation between 290–315 nm. However, due to the increased emission of halogenated chemicals like chlorofluorocarbons through anthropogenic sources, the likelihood of being exposed to the radiation is becoming higher. It has been suggested by Hollosy (2002) that a reduction by 1% in the ozone layer can increase the chances of getting UV-B exposure by 1.3–1.8%. This chapter focuses the effect of UV radiation on plants at the cellular level, beginning with its action spectra and moving towards its interaction with biomolecules, particularly in DNA, chromophores and other targets, like membrane proteins and other molecules.

9.2 Absorption Characteristics of Biomolecules

The biological effect of UV radiation arises due to photochemical absorption by nucleic acids, proteins and other molecules when irradiated with UV (Harm, 1980). The absorption centres for nucleotide bases are the chromophores constituting them. In DNA, these bases are adenine and guanine, which are purine derivatives while, for pyrimidine derivatives, they are thymine and cytosine. The absorption spectra of these component bases differs slightly, but can be ranged with their maxima between 260–265 nm. However, the absorption maxima for proteins is 280 nm. The absorbance maxima of proteins and nucleic acids, at equal concentration, shows lower absorbance by proteins (Figure 9.1). UVR is also absorbed by other molecules, such as porphyrins, carotenoids, steroids and quinones, which leads to biological consequences.

9.3 Action Spectrum

With technological advances, action spectroscopy has played an important role in characterization of bio-responses and, thus, has helped in obtaining and analysing the photobiological data that point towards the action spectra of UV. An action spectrum is a measure of relative effectiveness of different wavelengths within the spectral region of study to produce a given response (Diffey, 1991), and thus it serves as function to determine whether changes in ozone resulting in UV radiation quality are biologically significant or not.

The formation of photoproducts by UV absorption can show a resemblance between action and absorption spectra only if one chromophore is there. Gates (1928) showed that the absorption spectra of nucleotide bases in *Staphylococcus aureus* cells closely

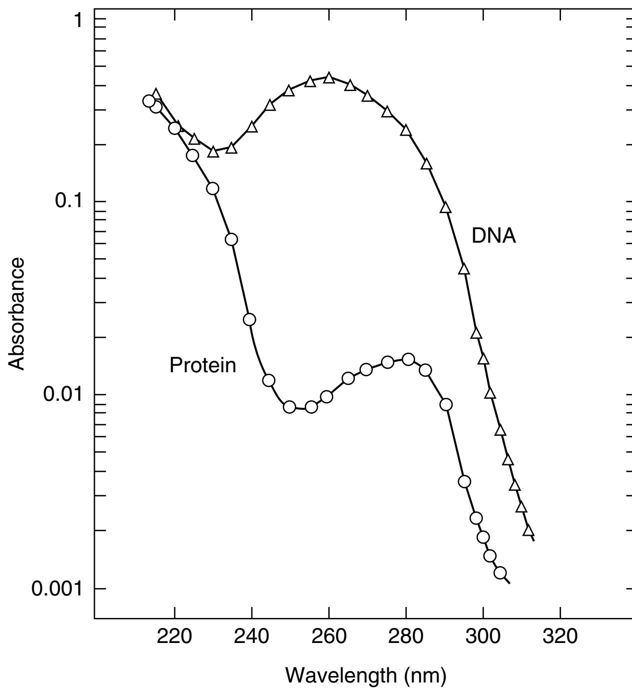


Figure 9.1 Absorption spectra of DNA and a protein at equal concentrations (reproduced from Harm, 1980).

matched the action spectra of UVR. The similarity between action and absorption spectra of DNA within the wavelength region 220–300 nm, which induces cell inactivation, chromosomal aberrations, mutations and so on suggested that main chromophore for all the effects within this region is DNA. However, in some cases, there is a difference in the absorption and the action spectra, so comparing the action spectra with the absorption spectra can give an insight of molecule responsible for the biological effects. This difference was later on suggested to originate due to absorption by some proteins.

Later, a new action spectrum for pyrimidine dimer was observed in alfalfa leaves, which indicated a less steep slope in short wavelengths when shielded DNA was considered (Quaite *et al.*, 1992). This shifting of spectrum towards shorter wavelengths was expressed as rapid amplification factor (RAF), which is the percentage increase in biologically effective radiation due to the total ozone column (Caldwell *et al.*, 1986). Thus, computing the RAF value can help in computing biological damage.

9.4 Targets of UV-B

9.4.1 Interaction with Nucleic acids

In order to produce a chemical change, UV must interact with the biomolecules and must be absorbed by the molecules. The first phase of interaction includes absorption of photons by molecules, leading to a photochemical reaction, followed by production

of an excited state, where one electron from the molecule reaches a higher energy level. Such transitions are efficient only if the energy of radiation is close to the energy difference of the atom. These photochemical reactions instantly lead to the formation of photoproducts, which are in a metastable state or a free radical. While the dark reactions occurring after the photochemical reaction may last from microseconds to hours, and may initiate the photobiological responses.

9.4.1.1 Deoxyribonucleic Acids

The most important targets of UV include DNA, and irradiation with UV-B and/or UV-C results in formation of photoproducts. The most common photoproducts arising due to UV irradiation includes cyclobutane-type pyrimidine dimers (CPDs) and pyrimidine (6,4) pyrimidone (6,4 PP) dimer (Hutchinson, 1987). Cyclobutane-type pyrimidine dimers arise from production of reactive excited states following UV absorption. The action spectra for dimer formation, at about 313 nm, resembles the extinction coefficient of monomers cytosine (C) or thymine (T). The second type of pyrimidine dimer formed by the UV is a thy (6–4) pyo photoproduct, which is formed between cytosines located 5' of adjacent pyrimidines.

UV exposure damages DNA double strands as well, and can break them (Ries *et al.*, 2000). It has been reported that UV-B absorption by DNA induces formation of covalent bonds between adjacent pyrimidines and forms CPD, resulting in blockage of transcription and replication, as these are not recognised by the RNA and DNA polymerase (Jansen *et al.*, 1998; Figure 9.2).

Sancar (2003) reported that ratio of CPD and 6,4 PP photoproducts varies according to UV-B exposures; lower doses induce 9 : 1 ratio, while of higher dose induces in the ratio 6 : 4. However, the photoproducts formed are reversed by photorepair and

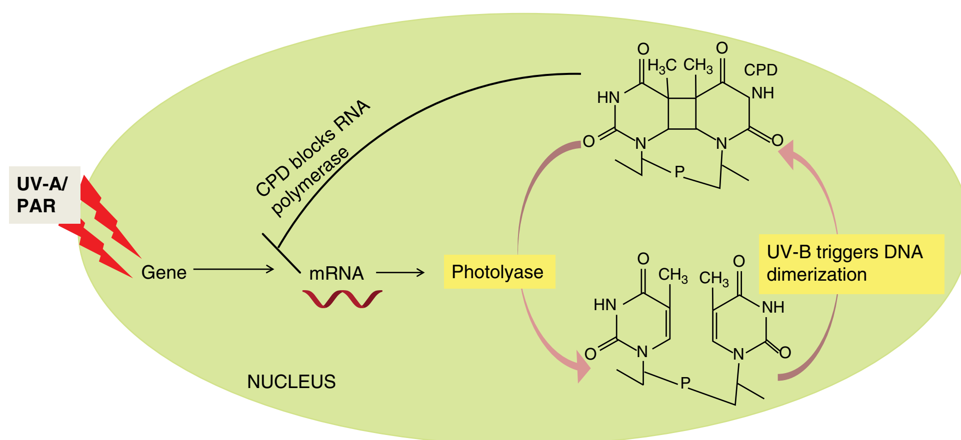


Figure 9.2 Diagram showing the regulation of cyclobutane-pyrimidine dimer (CPD) photoreactivation. Transcription of genes encoding photolyases is minimal in the dark, but is induced by UV-A and PAR, possibly involving a UV-A/B photoreceptor. Photolyase activity requires blue or UV-A radiation, but dimerization is mainly driven by UV-B. If dimerization is not reversed, it will block transcriptional activity by impeding RNA polymerases (Britt, 1996; Sancar, 1996).

nucleotide excision repair (NER). The CPD and 6,4 PP photolyase binds to the damage site of DNA, and uses 350–450 nm light as energy source to repair the injury caused by UV (Sancar, 2003; Weber, 2005). However, the efficiency of repairing, in the case of 6,4 PP, is higher than that of CPD photolyase photorepair (Chen *et al.*, 1994; Jiang *et al.*, 1997). NER is an ATP-dependent repairing pathway that repairs the damage by removing the lesions from bulky DNA. However, the repairing rate for 6,4 PPs is 10.7 times faster than for CPDs (De Lima-Bessa *et al.*, 2008).

Further studies have also pointed out that evolutionary conserved DNA repair pathways involve chromatin modifications and correct various DNA lesions (Schmidt and Jackson, 2013). Landry *et al.* (1997) reported that *Arabidopsis uvr2-1* mutant containing a lesion in CPD photolyase PHR1 was hypersensitive to UV-B, thus suggesting the requirement of the functional DNA repair system to maintain genome integrity. Another repairing system recognised in recent years in yeast, bacteria, plants and animals is a mismatch repair (MMR) system that corrects the mis-paired or unpaired DNA bases caused by UV radiation (Lario *et al.*, 2011). To date, several systems, including photolyase dependent photrepair, chromatin remodelling and histone acetylation, have been shown to be involved in DNA repair.

9.4.1.2 Ribonucleic Acids

The susceptibility of RNA modification by UV radiation has not been explored much. It has been reported that messenger RNA undergoes modification when exposed to UV radiation; however, due to rapid turnover and *de novo* synthesis capacity of mRNA, it is not the critical target of radiation. While some fascinating photobiological phenomenon has been suspected in bacterial transfer RNA, these RNA were reported to be photosensitive to UV due to presence of unusual nucleoside 4- thiouridine, which could be of ecological significance (Jagger, 1985).

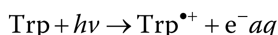
9.4.2 Proteins

Due to the higher abundance of chromophores (certain amino acids' side chains, as well as chromophores like flavin and porphyrins), proteins are major cellular targets of UV. Protein damage can be majorly through two pathways. The first of these is UV-B-mediated, which is also referred as the direct one, as particular amino acids, such as tryptophan (Trp), tyrosine (Tyr), histidine (His) and so on absorb the radiation and enter the excited state. Moreover, phtosensitized reactions can also take place by UV, by sensitizing endogenous species such as porphyrins (Silvester *et al.*, 1998; Afonso *et al.*, 1999), vitamins (Bova *et al.*, 1999; Korlimbinis and Truscott, 2006) or exogenous species like polyaromatic compounds and dye molecules (Pervaiz and Olivo, 2006; Konopka and Goslinski, 2007; Maisch *et al.*, 2007; Choudhary *et al.*, 2009; Phillips, 2010). These sensitizers, after absorbing radiation, are excited to the singlet state, which is short-lived and rapidly changes to the long-lived triplet state by intersystem crossing. The excited triplet state could either undergo a decay process and return to the ground state, or react with other species via two pathways (Type I and Type II), ultimately damaging the protein molecule. The following sections will deal with the damage to some specific amino acid induced by UV.

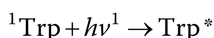
9.4.2.1 Tryptophan (Trp)

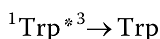
Bent and Hayon (1975) revealed that flash photolysis of Trp results in two relaxation channels:

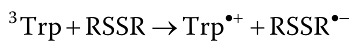
1) The first step includes ejection of electron-yielding solvated electrons, which have an absorption peak at 720 nm, while for the tryptophan radical cation ($Trp^{\bullet+}$), the absorption maximum lies at 560 nm. Thus, the radical cation forms deprotonates, yielding neutral $Trp^{\bullet}$ radicals having absorption maxima at 510 nm.



2) The second state gives rise to the triplet state ^{3}Trp, which has its absorption maxima at 450 nm. The so-formed triplet state transfers its electron to a nearby electron acceptor (Creed, 2008), thus forming the corresponding radical (RSSR) and a radical cation ($Trp^{\bullet+}$).







The radical cation deprotonates to form an indolyl radical (Davies and Gilbert, 1991), and subsequent reactions with O_2 form a ring C-3 peroxy radical that further undergoes atom abstraction reaction (Figure 9.3).

The other pathway depicted in Figure 9.3 shows the $^{1}O_2$-mediated oxidative damage. The two major products (i.e. N formylkynurenine and kynurenine) are most effective photosensitizing agents, and can produce other reactive species that can degrade these and other structures. A recent study by Grosvenor *et al.* (2009, 2010) with UV-A has shown the formation of nitrotryptophan, suggesting the involvement of reactive nitrating species. Several oxidation/degradation product formed by free Trp have been shown to be involved in the formation of adducts at Cys, His and Lys residues (Hood *et al.*, 1999; Vazquez *et al.*, 2001; Parker *et al.*, 2007; Mizdrak *et al.*, 2008), and they act as sensitizers and further exacerbate the damage induced by UV.

9.4.2.2 Tyrosine (Tyr)

Another aromatic residue with its absorption in UV region is tyrosine (Tyr-OH). The absorption maxima for Tyr is 220 nm at neutral pH, while it shows a shift with its maxima at 240 nm at alkaline pH, because of deprotonation of the OH group of Tyr, resulting in the formation of tyrosinate (Tyr -$O^{\bullet-}$). Photoexcited tyrosine can undergo similar electron transfer process to that followed by Trp. Alternatively, Tyr can photoionize by absorbing a second photon from the triplet state, which results in a solvated electron (e-aq) and a radical cation (Tyr-$OH^{\bullet+}$) that deprotonates to create the neutral radical

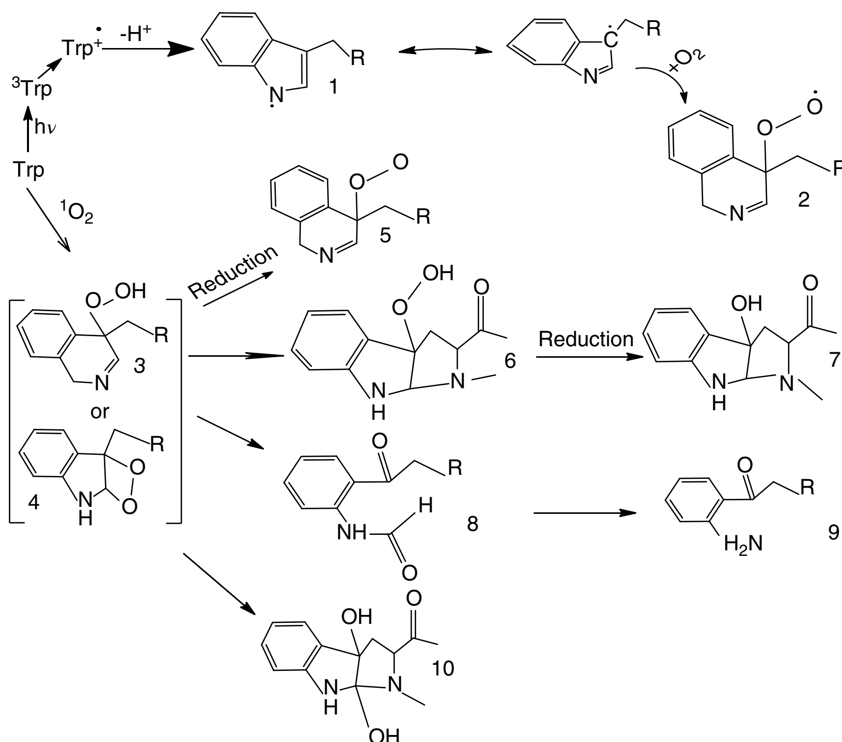
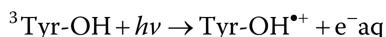
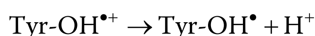


Figure 9.3 Mechanisms of oxidation of Trp by direct UV absorption and $^1O_2$-mediated pathways. Key to structures (where the *a*-amino acid moiety is represented by R CH(CO_2H)NH_2): **1**, indolyl radical; **2**, C-3 peroxyl radical; **3**, C-3 hydroperoxide; **4**, dioxetane intermediate; **5**, C-3 alcohol; **6**, 3α-hydroperoxypyrroloindole; **7**, 3α-hydroxypyrroloindole; **8**, *N*-formylkynurenine; **9**, kynurenine; **10**, 3α-dihydroxypyrroloindole.

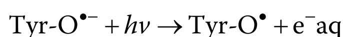
(Tyr-OH$^\bullet$) at neutral pH. At high pH, photoionization is monophotonic and results in a neutral radical (Tyr-O$^\bullet$) and a solvated electron (e^-aq).



$$^3\text{Tyr-OH} + h\nu \rightarrow \text{Tyr-OH}^{\bullet+} + e^-\text{aq}$$



$$\text{Tyr-OH}^{\bullet+} \rightarrow \text{Tyr-OH}^{\bullet} + H^+$$



$$\text{Tyr-O}^{\bullet-} + h\nu \rightarrow \text{Tyr-O}^{\bullet} + e^-\text{aq}$$

$$^3\text{Tyr-OH} + \text{RSSR} \rightarrow \text{Tyr-O}^{\bullet} + H^+ + \text{RSSR}^{\bullet-}$$

The triplet state Tyr is quenched by molecular O_2 or disulphide bridges, leading to the formation of a tyrosyl radical following rapid deprotonation of a radical cation. The Tyr-formed radical undergoes oxidation and dimerization to yield a di-tyrosine product and hydrogen atom abstraction reaction (Ostdal *et al.*, 1997; Irwin *et al.*, 1999; Ostdal *et al.*, 2002; Figure 9.4). The photo-oxidation of Tyr by $^1O_2$ yields endoperoxides that

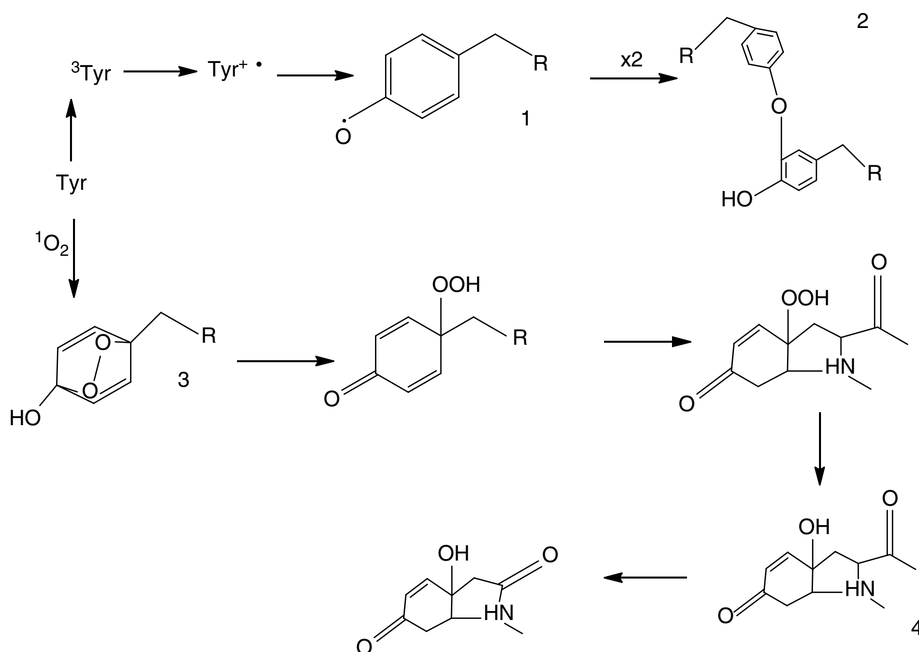


Figure 9.4 Oxidation pathways for Tyr via direct UV absorption or $^1O_2$ production. Key to structures (where the a-amino acid moiety is represented by R $CH(CO_2H)NH_2$): **1**, tyrosyl radical; **2**, a C–O linked isomer of a dityrosine crosslink; **3**, Tyr endoperoxide; **4**, HOHICA (3α-hydroxy-6-oxo-2,3,3α,6,7,7α-hexahydro-1*H*-indol-2-carboxylic acid).

rapidly decompose to form C1 hydroperoxide and cyclized products, which are further oxidised to decarboxylated keto compounds.

9.4.2.3 Phenylalanine (Phe)

UV mediated oxidation of Phe is quite simple, and excitation in first triplet state results in the formation of a benzyl radical (Bent and Hayon, 1975), while direct photoionization results in the formation of hydroxylated ring products (o-, m- and p- Tyr) following hydration (Figure 9.5).

9.4.2.4 Histidine (His)

The damage induced by UV at His residues is initiated by sensitization processes. A study by Huvaere and Skibsted (2009) demonstrated the formation of radicals on imidazole functions of His compounds, and it has been also demonstrated with other sensitizers (Agon *et al.*, 2006). There is a direct interaction between the triplet state flavin and His, followed by several protonations giving rise to an imino group (Huvaere and Skibsted, 2009). Flavin-mediated photooxidation of His occurs via production of $^1O_2$, and similar results have been obtained by other sensitizers (Agon *et al.*, 2006), while, oxidation mediated by $^1O_2$ results in formation of endoperoxides (Agon *et al.*, 2006), which undergo a decomposition reaction, yielding aspartic acid, asparagine and urea (Figure 9.6; Tomita *et al.*, 1968, 1969; Kai and Suzuki, 1996).

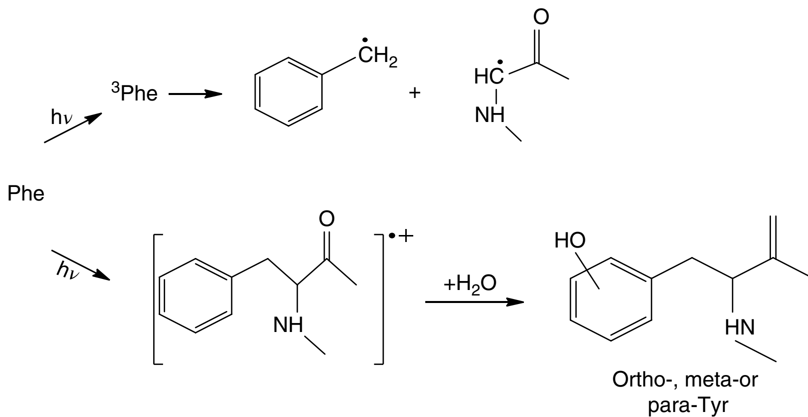


Figure 9.5 Direct UV absorption by Phe leads to formation of the triplet state and subsequent radical formation, or photo-ionization, followed by hydration, to form Tyr isomers.

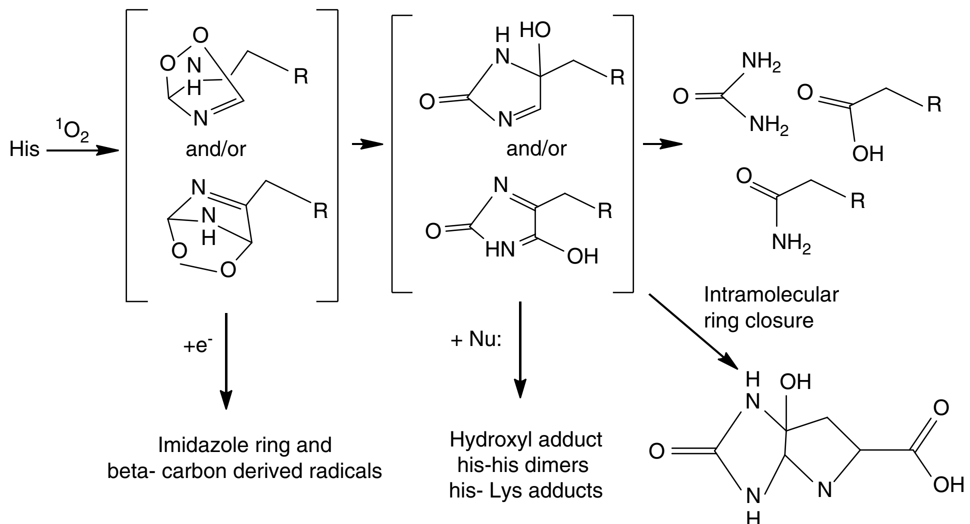


Figure 9.6 Reaction pathways for $^1O_2$-mediated oxidation of His residues (where the α-amino acid moiety is represented by R $CH(CO_2H)NH_2$).

Many His-derived products may undergo reaction and form dimers from N-acetyl-His (Agon *et al.*, 2006), His-His or His-Lys cross links, followed by nucleophilic addition of a His or Lys side chain to a keto group (Shen *et al.*,1996, 2000). Exposure to proteins with light can also lead to the formation of 2-oxo-His and a nitrated His derivative (Dyer *et al.*, 2009). The formation of these products suggests the involvement of radical species.

9.5 The Photosynthetic Machinery

Studies from past years have shown that UV has a damaging effect on plants which, in turn, decreases and degrades the crop qualitatively and quantitatively. Although there are several targets of UV, the photosynthetic apparatus, and especially photosystem II

(PSII), seems to be the most important target of UV, and damage to this apparatus contributes to overall UV-B damage. Reduction in photosynthesis can produce direct, as well as indirect, effects. The direct effects include decrease in CO_2 fixation and O_2 evolution, impairment of PSII (and to a lesser extent, to photosystem I (PSI)), reduction in dry weight, secondary sugars, starch and total chlorophyll, decrease in Rubisco activity and inactivation of ATP synthase. The indirect effects include induction of stomatal closure and, thus, decreased efficiency of gas exchange, changes in leaf thickness and changes in canopy morphology.

9.5.1 Photosystem I and II

PSII is a protein-pigment complex, catalysing transfer of electrons from water to plastoquinone. This core system is composed of two structurally and functionally similar proteins, D1 and D2, while other components of PSII include Q_A, Q_B and PQ electron acceptors and Tyr-Z redox active residues, as well as the Mn cluster of water oxidation. All these components are considered to be the most important target sites of UV-B (Jansen *et al.*, 1998; Figure 9.7).

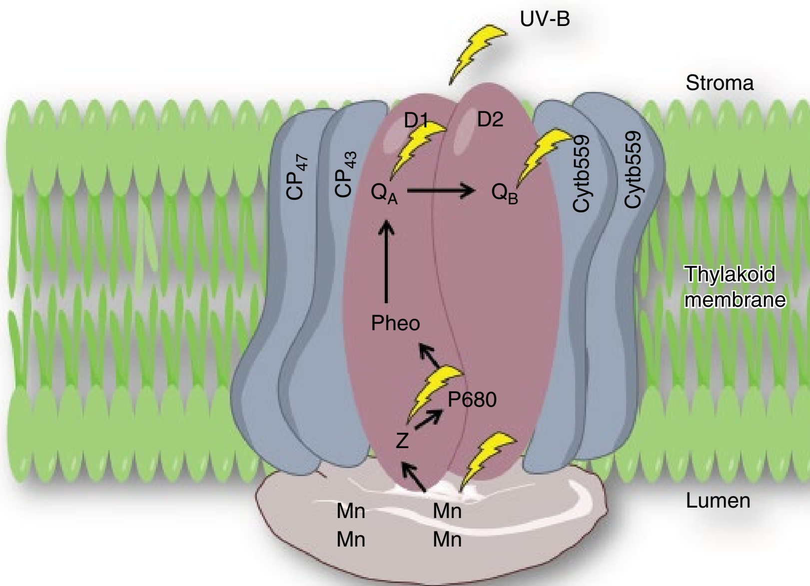


Figure 9.7 Schematic presentation of photosystem II (PSII), indicating photosensitizers proposed to be involved in its UV-B-mediated inactivation. P_{680} is the primary electron donor of PSII. Z is redox active tyrosine located on the D1 and D2 proteins, respectively; Z normally serves as the electron donor to P680. Electrons originate from water, the splitting of which is catalysed by a cluster of four manganese atoms. Extrinsic proteins are involved in stabilizing this reaction. On the acceptor site, a pheophytin (Pheo) serves as the primary electron acceptor. The plastoquinones, Q_A and Q_B, are the secondary electron acceptors. Photosensitizers that absorb in the UV-B range, and which have been proposed to play a role in PSII inactivation and/or D1-D2 degradation, are marked by arrows (Adapted from Jansen *et al.*, 1998).

As techniques have advanced, it has become quite easy to understand the damaging sites of UV. One such technique is Chlorophyll *a* fluorescence, which is very effective for assessing the performance of photosynthetic apparatus (Govindjee, 2004) – especially the OJIP test, which is a polyphasic transient based on the theory of energy flow in thylakoid membrane. J- step represents the accumulation of Q_A^- Q_B form (Strasser *et al.*, 2004) and I- step suggests accumulation of Q_A^- Q_B^-, whereas P- step suggests accumulation of Q_A^- Q_B^{2-} (Lazar, 1999; Strasser *et al.*, 2004).

A complete loss in J, I, P phase has been reported in wheat plants exposed to UV-B, suggesting an inhibition of electron transport beyond Q_A (Mathur and Jajoo, 2015). Using different variables like F_v/F_m, F_v/F_0, F_0/F_v, ψ_0, E_0, and some energy flux parameters like ABS/RC, DI_0/RC, ET_0/RC and TR_0/RC in this test, one can explain the energy flow through PSII. The effects of UV radiation on the several above mentioned variables have been summarised in Table 9.1.

A study by Albert *et al.* (2011) analysed the photosynthetic performance of leaves by chlorophyll *a* fluorescence (Figure 9.8), and reported that UV-B radiation is a significant stress factor for plants.

The PSI system is quite resistant to UV stress, and thus shows very much less or no damaging effect, compared with PSII (Bornman *et al.*, 1984; Mishra *et al.*, 2008). The lack of effect of UV-B on PSI has been demonstrated by adding artificial electron donor to UV-B treated chloroplast (Mishra *et al.*, 2008). Cytochrome b6/f complex mediates electron transport between the two photosystems; it oxidises plastoquinol produced by PSII and reduces plastocyanin, which further serves as electron donor to PSI.

Studies have reported that, like PSI, the cyt b6/f complex is the less affected component of thylakoid when exposed to UV-B (Strid *et al.*, 1990; Zhang *et al.*, 1994). However, the resistance capability is noteworthy, since cyt b6/f contains two quinone binding sites – one where the quinol oxidation occurs, and the other where the quinone reduction occurs (Hope, 1993). ATP synthase and ribulose 1,5-biphosphate carboxylase (Rubisco) are among the thylakoid membrane components which are adversely affected by UV-B radiation. Murphy (1983) reported a decrease in activity, as well as in the amount of ATP synthase. Rubisco is the main CO_2-fixing enzyme, consisting of two subunits of proteins; due to its subunits, cells are more prone to UV radiation. Decline in activity of Rubisco has been correlated with decreased mRNA levels (Jordan *et al.*, 1992).

9.5.2 The Light-Harvesting Complexes

The light-harvesting complex of PSII (LCH II) plays an important role in light absorption and energy transfer to the reaction centre, as well as in thylakoid organization. UV has been shown to decrease transcription of the *cab* gene, which is responsible for synthesis of Chl *a*/*b* binding protein of LHC II, leading to functional disconnection of LHC II from PSII (Lidon *et al.*, 2012; Ashraf and Harris, 2013). The function of harvesting light in cyanobacteria is performed by phycobilisomes, which are profoundly affected by UV radiation. The phycobiliproteins can be destroyed by UV-B, or the energy transfer towards the photosynthetic reaction centres can be impaired (Sinha *et al.*, 1995).

Table 9.1 Showing the effects of UV radiation on photosystem II with the help of different variables of OJIP transient.

Variables	Definition	UV-induced changes	Physiological relevance	References
F_0	Initial fluorescence	increases	Due to functional disconnection of light harvesting complex from PSII as well accumulation of inactive RC	Yamane *et al.*, 2000; Hollosy, 2002
F_v/F_m	Quantum efficiency of PSII photochemistry	decreases	Due to decrease in rate of primary charge separation or by disconnection of some minor antenna from PSII	Guo *et al.*, 2005; Guidi *et al.*, 2007
F_v/F_0	Size and number of active RC	decreases	Reflects impairment and down regulation of PSII photochemistry and low electron transport	Essemine *et al.*, 2012
RC/ABS	Density of active PSII reaction centre per chlorophyll and antenna size of chlorophyll molecules	decreases	Decreased active RC followed by decrease in size of chlorophyll antenna serving each RC	Mathur and Jajoo, 2015
F_0/F_m	Maximum quantum yield for heat dissipation by PSII	increases	Survival strategy to cope with excess energy	Mathur and Jajoo, 2015
TR_0/RC	Energy trapped per reaction centre	decreases	Indicate inefficient trapping due to inability of Q_A to reduce back for efficient trapping	Albert *et al.*, 2011
N	Turn over number	increases		
Ψ_0	Yield of electron transport per trapped exciton	decreases	Indicate towards inhibition of Q_A^- electron transfer	Lidon *et al.*, 2012
E_0	Quantum yield of electron transport	decreases		
Energy flux parameters per reaction centre				
ABS/RC	Light absorption	increases	Decreased value due to inactiveness of active RC which might be due to active participation in quenching sinks	Lidon *et al.*, 2012
DI_0/RC	Dissipation energy			
ET_0/RC	Maximum electron transport			
TR_0/RC	Trapped energy			
Energy flux parameters per cross-section				
ABS/CS	Efficiency of light absorption	decreases	due to inactivation of PSII RC	Yu *et al.*, 2013; Mathur and Jajoo, 2015
TR/CS	Trapping efficiency	decreases	Represents maximal rate of closure of RCs, i.e. inactiveness of RC	
ET/CS	Efficiency of electron transport	decreases	Lower energy absorption by antenna pigment and inactivation of reaction centre complexes	Yu *et al.*, 2013; Mathur and Jajoo, 2015
DI/CS	Dissipation	increases	Energy available for photochemistry was less under stress	Kruger *et al.*, 1997

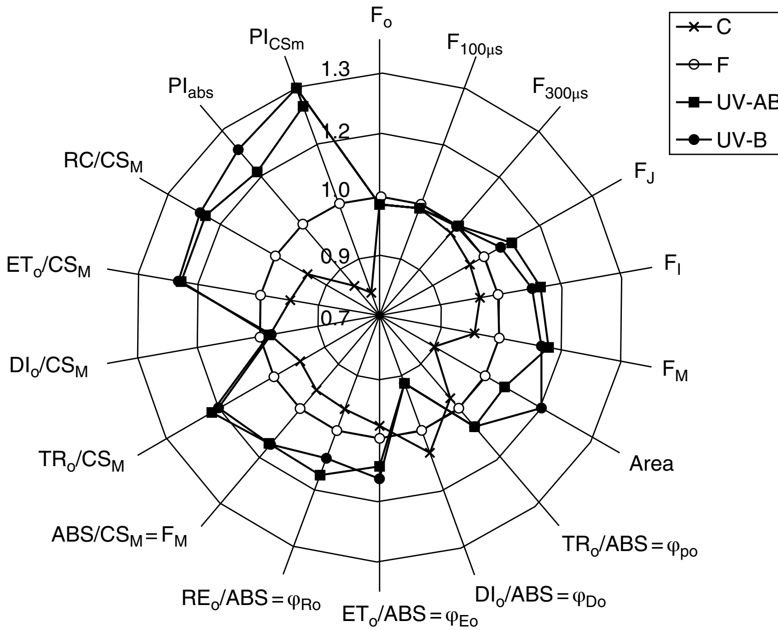


Figure 9.8 Radar plot of JIP test parameters from 45° fixed angle leaves. F_O is the minimal and F_M is the maximal fluorescence. F100 μs, F300 μs, F_J and F_I is the fluorescence measured after 100 μs, 300 μs, 2 ms and 30 ms. Area is the area under induction curve. Derived parameters are: quantum yields for trapping TRo/ABS, dissipation DIo/ABS, electron transport ETo/ABS and reduction offend acceptors REo/ABS. ABS is the absorption energy flux; CS is the excited cross-section of the leaf sample; TR is the excitation energy flux trapped by the RC and utilized for the reduction of Q_A to Q_A; DIo is the dissipation energy flux at the level of the antenna chlorophylls; ETo is the flux of electrons from QA^- into the intersystem electron transport chain; RC is the PSII reaction centre; RC/CS is the concentration of RC per excited CS of leaf sample; PIabs and PICSm are the performance index on absorption basis and sample per cross section (Adapted from Albert et *al.*, 2011).

9.6 Cell Division and Expansion

Regulation of cell division and cell expansion is a most important and central point, controlling organ size (Sugimoto-Shirasu and Roberts, 2003). However, these processes are very sensitive to UV-B, and can be affected through regulatory or stress mediated processes initiated by UV-B. Jiang *et al.* (2011) reported that UV-B mediated DNA damage slows down the G1-S step of cell cycle, thus impeding cell cycle progression. However, this arrest in the cell cycle process facilitates DNA repair before further replication occurs, but leads to decreased cell numbers or endoreduplication by downregulating the transcription factor E2Fe/DEL1 (involved in repressing endoreduplication process) (Radziejwoski *et al.*, 2011).

In order to compensate for the decreased cell numbers, there is an increase in ploidy level, which is UV-B mediated and, in turn, results in cellular expansion. Thus, a balancing system operates between cell division and cell expansion. Radziejwoski *et al.* (2011) reported an increase in ploidy level with decrease in leaf area in *Arabidopsis*. Moreover, some UV-B-mediated processes include inhibition of cell division, as well as cell

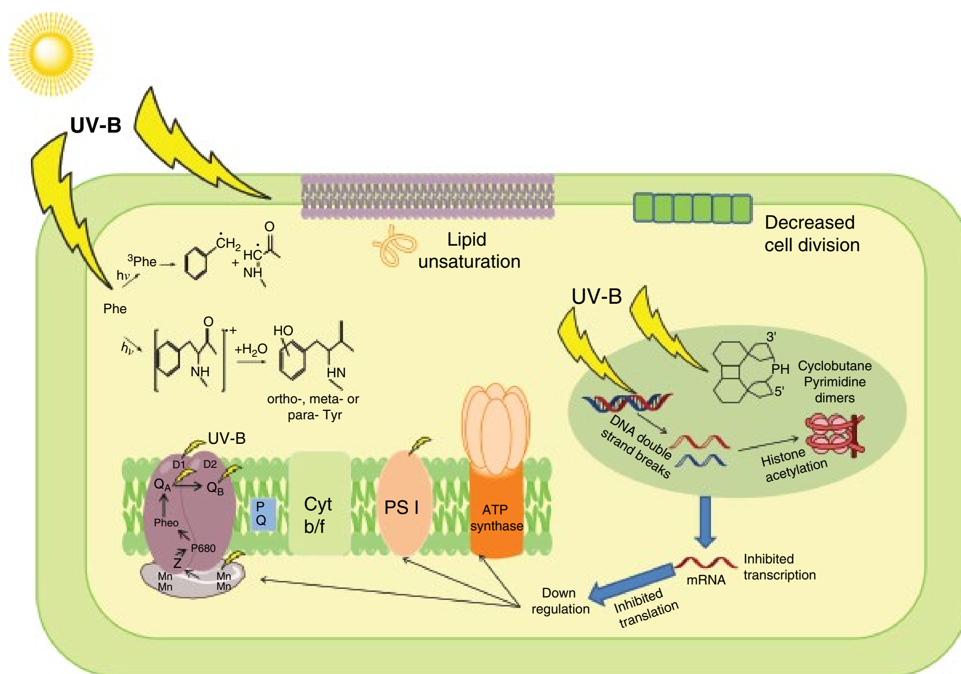


Figure 9.9 Showing the overall effect of UV-B on the cellular system.

expansion (Wargent *et al.*, 2009; Hectors *et al.*, 2010). Lake *et al.* (2009) reported that when *Arabidopsis* Col-0 wild was exposed to UV-B, the abaxial surface developed larger cells than the adaxial surface, but it was not compensated by an increase in the number of cells, and cell density was found to increase in UV-sensitive *fah* mutant. In another study by Wargent *et al.* (2009) on *Arabidopsis*, it was reported that inhibition in cell division did not compensate with cell expansion.

However, there is still need to study the mechanism lying behind UV-B affecting cell orientation and organization within a leaf, as well as the effects of UV-B on the differentiation process. UV-B has been reported to affect stomatal density as well, but no clear-cut conclusions have been drawn, as some studies have reported a decrease in stomatal index (stomata: epidermal cells) but not on stomatal density (Staxen and Bornman, 1994; Lake *et al.*, 2009; Jacques *et al.*, 2011). However, the decrease in stomatal index is attributed to the reduction in abscisic acid content, while others have reported a decrease in stomatal density (Gitz *et al.*, 2005) or even no effect (Kostina *et al.*, 2001; Kotilainen *et al.*, 2009). Thus, it can be concluded that UV induces change at the cellular level by affecting cell division, elongation, amino acids, DNA, RNA and many more (Figure 9.9).

9.7 Conclusion and Future Perspectives

This chapter has tried mapping UV research at the cellular level, beginning from the action spectra of UV, and then to its effect on biomolecules, photosynthetic machinery and ongoing cell changes. Researches are being conducted to study each and every

aspect of UV radiation with context to plants, but there are many loopholes still to be filled. In order to understand the significance of changes going inside the cellular system under UV radiation, future research must focus on several areas:

1) Basic research investigating the mechanistic action of UV on biochemical, physiological and physical processes.
2) Field validation of all the aspects covered, to test the response under environmental condition.
3) UV induces several changes at the cellular level, but no clear picture can be made due to seemingly confusing and contradictory plant responses being reported. Future research will need to disentangle these questions occurring within the plants with the help of model systems, and then compare the deviations from the system.
4) No clear evidences from past research can be drawn, regarding the action spectra of several biomolecules.
5) UV is known to affect the photosynthetic apparatus but, among the several targets, only PSII is being focused upon while, with respect to cyt b6/f complex, our knowledge is still limited. There seems a clear gap between interaction of UV and organisational level of cells.
6) Since plants are exposed to varying levels of UV, they might thus be adjusting continuously to cope with the changes. Therefore, elucidating the adaptive pathway also needs to be addressed.

Acknowledgements

The University Grants Commission, New Delhi is greatly acknowledged for financial support.

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10

Silicon: A Potential Element to Combat Adverse Impact of UV-B in Plants

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10.1 Introduction

Ultraviolet-B is the major part of sunlight which is in the wavelength band range from 280 nm to 315 nm; however, only wavelengths larger than 290 nm can reach the Earth's surface (Marcel *et al.*, 1998). Extensive use of CFCs lead to a serious reduction in the amount of ozone in the stratosphere. This ultimately has harmful effects on human and plant health, and is thus recognized as one of the major environmental threats (Mishra *et al.*, 2008). Those plants capable of photosynthesis need extensive sunlight to complete the photosynthesis process, and therefore, inevitably exposed to UV-B radiation (Marcel *et al.*, 1998). From the last few decades, due to these harmful impacts and losses in yield of crops caused by UV-B, it is recognized as a major environmental threat among abiotic stress factors. Furthermore, it has been well documented that UV-B hampers the physiological, biochemical and molecular characters of crop plants, and ultimately reduces the quality and quantity of yield (Rozema *et al.*, 1997; Yuan *et al.*, 1998; Teramura, 1983; Teramura *et al.*, 1990; Liu *et al.*, 2013).

Studies have also suggested that enhanced UV-B exposure drastically injures the anatomical structures, and also reduces the water balance capacity of plant cells (Day, 1993; Kakani *et al.*, 2003; Sarghein *et al.*, 2011). Kakani *et al.* (2003) further suggested that wide inter- and intra-specific variations may affect the extensive response of UV-B radiation in plants. Some plants, interestingly, show a stimulatory response under UV-B exposures. In contrast, most plants are sensitive to UV-B radiation and show negative traits. Additionally, it has been also noticed that negative or positive response of UV-B may also be influenced by numerous environmental factors, such as drought, the water system, and nutrient status, which drastically influence the growth level and sensitivity of crops (Balakumar *et al.*, 1993; Alexieva *et al.*, 2001). Furthermore, it has also been demonstrated that UV-B exposure severely influence the external and internal metabolic systems of plants, such as growth, development and morphology, as well as alterations in

UV-B Radiation: From Environmental Stressor to Regulator of Plant Growth, First Edition. Edited by Vijay Pratap Singh, Samiksha Singh, Sheo Mohan Prasad and Parul Parihar.
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Table 10.1 UV-B induced impacts on plants.

Concentration of UV-B	Plants species	Integrated precarious impacts imposed by UV-B in plants	Reference
2-11 $kJm^{-2}day^{-1}$	*Gossypium hirtusum*	Increased epicuticular wax content and stomatal index. Alteration in plant height, internode and branch length and leaf necrosis	Kakani *et al.*, 2003
13 $kJm^{-2}day^{-1}$	*Brassica napus*	Leaves shows a significant decrease in chloroplast, mitochondria and starch content	Fagerberg and Bornman, 2005
7.5 $kJm^{-2}day^{-1}$	*Pinus sylvestris* and *Pinus taeda*	The outer epidermal walls thickened and neddle cross section area and mesophyll areas decreased	Laakso *et al.*, 2000
3.6 $kJm^{-2}day^{-1}$	*Coleus forskohlii*	Levels of secondary metabolites increase,concentration of antioxidant enzymes increase as well as the protein and chlorophyll content decline	Swabha and Agrawal, 2015
12 $kJm^{-2}day^{-1}$	*Avena fatua* *Setaria viridis*	High level of UV-b irradiation caused leaf curling,delayed plant growth,Reduction in leaves number and fresh weight of leaves and stem	Golaszewska *et al.*, 2003
13.8 $kJm^{-2}day^{-1}$	*Vicia faba*	Small increase in epidermal transmittance,reduction in PAR,Amount of UV-absorbing compounds increase	Ryel *et al.*, 2010
12 $kJm^{-2}day^{-1}$	*Hordeum vulgare*	Induced DNA damage and flavonoid accumulation in epidermal and subepidermal mesophyll tissue.Growth and development of primary leaves slightly reduced	Schmitz-Hoerner and Weissenböck, 2003
6.5 $kJm^{-2}day^{-1}$	Gunnera magellanica	DNA damage and disruption of membrane integrityby lipid peroxidation. Increased UV-B concentration also decreased leaf expansion and generate a transient oxidative stress	Giordano *et al.*, 2004

transpiration and photosynthesis thereby, causing damage to DNA, proteins and membranes (Teramura *et al.*, 1994; Marcel *et al.*, 1998).

Some studies also revealed that exposure to UV-B radiation may also significantly reduce the biomass accumulation of plants (Teramura and Sullivan, 1994; Deckmyn and Impens, 1997). In addition, some morphological parameters, including plant height, leaf area and biomass accumulation, are also significantly affected by the exposure of UV-B radiation (Prasad *et al.*, 2005). At the same time, several studies also demonstrated the reduction of photosynthetic CO_2 assimilation, and particularly significant impacts on the action, and synthesis Rubisco under UV-B exposure (Strid *et al.*, 1994; Huang *et al.*, 1993; Nogués and Baker, 1995; Allen *et al.*, 1997; Bassman *et al.*, 2001; Tekeuchi *et al.*, 2002; Prasad *et al.*, 2005).

Table 10.2 Impact of silicon application in plants exposed to UV-B radiation.

Concentration of silicon in plants exposed to UV-B	Plant species	Ameliorating effects by exogenous application of silicon	Reference
5 mM Si	*Zea mays*	Show ameliorative effects on some aspects of UV-b stress like no significant decrease in growth and development and the ratio of chlorophyll *a/b* not significantly affected	Mihaličoá *et al.*, 2014
40 g^{-2} Si	*Oryza sativa*	Si decrease the biosynthesis of phenolic compound such as ferulic acid and p-coumaric acids and increase silica deposited and also decrease the activity of cinnamyl alcohol dehydrogenase (CAD)	Goto *et al.*, 2003
1.70 mM Si	*Glycine max*	Si application had positive effects on stomatal conductace, transpiration and photosynthesis of seedling, and decreases the concentration of intracellular CO_2, proline and H_2O_2.	Shen *et al.*, 2009
400 mg SiO_2 kg^{-1}	*Triticum aestivum*	Si increase the total biomass and chlorophyll content and reduce ROS generation by inducing the activity of antioxidant enzyme	Yao *et al.*, 2010

Thus, to protect the plants from the harmful impacts of UV-B radiation it is necessary to innovate appropriate methods which would not be only eco-friendly to the environment, but also beneficial for the growth and development of plants. There are several methods which are being used to alleviate the toxic effect of abiotic stresses; however, silicon supplementation is one of the most popular methods used by researchers from the last few decades, due to its capability to perform an excellent alleviator against any abiotic or biotic stress (Singh *et al.*, 2011; Tripathi *et al.*, 2012, 2013, 2015a).

Silicon (Si) is known as one of the most beneficial substances for the growth and development of plants, as it is the second most abundant element in the earth's crust (Epstein, 1999; Tripathi *et al.*, 2015a). There are many studies that have demonstrated that silicon works as an essential element in a number of plant species of the Poaceae and Cyperaceae, although this cannot be claimed for the all higher plants, due to lack of direct evidence of the molecular traits (Epstein, 1999). Similarly, a number of studies have also suggested the positive role of silicon supplementation in plants against drought, heavy metals, UV-B radiation, salt stress and some biotic stresses, including pests and pathogens (Neumann and Zur Nieden, 2001; Liang *et al.*, 2006; Richmond and Sussman, 2003; Li *et al.*, 2004; Gong *et al.*, 2005; Tripathi *et al.*, 2015b, 2015c). Although many studies have reported the significant response of silicon in plants against abiotic and biotic stress, however, very few attempts have been made to investigate the behaviour of silicon against UV-B radiation in plants. Thus in this chapter, we have summarized the silicon and UV-B interaction related studies.

10.2 The Role of Silicon Against UV-B Exposure on Morphology of Plants

UV-B induces many morphological impacts on plants, such as reduction in plant height, leaf length and leaf area (Reddy *et al.*, 2013), and induces axillary branching (Meijkamp *et al.*, 2001; Li *et al.*, 2010). UV-B also causes chlorosis and necrotic spots (Kakani *et al.*, 2003). In general, excess UV-B diminishes main plant growth and induces lateral branching, resulting in a more dense and smaller plant (Kakani *et al.*, 2003; Reddy *et al.*, 2013). Plants exposed to UV-B are shorter, due to the presence of shorter internodes (Zhao *et al.*, 2003). Leaf area is also a perceptive parameter which fluctuates upon UV-B exposure. Under enhanced UV-B radiation, leaf area decreases in order to develop defence mechanisms (Nogués *et al.*, 1998; Zhao *et al.*, 2003).

Besides these impacts, UV-B induces other morphogenetic changes, such as delayed seed setting, flowering and fruit ripening (Wang *et al.*, 2012; Zinser *et al.*, 2007). It has also been hypothesized that UV-B-driven morphogenic responses are outcomes of UV-B-influenced alterations in hormone metabolism and cell wall loosening (Casati and Walbot, 2003; Hectors *et al.*, 2007). UV-B radiation induces several changes in dicotyledones, compared with monocotyledons (Caldwell *et al.*, 2007; Kataria *et al.*, 2007). In various plant species, UV-B induces a reduction in plant biomass, which results in a decline in crop products (Kakani *et al.*, 2003; Ruhland *et al.*, 2005; Searles *et al.*, 2001). UV-B also influences root and rhizome development. Kumari *et al.* (2009) reported a decrease in root length due to UV-B in *Acorus calamus*.

Various species and their varieties, including *Zea mays* (Zancan *et al.*, 2006; Britto *et al.*, 2011; Campi *et al.*, 2012), *Oryza sativa* (Takeuchi *et al.*, 2002), barley (Bandurska *et al.*, 2012), *Triticum aestivum* (Agrawal *et al.*, 2007) and *Glycine max* (Galatro *et al.*, 2001; Gitz *et al.*, 2005; Chimphango *et al.*, 2007) have been used to evaluate the damaging effects of UV-B. UV-B-induced physiological effects are a source of reduction in photosynthetic activity, due to degradation in photosystem II proteins and pigments (chlorophyll and carotenoids), reduced Rubisco activity and stomatal functions (Sullivan *et al.*, 2003;Surabhi *et al.*, 2009; Cooley *et al.*, 2000).

UV-B provokes accumulation of flavonoids in the leaf epidermis, which protects against UV-B (Hollosy, 2002; Hassan *et al.*, 2013). These biomolecules are produced in response to ROS formation, which stimulates the oxidation of lipids and proteins, and causes DNA damage (Jain *et al.*, 2004; Jenkins, 2009; Hassan *et al.*, 2013). UV-B absorption in DNA induces the phototransformation due to which dimers are formed, such as cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidinone dimers (6-4 PPs), and it damages the efficiency of replication and transcription and, eventually, the endurance of organisms (Britt and May, 2003).

To avoid ROS generation and DNA damage upon UV-B exposure, plants develop several mechanisms, such as accumulation of surface wax, phenols and trichomes, which reduces the further penetration of UV-B (Caldwell *et al.*, 1983; Jenkins, 2009). DNA damage is improved with the help of antioxidants like ascorbic acid and α-tocopherol (Kliebenstein *et al.*, 2002; Jain *et al.*, 2004) and by ROS scavengers such as superoxide dismutase, ascorbate peroxidase, glutathione reductase and guaiacol peroxidase (Jain *et al.*, 2004; Hassan *et al.*, 2013). Defensive reactions arising at the cytological level stimulate a mending complex that includes photoreactivation,

base and nucleotide excision and recombination repair. Photoreactivation causes monomerization of dimers by the use of UV-A/blue light, where the photolyase splits the bonds between cyclobutane rings using the light energy, thus returning the bases' integrity. Excision repair takes place by endonucleolytic cleavage, thereby releasing the nucleotides damaged by UV-B so that the strand is resynthesized (Liu *et al.*, 2000).

10.3 The Defensive Role of Silicon Against UV-B Exposure on Physiological and Biochemical Traits of Plants

Incidence of enhanced UV-B (280–315 nm) radiation influences the diverse phase of a plant's physiological and biochemical processes (Yao *et al.* 2011). Numerous studies on UV-B application to plants have revealed that UV-B amends plant morphology, reduces growth, alters biosynthesis of secondary metabolites, induces oxidative stress, perturbs the common physiological route and can accelerate plant death (Frohnmeyer and Staiger, 2003; Bassman, 2004; Edreva, 2005).

There have been well-acknowledged effects of UV-B radiation with different plants: strawberry (*Fragaria ananassa*) and barley (*Hordeum vulgare*) (Valkama *et al.*, 2003), wheat (Tian and Lei, 2007), oats (Zuk-Golaszewska *et al.*, 2003), maize (Barsig and Malz, 2000), soybean (Yuan *et al.*, 2002), cotton (reduction in height, leaf area, total biomass and fibre quality (Gao *et al.*, 2003), rice (*Oryza sativa*) (Kumagai *et al.*, 2001), mustard (*Brassica juncea*), and mung bean (*Vigna radiate*) (increased proline content) (Saradhi *et al.*, 1995). The injuries induced by UV-B affect plant processes through direct damage (DNA damage, membrane changes and protein denaturation) or by diverse regulatory effects.

Direct damage could affect numerous physiological processes, including changes in the photosynthetic apparatus (Zlatev *et al.*, 2012). This often alters the levels of chlorophyll and carotenoids (Gaberscik *et al.*, 2002), affects the rate and duration of both cell division and elongation (Hopkins *et al.*, 2002), elevates the level of phenolic pigments (Hollosy, 2002), and also significantly induces change in UV-B engrossing compounds, for instance anthocyanin and flavonoids (Ravindran *et al.*, 2010).

Furthermore, research by Todorova *et al.* (2014) demonstrated that UV-B-treated tropical plants displayed elevated levels of malondialdehyde and anthocyanin contents, and POD and SOD actions in shoots, whereas chlorophyll *a* and *b* content, fresh weight, and shoot length declined. On the other hand, catalase and total phenolic content is not changed in shoots, and is only slightly affected in roots (Todorova *et al.*, 2014). Another similar investigation by Salama *et al.* (2011) was carried out on four annual desert plant species (*Malva parviflora* L., *Plantago major* L., *Rumex vesicarius* L. and *Sismbrium erysimoides* Desf.) to investigate the effects of UV radiation on photosynthesis and several metabolic actions. Elevated UV radiation brought on several modifications, such as reduced chlorophyll, protein and proline contents and increased carotenoid content. Conversely, reducing UV wavelength stimulated the level of proline in both roots and shoots. An enhanced level of proline defends plant cells against UV-induced damage.

Silicon is known as a beneficial element, reported to augment the tolerance of plants against UV-B induced stress. Plants absorbs silicon in the form of silicic acid,

which is found in soil, mostly in the form of silicates (Cooke and Leishman, 2011; Malčovská *et al.*, 2014). Furthermore, Malčovská *et al.* (2014) reported in their article about the role of silica supplementation that changed the content of pigments, oxidative status and phenolic metabolism of young maize seedlings exposed to UV-B.

Si leads to an increase in H_2O_2 content. Exoginous application of Si reduces the accumulation of cyclobutane pyrimidine dimmers (photoproduct) resulting from UV-B induced DNA damage (Chen *et al.*, 2016). Furthermore, research by Shen *et al.* (2010) showed UV-B light additionally impacted on the growth of soybean seedlings. Under drought condition, and silicon application considerably lessened the physiological and biochemical impact of UV-B stress conditions. Similar results were reported in rice plants (Goto *et al.*, 2003) and wheat seedling (Yao *et al.*, 2011) when using seedlings were supplemented to silica under UV-B stress silica to reduce UV-B stress.

10.4 Silicon Repairs Anatomical Structures of Plants Damaged by UV-B Exposures

The anatomical effects of UV-B have been seen in various plants in the form of increased amounts of wax on the leaf, and stomatal index on leaf surfaces. UV-B also affects the leaf thickness, by reducing the thickness of leaf tissue (Kakani *et al.*, 2003). Conversely, UV-B application on plants reduces the wax content and also lowers leaf reflectance. Fagerberg and Bornman (2005) described the effects of UV-B on the mature brassica leaf, and found an increase in the intercellular space in mesophyll tissues.

The impact of UV-B radiation on the anatomy of the epidermis in Scots pine and Norway spruce pine has been examined by light microscopy, but no ultrastructural studies have been performed (Turtola *et al.*, 2006). However, deciduous trees, such as *Quercus robur* and *Facus sylvatica*, showed the deposition of tannin and phenolics compounds with cellulose fibrils in the primary and secondary layer of the epidermal cell wall (Newsham *et al.*, 1997).The deposition of phenols causes the thickening of the epidermal cell wall. Laakso *et al.* (2000) illustrated the effect of UV-B on the leaf of Scots and Lobolly pine, and reported 15–22% greater thickness in the outer epidermis of UV-B treated seedlings. The needles of seedling also shows tannin accumulation (Laakso and Huttunen, 1998).

Silicon has several beneficial effects on plants under enhanced UV-B stress. The anatomical changes of the UV induced plants has been mitigated by the silicon treatment (Balakhnina and Borkowska, 2013). The thickness of palisade and mesophyll cells did not increase due to silicon treatment. Leaf epidermis, sclerenchyma and conduction tissue has higher UV absorbance, however, silicon-applicated plants showed lower UV absorbance range.

It has also been noticed in *Glycine max* seedlings and *Triticum aestivum*, that silicon application reduces the negative impact of UV radiation (Shen *et al.* 2010; Yao *et al.* 2011). Silica is deposited in the leaf epidermis, due to which a lower intensity of UV can penetrate the sclerenchyma,mesophyll and palisade cells (Goto *et al.*, 2003). The silicon layer behaves as a glass layer and diminishes the UV transmission in the leaf epidermis (Gatto *et al.* 1998). This reduction of transmission due to silica layer may be clarified by the absorption bands from interaction with electrons, impurities and the presence of OH- groups (Kitamura *et al.* 2007). Fang *et al.* (2006) described the countering effect of

silicon in UV-induced grass (*Phragmies australis*), in which the plants show less UV absorption due to the formation of a silicon layer.

10.5 UV-B-induced Oxidative Stress and Silicon Supplementation in Plants

Oxidative stress is a result of various biotic and abiotic stresses occurring in plants, including many sophisticated physiological and chemical mechanisms. These oxidative stresses generates as a result of excessive generation of Reactive Oxygen Species (ROS) and, subsequently, their accumulation in different organelles (Demidchik, 2015). Demidchik (2015) confirmed that synthesis of ROS occurred from the peroxidases and NADPH oxidases that ultimately damage many cell bio-polymers in plants and, hence, causes malfunctioning of the cell. The author also demonstrated that the accumulation of ROS also affects the channel proteins or signalling molecules, like activation of Ca^{2+} and K^+ permeable cationic channels of plasma membranes, and annexin, which triggers PCD (programmed cell death) by activating K^+ leakage. In the process of downregulation of various signalling and channel proteins, there is also a process governed by the plant which act as a defence mechanism – that is, extra- and intra- cellular antioxidants act against the stress signalling.

Generation of ROS is a normal process of a plant's metabolism, but their concentration enhances by increasing the level of toxicity, either from biotic or abiotic stress (Ren *et al.*, 2007; Shen *et al.*, 2014). These biotic and abiotic factors affect the metabolism of plants severely, and the most studied or known stress is environmental stress that causes the formation of ROS and leads to oxidative stress in plant cells (Shen *et al.*, 2010).

However, plants adopts a strategy to combat with these stresses by enhancing their antioxidative capacity and generating resistance against the damage (Monk *et al.*, 1989; Shen *et al.*, 2010). The most prominent effect of stress is lipid peroxidation, which can be characterized by leakage of ions due to oxidation of unsaturated fatty acids (UFA) in membrane and, consequently, increases the level of malondialdehyde (MDA; a primary indicator of stress in plants). However, it is also reported that the level of MDA in the plant declines with exposure to silicon (Shen *et al.*, 2010).

Thus, the studies of Shen *et al.* (2010) showed that silicon plays a vital role in the metabolism of higher plants, even under UV-B stress. Their results showed an optimistic approach towards the resistance mechanism against UV- B stresses in soybean seedlings. However, Li *et al.* (2004) observed very little change in the content of MDA and activities of SOD, and it was clear that the different types of peroxy radicals (e.g. of $O_2^{\bullet-}$, H_2O_2, etc.), as well as SOD activities, were also seen to be altered in the presence of UV-B radiations. Alteration in SOD activities leads to lipid peroxidation and, hence, affects the membrane permeability, which ultimately results in changing the structure of the cell membrane (Murphy, 1990).

Shen *et al.* (2014) also reported an increase in POD and SOD levels under UV-B stress, confirming the generation of ROS. In addition, Ren *et al.* (2007) demonstrated the adverse effect of UV-B radiation on the activities of antioxidant enzymes. However, they found that the application of silicon reduces the stress generated from UV-B radiation. Moreover, Epstein (1999), Gong *et al.* (2005) and Shen *et al.* (2014) found that treatment with silicon helps in the strengthening the cell wall, due to its deposition in the form of amorphous silica and phytolith (Shen *et al.*, 2014).

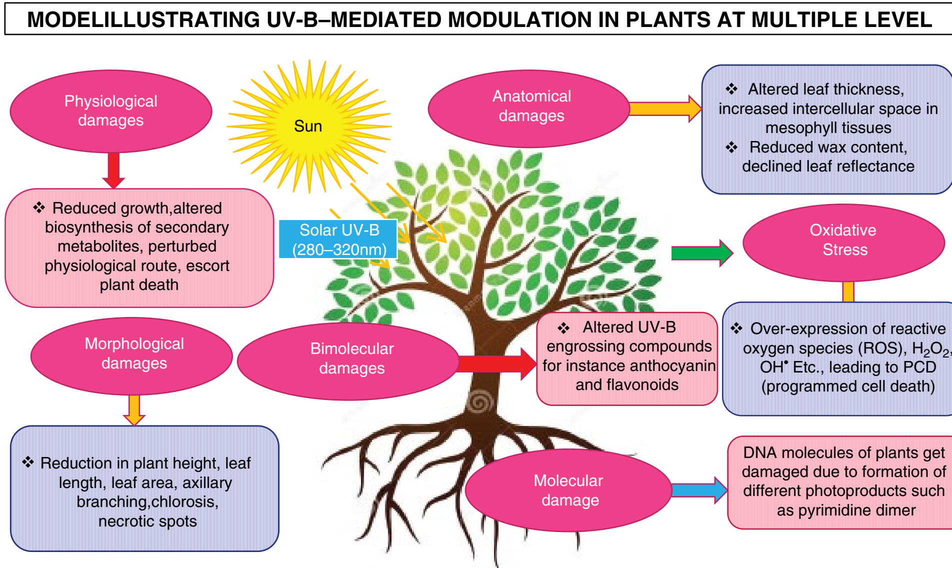

Figure 10.1 Stress responses in plant organelles against ambient UV-B radiations (modified after Nawker *et al.*, 2013; de Andrade *et al.*, 2015; Michaeli and Fromm, 2015). The figure describes that, after exposure to UV-B radiation, major alterations occur in the organelles of the plant cell. As indicated in the diagram, the photoreceptor for UV-B radiation is called as UVBR8, which is generally found in dimeric form. When UVBR8 enters into the nucleus, its monomeric form interacts with COP1 (Constitutive Photomorphogenic 1) and forms a complex of UVBR8-COP1. This complex blocks the expression of UV-B induced genes. However, HY5 (Elongated Hypocotyl 5) blocks the expression of flavonoid synthase and chalcone synthase pathways. A cascade of mitogen-activated protein kinases (MAPK) is also activated from exposure to UV-B stress and ultimately leads to PCD. In the chloroplast, an excessive amount of electrons is released and hence elevates the amount of ROS. Cytochrome C is released from the mitochondrial transmembranes, activating the activity of caspase and finally causing DNA laddering.? denotes that the photoreceptor for UV-B is unknown, its function in the UVR8 stress pathway is unclear and the roles of AtDAD1, AtDAD2 and AtBI are still in doubt.

Yao *et al.* (2010) demonstrated that environmental stress causes imbalance between ROS and antioxidant enzymes, which ultimately led to more production of ROS and, consequently, damages the molecules of plants cells. Shen *et al.* (2010) demonstrated that enhanced UV-B severely affects the cell organelles of the plant and is highly involved in the destruction of lipid membrane in the wheat seedlings. Also, Pei *et al.* (2010) reported that exposure of silicon in barley results in declining MDA levels and ROS production.

Plants depend on sunlight for making their food or obtaining energy and, hence, they are also known as photosynthetic organisms. However, their response occurs through various receptors called as photoreceptors. For example, UVR8 (UV Resistance Locus 8) proteins are the receptor for UV-B radiation. Exposure to UV-B radiation starts triggering the signalling molecules from UVR8 proteins that led to induction of secondary metabolite genes (Nawkar *et al.*, 2013; see Figure 10.1).

The formation of secondary metabolite genes is beneficiary up to certain concentrations, but higher doses may be lethal to the plant (Nawkar *et al.*, 2013). However, the genes involved in the MAPK cascade provide resistance against the harmful effect of UV-B radiations (Nawkar *et al.*, 2013). In addition to this, Nawkar *et al.* (2013) demonstrated

that the generation of ROS due to UV-B exposure generally occurs in mitochondria and chloroplasts. The authors found that in *Arabidopsis* metacaspase-8 (AtMC8), generation of ROS occurs from oxidative stress that triggers the *AtRCD1* (radical induced cell death) gene and, ultimately, the cell becomes susceptible to collapse. However, many studies revealed that jasmonic acid (JA) and salicylic acid (SA) regulate the level of ROS, and ultimately lead to the death of the cell (Nawkar *et al.*, 2013).

10.6 Silicon Supplementation and the Status of Antioxidant Enzymes in Plants Exposed to UV-B

Plants have a very beautiful and systematic mechanism to cope with stress. Antioxidative enzymes play a key role in their defence mechanism against the various oxidative stresses that occur. Plants play an efficient role in scavenging the oxygen radical, thereby saving themselves from the damaging effects of oxidative reactions (Murphy, 1990). Moreover, Shen *et al.*, (2010) described that some antioxidant enzymes, including superoxide dismutase, peroxidase and catalase, search the reactive oxygen species. However, the H_2O_2 formed from the free radicals is detoxified by APX, POD, CAT, which may cause damaging effect to nucleic acids (Shen *et al.*, 2010), chloroplasts (Shen *et al.*, 2010; Karuppanapandian *et al.*, 2011; Rico *et al.*, 2015), peroxisomes (Karuppanapandian *et al.*, 2011; Rico *et al.*, 2015), mitochondria (Karuppanapandian *et al.*, 2011; Rico *et al.*, 2015), plasma membranes (Karuppanapandian *et al.*, 2011;

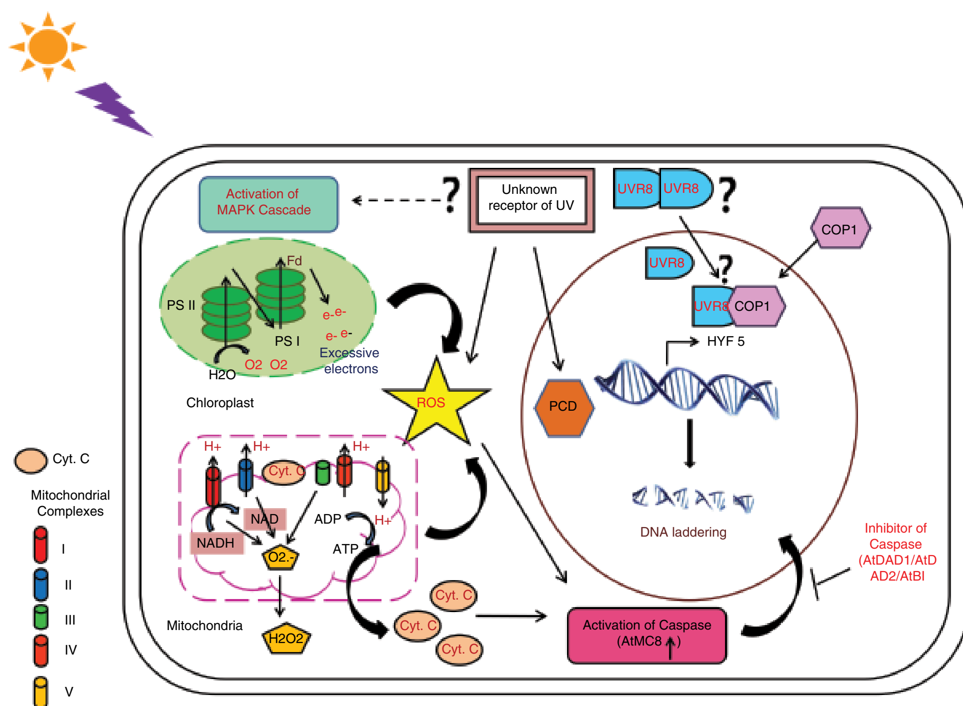

Figure 10.2 Model illustrating the overall damaging effects posed by UV-B (enhanced or ambient) on higher plants at multiple level.

Rico *et al.*, 2015), endoplasmic reticulum (Karuppanapandian *et al.* 2011; Rico *et al.*, 2015) and proteins (Shen *et al.*, 2010).

It has been mentioned earlier that plant also have defence strategies, such as antioxidant enzymes, to cope up with the stress generated from ROS. Silicon also has been shown to have a marked effect against UV-B radiation (Shen *et al.*, 2010). Gong *et al.* (2005) reported that silicon implementation in the leaves of wheat plants led to enhancement of various antioxidant enzymes (e.g. POD, CAT and SOD), while Gunes *et al.* (2007) also observed that, under UV-B stress conditions, barley seedlings showed marked phytotoxicity, although silicon treatment enhanced the levels of POD, SOD and CAT in the presence of stress.

The same result was obtained by Yao *et al.* (2010) in wheat seedlings – i.e. the damage was inhibited by silicon by following the stress resistance mechanism. The studies of Malčovská *et al.* (2014) showed that production of ROS in plant cells due to UV-B radiation not only causes damaging effects, but also affects second messenger; for example, after the exposure to UV-B radiation in plants, various transcripts, including PR-1 and Lhcb, start increasing and decreasing, respectively, and their pathways are mediated by superoxide radicals like hydrogen peroxide. The superoxide radicals directly trigger the regulation PDF by upregulating the PDF-1.2 transcript (Mackerness, 2000). Hideg *et al.* (2013) found that, from the assay of antioxidant genes, it could be concluded that, under increased and decreased UV-B doses, alteration occurs in the metabolism of ROS. However, the gene expression mechanism of ROS in low UV-B radiation exposure is unknown.

It was found that, UV-B radiation, enhances the level of hydrogen peroxide, while the content of hydrogen peroxide is seen to be slightly declined on applying silicon (Shen *et al.*, 2010b; Malčovská *et al.*, 2014). Malčovská *et al.*, (2014) found that, after exposure to UV-B radiation, silicon-treated seedlings of *Zea mays L.* were not affected by the increased superoxide radicals, while the studies of Yao *et al.* (2011) observed a reduction in the formation of superoxide radicals by enhancing the concentration of silicon.

Malčovská *et al.* (2014) demonstrated that, after the disruption of polyunsaturated lipid bilayer from ROS, TBARS were seen to be accumulated near the membrane. Actually, the level of TBARS determines the extent of the oxidative damage that occurs in the cell. In addition, they also found that silicon-treated seedlings were unaffected by UV-B radiations and, hence, the content of TBARS was also found to be unaltered. Accumulation of various phenol derivatives, such as phenylpropanoids and flavonoids, enhances the role of ROS-scavenging activities in UV-B radiation stress (Agati and Tattini, 2010; Fini *et al.*, 2011; Malčovská *et al.*, 2014).

10.7 Silicon and Level of Phenolic Compounds Under UV-B Stress

All plants have a tendency to produce vast numbers of different secondary metabolites, in which the highly studied and important group is the phenolic compounds (Michalak, 2006). These contain a single aromatic ring (C_6), with either one or more hydroxyl groups (Michalak, 2006). However, their formation occurs from cinnamic acid, whose precursor is L-phenylalanine ammonia-lyase (PAL).

Phenols can be categorized into different groups, according to the number of carbon atoms joined with the phenolic skeleton – for example, flavonoids, benzoic acids, phenyl propanoids and simple phenols (Rice-Evans *et al.*, 1997; Solecka, 1997; Chaudière and Ferrari-Iliou, 1999; Takahama and Oniki, 2000; Michalak, 2006). Many authors have studied the role of flavonoids, and revealed that they perform various metabolic functions in plants. It has been reported that, under various stresses, such as unfavourable environmental conditions and others, the amount of phenylpropanoid and phenolic compounds are elevated (Lavola *et al.*, 2000; Grace and Logan, 2000; Sakihama and Yamasaki, 2002; D'ıaz *et al.*, 2001; Michalak, 2006). In addition to this, Takahama and Oniki (2000) and Ruiz *et al.* (2003) also observed that, in cases of infection, injury or stresses, isoflavones and flavonoids are produced. Beside this, Sakihama and Yamasaki (2002) and Ruiz *et al.* (2003) also observed that the synthesis of flavonoids and isoflavones occurs in nutrient-deprived conditions and temperatures lower than required.

Phenolic compounds, especially the flavonoids (Winkel-Shirley, 2002) are indicators of stress generated in the plants (Madan *et al.*, 1995; Nayyar *et al.*, 2003; Yao *et al.*, 2010). From many researches, it has been concluded that phenolic compounds help in absorption and screening of UV-B radiations. They are generally found beneath the cuticle or cell wall or vacuoles of epidermal tissues and, hence, protect from damages by UV-B stress (Krauss *et al.*, 1997; Hutzler *et al.*, 1998; Winkel-Shirley, 2002; Schmitz-Hoerner and Weissenböck, 2003). Carlos *et al.* (2001) also believed that, as the radiation of UV-B increases, levels of phenolic compounds also rise in the epidermal cells.

In the case of any biotic and abiotic stresses, the level of phenol increases, acting as a defensive compound (Yao *et al.*, 2010). It is also thought that phenolic compounds are a fine line of defence against UV-B radiation, inhibiting the penetration of radiation into the cells or tissues (Mackerness, 2000; Hollósy, 2002; Goto *et al.*, 2003; Malčovská *et al.*, 2014). Pie *et al.* (2010) also observed that concentration of decreases with the exposure of silicon in plants. Alexieva *et al.* (2001) also reported that the levels of H_2O_2 and proline are also enhanced in the presence of UV-B radiation stresses. Shen *et al.* (2010) also demonstrated the same result, while proline levels decreased on supplementation of silicon. They observed that silicon had played a defensive role in soybean plants under UV-B stress, characterized by a decreased level of proline, compared with plants not treated with silicon (Shen *et al.*, 2010).

Kurkdjian and Guern (1989) observed the same interesting fact about proline involved in the altering of cytosolic acidosis (i.e. generated from various stresses). However, it has been also found that the damage can be reduced by the sequential removal of H^+ arising from the synthesis of proline (Shen *et al.*, 2010). In addition to this, Shen *et al.* (2010) also found that physiological and metabolic activities of plants under UV-B stress were ameliorated by the treatment of silicon. The damages incurred from UV-B radiation were seen by various stress markers, when applied separately.

Silicon has the capability to alter the phytotoxic effects on growth of roots and shoots, photosynthesis and other metabolic processes in plants under UV-B stress. However, Schaller *et al.* (2013) found that it is a double layer of silicon that protects the leaves of grasses from UV-B radiations, as compared to prolines. They also observed that the reflection or absorbance of UV-B radiation depends on the quality or quantity of phenols or silicon layer.

Lutts *et al.* (1999), Pie *et al.* (2010), de Lacerda *et al.* (2003) and Yao *et al.* (2010) reported that proline accumulation under UV-B stress conditions correlated with stress tolerance, and protects from many injury symptoms in the plant. Kauss *et al.* (2003) reported that, in cucumber plants, the cationic form of prolines is capable of polymerizing the orthosilicic form of silica into insoluble silica, which results in the increased cationic charge density. This accumulation of proline-rich proteins helps in the deposition of silica at particular developmental stages (Kauss *et al.*, 2003).

However, Goto *et al.* (2003) observed that, on exposure to UV-B radiation, the synthesis of phenolic compounds is linked with silicon concentrations. Moreover, it was found that, after exposure to UV-B, *Arabidopsis thaliana* and petunia plants synthesize flavonols having an increased level of hydroxylation (Ryan *et al.*, 2001). The process of hydroxylation only affects the antioxidant capacity of plants, not the property of UV absorption. A further role of flavonoids was also demonstrated by Bieza and Lois (2001) in *Arabidopsis* mutant, which showed a high tolerance level against UV-B stress. In addition to this, Winkel-Shirley (2002) also described that an increased level of various phenolic substances results in upregulation of the CHS (chalcone synthase) gene. However, there are still some studies occurring in this area, namely in the exposure of UV, biosynthesis of phenolics and flavonoids, and also their reaction against the defence from UV stress.

10.8 Conclusion and Future Perspectives

Being restricted in locomotion, plants inevitably experience or encounter a myriad of environmental insults. Since they are unable to move or escape from such environmental constraints, they have to adapt to the surrounding factors, as these factors influence their optimal growth and productivity. Among the various environmental stress factors influencing the growth and productivity of plants, light, in particular, has ultimate or utmost significance. It does not only serves as the source of energy, controlling a plethora of physiological and morphological processes, including photosynthesis and growth, but also provides an informational signal that directs the overall development or multiple phases of the plant, ranging from germination to the flowering phase.

Plants sense the intensity, duration and quality of light, and these cues are bestowed with the potential of directly influencing the germination, growth rate, productivity, reproduction and survival in plants. Plants are inevitably exposed to ultraviolet-B radiation, since they require the capture of sunlight for photosynthesis. UV-B (280–315 nm) is a fundamental stress signal which is explicitly discerned by plants in order to endorse UV acclimation. It is an inherent or intrinsic component of sunlight and, since plants evolved, the spectral composition of the total fraction of sunlight reaching the earth's surface has changed dramatically.

At the present time, UV-B radiation has become a major concern for crop scientists and producers. A rapid depletion has been observed in the stratospheric ozone layer, and this ultimately influences global crop productivity. Only a handful of studies are now available that mark the precarious impacts of elevated level of UV-B on terrestrial plants. Studies have showed that exposure to UV-B radiation induces profound indirect damaging effects on plants, including deformed/distorted morphological traits, such as impediment in plant height, decreased leaf area, reduced dry weight,

decreased biomass accumulation, intensified auxiliary branching, profound leaf curling and so on.

So far, the search for an appropriate stress alleviator has had mixed outcomes. An overwhelming number of studies are available that have reported how the profound reduction in the growth, productivity and dry matter of plant under UV-B exposure has been significantly ameliorated by exogenous application of Si in crop species (Gong *et al.*, 2005; Eneji *et al.*, 2005). In addition to this, distress caused by elevated levels of UV-B leads to overexpression of ROS and, thus, generation of ROS, UV-B exposure and stress could be closely linked or associated. Emerging evidence has revealed that silicon plays a significant role in phenolic metabolism, and will significantly ameliorate the overall damaging effects imposed by UV-B radiation by enhancing the agglomeration of phenolic compounds. Future studies are still required to illuminate the underlying pathways attributed to UV-B tolerance in several other crops and tree species.

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11

Sun-Screening Biomolecules in Microalgae: Role in UV-Photoprotection

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11.1 Introduction

Anthropogenically, as well as naturally released ozone-depleting substances (ODS), followed by increased incidence of harmful solar ultraviolet (UV) radiation (Williamson *et al.*, 2014) has fuelled several concern about their negative impacts on the living biota (Rastogi *et al.*, 2014a; Häder *et al.*, 2015). The obligate necessity of solar light for life-driven phenomena-photosynthesis concurrently exposes microalgae, including cyanobacteria, to lethal doses of UV-A (315–400 nm) and UV-B (280–315 nm) radiation in their natural habitats. Among solar UV radiation, UV-B radiation exerts more drastic effects on the normal metabolic processes of all sun-exposed organisms. UV radiation affects the normal physiology and biochemistry of an organism, either through direct effects on key cellular machinery such as DNA and proteins, or indirectly by the production of reactive oxygen species (ROS).

Microalgae are the important primary producers, and they play a significant role in maintaining the energy dynamics of an ecosystem. Besides their distinguished role in the sustainability of an ecosystem, they are important sources of several natural products of high economic value (Rastogi *et al.*, 2009, 2010a). A number of biological processes, such as growth and development, motility and orientation, pigmentation, photosynthesis, enzyme activity, N_2-fixation and CO_2 assimilation, are affected by the incidence of UV radiation. DNA is one of the prime targets of UV radiation, due to its absorbance in the UV range.

During the course of evolution a number of organisms have developed some specific defence mechanisms to protect and sustain in different sun-exposed environments with high UV fluxes. Adaptive diversification, and inclusive survival of microalgae/cyanobacteria in diverse ecological niches, has compelled them to synthesize an array of

UV-B Radiation: From Environmental Stressor to Regulator of Plant Growth, First Edition. Edited by Vijay Pratap Singh, Samiksha Singh, Sheo Mohan Prasad and Parul Parihar.
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biomolecules, each with specialized functions, to compete successfully on the planet. A number of small biomolecules have been reported in microalgae which have great potential to minimize the detrimental effects of UV radiation.

Mycosporine-like amino acids (MAAs) and scytonemin (Scy) are the important biomolecules that have great potential to absorb/screen harmful doses of short-wavelength solar UV radiation and defend organisms from UV photo-damage. Biosynthesis and/or accumulation of some UV-absorbing compounds have been reported in several microalgae, including cyanobacteria inhabiting diverse habitats. It has been shown that the biosynthesis of some UV-absorbing compounds, such as MAAs and Scy, is greatly induced under UV exposure, and these can be established as the key biomarkers of UV-stressed environment. Herein, we summarize the occurrence and biosynthesis of some chemical biomarkers in microalgae under intense UV radiation and their ecological importance, with special emphasis on photoprotective function.

11.2 Global Climate Change and UV Radiation

Destruction of the stratospheric ozone layer caused by anthropogenically released ozone-depleting substances (ODS) such as chlorofluorocarbons (CFCs), chlorocarbons (CCs), organo-bromides (OBs), and reactive nitrogen species such as nitrous oxide (N_2O), has fuelled serious concern about the increasing incidence of short-wavelength UV radiation on the Earth's atmosphere (Ravishankara *et al.*, 2009; Manney *et al.*, 2011; Williamson *et al.* 2014). It has been estimated that a single chlorine atom released by the breakdown of CFC molecules under strong UV irradiation, may destroy more than one lac ozone molecules (Figure 11.1).

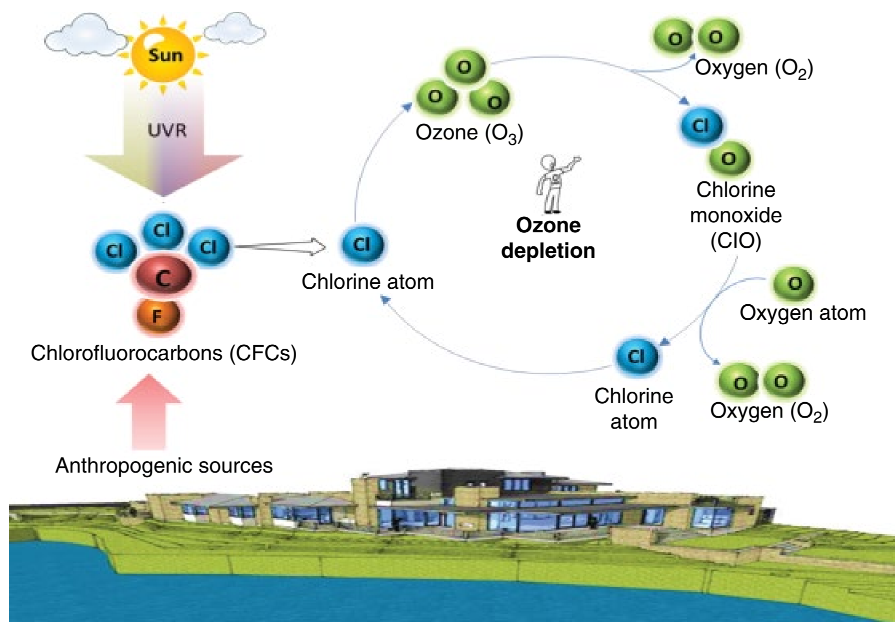


Figure 11.1 A generalized diagrammatic presentation of ozone depletion caused by anthropogenically released chlorofluorocarbons (Image by RP Rastogi).

The data obtained from Eldonet dosimeters (Häder *et al.*, 1999) have revealed the extreme UV-B (280–315 nm) irradiance in different parts of the Earth (Cabrol *et al.*, 2014). Besides the ODS, some other abiotic factors, such as aerosols and various tropospheric pollutants, cloud cover, sun-angle and surface reflectants also affect the intensity of UV-B radiation reaching the Earth's surface (Madronich *et al.*, 1998). Climate changes in the Arctic and/or Antarctic ecosystems are one of the major global environmental issues (Manney *et al.*, 2011).

Moreover, the global climate change and increase in short wavelength UV radiation can affect the normal physiology and biochemistry of all sun-exposed organisms of aquatic (Häder *et al.*, 2014) or terrestrial ecosystems (Bornman *et al.*, 2015). It has been established that UV-B radiation produces more adverse life-menacing effects, despite the fact that most of the extraterrestrial UV-B radiation is absorbed by the stratospheric ozone layer (McKenzie *et al.*, 2003). UV-C radiation (100–280 nm) does not play any damaging role on our ecosystems, since it is quantitatively absorbed by oxygen and ozone molecules in the Earth's atmosphere. However, UV-A radiation may exert harmful effects on living biota by the formation of reactive oxygen species (ROS), through indirect photosensitizing reactions (Rastogi *et al.*, 2010b).

11.3 Effects of UV Radiation on Microalgae

Microalgae, including cyanobacteria, play a major role in the primary productivity of an ecosystem. They are highly sensitive to daily fluctuating solar energy affecting their natural habitats. UV radiation coming from solar light triggers a number of detrimental effects on all photosynthetic life, including microalgae. Solar UV radiation drastically affects the integrity of prime cellular machinery proteins and DNA, and also a number of key cellular and/or metabolic activities, such as: growth and pigmentation; morphology and orientation; photosynthesis; N_2 and CO_2 metabolism; enzyme activity; and buoyancy (Brodhun and Häder, 1993; Lesser *et al.*, 1994; Malanga *et al.*, 1997;Beardall *et al.*, 2002; Buma *et al.*, 2006; Richter *et al.*, 2007; Sinha *et al.*, 2008; Ma *et al.*, 2012; Rastogi *et al.*, 2014a, 2014b) (Figure 11.2).

Several studies have been conducted to support the detrimental effects of UV radiation. UV-B-induced photo-degradation of photosynthetic pigments, followed by reduction in photosynthesis, has been reported in several microalgae (Ghetti *et al.*, 1999; White and Jahnke, 2002; Guan and Gao, 2008; Rastogi *et al.*, 2014b). A decrease in relative electron transport rate (rETR), followed by decrease in total photosynthetic yield, was found under UV radiation in the cyanobacterium *Anabaena variabilis* PCC 7937 (Figure 11.3A) (Singh *et al.*, 2013). UV radiation may destabilize the structural and functional integrity of photoharvesting phycobiliprotein pigments (Rastogi *et al.*, 2015a; Figure 11.3B). Furthermore, D1 and D2 proteins of the PSII reaction centre have also been found to be affected by UV radiation (Campbell *et al.*, 1998). Zhang *et al.* (2013) found marked reductions in oxygen evolution in cyanobacterium *Microcystis* sp. and green microalga *Chlamydomonas microsphaera*. The key N_2-fixing enzyme, nitrogenase, is highly sensitive to UV radiation (Kumar *et al.*, 2003).

Microalgae, including cyanobacteria, exhibit several morphological differentiations under UV radiation (Tian and Yu, 2009; Rastogi *et al.*, 2010c, 2014b). Tian and Yu (2009) observed that enhanced UV-B radiation caused ultrastructural changes and induced antioxidant systems in a green microalga, *Dunaliella salina*. Alterations in chloroplasts'

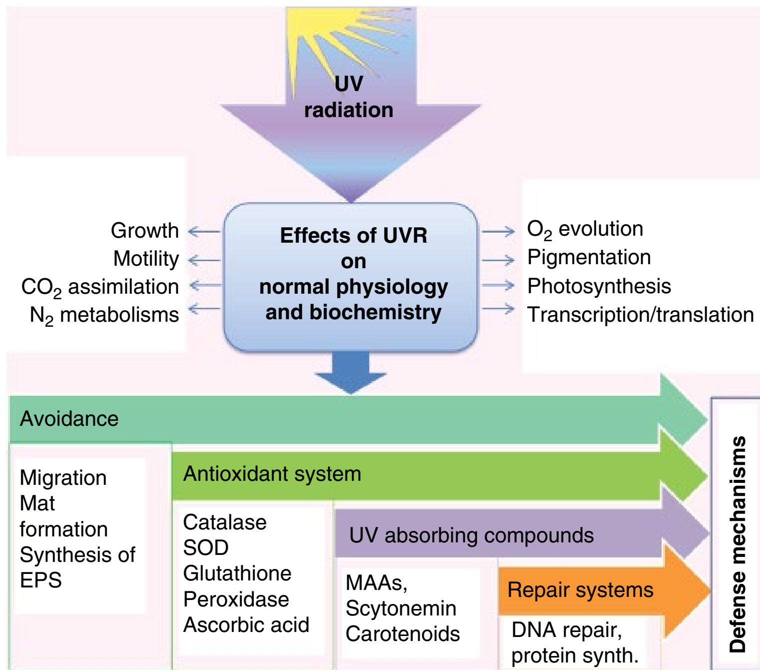
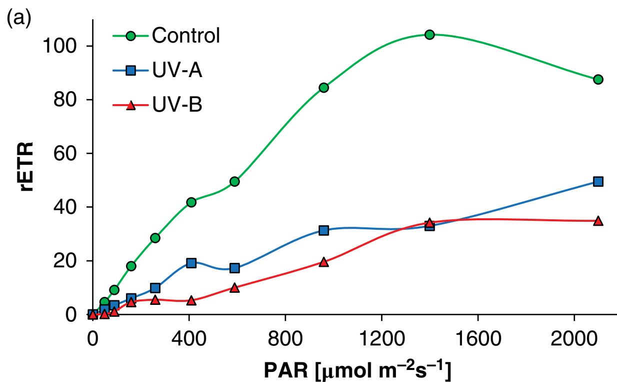


Figure 11.2 A simplified view for effects of UV radiation and probable defence mechanisms adopted by microalgae (Illustration by RP Rastogi).



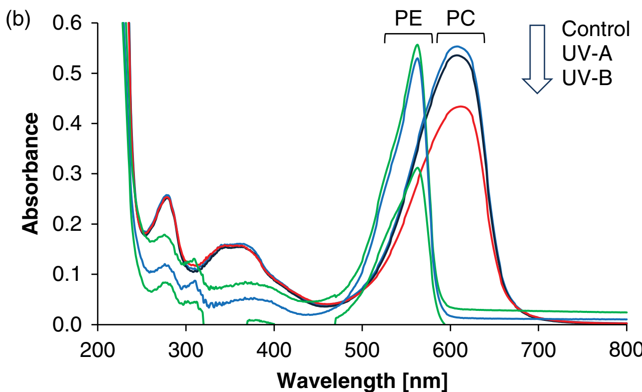


Figure 11.3 Effects of UV radiation on relative electron transport rate (A) and phycobiliproteins phycocyanin (PC) and phycoerythrin (PE) in the cyanobacteria *Anabaena variabilis* PCC7937 and *Lyngbya* sp. A09DM, respectively (adapted from Singh *et al.*, 2013; Rastogi *et al.*, 2015a).

function and integrity was found to be triggered by UV-B exposure in a microalga, *Chlorella vulgaris* (Malanga *et al.*, 1997). A significant disruption of the differentiation of heterocysts, and a reduction in filament length, was observed in the cyanobacterium *Anabaena siamensis* TISTR-8012 upon exposure to UV radiation (Rastogi *et al.*, 2014b). Gao *et al.* (2007) also observed a significant disruption of the differentiation of heterocysts and a reduction in trichome length of up to 49% in the cyanobacterium *Anabaena* sp. PCC 7120 after UV-B exposure.

The exact mechanisms of cell/filament breakage are not known. However, induced accumulation of ROS under UV radiation may result in filament breakage by oxidizing the sheath or membrane lipid peroxidation (Donkor *et al.*, 1993) or by selective lysis of damaged cells (Rastogi *et al.*, 2015b). Recently, Rastogi *et al.* (2010c) have proposed that fragmentation of the cyanobacterial filaments could be due to oxidative stress, via generation of ROS. UV-B radiation leads to generation of excess ROS and disrupts the homeostasis of the cell (Malanga *et al.*, 1997; Rijstenbil, 2002). Moreover, several studies have revealed the UV-B-induced overproduction of ROS, and its harmful oxidative effects, in different microalgae (Malanga *et al.*, 1997; Rijstenbil, 2002; Richter *et al.*, 2003) and cyanobacteria (Rastogi *et al.*, 2010c, 2015b; Rastogi and Madamwar, 2015).

Besides proteins and lipids, the hereditary material, DNA is one of the prime targets for UV damage. Solar UV-B radiation damages cellular DNA by inducing the formation of cyclobutane purine/pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts (6-4 PPs) and their Dewar isomers (Rastogi *et al.*, 2010b; Cadet *et al.*, 2015). UV radiation also induces single- and/or double-strand DNA breaks, leading to loss of genetic information in several microalgae (Buma *et al.*, 2006) and cyanobacteria (Rastogi *et al.*, 2011, 2014c). Moreover, some organisms, including microalgae and cyanobacteria, have developed various strategies to protect themselves from UV-induced damages (Rastogi *et al.*, 2011; Dolinko *et al.*, 2015; Rastogi and Madamwar, 2015).

11.4 UV-induced Defence Mechanisms

In response to several harmful effects of UV radiation, some species/strains of microalgae have devised specific defence mechanisms to survive in harsh environments with high UV-B fluxes (Figure 11.2). A number of physiological (e.g. migration and mat formation) and biochemical (e.g. synthesis of exopolysaccharides, UV absorbing compounds, antioxidants, DNA repair and protein resynthesis) defence mechanisms have been reported in microalgae (Malanga *et al.*, 1997; Zhu and Green, 2010; Rastogi *et al.*, 2014a, 2015b). Biosynthesis or accumulation of some UV-absorbing compounds plays an important role in UV photoprotection of microalgae (Carreto *et al.*, 1990). In the subsequent section, occurrence of some sun screening biomolecules has been discussed as the key biomarkers in cells under UV-exposed environment.

11.5 Sun-Screening Biomolecules as Key UV Photoprotectants

Among different physiological and/or biochemical defence strategies, as discussed above, synthesis of certain UV-absorbing/screening compounds has been recognized as being crucial against short-wavelength UV radiation in several organisms. Microalgae

have assimilated a number of UV-photoprotective compounds to overcome the toxicity of UV radiation. MAAs and Scy are prominent photoprotectants in microalgae that confer protection against UV radiation.

11.5.1 Mycosporine-Like Amino Acids (MAAs)

MAAs have been reported in different taxonomic groups, including micro/macroalgae, lichen symbionts and several aquatic animals (Sinha *et al.*, 2007). It has been considered that, in higher animals, occurrence of MAAs can be attributed either to their ingestion through the food chain or their synthesis by symbiotic algal partners. MAAs (Figure 11.4) are small, colourless, hydrophilic compounds, chemically composed of a cyclohexenone or cyclohexenimine chromophore, conjugated to the nitrogen substituent of an amino acid or its imino alcohol. Strong UV absorption (λ_{max}: 310-362 nm) properties of different MAAs vary, due to variations in the attached side groups and nitrogen substituents.

A number of MAAs have been reported from cyanobacteria and eukaryotic microalgae (Table 11.1; Xiong *et al.*, 1999; Sinha *et al.*, 2003a; Rastogi *et al.*, 2010a, 2012; Llewellyn and Airs, 2010; Carignan and Carreto, 2013).

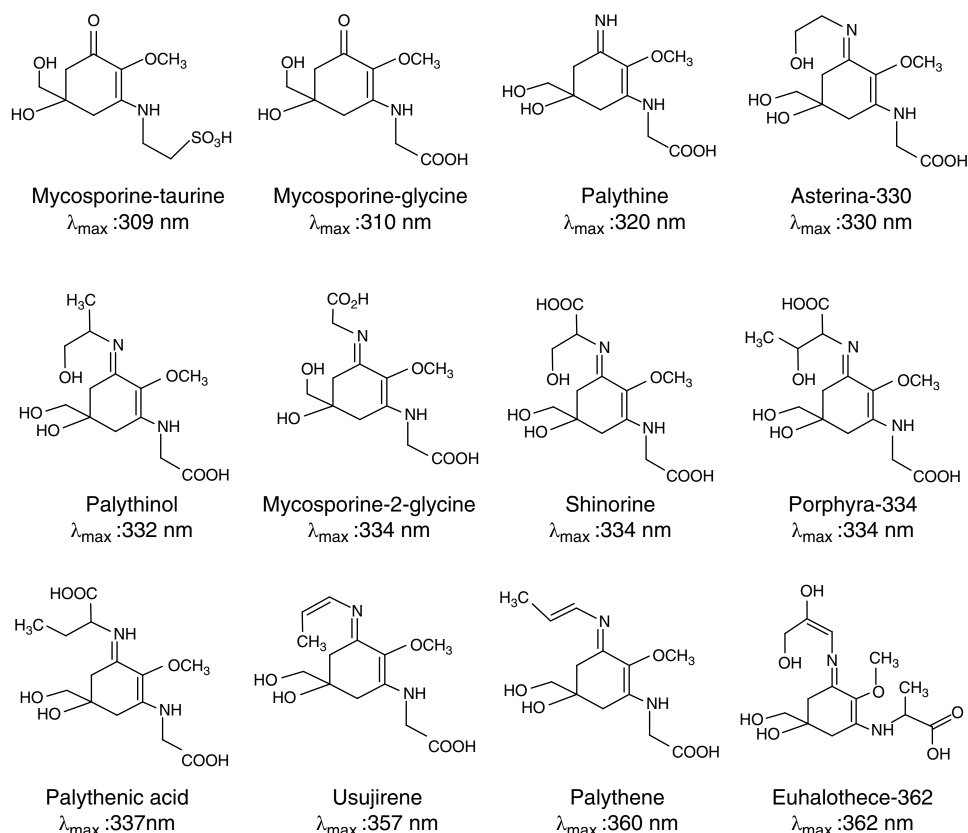


Figure 11.4 Chemical structure of some common mycosporine-like amino acids reported in microalgae.

Table 11.1 Occurrence of some common UV-absorbing MAAs in different microalgae.

Microalgae	Mycosporine-like amino acids										
	MG	AS	PL	PR	SH	PE	M2G	PT	E-362	PA	US	Ref
Anabaena doliolum	+			+	+							Singh *et al.*, 2008
Aphanothece halophytica					+	+						Oren, 1997
Arthrospira sp.	+											Rastogi and Incharoensakdi, 2014c
Aulosira fertilissima				+	+							Mushir and Fatma, 2011
Calothrix sp.	+		+		+							Garcia-Pichel and Castenholz, 1993
Euhalothece sp.							+		+			Kedar *et al.*, 2002; Volkmann *et al.*, 2006
Gloeocapsa sp.	+	+	+		+							Garcia-Pichel and Castenholz, 1993; Garcia-Pichel *et al.*, 1993; Sommaruga and Garcia-Pichel,1999
Lyngbya aestuarii		+			+							Garcia-Pichel and Castenholz, 1993; Karsten *et al.*, 1998
Lyngbya sp.		+						+				Rastogi and Incharoensakdi, 2014a
Nodularia sp.				+	+							Sinha *et al.*,2003a
Nostoc commune	+				+							Böhm *et al.*,1995; Sinha *et al.*, 2003b
Rivularia sp.	+											Rastogi *et al.*,2014c
Scytonema sp.	+				+							Garcia-Pichel and Castenholz, 1993; Rastogi *et al.*, 2010d
Dunaliella salina			+	+	+							Tian and Yu, 2009
Gymnodinium catenatum	+			+	+							Vale, 2015
Alexandrium excavatum	+	+		+	+	+		+		+		Carreto *et al.*, 1990
Alexandrium tamarense	+		+	+	+	+		+		+	+	Carreto *et al.*, 2001; Llewellyn and Airs, 2010
Gymnodinium galatheanum	+			+	+			+		+		Llewellyn and Airs, 2010
Gymnodinium venificum	+			+	+	+		+		+		Llewellyn and Airs, 2010
Gyrodinium dorsum				+	+			+				Klisch and Häder, 2000

MG: mycosporine-glycine; AS: asterina-330; PL: palythinol; PR: porphyra-334; SH: shinorine; PE: palythene; M2G: mycosporine-2-glycine; PT: palythine; E362: euhalothece-362; PA: palythenic acid; US: Usujirene.

MAAs have been reported to occur predominantly in members of the Dinophyceae, Bacillariophyceae and Haptophyceae (or Prymnesiophyceae). Recently, Rastogi and Incharoensakdi (2014a, 2014b, 2014c) have reported the synthesis of different MAAs in filamentous and unicellular cyanobacteria. Two different UV-absorbing compounds with absorption maxima at 324 nm and 322 nm were found in the green microalga *Tetraspora* sp. CU2551 (Rastogi and Incharoensakdi, 2013). Some species of dinoflagellates, such as *Alexandrium excavatum* (Carreto *et al.*, 1990) and the prymnesiophyte *Phaeocystis pouchetii* (Marchant *et al.*, 1991) are known to produce MAAs in high concentrations.

Jeffrey *et al.* (1999) have investigated more than 150 species (206 strains) of microalgae, including several green algae, for the presence of UV-absorbing compounds, and have found compounds with absorption maxima between 330–340 nm. Some novel glycosylated MAAs have also been reported from different cyanobacteria (Böhm *et al.*, 1995; Matsui *et al.*, 2011; Nazifi *et al.*, 2014). However, their biosynthetic pathway and exact biological functions are still unknown.

Biosynthesis of MAAs in microalgae and various other organisms may serve as passive defence mechanisms that allow them to capture photons, preventing their interaction with key cellular machinery such as proteins and DNA. MAAs can prevent three of every ten photons from striking cytoplasmic targets in cyanobacteria (Garcia-Pichel *et al.*, 1993). The role of MAAs as potent photoprotectants has already been reported in different microalgae, including cyanobacteria (Garcia-Pichel *et al.*, 1993; Xiong *et al.*, 1997; Klisch and Häder, 2000; Suh *et al.*, 2003; Rastogi *et al.*, 2015c; Rastogi and Madamwar, 2015). It has been suggested that MAAs effectively dissipate absorbed radiation as heat, without producing ROS (Conde *et al.*, 2000, 2007). The genetic basis of MAAs biosynthesis has been elucidated in some cyanobacteria (Portwich and Garcia-Pichel, 2003; Balskus and Walsh, 2010; Gao and Garcia-Pichel, 2011; Spence *et al.*, 2012).

11.5.2 Scytonemin

In contrast to MAAs, Scy is predominantly produced by cyanobacteria and algal symbionts of some lichens. It is a pale yellow to brown, lipid-soluble, dimeric phenolic compound, located in the extracellular polysaccharide sheath (Figure 11.5A) of some cyanobacteria (Rastogi *et al.*, 2015d), and it acts as a passive UV sunscreen (Garcia-Pichel and Castenholz, 1991). Scy has an UV absorption maximum at 386 nm, but it also absorbs significantly at 251, 278 and 300 nm (Figure 11.5B), and provides photoprotection to the organisms inhabiting under UV-A/B-exposed environments. Scy exists in both an oxidized (Mw 544 Da) and a reduced (Mw 546 Da) form. However, four different derivatives of Scy, such as dimethoxyscytonemin, tetramethoxyscytonemin, scytonin and scytonemin-3a-imine, have also been reported from different cyanobacteria (Bultel-Poncé *et al.*, 2004; Grant and Louda, 2013; Rastogi *et al.*, 2014d).

The biosynthesis of Scy is regulated by a number of genes (Rastogi *et al.*, 2010a). Several cyanobacterial genomes have been investigated to identify the genes responsible for Scy biosynthesis (Soule *et al.*, 2007, 2009; Sorrels *et al.*, 2009). A gene cluster consisting of 18 unidirectionally transcribed ORFs (NpR1276–NpR1259), including eight genes involved in the biosynthesis of tryptophan and tyrosine, was identified in the cyanobacterium *Nostoc punctiforme* ATCC 29133 (Soule *et al.*, 2007). Tryptophan and tyrosine derivatives are considered as key precursors for Scy biosynthesis. Moreover,

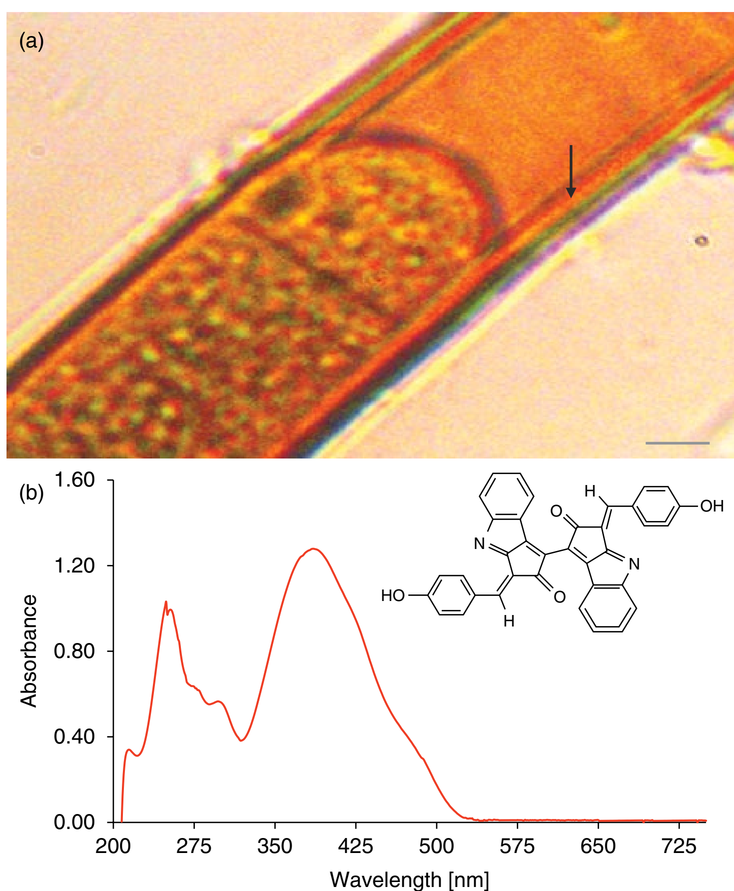


Figure 11.5 Occurrence of scytonemin in the extracellular polysaccharide sheath of *Lyngbya* sp. (A) shows the UV-absorption maximum at 386 nm (for details, see Rastogi and Incharoensakdi, 2014a) (Image by RP Rastogi).

Balskus and Walsh (2009) presented a probable route for Scy biosynthesis, and recognized the acycloin reaction as a key step in assembling the carbon framework of this ecologically and evolutionarily important pigment molecule.

Besides MAAs and Scy, some other UV-absorbing biomolecules, such as biopterin glucoside (λ_{max}: 362 nm), pteridines and prenostodione (λ_{max}: 318 nm) have also been reported in some cyanobacteria (Matsunaga *et al.*, 1993; Ploutno and Carmeli, 2001). The carotenoid pigments found in microalgae also play an important role in the quenching of ROS. A significant increase in the outer-membrane carotenoids echinenone and myxoxanthophyll was found in the cyanobacterium *Nostoc commune* after a few hours of UV-B irradiation (Ehling-Schulz *et al.*, 1997). However, the role of carotenoids as photoprotective compounds is still controversial. Some other groups of compounds, such as polyamines (PAs) found in microalgae, also play an important role in photoprotection. The diamine putrescine, triamine spermidine and tetramine spermine are the common PAs existing inside the cells. However, spermidine signifies the major PA in cyanobacteria (Jantaro *et al.*, 2003; Incharoensakdi *et al.*, 2010).

11.6 UV-Induced Biosynthesis

The biosynthesis of UV-absorbing substances such as MAAs and Scy can be influenced by various factors, such as irradiance, heat, ionic stress and different nutrients. Earlier studies have shown that the microalgae increase their MAA (Gröniger and Häder, 2002; Rastogi and Incharoensakdi, 2013, 2015; Vale, 2015) and Scy (Rastogi *et al.*, 2013, 2014d) contents in response to UV radiation, and are able to adapt to changing daily solar radiation in their natural habitat. Elevated levels of PAR, UV-A and UV-B irradiation have been found to induce MAAs (Carreto *et al.*, 1990; Rastogi *et al.*, 2010d).

The UV-B-induced synthesis of shinorine and porphyra-334 has been reported in three cyanobacterial strains, such as *Nodularia baltica*, *N. harveyana* and *N. spumigena* (Sinha *et al.*, 2003a). The conversion of mycosporine-glycine into two MAAs, porphyra-334 and shinorine, in response to PAR and UVR, was observed in the cyanobacteria *Anabaena doliolum* (Singh *et al.*, 2008), *Scytonema* sp. (Rastogi *et al.*, 2010d) and *Fischerella muscicola* TISTR 8215 (Rastogi and Incharoensakdi, 2015). Photoinduction of MAA synthesis was also found in some dinoflagellates, such as *Gyrodinium dorsum* (Riegger and Robinson, 1997; Klisch and Häder, 2000) and *Gymnodinium galatheanum* (Llewellyn and Airs, 2010). Two MAAs with absorption maxima at 324 nm and 322 nm were found to be accumulated after UV irradiation in a green microalga *Tetraspora* sp. CU2551 (Rastogi and Incharoensakdi, 2013).

Recently, we have reported the UV-induced synthesis of MAAs (Figure 11.6A), palythine (λ_{max}: 319 nm) and asterina (λ_{max}: 330 nm) in the cyanobacterium *Lyngbya* sp.

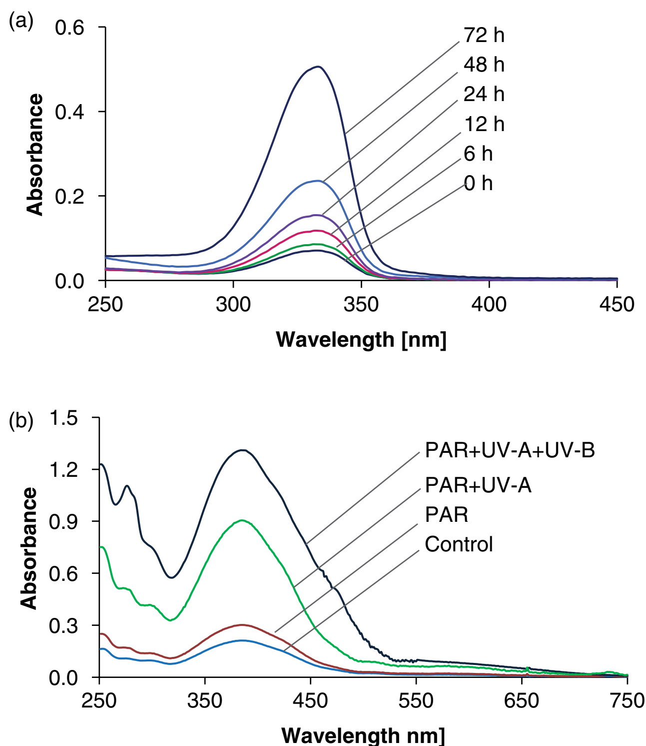


Figure 11.6 UV-induced biosynthesis of shinorine (A) and scytonemin (B) in the cyanobacteria *Gloeocapsa* sp. CU2556 and *Lyngbya* sp. CU2555, respectively (adapted from Rastogi and Incharoensakdi, 2014a, 2014b).

(Rastogi and Incharoensakdi, 2014a), as well as shinorine (λ_{max}: 333 nm) and an unknown MAA designated as M-307 (λ_{max}: 307 nm) in a unicellular cyanobacterium, *Gloeocapsa* sp. CU2556 (Rastogi and Incharoensakdi, 2014b).

Like MAAs, the biosynthesis of Scy is also induced under high photon fluence rates of UV radiation. Short wavelength UV-B radiation can induce the biosynthesis of Scy; however, UV-A radiation is highly effective in inducing the biosynthesis of scytonemin (Garcia-Pichel and Castenholz, 1991; Dillon *et al.*, 2002). Recently, induction of scytonemin synthesis (Figure 11.6B) under UV stress was observed in different cyanobacteria, such as *Rivularia* sp. HKAR-4 (Rastogi *et al.*, 2013), *Lyngbya* sp. CU2555 (Rastogi and Incharoensakdi, 2014a) and *Scytonema* sp. R77DM (Rastogi *et al.*, 2014d).

Induction of the synthesis of some carotenoids under UV radiation was observed in several cyanobacteria (Wachi *et al.*, 1995; Ehling-Schulz *et al.*, 1997; Ehling-Schulz and Scherer 1999). PAs were observed to play a crucial role in mitigating the UV-induced oxidative stress in cyanobacteria (Jantaro *et al.*, 2014). Moreover, there is a clear direct correlation between solar UV radiation and increased biosynthesis/accumulation of UV absorbing/screening compounds in the microalgae inhabiting diverse habitats.

11.7 Photoprotective Function

As discussed above, MAAs can dissipate absorbed radiation as heat without producing ROS (Conde *et al.*, 2000, 2007), and protect the cellular machinery from harmful effects of UV radiation. Several MAAs have been found to act as strong antioxidants (Rastogi and Incharoensakdi, 2014b, 2014c), and also have the ability to block the formation of UV-induced thymine dimers (Misonou *et al.*, 2003). Moreover, strong UV-absorption maxima, high molar extinction coefficients, resistance to several abiotic stressors, potential antioxidant properties, and the ability to prevent UV-induced skin damage, convenes strong support in favour of MAAs as photoprotective compounds (Dunlap and Yamamoto, 1995; Conde *et al.*, 2007; Gröniger *et al.*, 2000; De la Coba *et al.*, 2009; Rastogi and Incharoensakdi, 2014a, 2014b, 2014c).

The cyanobacterial pigment Scy is also highly stable against different abiotic stressors (Rastogi and Incharoensakdi, 2014a), and performs its UV absorbing/screening function without any further metabolic investment (Brenowitz and Castenholz, 1997). The role of Scy as a potential UV sunscreen has been observed in different cyanobacteria (Garcia-Pichel *et al.*, 1992; Rastogi *et al.*,2014a, 2014d). Scy display strong photoprotective function to sustain cyanobacterial life under intense UV radiation, even under the prolonged physiological inactivation (Garcia-Pichel and Castenholz, 1991; Garcia-Pichel *et al.*, 1992). Scy play an important role in photoprotection by reducing the formation ROS and thymine dimers in microalgae (Matsui *et al.*, 2012; Rastogi *et al.*, 2013, 2014d; Rastogi and Incharoensakdi, 2014a).

The antioxidant function of some carotenoids has also been observed in *Trichodesmium* sp. (Kelman *et al.*, 2009). A number of carotenoids, such as canthaxanthin, echinenone, myxoxanthophyll and zeaxanthin, have been reported in cyanobacteria, with protective function against photooxidative damage (Gotz *et al.*, 1999; Kerfeld, 2004; Latifi *et al.*, 2009; Rastogi *et al.*, 2010a). The polycationic molecules, PAs, act as free radical scavengers in cyanobacteria. The PA spermine acts as free radical scavenger, and has been shown to afford the protection of ROS-induced DNA damage (Ha *et al.*, 1998).

UV-induced cell damage was found in *Synechocystis* cells due to a decrease in spermidine content (Jantaro *et al*., 2011). Recently, exogenous spermidine was found to alleviate UV-induced growth inhibition of *Synechocystis* PCC 6803, via reduction of H_2O_2 and malonaldehyde levels (Jantaro *et al*., 2014).

11.8 Conclusion

It is now a well-established fact that certain UV-absorbing compounds are the sole chemical components that provide photoprotection against the direct effects of short-wavelength solar radiation, enabling microalgae to survive and maintain the energy dynamics of an ecosystem. Sun-screening small biomolecules produced in some microalgae play a crucial role in photoprotection in environmentally stressed conditions. Overall, high stability against different abiotic factors, ROS scavenging capacity, and increased synthesis under UV stress, directly indicate that these secondary compounds may act as potential photoprotectants under a UV-stressed environment.

Acknowledgements

Rajesh P Rastogi is highly thankful to the University Grant Commission, New Delhi, India for financial assistance in the form of Dr. DS Kothari. Ravi R Sonani gives thanks to Department of Science and Technology, New Delhi, India for INSPIRE fellowship.

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12

Plant Response: UV-B Avoidance Mechanisms

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12.1 Introduction

Photosynthetic organisms need sunlight and are, thus, inevitably exposed to UV radiation. This constitutes three categories on the basis of wavelength band ranges (namely, UV-A, 315–400 nm; UV-B, 280–315 nm; and UV-C, 100–280 nm), though only wavelengths greater than 290 nm can reach the earth's surface. The ozone layer, at an altitude of 15–35 km (stratospheric ozone), effectively absorbs the wavelength of some range of UV-B, and all UV-C rays. While most of the UV-B light is absorbed by the ozone layer, some can penetrate through it into the troposphere (Björn, 2008; Green, 1983). Ozone layer thinning has resulted in an increase of UV-B radiation on the earth's surface, which has been recognized as one of the serious global environmental problems, and surface UV-B radiation will continue to increase in the next few decades (Chen, 2009).

Terrestrial UV-B levels are influenced by solar peak angle, latitude, altitude, unevenness in cloud cover, time of the day and season of the year, shade, aerosols and surface reflectivity (McKenzie *et al.*, 2003). UV-A and UV-B represent approximately 6% and 0.15%, respectively of the energy in solar radiation at surface level (Frederick *et al.*, 1989). Solar UV radiation is of particular importance because a number of plant biomacromolecules, including DNA, RNA, lipids and proteins, absorb in this region of the UV spectrum. Furthermore, UV-B photons have the highest energy of all wavelengths in sunlight and, hence, the potential to cause cellular damage through photochemical reactions (Caldwell and Flint, 1994; Jansen *et al.*, 1998; Ballaré, 2003).

Recent reports indicate that the molecular mechanism of UV-B damage and repair has been studied in detail with genetic tools such as *Arabidopsis*, including involvement of the MAPK gene protecting against higher doses of UV-B and targets of UV-B in plants such as DNA, lipids, and protein (Nawkar *et al.*, 2013). Thus, it is essential to understand the effect and repair mechanisms of ambient UV-B, prior to the evaluation of enhanced UV-B.

UV-B Radiation: From Environmental Stressor to Regulator of Plant Growth, First Edition. Edited by Vijay Pratap Singh, Samiksha Singh, Sheo Mohan Prasad and Parul Parihar.
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Another issue that may seem more relevant to the human population is the effect of UV on plants and food crops. Greater exposure of crops to UV-B can result in decreased yield. Several efforts have been made to develop UV-tolerant cultivars of rice, as some varieties have greater tolerance towards UV radiations. With this approach, scientists can find the most tolerant and economical species to use in rice farming, thereby increasing the UV resistance of much of the earth food supply.

Krupa and Kickert (1989) found no reports of O_3/UV-B interactions in their review. They assessed potential risks on a geographical basis, using distributions of major crops, tropospheric ozone concentrations and UV-B irradiance. They suggested that relationships might involve periodic exposure to the two stresses (i.e. ozone and UV-B), so that peaks of O_3 would coincide with lower UV-B irradiance, and vice versa. Runeckles and Krupa (1994) have developed this concept further, arguing that, as tropospheric O_3 increases, UV-B irradiance at the surface can be reduced, since O_3 absorbs some UV. In theory, this effect could be important, in spite of the comparatively small contribution of tropospheric O_3 to the total stratospheric atmospheric O_3, because scattering by mist particles and water molecules in the troposphere increases the radiation path length (Bruhl and Crutzen, 1989).

In an attempt to quantify this effect, Albar (1992) compared the measured solar spectral irradiance at the ground near Nottingham, England, on two days (12 and 18 July 1990) when the tropospheric O_3 concentration was 51 and 84 nmol/mol, respectively, and stratospheric O_3 was constant. It was about 20–40% greater in the UV-B waveband on the low O_3 day than on the high O_3 day, apparently supporting the hypothesis of Runeckles and Krupa (1994). However greater increase was also found at longer wavelengths (by about 20–25%), probably a consequence of less aerosol (dust) being present on the low O_3 day. Therefore, variations in aerosol from day to day seem likely to be more important than variations in tropospheric O_3 in modulating the intensity of UV-B at the ground.

Photoreceptive cells are responsive to some of the selected solar spectrum, but they are also sensitive to some other wavelengths of solar radiation (Figure 12.1). The largest family characterized for plant photoreceptors comprises *Arabidopsis thaliana* (*Arabidopsis*), five members of phytochromes (PHY A-E) which mediate responses to red and far red light (600–750 nm). Blue light (400–500 nm) and ultraviolet-A radiation are perceived by cryptochromes (CRY1 and CRY2), phototropins (PHOT1 and PHOT2)

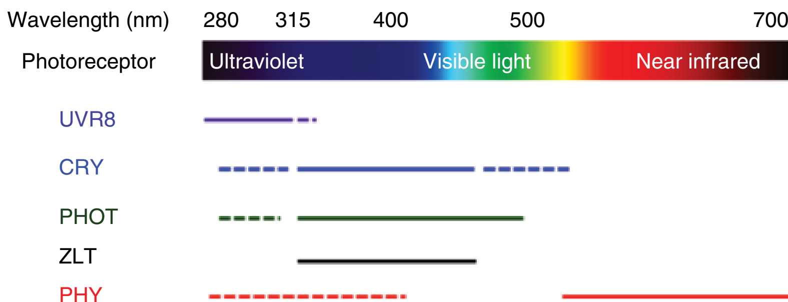

Figure 12.1 Plant photoreceptors and their absorption in the solar spectrum. Regions of maximal absorption are indicated by solid lines, while sensitivity towards other wavelengths is indicated with dashed lines (Courtesy of Morales *et al.*, 2013).

and the zeitlupe proteins (ZTLs), while UV-B radiation is sensed by UV Resistant Locus 8 (UVR8) (Figure 12.1; Heijde and Ulm, 2012).

12.2 Ultraviolet Radiation: Common Source, Classification and Factors

UV rays were discovered by JW Ritter, a German physicist, in 1801.

12.2.1 Common Sources of UVR

The natural source of UV rays is the sun, while man-made sources include UV lamps, and welding instruments are also producers of UV radiation.

- **Sunbeds**: these are designed to produce a tan by emitting UVA and some UVB. Regular use of a sunbed may contribute significantly to a person's annual UV skin exposure. The use of eye protection, such as goggles or sunglasses, is mandatory. Working staff in tanning salons also be exposed to UV-B light.
- **Medical exposure**: in some medical and therapeutic diagnostics, UV lights are used. Exposures vary considerably, according to the type of treatment.
- **Industrial/commercial exposures**: the most significant source of potential exposure is welding. The levels of UV around welding equipment are very high, and the potential for acute injury to the eye and the skin is great. Skin and eye protection is compulsory for this work. Many industrial and commercial processes involve the use of UV-producing lamps. While the probability of harmful exposure is low, because of protection provided with the lamp, unusual exposure can occur in some cases.
- **Lighting**: fluorescent lamps are common in the workplace, and are often used in the home. These lamps emit small amounts of UV, and typically contribute only a few percent to a person's yearly UV exposure. Halogen lamps, made of tungsten, are increasingly used in the home and in the workplace for a variety of lighting and display purposes. Uncovered lamps can emit UV radiations sufficient to cause acute injury at short distances. UV filters on the lamps can considerably reduce these radiations. Black lights, which emit mainly UVA, are frequently used for special effects (e.g. in discothèques), and also for the authentication of bank notes and documents. These lamps have not caused significant UV exposure to humans.

12.2.2 Classification

UV radiation falls into three types, on the basis of wavelength and intensity:

1) **UV-A (315–400 nm)** are less absorbed by the stratospheric ozone layer. The maximum part of UV-A radiation is able to reach the earth's surface, and can cause tanning, skin aging, eye damage, and immune suppression in animals; while, in plants, it can influence plant morphology, plus some specific effects (e.g. stomatal opening and induction of pigment formation).
2) **UV-B (280–315 nm)** is strongly absorbed by the ozone layer but, if it reaches the earth's surface, it can contribute to snow blindness, sunburns, immune reductions and a variety of skin problems, including skin cancer and premature aging. In plants,

it induces many morphological, physiological and molecular changes, including leaf structure alteration, antioxidative machinery and DNA damage.
3) **UV-C (100–280 nm)** is completely absorbed by the ozone layer, so that the levels of UV-C radiation reaching the earth's surface are very small. However, it is lethal in nature and can change the expression pattern of genes in animals as well as in plants. Artificial UV-C can cause severe damage to exposed tissues.

12.2.3 Environmental Factors Affecting UV Level

- **Sunlight**: diurnal variations and seasonal variations both have an impact on UV radiation levels.
- **Latitude**: at lower latitudes, exposure to UV radiation is much higher.
- **Cloud cover**: UV levels are mostly less in a cloudy sky, but sometimes they may be high, due to scattering of UV radiation through water molecules and tiny particle present in the clouds.
- **Altitude**: for every 1000 metres increase in elevation, UV levels increase by 10–12%.
- **Stratospheric ozone**: all UV-C, and 90% of the UV-B radiations, are absorbed by the ozone layer, water vapour, and carbon dioxide. UV-A radiation is less absorbed by the atmosphere. Hence, the UV radiation reaching the earth's surface is largely composed of UV-A, with a small quantity of UV-B.

12.3 UV-B and Human Health

12.3.1 Effects on the Skin

UV-B radiations are responsible for important biological effects on human beings. Most of the common symptoms are skin cancers and premature ageing of the skin (Urbach, 1997). Red spots on the skin, from minor to large burning, are the main detrimental effect in the short term, but the effects in the long term usually result in abnormalities. However, UV radiation becomes a risk for the health when human beings keep on exposing themselves for years, ignoring their type of skin. The risk caused by UV radiation goes down in proportion to the grade of natural pigmentation on human skin, being a minimum for people of black skin, and maximum in those with very white skins.

12.3.2 Effects on the Eyes

There are many reports regarding the damage that UV-B radiation can cause on eye structure. Cataracts are one of the most common effects, while increasing incidence of UV melanoma, commonly known as ocular cancers, is being attributed to increasing levels of UV.

12.4 UV-B and Plant Responses

12.4.1 Morphological Responses

12.4.1.1 Visible Symptoms

Plants under stress show unusual growth patterns and coloration, and UV-B radiation is also known for producing these symptoms. Changes in leaf colour and form are

reported in several species. Initially, bronze or brown spots appear on the leaf surface, that later result in chlorosis, necrosis, and desiccation of the leaves (Ambler *et al.*, 1975; Strid and Porra, 1992; Dai *et al.*, 1994; Visser *et al.*, 1997; Krizek *et al.*, 1993). Under high UV-B irradiation, the silicon-deficient leaves exhibit obvious brown spots and strips of UV damage symptoms (Li *et al.*, 2004). There is a decline in plant height, shoots and roots, as well as in leaf area and fresh mass of leaves. Additionally, it causes leaf curling. The content of chlorophyll varies considerably; in some plants it shows chlorotic patches within 4–5 days after exposure, and later on, these patches turn into necrotic patches and result in early senescence of leaves (Kakani *et al.*, 2003). Visual symptoms, consisting of chlorotic or necrotic patches on leaves exposed to UV-B, are not unique. Both vegetative and reproductive morphology are altered by UV-B radiation (Kakani *et al.*, 2003).

12.4.1.2 Plant Growth and Leaf Phenology

A number of studies have been done on the impact of UV-B radiation on plant growth. (Robson *et al.*, 2015). Overall, enhanced UV-B radiation reduces main stem and branch elongation growth rates, resulting in more 'squashed plants' with a shorter height. Decreased plant height is mainly due to shorter internodes, rather than to fewer nodes (Searles *et al.*, 1995; Li *et al.*, 1998; Gonzalez *et al.*, 1998b; Zhao *et al.*, 2003). Mark and Tevini (1996) speculated that the mechanism for reduced stem elongation by UV-B might be due to changes in the phyto-hormone level, especially IAA, which plays a role in stem elongation. Some studies also indicated a breakdown of IAA on exposure to UV-B (Ros and Tevini, 1995; Huang *et al.*, 1993). Gonzalez *et al.* (1998a) pointed out that the shorter internodes for UV-B treated pea plants were due to fewer cells rather than reduced cell length. Other UV-B-induced effects on stems include coiling of both attached and detached tendrils in peas, which could be used as markers for selecting UV-B tolerant genotypes (Brosché and Strid, 2000).

Similar to plant height, leaf area is also a very sensitive growth parameter that responds to elevated UV-B. Under most experimental conditions, leaf area was less due to both smaller leaves and lesser numbers (Nogués *et al.*, 1998; Zhao *et al.*, 2003), which serves as a protective mechanism (Bornman and Teramura, 1993). The reduction in leaf area is caused by a reduction in cell size and/or a change in leaf structure (Tevini *et al.*, 1983), reduction in cell numbers (Gonzalez *et al.*, 1998a) and by both cell division and cell expansion (Hofmann *et al.*, 2001). The UV-B effect on cell division was greater than on cell expansion (Nogués *et al.*, 1998; Hofmann *et al.*, 2001). In contrast, Nedunchezhian and Kulandaivelu (1997) reported that, under field conditions, slightly enhanced UV-B radiation (1.8 $kJ\,m^{-2}day^{-1}$) increased the leaf area of cow pea. Even high UV-B-treated (13.4–63.3 $k\ Jm^{-2}\ day^{-1}$) broad bean and wheat plants had higher leaf area than the control plants (Al-Oudat *et al.*, 1998). Along with reduced leaf area, heliotropism (plant having acquired adaptation to higher salt concentrations) also helps to reduce the amount of UV-B intercepted by leaves, and could be used to characterize tolerant and susceptible cultivars to UV-B (Grant, 1999).

In the case of diatoms, UV-B depressed the growth of all tested marine diatoms. However, low levels of UV-B resulted in a slight increase of biomass production (dry weight), compared with non-UV-B-treated cells (Döhler, 1984). UV-B caused alteration in the biomass translocation pattern, with more retention of biomass in belowground parts, leading to an increment in root/shoot ratio in the bean *Dolichos lablab* (Singh *et al.*, 2011). It has been noted elsewhere that UV-B is more damaging to broader-leafed

species than to thin-leaved species, predominantly because the former have a greater area for UV-B to damage (Sullivan and Teramura, 1992; Liakoura *et al.*, 1997; Nagel *et al.*, 1998; Keiller and Holmes, 2001).

12.4.1.3 Reproductive Morphology

In recent years, plant UV-B research has experienced a substantial conceptual change, from a stress-dominated view towards a more regulatory perspective. UV-B radiation affects plant sexual reproduction but, at present, general patterns about the nature of these effects and their underlying mechanisms remain elusive. Effects of UV-B radiation on pollination can be direct (due to UV-B effects on pollinators), or indirect (due to pollinators responding to UV-B-mediated changes in the plants).

In the case of annual species, a literature survey revealed that, as UV-B doses increase, there is a tendency to delay the onset of flowering and to decrease fruit and/or seed production (Llorens *et al.*, 2015). Reproductive morphology includes flower length, petal and sepal area, as well as length, inflorescence and gynoecium length and so on. Under UV-B exposure, all the reproductive parts become reduced in shape and size (Kakani *et al.*, 2003).

Flower morphology, pollen production, pollen germination, pollen tube lengths, and pollen morphology are all negatively affected by UV-B treatments, alone or in combination with other abiotic stresses such as temperature, CO_2, and so on, in soybean (Koti *et al.*, 2005). In entomophilous alpine plants, pollen grains normally protected by flower structures (such as bracts or petals) become more sensitive to UV-B radiation once removed from such structures, compared with pollen grains originating in UV-B-exposed anthers (Zhang *et al.*, 2014). UV-B can also affect flower size and number, and this can have important implications for pollination because, in general, pollinators prefer large flowers and/or a high density of flowers (Conner and Rush, 1996; Strauss and Whittall, 2006; Essenberg, 2012; Barbir *et al.*, 2014).

Therefore, it can be concluded that most floral parts are effectively UV-B protected, in accordance with the idea that UV-B stress is rare (Hideg *et al.*, 2013). However, pollen is an exception to this, since many studies show that it is sensitive to UV-B radiation.

12.4.1.4 UV-B-induced photomorphogenesis

At higher altitudes, or geographical latitudes where the UV levels are comparatively more, plants or crops in these areas have more tolerance to UV radiation, compared with plants growing in plains (Jordan, 1996). Such differential UV-B tolerance has been shown between different *Arabidopsis* ecotypes (Torabinejad and Caldwell, 2000). The perception of UV-B radiation has either been related to the action of phytochromes/cryptochromes, as they absorb UV-B to some extent, or attributed to aromatic amino acids, DNA and phospholipids (Beggs *et al.*, 1986). The most frequently reported UV-B-induced morphological changes are a decrease in leaf area and/or an increase in leaf thickness (Jansen 2002; Furness *et al.* 2005; Hectors *et al.* 2007; Yang *et al.* 2008; Wargent *et al.* 2009; Klem *et al.* 2012; Robson and Aphalo, 2012).

12.4.2 Leaf Ultrastructure and Anatomy

Since the adaxial surfaces of leaves receive higher levels of UV-B radiation than the abaxial surfaces, most of the ultrastructural studies have focused on cells of the adaxial side (Kokilavani *et al.* 2013; Kokilavani and Rajendiran, 2015). Detailed ultrastructure

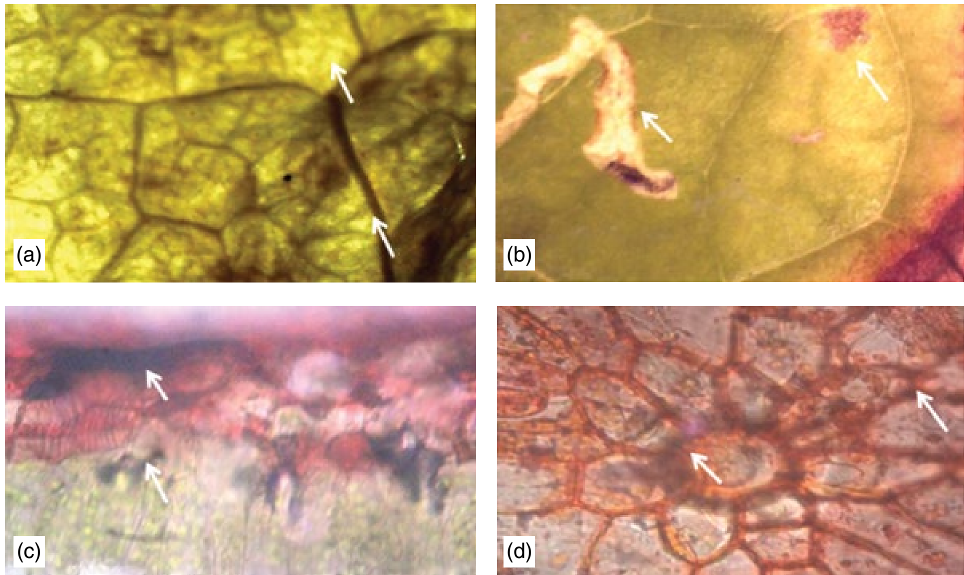

Figure 12.2 Epidermal and anatomical characteristics of first fully expanded leaves of *Vigna unguiculata* (L.). (**A**) shiny adaxial surface under UV-B; (**B**) UV-B adaxial – brittle and dead; (**C**) UV-B adaxial – multiseriate epidermis; (**D**) UV-B adaxial – broken trichome.

observations have showed that UV-B exposure caused some changes in guard cells, epidermal cells and palisade cells. In guard cells, a deviation from the control cells was observed in plastids, which contained more thylakoid membranes and smaller starch granules in potato (Santos *et al*., 2004). PAR + UVB caused changes in the ultrastructure of leaves of *Oryza sativa*, mesophyll cells, which included increased thickness of the cell wall and plastoglobuli, reduced intracellular spaces, changes in the cell contour, and destruction of chloroplast and mitochondria internal organization (Almeida *et al*., 2012). A change in leaf ultrastructure due to enhanced UV-B modifies the light attenuation by the leaf, which, in turn affects photosynthesis.

Of the incident solar UV-B radiation, leaves reflect 3–6% (Gao *et al*., 1996; Yang *et al*., 1995) to 10–40% from *Pubescentor glaucous* surfaces (Robberecht and Caldwell, 1980), and leaf epidermis transmits anywhere between <0.1 and 5% of the incident UV-B radiation (Robberecht and Caldwell, 1980; Yang *et al*., 1995). UV-B exposure induces various types of malformations in the leaf architecture, and causes injuries to epidermis and cuticle present on the adaxial surface (Figures 12.2 and 12.3; Kokilavani and Rajendiran, 2015). Leaf thickness is also influenced by UV-B exposure. Santos *et al*. (2004) showed that leaf thickness is increased significantly by exposure to UV-B radiation (Figure 12.4). However, the stomatal density is significantly reduced in elevated UV-B (Morsky *et al*., 2013; Figure 12.3).

12.4.3 Crop Yield

The final yield or biomass is influenced by various parameters that are affected by UV-B radiation, including morphological change in floral or reproductive organs, decrease in chlorophyll concentration, photosynthesis, leaf area, and fruit retention (Kakani

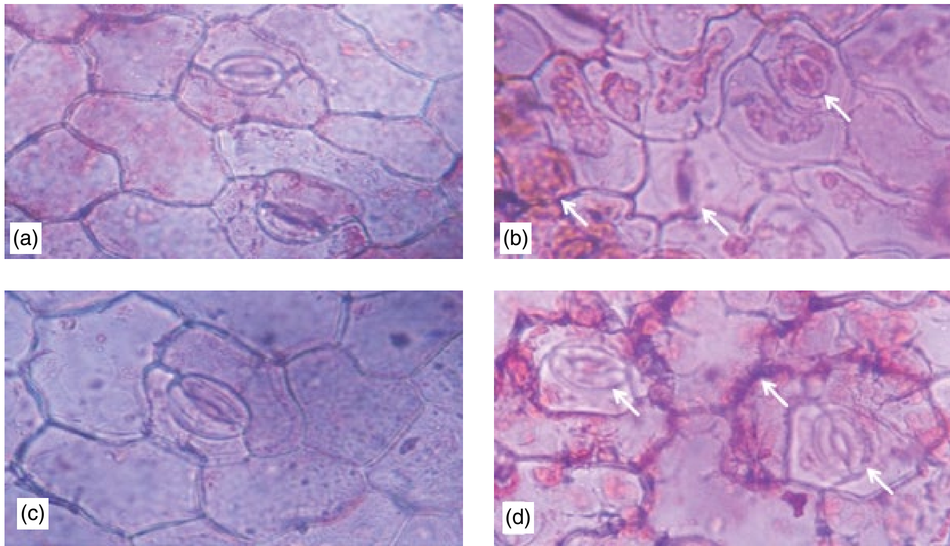

Figure 12.3 Epidermal and anatomical characteristics of first fully expanded leaves of *Vigna unguiculata* (L.). (**A**) control adaxial – normal stomata; (**B**) UV-B adaxial – abnormal stomata; (**C**) control abaxial – normal stomata; (**D**) UV-B abaxial – abnormal stomata.

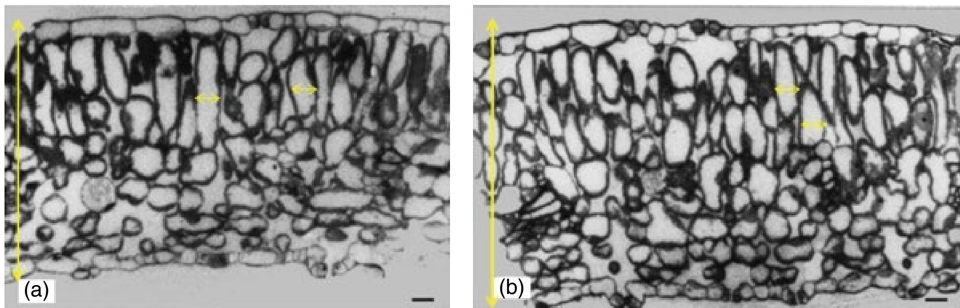

Figure 12.4 Transverse section of potato leaves. In comparison with control (**A**), UV-B exposed leaf (**B**) appeared thicker, but the gross anatomy was maintained (scale bar for **A** and **B**: 1 µm). (Courtsey of Santos *et al*., 2004).

et al., 2003; Yao *et al*., 2007; Wolf *et al*., 2010; Klem *et al*., 2012). Teramura and Sullivan (1991) reported that approximately two-thirds of some 300 species and cultivars tested appeared to be susceptible to damage from increased UV-B radiation. Cotton flowers exposed to UV-B treatments were smaller, due to reduced petal and bract size, and reduced anther number (Kakani *et al*., 2003).

In vitro experiments showed that pollen germination was also inhibited by exposure to enhanced UV-B (Chang and Campbell, 1976; Caldwell, 1979; Flint and Caldwell, 1984). A significant correlation ($R^2 = 0.7$) was found between leaf area and final biomass, as UV-B induced changes in leaf ultrastructure, pigments and canopy photosynthesis. No significant difference in flower yield was observed in UV-B-exposed plants during different stages, in comparison with control plants, showing that, in *Chrysanthemum*,

elevated UV-B did not decrease flower yield (Yao *et al.*, 2015). Some studies have shown that female flowers are more tolerant to UV-B, compared with males, as decreased leaf thickness and biomass are recorded in male flowers (Tendry *et al.*, 2015).

12.4.4 Photosynthesis

12.4.4.1 Pigments

The contents of chlorophyll *a*, *b*, and total chlorophyll are decreased compared with control values, and with increasing UV radiation levels (Salama *et al.*, 2011). Elevated UV-B transiently increases the amount of cell wall-bound UV-B-absorbing pigments in *Eriophorum russeolum* leaves during the first year of exposure (Morsky *et al.*, 2013). Ultrastructural damage to chloroplasts and changes in photosynthetic pigments result in reduction of photosynthesis (Sullivan and Rozema, 1999). Pigment concentration decreases significantly in *Ulva* sp, depending upon the exposure time to UV (A and B) (Eswaran *et al.*, 2001).

As a consequence of decrease in pigment concentration due to ultrastructural damage to photosynthetic machinery, reduced photosynthetic rate has been observed (Sullivan and Rozema, 1999). Algae exposed to UV-B stress showed a marked decrease in the pigment content (chlorophyll *a*, chlorophyll *c*, + *c2* and carotenoids) (Döhler, 1984). Total chlorophyll and carotenoid contents were significantly affected due to UV-B exposure, and it was dependent on the age of the plant, the treatment dose and the duration and their interaction. Total chlorophyll and chlorophyll *a*/*b* ratio decreased significantly in UV-B treated plants (Salama *et al.*, 2011; Singh *et al.*, 2015). Strid and Porra (1992) suggested that decline in chlorophyll level might be due to inhibition of the cab gene, which codes for chlorophyll protein.

Supplemental UV-B radiation influenced shoot tissue carotenoid concentrations in some, but not all, of the bunching onions. Xanthophyll carotenoid pigments lutein and β-carotene and chlorophylls *a* and *b* in shoot tissues differed between UV-B radiation treatments and among cultigens (Abney *et al.*, 2013). Cultigen 'Pesoenyj' responded to supplemental UV-B radiation with increases in the ratio of zeaxanthin + antheraxanthin to zeaxanthin + antheraxanthin + violaxanthin, which may indicate a flux in the xanthophylls carotenoids towards deepoxydation, commonly found under high irradiance stress. Increase in carotenoid concentrations would be expected to increase crop nutritional values (Abney *et al.*, 2013).

12.4.4.2 Photosynthetic Machinery

Solar radiation is used by the pigments presents in the photosystems for the excitation of electrons. Photosystems constitute three types of pigments associated with the proteins in core complexes. Carotenoids protect chlorophyll from light saturation during the process of photosynthesis. UV-B damages these systems and, ultimately, leads to reduced photosynthesis. The photosynthetic machinery of bean plants was found to be the potential target of UV-B, as photosynthetic rate is decreased by 88.6% at 30 days after germination (Singh *et al*, 2011). It is known from various reports that PSII is more vulnerable to UV-B effect (Briggs and Christie, 2002; White *et al.*, 2002; Sicora *et al.*, 2006; Kataria *et al.*, 2014). Within PSII, major components such as the D1/D2 of the PSII RC (reaction centre) and the OEC (oxygen evolving complex), as well as the LHC (light harvesting complex), are highly affected.

Allen *et al.* (1998) summarized the mechanisms of UV-B-induced inhibition of photosynthetic capability, and concluded that ambient UV radiation is not a direct threat to photosynthetic productivity in crops and natural vegetation. However, a number of studies have demonstrated that, in the photophosphorylation processes, photosystem II (PSII) is the most sensitive component of the thylakoid membrane of the photosynthetic apparatus on exposure to UV-B radiation (Brandle *et al.*, 1977; Noorudeen and Kulandaivelu, 1982; Renger *et al.*, 1989; Kulandaivelu *et al.*, 1991; Melis *et al.*, 1992; Chaturvedi *et al.*, 1998; Correia *et al.*, 1999; Bolink *et al.*, 2001; Savitch *et al.*, 2001). Several other investigations (Ziska and Teramura, 1992; Middleton and Teramura, 1993; Allen *et al.*, 1997) suggest that UV-B inhibition of PSII photochemistry is not a ubiquitous primary limitation to photosynthesis.

In the Calvin cycle during CO_2 fixation, enhanced UV-B radiation caused reductions in both Rubisco activity and content in many field crops, including soybean (Vu *et al.*, 1982, 1984), pea (Strid *et al.*, 1990; Mackerness *et al.*, 1997), cowpea (Nedunchezhian and Kulandaivelu, 1991; Kulandaivelu and Nedunchezhian, 1993), rice (He *et al.*, 1993, 1994; Huang *et al.*, 1993), corn (Correia *et al.*, 1999) and rapeseed (Savitch *et al.*, 2001). In addition, RuBP regeneration (Allen *et al.*, 1997; Savitch *et al.*, 2001) and the amount of sedoheptulose 1, 7-bisphosphatase were also decreased by UV-B radiation (Allen *et al.*, 1998).

Caldwell *et al.* (1994) pointed out that UV-A appeared to be particularly effective in mitigating UV-B damage when PAR was low. Savitch *et al.* (2001) investigated the effect of UV-B, with or without UV-A radiation, on the mechanisms of UV-B-reduced photosynthesis of *Brassica napus*, using 200 $\mu mol\ m^{-2}\ s^{-1}$ PAR, and suggested that the decrease in the CO_2 assimilation capacity for PAR + UV-B treated plants was not associated with limitation at the level of PSII electron transport but, rather, with a decreased capacity for sucrose biosynthesis, limited triose-P utilization, and a decreased capacity for RuBP regeneration. In contrast, decreased CO_2 assimilation capacity for PAR + UV-A + UV-B treated plants was associated with an inhibition of PSII photochemistry and a decreased supply of ATP. Therefore, UV-A radiation appeared to induce feedback-limited photosynthesis, and did not enhance resistance of the crop to UV-B radiation (Savitch *et al.*, 2001).

12.4.5 Biochemical Responses

12.4.5.1 ROS Production in Plants

Elevated UV-B radiation exposure during vigorous growth, bud and flower stages significantly increased H_2O_2 concentration in flowers over the control, and the H_2O_2 concentration induced by elevated UV-B radiation applied during the flower stage was the most. The rate of $O_2^{\bullet-}$ production and MDA concentration in flowers were not significantly affected by elevated UV B radiation applied during different growth stages. Leaf MDA contents were decreased significantly by all doses of UV irradiation both in *Portulaca grandiflora* and *P. oleracea* genotypes (Peykarestan *et al.*, 2012). Elevated UV-B radiation applied during vigorous growth, bud and flower stages significantly increased PAL (phenylalanine ammonia lyase) activity in flowers over the control, but there was no obvious difference in PAL activity during the three growth stages in *Pistacia vera* L. (Nadernejad *et al.*, 2012).

12.4.5.2 Free Radical Scavenging Mechanism

UV-B irradiation causes generation of ROS in plant cells. At modest concentrations, ROS are known to play an important role as signalling molecules in plant cells. However, an excess of ROS damages cell components, resulting in premature senescence and/or apoptosis. UV-B-induced ROS production alters the pattern of gene expression, such as anti-oxidative enzymes (Egert and Tevini, 2003) and pathogenesis-related gene PR-1 (Green and Fluhr, 1995).

Superoxide dismutase, peroxidase and catalase are key enzymes of the antioxidant defence system. Superoxide dismutase accelerates the conversion of superoxide to hydrogen peroxide, while catalase and peroxidase catalyze H_2O_2 breakdown. In addition, UV-B also promotes the biosynthesis of natural sunscreens, such as flavonoids or anthocyanins (Landry *et al.*, 1995; Reddy *et al.*, 1994; Schenke *et al.*, 2011). When plant cells are exposed to UV-B, the activity of NADPH oxidase, SOD (superoxide dismutase) and peroxidase are enhanced, while catalase activity is decreased in *Arabidopsis thaliana* (Rao *et al.*, 1996).

Multiple sources of UV-B-induced ROS have been proposed, such as peroxidase, and several other unknown factors produce ROS (Mackerness *et al.*, 2001; Egert and Tevini, 2003; Yannarelli *et al.*, 2006). The thylakoid membrane was proposed as a source of free radicals (mainly hydroxyl radical) generated via the cleavage of hydrogen peroxide (H_2O_2) (Hideg and Vass, 1996). According to Kovacs *et al.* (2002), UV-B irradiation elicits multilevel stress. It has been found that, besides causing immediate free radical production in UV-B-irradiated thylakoid membranes, it also initiates radical-yielding reactions detectable in leaves even minutes after the cessation of the irradiation (Hideg and Vass, 1996).

12.4.6 Molecular Responses

12.4.6.1 UV-B and Genes

Plants are inevitably exposed to UV-B, which penetrate and damage the genome. UV photons induces oxidative damage (pyrimidine hydrates), crosslinks (both DNA-protein and DNA-DNA) and generation of photoproducts of DNA that are responsible for retarding the growth and development of plants (Tuteja *et al.*, 2001, 2009; Stapleton, 1992; Britt, 1999). UV-B radiation damages nuclear, chloroplast and mitochondrial DNA by inducing various DNA lesions. The primary UV-induced DNA lesions include cyclobutane pyrimidine dimers (CPDs), while secondary lesions include 6-4 pyrimidine-pyrimidone photoproducts (6-4 PP). CPDs are adjacent pyrimidines covalently linked between C-5 and C-6 carbon atoms, and 6-4 PPs are formed by covalent linkage between the C-4 position of a pyrimidine to the C-6 position of an adjacent pyrimidine. CPDs accounts for approximately 75% of UV-B-mediated total DNA damage.

Minor DNA damage includes oxidized or hydrated bases, single-strand breaks, and so on (Ballaré *et al.*, 2001; Takahashi *et al.*, 2011). The CPDs are probably the most cytotoxic lesions but the 6-4 PPs may have more serious, potentially lethal and mutagenic effects. The structural distortions within DNA due to CPDs is the slight bending on the DNA helix, but 6-4PPs can produce much more bending, and also unwinding of the DNA (Tuteja *et al.*, 2009). They can also impede transcriptional processes, resulting in error-prone replication. Indirect generation of ROS in nucleus due to UV-B can

accrue base and nucleotide modifications, especially in high guanosine content sequences and strand breaks (Tuteja *et al.*, 2001).

12.4.6.1.1 Genes Damaged by UV Radiation

The regulation of gene expression is one of the earliest responses observed in plants exposed to UV-B (Hofmann *et al.*, 2007; Jenkins, 2009). UV-B-induced changes in gene expression, as determined indoors, have often been obtained using irradiation protocols where plants do not receive any UV-B before the actual UV-B treatment. These irradiation conditions are very different from those present in the natural environment, where plants are constantly exposed to UV-B and, therefore, become acclimated (Casati *et al.*, 2011). There is evidence that most UV-B regulated genes (*CHS*, *TT7*, *ATR4*, *F7A740* (At5g01520), *LOX*, *AOC3*, *AIF1*, *PMI2*, *SIGE*, *STO*) are transiently expressed (Brosché *et al.*, 2002; Ulm *et al.*, 2004; Kilian *et al.*, 2007; Favory *et al.*, 2009; Morales, 2014) and that, after UV-B acclimation, fewer genes are expressed to maintain this state (Hectors *et al.*, 2007; Jenkins, 2009). To test the possible roles of plant photoreceptors on the regulation of genes induced by solar UV, the expression of genes upregulated by solar UV-B plus UV-A in wild-type (Ler) after 12 hours outdoors was compared with available transcriptome data from experiments with photoreceptor mutants in *Arabidopsis* (Figure 12.5). A list of genes and their respective treatments is shown in Table 12.1.

The photosynthesis-associated genes are downregulated under UV-B exposure, negatively affecting photosynthesis. The genes for Rubisco synthesis (rbcL, rbcS), the D1 protein (psbA) of photosystem II, and the chlorophyll *a*/*b* binding protein (Lhcb) downregulate upon exposure to UV-B in pea and wheat (Jordan, 1996; Mackerness *et al.*, 1997). Casati and Walbot (2004) reported that transcripts encoding proteins related to photosynthesis and CO_2 fixation (Transketolase gene TKT), such as Rubisco, and proteins of both photosystems I and II, were downregulated by UV-B radiation in maize (*Zea mays*). The expression of transport and transcriptional regulation genes, phosphate transporter (AtPT2) and myb family transporter protein (MYB34), respectively was downregulated in *Arabidopsis* in response to UV-B (Ulm *et al.*, 2004).

Expression of genes associated with the Calvin-Benson cycle, photosynthesis, photorespiration, cell wall synthesis, lipid metabolism and starch synthesis were significantly reduced by UV-B irradiation in *Arabidopsis thaliana* (Kusano *et al.*, 2011). There is evidence that most UV-B regulated genes are transiently expressed (Brosché *et al.*, 2002; Ulm *et al.*, 2004; Kilian *et al.*, 2007; Favory *et al.*, 2009) and that, after UV-B acclimation, fewer genes are expressed to maintain this state (Hectors *et al.*, 2007; Jenkins, 2009).

12.4.6.1.2 DNA Damage

UV-B radiation can penetrate and damage the plant genome by inducing oxidative damage (pyrimidine hydrates) and crosslinks (both DNA-protein and DNA-DNA), which are responsible for retarding the growth and development of plants (Tuteja *et al.*, 2001, 2009; Stapleton, 1992; Britt, 1999). UV-B radiation damages nuclear, chloroplast and mitochondrial DNA by inducing various DNA lesions. These include the generation of cyclobutane pyrimidine dimers (CPDs) (as the primary UV-B-induced DNA lesions accounting approximately 75% of UV-B-mediated total DNA damage) and other photoproducts, pyrimidine (6-4) pyrimidone dimers as the major lesions, while minor damages include oxidized or hydrated bases, single-strand breaks, and others (Ballaré *et al.*, 2001; Takahashi *et al.*, 2011). For instance, cyclobutane pyrimidine dimers

Table 12.1 List of treatment and genes affected by UV radiation (Courtesy of Morales *et. al.*, 2014).

S. No.	Type of treatments and genes
1	*G. cichoracearum* (18 hours) (col-0)
2	*G. cichoracearum* (36 hours) (col-0)
3	*G. cichoracearum* (96 hours) (col-0)
4	IAA (Col)
5	IAA (hy5)
6	IAA (hy5hyh)
7	IAA (hyh)
8	high light (Col-0)
9	high light (cry1)
10	high light(hy5)
11	blue light (Col-0)
12	red light (1 hour) (pif1pif3pif4pif5)
13	red light (45 hours) (pif1pif3pif4pif5)
14	red light (1 hour) (Col-0)
15	red light (45 hours) (Col-0)
16	shift UV > 345 nm to UV > 305 nm (Col-0) 1 hour
17	shift UV > 345 nm to UV > 305 nm (Cop 1–4) 1 hour
18	shift UV > 345 nm to UV > 305 nm (uvr 8–6) 1 hour
19	shift UV > 345 nm to UV > 305 nm (Col-0) 6 hours
20	shift UV > 345 nm to UV > 305 nm (Cop 1–4) 6 hours
21	shift UV > 345 nm to UV > 305 nm (uvr 8–6) 6 hours
22	UV > 305 nm (Col-0)
23	UV > 305 nm (cop 1–4)
24	UV > 305 nm (uvr 8–6)
25	UV 312 nm (Col-0) 24 hours
26	UV 312 nm (sng 1-1) 24 hours
27	UV 312 nm (tt4) 24 hours
28	White + far-red (Col-0) 1 hour
29	White + far-red (pifq) 1 hour
30	White + far-red (Col-0) 3 hours
31	White + far-red (pifq) 3 hours
32	White + far-red (Col-0) 24 hours
33	White + far-red (pifq) 24 hours
34	UV 305 nm (cop 1-4vs Col-0)
35	UV 327 nm (cop 1-4vs Col-0)

(*Continued*)

Table 12.1 (Continued)

S. No.	Type of treatments and genes
36	UV 345 nm (cop 1-4vs Col-0)
37	UV 305 nm (cop 1-4vs Col-0)
38	UV 345 nm (cop 1-4 vs Col-0) 1 hour
39	UV 345 nm (cop 1-4 vs Col-0) 6 hours
40	high light (cry1 vs Col-0)
41	IAA exp (hy5 vs Col-0)
42	IAA hy5 vs IAA Col-0
43	strat (pifqvs Col-0)
44	dark (pifqvs Col-0)
45	red light (pifqvs Col-0)
46	UV > 345 nm (uvr 8-6 vs Col-0) 1 hour
47	UV > 305 nm (uvr 8-6 vs Col-0) 6 hours
48	UV > 305 nm (uvr 8-6 vs Col-0) 1 hour
49	UV > 345 nm (uvr 8-6 vs Col-0) 6 hours
50	WL (sng 1-1 vs Col-0) 24 hours
51	UV 312 nm (sng 1-1 vs Col-0) 24 hours
52	WL (tt4 vs Col-0) 24 hours
53	UV 312 nm (tt4 vs Col-0) 24 hours
54	Hight light (hy5 vs Col-0)
55	UV 305 nm (hy5-1 vs Ler-0)
56	UV 327 nm (hy5-1 vs Ler-0)
57	IAA exp (hy5hyh vshyh)
58	IAA hy5hyh vshyh

immediately formed after DNA molecules absorb UV-B energy lead to mutation or cell death, unless they are correctly repaired by certain enzymes, such as photolyases (Garinis *et al.*, 2005; Figure 12.6).

12.4.6.2 UV and Proteins

Leaf protein contents in *Portulaca oleracea* genotype are slightly decreased after different levels of UV irradiation of seeds, compared with non-irradiated control (Peykarestan *et al.*, 2012). Trentin *et al.* (2015) reported the significance of a gamma-glutamyl cycle in plants under UV-B, through a *ggt1* mutant in *Arabidopsis thaliana*. The GGT1 gene encodes for gamma-glutamyl transferase enzyme of the cycle bound to the cell wall in extracellular glutathione degradation and recovery, and may be implicated in redox sensing and balance under UV-B irradiation. Compound polyamine synthesis through SAMS, detoxification of methylglyoxal and accumulation of glutathione through glyoxalase I provides UV-B avoidance to plants.

Figure 12.5 Heat map comparing the expression of solar UV-induced genes in wild-type Ler exposed for 12 hours to solar UV, with microarray data of photoreceptor mutants available in the *Genevestigator* database (Hruz *et al*., 2008). The gene expression responses are calculated as log2 ratios between the signal intensities from treated genotypes vs. controls. Red and green colours are used to indicate upregulation and downregulation of genes, respectively.

12.4.6.2.1 Amino acids

Among the 20 naturally occurring amino acids, only tryptophan and tyrosine, with maximal absorption wavelengths of 280 nm and 275 nm, respectively, are potentially capable of perceiving UV-B. The three amino acid residues whose side chains absorb in the UV range are the aromatic residues tryptophan (Trp), tyrosine (Tyr) and phenylalanine (Phe). Several reviews have been published on the photochemistry and photophysics of Trp (Bent and Hayon, 1975a; Creed, 1984b), Tyr (Bent and Hayon, 1975b; Creed, 1984a), Phe (Bent and Hayon, 1975c), and cystine (name given to each bridged cysteine in a disulphide bridge) (Creed, 1984a). Excitation to higher energy states is followed by relaxation to

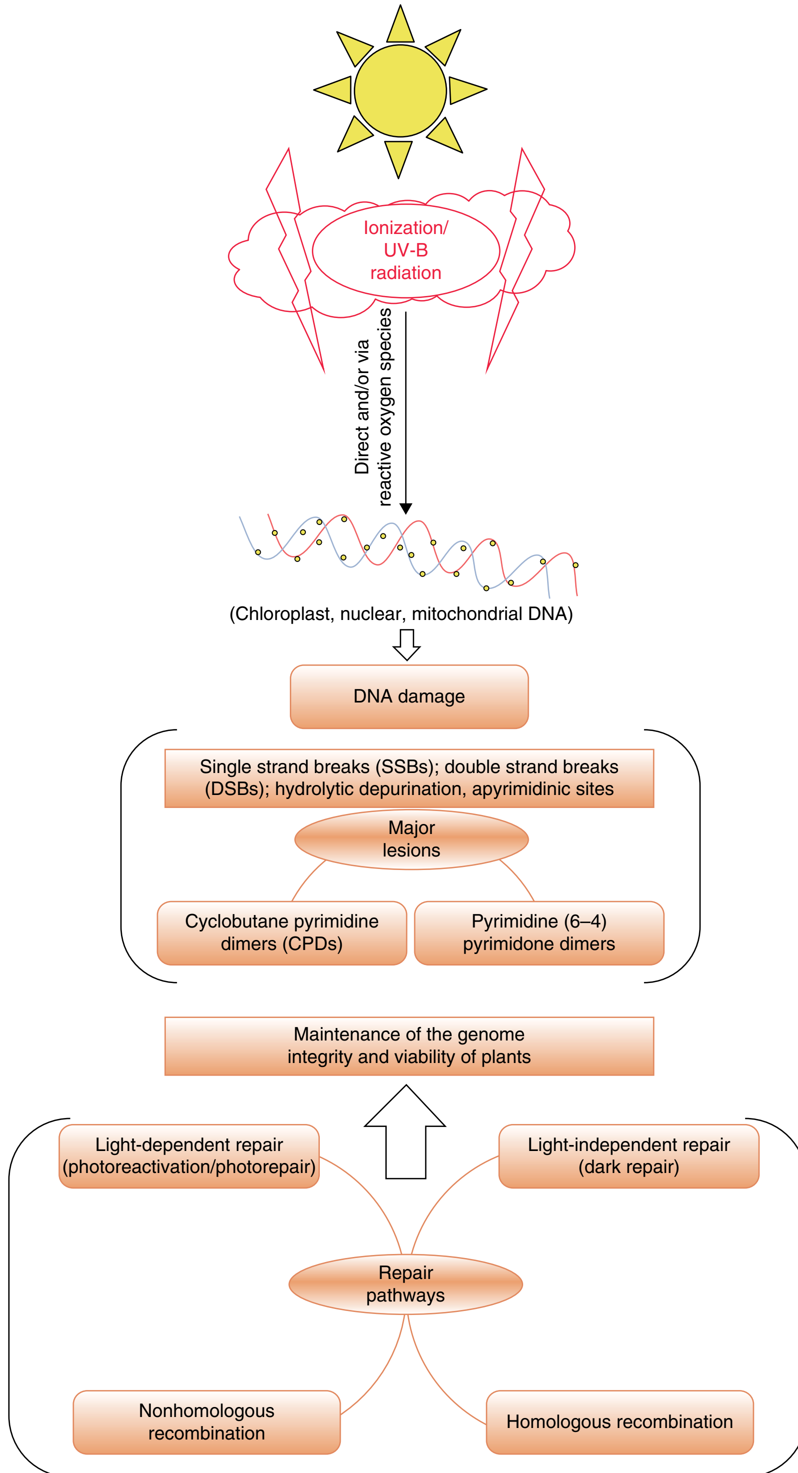

Figure 12.6 Mechanism of DNA damage and repair (Courtesy of Gill *et al*., 2015).

ground-state (e.g. fluorescence, phosphorescence), or to excited-state photochemical or photophysical processes, such as photoionization (Creed, 1984b). Lipids and proteins are also damaged by UV-B irradiation directly (Kramer *et al.*, 1991; Caldwell, 1993).

Tryptophan Flash photolysis studies have revealed two non-radiative relaxation channels from the singlet excited state of tryptophan (Bent and Hayon, 1975a):

Electron ejection to the solvent, yielding solvated electrons, $e^{-}aq$, which have a broad absorption peak centred at ≈ 720 nm, and the tryptophan radical cation $Trp^{\bullet+}$, which has its maximum absorption at ≈ 560 nm. $Trp^{\bullet+}$ deprotonates rapidly, yielding the neutral radical $Trp^{\bullet}$, which has its maximum absorption at ≈ 510 nm.

$$Trp + h\nu \rightarrow Trp^{\bullet+} + e^{-}_{aq} \quad (1)$$

$$Trp^{\bullet+} \rightarrow Trp^{\bullet} + H^{+} \quad (2)$$

4) Intersystem crossing, yielding the triplet-state ^3Trp, which has its maximum absorption at ≈ 450 nm. The triplet state tryptophan can transfer an electron to a nearby disulphide bridge to give $Trp^{\bullet+}$ and the disulphide bridge electron adduct RSSR•−, where the latter has its maximum absorption at ≈ 420 nm (Bent and Hayon, 1975a).

$$^1Trp + h\nu \rightarrow Trp^{*} \quad (3)$$

$$^1Trp^{*3} \rightarrow Trp \quad (4)$$

$$^3Trp + RSSR^{\bullet+} \rightarrow Trp + RSSR^{\bullet-} \quad (5)$$

Tyrosine Another aromatic residue with non-negligible absorption in the near-UV region is tyrosine (Tyr-OH). At neutral pH, tyrosine has absorption maxima at 220 nm ($\epsilon \approx 9000\,M^{-1}cm^{-1}$) and 275 nm ($\epsilon \approx 1400\,M^{-1}cm^{-1}$) (Creed, 1984a). At alkaline pH, the OH group of the tyrosine side chain deprotonates. The resulting tyrosinate ($Tyr\text{-}O^{\bullet-}$) has a slightly red-shifted absorption compared with tyrosine, with maxima at 240 nm ($\epsilon \approx 11000\,M^{-1}cm^{-1}$) and 290 nm ($\epsilon \approx 2300\,M^{-1}cm^{-1}$) (Creed, 1984a). Photoexcited tyrosine can fluoresce, decay non-radiatively, or undergo intersystem crossing to the triplet state, from which most of the photochemistry proceeds.

Alternatively, at neutral pH, tyrosine can be photoionized through a biphotonic process that involves absorption of a second photon from the triplet state. This results in a solvated electron (e^{-}_{aq}) and a radical cation ($Tyr\text{-}OH^{\bullet+}$) that will rapidly deprotonate to create the neutral radical ($Tyr\text{-}OH^{\bullet}$). Photoionization of tyrosinate at high pH is monophotonic, and results in a neutral radical ($Tyr\text{-}O^{\bullet}$) and a solvated electron (e^{-}_{aq}).

$$^3Tyr - OH + h\nu \rightarrow Tyr - OH + e^{-}_{aq} \quad (6)$$

$$Tyr - OH^{\bullet+} \rightarrow Tyr - OH^{\bullet} + H^{+} \quad (7)$$

$$Tyr - O^{\bullet-} + h\nu \rightarrow Tyr - O^{\bullet-} + e^{-}_{aq} \quad (8)$$

The triplet state tyrosine is rapidly quenched by molecular oxygen or by nearby residues like tryptophan or disulphide bridges (Bent and Hayon, 1975b):

$$^{3}\mathrm{Tyr}-\mathrm{OH}+\mathrm{RSSR}\rightarrow \mathrm{Tyr}-\mathrm{O}^{\bullet}+\mathrm{H}^{+}+\mathrm{RSSR}^{\bullet -} \quad (9)$$

12.5 UV-B Avoidance and Defence Mechanism

Avoidance means bypassing any aspect related to a given condition. Plants use certain signalling mechanisms to enhance avoidance at morphological, physiological, biochemical and molecular levels. They show adaptation to environmental stresses, sometimes referred to as 'plant memory'. There is growing evidence that plants memorize exposure to biotic or abiotic stresses through epigenetic mechanisms at the cellular level (Xing *et al.*, 2014).

UV-B radiation is a key environmental signal that is specifically perceived by plants to promote UV acclimation and survival in sunlight (Heijde and Ulm, 2012). Plants are able to sense UV-B through the UV-B photoreceptor UVR8. UV-B photon absorption by a UVR8 homodimer leads to UVR8 monomerization, and interaction with the downstream signalling factor COP1 (Ulm and Jenkins, 2015). High UV-B levels will trigger signalling responses that contribute to acclimation and plant survival (Hideg *et al.*, 2013). Scattering and reflection of UV-B radiation is achieved by epidermal and cuticular structures, and other leaf optical properties such as waxy layer, leaf hairs and leaf bladders.

UV-B radiation is absorbed by pigments (flavonoids, anthocyanins), particularly in the epidermal cells, photoreactivation enzymes (photolyases). Monomerization of dimers formed by the DNA-absorption of UV-B photons (photo repair) is a rapid process, but it needs sufficient PAR. Excision repair of DNA damage caused by UV-B radiation is a slow process, and also occurs in the dark. Free radicals formed by absorption of UV-B photons are scavenged by superoxide dismutase (SOD) and catalase. Flavonoids are also involved in neutralizing radicals. Polyamines may ameliorate UV-B damage to membranes.

A relationship between acclimation and UV response of plants can be evaluated, as acclimation to UV-B involves a combination of protective, as well as repair, measures. These include the accumulation of UV-B-absorbing 'sunscreen' metabolites in the vacuoles of epidermal cells, increased levels of antioxidants, protection of the photosynthetic apparatus, and increased levels of DNA repair enzymes.

12.5.1 Avoidance at Morphological Level

Leaf morphological and anatomical changes, such as increased epicuticular wax content, increase in cuticle thickness, wider epidermis and palisade layers, are some of the modifications plants employ to avoid UV-B exposure. Some of the avoidance mechanisms are as follows.

12.5.1.1 Epicuticular Waxes

Plants, in general, possess a suite of mechanisms that act either to prevent absorption of damaging and excess radiation or to mitigate against the damage that such radiation can

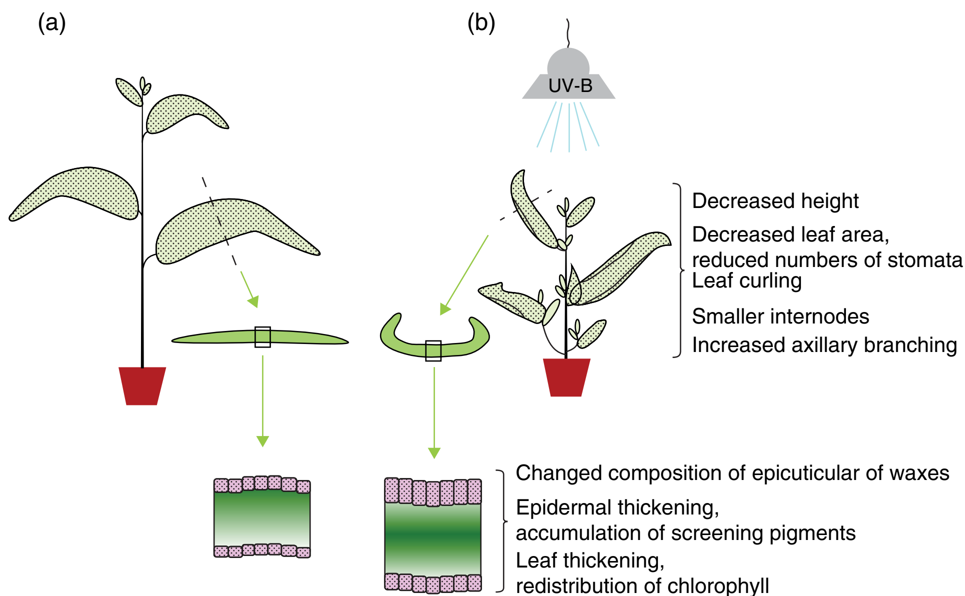

Figure 12.7 Diagram showing UV-B-induced changes in leaf and plant morphology. Part (a) is the control; part (b) is a plant exposed to supplementary UV-B (from Jansen *et al.*, 1998).

cause once it is absorbed. An epicuticular wax layer is an important leaf surface character that responds to environmental stresses (Bondada *et al.*, 1996; Rao and Reddy, 1980; Baker, 1982) and acts as an interface between environment and leaf internal structures, providing the first line of defence. Increased wax provides protection by reflecting 10–30% of the incident UV-B radiation in many plants (Caldwell *et al.*, 1983; Holmes, 1997). Enhanced UV-B radiation not only alters the quantity, but also the chemical composition of leaf surface wax (Tevini and Steinmuller, 1987; Barnes *et al.*, 1996) that modifies leaf reflectance of UV-B. In cotton, exposure to UV-B resulted in 200% increase of epicuticular wax content (Kakani *et al.*, 2003; Figure 12.7)

12.5.2 Avoidance at Biochemical Level

12.5.2.1 Possible Role of Pectin Endocytosis in UV-B Avoidance

Polygalacturonic acid and galacturonic acid are reported to generate superoxide upon UV-B irradiation in the presence of hydrogen peroxide. The superoxide anion radical was observed *in vitro* by using the isolated cell wall from *Pisum sativum* leaves with the electron paramagnetic resonance method (Pristov *et al.*, 2013). In root cells, pectin is a major component of the polygalacturonic acid cell wall. Pectin, crosslinked with boron and calcium, has been shown to be internalized and transported by endocytosis (Baluška *et al.*, 2002). For root tropisms, endocytic vesicle recycling is a critical process, and is especially active in the root apex transition zone, which is located in between the apical meristem and basal elongation zone (Baluška *et al.*, 2010; Baluška and Mancuso, 2013).

During trophic movements, the rate of endocytic recycling is increased in order to relocalize many proteins, allowing transport of critical biomolecules such as auxin. As Figure 12.8 shows, internalized endosomal cell wall pectin is likely to affect cellular redox

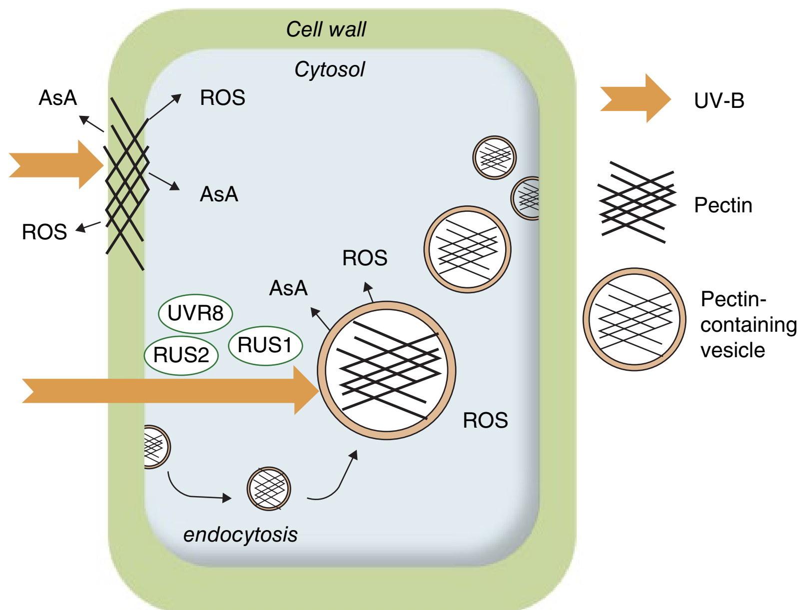

Figure 12.8 Possible role of pectin as a redox regulator during UV-B response in cells of the root apex transition zone. AsA indicates ascorbic acid; for other details, see the text above (courtesy of Yokawa and Baluška, 2015).

balance, as it produces superoxide under UV-B stress (Pristov *et al.*, 2013). Furthermore, D-galacturonic acid, released by the degraded cell wall, is known to be re-utilized, not only for forming new cell wall, but also as a substrate of L-ascorbic acid biosynthesis (Valpuesta and Botella, 2004). L-ascorbic acid is well known as a reducing agent, controlling cellular redox homeostasis via glutathione-ascorbate cycle (Foyer and Noctor, 2011). It is reasonable to expect the importance of crosslinked cell wall pectin for ROS homeostasis, because UV-B easily breaks down cell wall components, and this might act as a cue for the ascorbate biogenesis at irradiated organ sides, in order to recover the cellular redox balance. Of course, internalized cell wall pectin within endocytic vesicles and endosomes might also be a potential source for the L-ascorbate synthesis.

12.5.3 Avoidance at the Molecular Level

12.5.3.1 DNA Repair

The repair of DNA damage is essential for the survival of organisms, otherwise genomic integrity will not be maintained (Ries *et al.*, 2000). To this end, coordination between DNA replication and repair has been considered essential for the maintenance of the genome (Kimura *et al.*, 2004). UV radiation-induced DNA damage and repair has been well studied, but information on the underlying mechanisms in plant system is still lacking (Kimura *et al.*, 2004).

DNA repair is performed in two conditions:

1) In light conditions, photoreactivation catalyses dimer monomerizations.
2) During dark conditions, nucleotide excision repair (NER) excises helix-distorting lesions, and base excision repair (BER) repairs oxidized or hydrated bases.

The photorepair (photoreactivation) repairs UV-B-induced low frequencies of DNA damage, such as CPDs, where the photolyase mediates the major processes by absorbing blue/UV-A (320–400 nm) light. Similar reaction mechanisms for CPDs and 6-4 PP involve repair mediated by CPD photolyases and 6-4 photolyases, respectively (Bray and West, 2005; Waterworth *et al.*, 2002). Some credible work has been performed on *Oryza sativa*, where photolyase has been evidenced for its capability to repair DNA damage (Hidema *et al.*, 2005; Teranishi *et al.*, 2012).

Nucleotide excision repair under dark conditions releases the damaged nucleotides, and achieves resynthesis of strands through endonucleolytic cleavage (Liu *et al.*, 2000). Ries *et al.* (2000) correlated the amount of CPDs formed with the increased homologous recombination frequency, confirming its role in UV-induced DNA repair. Short-patch BER (DNA polymerase beta dependent) removes damaged bases by DNA glycosylases (Tuteja *et al.*, 2001). Long-patch BER (DNA polymerase delta/epsilon dependent) displaces the strand in 5’-3’ direction after a nick translation reaction (Kunkel and Bebenek, 2000; Kimura and Sakaguchi, 2006). Mismatched Repair (MMR) systems have been evolved in plants, as reported in *Arabidopsis* (Leonard *et al.*, 2003) for repair of UV-B-induced recombination, and is a light-independent repair mechanism.

12.5.3.2 Genes and Avoidance

The UV-B specific signalling components orchestrate plant protection against UV-B irradiation. The UV-B photomorphogenic pathway promotes photomorphogenic responses characterized by the inhibition of hypocotyl growth, cotyledon expansion, and stomatal opening (Ulm and Nagy, 2005; Jenkins, 2009). UV Resistance Locus 8 (UVR8) is a key photoreceptor of the photomorphogenic pathway, regulating a range of genes with important roles in UV protection and the repair of UV damage (Brown *et al.*, 2005; Favory *et al.*, 2009). Cloix and Jenkins (2008) observed the association of UVR8 chromatin with the promoter region of Elongated Hypocotyl 5 (HY5).

HY5 and COP1 (Constitutive Photomorphogenic 1, a central regulator of photomorphogenesis), are principal genes identified as mediators of UV-B photomorphogenic responses (Oravecz *et al.*, 2006; Tohge *et al.*, 2011). The UVR8-COP1-HY5 pathway initiates with UV-B perception by UVR8 in cytosol, further initiating a signalling cascade principally involving a bZIP transcription factor (HY5) and E3 ubiquitin ligase (COP1) in the nucleus. Different responses mediated by the pathway involve hypocotyl growth inhibition (Favory *et al.*, 2009), stomatal closure (Tossi *et al.*, 2014), phototropic bending (Vandenbussche and Straeten, 2014) and leaf development (Wargent *et al.*, 2009). To balance the UV-B response, a negative feedback loop involves the action of the Repressor of UV-B Photomorphogenesis (RUP) 1 and 2 gene, induced by UV-B through UVR8 (Gruber *et al.*, 2010; Heijde and Ulm, 2013).

12.5.3.3 UV-B Perceived by UVR8 Strongly Inhibits Shade Avoidance

In plants, UV-B is perceived by the photoreceptor protein UVR8. Along with regulating photoprotective responses, UV-B dramatically inhibits stem elongation. When grown in dense stands, plants use reflected far-red light signals from neighbours to detect the threat of shading. In many species, these signals drive rapid elongation responses to overtop competitors. UV-B perceived by UVR8 provides a potent sunlight signal that inhibits shade avoidance.

UVR8 activation stimulates multiple pathways that converge to block biosynthesis of the plant growth hormone auxin. Understanding how UV-B regulates plant architecture

is central to our understanding of plant growth and development in sunlight. During emergence from the soil, seedlings are exposed to a drastic step change in UV-B and, afterwards, acclimation adjustment depends on gradual changes in UV-B (Mazza and Ballare, 2015). Therefore, after emergence, for plants growing in sunlight, long-term acclimation is the most important response for coping with UV exposure. Thus, in two of the experiments, patterns of gene expression with relevance for long term acclimation of plants to solar UV were determined in the presence of solar UV-A and high PAR.

12.5.4 UV-B and Secondary Metabolites

Another adaptive mechanism against enhanced UV-B radiation is increased production of secondary metabolites in leaf tissues. Some studies indicated that UV-B-absorbing compounds increase from 10% to 300% in agronomic crops. It is well known that one of the most effective defensive mechanisms against UV-B radiation in higher plants is the accumulation of a diverse range of phenolic metabolites (e.g. Tegelberg *et al*., 2001; Mpoloka, 2008; Nybakken *et al*., 2012).

The UV-B radiation transmitted after reflection by the epicuticular wax layer reaches the epidermal layer. This layer is known to accumulate most of the secondary metabolites, such as phenolics and flavonoids that absorb/screen UV-B radiation and shield the underlying tissues against harmful UV-B radiation (Cen and Bornman, 1993; Cen *et al*., 1993; Liu *et al*., 1995; Olsson *et al*., 1998). Accumulation of leaf condensed tannins, salicylates and flavonoids were increased more in females than in males by elevated UV-B, and facilitated by warming. Leaf hyperin and salicortin are increased by UV-B exposure in *Saiyx myrsinifolia*, while chlorogenic acid concentrations are increased by elevated temperature, but these have been observed for female only (Randriamanana *et al*., 2015).

12.5.4.1 Plant Phenolics

Plant phenolics, like many phenylpropanoid derivatives, selectively absorb in the UV-B region of the spectrum, which makes them ideally suited for a role in UV-protection (Winkel-Shirley, 2002). Flavonoids are accumulated in different plants, such as *Ginkgo biloba* and *Lonicera japonica* under UV-B exposure (Sun *et al*., 2010; Ning *et al*., 2012). Hydroxycinnamic acid derivatives and cinnamic acid derivatives levels in willow plants increase upon UV-B exposure (Tegelber and Julkunen-Tiitto, 2001; Turtola *et al*., 2005).

The flavonoids like quercetin and kaempferol have similar extinction coefficients in the UV region of the spectrum, and the ratio of quercetin to kaempferol increases in many UV-B-exposed plants (Reifenrath and Muller, 2007). Flavonols kaempferol-3-O-b-D-glucuronopyranoside and quercetin-3-O-b-D-glucuronopyranoside increase on UV-B irradiation through the UVR8-HY5-COP1 signalling pathway (Bidel *et al*., 2015).

UV-B radiation increases some other flavonoids in plants, such as ferulic acid in sweet basil (Nitz and Schnitzler, 2004), rosmarinic acid and vanillic acid in rosemary (Luis *et al*., 2007) and chlorogenic acid in birch (Lavola *et al*., 1997). In *Pinus sylvestris*, subjected to UV-B irradiation, enhanced accumulation of diacylated flavonols (dicoumaroyl-trifolin, dicoumaroyl-isorhamnetin, dicoumaroyl-astragalin and dicoumaroyl-isoquercitin) has been observed (Lavola *et al*., 2003). Flavonoids absorb light strongly in the UV-B region while not interrupting visible (PAR) wavelengths, when they accumulate in epidermal layers of leaves and stems under UV-B exposure (Lake *et al*., 2009).

12.5.4.2 Anthocyanin

Anthocyanin is a water-soluble blue red flavonoid pigment, and is found in almost all tissues of the plant kingdom. Its increased synthesis is often observed during different kinds of environmental factors, including visible and UV-B radiations. The subsequent production and localization of anthocyanin in root stem, and especially leaf tissue, may allow the plant to develop resistance to UV-B radiations (Chalker-Scott, 1999). There is strong evidence that anthocyanins protect plant tissues from excessive solar radiation. The red flavylium form of anthocyanins (AHD) dissipates more than 99% of the absorbed radiation energy into heat through a series of ultra-fast excited-state mechanisms, among which the major role is played by excited-state proton transfer to water. According to Costa *et al.* (2015), the less exuberant (colourless) hemiketal form of three common anthocyanins (pelargonin, cyanin, and malvin) is equally efficient at dissipating absorbed UV-B radiation into heat by fast photo-tautomerization, which yields the cis-chalcone form in its ground state in a few picoseconds.

Anthocyanins were reported to increase after UV irradiation in peach, apple, strawberry and berry (Marais *et al.*, 2001; Kataoka and Beppu, 2004; Higashio *et al.*, 2005; Huyskens-Keil *et al.*, 2007). The activity of enzymes involved in phenylpropanoid and flavonoid pathways, namely phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), chalcone isomerase and dihydroflavonol-4-reductase, were found to increase in UV-B irradiated plants, enhancing phenolic accumulation (Tomas-Barberan and Espin, 2001; Treutter, 2005). Light-dependent regulation of chalcone synthase (CHS), a key structural gene of the anthocyanin biosynthesis pathway, is through a photomorphogenic pathway involving UVR8 induced by UV-B (Jenkins and Brown, 2007; Pandey and Pandey-Rai, 2014). An increment in anthocyanin with hydroxycinnamic and hydroxybenzoic acids has been observed in UV-B-exposed *Ribes nigrum* L. (blackcurrant) (Huyskens-Keil *et al.*, 2007). Zavala *et al.* (2015) suggested that constitutive and UV-B-induced isoflavonoids increase plant resistance and enhance defence in soybean.

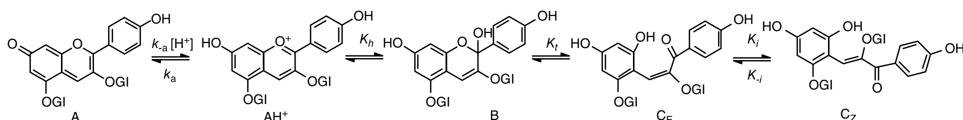

Figure 12.9 Multi equilibria of anthocyanins in aqueous solution, illustrated with pelargonin.

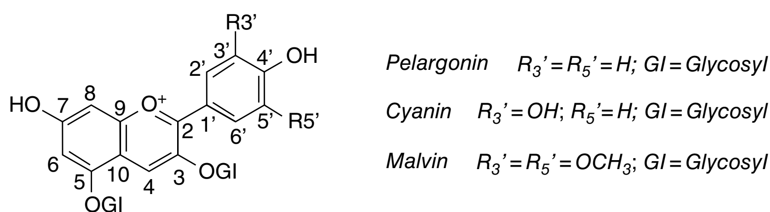

Figure 12.10 Molecular structures of pelargonin, cyanin and malvin.

12.5.4.3 Alkaloids

Alkaloids are nitrogen-containing secondary metabolites of plants, functioning in defence against stress. Two quite distinct alkaloids are nicotinamide and the derived metabolite trigonelline, induced by UV-B irradiation accumulated in plants (Kalbin *et al.*, 1997). Brachycerine, an indole alkaloid produced in *Psychotria brachyceras*, has been reported to accumulate in UV-B exposed plants (Gregainini *et al.*, 2003). Nascimento *et al.* (2013) reported significant increases in transcription after UV-B exposure in genes related to brachycerine synthesis – tryptophan decarboxylase, ACC oxidase, UDP-glucose glucosyltransferase, lipase, and serine/threonine kinase in *Psychotria brachyceras*. The induction of the monoterpenoid indole alkaloids camptothecin and 10-hydroxycamptothecin by UV-B radiation were evaluated in the tree *Camptotheca acuminate* (Pi *et al.*, 2010). Ramani and Chelliah (2007) showed transcriptional activation of tryptophan decarboxylase (Tdc) and strictosidine synthase (Str) genes, participating in the biosynthesis of terpenoid indole alkaloids, and subsequent accumulation of catharanthine and vindoline.

12.5.4.4 Isoprenoids

Isoprenoids are a large group of C5-isoprene units containing compounds accumulated in plants, including carotene, xanthophylls, terpens and so on. Excess energy imposed on the photosynthetic apparatus can be thermally deactivated through quenchers like xanthophylls (Lidon and Ramalho, 2011). The terpene emission potentials explain protection from the effects of UV-B radiation through these UV-B-absorbing compounds in plants (Penuelas and Munné-Bosch, 2005; Albert *et al.*, 2008; Llusia *et al.*, 2012).

Carnosic acid, a ROS- scavenging terpene present in rosemary (*Rosmarinus officinalis*) doubled its level in leaves exposed to chronic UV-B (Luis *et al.*, 2007). Glycyrrhizin, a bioactive glycosidic triterpenoid of *Glycyrrhiza uralensis*, accumulated with exposure to UV-B consistent with other reports on terpens under UV-B irradiation (Afreen *et al.*, 2005).

12.5.4.5 Glucosinolates

The glucosinolates is a group of sulphur-rich, amino acid-derived metabolites found exclusively in cruciferous plants against biotic and abiotic stress. Some researchers noticed the effect of UV-B on glucosinolate metabolism, and genes related to its biosynthesis were found to be differentially regulated by UV-B (Hectors *et al.*, 2007). Schreiner *et al.* (2009) studied the increase in the glucotropaeolin concentration of *Tropaeolum majus* due to UV-B. The remarkable studies on glucosinolates were in biotic stress, compared with few in abiotic stresses like UV-B.

12.6 UV-B and its Significance

12.6.1 Ecological Significance

Research into the effects of UV radiation on terrestrial ecosystems remains a relatively new discipline that is currently split into two broad themes: the effects of increased UV-B radiation resulting from ozone depletion; and the role of UV radiation (largely UV-B) in the context of many plants and animals. There is increasing indication that

UV radiation affects many trophic relations and, in turn, sways a variety of ecosystem functions. A variety of ecological processes have often been classified into direct and indirect effects. Current exposure of UV which affects/alter the normal mechanism of an organism is called a direct effects – that is, some plant diseases can be minimized by UV exposure, as it kills the fungal spores which infect the plants. Indirect effects can be categorized as whenever UV tolerant plants also become tolerant to certain plant diseases, ultimately leading to disease-resistant plants.

Disease resistance in plant persists in plants for a longer time if there is no exposure to UV light, because of the adaptation of plants to UV radiation. It includes effects owing to altered plant chemistry and changes in tissues not directly exposed to radiation. Increases in UV-B radiation can damage many organisms, but the effects of solar UV on many ecological processes also depend on the use of UV-B and UV-A by microbes, plants and animals as a source of information about their environment. Solar UV radiation penetrates to ecologically significant depths in aquatic systems, and can affect both marine and freshwater systems, from major biomass producers (phytoplankton) to consumers (e.g. zooplankton, fish, etc.) higher in the food web (Hader *et al.*, 2007).

Some ecosystem studies show that ambient solar UV radiation can be an important determinant of reproductive effort, though solar UV appears to have little effect on the growth and development of the dominant dwarf shrubs over three years (Phoenix *et al.*, 2002). However, the possible interactions between UV-B and additional potential stresses found in natural environments have rarely been studied experimentally. Because the reported effects of increased UV-B on plant growth and fitness have been highly variable, studies that focus on factors that may lead to these differences in results are important for the formulation of accurate predictions about future plant success under varying UV-B levels (Conner and Neumeier, 2002; Nigel and Dylan, 2003).

12.6.2 UV-B and Plant Competition

In forests, grasslands and so on, overall primary plant productivity may not be greatly affected by ozone reduction, even if the growth of some plants is diminished. However, since plant species differ greatly in their growth responsivity to UV-B, it is anticipated that a productivity reduction in one species will probably lead to increased productivity in another, more UV-tolerant species. This is likely because more resources (e.g., light, moisture and nutrients) will be available to the tolerant species. Thus, the overall productivity of the system may well remain about the same, while species composition may change.

However, a change in the balance of species could have far-reaching consequences for the character of many ecosystems. Although the response of individuals of the same species is expected to be uniform, UV-B may influence intraspecific competition less than interspecific competition (Furness *et al.*, 2005). Even though UV-B effects on shoot morphology under realistic sub-ambient or enhanced solar UV-B scenarios are often subtle, and have minimal impact on the overall performance of isolated plants, they could have considerable ecological consequences, by changing competitive interactions within mixed plant associations (Barnes *et al.*, 1996; Caldwell *et al.*, 1999) via alteration of canopy structure and light-interception patterns (Caldwell *et al.*, 1994; Furness, 2003; Barnes *et al.*, 1990; Ryel *et al.*, 1990).

Genetic variation in sensitivity to UV-B radiation has implications for plant competition and, thus, for plant ecosystem dynamics and community structure, in both natural and managed ecosystems. The plant species susceptible to UV-B will not be sustained, while the tolerant will dominate, and eventually the ecosystem composition will change (Fox and Caldwell, 1978). There might be a scenario where certain ecosystems will have only a UV-B resistant plant community, ultimately leading to a global change in ecosystem structure and function. High UV-B also leads to increase in plant aboveground biomass, with increased numbers of dense trees at the community level. In some experimental conditions, it has been observed that the same species either benefited or faced disadvantage due to UV supplement, depending upon which competitor species was around (Fox and Caldwell, 1978). Almost all plant species face some critical competitive stage in their life cycle and, at that time, UV-B radiation may play a crucial role in context, with competitive exclusion.

12.7 Conclusion and Future Perspectives

The UV radiation that reaches the earth's atmosphere is mainly from solar radiations. The ozone layer in the stratosphere plays a very crucial role in screening UV radiation, with a maximum for UV-C, followed by UV-B and UV-A. Human beings suffer deleterious symptoms from UV, especially on the skin and eyes, which may lead to malignancy. On the other hand, in plants, visible symptoms, including reduced chlorophyll content and plant growth, changes in leaf phenology and photo morphogenesis are induced by UV-B. There are changes in anatomical structures, including hampered epidermis and palisade parenchyma. Grain and flower yield are also negatively affected by UV-B, both qualitatively and quantitatively. The photosynthetic rate is also decreased, as UV-B destroys the D1 and D2 protein of PSII complex. At the biochemical level, there is increased production of reactive oxygen species, high MDA level and PAL activity. At the molecular level, DNA damage as affected by UV-B radiations leads to changes in gene expression pattern and functioning of amino acids.

Plants have developed some avoiding mechanisms to cope up with UV radiations, as increased epicuticular wax content in high altitude plants, pectin endocytosis, and avoidance by anthocyanin. Other secondary metabolites that accumulate in tissues upon exposure to UV-B radiation include alkaloids (nicotinamide, trigonelline, brachycerine), isoprenoids (xanthophylls, carotenoids, terpenes) and glucosinolate (glucotropaeolin). UV-B radiation can penetrate and damage the plant genome by inducing oxidative damage (pyrimidine hydrates) and crosslinks (both DNA-protein and DNA-DNA) that are responsible for retarding the growth and development of plants. Cyclobutane pyrimidine dimers (CPDs) and 6-4 pyrimidine-pyrimidone photoproducts (6-4 PP) are the primary UV-induced DNA damages, out of which CPDs account for approximately 75% of total DNA damage induced by UV-B, but the 6-4 PPs may have more serious, potentially lethal and mutagenic effects.

The genes related to photosynthesis – for example, chl *a/b* (Lhcb), Rubisco synthesis (rbcL, rbcS), and the D1 protein (psbA) of photosystem II and PSI are also downregulated upon exposure to UV-B. Plants are able to sense UV-B through the UV-B photoreceptor UVR8. UV-B photon absorption by a UVR8 homodimer leads

to UVR8 monomerization and interaction with the downstream signalling factor COP1. High UV-B levels trigger signalling responses that contribute to acclimation and plant survival.

There is also some indication that UVB may influence the composition of plant community structure if there is an increase in dominant vegetation types that are resistant to UV-B and other environmental stresses, leading to changes in global ecosystem. There is increasing concern that UV radiation may affect many trophic relations and, in turn, may influence a variety of ecosystem functions.

Global climate changes in coming decades, such as elevated CO_2 and higher temperatures, will most likely be superimposed on the predicted increase in UV-B radiation. Consequently, studies of combined impacts of environmental factors and UV-B radiation will be necessary if plant breeders are to select crop varieties that are better adapted to cope up with these stresses, and for ecologists to predict the likely outcomes for natural ecosystems.

Acknowledgments

Authors are grateful to the director, CSIR-NBRI for providing necessary facilities. SKG and MS are grateful to CSIR and UGC, respectively, for senior research fellowship.

Box 12.1 Targets of UV-B

Morphological
- Visible symptoms
- Plant growth and leaf phenology
- Reproductive morphology
- Photo morphogenesis

Anatomical
- Epicuticular wax layer
- Epidermis
- Stomata

Yield

Photosynthesis
- Pigments
- Photosynthetic machinery

Biochemical response
- Reactive oxygen species
- Fee radical scavenging mechanism

Molecular response: gene and protein
- Change in gene expression
- Protein expression

Box 12.2 UV-B avoidance mechanism
Morphological level • Epicuticular wax **Biochemical level** • Pectin endocytosis **Molecular level** • Anthocyanin • Amino Acids • Nucleic Acids **Secondary metabolites** • Plant phenolics • Anthocyanin • Alkaloids • Isoprenoids • Glucosinolates

Box 12.3 - UV- B and its significance
Ecological Significance **Plant Competition and UV- B**

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13

Impact of UV-B Exposure on Phytochrome and Photosynthetic Machinery: From Cyanobacteria to Plants

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13.1 Introduction

In the past few decades, depletion of stratospheric ozone due to anthropogenic pollutants has emerged as a matter of serious concern, and the reason for the concomitant increase in solar UV-B radiation (280–315 nm). UV-B, despite constituting less than 0.5% of total solar radiation, holds significant potential to cause biological damage, due to its high energy. Most of the dangerous UV-B radiation is filtered through the ozone layer. As the absorption coefficient of ozone at wavelengths longer than 290 nm decreases, a significant amount of biologically effective radiation reaches the earth, specifically in the range of 280–315 nm. At 330 nm, absorption coefficient of ozone is nearly zero, therefore, reduced ozone levels will lead to a selective increase in the UV-B radiation reaching the earth's surface (Bornman, 1989).

Although enhanced UV-B radiation reaching earth surface poses a significant threat to all living organisms, photosynthetic organisms seem to be the prime targets, due to their mandatory requisite of sunlight for survival. In photosynthetic organisms, a large number of physiological processes are negatively affected, but photosynthesis seems to be one of the most important processes influenced by UV-B radiation. Studies suggest a prominent effect of UV-B on photosynthesis of land plants (Teramura and Sullivan, 1994), specifically on the reaction centre of PSII. Moreover, earlier it was thought that UV-B cannot penetrate ocean water in significant amounts, but now it is well documented that, up to a depth of 10 m, harmful doses may reach (Wood, 1987). Therefore, not only terrestrial photosynthetic organisms, but also marine and freshwater forms, are affected by harmful UV-B.

UV-B Radiation: From Environmental Stressor to Regulator of Plant Growth, First Edition. Edited by Vijay Pratap Singh, Samiksha Singh, Sheo Mohan Prasad and Parul Parihar.
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UV-B irradiation is known to affect life processes via two ways – direct and indirect damage. Major direct effects of UV-B on photosynthetic functions are:

1) destruction of PSII (Renger *et al.*, 1989; Greenburg *et al.*, 1989a, 1989b; Melis *et al.*, 1992);
2) reduced Rubisco activity (Jordan *et al.*, 1992; Strid *et al.*, 1990); and
3) declined CO_2 fixation and O_2 evolution (Sullivan and Teramura, 1990; Ziska *et al.*, 1992).

Indirect effects of UV-B include induced stomatal closure (Negash and Bjorn, 1986), altered leaf thickness/anatomy in plants (Bornman and Vogelmann, 1991), changes in canopy morphology, and so on (Barnes *et al.*, 1990; Ryel *et al.*, 1990).

In this chapter, significant effort has been made to survey existing information about the effects of UV-B on photosynthesis and the photosynthetic apparatus of organisms, ranging from primitive photoautotrophs like cyanobacteria to higher plants.

13.2 Effect of UV-B Irradiation on Photosynthetic Machinery of Cyanobacteria

13.2.1 Pigments

UV-B irradiation causes damage to photosynthetic pigments, either through degradation or inhibition of enzymes involved in their biosynthetic pathway (Ranjbarfordoei *et al.*, 2011). In cyanobacteria, the photosynthetic pigment includes chlorophyll *a*, carotenoids, and phycobiliproteins such as phycoerythrin (PE), phycoerythrocyanin, phycocyanin (PC) and allophycocyanin (APC). UV-B irradiation causes reduction in phycobiliprotein content, and leads to disassembly of the phycobilisome complexes (Sinha *et al.*, 1995; Lao and Glazer, 1996; Banerjee and Häder 1996; Nedunchezhian *et al.*, 1996; Pandey *et al.*, 1997).

Sinha *et al.* (1997) observed the patterns of fluorescence emission spectra of the phycobiliproteins after UV-B irradiation, and suggested that the energy transfer from the accessory pigments to the photosynthetic reaction centre is impaired. Intense UV-B radiation photobleaches photosynthetic pigments, disintegrates phycobilisome and, subsequently, bleaches PC and PE (Sinha *et al.*, 2005). In *Anabaena* sp. PCC7120, the loss of the '*a*' and '*b*' monomers of PC, rod-core and core-membrane linker polypeptides, after 60 min of UV-B irradiation, was observed on SDS-PAGE analysis (Sinha and Häder, 2003). Rinalducci *et al.* (2006) studied the effects of UV-B radiation (1.3 Wm^{-2}) on the biliproteins of *Synechocystis* 6803, and revealed rapid destruction of beta-phycocyanin and a slower damage of the other biliproteins, alpha-phycocyanin and both alpha and beta-allophycocyanin.

Apart from phycobiliproteins, UV-B radiation negatively affects the chlorophyll and carotenoid contents of cyanobacteria. After UV-B exposure, a significant reduction of chlorophyll *a* content was observed in the cyanobacterium *Spirulina platensis* (Gupta *et al.*, 2008). UV-B (8 Wm^{-2}) exposure for six hours in the marine cyanobacterium *Phormidium tenue* decreases the levels of photosynthetic pigments chlorophyll *a*, myxo-xanthophylls, and β-carotene by 74%, 81%, and 86% respectively (Bhandari and Sharma, 2011). In the rice-field cyanobacterium *Aulosira fertilissima*, photosynthetic

pigments were completely bleached after 2–3 hours of UV-B irradiation (5 Wm^{-2}), suggesting the loss of effective energy transfer from the accessory pigments to PSII (Banerjee and Häder, 1996). In *Nostoc muscorum*, after 70 minutes of UV-B (3.5 Wm^{-2}) exposure, PC, carotenoids, and chlorophyll *a* content were found to be reduced by 86.4%, 81.25%, and 76.85%, respectively (Agrawal, 1996). Similarly, Rai *et al.* (2013) and Shrivastava *et al.* (2015) found decrease in pigment content following UV-B stress.

13.2.2 Photosynthetic Electron Transport System

In the cyanobacterium *Synechocystis salina*, UV-B-induced inhibition of oxygen evolution is a result of a decrease in the number of functionally active PSII centres (Apostolova *et al.*, 2014). Rajagopal *et al.* (2005) studied the effect of UV-B radiation on intact cells of *Spirulina platensis*, and reported significant decrease in photosystem II activity, but no loss in photosystem I activity. Additionally, the results clearly demonstrated that the photosystem II core antennae of chlorophyll proteins CP47 and CP43 are affected by UV-B exposure. Studies in the bloom-forming cyanobacterium *Microcystis aeruginosa* suggest that PSII is more sensitive to UV-B exposure than PSI, and that the oxygen-evolving complex of PSII is the most important target of UV-B damage in cyanobacteria (Jiang and Qiu, 2011). A marked reduction in oxygen evolution (33–38% relative to the control) in two *Microcystis* strains under 0.372 Wm^{-2} UV-B for four days was reported (Zhang *et al.*, 2013).

In *Spirulina platensis*, a severe reduction in the O_2-evolving activity (56% after three hours of UV-B) was accompanied by a significant loss of *de novo* synthesis of D1 protein (up to 40% of the initial value) at the PSII level (Wu *et al.*, 2011). Additionally, D1 and D2 polypeptides, the major constituents of the PSII reaction centres, were also degraded, even after the exposure of cyanobacteria to intermediate levels of UV-B radiation (Wu *et al.*, 2011). In *Synechococcus* sp. WH8102, a dramatic reduction in the amount of D1 protein was observed, with the rapid photoinactivation of the PSII reaction centres after short-term exposure (five hours) to UV-B (0.86 Wm^{-2}). Shrivastava *et al.* (2015) reported a drastic decrease in PSII activity in three species of *Anabaena*, with maximum reduction in *A. doliolum*, and appreciable inhibition in PSI activity on the first day of treatment, followed by a gradual increase on later days. Rai *et al.* (1995) observed significant inhibition of carbon fixation, O_2 evolution and the photosynthetic electron transport chain of the cyanobacterium *A. doliolum* by UV-B and copper.

The cytochrome b6/f complex (Cyt b6/f), together with photosystem II (PSII) and photosystem I (PSI), is a key integral membrane protein complex, constituting the photosynthetic electron transport chain in the thylakoid membrane. The cytochrome b6/f complex contains two quinone binding sites – one where quinol oxidation occurs, and the other where quinone reduction occurs (Hope, 1993) and, due to this reason, it was found to be the thylakoid component least affected by UV-B (Strid *et al.*, 1990; Zhang *et al.*, 1994). Eichhorn *et al.* (1993) reported UV-B induced reduction in Cyt b6/f content, and this decline was probably associated with a lower Chl *a* : Chl *b* ratio, which causes a reduction in electron transport capacity (Watanabe *et al.*, 1994).

Earlier, it was observed that inactivation of the plasma membrane-bound ATPase by UV irradiation was optimal at 290 nm (Imbrie and Murphy, 1982). Enhanced UV-B radiation reduces ATPase and photophosphorylation activities, probably because of modification in the transformation efficiency of electric energy into active chemical

energy, hence leading to a decrease in the photochemical capacity and CO_2 assimilation (Yu *et al.*, 2013; Zhang *et al.*, 1994; Yang *et al.*, 2013).

13.2.3 Photophosphorylation and CO_2 Fixation

UV-induced inhibition of $^{14}CO_2$ uptake in various rice field cyanobacteria could be due to the effect on the photosynthetic apparatus, leading to a reduction in the supply of ATP and $NADPH_2$ (Sinha and Häder, 1996). A disruption of the cell membrane and or alteration in thalakoid integrity, as a result of UV-B irradiation, partly or wholly destroy the components required for photosynthesis and, thus, affects the rate of CO_2 fixation (Sinha and Häder, 1996; Sinha *et al.*, 1997).

It is known that the Calvin cycle and the pentose phosphate pathway (PPP) perform most of the carbohydrate metabolism in cyanobacteria, since the Krebs cycle is incomplete in cyanobacteria. The expression levels of transaldolase and transketolase increased more than twofold when the photosystem was damaged by UV-B (Babele *et al.*, 2015). This refers to an enhanced PPP, particularly when the Calvin cycle, where these enzymes also have a role in the reductive biosynthetic process, is scaled down. PPP is the main NADPH-producing pathway (Kumar *et al.*, 1996). Operons composed of genes encoding carbon dioxide-concentrating mechanism proteins (fructose-1,6-bis phosphate aldolase, glucose6-phosphateisomerase, and phospho-glucomutase/ phosphomannomutase) were found repressed under UV-B stress in *Anabaena* L31 (Huang *et al.*, 2002; Babele *et al.*, 2015).

Proteome analysis of cyanobacteria revealed that the majority of energy metabolism proteins remain embedded in integral membranes, and only a few occur in the soluble fraction (Anderson *et al.*, 2006). Consistent with the downregulation of proteins of carbohydrate metabolism, the transcript allowing ATP synthesis by F0F1 ATP synthase was found to be downregulated in the presence of UV-B stress (Babele *et al.*, 2015). This enzyme is of great significance to the test cyanobacterium, as it uses a proton gradient to drive ATP synthesis, and hydrolyzes ATP to build the proton gradient (Babele *et al.*, 2015).

13.3 Effect of UV-B Irradiation on Photosynthetic Machinery of Algae

All aquatic organisms, including algae, appear to be vulnerable to UV-B, but to different extents (Sinha and Hader 2002). In spite of many studies on the effects of the UV-B on photosynthetic organisms, relatively limited information is available on green algae. Out of 16 freshwater microalgae species employed in an UV simulation study, half were described as UV-tolerant, and most of these showed higher oxygen evolution under treated conditions (Xiong *et al.*, 1997). A number of green algae appear to be well adapted to high levels of UV irradiation, which might be due to the presence of highly efficient avoidance mechanisms. Surface mucilage sheaths and internal UV-absorbing compounds may shield cytoplasm and the chloroplast from detrimental irradiation, and facilitate organisms to survive under UV irradiation.

Unicellular freshwater green algae occur in shallow lakes or small ponds, distributed all over the world, even at high altitudes. They are compelled to face high UV irradiation

in their natural habitats (Sommaruga and Psenner, 1995). As with other photosynthetic organisms, photosynthesis is very sensitive to UV-B induced damage in green algae. The primary light-harvesting antenna of PSII is the chlorophyll *a*/*b* protein complex in green algae (Kouril *et al.*, 2012). The photosynthetic pigment can act as photosensitizer, and produces ROS under excess of UV-B/visible light (Rinalducci *et al.*, 2006; Triantaphylide and Havaux, 2007). Enhanced UV-B generally decreases the chlorophyll content and inhibits photosynthesis, resulting in lower biomass production in algae (Xue *et al.*, 2005).

In *Dunaliella bardawil*, UV-B causes major damage to cells with respect to photobleaching, but it does not have a significant effect on F_v/F_m ratio and, thus, on PSII (White and Jahnke, 2002). UV-B exposure does not appear to inhibit photosynthesis process via damage to electron transport but, rather, as a result of damage to components of carbon fixation, particularly Rubisco (Lesser, 1996; Allen *et al.*, 1998).

In the green alga *Selenastrum capricornutum*, enhanced UV-B irradiation increased the cell size, whereas photosynthetic parameters remained unaffected (Hessen *et al.*, 1997). The effects of UV-B irradiation on growth and photosynthesis in *Scenedesmus quadricauda* turned out not to be very prominent (Germ *et al.*, 2002). Antarctic *Scenedesmus* sp. copes with UV-B by enhanced replacement of the damaged D1 protein or Rubisco, and repair of DNA damage (Lesser *et al.*, 2002).

In the unicellular freshwater green alga *Micrasterias denticulata* (at high altitude), detailed studies with UV-B irradiation at different cut-off wavelengths, together with simulated sunlight, were performed (Lütz *et al.*, 1997). The authors were able to detect a marked resistance against UV-B irradiation and, initially, no marked changes to control cells were observed. Further photosynthetic activity was recorded, together with the ultrastructural analysis. Oxygen production responded more severely to UV-B irradiation, but it was not reduced for the first 15 minutes at a cut-off wavelength of 275 nm. With decreasing UV cut-off wavelengths, breakdown products of chlorophyll *a* and chlorophyll *b* were detected by HPLC separation analysis (Lütz *et al.*, 1997). The green alga *Chlamydomonas nivalis* adapts to unfavourable conditions by the ability to form red-coloured hypnoblasts (Leya *et al.*, 2004). Several authors claim that the carotenoids causing the red colour of these algae protect the cells from UV irradiation (Gorton and Vogelmann, 2003). In the unicellular *Haematococcus pluvialis*, production of asthaxanthin takes place to protect the chloroplasts (Hagen *et al.*, 1994).

The effect of solar UV-B radiation on the physiology of the green macroalga *Ulva lactuca* was investigated, and changes in the activity and concentration of photosynthetic and xanthophyll cycle pigments were determined (Bischof *et al.*, 2002). Exclusion of UV-B radiation from the natural solar spectrum resulted in an elevated activity of Rubisco, and increases in the amount of the photosynthetic pigment lutein and the ratio of zeaxanthin content to the total xanthophyll content, indicating adverse effects of UV-B on the efficiency of photoprotection under high irradiance of PAR. The results confirm a marked impact of prevailing UV-B levels on macroalgal physiology under field conditions (Bischof *et al.*, 2002).

To gain insight, simultaneous investigation of photosynthesis and ultrastructure in *Prasiola crispa* (supralittoral green alga) in response to UV-B ($2.0\,W\,m^{-2}$) exposure was undertaken (Holzinger *et al.*, 2006). It was found that six hours of exposure did not lead to significant alterations in the ultrastructure, but 24 hours caused slight

alterations to chloroplasts, with slight dilatations in thylakoids and an apparent reduction in the number of plastoglobuli.

Despite the fact that a decrease in the optimum quantum yield (F_v/F_m) has been reported by Holzinger *et al.* (2006), even $2.0\,W\,m^{-2}$ UV-B did not significantly alter the photosynthetic performance of the closely related *Prasiola crispa* spp. *antarctica* (Lud and Buma, 2001). *Prasiola crispa* is an ecologically interesting genus, due to its capability to grow subaerially on various hard substrata, often several metres above sea level. As a consequence of living under almost terrestrial conditions, *Prasiola crispa* experiences higher UV irradiation than submerged species. Hence, the genus has developed a range of morphological, physiological and biochemical protective mechanisms (Jacob *et al.*, 1992), or the formation of UV-sunscreens such as MAAs, to cope with UV radiation (Hoyer *et al.*, 2001; Karsten *et al.*, 2005).

13.4 Effect of UV-B Irradiation on Photosynthetic Machinery of Higher Plants

13.4.1 Pigments

Light is the ultimate energy source that regulates various events in the life cycle of plants. For perception of light of a specific wavelength, plants have evolved exquisite sensory systems known as photoreceptors or pigments. These photoreceptors can be ‘photosynthetic’ (such as chlorophylls and carotenoids), powering photosynthesis by absorbing light, or ‘photomorphogenic’ (such as phytochromes), initiating the developmental switch from dark to light growth. The effect of prolonged exposure of ultraviolet radiation on these essential pigments is reviewed here.

13.4.1.1 Phytochrome

Phytochromes are bluish protein pigments that play an important role in the regulation of plant growth and development. Their two photo-reversible forms, ‘Pr’ (red absorbing) and ‘Pfr’ (far-red absorbing), change back and forth upon absorption of red (R)/far-red (FR) light, to mediate photomorphogenic responses such as seed germination, leaf expansion, chloroplast development, flowering and so on. Pratt and Butler (1970) reported that phytochromes can also absorb in the UV-B region and can undergo photoconversion. Thus, the role of UV-B in phytochrome-mediated photomorphogenic responses is quite conceivable.

There are several accounts that have witnessed UV-B induced photomorphogenic responses in plants (Tevini and Teramura, 1989; Jansen, 2002; Jenkins, 2009; Jiang *et al.*, 2012; Heijde and Ulm, 2012), although the mechanisms that mediate these responses, and the direct involvement of phytochromes in these effects, are unclear. Kim *et al.* (1998) showed that UV-B at low doses elicited photomorphogenic responses (e.g. inhibition of hypocotyl and stem elongation), while higher doses resulted in damaging effects. They also proposed that either phyA or phyB was required for UV-B-induced elongation inhibition in *Arabidopsis*.

On the other hand, some evidence suggests that phytochromes are not directly involved in UV-B induced inhibition in stem elongation. For instance, no difference in UV-B induced stem elongation response was reported in wild type and phytochrome-deficient

mutants (phyA or phyB) of cucumber and tomatoes (Ballare *et al.*, 1991; Bertram and Lercari, 2000). The findings of Boccalandro *et al.*, (2001) also discerned the existence of a distinct photoreceptor that perceives UV-B signal and enhances a de-etiolation response in *Arabidopsis*. Furthermore, the presence of many photosensors (e.g. phytochromes, cryptochromes and UVR8) with overlapping absorption spectra, and multiple mechanisms of action, make the study of photomorphogenesis more complicated. Therefore, whether it is phytochrome, any other photoreceptor or the interaction between photoreceptors that drives UV-B-induced photomorphogenic responses is still an open question.

13.4.1.2 Chlorophylls, Carotenoids and Other Pigments

UV-B radiation has a great impact on the pigments of plant photosynthetic apparatus – chlorophylls and carotenoids. A marked reduction in chlorophyll *a*, *b* and total chlorophyll ($a + b$) contents due to UV-B radiation was observed in a variety of plant species, including pea (Vu *et al.*, 1982, 1983), pepper (Hoffmann *et al.*, 2015), sweet almond (Ranjbarfordoei *et al.*, 2011), barley, corn, bean and radish (Tevini *et al.*, 1981). Carotenoids, which are considered to be directly associated with the photoprotection of photosynthetic function (Middleton and Teramura, 1993) and are relatively less affected upon UV-B exposure (Pfundel *et al.*, 1992), also recorded a significant decrease in some plant species (Hoffmann *et al.*, 2015; Muzafarov *et al.*, 1995). According to Hideg *et al.* (2013), UV-B radiation generates free radicals that cause degradation of chl *a*, *b* and carotenoids, and leads to the concomitant impairment of photosynthetic machinery.

Other leaf pigments, such as UV-B-absorbing flavonoids (including anthocyanin, flavones and flavonols), are also affected by UV-B. These pigments act as a UV-screen, which protects the photosynthetic apparatus from the damaging effect of UV radiation (Olsson *et al.*, 1999; Feng *et al.*, 2007). UV-B treatment causes a significant increase in flavonoids content (Tevini *et al.*, 1991) by activation of chalcone synthase and 'group I' enzymes involved in the flavonoid synthesis pathway (Wellmann, 1971; Ragg *et al.*, 1981; Chappell and Hahlbrock, 1984; Stafford, 1990).

13.4.2 Photosystem II

Of the two photosystems, PSII is extensively explored, and a detailed description of UV-B effects on PSII is given below. Reports suggest that PSII is the prime site of UV-B-induced damage, whereas PSI is less affected (Kulandaivelu and Noorudeen, 1983; Iwanzik *et al.*, 1983; Renger *et al.*, 1982). Other than PSII, one of the contributing factors to reduced photosynthetic capacity is the structural disturbance to membranes. Studies have demonstrated that dilation of the thylakoid membrane and rupture of the chloroplast double membrane are responsible for alterations in membrane permeability (Brandle *et al.*, 1977; Bornman *et al.*, 1983). Components of PSII which are prime target sites of UV-B in sequential manner are:

1) O_2-evolving complex
2) plastoquinone: the electron acceptor
3) tyrosine residues: the electron donor; and
4) light harvesting system.

This section of the chapter will provide details of UV-B targets of photosystem II and how they are affected.

13.4.2.1 Oxygen-evolving Complex

Studies on PSII membrane fragments of *Spinacea oleracea* displayed that the oxidizing side of PSII, specifically the oxygen-evolving system, is one of the major and foremost targets of UV-B (Renger *et al.*, 1989). Some of the supporting evidences are as follows:

1) Change of the re-reduction kinetics of $P680^+$ from nanoseconds to microseconds suggests reduced donation of electron from the Mn cluster of P680 (Renger *et al.*, 1989; Post *et al.*, 1996; Larkum *et al.*, 2001).
2) Limited e^- transport at the donor side, due to arrested rise in variable fluorescence (Kulandaivelu and Noorudeen, 1983; Iwanzik *et al.*, 1983; Bornman *et al.*, 1984; Tevini and Pfister, 1984).
3) Artificial e^- donors induced reestablishment of PSII activity in PSII centres with depressed O_2-evolving capacity (Bornman *et al.*, 1984; Renger *et al.*, 1989).
4) UV-B induced loss of multiline EPR signal, arising from S2 redox state of Mn cluster (Vass *et al.*, 1995, 1996).
5) One of the persuasive pieces of evidence in support of above is increased stability of TyrZ from microseconds to milliseconds in UV-B-irradiated PSII membrane, which clearly displays that e^- transfer between catalytic Mn cluster and TyrZ is stopped (Vass *et al.*, 1996).

Also, the highly resistant reaction centre of purple bacteria, which lacks a water-oxidizing complex, closely resembles PSII, further confirming the abovementioned findings (Tandori *et al.*, 1996). Thus in the light of the above evidence, it can be concluded that the primary effect of UV-B irradiation is inactivation of the Mn cluster of the water-oxidizing complex.

13.4.2.2 Plastoquinones and Redox-active Tyrosines

Followed by impairment of the O_2 evolving complex, the next targets of UV-B are quinone e^- acceptors and tyrosine donors. Since oxidized PQ absorbs at 250–260 nm, and the action spectrum of PSII damage peaks between these values, this clearly indicates the effect of UV-B on plastoquinones (Jones and Kok, 1966; Bornman *et al.*, 1984; Amesz, 1977; Crane, 1959). A large body of evidence supports UV-B-induced damage of quinones, including:

1) reduced yield of absorption change at 263 nm (Melis *et al.*, 1992);
2) loss of flash-induced Chl*a* fluorescence (Tevini *et al.*, 1988; Yerkes *et al.*, 1990); and
3) decrement in extent of flash induced absorption change at 320 nm (Iwanzik *et al.*, 1983; Renger *et al.*, 1986; Melis *et al.*, 1992).

Similar to plastoquinones, tyrosines absorb at approximately 280 nm in the neutral form and between 250–300 nm in the oxidized form, which values lie in the UV-B range (Diner and Vitry, 1995; Dekker *et al.*, 1984; Gerken *et al.*, 1988). Damage to the redox function of TyrZ and TyrD is indicated by loss of EPR signals arising from them (Vass *et al.*, 1995,1996; Yerkes *et al.*, 1990).

Although the abovementioned studies provide information regarding UV-B-induced damage of the photosynthetic apparatus, for clear understanding, a proper mechanism underlying UV-B induced damage is required, which is explained here in brief. The foremost action of UV-B irradiation is inactivation of the O_2-evolving complex, which is attributed to changes in the protein-binding site of the catalytic Mn cluster. S state (oxidation state of water oxidizing complex) dependence is observed from experiments and, specifically, S_2 and S_3 are the most sensitive ones. Higher UV-B sensitivity of S_2 and S_3 is indicative of Mn(III) and Mn(IV) as 1° sensors of UV-B-induced damage (Vass *et al.*, 2001). Furthermore, UV-B-induced splitting of disulfide bridges in the 33 Kda water-soluble protein subunit of the water-oxidizing complex suggests another possibility for Mn site inactivation (Ferreira *et al.*, 2004; Ono and Inoue, 1984; Vass *et al.*, 1992; Creed, 1984). Nothing is known about the mechanism behind the degradation of quinones and tyrosines, but a direct destruction of these molecules could lead to their inactivation.

13.4.2.3 D1 and D2 Proteins

One of the significant impacts of UV-B is the destruction of the PSII reaction centre protein complex, specifically the core component (i.e. D1 and D2 subunits). Both *in vivo* as well as isolated thylakoid preparations demonstrate UV-B induced damage to D1 and D2 subunits (Barbato *et al.*, 1995; Greenberg *et al.*, 1989a, 1989b; Jansen *et al.*, 1993a, 1993b; Melis *et al.*, 1992; Trebst and Depka, 1990; Friso *et al.*, 1993, 1994, 1995; Spetea *et al.*, 1995, 1996).

The UV-B-induced damage site of D1 is likely to be located at the middle, or close to the lumenal end, of the second transmembrane helix (Friso *et al.*, 1993), which is found to be in close association with the putative binding site of the catalytic cluster of water oxidation (Svensson *et al.*, 1990; Zouni *et al.*, 2001; Kamiya *et al.*, 2003). The UV-B induced damage of D2 protein is not explored to the extent that of D1 has been. Since D2 degradation was not seen in an isolated PSII reaction centre complex lacking Q_A, Q_A seems to be a plausible sensitizer of D2 degradation (Friso *et al.*, 1994). Moreover, visible light accelerates degradation of D2 protein, which is attributed to an enhanced reduction level of Q_A (Jansen *et al.*, 1996). It is noteworthy that UV-B induced protein degradation does not involve proteases in UV-B mediated polypeptide cleavage.

13.4.3 Photosystem I

Uneven distribution of effect of UV-B irradiation has been demonstrated by various studies to display minor or no effects on PSI (Kulandaivelu and Noorudeen, 1983; Iwanzik *et al.*, 1983; Turcsányi and Vass, 2001; Brandle *et al.*, 1977), which is possibly due to lack of a water-oxidizing complex in PSI, and absence of redox-active tyrosine (Hansson and Wydrzynski, 1990). Insignificant decrease in PSI activity is an indication of acclimation response, as it readjusts the PSI/PSII ratio upset by UV-B induced damage of PSII.

13.4.4 Cytochrome b6/f Complex, ATP Synthase and Rubisco

Cytochrome b6/f complex is the thylakoid component least affected by UV-B irradiation (Strid *et al.*, 1990), while ATP synthase and Rubisco are the most adversely affected components of the thylakoid membrane. Not only the amount, but also activity of ATP

synthase, is reported to be reduced in pea plants during UV-B irradiation (Zhang *et al.*, 1994). Similar results are found for Rubisco, which shows declined activity as well as decreased amounts of both subunits and corresponding mRNA levels (Vu *et al.*, 1983; Jordan *et al.*, 1992).

13.4.5 Net Photosynthesis

In addition to photosynthetic apparatus, the effect of UV-B irradiation on different aspects of net photosynthesis has been explored. Studies have demonstrated reduction in net photosynthesis (measured as CO_2 uptake), decreased leaf transpiration (Teramura *et al.*, 1980), reduced dry weight and total chlorophyll in *Glycine max*, *Avena sativa* and so on (Basiouny *et al.*, 1978). Reduced O_2 evolution (measure of net PS) and reduction in organic acids and soluble sugars are indicative of UV-B sensitivity of 1° carbon metabolism. In UV-B exposed seedlings, glycerate, succinate and fumarate were the significantly reduced organic acids whereas fructose, glucose and sucrose were the significantly affected soluble sugars (Takeuchi *et al.*, 1989).

13.5 Conclusion and Future Perspectives

A generalized model (Figure 13.1) clearly displays the major photosynthetic targets of UV-B irradiation in photosynthetic organisms, ranging from primitive cyanobacteria to higher plants. During the last two decades, rigorous research has resulted in a substantial increment in our knowledge of physiological, as well as molecular, aspects of UV-B-induced effects on photosynthesis. However to gain insight into complex interaction of UV-B with various other stresses, such as salinity, temperature, metal and so on, further research is required. Elucidation of the molecular mechanism involved in protection of photosynthesis will increase our understanding why certain species are capable to withstand enhanced UV-B.

One more important perspective that needs to be investigated is the significance of UV-B damage exerted on the photosynthetic apparatus, in relation to damage caused at the level of nucleic acids. It is noteworthy that most of the UV-B responses are investigated under lab conditions. This situation questions whether or not similar changes in gene/protein expression occur under natural conditions after exposure to UV-B radiation. Hence, the search must continue further, to completely explore UV-B-induced global effects.

Acknowledgements

LC Rai is thankful to DST for the project and JC Bose National Fellowship and DAE for Rajaramanna fellowship. Alok Kumar Shrivastava is thankful to ICAR for RA, Chhavi Agrawal to DBT for SRF, Sonia Sen to CSIR for SRF, Antra Chatterjee and Shivam Yadav to UGC for JRF, and Shweta Rai to DST for financial support. Ruchi Rai and Shilpi Singh thanks DST for WOSA.

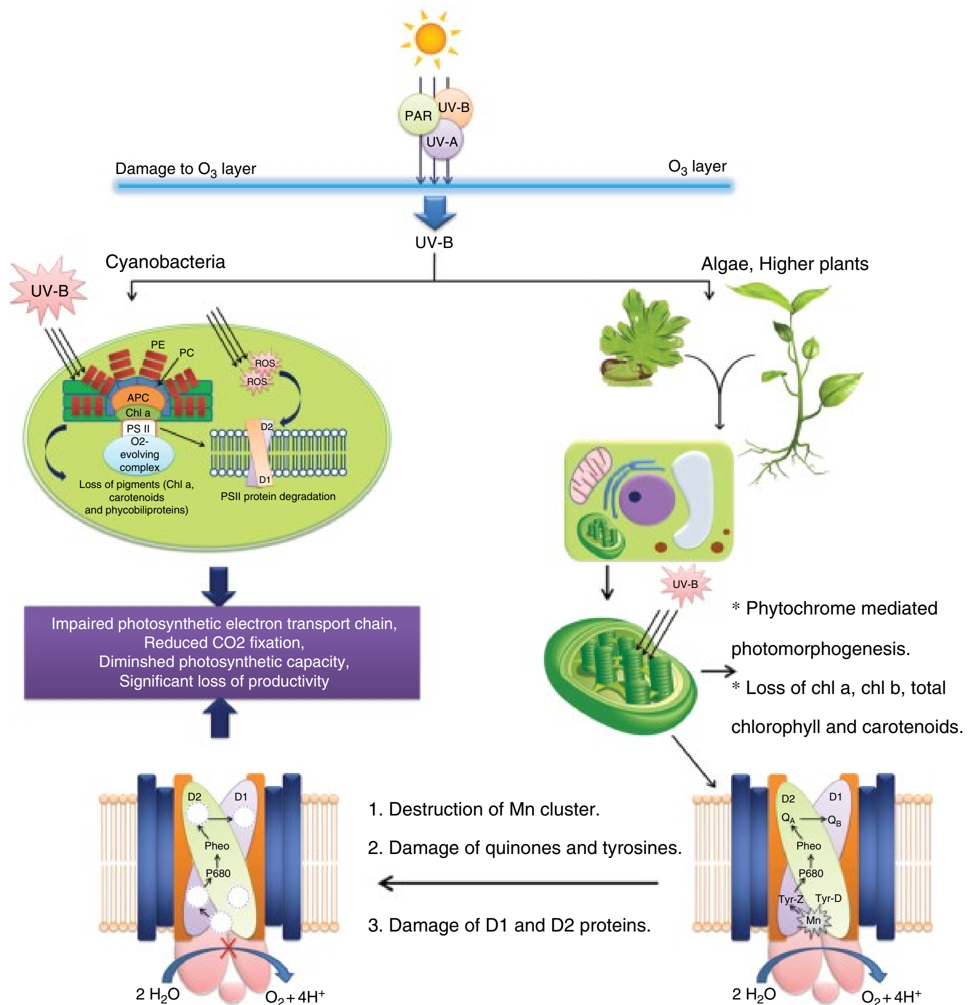

Figure 13.1 A generalized model for UV-B irradiation effects on photosynthetic machinery.

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14

Discovery of UVR8: New Insight in UV-B Research

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14.1 Introduction

Despite being a minor component (<0.5% of solar energy at the earth's surface) of sunlight, UV-B radiation has a significant impact on all living organisms that can be attributed to its relatively high energy (Caldwell *et al.*, 1998, 2007). UV-B is widely known to cause macromolecular destruction and to severely inhibit life processes. For example, in plants, reports suggest that UV-B irradiation can cause DNA damage, generate ROS and impair photosynthetic processes (Dai *et al.*, 1997; Jansen *et al.*, 1998; Brosché and Strid, 2003; Jenkins and Brown, 2007). Being sessile, and having an obligate necessity for sunlight for photosynthesis, exposure of plants to UV-B is inescapable. Thus, plants have evolved various strategies to avoid UV-B and repair damage.

Apart from this, studies suggest that UV-B is not solely a damage-causing agent. UV-B is also involved in some developmental processes and UV-protective responses, by acting as an information signal (Jansen *et al.*, 1998; Frohnmeyer and Staiger, 2003; Paul and Gwynn-Jones, 2003; Ulm and Nagy, 2005; Jenkins and Brown, 2007). Wellmann (1976, 1983) discovered that low doses of UV-B induces photomorphogenic responses that could not be explained by the action of known photoreceptors.

One of the studied photomorphogenic responses to UV-B is the biosynthesis of flavonoid compounds, one of the components of UV- absorbing sunscreen (Caldwell *et al.*, 1983; Rozema *et al.*, 1997; Winkel-Shirley, 2002). Studies of UV-B-mediated photomorphogenic responses prompted researchers to investigate UV-B photoreceptors. However, research over several decades was unable to find any of the UV-B photoreceptors and, thus, the UV-B signalling mechanism remained unknown. Kliebenstein *et al.* (2002) applied the genetic approach and isolated for the first time an *Arabidopsis thaliana* mutant of UV resistance locus (UVR8), which proved to act as a UV-B photoreceptor (Rizzini *et al.*, 2011).

In view of the above, the present chapter focuses on facts about the discovery of UVR8 and reviews in brief about its structure and physiological roles.

UV-B Radiation: From Environmental Stressor to Regulator of Plant Growth, First Edition. Edited by Vijay Pratap Singh, Samiksha Singh, Sheo Mohan Prasad and Parul Parihar.
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14.2 Photoperception in Plants

Plants are known to use multiple sensory proteins in order to respond to environmental stimuli, each being specific for a certain stimulus. For light-sensing, there exists a wide variety of highly sophisticated and sensitive photoreceptors that can perceive even trivial changes in light quality.

The first photoreceptor identified was a red, far-red reversible chromoprotein – phytochrome. Subsequently, a blue light receptor cryptochrome was identified that mediates various responses. Plants employ these photoreceptors for specific light perception, so as to optimize photosynthetic processes by regulating phototropism, stomatal conductance, chloroplast distribution and so on in response to weak or strong light intensity.

Arabidopsis possess around 13 photoreceptors for light-sensing, each being specific for a particular wavelength. These include five red/far-red perceiving phytochromes (phy A-E), two cryptochromes (cry1 and cry2), two phototropins (phot1 and phot2) and three members of the Zeitlupe family (ZTL, FKF1 and LKP2) to perceive blue light (Kami *et al.*, 2010; Heijde and Ulm, 2012).

14.3 Discovery of UVR8: UV-B Photoreceptor

The ability of plants to specifically perceive UV-B photons prompted researchers to explore the perception mechanism behind exact discrimination of UV-B from other light qualities. However, UV-B induced responses have been observed in different plant species (Ballaré *et al.*, 1991, 1995; Beggs and Wellmann, 1994; Frohnmeyer *et al.*, 1999; Boccalandro *et al.*, 2001; Brosche *et al.*, 2002; Brown *et al.*, 2005), and the nature of UV-B perception was uncertain for many years. Continuous research finally resulted in identification of UV-B photoreceptor UVR8, after application of the genetic approach. Kleibenstein *et al.* (2002) isolated a UVR8 mutant, which was later demonstrated to act as a UV-B photoreceptor (Rizzini *et al.*, 2011).

The research was undertaken jointly by between researchers and scientists at the University of Glasgow and at the Scripps Research Institute in California. Initially, the Arabidopsis UVR8-1 mutant was isolated during a screen for plants displaying hypersensitivity to UV-B. These mutants displayed altered gene regulation following UV-B exposure. A decreased abundance of genes encoding flavonoid biosynthesis enzyme CHS (chalcone synthase) and reduced levels of protective flavonoids was observed in the mutants.

Based on the phenotypic observation, Kleibenstein *et al.* (2002) suggested that UVR8 probably has a role in UV-B signalling. Various studies were conducted to identify UV-B photoreception (Brown *et al.*, 2005; Oravecz *et al.*, 2006; Favory *et al.*, 2009), but none of them provided convincing evidence. Rizzini *et al.* (2011) were the first to observe UV-B-induced dissociation of homodimer to monomers, accompanied by some conformational changes which expose an epitope present at the C-terminus.

Furthermore, co-immuniprecipitation and biomolecular fluorescence complementation studies proved the interaction of UVR8 with COP1, a component of an E3 ubiquitin ligase complex and primary signalling partner of UVR8. Rizzini *et al.* (2011) also

showed that this interaction was UV-B dependent. The abovementioned evidences, however, supported the hypothesis, but were not able to prove it convincingly.

In order to get substantial evidence, UVR8 must mediate UV-B responses in heterologous systems. Thus, both proteins (UVR8 and COP1) were expressed in yeast and mammalian cells, where UVR8 displayed interaction with COP1 in a UV-B-dependent manner (Rizzini *et al.*, 2011; Cloix *et al.*, 2012; Crefcoeur *et al.*, 2013).

The abovementioned studies together provided persuasive evidences in order to prove that UVR8 is a photoreceptor involved in UV-B sensing.

14.4 UVR8 Structure

UVR8 from *Arabidopsis* is a 440 amino acid seven-bladed β-protein with a molecular mass of ≈ 47 Kd (Christie *et al.*, 2012; Wu *et al.*, 2012). Biochemical and molecular studies performed on purified recombinant protein expressed in *E. coli* demonstrated that it exists as homodimer, and undergoes quick monomerization following UV-B exposure (Christie *et al.*, 2012; Wu *et al.*, 2012). Two independent research groups resolved the crystal structure of the β-propeller core of UVR8 (Christie *et al.*, 2012; Wu *et al.*, 2012), and provided a detailed mechanism behind UVR8-mediated UV-B perception.

14.4.1 Salt Bridge Interactions Mediate UVR8 Dimerization

The interface of the UVR8 dimer is the crucial region responsible for the interaction of two monomers. Localisation of charged amino acid residues at the dimer interface creates areas of balancing electrostatic potential on the opposing monomers. Amino acids dominating this interface – basic Arg and acidic Asp and Glu – create a web of salt bridges that hold the monomers. Out of various amino acids interaction, double hydrogen bonded interactions are more critical, such as Arg 286 with Asp 107. Similarly, the double hydrogen bonded salt bridge between R-146 and Glu-182 holds significance in view of UVR8 dimerization (Christie *et al.*, 2012; Wu *et al.*, 2012).

14.4.2 Chromophore and Key Tryptophan Residues

To perform photoreception, a light-reactive chromophore is required. In general, photoreceptors possess bound cofactors as chromophores, but UVR8 does not have a prosthetic cofactor, and employs specific amino acid residues for UV-B perception. Arabidopsis UVR8 has 14 Trp residues for photoreception – one in the C- terminal region (W400), six in the β-propeller core of the protein, and seven in the dimer interface (Figure 14.1). The six Trp residues (W39, W92, W144, W196, W300 and W352) of the β-propeller core are each located on different blades of the propeller and, along with a Tyr (Y248), form a ring of aromatic residues. This structure ultimately leads to the formation of hydrogen bonds and hydrophobic interactions between adjacent blades, and maintains the core structure. Three residues (triad) (W233, W285, W337) of the seven residues located in the dimer interface are highly conserved, and are positioned in such a way as to allow overlapping of electronic orbitals. In the vicinity of the triad on the opposite monomer, W94 is present, creating a cross-dimer pyramid arrangement, and each dimer contains two such dimers (Christie *et al.*, 2012).

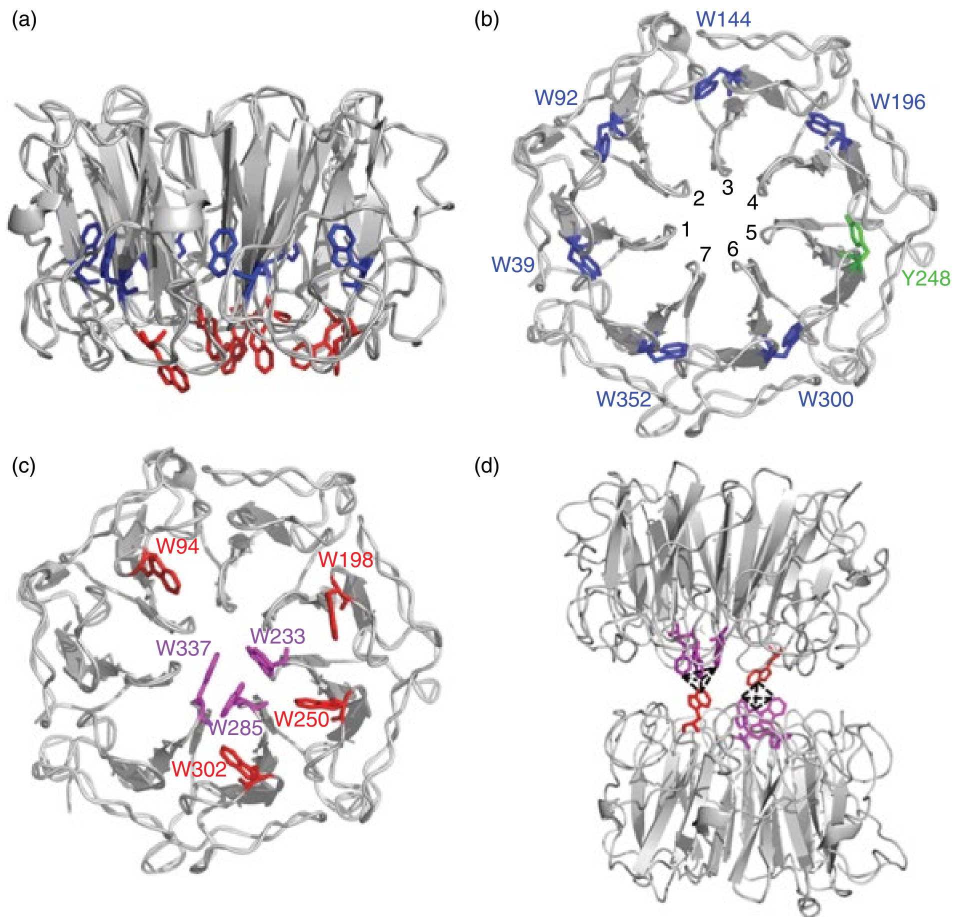

Figure 14.1 UVR8 structure and distinct groups of Trps.
(A) The arrangement of all UVR8 tryptophan residues (Trp) (except W400) in the monomer (side view). Trps in the protein core and at the dimer interaction surface are shown in blue and red, respectively.
(B) The Trps in the core (dimer interaction surface view). Each Trp is associated with a different propeller blade (numbered).
(C) The Trps at the dimer interaction surface. (Triad-magenta)
(D) Two pyramid clusters of excitonically coupled Trps in UVR8 dimer, each consisting of the triad Trps together with W94 on the opposing monomer.From O'Hara and Jenkins (2012).

Similar to the core arrangement, the dimer interface also contains an aromatic shield, formed by the remaining three Trp amino acids of the dimer interface (W198, W250 and W302), along with Tyr residues Y201, Y253 and F305, although its functional importance is not yet known. Wu *et al.* (2012) performed a fluorescence emission experiment in wild and various Trp residue mutants ($UVR8^{W285A}$,$UVR8^{W285F}$,$UVR8^{W233F}$, $UVR8^{W233A}$,$UVR8^{W337F}$ and $UVR8^{W9F}$), and found W285 and W233 as the chief chromophores. Circular dichroism spectroscopy results also supported these findings (Christie *et al.*, 2012).

In summary, combining studies on UVR8 to the present date, we find that Trp-285 and Trp-233 are key chromophore components responsible for UV-B sensing. Dimer dissociation is a result of destabilization of intermolecular hydrogen bonds, due to disrupted cation-pi interaction between Trp-285, Trp-233 and surrounding residues.

14.5 Physiological Roles of UVR8

Although UVR8 functions are yet not completely explored, physiological responses elicited by UVR8 are summarized in table 14.1. Major ones are discussed in this section.

14.5.1 Photomorphogenic Response Regulation by UVR8

UV-B elicits photomorphogenic responses (hypocotyl extension, cotyledon opening and phototropism) at low fluence rates in seedlings (Ballaré *et al.*, 1995; Kim *et al.*, 1998; Eisinger *et al.*, 2003; Conte *et al.*, 2010), as well as in older plants (leaf expansion, stem elongation and branching) (Hectors *et al.*, 2007; Wargent *et al.*, 2009). Several research groups worked on regulation of UV-B responses, and found that, at least in part, UVR8 signalling is involved in these responses. Reports suggest that UVR8 is involved in hypocotyl extension, leaf expansion and enhances stomatal index in *Arabidopsis*, thus confirming its role in photomorphogenesis (Favory *et al.*, 2009; Wargent *et al.*, 2009). However the mechanism behind these responses is yet unclear. De Veylder *et al.* (2011) proposed UVR8-regulated endoreduplication control as one of the possible mechanisms. However, extensive research in this direction is required.

Table 14.1 Physiological responses mediated by UVR8.

No.	Physiological response	References
1.	UV-B tolerance	Kliebenstein *et al.*, 2002; Brown *et al.*, 2005; Brown and Jenkins, 2008; Favory *et al.*, 2009
2.	Hypocotyl growth inhibition	Favory *et al.*, 2009 ; Cloix *et al.*, 2012; Huang *et al.*, 2013
3.	Gene regulation	Kliebenstein *et al.*, 2002; Brown *et al.*, 2005; Brown and Jenkins, 2008; Favory *et al.*, 2009; Grüber *et al.*, 2010; Fehér *et al.*, 2011; Morales *et al.*, 2013
4.	Flavonoid biosynthesis	Kliebenstein *et al.*, 2002; Favory *et al.*, 2009; Grüber *et al.*, 2010; Morales *et al.*, 2013; Demkura and Ballaré, 2012
5.	Leaf expansion	Favory *et al.*, 2009; Morales *et al.*, 2013; Wargent *et al.*, 2009
6.	Endoreduplication in epidermis	Wargent *et al.*, 2009
7.	Light entrainment of circadian clock	Fehér *et al.*, 2011
8.	Enhanced photosynthetic efficiency	Davey *et al.*, 2012
9.	Plant pathogen cross-resistance	Demkura and Ballaré, 2012
10.	Reduced plant herbivory	Demkura *et al.*, 2010; Ballaré *et al.*, 2012

14.5.2 Regulation of Flavonoid Biosynthesis

Flavonoids, specifically colourless flavonols, are widely known for their absorption properties. Chalcone synthase (*CHS*) is one of the crucial biosynthetic enzymes of the flavonoid biosynthesis pathway, and its increased level is seen during UV-B irradiation (Jenkins, 2008). Various studies demonstrated that an increase in abundance of *CHS* under UV-B is UVR8 -, COP1- and HY5- dependent (Kleibenstein *et al.*, 2002; Brown *et al.*, 2005; Favory *et al.*, 2009; Stracke *et al.*, 2010).

However, the exact mechanism of transcriptional control over *CHS* in response to UV-B is unknown. HY5 is known to directly bind to *CHS* promoter, and also to the promoter elements of the UV-B-activated MYB12 gene (Lee *et al.*, 2007; Stracke *et al.*, 2010), and UV-B activation of MYB12 is UVR8-, COP1- and HY5- dependent (Oravecz *et al.*, 2006; Favory *et al.*, 2009; Stracke *et al.*, 2010). Likewise, UVR8 also binds to MYB12 promoter regions (Cloix and Jenkins, 2008). Altogether, this suggests UVR8 and HY5-mediated regulation of MYB12 and, thus, flavonoid biosynthesis.

14.5.3 Plant-Pathogen and Plant-Herbivore Interactions

Recent studies have suggested that light, including UV-B, is involved in plant immune responses against pathogens and herbivores. UV-B is known to confer cross-resistance to a fungal pathogen in *Arabidopsis*, and the role of UVR8 in this phenomenon has been proposed (Demkura and Ballaré, 2012). UVR8-mediated UV-B perception induces flavonoid and sinapates accumulation, but only sinapate is involved in cross-resistance against pathogens (Demkura and Ballaré, 2012). Thus, cross-resistance against pathogens can be partially attributed to accumulation of phenylpropanoids.

Similarly, UV-B is also seen to reduce plant herbivory by insects (Kuhlman and Müller, 2011; Ballaré *et al.*, 2012). UVR8 is reported to be probably involved in reduced herbivory, as low UV-B irradiances induces these effects (Demkura *et al.*, 2010; Ballaré *et al.*, 2012).

14.6 Conclusion and Future Perspectives

The discovery of UVR8 as a UV-B photoreceptor enhanced our understanding regarding plant UV-B responses, and begun a new era in UV-B perception research. However, the UVR8 story is still in its initial phase and needs to be extensively explored. Studies in last few years have succeeded in exploring the mechanistic details of UV-B perception and signalling mediated by UVR8. UV-B perception through UVR8 and further UVR8-COP1 interaction is the crucial and central phenomenon of UV-B signalling. However, although these studies have answered many questions, they have simultaneously raised various questions such as: whether there are other photoreceptors in addition to UVR8? How is gene expression regulated via UVR8? Whether and how UVR8 signalling is linked with UVR8 mediated other responses? Answers to these questions will definitely provide a better understanding of the captivating story of UV-B perception and signalling in plants.

Additionally, application-based potentials of UVR8 also needs to be investigated. Alterations in UVR8 properties and amounts might be helpful in crop improvements, specifically in the production of abiotic stress-tolerant crop species. The function of

UVR8 as an optogenetic light switch can be utilized in the field of optogenetics, and can be applied in synthetic biology and medicine.

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15

UVR8 Signalling, Mechanism and Integration with other Pathways

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15.1 Introduction

Development of plants under natural condition is often interrupted by episodic situations of environmental stresses, or cues which prompt them to evolve numerous strategies to exist under such adverse conditions. Each source of stress prompts a particular response that includes a specified set of signalling factors for the perception of stress, transcriptional regulators and downstream responsive genes for stress acclimation and tolerance. To some extent, a crosstalk occurs between the networks responsive to stress, and the metabolic and developmental pathways of the plant.

Plants use a wide variety of extremely sensitive and complex photoreceptors to cope with alterations in light quality, quantity, direction and duration. UV-B, a part of the solar spectrum, contributes less than 0.5% of solar energy at the earth's surface (Blumthaler, 1993), which generally depends on factors such as altitude, latitude, stratospheric ozone, solar angle and troposphere pollution (McKenzie *et al*, 2007; Paul and Gwynn- Jones, 2003). As a potential abiotic stress factor, it has significant biological effects arising from reactive oxygen species generation and DNA damage. However, at a low fluence rate, it serves as an environmental stimulus, mediating important physiological responses in plants such as photomorphogenesis, photo-protection, circadian rhythm and many more.

UVR8 (UV Resistance Locus 8) was identified as a distinct photoreceptor responsible for UVB responses in plants. However, before the identification of UVR8 as the UV-B photoreceptor, UV-B perception in plants was not very clear. It has been suggested that, before the formation of the dioxygen-rich and ozone-containing stratosphere, UVR8 appeared in plants to assist their survival against the UV-B fraction of sunlight (Kaiserli and Jenkins, 2007; Rizzini, *et al.*, 2011). An action spectrum for UVR8 function revealed that although the major UV absorption of the UVR8 protein is around 280 nm, and the most physiologically pertinent responses are induced by absorption occurring with a minor absorption peak at ≈ 300 nm.

UV-B Radiation: From Environmental Stressor to Regulator of Plant Growth, First Edition. Edited by Vijay Pratap Singh, Samiksha Singh, Sheo Mohan Prasad and Parul Parihar.
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UVR8 is a β-propeller protein, in which intrinsic Trp residues are the basis of UV-B photoreception (Christie *et al.*, 2012; Liu *et al.*, 2014). The 440-residue-long UVR8 from *Arabidopsis* exists as a stable homodimer, but dissociates into monomers after UVB irradiation (Rizzini *et al.*, 2011; Wu *et al.*, 2012; Christie *et al.*, 2012) and triggers signal transduction, leading to altered gene expression, followed by acclimation responses. Apart from its reported presence throughout plant bodies (Rizzini *et al.*, 2011), the UVR8 protein is mostly found to be located in the cytoplasm and a little amount in the nucleus, even in the absence of UV-B. When plants are exposed to UV-B, UVR8 accumulates within minutes in the nucleus, while a large amount of UVR8 remains in cytoplasm (Kaiserli and Jenkins, 2007). UV-B-specific responses can be in deployed as required in plants, which is the fundamental advantage of the UVR8-mediated UVB perception and signalling pathway.

15.2 UVR8-Arbitrated Signalling

The dimer of UVR8 becomes monomerized after perception of UVB, and the signalling pathway begins, and transducing this primary event into appropriate changes in gene expression. UV-B perception is translated into plant photomorphogenesis and UV-B acclimation via molecular signalling, with the help of UVR8. Very little information is available on molecular players associated in UVR8-mediated UV-B signal transduction. However, there is firm evidence that COP1, FHY3 and HY5 are three common elements in light signalling which play key role in promoting UV-B-induced photomorphogenesis. Transcription factors Far-Red Elongated Hypocotyl 3 (FHY3), Constitutively Photomorphogenic 1 (COP1) and Elongated Hypocotyl 5 (HY5) (Stracke *et al.*, 2010; Huang *et al.*, 2012; Binkert *et al.*, 2014) and the negative regulators Repressor of UV-B Photomorphogenesis 1 (RUP1) and RUP2 (Gruber *et al.*, 2010; Heijde and Ulm, 2013) are associated with the UVR8 signalling pathway. Figure 15.1 depicts the UVR8-mediated signal perception and its positive and negative feedback regulation.

15.2.1 Constitutively Photomorphogenic 1 (COP1)

COP1 has been reported to work as an E3 ubiquitin ligase, and it assists in selection of protein ubiquitination and degradation (Lau and Deng, 2012). This phenomenon alters a lot of light signalling proteins, together with the HY5 (transcription factor) for suppressing seedling photomorphogenesis in darkness. Due to this, COP1 mutant seedlings, known as constitutively photomorphogenic, display a light-grown phenotype, even when developed in darkness (Deng *et al.*, 1991). In contrast to the role of COP1 as an E3 ubiquitin ligase, UVR8-COP1 interaction does not lead to degradation of UVR8 (Favory *et al.*, 2009).

Interestingly, UV-B exposure is acknowledged to increase intensity of COP1 protein at the post-transcriptional stage in a UVR8-dependent fashion, possibly because of diminished autoubiquitination of COP1 (Favory *et al.*, 2009). In addition, transcription factors FHY3 and HY5 act in UVB-mediated UVR8 signalling perception by enhancing the COP1 transcript under UV-B (Huang *et al.*, 2012). Because UVR8-COP1 interaction is seen only upon UV-B exposure (Rizzini *et al.*, 2011; Cloix *et al.*, 2012), and accumulation of COP1 in the nucleus follows UV-B exposure, it can be suggested that COP1 interacts only with the UVR8 monomer, and that UV-B signalling takes place in the nucleus (Oravecz *et al.*, 2006; Favory *et al.*, 2009).

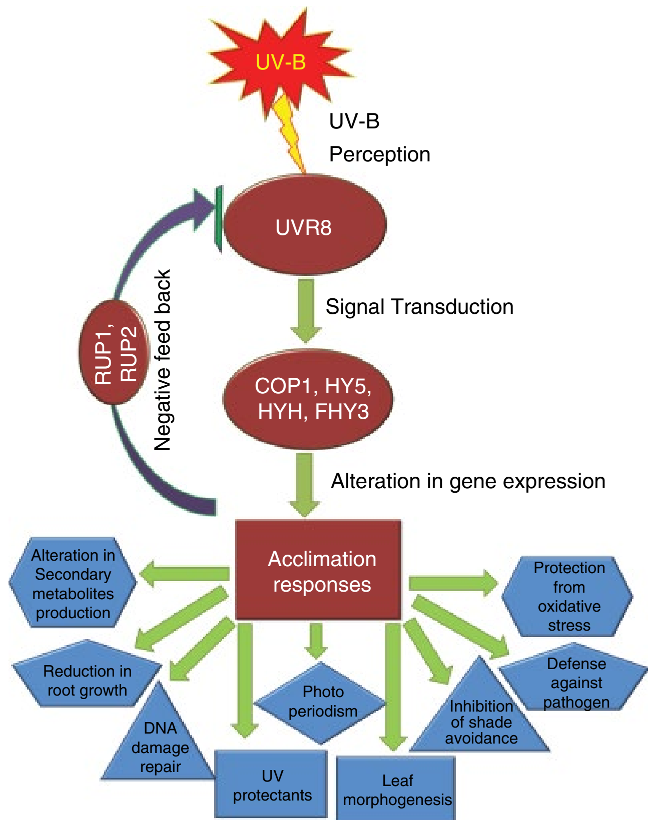

Figure 15.1 Diagrammatic representation of UVR8-mediated UVB perception, signalling and physiological responses (modified from Tilbrook *et al.*, 2013).

As stated above, transcription factor FHY3 participates in expression of *COP1* under UV-B, and it has been found that the expression of FHY3 is repressed by far-red light (Lin *et al.*, 2007) and induced by UV-B (Huang *et al.*, 2012). FHY3 positively regulates expression of COP1 gene in the presence of UVR8. Mutants of FHY3 show downregulated expression of the UV-B-induced gene and inhibited physiological responses (Huang *et al.*, 2012). The binding site for FHY3 on the COP1 promoter has been demonstrated both *in vitro* as well as *in vivo*, which is adjacent to the binding site for HY5 (Huang *et al.*, 2012).

15.2.2 Elongated Hypocotyl 5 (HY5) and HYH

Transcription factor HY5 plays a significant role in UV-B signalling (Stracke *et al.*, 2010; Huang *et al.*, 2012). HY5 responds to a wide range of light to alter the expression of the light-responsive gene. The primary importance for HY5 in UV-B signalling was anticipated once HY5 was identified as one of many genes subject to expression induction upon UV-B exposure (Ulm *et al.*, 2004). UVR8- and COP1-dependent UV-B induction of HY5 expression has been demonstrated several times (Brown *et al.*, 2005; Favory *et al.*, 2009). Interestingly, it has been found that UVR8 has association with chromatin adjacent to HY5 genomic locus (Brown *et al.*, 2005; Cloix and Jenkins, 2008).

Successive experiments have demonstrated that, in *hy* mutant, under UV-B exposure, a set of UV-B responsive genes were not expressed (Brown and Jenkins, 2008; Stracke *et al.*, 2010). The central role of HY5 in the UV-B acclimation response is further highlighted by the UV-B stress hypersensitivity of *hy5* seedlings (Oravecz *et al.*, 2006; Huang *et al.*, 2012). it was found that HY5 interacts with a positive regulator of photomorphogenesis HY5 Homolog (HYH) (Holm *et al.*, 2002). This suggests the involvement of HYH in UVR8-mediated UV-B signalling.

HY5 and HYH are frequently mentioned as regulating the majority of the UV-B transcriptional response (Brown and Jenkins, 2008). The function of HY5 in UVR8-mediated UV-B signalling is most important, as it is required for COP1 and proteasome-mediated degradation in darkness (Osterlund *et al.*, 2000). Under UV-B, however, COP1 is required for HY5 expression induction (Oravecz *et al.*, 2006). Furthermore, after HY5 expression is induced, it is involved in a positive feedback loop promoting *COP1* expression, by binding to one out of three ACGT-containing elements (ACEs) present within the promoter of COP1 (Huang *et al.*, 2012).

HY5 plays a major role in light responses of young seedlings, and the importance of this role is reduced in adult plants (Hardtke *et al.*, 2000). This functional shifting is confirmed by the presence of higher amounts of HY5 in the seedlings, compared with mature plants. Thus, an additional distinctive attribute of HY5 is its reengagement, maintaining a functional significance even in older seedlings and mature plants in UVR8-mediated UV-B signalling (Ulm *et al.*, 2004; Oravecz *et al.*, 2006).

15.2.3 Repressor of UV-B Photomorphogenesis 1 (RUP1)and RUP2

Signalling pathways, by and large, include negative feedback loops. The importance of a negative feedback loop in UV-B signalling is highlighted by the dwarf and overly-photomorphogenic phenotype of *Arabidopsis* UVR8 overexpression plants, when they are grown under sun-simulating conditions (Favory *et al.*, 2009). UV-B induced proteins RUP1 and RUP2 are highly homologous to WD40-repeat proteins, and negative regulators of UVR8-mediated UV-B signalling (Gruber *et al.*, 2010). RUP1 and RUP2 are phylogenetically linked to light signalling components COP1 and the SPA proteins (Gruber *et al.*, 2010).

Under UV-B, expression of RUP1 and RUP2 is provoked in a UVR8-, COP1-, and HY5-dependent manner (Gruber *et al.*, 2010). Overexpression of RUP1- and RUP2-promotes early flowering and stops the inhibition of hypocotyl growth under UV-B minus light conditions, regardless of whether plants are grown under short-day or long-day photoperiods (Wang *et al.*, 2011). Overexpression of RUP2 reduces UV-B-induced photomorphogenesis and puts off UV-B acclimation (Gruber *et al.*, 2010), which agrees with the role of RUP1 and RUP2 as negative regulators of UV-B signalling. Furthermore, induction of UV-B responsive gene HY5 and CHS is higher in *rup1 rup2* seedlings (double mutant of RUP1 and RUP2 genes *Arabidopsis*), and demonstrates enhanced UV-B tolerance. This implies a UV-B over-responsiveness in *rup1 rup2* plants, and highlights the important roles that RUP1 and RUP2 play in achieving a balance between UV-B responses and plant growth (Gruber *et al.*, 2010).

Both transcription factor RUP1 and RUP2 have shown their direct interaction with UVR8 (Gruber *et al.*, 2010). Interaction of UVR8-RUP1/RUP2 increases under UV-B, because of induction of RUP1 and RUP2 expression and protein accumulation (Gruber

et al., 2010). That reversion of UVR8 to the dimer form is faster *in vivo* than *in vitro* is well known after post-UV-B exposure (Heijde and Ulm, 2013; Heilmann and Jenkins 2013).

A most important role of RUP1 and RUP2 in UVR8 redimerization was found which is independent of COP1 (Heijde and Ulm, 2013). In double mutant *rup1 rup2*, a block in redimerization of UVR8, interaction of UVR8-COP1 remains longer after UV-B exposure (Heijde and Ulm, 2013). Hence, RUP1 and RUP2 serve as negative regulators of UV-B signalling by assisting UVR8 redimerization after UV-B exposure, which further interrupts the key interaction of UVR8 with COP1 (Heijde and Ulm, 2013).

15.3 Molecular Mechanism of Photoreceptor-Mediated Signalling

The explicit molecular mechanism of the UV-B-induced reaction is as yet unclear, but different authors have tried to unravel the mechanism behind UVR8-mediated UV-B signalling.

Christie *et al.* (2012) studied UVR8 homodimer using crystallographic and solution structure, along with mutagenesis and far-UV circular dichroism spectroscopy, which further unveiled the mechanisms of UVR8-mediated UV-B perception and signal transduction. It was proposed that β-propeller subunits constitute the multitude of tryptophan residues, forming a dimer interface bound together by a complex salt-bridge network. A Trp pyramid, formed by Trp-233, Trp-285, Trp-337 and Trp-94 (tryptophan intrinsic to UVR8), provide a 'UV-B antenna' involved in UV-B sensing. Perception of a UV-B signal results in effective transfer of an excited electron from the excitonically coupled Trp pyramid to adjacent arginine(s), leading to charge neutralization, consequent breakage of cross-dimer salt bridges and, thus, dimer destabilization and dissociation.

These findings revealed that the Trp pyramid is important for UVR8 photoperception, and W285 emerged as a major UV-B sensor. Apart from this, W233 is a key player in photoreception, especially maintaining excitation coupling. On the other hand, W337 and W94 play auxiliary roles. A conserved Gly-Trp-Arg-His-Thr sequence repeat generates a 'triad' of closely packed tryptophans – W233, W285 and W337 – which are implicated in UVR8 photoreception. W285 piles with adjacent R286, which is found to be important for dimerization. ThisW285-R286 serves as a link between UV-B photoreception and salt-bridge status. In conclusion, the complex packing assembly of the conserved aromatic cluster surrounding the Trp pyramid, and the interconnectivity of the conserved salt bridges that zip together the dimer interface, suggest that UVR8 has evolved a robust, concerted mechanism for UV-B perception and signalling.

Wu *et al.* (2012) performed structural and biochemical analyses to reveal the mechanism for ultraviolet-B sensing by UVR8. Observations revealed that UVR8 uses two tryptophan residues, Trp 285 and Trp 233, as the chromophore for ultraviolet-B perception, because the absorption wavelengths for tryptophan coincide with ultraviolet-B. Consequently, the UV-B-sensing mechanism of UVR8 differs markedly from other photoreceptors that depend on an external cofactor for UV-B perception.

The authors' experimental findings, in conjunction with knowledge of tryptophan fluorescence, yields a mechanistic model of ultraviolet-B perception by UVR8. Ultraviolet-B irradiation results in excitation of the Trp 285 and Trp 233 indole rings, which is thought to disrupt the P-bond over the indole rings, leading to destabilization and disruption of the intramolecular cation-π interactions. Such disruption triggers pronounced conformational changes in the side-chain of Arg 286 and Arg 338, which would no longer be able to maintain intermolecular hydrogen bonds with Asp or Glu residues from the neighbouring UVR8 molecule, causing dissociation of the UVR8 homodimer. Furthermore, the excited indole rings are known to undergo a process of excited-state proton transfer, which allows the indole ring to carry a positive charge and completely destroy the cation-π interactions. Notably, excited-state proton transfer leads to quenching of intrinsic tryptophan fluorescence, thus resulting in slow decrease of fluorescence signal.

Asp 129, Glu 182 and Arg 234 are found to be located adjacent to Trp 233 and Trp 285, which further serve as proton donors. The ultraviolet-B perception involves no covalent modification of UVR8, such as tryptophan oxidation or cross-linking, which further allows re-formation of homodimers. This study further elucidates that for the detection of UV-B, one of the most sensitive methods is measurement of intrinsic tryptophan fluorescence.

Voityuk *et al.* (2014) suggested a mechanism for the photodissociation of UVR8 through high-level quantum chemical calculations which includes mainly three steps:

i) After dissociation of dimer into monomers, tryptophan residues intrinsic to the monomer form a broad light-harvesting system, in which the L_a excited state of Trp233 experiences strong electrostatic stabilization by the protein environment.
ii) Charge separation results in fast decay of the locally excited state, which further generates the radical ion pair Trp285(+)-Trp233(−), with a dipole moment of $\approx$ 18 D.
iii) The dipole moment generated leads to breakdown of the salt bridges between the two monomer subunits.

Yin *et al.* (2015) recently reported that two separate domains of UVR8 interact with COP1: the β-propeller domain of UVR8 mediates UV-B-dependent interaction with the WD40 repeats-based predicted β-propeller domain of COP1, whereas UVR8 C-terminal C27 domain interacts with COP1 and, hence, regulates its activity. Upon UV-B irradiation, light is absorbed by one or more Trp residues, which are situated adjacent to Arg residues which form salt bridges across the dimer interface. This light absorption induces the disruption of the salt bridges and, thus, leads to the instant dissociation of UVR8 homodimers. Subsequently, the UV-B light-activated UVR8 monomer, with its seven-bladed β-propeller domain (C27), binds to the COP1 WD40 domain (a structurally related seven-bladed β-propeller), thus initiating UV-B signalling pathway. The activated UVR8-COP1 stabilizes the bZIP transcription factor HY5, which further induces expression of RUP1 and RUP2 genes, forming a negative feedback loop.

The basic leucine-zipper transcription factor HY5 plays an important role in de-etiolation, the process by which plants adjust from growth in darkness to growth in light. In darkness, HY5 is ubiquitinated by COP1 and degraded by the proteasome. In light, HY5 is stabilized, and acts as a promoter of photomorphogenesis (Saijo *et al.*, 2003; Osterlund

et al. 2000; Yi and Deng, 2005). RUP1 and RUP2 are WD40-repeat proteins that are phylogenetically and structurally related to COP1. Their interaction with the C27 domain of UVR8 facilitates disruption of the UVR8-COP1complex, which further promotes UVR8 redimerization.

RUP1/RUP2-UVR8-COP1 complex formation occurs transiently when RUP1 and RUP2 attach to the C27 domain of UVR8, while UVR8 and COP1 still interact via their β-propeller surfaces (Figure 15.2). Furthermore, it was concluded that the difference in the UV-B dependence of the UVR8-COP1 and UVR8-RUP1/RUP2 interactions is due to at least two differences in their modes of interaction: the β-propeller surface exposed in the UVR8 monomers have propensity to interact with COP1 but not with RUP1 and RUP2; and COP1 and RUP1/RUP2 have a distinct ability to interact with the UVR8 C-terminal 44 amino acids. The interaction of COP1 requires UV-B activation and monomerization of UVR8 but, in the case of RUP1 and RUP2, it occurs also with the nonactivated homodimeric UVR8.

Zeng *et al.* (2015) accomplished crystallization of UVR8 construct from *Arabidopsis* (12–381 residues) to study light-induced structural alteration by employing temperature-scan cryo-crystallography technique. A difference in dark data set F_{DARK} (data obtained from crystal without UVB illumination) and light data set F_{UV} (after UVB

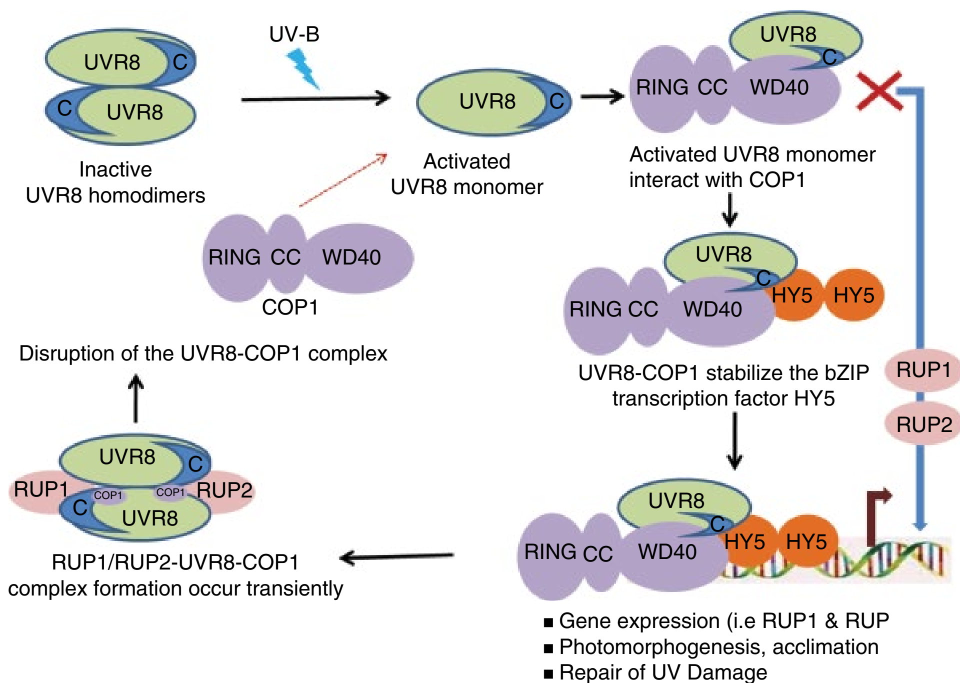

Figure 15.2 Working model of molecular mechanism of UVB perception, depicting formation of monomer from UVBR8 dimer, followed by interaction with the WD40 domain of COP1 (violet colour) and C terminal C27 amino acid fragment of UVR8 (blue colour). UVR8-COP1 complex stabilizes the bZIP transcription factor HY5 (orange in colour), which further induces expression of RUP1 and RUP2 genes (pink in colour) and leads to feedback regulation (represented by red cross) (scheme adapted from Yin *et al.*, 2015).

illumination, structural changes were cryo-trapped by lowering the temperature of the illuminated crystal to 100 K) was outlined at 120 K. $F_{UV} - F_{Dark}$ difference Fourier maps were drawn, using the phases from the dark structure, which further revealed that two clusters of strong positive and negative difference densities exist at the dimer interface specifically associated with Trp 285/Trp 233 and a water molecule.

The indole rings of Trp 285 and Trp 233 displace to collide with each other, due to strong attraction. This leads to rotation of the indole ring of Trp 233 at about 10° while moving towards Trp 285. A crossover pattern is generated when the indole ring of Trp 285 tilts about 30°, with associated positive and negative difference densities (-14σ at the peak). Such cooperative motions of the indole rings are associated with torsional motions about the Cα–Cβ and Cβ–Cγ bonds in Trp 285 and Trp 233. This event results in exhausted backbone conformations around Trp 285 and Trp 233 residues.

Interestingly, $F_{UV} - F_{Dark}$ difference Fourier maps disclose an 'epicentre water' molecule in the dark structure with significant negative density. This water molecule belongs to a hydrogen-bonding network at the dimer interface, including Trp 285/Arg 286 in one subunit and Asp 96/Trp 94/Asp 107 in another subunit. Rotation of the indole ring of Trp 285 results in ejection of this water molecule, which subsequently leads to loosening of inter-subunit interactions – for example, breakdown of hydrogen bonds and salt bridges at the dimer interface results in dimer dissociation.

Heilmann and Jenkins (2013) demonstrated the kinetics and mechanism of regeneration of the UVR8 photoreceptor in plants by utilizing inhibitors of protein synthesis and degradation. This study revealed that the regeneration of UVR8 dimer occurs due to reversion of the monomeric form to the dimer form, but not by rapid *de novo* synthesis following destruction of the monomer. Additionally, regeneration of dimeric UVR8 in darkness following UV-B exposure occurs much more rapidly *in vivo* than *in vitro* with illuminated plant extracts or purified UVR8, therefore indicating that rapid regeneration requires intact cells. It is concluded that the process of reversion from monomer to dimer is complex, and is facilitated by several factors: the presence of intact cells; protein synthesis in response to UV-B; and interaction of the C-terminal region of UVR8 with proteins, including COP1.

15.4 UVR8 Involvements in Different Pathways

UV-B accelerates a large number of physiological responses modulating growth and development at various stages of the plant life cycle, which can be seen in both the natural environment and under controlled laboratory conditions (Jenkins, 2009; Ballaré *et al.*, 2012; Heijde and Ulm, 2012; Wargent and Jordan, 2013). Microarray and reverse transcription PCR analyses of *uvr-8* (mutant of *uvr8* in *Arabidopsis)* and wild type plant show that UVR8 regulates the gene concerned with protection against oxidative stress (PDX1), photo-oxidative damages (ELIP1, SIG5), UV protectant (flavonoid, PHR1), a number of genes encoding signalling components, transcription factors, transporters, proteases, and several proteins with unknown functions (Brown *et al.*, 2005).

Davey *et al.* (2012) observed more substantial photo-inhibition in *uvr8*, indicating that PSII is more sensitive to UV-B induced damage. This is because *uvr8* mutants may be less able than the wild type to replace damaged PSII reaction centres. The protection offered by plants to photosynthetic machinery against low doses of UVB through

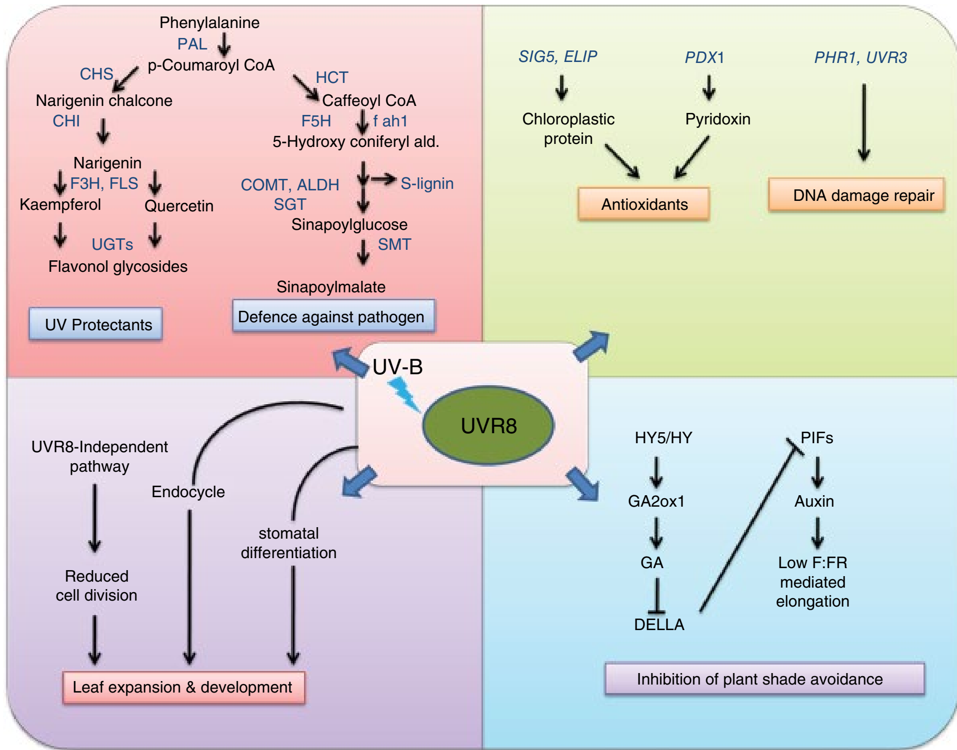

Figure 15.3 Hypothetical model depicting integration of UVR8 with other metabolic pathways leading to different physiological responses.

UVR8-regulated gene expression can be directly via induction of chloroplastic proteins, and indirectly via regulating the phenylpropanoid and other secondary metabolites pathway, photomorphogenesis and DNA repair. Specifically, UVR8 accumulates fewer UV-B absorbing flavonoids, which makes PSII more susceptible to UV-B damage (Booij-James *et al.*, 2000). Alternatively, UVR8 plays key signalling role in plant acclimation and whole plant responses to UV-B, as depicted in Figure 15.3.

15.4.1 Protection from Photo-Inhibition and Photo Oxidative Stress

In response to oxidative stress, plants synthesize antioxidants such as vitamins C and E, glutathione and carotenoids (Chen and Xiong, 2005). Pyridoxine (vitamin B6), one of the essential antioxidant that provides protection from UV-B (Ristilä *et al.*, 2011; Brosché *et al.*, 2002; Ulm *et al.*, 2004; Kalbina *et al.*, 2008) involves, two proteins in its biosynthesis – Pyridoxine Biosynthesis 1 (PDX1) and PDX2 (Denslow *et al.*, 2007). A literature study revealed that *Arabidopsis thaliana* leaves after exposure to UV-B rapidly accumulate PDX1 and vitamin B6 (Ristilä *et al.*, 2011; Figure 15.3). Additionally, a low fluence dose of UV-B might be involved in the regulation of transcripts of PDX1.3 (homologue of PDX1) (Ristilä *et al.*, 2011).

UVR8 maintains photosynthetic competence by regulating expression of genes in response to UV-B, in which some are encoding chloroplastic proteins (SIG5 and ELIP1)

(Davey *et al*, 2012). Among these, SIG5, which encodes the plastid RNA polymerase sigma factor, regulates PsbD (a transcript of psbd-BLR-P encoding the PSII D2 proteins) (Kanamaru *et al*, 2004,) and is reported to be downregulated in *uvr8* mutant plants more than in the wild type (Brown and Jenkins, 2008).

ELIPs (Early Light Inducible Protein) are thylakoid proteins encoded by one of the major light-responsive nuclear genes, leading to tolerance to photoinhibition and photooxidative stress. Their elevated expression in plants exposed to stress has been reported (Adamska *et al.*, 2001). They are absent in mature plants unless the plants are exposed to stress such as UV-B (Adamska *et al.*, 1992). ELIP transcripts and proteins are induced in the first hour of growth of etiolated seedlings (Pötter and Kloppstech, 1993), when the photosynthetic system is more susceptible to photooxidative stress. Brown and Jenkins (2008) suggested that ELIP1 expression is regulated by HY5 transcription factor and, hence, is controlled by the UVR8-dependent UV-B signalling pathway. This inducible property, with the ability to bind the pigments, suggests that ELIPs may have a photo-protective function, and are regulated by UVR8 (Figure 15.3).

15.4.2 Flavonoid and Alkaloid Pathways

Transcriptome analyses of *uvr8* mutants revealed that UVR8 is required for the induction of genes with significant role in flavonoid and alkaloid pathways (Kliebenstein *et al.*, 2002; Brown *et al.*, 2005; González Besteiro *et al.*, 2011; Demkura and Ballaré, 2012). These are gene expression responses that provide UV protection, the best studied being the UV-B induction of *CHS* (Chalcone Synthase) and other genes involved in flavonoid biosynthesis which have free radicals-scavenging activity, and also work as a sunscreen by absorbing UV radiation (Jenkins *et al.*, 1997, 2001). *uvr8-2* (mutant of *uvr8* in *Arabidopsis thaliana*) fails to induce the expression of *CHS*, the first enzyme committed in the flavonoid pathway (Brown *et al.*, 2005; Cloix *et al.*, 2012), and also shows phenotypic differences from its wild type when exposed to UV-B (Brown and Jenkins, 2008).

Heijde *et al.* (2013) reported that plants overexpressing UVR8^{W285A} showed higher *CHS* mRNA levels than the wild-type plants. Kinetically different phototransduction pathways occur for UV-B and UV-A/blue light (*cry1*) for regulation of *CHS* in *Arabidopsis* cells (Jenkins *et al.*, 2001). Additionally, UV-B and UV-A/blue light signalling pathways are also pharmacologically distinct in controlling *CHS* expression, in a way that UV-B induction of *CHS* is inhibited by calmodulin antagonist W-7, but this is not the case in UV-A/blue light (*cry1*) mediated *CHS* induction (Christie and Jenkins, 1996). Brown *et al.* (2005) screened mutants defective in expression of the gene encoding *CHS*, by developing transgenic *Arabidopsis* line expressing luciferase driven by the *CHS* gene promoter. Further, genetic analysis of mutants defective only in their response to UV-B found them to be allelic with the *uvr8-1* mutant (*UV resistance locus 8-1 Arabidopsis* mutant hypersensitive to UV-B contains a single recessive mutation at the bottom of chromosome 5).

In addition, UVR8 was indicated as a positive regulator of the UV-B induction of kaempferol-3-glucoside, quercetin and quercetin-3-glucoside in plants that receive both solar UV-A and UV-B (Morales *et al.*, 2013; Figure 15.3). Hence, UVR8 is required for UV-B induction of phenolics in the leaf epidermis, and to increase the content of epidermal flavonoids in plants exposed to solar UV-B radiation (Figure 15.3).

15.4.3 DNA Damage Repair

UV-B works as a DNA damaging agent by generating two photoproducts, pyrimidine photoaducts (6-4 PP) and, mainly, cyclobutane pyrimidine dimer (CPDs). The main repair pathway for CPD and 6-4 PP in prokaryotes and eukaryotes includes photolyases (Britt, 2004). Light-dependent photolyases bind with dimers and, upon absorption of a photon of the appropriate wavelength (350–450 nm), directly reverse the damage in an error-free manner and restore the native form of the DNA (Jansen *et al.*, 1998).

In *Arabidopsis*, a variety of UV-B-hypersensitive mutants deficient in DNA repair have been identified (*uvr1* (Britt *et al.*, 1993), *uvr2* (Jiang *et al.*, 1997; Landry *et al.*, 1997), *uvr3* (Jiang *et al.*, 1997; Nakajima *et al.*, 1998), and *uvh1* (Harlow *et al.*, 1994). Microarray and transcriptomic analyses of *Arabidopsis* have revealed that UVR8 regulates sets of genes that have an important role in UV protection and repair of UV damages, including type II photolyase PHR1. Moreover, the *uvr8-2* mutant of *Arabidopsis* fails to accumulate PHR1 and, thus, is found to be highly sensitive to UV-B, suggesting that *uvr8* regulates the *phr1* expression (Brown *et al.*, 2005). A functional DNA repair system is crucial to maintain genome integrity and therefore supports light-induced stress tolerance.

15.4.4 Defence Against Pathogens

UVR8 should be included in the list of environmental signals that regulate the expression of plant defence in canopies. Sinapates serve as a precursor for syringyl-type lignin synthesis, which plays a role in cell wall fortification and has potential to prevent fungal hyphae penetration into plant cell (Kishimoto *et al.*, 2006; Quentin *et al.*, 2009; Lloyd *et al.*, 2011). A *fah1-7* mutant (no ability to convert ferulic acid into 5-hydroxyferulic acid) with sinapate deficiency shows increased susceptibility to *Botrytis cinerea*. This mutant was found to be significantly different from the wild type, as it did not express the resistance phenotype induced by UV-B radiation (Meyer *et al.*, 1996).

Arabidopsis, Col 0 (*uvr8-6* mutant in *Columbia*) and Lur 0 (*uvr8* mutant in *Landsberg irecta*), when exposed to UV-B radiation, results in induced sinapate levels, mediated by UVR8. Such results go in accordance with the finding of Favory *et al.* (2009), who demonstrated that *fah1* transcription is abruptly upregulated by UV-B radiation, interceded by UVR8. Hence, it can be concluded that UV-B radiation enhances *Arabidopsis* resistance to fungal infection, which might be because of induction of increased sinapate level through a UVR-8 dependent phenomenon (Demkura and Ballaré, 2012; Figure 15.3). Interestingly, UV-B induced defence system might be utilized in an agricultural system which prefers light environment manipulation, as reported by Wargent *et al.* (2006).

Apart from this, after 12 hours of solar UV exposure, UVR8-dependent jasmonic acid (JA) biosynthesis and signalling genes transcript accumulation has been observed. These JA biosynthesis genes (*Allene Oxide Synthase [AOS]*, *Allene Oxide Cyclase1 [AOC1]*, *AOC3*, and *Oxophytodienoate Reductase 3*) and JA signalling transcription factors (*WRKY70*, *Jasmonate Zim Domain 1[JAZ1]*, *Syntaxin Related Protein 1*) were accumulated at lower levels in *uvr8-2* than in the wild type (Morales *et al.*, 2013). Such findings suggest that the UVR8 pathway interacts with the JA signalling pathway, and could induce resistance against pathogens and herbivores under natural sunlight conditions (Izaguirre *et al.*, 2003; Demkura *et al.*, 2010; Demkura and Ballaré, 2012).

15.4.5 Inhibition of Plant Shade Avoidance

UVR8 perceives a distinct signal from sunlight that impedes shade avoidance responses in *Arabidopsis thaliana* by estranging auxin and gibberellins. UVR8 interaction with COP1 leads to elevated levels of HY5 and HYH which, in turn, leads to increased *GA2ox1* (Gibberellin 2 oxidase) transcript. This further decreases GA (Giberellic acid) and increases DELLA protein (negative regulator of GA) stability, followed by suppression of PIFs (Phytochrome Interacting Factor 4 and Phytochrome Interacting Factor 5) (de Lucas *et al.*, 2008; Feng *et al.*, 2008).

In a parallel HY5/HYH-independent pathway, perception of UV-B by UVR8 inhibits low R: FR-mediated induction of Indole-3-pyruvate monooxygenase genes *YUCCA2*, *YUCCA5*, *YUCCA8*, and *YUCCA9* (genes which are involved in auxin biosynthesis and convert indole-3-pyruvic acid (IPA) into indole-3-acetic acid (IAA)) and the auxin-responsive genes IAA29 and GH3.3, thus inhibiting auxin biosynthesis. ΔC27UVR8 plants (ΔC27UVR8 plants express a deletion mutant of UVR8 that is unable to bind COP1) exhibit less inhibition of YUCCA8 and YUCCA9 transcript abundance, compared with wild type (WT) plants. This occurs due to UV-B-mediated turnover of PIF4 and PIF5. Hayes *et al.* (2014) reported no physical interaction between UVR8 and PIF4 or PIF5 in yeast two hybrid, and subsequently suggested occurrence of an unknown pathway which may link UVR8 activation to PIF degradation. Reduction in the abundance and activity of PIF4 and PIF5 under UV-B exposure decreases auxin activity, which further inhibits elongation, and shade avoidance is suppressed (Figure 15.3).

15.4.6 Regulation of Leaf Morphogenesis

UV-B inhibits leaf growth and shape in various plant species (Liu *et al.*, 1995; Searles *et al.*, 2001). Epidermis plays an important role in controlling leaf growth and shape (Dale, 1988; Savaldi-Goldstein *et al.*, 2007), and leaf growth can be analyzed by the number of epidermal cells per leaf and epidermis cell area (μm^2). A significant reduction in the number of epidermal cells per leaf was observed under UV-B exposure by about 1.6-fold *uvr8-2* and about 2.2-fold in wild type, suggesting that UV-B-mediated effects on epidermal cell division are mostly independent of UVR8. Nevertheless, it has been seen that overall leaf growth under UV-B irradiation in wild-type plants is found to be increased when compared with *uvr8-2* mutant, because of a UVR8-dependent enhancement of cell area in wild-type plants compared with the mutant plants.

The regulation of endopolyploidy, which can be correlated with increased cell size, requires UVR8 in response to UV-B (Wargent *et al.*, 2009). Also, a lower density of stomata has been found in *uvr8* mutant, compared with the wild type, suggesting that UVR8 has a regulatory role in other developmental events. The above findings indicate that UVR8 is a key signalling component in the whole plant in response to UV-B, regulating an important morphogenetic activity in the leaf.

15.4.7 Regulation of Root Growth and Development

Previous studies have revealed that, under drought conditions, UVR8 drives reduction of plant growth (Kliebenstein *et al.*, 2002; Brown *et al.*, 2005; Favory *et al.*, 2009; Fasano *et al.*, 2014). Fasano *et al.* (2014) performed phenotypic analysis of *uvr8* overexpressing plants by generating transgenic lines (*35-UVR8*) of *Arabidopsis* with

overexpressed *uvr8* gene, under the control of the 35S CaMV promoter. Overexpressed *uvr8* in *Arabidopsis* impaired negative effect on vegetative growth of the root under light exposure. The primary root length of seven-day-old *uvr8*-overexpressing seedlings was 18% reduced, compared with the control. This inhibition in root elongation is caused by a reduction in cell expansion, not in cell numbers. Additionally, a reduction in primary root length of about 13% and lateral root density of about 60% was also observed.

It is to be noted that lateral root growth is auxin-dependent. Fasano *et al.* (2014) reported a 2.2-fold increase in flavonoids level, and a marked decrease in IAA conjugates content in UVR8-overexpressing plants. (Casimiro *et al.*, 2001; Bhalerao *et al.*, 2002; De Smet and Jürgens, 2007). Thus, it can be concluded that decrease in cell expansion in 35-UVR8 could be correlated with an enhanced flavonoids level, which subsequently leads to a change in polar auxin transport and homeostasis. UVR-8 may regulate the flavonoids level and auxin transport in roots, which further plays an important role in root development, as well as acting as a common link between the light- and hormone-signalling pathways.

15.4.8 Circadian Clock

A mutual interaction between circadian clock and photomorphogenic UV-B light has been reported. In *Arabidopsis*, Feher *et al.* (2011) demonstrated the contribution of low-intensity, non-damaging UV-B for the light-mediated entrainment of the circadian clock. Such phenomena include UVR8 and COP1, although HY5 and HYH are not involved. Transcription activation of responsive clock genes was found to be required for photomorphogenic UV-B-mediated circadian rhythm. In the arrhythmic early flowering 3-4 mutant (*elf-3-4*), non-gated UV-B-induced high-level gene expression has been found, which was independent of the time of the UV-B pulse. Nevertheless, *elf-3-4* displayed similar tolerance behaviour to that shown by wild type. These findings suggest that temporal restriction of low-intensity UV-B responses by the circadian clock might be utilized for saving resources during acclimation, and not for increasing stress tolerance.

15.5 Conclusion and Future Perspectives

The UVR8 UV-B photoreceptor is important for the acclimation of plants to potentially damaging UV-B radiation and, hence, it contributes to developing strategies for plant survival. Research on the basis of biochemical, genetic and molecular approaches in *Arabidopsis thaliana* has been essential for initial mechanistic characterization of the UVR8 mediated molecular UV-B signalling pathway. Subsequent Trp involvement in UV-B perception, salt bridge breakage and UVR8-COP1 interaction have emerged as a fundamental mechanism for UV-B signalling. A number of questions might be raised concerning the current knowledge of UVBR8 signalling mechanism and its involvement in other metabolic pathways. What is the range of physiological UV-B responses mediated by UVR8? Is there any other photoreceptor/(s) responsible for UV-B perception in plant (and, if yes, what is the basis of their molecular function)? What is the mechanism behind UVR8-mediated regulation of gene expression?

Such novel findings can be employed in several applications, such as in the field of optogenetics, where various photoreceptors are used to generate light-controlled modules that control the localization together with function of diverse proteins (Toettcher *et al.*, 2011; Müller and Weber, 2013). UVR8 specificity and sensitivity to UV-B are providential summation in the optogenetic study. First reports of implementations of UVR8 in novel optogenetic systems include exploitation of UV-B to control nuclear retention, chromatin association, protein secretion and gene expression in mammalian cells (Chen *et al.*, 2013; Crefcoeur *et al.*, 2013; Müller *et al.*, 2013). In agriculture, UVR8-mediated UV-B signalling might be utilized to extenuate the undesirable effects, or to grasp the desirable effects of UV-B exposure, in improvement of crop productivity and quality (Wargent and Jordan, 2013). For example, in terms of increasing nutritional value in crops, the alteration in plant secondary metabolism in response to UV-B could be considered as a novel approach (Jansen *et al.*, 2008; Schreiner *et al.*, 2012). With our current understandings of UVR8-mediated acclimated responses, research areas, including manipulation of plant growth as well as plant tolerance to abiotic and biotic stress, can be elaborately explored.

Acknowledgements

LC Rai is thankful to DST for project and JC Bose National Fellowship and DAE for Raja Ramanna Fellowship. Alok Kumar Shrivastava is thankful to ICAR for RA, Sonia Sen to CSIR for SRF, Antra Chatterjee and Shivam Yadav to UGC for JRF, and Shweta Rai to DST for financial support. Ruchi Rai and Shilpi Singh thanks DST for WOSA.

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UV-B Radiation: From Environmental Stressor to Regulator of Plant Growth, First Edition. Edited by Vijay Pratap Singh, Samiksha Singh, Sheo Mohan Prasad and Parul Parihar.
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