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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

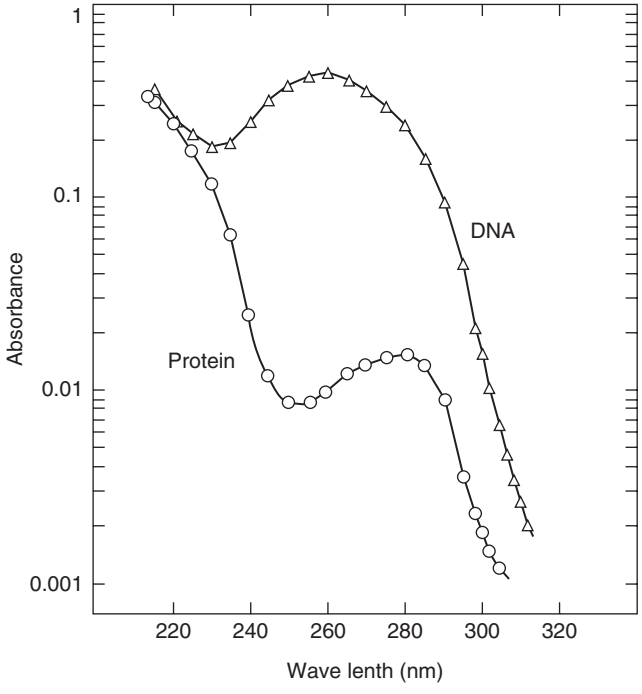

Figure 1.1 Absorption spectra of protein and DNA at equal concentrations (adapted from Harm, 1980).

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.

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