

EFFECTS OF UV-B RADIATION ON MORPHOLOGICAL, PHYSIOLOGICAL AND BIOCHEMICAL ASPECTS OF PLANTS : AN OVERVIEW

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Abstract

Origin of life was never be thought without considering the role of UV radiation but once the “boon”, is slowly becoming “curse” for life. Plants are exposed to many factors but the problem of enhanced UV-B is created by the anthropogenic activities resulted in ozone layer depletion. However, plants are performing well to cope up with such problems, but the changing climatic conditions and their interactive effects needs attention. Focusing on this, the main objective of this study is to summarize the plant responses observed under various studies against enhanced UV-B. Depletion of stratospheric ozone layer has created the threat of enhanced UV-B which causes the reduction of productivity by reducing the rate of photosynthesis, plant growth, and overall yield in most of the species. So, the scope of further research should be focused on the selection of tolerant species for improving knowledge, scope of genetic improvement and other responses against combination of stresses for better understanding and management of plants with changing climate.

Keywords: Photosynthesis, Lipid peroxidation, Ozone layer, UV-B, Climatic.

Abbreviations: APX - ascorbate peroxidase; CAT - catalase; ·OH - hydroxyl radical; LPO - lipid peroxidation; MDA - malondialdehyde; ROS - reactive oxygen species; SOD - superoxide dismutase; UV-B - ultraviolet B radiation; UV-B_{BE} - biologically effective UV-B.

Introduction

Although the Earth is now survivable but origin of life was dated back only few billion years ago and was recorded with the help of some microbial evidences. This origin of life is mainly supported by the solar spectrum which is the driving force for entire ecosystems of Earth and is comprised of electromagnetic spectrum including different wavelengths ranging from radio waves to gamma waves. These

electromagnetic waves helped in the formation of early atmosphere on the Earth and ultimately lead to the origin of early life.

The more energetic regions of the spectrum are at shorter wavelengths, but the region of our interest in the spectrum is “**Ultraviolet Radiation**” which lies at the short wavelength end. Ultraviolet, visible and infra red rays are critically very important for life on the Earth. Infra red light and visible light are the part of solar spectrum, which are responsible for increasing the temperature of the Earth up to the survival range.

Ultraviolet radiation (UV) is a part of the non ionizing region of the electromagnetic spectrum which comprises approximately 8-9% of the total solar radiation. UV is traditionally divided into three wavelength ranges: **UV-A (320-400 nm)** represents approximately 6.3% of the incoming solar radiation and is comparatively less harmful part of UV- radiation (Table.1). **UV-B (280-320 nm)** is of particular interest because this wavelength represents only 1.5% of the total spectrum, but can induce a damaging effect in plants, animals as well as at ecosystem level (Table.1). **UV-C (200-280 nm)** is extremely harmful to organisms (Table.1).

History of Ultra violet radiation

Ancient civilization thought that sun is the ultimate source of visibility, warmth, health and vitality and their understanding about sun was immersed in the mythology and cultural traditions. But their newer generations were not satisfied by the explanations, so they lead to the beginning for search of new thoughts, believes and understanding. By the start of 9th century, the newer knowledge and principles led to the realization that sunlight is not a single stimulus of single wavelength but it is a collection of stimuli of different wavelengths. In that series the discovery of UV radiation prior to 1920 took place. First of all Sala (1614) made an observation in which he found that silver nitrate crystal turned black when exposed to sunlight. Scheele (1777) also found the same phenomenon, when he directed sunlight through a prism onto a paper, a coloured spectrum was also observed by him. Ritter (1801) noticed invisible rays beyond violet end of the spectrum and called it deoxidizing rays, later it was termed chemical rays. Becquerel and Draper (1842) independently observed that wavelength between 240 to 400 nm induce in or photochemical reaction. This was the first indication of spectral extent of UV radiation.

Maxwell (1865) proposed the theory of electromagnetic waves. After that a revolution in the field of solar spectrum studies occurred and by 1920, the existence of UV radiation, its properties and relationship to sunlight were well established.

UV-B and life of terrestrial plants

Evolution of plants from the early Archean era began as single photosynthetic cell (Cockell and Horneck, 2001). The effect of ultraviolet radiation is detrimental to the terrestrial plants. It has been demonstrated with some most important biochemical machinery i.e. DNA and PS-II (Singh et al., 2008).

During the course of evolution, when stratospheric ozone was not formed; life was not possible on land so the first life was originated under water but with the help of UV-C and other solar radiations stratospheric ozone layer was formed. After that, all of the UV-A and minor part of UV-B were allowed to reach the Earth's environment and UV-C was fully absorbed and scattered. Therefore, it can be said that UV radiation has regulatory properties and hence, it altered the Earth's environment over geological time periods and it is essential to understand the evolutionary history of the Earth and also the selection pressure developed by UV-B on terrestrial plants. In fact, Sagan (1973) first considered the UV radiation as a selection pressure on the early photosynthetic organisms.

The terrestrial plants coevolved under different solar UV-B levels and may have experienced significantly higher UV-B irradiance during course of evolution than current surface UV-B level (Cockell and Horneck, 2001; Rozema et al., 2002). So, the UV-B tolerance acquired earlier; probably helps to explain why plants are distributed at lower latitudes or higher elevations, where UV-B irradiance is greater, are less sensitive to high levels of the UV-B radiations than those at higher latitudes and/or lower elevations (Turunen and Latola, 2005).

UV-B environment of terrestrial plants is quite variable in both time and space and thus, organisms experiences different UV-B doses and adapt to UV-B radiation at different levels (Rozema, 2000). In this context, it is expected that terrestrial plants responds differently to increasing solar UV-B. And because of the increasing consequences of ozone layer depletion and UV-B irradiances, focus from the point that UV-B radiation is a component of sunlight which is involved in the evolution of life on Earth, may have distracted (Cockell and Horneck, 2001).

Ozone and UV-B irradiance

UV radiations are measured in two terms i.e. irradiance and fluence rate. Irradiance is the radiation falling on a flat surface per unit area per second and when it is multiplied by time then is called as dose. Fluence rate is the radiation falling on sphere per unit cross section per second and when fluence rate is multiplied with time, it is called as fluence. Ozone is a form of oxygen which plays a vital role in the atmosphere. It is known that the protection of life on the Earth from UV-B and UV-C radiations is a result of the absorbance of these radiations by stratospheric ozone layer. While some ozone is also found in the tropospheric region, but its concentration is very low as compared to stratospheric ozone concentration. In troposphere, ozone is an air pollutant and is a green house gas which causes harmful effects on both living system and environment. But in stratosphere, it forms a thick covering around the Earth which protects the living organisms from the harmful rays of solar spectra. However, due to anthropogenic activities this protective layer is depleting and it is termed as Ozone Hole. The thinning of ozone layer less than 220 DU caused by ozone depleting substances such as halocarbons is called as Ozone hole.

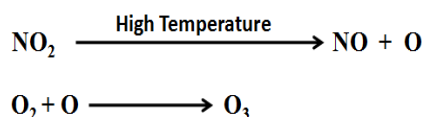
The stratospheric ozone layer efficiently filters out most of the detrimental shortwave UV radiation shorter than 280 nm. The absorbance coefficient of ozone decreases rapidly at wavelengths longer than 280 nm and approaches zero at about 330 nm. This is referred to as "Column Ozone" since it is the total amount of ozone in a column between the Earth's surface and the top of the stratosphere; normally expressed as "Dobson Unit" and abbreviated as "DU". Therefore, UV-A radiation is virtually unaffected by changes in ozone concentration. At the Earth's surface, radiation becomes significant between wavelengths 290-315 nm, increasing rapidly within this range by about three orders of magnitude. A small decrease in ozone levels may cause a large relative increase in biologically effective UV-radiation. In general, each 1% decrease in ozone concentration causes an increase of 1.3 to 1.8% in UV-B radiation reaching the biosphere.

The amount of radiation passing through the ozone column is dependent not only on its concentration in the atmosphere, but also dependent on the elevation above the sea level and angle of the Earth's surface. The higher the elevation above the sea surface the shorter the path through the atmosphere that the radiation has to travel; which ultimately results in increase in irradiance.

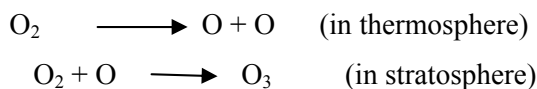
Ozone Layer Formation

Formation of stratospheric ozone is a natural process, whereas formation of tropospheric ozone is due to anthropogenic activities and tropospheric ozone acts as air pollutant (secondary pollutant) and also as one of the green house gases.

Main sources of tropospheric ozone are automobiles, xerox machines, biomass burning etc. In automobiles emissions, nitrogen dioxide breaks in presence of light into nitric oxide and nascent oxygen which combines, with molecular oxygen to form ozone.



Stratospheric ozone is formed by the photolysis of molecular oxygen in thermosphere and formation of ozone takes place in stratosphere because of the favorable conditions like sufficient oxygen densities.



Ozone layer formation and degradation in stratosphere is a natural phenomenon and a balance is maintained between molecular oxygen (O_2), hydroxyl ion ($\cdot\text{OH}$) and peroxy radical ($\text{HO}_2\cdot$), which maintains the thickness of ozone in stratosphere. But any disturbance in the surrounding atmosphere like entry of ozone depleting substances

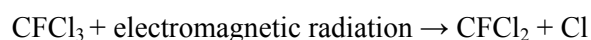
causes disturbance in these natural processes and depletion of ozone layer starts. Through various studies it was found that 5% decrease in ozone layer thickness causes 10% increase in UV-B radiation penetration.

Ozone Layer Depletion

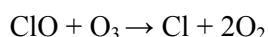
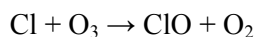
The process of ozone layer depletion was first reported by Farman and his team at South Pole especially in spring season in 1982 and was later confirmed by NASA in 1987. The ozone layer depletion process begins when CFCs, NO_x and other ozone depleting substances (ODS) are emitted into the atmosphere. When this ODS reaches up to stratospheric region, ozone depletion results. In the presence of strong UV-radiation, ODS molecules break apart into chlorine atoms and other halogens and these atoms are actually involved in destruction of ozone. Ozone is continuously produced and destroyed in a natural cycle and the overall amount of ozone is essentially stable. This ozone production and destruction are balanced and ozone levels remain stable. But increase in stratospheric chlorine and bromine upsets that balance leading to decrease in ozone production and creating ozone levels fall.

Ozone depletion represents two distinct but related phenomena observed in late 1970s i.e. a steady decline of about 4% per decade in the total volume of ozone in Earth's stratosphere (the ozone layer) and much larger decrease in stratospheric ozone layer over Earth's polar regions. The latter phenomenon results into **Ozone Hole**. CFCs and other contributing substances are referred as ozone depleting substances (ODS). The ozone layer inhibits the penetration of most harmful UV-B (280-320 nm) of UV-radiation from passing through the atmosphere. The observed decrease in ozone concentration have generated a worldwide concern, leading to the adoption of the Montreal Protocol that bans the production of CFCs, halogens and other ozone depleting substances such as carbon tetrachloride and trichloroethane.

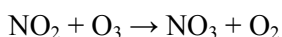
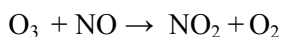
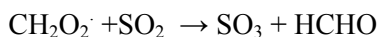
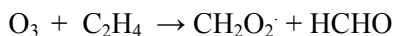
Ozone can be destroyed by a number of free radicals such as hydroxyl free radical (OH), nitric oxide free radical (NO), chlorine free radical (Cl), peroxy radical (HO₂) superoxide radical (O₂⁻) and these are found in certain organic compounds especially CFCs. The Cl and Br atoms are liberated from the parent compounds by the action of ultraviolet radiation.



These free radicals can then destroy ozone molecules through variety of reactions. The simplest example of such reactions is, a chlorine atom reacts with ozone molecules to form ClO and leaving a normal oxygen molecule. The chlorine monoxide (ClO) reacts with second molecule of ozone to form another chlorine atom and two molecules of oxygen.



Other reactions involving ozone destruction are:



Consequences of Ozone layer depletion

In the last few decades, there has been an average of 7% increase in biologically active UV-B radiation in northern mid latitude due to depletion in stratospheric ozone layer (Yang et al., 2007).

The amount of UV-B radiation that penetrates through the ozone layer decreases exponentially with the slant path thickness/density of the layer. Correspondingly, a decrease in atmospheric ozone is expected to give rise to significantly increased levels of UV-B near the surface.

According to IPCC 2007 report, it has been predicted that if the current rate of anthropogenic pollution and green house gases emission continues, it will result into tremendous stress on living system by increasing the concentration of heat trapping gases which will lead to rise in global temperature from 0.4°C to 1.1 °C by 2025 and up to 5.8 °C by 2100. However, the proposed CO₂ concentration is 720 to 1020 ppm up to 2100. These climate change models revealed that this climate change will have more adverse effect than beneficial on ecosystem.

Clouds, Aerosols, Air Pollutants and UV-B irradiance

Stratospheric ozone determines the amount of UV-B radiation that reaches the surface of the Earth, its level is significantly affected by variations in latitude and altitude. Level of UV-B over tropical latitude is higher than in temperate region.

Atmospheric UV-B absorption is determined by the solar angle and ozone itself (thinner in equatorial region). UV-B radiation is relatively high in tropical area and low in polar area. Other than geographical factors, atmospheric pollutants like Smoke, aerosols and weather factors like cloud, haze; greatly decreases the level of UV-B reaching the Earth's surface. Clouds have a large effect on irradiance on the Earth's surface.

Clouds and aerosols scatter radiation, but other air pollutants like sulphur dioxide absorbs and scatters UV. However, the absorbance of UV radiation by gases other than ozone is generally a small factor except in highly polluted areas. Thin cloud layers actually scatter a significant portion of the UV-B towards the Earth, while thick cloud layers scatter most of the UV-radiation back to the space. Thus, it can be said that clouds are not necessarily good protector from sun burn. Depending upon the type and height of clouds, light water content and particle distribution, cloud cover can attenuate over 70% of the incident UV-B radiation (McKenzie et al., 2007).

Steps for recovery of ozone layer

In 1972, it was warned that nitrous oxide and CFCs (Chlorine containing compounds) causes the breakdown of large amount of ozone in the stratosphere (Molina and Rowland, 1974; Velders et al., 2007). This fact causes the depletion of stratospheric ozone layer, increasing the UV-B radiation at the ground level, especially in Antarctica and Arctic region as well as at high altitude area. After which the British-Antarctica Survey was done by Farman et al. (1985) and the result of the survey showed a springtime ozone hole in Antarctic ozone layer. In the same year, 20 nations signed the Vienna Convention, which established a frame work for negotiating an international regulation on ozone depleting substances.

On September 16, 1987, the Montreal Protocol on “Substances that deplete the Ozone layer” was signed and applied on January 01, 1989 (Veldars et al., 2007). The ozone depletion is a global phenomenon and according to European Ozone Research Coordinating Unit (EORCU) its amount reaches approximately 0.6% per year. Despite reductions in production and use of ozone depleting chemicals, the potential of ozone depletion by anthropogenic emissions or natural causes (e.g. Volcanoes) still remains. Recently, in 2016, a springtime recovery in ozone layer is reported by Solomon et al. (2016) over 1.5 million sq. miles.

Effects of UV-B on Plants

Depletion of ozone layer leads to the increase in ultraviolet radiation (UV-B) reaching to the Earth surface (Madronich et al., 1998). Increase of UV-B radiation will alter the growth and metabolism of plants, thus UV-B radiation acts as an environmental stress/ abiotic factor on plants, which ultimately causes the slowing of plant growth, damages the photosynthetic pigments, lowers the carbon assimilation, altering the biomass allocation ultimately results in reduction of biomass and productivity (Tevini and Teramura, 1989).

All the plants show various responses to UV-B, some plants can tolerate this stress and some becomes sensitive and cannot tolerate such situation. These plants will acquire different defense mechanisms like increased thickness of leaves, production of more flavonoids, stimulation of the antioxidant formation; activation of the reactive species to quench free radicals, etc to protect the plants from such kind of environmental stresses. (Caldwell et al., 2003; Agrawal and Mishra, 2008). Rise in UV-B radiation in the environment results into different physiological responses (Tevini and Teramura, 1989). Changes observed after supplemental UV-B radiation includes epidermal deformation (Tevini and Steinmuller, 1987), changes in stomatal conductance, changes in ultra structure of leaves, increased level of flavonoids (Tevini et al. 1981, 1991; Beggs and Wellman, 1985), reduction in percentage of pollen germination (Flint and Cladwell, 1984), biomass reduction (Tevini et al., 1981; Lydon et al., 1986; Sullivan and Teramura, 1988). These changes could be the result of DNA damage, photosynthetic damage, alteration in membrane, destruction of protein, hormone inactivation (Tevini et al. 1989, 1991b), and signal transduction through

phytochrome (which photoconverts in response to UV-B)(Pratt and Butler, 1970), or signal transduction via a UV-B photo receptor.

Morphological changes induced by UV-B

Morphology of plants is considered to be a very effective indicator of UV-B damage. Measurements of other parameters like chlorophyll, carotenoids, phenols, lipid peroxidation, etc. have also proved to be useful indicators of UV-B tolerance and sensitivity.

Solar radiation comprised of various radiation, among which the percentage of UV-B is very less but the range of its morphological effects on plants is very diverse. And these changes includes from increase in leaf thickness, discoloration of leaves, increase in leaf serration to changes in root-shoot ratio (Jansen et al., 2002).

As a result of UV-B stress, initially bronzing, cupping, glazing of leaves are observed which is followed by development of irregular patches and with prolonged exposure, these patches gets converted into brown spot and dies (Singh et al., 2008). Zhao et al. (2003) has reported that these chlorotic and necrotic spots are formed due to decrease in chlorophyll content. It was also reported that leaf size decreases, leading to less leaf area and lesser branches, extension rate of stem also declines (Kakani et al., 2003; Reddy et al., 2003; Zhao et al., 2003) but sometimes opposite trends was observed like intense branching, more number of internodes, increased plant height and dense canopy. Exposure to UV radiation also leads to increase in cuticular wax deposition.

Kakani et al. (2003) had reported that prolonged exposure of UV-radiation leads to delayed flowering in different crops; opposite to that Sinclair et al. (1990) has told that early bud or flower development or time of first flower is not affected by UV-B.

Broad leaf plants are more sensitive in comparison to narrow leaf plants. Moreover, the members of family Cucurbitaceae and Brassicaceae are more sensitive. Increased level of UV-B also induces some common morphological changes such as reduction in leaf area, thickening of the leaves, curling or cupping of the leaves, increases in branching, tillering and number of leaves, decreases in number of fruits and flowers and seedling.

Reduction in leaf area occurs due to destruction of photosynthetic pigments but to cope up with the situation and to increase photosynthesis, number of leaves increases into the affected plants, which ultimately leads to increased number of branching in dicots and increased number of tillers in monocots. Most of the energy is lost in repair mechanisms, leading to the reduction in flowering and fruiting. Moreover, thickening of leaves are a defense mechanism acquired by the plants in order to increase the path length of UV radiation.

Due to conversion of Indole-acetic-acid into 3-methylene oxindole curling of leaves takes place.

By this conversion, less growth of the upper side and normal growth of the lower side of the leaves take place, this ultimately causes the cupping of leaves (Kakani et al., 2003).

Physiological changes induced by UV-B

The process of conversion of CO₂ and water into carbohydrate in the presence of sunlight is termed as "Photosynthesis". Photosynthetic apparatus is comprised of two photosystems: PS-I and PS-II. Although UV-B encroaches most of the aspects of photosynthesis such as damages to ultra structure of chloroplast and light harvesting complex, decrease in the activity of Rubisco, decline in the oxygen evolving and CO₂ fixation, reduction in the chlorophyll and starch content. And the photosynthetic responses of plants towards UV-B radiation depend on plant species, cultivars, experimental conditions, UV-B dosage, and the ratio of PAR to UV-B radiation.

But the main target of UV-B is PS-II (Fiscus and Booker, 1995). PS-II is a complex of protein and pigment which transports the flow of electron(s) from splitting of water to plastoquinones (Barber et al., 1997; Mattoo et al., 1999). PS-II is comprised of two proteins, namely D1 and D2, which forms the core of PS-II. These two proteins are very sensitive against UV-B and UV-B driven degradation of D1 and D2 protein leads to impairment of PS-II, which can be measured in terms of decreased oxygen evolution or variable chlorophyll fluorescence. This variable chlorophyll fluorescence is often intended as a measure of radiation-damage to PS-II.

According to previous reports, almost all components from Mn binding sites to plastoquinone acceptor sites within PS-II on thylakoid membrane are sensitive against UV-B. In addition, some indirect effects of UV-B are also observed which alters the rate of photosynthesis, such as, stomatal closure, changes in leaf thickness and anatomy, decrease in individual leaf area and total canopy leaf area. According to Van Rensen et al. (2007) damage caused by UV-B radiation occurs first on the acceptor side of photosystem II and only later on the donor side.

Due to elevated UV-B, changes in ultra structure of leaves takes place, which modifies the light attenuation by leaf and the total UV-B radiation in turn, affect photosynthesis. Leaf reflects 3-6% (Gao et al., 1996, Yang et al., 1995) to 10-40% from pubescent or glaucous surface (Robberecht and Caldwell, 1980). An increase in incident UV-B radiation would increase the amount transmitted if no additional reflection occurs at leaf surface. Plant species differed in their anatomical responses to UV-B radiation, while increase in leaf thickness due to UV-B was common (Nagel et al., 1998; Bornman and Vogelman, 1991). The palisade cells from UV-B irradiated leaves of species were wider. The increase in cell number would increase the cell wall surface area, which blocks and prevents the harmful UV-B radiation from reaching the abaxial photosynthetically active mesophyll. The increased palisade cell number would also increase the amount of air cell wall interfaces, an important parameter that affects reflectance (Knipling, 1970) and transmission of the incident radiation through the leaf surface (Bornman and Vogelman, 1991).

Other changes produced by elevated UV-B include more trichomes on the abaxial leaf surface (Barnes et al., 1996), a reduction in number and diameter of xylem tubes, decreased stomatal frequency and distorted leaf area (Lingakumar and Kalandaivelu, 1993). These adverse effects of UV-B on leaf anatomy would inhibit the uptake of CO₂ and in turn more assimilate production. UV-B exposure also reduces the CO₂ fixation by decreasing the activity and concentration of Rubisco. This decline in the activity of Rubisco is due to the decrease in soluble protein.

Moreover, stomatal conductance is the single and most variable factor which governs the responses of plants to UV-B. Therefore, intra- and inter specific variations in the impacts of UV-B often correlate with the intrinsic capacity of stomatal conductance; plants with higher rates of stomatal uptake exhibiting greater effects (Mansfield and Freer-Smith, 1984). Furthermore, since exposure generally results in the decline in stomatal apertures, plants that display most rapid stomatal closure in response to UV-B are often reported to be resistant. However, stomatal closure leads to reduction of evapotranspirational loss of water and water use efficiency gets increased, which ultimately leads to increased plant growth.

Factors such as volume of leaves, intercellular air spaces and exposed mesophyll cell surface area play an important role in determining the variable UV responses. Furthermore, thinner leaves are generally found to be more sensitive to UV-B than thicker leaves.

Chlorophyll fluorescence is an effective physiological indicator of plant response to stress. A decrease in Fv/Fm ratio under UV-B; reflects photo inhibition. Light-dependent inactivation of the photosystem II (PSII) reaction centre is associated with a decline in Fm and Fv/Fm and with an increase in initial fluorescence as is observed in UV-B-exposed plants (Long and Humphries, 1994). A diminished electron flow from the water-splitting complex could cause impairment on the donor side, probably lowering Fm in UV-B-exposed plants (Demmig-Adams et al., 1989). Fv, which characterizes the photosynthetic competence of a leaf or chloroplast, reduced under ambient and UV-B levels to a similar extent. The impact of UV-B radiation on PSII activity is commonly measured as a decrease in oxygen evolution or variable fluorescence (Vass et al., 1996). The decrease in Fv/Fm ratio was reported to be accompanied by an elevated production of superoxide radicals (Jin and Tao, 2000) which corresponded directly with higher MDA content under UV-B stress. UV-B caused more membrane damage measured in terms of MDA content with a higher magnitude of damage under UV-B.

Although, photosynthesis is directly related to the biomass accumulation in plants but reduction in biomass is not necessarily related with the UV induced reduction in photosynthesis. To cope up with prevailing climatic conditions, light and UV-B stress, plant induces the synthesis of various secondary metabolites, which also alters the physiological processes and might be a reason for reduction of biomass.

Biochemical responses against UV-B and defense mechanisms adapted by the plants

UV-B is well known for its deleterious effects and severe consequences on various physiological and biochemical characteristics of economically important plants (Miller et al., 1994; Rao et al., 1996; Ambhast and Agrawal, 2003). UV-B penetrates through leaves and is absorbed by chromophores associated with the photosynthetic apparatus. Leaves absorb over 90% of incident UV-B. Leaf surface reflectance in the wavelength is generally below 10% and there is negligible transmission of UV-B through leaves (Robberecht and Caldwell, 1980; Cen and Bornman, 1993; Gonzalez et al., 1996). Cell components which absorb UV-B directly include nucleic acids, proteins, lipids and quinones (Jordan, 1996). Water soluble phenolic pigments such as flavonoids are also found in leaves, which strongly absorb UV-B radiations and protect the plants. UV-B stress leads to the production of reactive oxygen species. Rao et al. (1996) proposed that UV-B activates membrane localized NADPH oxidase, which then leads to the generation of ROS. Plants comprised of several strategies to acclimatize and metabolise ROS. These includes active defence systems using low molecular weight antioxidants such as ascorbic acid, phenols, flavonoids, glutathione, carotenoids, etc and high molecular weight enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POD), catalase (CAT), etc (Miller, 2002; Cervilla et al., 2007).

Increased levels of UV-B radiations are responsible for the increased reflectivity of the plants surface i.e. the leaves becomes more shiny and glabrous because of the increased deposition of waxy material. It is a common type of defense mechanism acquired by the plants to protect them from harmful UV-B radiation. Excess UV-B exposure also induces the bronzing and reddening of leaves, due to more production of polyphenolic compounds like flavonoids.

UV-B and photosynthetic pigments

Chlorophyll a and b are the main photosynthetic pigments in all higher plants whereas carotenoids are the main UV-protecting pigments. Carotenoids act as quencher in plants during stress. Carotenoids are effective scavengers of ROS and thus protect chlorophylls against photo oxidative damage caused by UV-B irradiation by dissipating excess excitation energy (Yamamoto and Bassi, 1996). Along with carotenoids other pigments like xanthophylls, anthocyanin etc are also found in plants and they also protect the plants during stress.

Elevated UV-B radiation reduces the chlorophyll content in many plant species, this occurs due to lipid peroxidation in chloroplast membrane. The photo reduction of protochlorophyllide to chlorophyllide by protochlorophyllide oxidoreductase is one of the possible targets of UV-B (Marwood and Greenberg, 1996). Because this reaction is light driven, it is possible that UV-B can damage this enzyme resulting in lowering rate of chlorophyll accumulation. UV-B down regulates the expression of genes, which encodes for chlorophyll a/b binding proteins (Casti and Walbot, 2003).

Chlorophylls are relatively photostable except at very high white light illumination. Chlorophyll destruction was a function of UV-B fluence rate; carotenoids are similarly affected in sensitive species. Decrease in chlorophyll content was evident on exposure to elevated UV-B radiation in most of the crop species reviewed, ultrastructural damage to chloroplast and changes in photosynthetic pigments results in the reduction of photosynthesis (Sullivan and Rozema, 1999). Chlorophyll reduction on exposure to UV-B in major crops species ranged from as low as 10% (Mirecki and Teramura, 1984; Tevini et al., 1981; Pal et al., 1999) to as high as 70% (Tevini et al., 1981; He et al., 1993) and reduction being higher among the dicots species (10-78%) compared to that in monocot species (0-33%).

The chlorophyll components, thylakoids and grana were sensitive to the incoming solar radiation (Tevini et al., 1991; He et al., 1994; Cassi-Lit et al., 1997). An increase in UV-B radiation resulted in rupture of the thylakoid and grana due to disintegration of the membrane. Photo bleaching was dependent on the length and intensity of UV-B radiation (Huang et al., 1993). Most higher plants accumulate UV-absorbing pigments in their leaves particularly phenylpropanoids after irradiation with UV-B (Wellman, 1983; Tevini et al., 1983; Beggs et al., 1986), these compounds were commonly located at upper epidermal layer of the leaves. These UV-absorbing compounds are mainly phenylpropanoids such as cinnamoyl esters, flavones, flavonols, etc.

UV-B and permeability

An early consequence of UV-B radiation is an increase in ion permeability of thylakoid membranes, UV-B induced leakiness is also observed in the plasma membrane which is explained by association with specific ion channels. UV-B induced loss of K^+ ions from guard cell may be responsible for the observed loss of stomatal conductance in irradiated plants (Negash and Bjorn, 1986). UV-B induced damage of membrane is mainly focussed on the transport phenomenon (Murphy et al., 1985).

UV-B and phenols

Increase in phenolic content is an adaptive response, acting as a screen that provides protection against UV-B penetration in leaf tissues and as antioxidants protecting cells from reactive oxygen species (Bornman et al., 1998; Rozema et al., 2002). Phenolic compounds of plants have been generally reported to be responsible for different biological properties. Hence, enhancement in the level of phenolics in UV-B-exposed plants plays a significant role in plant functions. The activation of the genes of phenyl propanoid pathway producing phenolic compounds is a common response to UV-B stress in plants. These phenolic compounds are produced from aromatic amino acids (phenylalanine and tyrosine) via the phenyl propanoid pathway. These compounds can mitigate the UV-induced damage by protecting the photosynthetic pathway and cellular components. Increase in concentrations of UV-B absorbing compounds including phenolic acid and flavonoids are the most consistent response of plants to UV-B supplementation. Many studies have shown that plant mutants with

elevated accumulation of UV-B absorbing phenolics compounds including flavonoids display remarkable tolerance to UV-B (Bieza and Lois, 2001). Phenolics may have a multifunctional role in UV-B defence. They screen out harmful UV-B radiation and emit less-harmful longer-wavelength fluorescent radiation and also act as antioxidants directly scavenging the active oxygen species induced by UV-B radiation (Rozema et al., 2002).

UV-B and flavonoids

Flavonoids, the most common group of polyphenolic compounds that are found ubiquitously in plants. Flavonoids are potential antioxidants and have aroused considerable interest recently because of their potential beneficial effects on human health in fighting diseases. The capacity of flavonoids to act as antioxidants depends upon their molecular structure. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities. Quercetin, the most abundant dietary flavonol, is a potent antioxidant because it has all the right structural features for free radical scavenging activity. Flavonoids helps in reproduction by providing colours to the petals, helps in signal transduction, help in nectar guiding, provides allelopathy and also interacts with microbes and helps in root nodule and mycorrhizal association formation. Flavonoids are confined to the epidermal part of the plants and it remains bound inside the vacuoles and is also found in root hairs, glandular hairs or trichomes of the plants.

There are two types of flavonoids that are found in plants: constitutive flavonoids and induced flavonoids. Constituted flavonoids (e.g. Hydroxy cinnamic acid) are normally present in plants; these are simple and are able to remove small amount of UV radiation. But high amount of UV radiation induced flavonoids (e.g. Quercetin-upper part of plant and Kaempferol- lower part of the leaf); these are specialized flavonoids known as flavonoids glycosides.

Plants respond to UV B by balancing reactions that lead to damage, repair, and acclimation. UV-B attenuation is mainly attributed to flavonoids and related phenolic compounds that absorb UV-B radiation effectively while transmitting PAR to the chloroplasts (Caldwell et al., 1983; Li et al., 1993; Reuber et al., 1996). Levels of these complex phenolic compounds vary considerably between plant species, with developmental stage, and with differing environmental conditions such as visible radiation levels, water, and nutrient supply (Caldwell, 1971; Murali and Teramura, 1985). In addition, exposure to UV-B radiation may increase the concentration of UV-B-absorbing compounds in the epidermis, rendering some plants less susceptible to photosynthetic damage due to UV-B exposure. Oilseed rape plants when pre-adapted to grow in UV-B, developed tolerance (Wilson and Greenberg, 1993). These plants have elevated levels of flavonoids in their epidermal cells. Increased amounts of UV protective compounds have been commonly shown in the literature (Tevini et al., 1991; Ziska and Teramura, 1992; Santos et al., 1993), while stimulation in leaf respiration has previously been observed (Sisson and Caldwell, 1976; Ziska et al., 1991) but not

discussed (Gwynn-Jones, 2001). But now it is hypothesized that a stimulation of leaf respiration represents increased resource demands for protection and repair (cuticular thickening, flavonoid biosynthesis and photoreactivation). The stimulation of respiration in non-growing mature leaves supports this view as it can be used to reflect maintenance of respiration.

UV-B and ascorbic acids

Ascorbic acid is one of the most powerful antioxidants that scavenges the H_2O_2 and other ROS profoundly. It is commonly called as Vitamin-C. Protective effect of ascorbic acid is possibly more related to its participation in direct scavenging of O_2 and $\cdot OH$, and also the removal of H_2O_2 through Asada-Halliwell pathway thus, reducing ROS induced damage to essential proteins and/or nucleic acids. Significantly, higher ascorbic acid content in *V. acotifolia* leaves before (in control plants) and after UV-B exposure showed its greater capability to scavenge ROS.

There may be two possibilities regarding increased ascorbic acid content; either its synthesis has been amplified or its regeneration through Asada-Halliwell pathway.

UV-B and lipids

Oxidative damage to cellular membrane due to oxyradicals is measured in terms of lipid peroxidation (LPO) that accessed via increase in MDA content. Single or conjugated double bonded lipids can be photo chemically modified by the action of UV-B. Plant cell membranes are composed of phospholipids and glycolipids which are the main components of plant cell membrane and are made up of unsaturated fatty acids, which are destroyed by UV-B in presence of oxygen (Kramer et al., 1991; Panagolpoulas et al., 1990).

Oxidative damage can be detected by lipid peroxidation. Hydroxyl free radicals and nascent oxygen reacts with methylene group forming dienes, lipid peroxy radicals and hydroperoxides (Smirnoff, 1995). The peroxy radicals can extract hydrogen from other polyunsaturated fatty acids, leading to a chain reaction of peroxidation. The peroxidation of membrane lipids leads to break down of their structure and function. One of the products of lipid peroxidation is malondialdehyde, MDA, which is often used as a measure of peroxidation.

Other hydrocarbons such as ethylene, ethane and pentane can be measured (Halliwell, 1987). Hydroxyl radical can denature proteins and reacts with bases in DNA causing mutation.

UV-B and proteins

As we know that UV radiation causes modification and destruction of amino acids and is also responsible for inactivation of whole proteins and enzymes (Grossweiner., 1984; Prinsze et al., 1990). Due to UV radiation induced photolysis of aromatic amino acids or disulfide groups, the active sites of these proteins and enzymes becomes inactive. The absorption of UV-B within protein matrix causes damage far

away from the actual site by energy migration to functionally important amino acids of an active centre as suggested for the sensitization of cysteine destruction by aromatic residues.

Characteristic examples of this effect include Ribulose-1, 5-bisphosphate carboxylase (Rubisco) (Vu et al., 1984; Strid et al., 1990), ATPase violaxanthin de-epoxidase (Pfundel et al., 1992), and protein subunit of the photosystem I and II as well as insulin, pepsin, trypsin, myosin.

UV-B and plant growth regulators

Plant growth regulators are one of the most important regulators in plant growth and development and any alteration in their concentration influences or alters the processes which are dependent on them (Yang et al., 1993). Photolytic degradation of Indole-acetic acid (IAA) has been observed in sunflower seedling (Ros and Tevini, 1995).

The reduced concentration of IAA and growth inhibitors IAA photoproduct, 3-methylene oxindole, is responsible for the inhibition of hypocotyls growth. Moreover, the action of peroxidase functioning as IAA oxidase may also inhibit the elongation since, the cell wall extensibility is reduced.

Effects of UV-B on phenology and growth

Growth parameters such as plant height and leaf area are reduced in UV-B sensitive plants to various extents, depending on plants species and cultivar (Lydon et al., 1988; Murali and Teramura, 1986). Increased levels of UV-B radiation delayed both seedling emergence and flowering of several crop plants under controlled condition (Basiouny, 1986; Saile-Mark and Tevini, 1997) and in field (Li et al., 1998). Both artificially and naturally supplied levels of UV-B radiation decreased stem length, leaf area, chlorophyll and carotenoids content in cucumber seedling.

Mark and Tevini (1996) observed that the mechanism of reduction in stem elongation by UV-B might be due to changes in the phytohormones levels, especially IAA which plays important role in stem elongation. Few studies told that breakdown of IAA by UV-B exposure (Ros and Tevini, 1995; Huang et al., 1993) which results into reduced cell length and plant height (Gonzalez et al., 1998). Effects on the stem also include coiling of both attached and detached tendrils.

Similarly, leaf area is also a very sensitive growth parameter that responded to elevated UV-B radiation. Under most of the experimental studies, leaf area decreases in exposed plants and leaves becomes smaller in size (Nogues et al., 1998; Zhao et al., 2003) that serves as a protective mechanism (Bornman and Teramura, 1993), reduction in cell number (Gonzalez et al., 1998a) and by cell division and cell expansion (Hofmann et al., 2001). In contrast, Nedunchezian and Kulandaivelu (1997) reported that slightly elevated UV-B radiation increases leaf area in cowpea. Even high UV-B treated broad beans and wheat plant had higher leaf area. Out of few studies, in 54%

plants, reduction in biomass was observed in elevated UV-B, in 35% studies, no effect was observed on dry weight and in few cases, increase in crop dry matter accumulation was observed. These differences were probably associated with genotypes, UV-B doses and PAR: UV-A: UV-B ratio. Crop biomass production in response to UV-B radiation was highly UV-B dosage dependent.

In some investigation it is observed that the effect of UV-B /PAR ratio on the sensitivity of rye to increased UV-B radiation under controlled conditions, and concluded that plants grown under higher total irradiance levels developed leaves that were more tolerant to UV-B damage, while low PAR levels increases the sensitivity of plants to the UV-B radiations. The effects of elevated UV-B radiation on plants growth and dry mass accumulation in field conditions were usually much smaller than on those in growth chamber (Caldwell et al., 1994; Olszyk et al., 1996).

Effects of UV-B on crop yield and biomass partitioning

Reduced rate of photosynthesis alters the processes of carbon assimilation in different parts of the plant. The UV-B exposure to foliage causes the accumulation of carbohydrates in the source leaves and reduced translocation to the sink which is present distantly. The phloem loading is an active process which requires energy and increased concentration of UV-B reduces this process. UV-B is responsible for the oxidation of sensitive proteins involved in phloem loading. The stored carbohydrates are reported to be modified in wheat and barley. This stored carbohydrate is affected by both reduction in rate of carbohydrate production and by a shift of carbohydrate to repair and replacement processes. The translocation of photosynthates from source to sink is inhibited by UV-B, leading to the alterations in biomass partitioning.

Due to varied impact of UV-B on the translocation of photoassimilates from leaves the relative effects on the growth of different parts of the plant is observed. Photosynthates allocation patterns directly affect the growth and reproduction of the plants. Partitioning of reducing sugars, sucrose and starch varies along with the growing stages of the plants. The most UV-effective period occurred during the transition time between vegetative and reproductive phases (Teramura and Sullivan, 1987).

At flowering and fruiting stages, the reproductive organ requires high concentration of photosynthates and that's why the photosynthates are diverted from leaves and roots towards reproductive organs. In cereal crops, flag leaves are the active assimilatory source during anthesis stage of the plant. Flag leaves are the penultimate leaf sheath and peduncle photosynthesis provides assimilates for the grain but flag leaf blade and spikes are the most important contributors to grain filling. In a observation it is found that an increase in concentration of carbohydrates of wheat flag leaves fumigated with ozone at the time of anthesis due to interference in transport processes (phloem loading) caused by membrane damage of mesophyll or companion cells. Senescence of flag leaves due to UV-B radiation results into loss of chlorophyll and soluble protein. The duration of assimilate production and grain filling is a key factor

behind the yield reduction and is altered by UV-B. However, UV-B also affects the rate of grain filling and reduces the yield.

Conclusions

The impact of UV-B on biological systems mainly on plants is found to be severe because of the sessile nature of the plants. These impacts are the mixed result of damage, repair and acclimatization adapted and showed by the plants. The acclimatization mechanism of plants diverts the focus from many important targets of UV-B, which also requires brief study to understand their ecological relevance. The strong defense strategies of plants reduce the damaging effects induced by UV-B because plants have to acclimatize themselves continuously from the varying UV-B levels. Thus, future studies should be targeted not only on the activities showing greater changes but also on the minor variations. While evaluating the effects of stress, other climatic conditions and their interactions as well as its impacts on plants and community should be taken care too. In addition to this, the future perspective should not be limited up to the defense mechanism but also on the acclimatization processes towards increasing UV radiations.

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Table 1: Distribution of solar irradiance energy

Spectral Regions	Wavelength (nm)	% of Total Energy
Infra red	≥ 700	49.4
Visible	400-700	42.3
UV-A	320-400	6.3
UV-B	290-320	1.5
UV-C	≤ 290	0.5

Table 2: Defense responses showed by plants against enhanced UV-B

S.No.	UV-B induced damages	Repair and defense strategies
1.	Genetic material	Photoreactivation via photolyases Excision repair via by passing damage, removing damaged part, gap filling from sister duplex
2.	Physiology	Increasing stomatal closure, water use efficiency, reducing evapotranspiration.
3.	Leaf surface (chlorosis, necrosis, photobleaching)	Increased reflectance through cuticle, wax, hair and trichomes formation
4.	Epidermal region	Increased production of pigments (flavonoids, carotenoids, anthocyanin)
5.	Oxidative stress	Formation of antioxidants (SOD, APx, GSH) and compounds (ascorbates, α -tocopherols, polyamines)
6.	Pest attacks	Phytoalexins and anti fungal compounds formation

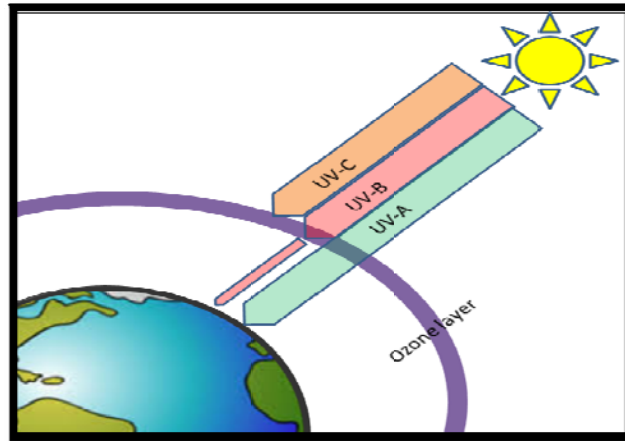


Fig.1: Imaginary diagram of UV radiation reaching to the Earth's surface

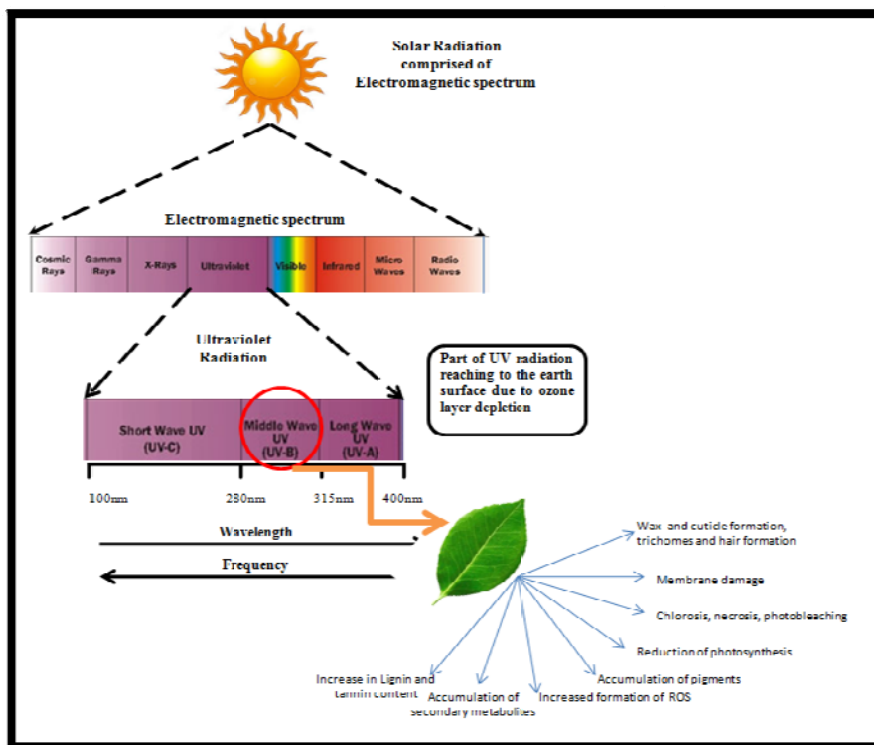


Fig.2: Portion of electromagnetic spectrum comprising ultraviolet region, which causes maximum damage to the vegetation and the main responses shown by the plants against UV-B

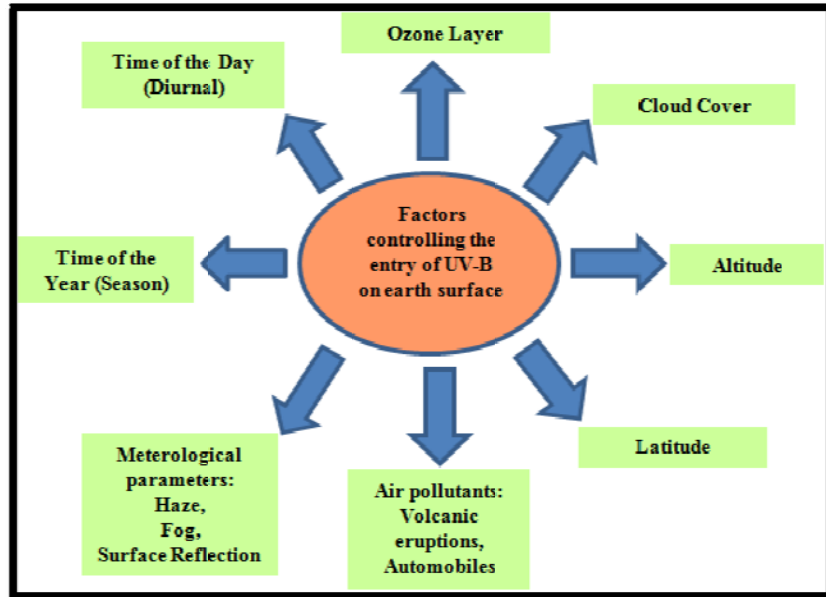


Fig.3: Factors controlling the entry of UV-B on Earth's surface



Fig.4: Bronzing of leaves of *Hordeum vulgare L.* under elevated UV-B

REFERENCES :

- Agrawal, S.B. and Mishra, S. (2007). Effect of supplemental ultraviolet-B and cadmium on growth antioxidants and yield of *Pisum sativum* L. *Ecotoxicol. Environ. Saf.* 72:610–618.
- Ambasht, N.K. and Agrawal, M. (2003). Interactive effects of ozone and ultraviolet-B singly and in combination on physiological and biochemical characteristics of soybean plants. *J. Plant Biol.*, 30:37-45.
- Barber, J., Nield, J., Morris, E.P., Zheleva, D., and Hankamer, B. (1997). The structure, function and dynamics of photosystem 2. *Physiol. Plant.* 100:817–827.
- Barnes, J.D., Percy, K.E., Paul, N.D., Broadbent, P., McLaughlin, C.K., Mullineaux, P.M., Creissen, G., Wellburn, A.R., (1996). The influence of UV-B radiation on the physico-chemical nature of tobacco (*Nicotinia tabacum* L.) leaf surfaces. *J. Exp. Bot.* 47:99-109.
- Basiouny, F.M., (1986). Sensitivity of corn, oats, peanuts, rice, rye, sorghum, soybean and tobacco to UV-B radiation under growth chamber conditions. *J. Agron. Crop Sci.* 157:31–35.
- Beggs, C., Wellmann, E., Grisebach, H. (1986). Photocontrol of flavonoid synthesis *In RE* Kendsrick, G Kronenberg, eds, *Photomorphogenesis in Plants*. Nijhoff/W. Junk Publishers, Dordrecht, The Netherlands, 467–499
- Beggs, C.J., Wellman, E. and Stolzer-Jehle, A. (1985). Isoflavonoid formation as an indicator of UV stress in beans (*Phaseolus vulgaris*. L) leaves. *Plant Physiol.* 79:630-635.
- Bieza, K., and Lois, R. (2001). An *Arabidopsis* mutant tolerant to lethal ultraviolet-B levels shows constitutively elevated accumulation of flavonoids and other phenolics. *Plant Physiology* 126: 1105–1115.
- Bornman, J.F., Teramura, A.H. (1993). Effects of ultraviolet-B radiation on terrestrial plants. In: Young A.R., Bjorn L.O., Moan J., Nultsch W. (Eds), *Environmental UV photobiology*. Plenum Press, New York, USA, 427–471.
- Bornman, J.F., Reuber, S., Cen, Y.-P., Weissenböck, G., (1998). Ultraviolet radiation as a stress factor and the role of protective pigments. In: Lumsden, P.J. (Ed.), *Plants and UV-B. Responses to Environmental Change*. Cambridge University Press, Cambridge, 157-170.
- Bornman, J.F., Vogelmann, T.C., (1991). Effect of UV-B radiation on leaf optical properties measured with fiber optics. *J. Exp. Bot.* 41:547–554.
- Caldwell, M. M. (1971). Solar UV irradiation and the growth and development of higher plants. In: A. C. Giese (Ed). *Photophysiology*. Academic Press. N.Y. 6:131–177.
- Caldwell, M. M., Robberecht, R. and Flint, S. D. (1983). Internal filters: prospects for UVacclimation in higher plants. *Physiol. Plant.* 58:445-450.
- Caldwell, M.M., Ballare, C.L., Bornman, J.F., Flint, S.D., Bjorn, L.O., Teramura, A.H., Kulandaivelu, G., and Tevini, M. (2003) Terrestrial ecosystems, increased solar ultraviolet radiation and interactions with other climate change factors. *Photochem. Photobiol. Sci.* 2:29-38
- Caldwell, M.M., Flint, S.D., (1994). Stratospheric ozone reduction, solar UV-B radiation and terrestrial ecosystems. *Climatic Change* 28:375-394.
- Casati, P. and Walbot, V. (2003) Gene expression profiling in response to ultraviolet radiation in maize genotypes with varying flavonoid content. *Plant Physiol* 132:1739 1754.

- Cassi-Lit, M., Whitecross, M.J., Nayudu, M., Tanner, G.J., (1997). UV-B irradiation induces differential leaf damage, ultrastructural changes and accumulation of specific phenolic compounds in rice cultivars. *Aust. J. Plant Physiol.* 24:261–274.
- Cen, Y.P., Bornman, J.F., (1993). The effect of exposure to enhanced UV-B radiation on the penetration of monochromatic and polychromatic UV-B radiation in leaves of *Brassica napus*. *Physiol. Plant* 87:249–255.
- Cervilla, L.M., B. Blasco, J.J. Rios, L. Romero and J.M. Ruiz, (2007). Oxidative stress and antioxidants in tomato (*Solanum lycopersicum*) plants subjected to boron toxicity. *Ann. Bot.*, 100:747-756.
- Cockell, C.S., Horneck, G., (2001). The history of the UV radiation climate of the Earth theoretical and space-based observations. *Photochemistry and Photobiology* 73:447-451
- Day, T.A., and Vogelmann, T.C. (1995). Alterations in photosynthesis and pigment distribution in pea leaves following UV-B exposure. *Physiol Plant* 94:433–440.
- Demmig-Adams, B., Winter, K., Winkelmann, E., Krüger, A., Czygan, F.C. (1989). Photosynthetic characteristics and the ratios of chlorophyll, 0-carotene, and the components of the xanthophyll cycle upon a sudden increase in growth light regime in several plant species. *Bot. Acta.* 102:319–325
- Fiscus, E. L. and Booker, F. L. (1995). Is increased UV-B a threat to crop photosynthesis and productivity. *Photosynth. Res.* 43:81-92.
- Friso, G., Barbato, R., Giacometti, G. M. and Barber, J. (1994). Degradation of the D2 protein due to UV-B irradiation of the reaction center of photosystem II. *FEBS Lett.* 339:217–221.
- Gao, W., Grant, R.H., and Heisler, G.M. (1996). Spectral radiative properties of various tree species in ultraviolet wavelengths and irradiance modeling implications. In: Proceedings of the 22nd Conference on Agricultural and Forest Meteorology with Symposium on Fire and Forest Meteorology, American Meteorological Society, Boston, MA, 417–418.
- Gonzalez, R. (1996). Responses to ultraviolet-B radiation (280–315 nm) of pea (*Pisum sativum*) lines differing in leaf surface wax, *Physiol. Plant.* 98:852–860
- Gonzalez, R., Mepsted, R., Wellburn, A.R. and Paul, N.D. (1998). Non-photosynthetic mechanisms of growth reduction in pea (*Pisum sativum* L.) exposed to UV-B radiation. *Plant Cell Environ.* 21:23–32.
- Greenberg, B. M., Wilson, M. I., Gerhardt, K. E. and Wilson, K. E. (1996). Morphological and physiological responses of *Brassica napus* to ultraviolet-B radiation: photomodification of ribulose-1,5-bisphosphate carboxylase/oxygenase and potential acclimation processes. *J. Plant Physiol.* 148:78–85.
- Gwynn-Jones, D. (2001). Short-term impacts of enhanced UV-B radiation on photoassimilate allocation and metabolism: a possible interpretation for time dependent inhibition of growth. *Plant Ecol.* 154:67–73
- Halliwell, B. (1982). Ascorbic acid and the illuminated chloroplast. In: *Ascorbic acid: Chemistry, metabolism and uses* (Eds.: P.A. Seib and B.M. Tolbert). American Chemical Society, Washington. 263-274.
- Halliwell, B., Gutteridge, J.M.C. and Aruoma, O.I. (1987). The deoxyribose method: a simple 'test tube' assay for determination of rate constants for reactions of hydroxyl radicals. *Anal. Biochem.*, 165:215-219.

- He, J., Huang, L.K., Chow, W.S., Whitecross, M.I., Anderson, J.M., (1993). Effects of supplementary ultraviolet-B radiation on rice and pea plants. *Aust. J. Plant Physiol.* 20:129–142.
- He, J., L.K. Huang, W.S. Chow, M.I. Whitecross and J.M. Anderson (1994). Responses of rice and pea plants to hardening with low doses of ultraviolet-B radiation. *Aust. J. Plant Physiol.*, 21: 563-574.
- Hofmann, R.W., Campbell, B.D., Fountain, D.W., Jordan, B.R., Greer, D.H., Hunt, D.Y., Hunt, C.L., (2001). Multivariate analysis of intraspecific responses to UV-B radiation in white clover (*Trifolium repens* L.). *Plant Cell Environ.* 24:917–927.
- Huang, L.K., He, J., Chow, W.S., Whitecross, M.I., Anderson, J.M., (1993). Responses of detached rice leaves (*Oryza sativa* L.) to moderate supplementary ultraviolet-B radiation allow early screening for relative sensitivity to ultraviolet B irradiation. *Aust. J. Plant Physiol.* 20:285–297.
- Jansen, M. A. K., Greenberg, B. M., Edelman, M., Mattoo, A. K. and Gaba, V. (1996). Accelerated degradation of the D2 protein of photosystem II under ultraviolet radiation. *Photochem. Photobiol.* 63:814–817.
- Jansen, M.A.K. (2002). Ultraviolet-B radiation effects on plants: Induction or morphogenic responses. *Physiol. Plant.* 116: 423-429
- Jin, Y.H., Tao, D.L. (2000). PS II photoinhibition and O₂ production. *Acta. Bot. Sin* 42:10 14
- Jordan, B.R. (1996). The effects of ultraviolet-B radiation on plants: a molecular perspective. *Adv Bot. Res.* 22:98–138
- Kakani, V. G., Reddy, K. R., Zhao, D. and Sailaja, K. (2003). Field crop responses to ultraviolet-B radiation: a review. *Agric. For. Meteorol.* 120:191–218.
- Knipling, E.B. (1970). Physical and physiological basis for the reflection of visible and near-infrared radiation from vegetation *Remote Sens. Environ.* 1:155–159.
- Kramer, G.F., Krizek, D.T., and Mirecki, R.M. (1992). Influence of photosynthetically active radiation and spectral quality on UV-B induced polyamine accumulation in soybean. *Phytochemistry* 31:1119–1125.
- Kramer, G.F., Norman, H.A., Krizek, D.T., Mirecki, R.M., (1991). Influence of UV-B radiation on polyamines, lipid peroxidation and membrane lipids in cucumber. *Phytochem.* 30:2101–2108.
- Li, J., Ou-Lee, T. M., Raba, R., Amundson, R. G. and Last, R.L. (1993). *Arabidopsis* flavonoid mutants are hypersensitive to UV-B irradiation. *Plant Cell* 5:171-179.
- Li, Y., Yue, M., Wang, X.L., (1998). Effects of enhanced ultraviolet-B radiation on crop structure, growth and yield components of spring wheat under field conditions. *Field Crops Res.* 57:253–263.
- Lingakumar, K., and Kulandaivelu, G. (1993). Changes induced by ultraviolet-B radiation in vegetative growth, foliar characteristics and photosynthetic activities in *Vigna unguiculata*. *Aust. J. Plant Physiol.* 20:299–308.
- Long, S.P., and Humphries, S. (1994). Photoinhibition of photosynthesis in nature. *Annu. Rev. Plant Physiol. Plant. Mol. Bio.* 45:633–662
- Lydon, J., Teramura, A.H., and Summers, E.G. (1988). Effects of ultraviolet-B radiation on the growth and productivity of field grown soybean. In stratospheric ozone reduction, solar

- ultraviolet radiation and plant life, R.C. Worrest and M.M. Caldwell, eds (Berlin, Heidelberg :Springer-verlag), 313-325.
- Madronich, S., McKenzie, R.L., Bjorn, L.O., and Caldwell, M.M. (1998). Changes in biologically active ultraviolet radiation reaching the Earth's surface. *Journal of Photochemistry and Photobiology B: Biology* 46:5-19.
- Mansfield, T.A. and Freer-Smith, P.H. (1984). The role of stomata in resistance mechanism. In: *Gaseous Air Pollutants and Plant Metabolism* (Ed. By M.J.Koziol and F.R. Whatley). Butterworths, London.
- Mark, U., Tevini, M., (1996). Combination effects of UV-B radiation and temperature on sunflower (*Helianthus annuus* L., cv. Polstar) and maize (*Zea mays* L., cv. Zenit 2000) seedlings. *J. Plant Physiol.* 148:49–56.
- Marwood, C.A. and Greenberg, B.M. (1996) Effect of supplementary UV-B radiation on chlorophyll synthesis and accumulation of photosystems during chloroplast development in *Spirodela oligorrhiza*. *Photochem Photobiol* 64:664–670
- Mattoo, A. K., Giardi, M.-T., Raskind, A. and Edelman, M. (1999). Dynamic metabolism of photosystem II reaction center proteins and pigments. *Physiol. Plant.* 107:454–461.
- McKenzie, R.L., Aucamp, P.J., Bais, A.F., Bjorn, L.O., and Ilyas, M. (2007). Changes in biologically active ultraviolet radiation reaching the Earth's surface. *Photochem. Photobiol. Sci.* 6: 218-231
- Miller, J.E., Booker, F.L., Fiscus, E.L., Heagle, A.S., Pursley, W.A., Vozzo, S.F., Heck, W.W., (1994). Ultraviolet-B radiation and ozone effects on growth, yield, and photosynthesis of soybean. *J. Environ. Qual.* 23:83–91.
- Mirecki, R.M., and Teramura, A.H. (1984). Effects of ultraviolet-B irradiance on soybean. The dependence of plant sensitivity on the photosynthetic photon flux density during and after leaf expansion. *Plant Physiol* 74:475–480
- Molina, M.J., and Rowland, F.S. (1974). Stratospheric sink for chlorofluoromethanes chlorine atomic-catalysed destruction of ozone. *Nature* 249:810–812.
- Murali, N. S. and A. H. Teramura. (1985). Effect of UV-B irradiance on soybean. VI. Influence of phosphorus nutrition of growth and flavonoid content. *Plant Physiol.* 63:413–416.
- Murali, N.S., and Teramura, A.H., (1986). Effectiveness of UV-B radiation on the growth and physiology of field grown soybean modified by water stress. *Photochem. Photobiol.* 44:215–219.
- Murphy, T.M., Hurrell, H.C. and Sasaki, T.L. (1985). Wavelengths dependence of ultraviolet radiation induced mortality and potassium ion efflux in cultured cells of *Rosa damascene*. *Photochemistry and Photobiology*, 42:281-286.
- Nagel, L.M., Bassman, J.H., Edwards, G.E., Robberecht, R., Franceschi, V.R. (1998). Leaf anatomical changes in *Populus trichocarpa*, *Quercus rubra*, *Pseudotsuga menziesii* and *Pinus ponderosa* exposed to enhanced ultraviolet-B radiation. *Physiol. Plant* 104:385–396.
- Nedunchezian, N., and Kulandaivelu, G., (1997). Changes induced by ultraviolet-B (280–320 nm) radiation to vegetative growth and photosynthetic characteristics in field grown *Vigna unguiculata* L. *Plant Sci. Limerick* 123:85–92.
- Negash, L., and Björn, L.O. (1986). Stomatal closure by ultraviolet radiation. *Physiol. Plant.* 66:360–364.

- Nogues, S., Allen, D.J., Morison, J.I.L., and Baker, N.R. (1998). Ultraviolet-B radiation effects on water relations, leaf development, and photosynthesis in droughted pea plants. *Plant Physiol.* 117:173–181.
- Olszyk, D., Dai, Q.J., Teng, P., Leung, H., Luo, Y., and Peng, S.B. (1996). UV-B effects on crops: Response of irrigated rice ecosystem. *J. Plant Physiol.* 148: 26-34
- Pal, M., Sengupta, U.K., Srivastava, A.C., Jain, V., and Meena, R.C., (1999). Changes in growth and photosynthesis of mungbean induced by UV-B radiation. *Indian J. Plant Physiol.* 4:79–84.
- Panagolopoulos, I., Bornman, J.F. and Bjorn, L.O. (1990). Effects of ultraviolet-B radiation and visible light on growth, fluorescence induction, ultraweak luminescence and peroxidase activity in sugar beet plants. *J. Photochem. Photobiol.B: biology* 8:73-87.
- Pfundel, E.E., Papan, R.S., Dilley, R.A. (1992). Inhibition of violaxanthin deep oxidation by ultraviolet-b radiation in isolated chloroplasts and intact leaves. *J. Plant Physiol.* 98, 1372–1380.
- Pratt, L.H., and Butler, W.L. (1970). Phytochrome conversion by ultraviolet light. *Photochem. Photobiol.* 11:503-509.
- Prinsze, C., Dubbleman, T.M.A.R., and Steveninck, J.V. (1990). Protein damage induced by small amounts of photodynamically generated singlet oxygen or hydroxyl radicals. *Biochem. Biophys. Acta*, 1038:152-157.
- Rao, M.V., Paliyath, G., and Ormrod, D.P. (1996) Ultraviolet-B- and ozoneinduced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana*, *Plant Physiol.* 110:125–136
- Reddy, K. R., Kakani, V. G., Zhao, D., Mohammeda, A. R. and Gao, W. (2003). Cotton responses to ultraviolet-B radiation: experimentation and algorithm development. *Agr. Forest Meteorol.* 120:249–265.
- Reuber, S., Bornman J. F. and Weissenbock, G. (1996). A flavonoid mutant of barley (*Hordeum vulgare* L.) exhibits increased sensitivity to UV-B radiation in the primary leaf. *Plant Cell Environ.* 19:593-601.
- Robberecht, R., and Caldwell, M. M., (1980). Leaf ultraviolet optical properties along a latitudinal gradient in the Arctic-alpine life zone. *Ecology* 61:612–619
- Ros, J. and Tevini, M. (1995). Interaction of UV radiation and IAA during growth of seedlings and hypocotyl segments of sunflower, *J. Plant Physiol.* 146:295–302
- Rozema, J. (2000). Effects of solar UV-B radiation on terrestrial biota. In: Hester, R.E., Harrison, R.M. (eds.) Causes and environmental implications of increased UV-B radiation. *Environmental sci. and technology*, R.S.C, Cambridge 14:86-105.
- Rozema, J., and Bjorn, L.O. (2002). Evolution of UV-B absorbing compounds in aquatic and terrestrial plants. *Journal of Photochemistry and Photobiology B: Biology* 66:1-2.
- Saile-Mark, M., and Tevini, M. (1997). Effects of solar UV-B radiation on growth, flowering and yield of central and southern European bush bean cultivars (*Phaseolus vulgaris* L.). *Plant Ecol.* 128: 115 – 125
- Santos, I., Almeida, J. M. and Salema, R. (1993). Plants of *Zea mays* L. developed under enhanced UV-B radiation. I. Some ultrastructural and biochemical aspects. *J. Plant Physiol.* 141, 450–456
- Sinclair, T.R., N'Diaye, O., and Briggs, R.H. (1990). Growth and yield of field-grown soybean in response to enhanced exposure to ultraviolet-B radiation. *J. Environ. Qual.* 19:478-481

- Singh, S.K. (2008). Developing screening tools for abiotic stresses using cowpea (*Vigna unguiculata* (L.) Walp.) as a model crop. Ph.D. Dissertation, Mississippi State University, MS
- Singh, S.K., Surabhi, G.K., Gao, W., and Reddy, K.R. (2008). Assessing genotypic variability of cowpea (*Vigna unguiculata* (L.) Walp.) to current and projected ultraviolet-B radiation. *J. Photochem. Photobiol. B: Biology* 93:71 - 81
- Sisson, W. B., and Caldwell, M. M. (1976). Photosynthesis, dark respiration, and growth of *Rumex patens* L. exposed to ultraviolet irradiance (288 to 315 nanometers) simulating a reduced atmospheric ozone column. *Plant Physiol.* 58:563–568.
- Smirnoff, N. (1995). *Environment and Plant Metabolism, Flexibility and Acclimation*. Bios Scientific Publishers, Oxford, UK
- Strid, A., Chow, W.S., and Anderson, J.M. (1990). Effects of supplementary ultraviolet-B radiation on photosynthesis in *Pisum sativum*. *Int. J. Biochem. Biophys.* 1020:260–268.
- Strid, A., W. S. Chow and J. M. Anderson. (1994). UV-B damage and protection at the molecular level in plants. *Photosynth. Res.* 39:475–489.
- Sullivan, J., and Rozema, J. (1999). UV-B effects on terrestrial plant growth and photosynthesis. In: Rozema, J. (Ed.), *Stratospheric Ozone Depletion, the Effects of Enhanced UV-B Radiation on Terrestrial Ecosystems*. Backhuys, Leiden, 39-57.
- Sullivan, J.H., and Teramura, A.H. (1988). Effects of UV-B irradiation on seedling growth in the Pinaceae. *Am.J.Bot.* 75:225-230.
- Teramura, A. H. and J. H. Sullivan. (1994). Effects of UV-B radiation on photosynthesis and growth of terrestrial plants. *Photosynth. Res.* 39:463–473
- Teramura, A.H., and Sullivan, J.H. (1987). Soybean growth responses to enhanced levels of ultraviolet-B radiation under greenhouse conditions. *Am. J. Bot.* 74:975–979.
- Tevini, M., and Steinmuller, D. (1987). Influence of light, UV-B radiation, and herbicides on wax biosynthesis of cucumber seedlings. *J. Plant Physiol.* 131:111-121.
- Tevini, M., And Teramura, A.H. (1989). UV-B effects on terrestrial plants. *Photochem. Photobiol.* 50:479-487.
- Tevini, M., Braun, J. and Fieser, G. (1991). The protective function of the epidermal layer of rye seedlings against ultraviolet-B radiation. *Photochem. Photobiol.* 53:329-333.
- Tevini, M., Iwanzik, W., and Thoma, U. (1981). Some effects of enhanced ultraviolet irradiation on the growth and composition of plants: barley, maize, kidney beans, radishes. *Planta* 153:388–394.
- Tevini, M., Mark, U., Fieser, G., and Saile, M. (1991). Effects of enhanced solar UV-B radiation on growth and function of selected crop plant seedlings. In *photobiology*, E. Riklis, ed. (New York : Plenum Press), 635-649.
- Tevini, M., Thoma, U., and Iwanzik, W., (1983). Effects of UV-B radiation on germination, seedling growth, leaf anatomy and pigments of some crop plants. *Z. Pflanzl. physiol.* 109:435–448.
- Turunen, M., and Latola, K. (2005). UV-B radiation and acclimation in timberline plants. *Environmental pollution* 137:390-403.
- Van Rensen, J. J. S., W. J. Vredenberg and G. C. Rodrigues. (2007). Time sequence of the damage to the acceptor and donor sides of photosystem II by UV-B radiation as evaluated by chlorophyll a fluorescence. *Photosynth. Res.* 94:291–297.

- Vass, I., Kirilovsky, D., and Etienne, A.L. (1999). UV-B Radiation-induced donor- and acceptor-side modifications of photosystem II in the Cyanobacterium *Synechocystis* sp. PCC 6803. *Biochemistry* 38(39):12786–12794.
- Vass, I., Sass, L., Spetea, C., Bakou, A., Ghanotakis, D.F., Petrouleas, V. (1996). UV-B induced inhibition of photosystem II electron transport studied by EPR and chlorophyll fluorescence. Impairment of donor and acceptor side components. *Biochemistry* 35:8964–8973.
- Velders, G. J. M., Andersen, S. O., Daniel, J. S., Fahey, D. W., and McFarland, M. (2007). The importance of the Montreal Protocol in protecting climate, *Proc. Natl. Acad. Sci.*, 104:4814–4819.
- Vu, C.V., Allen, L.H., and Garrard, L.A. (1984). Effect of UV-B radiation (280 nm-320 nm) on ribulose-1, 5-bisphosphate carboxylase in pea and soybean. *Environ. Exp. Bot.* 24:131-143.
- Wellman, E (1983). UV radiation: Definitions, characteristics and general effects. In: Shropshire, W., Mohr, H. (Eds.), *Encyclopedia of plant physiology, new series*, Springer-Verlag, Berlin, 16B: 745-756.
- Wilson, M. I. and B. M. Greenberg. (1993). Protection of the D1 photosystem II reaction center protein from degradation in ultraviolet radiation following adaptation of *Brassica napus* L. to growth in ultraviolet-B. *Photochem. Photobiol.* 57:556–563.
- Yamamoto, H. Y., and Bassi, R. (1996). Carotenoids: localization and function. In: *Oxygenic Photosynthesis: The Light Reactions* (Ort. D. R. and Yocum C. F., eds.). Kluwer Press, Dordrecht, 539-563.
- Yang, S.H., Wang, L.J., Li, S.H., Duan, W., Loescher, W., and Liang, Z.C. (2007). The effects of UV-B radiation on photosynthesis in relation to photosystem II photochemistry, thermal dissipation and antioxidant defenses in winter wheat (*Triticum aestivum* L.) seedlings at different growth temperatures. *Funct. Plant Biol.* 34:907–917.
- Yang, X., Heisler, G.M., Montgomery, M.E., Sullivan, J.H., Whereat, E.B., Miller, D.R. (1995). Radiative properties of hardwood leaves to ultraviolet irradiation. *Int. J. Biometeorol.* 38:60–66.
- Zhao, D., Reddy, K.R., Kakani, V.G., Reed, J., and Sullivan J. (2003). Growth and physiological responses of cotton (*Gossypium hirsutum* L.) to elevated carbon dioxide and ultraviolet-B radiation under controlled environment conditions. *Plant Cell Environ.* 26:771–782
- Ziska, L. H. and A. H. Teramura. (1992). CO₂ enhancement of growth and photosynthesis in rice (*Oryza sativa*). Modification by increased ultraviolet-B radiation. *Plant Physiol.* 99(2):473–481.
- Ziska, L. H., A. H. Teramura and J. H. Sullivan. (1991). Physiological sensitivity of plants along an elevational gradient to UV-B radiation. *Am. J. Bot.* 79(8):863–871.

