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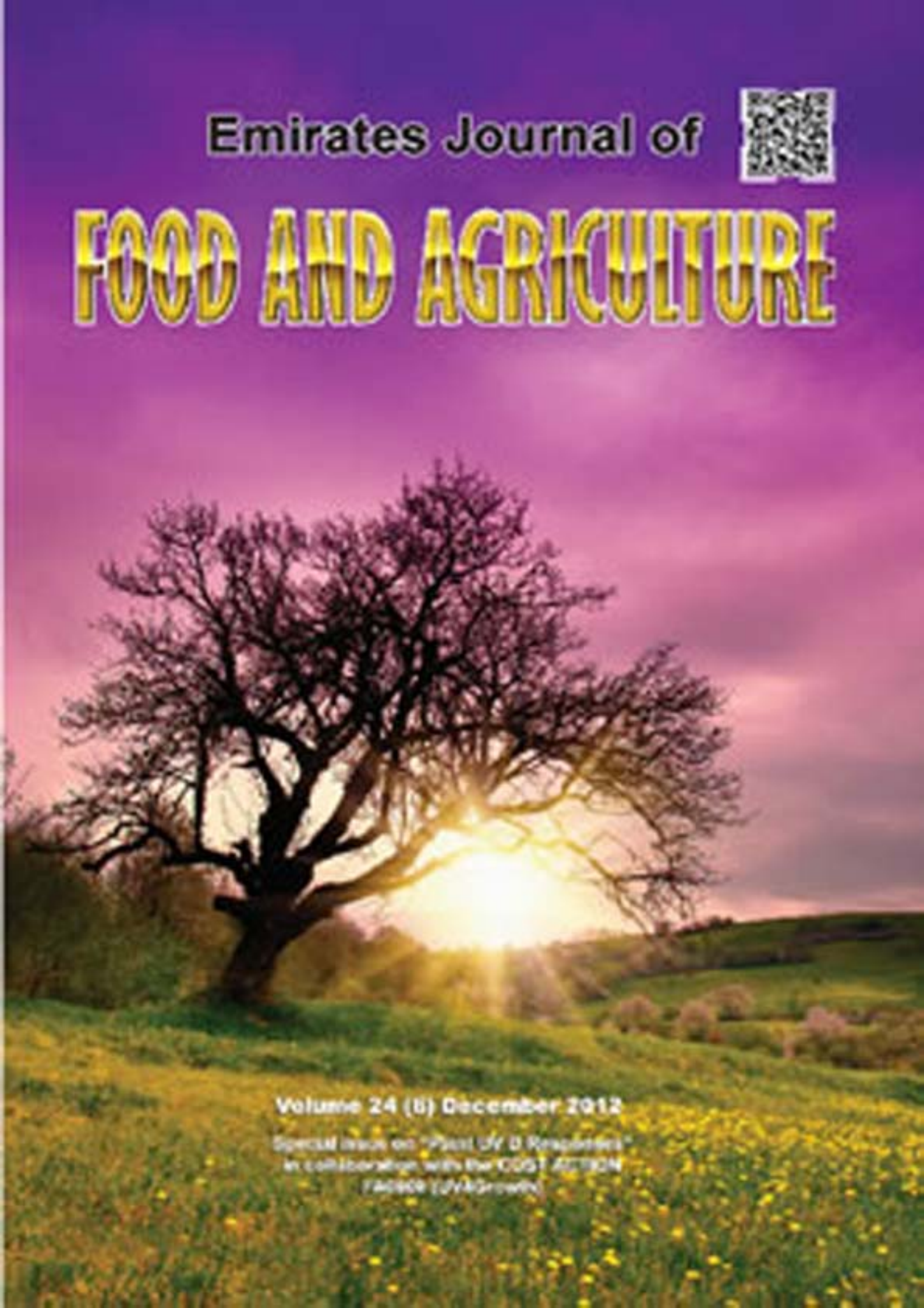

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EDITORIAL

UV-B radiation: "When does the stressor cause stress?"

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Exposure to UV-B radiation can cause stress in plants

Solar UV-B radiation (280 - 315 nm) has long been recognized as being potentially damaging to living organisms. Indeed, the literature on plant UV-B radiation effects has for decades been dominated by reports on UV-B mediated stress, including growth retardation, macroscopic injuries and oxidative damage (Caldwell et al., 1994; Searles et al., 2001). These negative effects comprise damaging effects on genetic material (formation pre-mutagenic cyclobutane pyrimidine dimers [CPD] and pyrimidine [6-4] pyrimidinone dimers), photosynthetic performance (in-tandem degradation of D1-D2 core proteins of photosystem II, inactivation RUBISCO, altered stomatal function) and a range of other cellular targets (see Jansen et al., 1998; Searles et al., 2001; Jordan 2002; Rozema et al., 2005; Jenkins, 2009). In this issue Lidon et al. (2012a) review our current understanding of the deleterious effects of UV-B on photosynthesis. UV-B damage is paralleled by the formation of lipid peroxidation products such as malondialdehyde (MDA) (Hideg et al., 2003; Lidon and Ramalho, 2011) and increased oxidation of antioxidants such as glutathione (Kalbin et al., 1997), both of which reflect the oxidative character of the UV-caused stress conditions. Consistently, UV-induced ROS have been measured using EPR spin trap reporters in both leaves (Hideg and Vass, 1996) and in isolated thylakoids (Lidon et al., 2012b) exposed to high doses of UV-B.

UV-B acclimation

Many studies have failed to find substantial, negative effects when plants are grown for prolonged periods under realistic levels of UV-B (Ballaré et al., 2011). Consistently, in this issue, Costa et al. (2012) conclude that there is no evidence that increases in UV-B influence wheat production. A major factor responsible for this lack of UV-B damage is the capability of plants to acclimate to ambient levels of UV-B. UV-B acclimation refers to the physiological adjustments that generate tolerance to transitory stress conditions. In the case of UV-B exposure, key components of the acclimation response are the increased capability of photorepair and the accumulation of UV-B absorbing flavonoids and other phenolics. These pigments have long been thought to accumulate mostly in the vacuoles of epidermal cells and to protect underlying tissues by absorbing UV-B photons. More recently, it has been argued that the main protective role of these phenolics is associated with their antioxidative capabilities (Agati and Tattini, 2010), and this fits the observation that flavonoids can be found in tissues not directly exposed to UV-B and also in sub-cellular domains as far apart as chloroplasts, vacuoles and nuclei, and roots and leaves. The UV-B induced increase in antioxidative defenses is further demonstrated by increases in both the reduction state and pool-size for antioxidants such as ascorbate, glutathione, xanthophylls, and tocopherol (Jansen et al., 2008). Moreover, numerous studies have reported upregulation of enzymatic antioxidant activities, including Cu or Zn superoxide dismutase (SOD), ascorbate peroxidase (APX), dehydroascorbate reductase (DHR), glutathione peroxidase (GPX), glutathione reductase (GR) and catalase activities (Hideg et al., 2006; Agrawal and Rathore, 2007; Xu et al., 2008). In this issue, Pessoa (2012) further highlights a range of UV-induced biochemical protection responses in algae and aquatic macrophytes. Interestingly, UV-protection appears to be largely dependent on physiological UV-acclimation. Few studies have reported evidence for UV-B driven genetic adaptation. In this issue Biswas and Jansen (2012) report that adaptation of local *Arabidopsis thaliana* accessions comprises the altered regulation of UV acclimation, thus again emphasize the relative importance of induced, physiological processes for UV-B protection.

Is the concept of "UV-B stress" still relevant?

Because of effective acclimation responses, UV-B mediated stress is in many circumstances a

potential oxidative stress, and most studies report, at most minor, effects on plant growth (Ballaré et al., 2011). This triggers the question whether "UV-B stress" is still a relevant concept. To address this question COST-Action FA0906, UV4Growth, organised a conference to review the roles of antioxidants, pro-oxidants and stress in plant responses to UV-B (Copenhagen, February 2012). Discussions revealed three key areas where the concept of oxidative UV-B stress is particularly relevant.

1) Oxidative UV-B stress and cross-tolerance

All stresses, biotic or abiotic, may cause a degree of oxidative stress. Understanding UV-B mediated stress and stress-defence responses, including ROS formation, activation of molecular targets, and induced antioxidant defences, therefore, has a generic relevance. Indeed, upregulation of antioxidant defences may result in a degree of cross-tolerance towards other stressors. UV-B acclimation has been shown to increase tolerance to, for example, low temperatures (Chalker-Scott and Scott, 2004), and drought (Manetas et al., 1997; Poulson et al., 2006). The reverse is also true, in this issue, Majer and Hideg (2012) show that a high light treatment can protect tobacco against subsequent UV-B exposure, notwithstanding subtle differences in the properties of high light and UV-B induced antioxidative defences. Such cross-tolerances are of particular interests to horticulturists. It is, however, not just the UV-induced upregulation of the antioxidant defence system that plays a part in cross-tolerances, other UV-induced physiological and morphological adjustments are also likely to contribute to such tolerances. Indeed, Kravets et al. (2012) report that both pre-exposures to UV-B or heat can induce UV-tolerance in barley cultivars, and this is linked to the development of complex changes at an anatomical, cytological, physiological level. Despite the relevance and importance of concurrent exposure to enhanced UV-B radiation and other global change factors (water availability, increased temperature, CO2, available nitrogen and altered precipitation), Zlatev et al. (2012) conclude in this issue that such responses are not fully understood so far.

2) UV-B stress as a relevant environmental factor

In general, realistic UV-B studies tend to show no negative impacts of UV-B radiation on plant growth (Ballaré et al., 2011). However, a number of field-based studies have shown that UV-B can cause stress under realistic conditions, especially when plants are simultaneously challenged by other environmental conditions, such as extreme climatic conditions of the polar zones, nutrient deficiencies or drought (Albert et al., 2010; Lau et al., 2006; Belnap et al., 2008). In this issue, Doupis et al. (2012) analyse the responses of grapevines to combinations of drought and UV-B stress, while Reboredo et al. (2012) present data on interactions between CO_2 and UV-B and ABA and UV-B. It appears that simultaneous exposure to multiple stressors can at times overwhelm oxidative defence capacity. This concept of UV-B stress in plants already challenged by another environmental parameter will be particularly relevant for plants growing near the limit of their distribution and/or subjected to changes in climate. Thus, the study of oxidative UV-B stress has clear (but under explored) links to evolutionary plant ecology.

3) UV-B as an exploitable regulator in horticulture

UV-B can induce a range of specific plant responses, some of which are particularly desirable from a horticultural perspective. For example, the potential to increase the content of specific phenolic, terpenoid and alkaloid compounds metabolites with nutraceutical or pharmaceutical value, is recognized as a useful tool for commercial plant manipulation (Jansen et al., 2008; Zhang and Bjorn 2009; Schreiner et al., 2012). UV-B can also increase development of colour in, for example, salad leaves (Park et al., 2007) or fruits (Dong et al., 1995), and control plant disease-tolerance and morphology (Wargent et al., 2006). In this issue, Jug and Rusjan (2012) describe several positive effects of UV-B radiation on grapevine biochemistry and physiology, while Ribeiro et al. (2012) review the use of post-harvest UV-B applications. Some of the reported UV-B responses are known to be mediated by a dedicated UV-B photoreceptor, UVR8, which operates under low UV-B levels (Jenkins 2009; Heijde and Ulm, 2012). Here, Krasylenko et al. (2012) report on the possible involvement of cytoskeleton components in further downstream signaling. Exploitation of the specific, low UV-B effects requires precision manipulation (wavelength selective cladding materials, UV-reflective mulches and/or supplemental UV-B light systems in pre- or post-harvest settings) whereby general, oxidative stress must be avoided. Clearly, a solid understanding of physiological and environmental conditions that cause UV-B stress is required in order to establish a (stress-free) window-of-opportunity for horticultural exploitation.

In summary

During the last decade it has become clear that UV-B mediated stress in plants is a relatively rare

event (Ballaré et al., 2011), and emphasis has increasingly shifted towards perception and signaling of low UV-B levels (Jenkins, 2009; Ulm and Heijde, 2012). Paradoxically, this shift has triggered new research questions for those researchers investigating UV-B stress, as there is now a clear need to accurately delineate the conditions that cause UV-B stress. Identifying the environmental conditions where UV-B causes oxidative stress will contribute both to our understanding of the ecological role of UV-B in a changing and/or hostile climate, as well as to the development of horticultural practices that exploit low UV-B effects. The manuscripts in this special issue reflect the various aspects of UV-B stress biology, and result from discussions between researchers at the COST-Action UV*4*Growth network meeting, held in February 2012 in Copenhagen, Denmark.

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As precursor of advanced multidisciplinary research COST has a very important role for the realisation of the European Research Area (ERA) anticipating and complementing the activities of the Framework Programmes, constituting a "bridge" towards the scientific communities of emerging countries, increasing the mobility of researchers across Europe and fostering the establishment of "Networks of Excellence" in many key scientific domains such as: Biomedicine and Molecular Biosciences; Food and Agriculture; Forests, their Products and Services; Materials, Physical and Nanosciences; Chemistry and Molecular Sciences and Technologies; Earth System Science and Environmental Management; Information and Communication Technologies; Transport and Urban Development; Individuals, Societies, Cultures and Health. It covers basic and more applied research and also addresses issues of pre-normative nature or of societal importance.

UV4Growth is a COST Action that brings together 184 researchers from 25 countries in Europe and the rest of the world. The network coordinates, and enhances nationally-funded plant UV-B research by forming an interdisciplinary research and training network that develops an integrated vision on the regulatory role of UV-B across organisational levels. Specifically, UV4Growth focuses on the role of UV-B as a specific regulator of plant growth and food quality against a backdrop of climate change. The network can be contacted via the Chair; Dr. Jansen, at uvforgrowth@gmail.com.

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REVIEW ARTICLE

Plant physiological responses to UV-B radiation

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Abstract

During the last few decades, there has been considerable concern over the depletion of stratospheric ozone as a result of anthropogenic pollutants. This has resulted in a concomitant increase in solar ultraviolet-B radiation (280–320 nm). High levels of UV-B radiation are responsible for multiple biologically harmful effects in both plants and animals. Many different plant responses to supplemental UV-B radiation have been observed, mostly injurious but sometimes beneficial. UV-B can influence plant processes either through direct damage or via various regulatory effects. In plants, direct effects include DNA damage, membrane changes and protein denaturation, which often cause heritable mutations affecting various physiological processes, including the photosynthetic apparatus. These could adversely affect plant growth, development and morphology, especially the productivity of sensitive crop species. This paper reviews the current knowledge about the plant physiological responses to UV-B stress.

Key words: UV-B radiation, Plant growth, Photosynthesis, Morphology, Oxidative stress

Introduction

Abiotic stresses are serious threats to agriculture and result in the deterioration of the environment and of crop loss worldwide, reducing average yields for most major crop plants by more than 50% (Wang et al., 2003). During the last few decades, there has been considerable concern over the depletion of stratospheric ozone as a result of anthropogenic pollutants such as halogenated hydrocarbons and other ozone depleting chemicals reaching the stratosphere (Molina and Rowland, 1974; Rowland, 1996; Madronich et al., 1998). Also greenhouse gases which cause cooling of the stratospheric ozone layer above the arctic, appear to be an indirect factor leading to ozone depletion (Shindell et al., 1998). A decrease in the ozone layer could lead to a significant increase in Ultraviolet-B (UV-B) radiation (280–320 nm) and shifts in the spectral UV-composition reaching the surface of the Earth (Blumthaler and Amback, 1990; Ajavon et al., 2007). This is predicted to continue in the future (Caldwell et al., 2003; McKenzie et al., 2003).

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. The ultraviolet radiation that is present in sunlight is divided into three classes: UV-A, UV-B and UV-C. The UV-A, with wavelengths from 320 to 390 nm, is not attenuated by ozone and thus is not affected by depletion of the stratospheric ozone layer. The UV-C, with wavelength shorter than 280 nm, does not reach ground level and this is not expected to change. It is the UV-B radiation that has received most attention because UV-B is absorbed by ozone. The daily fluence at the earth's surface increases as stratospheric ozone decreases (Ormrod and Hale, 1995). Although UV-B is only a minor component of the total solar radiation (less than 0.5%), due to its high energy, its potential for causing biological damage is exceptionally high and even small increases could lead to significant biological damage.

Different plant responses to supplemental UV-B radiation have been established, mostly injurious but sometimes beneficial. UV-B can influence plant processes either through direct damage or via

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various regulatory effects (Rozema et al., 1999; Potters et al., 2009). The injury can be classified into two categories: direct injury to DNA, which can cause heritable mutations, and direct and indirect injury to plant physiological functions (Ormrod and Hale, 1995; Lidon, 2012). The effects of UV-B that ultimately result in changed plant growth and productivity are initially felt at the cellular level, where both general and specific, and direct and indirect effects are found. The direct effects of UV-B can include DNA injury, membrane changes and protein denaturation.

In plants, wide inter- and intraspecific differences have been reported in response to UV-B irradiation with respect to growth, production of dry matter and physiological and biochemical changes (Kramer et al., 1991; Mpoloka, 2008; Fedina et al., 2010). Some plant species are unaffected by UV-B irradiation and several are apparently stimulated in their growth, but most species are sensitive and damage results, such as rice and maize (Teramura, 1983; Teramura and Sullivan, 1994; Hidema et al., 2007; Du et al., 2011; Lidon, 2012). On the other hand, plants have developed protective mechanisms against UV-B stress, such as enhancement of the antioxidant system (Brosché and Strid, 2003) and accumulation of UV-absorbing compounds (Frohnmeyer and Staiger, 2003; Fedina et al., 2007). Furthermore, numerous environmental factors such as water deficit, high temperature, ambient levels of visible radiation and nutrient status have also been shown to weaken or enhance the responses of plants to UV-B radiation (Murali and Teramura, 1985; Balakumar et al., 1993; Takeuchi et al., 1993; Mark and Tevini, 1996). Understanding the mechanism(s) by which physiological processes are damaged, repaired, and/or protected is important for understanding the ecophysiological role of UV-B radiation.

It has now been shown, that a natural balance of UV-B/UV-A/PAR is necessary for the adequate function of UV-B protection mechanisms (Rozema et al., 1997). Recent studies under semi-natural field conditions revealed that UV-B radiation is not as detrimental for plant growth and physiology, as previously believed (Björn et al., 2002). Furtheore, UV-B radiation effects are species specific and depend on interactions with other environmental parameters (Sullivan and Teramura, 1990; Gwynn-Jones, 2001; Kyparissis et al., 2001).

The present review surveys current knowledge about the plant physiological responses to UV-B stress based on physiological, biochemical and biophysical information. The interactions of UV-B stress with other environmental stresses are also discussed.

Photosynthesis and Respiration

Photosynthesis is sensitive to increased UV-B radiation, but the environmental relevance of UV-B effects on photosynthesis is not clear. Many studies have demonstrated detrimental effects of UV-B radiation on photosynthesis under laboratory conditions in both C3 and C4 plants (Krupa and Kickert, 1989; Groth and Krupa, 2000; Reddy et al., 2003), but the action spectrum of the UV-B effect does not suggest a specific target molecule (Renger et al., 1989; Fedina et al., 2010). At the whole-plant level, the effect of UV-B stress is usually perceived as a decrease in photosynthesis and growth, and is associated with alterations in carbon and nitrogen metabolism (Teramura and Sullivan, 1994; Julkunen-Tiitto et al., 2005; Lidon, 2012). Treatment with UV-B can affect stomatal conductance, altering the rate of water loss by transpiration and uptake rate of CO_2 for photosynthesis (Yao and Liu, 2006). Stomatal closure by enhanced UV-B and increased leaf diffusive resistance has been demonstrated with the action spectrum peaking below wavelength of 290 nm (Tevini and Teramura, 1989). It is assumed that stomatal closure is generating by a loss of turgor pressure with ion leakage from the guard cells.

It is demonstrared that transpiration is reduced in some UV-B sensitive seedlings (Tevini and Teramura, 1989; Yao and Liu, 2006). The time course for stomatal closure is rapid even at low UV-B levels. Stomatal opening is slowed by higher UV-B levels.

Direct injuries to the photosynthetic apparatus have been studied extensively. These effects include inactivation of photosystem II (PSII), reduced activity of Rubisco, decreased levels of chlorophylls and carotenoids, down-regulation of transcription of photosynthetic genes, and decreased thylakoid integrity and altered chloroplast ultrastructure (Friso et al., 1994; Strid et al., 1994; Teramura and Sullivan, 1994; Greenberg et al., 1996; Jansen et al., 1996; Vass et al., 1999).

Effects on PSII have drawn considerable attention (Jansen et al., 1996). PSII is a highly structured protein-pigment complex, the reaction center core of which is formed by the D1 and D2 similar proteins (Barber et al., 1997; Mattoo et al., 1999). The D1 and D2 reaction center proteins are extremely UV sensitive and degradation is driven by UV-B fluence rates as low as 1 μmol m^{-2} s^{-1} (Jansen et al., 1996). UV-driven D1-D2 degradation is strongly accelerated in the presence of a

background of visible radiation. The accelerated turnover of D2, as well as D1, under mixtures of UV-B radiation and photosynthetically active radiation (PAR), contrasts with the stability of the D2 protein under excessive flux densities of PAR alone (Jansen et al., 1996; Babu et al., 1999). The UV-B-driven degradation of the D1-D2 proteins may be, but is not necessarily, accompanied by a loss of PSII functionality, i.e. a decrease in oxygen evolution or in variable chlorophyll fluorescence.

The reduction in photosynthetic activity in the UV-B sensitive rice cultivar could be due to a decrease of Rubisco content, Rubisco activation and electron transport rate (Fedina et al., 2010). DNA lesions, such as CPD interfere with DNA replication and transcription (Britt, 1999).

Skórska (2011) established that after 60 min of UV-B irradiation the values of chlorophyll fluorescence parameters for cucumber leaves decreased by 4% to 44% versus the control. There were large decreases in F_v/F_o (20%) and vitality index - Rfd (33%). In the UV-B-treated cucumber leaves the Y value slightly decreased immediately after and especially 24 h after the end of the stress treatment.

Similar changes were observed for electron transport rate (ETR). In peppermint most of the measured parameters remained almost the same or even increased as in the F_v/F_m and F_v/F_o values. According 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. The decrease of F_v/F_o, attributed to inhibition of photosynthetic electron transport at the acceptor side, was observed only in the cucumber leaves subjected to UV-B. In peppermint leaves it increased, probably due to the higher tolerance of this species to UV-B. It is worth pointing out that changes indicating recovery were observed 24 h after the end of the UV-B stress treatment, suggesting that the damage to the acceptor side of photosystem II was reversible. On the other hand, damage to the donor side, reflected by the Y, ETR and Rfd parameters, seemed irreversible. Jordan et al. (1994) studying etiolated tissue indicated a strong link between the photosynthetic apparatus and UV-B-induced gene expression. The redox potential of photosystems regulates chloroplast gene expression through the redox state of the plastoquinone pool (Tullberg et al., 2000). This may be connected with its interaction with UV-B signal transduction and gene expression. Mackerness et al. (1996) showed that amelioration of UV-B effects on gene expression by strong irradiation involved photosynthetic electron transport and photophosphorylation. This may, in part, account for the lack of UV-B effect on gene expression in etiolated tissue when photosystems are not functional.

Many of the detrimental UV-B effects on photosynthesis observed under laboratory conditions are not obvious under field conditions (Fiscus and Booker, 1995; Rozema et al., 1997; Jansen et al., 1998). Plants respond to UV-B by balancing reactions that lead to damage, repair, and acclimation. A likely reason underlying the discrepancy between laboratory and field studies is a failure to take into consideration the naturally occurring tolerance mechanisms (Fiscus and Booker, 1995; Gonzalez et al., 1998; Jansen et al., 1998). In a converse manner, the effects of UV-B on photosynthesis offer a convenient means to screen for repair and acclimation responses that can confer UV tolerance. Booij-James et al. (2000) have assessed the role of UV-screening pigments in protecting chloroplast metabolism against UV-B radiation in the presence or absence of a background of PAR using the UV-sensitive D1-D2 protein degradation assay as a sensor for UV penetration. In comparison to the more common measurements of photosynthetic electron flow and/or efficiency of photosynthetic light utilization, this assay has several advantages: (a) it is only to a minor extent affected by non-physiological UV-C wavelengths (Greenberg et al., 1989; Jansen et al., 1996a); (b) in healthy plants, the response is triggered by a low threshold fluence (1 μmol m^{-2} s^{-1}) of UV-B (Jansen et al., 1996b); (c) the degradation response is not diminished by a physiologically relevant background of PAR (Jansen et al., 1996a; Babu et al., 1999); and (d) the measured bonafide *in vivo* pulse-chase response directly reflects damage, i.e. not a steady-state balance comprised of damage and repair reactions. 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

light supplemented with UV-B, developed tolerance to UV-B (Wilson and Greenberg, 1993). These plants, which had elevated levels of epidermal flavonoids, were also observed to have an increased half-life of the UV-B-sensitive PSII D1 protein. Arabidopsis mutants defective in the production of flavonoids have been successfully used in assessing the general effects of UV-B on plant growth, oxidative damage (Landry et al., 1995), and DNA repair (Landry et al., 1997). Although these studies have clearly demonstrated a general relationship between UV tolerance and flavonoid content, questions remain concerning (a) the extrapolation to different species, cultivars or ecotypes; (b) the protection of specific molecular targets; and (c) the relative contribution of specific phenols to the screening capacity.

Increases in the 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).

From this evidence, 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, as pointed Gwynn-Jones (2001) supports this view as it can be used to reflect maintenance respiration. Maintenance respiration can be closely correlated with plant nitrogen content and may account for stimulation of nitrogen commonly observed in leaf tissue at enhanced UV-B (Gwynn-Jones, 1999). Marked changes in the carbohydrate allocation between root and shoot of *C. purpurea* with UV-B exposure also provide supportive evidence for this hypothesis. The soluble carbohydrate:starch ratio was higher in young leaves, the stem and overall in the shoot, whilst the amount of soluble carbohydrates within the roots was reduced at enhanced UV-B.

The results partially agree with a previous study by Phoenix et al. (2000), a long-term stimulation of soluble leaf carbohydrates was observed in the dwarf shrub *Vaccinium ulginiosum*, although root and rhizome carbohydrates were not measured. The findings of both studies might be explained by increased respiratory demand in the leaves influencing photoassimilate allocation.

Oxidative stress

Under elevated UV-B radiation plant cells produce reactive oxygen species (ROS) that induces oxidative damage to DNA, functional and structural proteins, lipids and other cell compounds (Panagopoulos et al., 1990; Foyer et al., 1994; Smirnoff, 1998; Mahdavian, 2008). As a consequence, this environmental stress often activates similar cell signaling pathways (Knight and Knight, 2001; Zhu, 2001, 2002) and cellular responses, such as the production of stress proteins, up-regulation of antioxidants and accumulation of compatible solutes (Vierling and Kimpel, 1992; Cushman and Bohnert, 2000).

To cope with oxidative stress, various ROS-scavenging systems in plants are involved (Bowler et al., 1992). Enzymatic ones include superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6) and Halliwell/Asada pathway enzymes (Foyer et al., 1994). 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).

Plants respond to UV-B oxidative stress by activation of antioxidant enzymes or changes in the contents of antioxidants. The activities of antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidases (APX), and glutathione raductase (GR) are enhanced by UV-B treatment in *Arabidopsis* (Rao et al., 1996), cucumber (Tekchandani and Guruprasad, 1998), wheat (Sharma et al., 1998) and cyanobacterium (Prasad and Zeeshan, 2005). Similarly, a significant increase in enzymes such as peroxidase, polyphenol oxidase, ascorbate peroxidase, catalase, and glutathione reductase showed enhanced activity in UV-B and UV-C treated pepper plants (*Capsicum annuum*) (Mahdavian et al., 2008). In addition, the coordination among enzymatic activities, antioxidant substrate flux, and gene expression in roots might be different from that of leaves, even though these two organs share almost the same enzymatic machinery. In leaves of the Landsberg erecta strain of A. *thaliana,* it has been reported that UV-B irradiation enhances guaiacol peroxidase, APX, and SOD activities, but not GR or CAT activity (Landry et al., 1995; Rao et al., 1996).

Santos et al. (2004) have emphasized that UV-B radiation interferes with the SOD similarly as do other stresses and also affects the isoenzymes of SOD differently. Agarwal (2007) established that tolerance of *Cassia auriculata* L. seedlings to UV-B is due to the enhancement of SOD activity and other antioxidative enzymes. The same results were reported by Santos et al., (2004) in potato, Mackerness et al. (1999) in pea, Kondo and Kawashima (2000) in cucumber, and Prasad and

Zeeshan (2005) in *Plectonema boryanum.* Increases in activities of CAT and POX by UV-B radiation have been observed in several species including *Cassia* species (Agarwal and Pandey, 2003), cucumber (Krizek et al., 1993; Jain et al. 2004), sugarbeet (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). Increasing trend of GR and APX activity was also consistent with other studies performed under UV-B stress (Selvakumar, 2008). Induction of APX and GR due to UV-B indicates a preferential synthesis/activation of these enzymes, playing a crucial role in scavenging of H_2O_2.

Through these pathways chloroplasts are shielded against oxidative burst, with very little damage being caused to the photosynthetic apparatus (Fiscus and Booker, 1995), which allows synthesis and mobilization of photoassimilates. Moreover, enhanced UV-B irradiation might arrest plant growth (Strid et al., 1994; Tevini, 2004), as it inhibits photosynthesis (Teramura and Sullivan, 1994; Ambasht and Agarwal, 1998; Niyogi, 1999) and suppresses isoprenoid synthesis (Kulandaivelu et al., 1991). Thus, depending on the intensity of UV-B irradiation, the potential primary catabolisms involved in uncontrolled tissues injury are photoxidation and ROS production when the antioxidant systems become inhibited (Lidon and Henriques, 1993; Foyer et al., 1994; Caldwell et al., 2003).

It was found that during the beginning of the vegetative growth supplemental UV-B irradiation becomes lethal in directly exposed leaves of *Oryza sativa* L. cv Safari, but does not limit subsequent growth until the end of the life cycle (Lidon and Silva, 2011; Lidon, 2012). Following the sensitivity and recover of this genotype, the induced damages of ROS amplification by UV-B irradiation were timely followed and compared in leaves directly stressed and grown after irradiation. It is pointed that under UV-B stress the rates of ascorbate peroxidation in the xanthophyll cycle drive the availability of the ascorbate pool for the Asada-Haliwell cycle, concomitantly determining the extent of oxidative burst and thylakoid degradation through proteolysis and lipid peroxidation.

Increased content of non-enzymatic antioxidants was observed in pepper plants (Mahdavian et al., 2008), *Cassia auriculata* (Agarwal, 2007) and medical plant *Acorus calamus* (Kumari et al., 2010) after exposure with UV-B irradiation. Increase in ascorbic acid in plants at early age after UV-B exposure was also manifested in several studies suggesting its induction due to UV-B stress (Costa et al., 2002; Nasibi and Kalantari, 2005). Decline in ascorbic acid under UV-B stress was also reported by Agrawal and Rathore (2007) in wheat and mung bean. The reduction in ascorbic acid at later stages of observations could be explained due to increased activity of APX after UV-B exposure resulting into more consumption of ascorbic acid for effective quenching of oxyradicals. Ascorbic acid is postulated to maintain the stability of plant cell membranes against oxidative damage by scavenging cytotoxic free radicals. Conklin et al. (1996) have shown that an ascorbic acid deficient Arabidopsis mutant was very sensitive to a range of environmental stresses, an observation which demonstrates the key protective role for this molecule in *Arabidopsis* foliar tissues.

Synthesis of phenolic substances such as anthocyanin and flavonoids have been observed in UV-B treated *Arabidopsis thaliana* (L.) Heynh. seedlings (Winkel-Shirley, 2002). A role for flavonoids in UV protection is also supported by isolation of *Arabidopsis* mutant that is tolerant of extremely high UV-B levels (Bieza and Lois, 2001).

In addition, the metabolism of phenolic compounds also includes the action of oxidative enzymes such as POX (EC 1.11.1.7) and PPO (polyphenol oxidase, EC 1.10. 3.1), which catalyze the oxidation of phenols to quinones (Thypyapong et al., 1995). Agarwal (2007) found in *Cassia auriculata* a decrease in total phenol contents as well as the enhanced PPO activity under UV-B radiation. Also, phenol contents decreased with successive growth stage of bean plants after UV-B treatment (Singh et al., 2011). Whereas, Balakumar et al. (1997) reported for increases in phenol content and a decreases in PPO activity in *Licopersicon esculentum* after UV-B treatment. It seems possible that oxidoreductases PPO and POX involved in phenol oxidation may play an important role as defense against UV-B oxidative stress. In addition, phenols can protect DNA from UV-B induced damage (Mazza et al., 2000).

A decrease in photosynthetic pigments has been evident during exposure to enhanced UV-B radiation in most of the crop species (Kakani et al., 2003; Agrawal and Rathore, 2007). Carotenoids play an important role against UV-B damage in higher plants. Carotenoids, the scavengers of singlet oxygen species formed during intense light, are involved in the light harvesting and protection

of chlorophylls from photoxidative destruction. Significant reduction in carotenoid content was observed in UV-B treated bean plants (Singh et al., 2011).

Proline accumulation was also higher under UV-B stress condition, which might protect the plant cells against peroxidative process (Pardha Saradhi et al., 1995). Increment of proline under UV-B stress was observed in maize (Carletti et al., 2003) and pea (Singh et al., 2009).

Sensitivity to UV-B irradiation varies widely among plant species and genotypes (Alexieva et al., 2001; Yanqun et al., 2003; Zu et al., 2004). For instance, Sato and Kumagai (1993) working with Japanese lowland and upland rice groups, examined interactions between UV-B radiation and 198 rice cultivars, concluding that in similar ecotypes and groups the resistance of these genotypes vary broadly. Varying responses in antioxidants under UV-B exposure have been reported, depending on intensity of radiation and duration of irradiation period (Rao et al., 1996; Kubo et al., 1999). For example, increased ascorbate peroxidase activity was reported in *A. thaliana* under enhanced UV-B radiation at the level of 18 KJ m^{-1} d^{-1} (Rao et al., 1996). UV-B induced increment in ascorbic acid at 15 days after germination of bean plants whereas reduction was observed at 30 days after germination (Singh et al., 2011).

Under natural UV-B irradiation the sensitivity of genotypes depends of the activation of protective mechanisms, such as UV-B filters, quenchers of ROS (Bjorn et al., 2002; Caldwell et al., 2003) or antioxidant enzymes and some metabolites of the Asada-Haliwell and xanthophyll cycles (Lidon and Henriques, 1993; Asada, 1999; Mackerness, 2000).

Pigments and UV-B absorbing substances

Plants exhibit a wide range of responses to UV-B, including physiological responses which help to protect them from damaging UV-B wavelengths (Tevini and Teramura, 1989; Stapleton, 1992). The best studied direct UV-B protection mechanism, mediated by a photoreceptors is the differential production of UV-B absorbing compounds, such as phenolic compound, flavonoids, and hydroxycinnamate esters in the leaves, particularly in the epidermis (Fohnmeyer et al., 1997; Meijkamp et al., 2001; Caldwell et al., 2003; Fedina et al., 2007). This type of response is not a damage response and involves the stimulation of expression of particular genes by UV-B, implying specific UV-B light detection systems and signal transduction processes, which lead to the regulation of transcription (Jenkins et al., 1997). The largest concentration of these pigments is located in the epidermal and subepidermal cell layers, absorbing and effectively reducing the penetration of UV-B deeper into the mesophyll cells of the leaf with little effect on the penetration of visible or the photosynthetically active radiation (Bornman et al., 1997; Fedina et al., 2007; Fedina et al., 2010), thus acting to screen out the UV-B. The epidermis blocks transmittance of 95 to 99% of incoming UV radiation (Robberecht and Caldwell, 1986).

lnduction of flavonoids in rye seedlings can prevent UV-B-induced damage to photosynthesis (Tevini et al., 1991), which suggests that UV radiation protection is one of the functions of these pigments. This could be tested directly using mutants that are defective in the accumulation of flavonoids.

Species with higher contents of these compounds prior to the onset of UV-B treatment (Gonzales et al., 1996) or species that can rapidly accumulate these compounds (Murali and Teramura, 1986) are protected against UV-B damage and would be UV-B tolerant. However, such a trend was not observed in many studies. Smith et al. (2000) established that mean contents of UV-B absorbing compounds did not differ significantly between the tolerant and sensitive groups, not did an ability to increase the content of UV-B screening pigments in response to UV-B necessarily reduce sensitivity.

Fedina et al. (2010) established that there were no significant correlation between sensitivity to UV-B and accumulation of UV-absorbing compounds in three rice cultivars. Similar results were observed in rice by Teranishi et al. (2004) and in cucumber by Adamse and Britz (1996). Hada et al. (2003) reported that excess accumulation of anthocyanins reduced the amount of blue and UV-A radiation, which is utilized by cyclobutane pyrimidine dimers photolyase for monomerization of dimers and thus lowered CPD photorepair in purple rice leaves.

Beggs and Wellmann (1994) suggest that the synthesis of isoflavonoids in legumes may be induced by DNA damage because the wavelength dependency of the response is similar to that for DNA absorption and acceleration of DNA repair by photoreactivation. It is hypothesized that DNA damage is the sensory mechanism for the response to short UV wavelength. After UV-B exposure, some flavonoids are selectively produced (Markham et al., 1998). This accumulation does not relate to any enhanced capability to absorb UV-B, but rather reflects a greater potential to dissipate energy or produce greater antioxidant capacity.

Flavonoids absorb specifically in the UV region and not in the PAR region (Ballaré et al., 1992). At higher PAR levels, the interaction between UV-B and PAR effects may lead to compensation of negative UV-B effects (Cen and Bornman, 1990; Ballaré et al., 1992; Adamse and Britz, 1996). Firstly, radiation with a wavelength range between 300 and 500 nm is required for the activity of the enzyme DNA photolyase, repairing DNA dimers induced by UV-B (Jordan, 1993; Taylor et al., 1997). Secondly, some UV-B effects such as reduced plant height, thicker leaves and enhanced concentrations of phenolics, which have a protective function against UV-B, are also observed in response to enhanced PAR levels (Cen and Bornman, 1990; Ballaré et al., 1992). In most cases, PAR levels in the greenhouse and in climate chambers are lower than outside. Also, the light spectrum inside differs from the spectral composition of the light outside. Thus, when results from greenhouse experiments are extrapolated to the field situation, this may lead to an overestimation of UV-B effects on growth in the field (Rozema et al., 1997; Caldwell et al., 2003).

In addition to enhanced antioxidant capacity provided by specific flavonoids, plant cell produces a range of alternative antioxidant systems to protect against free radicals generated by UV-B (Strid, 1993). Thus, increased UV-B induces the rapid synthesis of antioxidant enzymes (SOD, CAT, and GPX) to cope with the free superoxide radicals. It is supposed that peroxidases under UV-B stress can use flavonoids as substrates to detoxify hydrogen peroxide.

Anthocyanins absorb also in the UV region of the spectrum of 270-290 nm. Therefore, they have been empirically implicated in UV-B protection of young leaves (Lee and Lowry, 1980). More recently Burger and Edwards (1996) provided experimental evidence that the anthocyanin-rich red varieties of *Coleus* were less damaged by UV-B radiation, compared to anthocyanin-less green varieties. In addition, Stapleton and Walbot (1994) showed that the DNA of maize varieties containing anthocyanins was better protected against UV-B radiation damage. However, Woodall and Stewart (1998) questioned the above on the basis that anthocyanins do not absorb appreciably in the UV-B (290-315 nm) spectral band, unless they are acylated with aromatic organic acids (Markham, 1982). In this case, their 270-290 nm UV peak is shifted to the UV-B region. However, this shift does not necessarily result in a considerable increase in their specific absorbance in the UV-B region of the spectrum (Woodall and Stewart, 1998). In anthocyanins, the UV and visible absorption coefficients are almost the same (Woodall and Stewart, 1998).

Mendez et al. (1999) assume that if anthocyanins in *Pinguicula vulgaris* are indeed acylated, their normalized absorbance at 300 nm would be as low as 0.20 and 0.44 for the control and UV-B treated plants respectively. Since the corresponding total normalized absorbances at this wavelength are 13.83 and 14.67, the relative contribution of anthocyanins to UV-B attenuation would be 1.4% for the controls and 3% for the UV-B treated plants. Authors therefore assume that the UV-B induced increase in anthocyanins of *Pinguicula vulgaris* cannot afford signifcant protection against UV-B radiation damage since the absorbances of other co-occurring phenolics are much higher. Absorption of visible light by epidermal anthocyanins could reduce the photosynthetically active radiation reaching the mesophyll and, accordingly, suppress the already low (Mendez and Karlsson, 1999) photosynthetic rates of this plant. However, corresponding reductions in growth or reproduction were not observed. On the other hand, anthocyanins may protect against photoinhibition by visible radiation, as suggested by Gould et al. (1995). Although previous attempts to verify this hypothesis were negative (Burger and Edwards, 1996), the results of Mendez et al. (1999) clearly showed that the anthocyanin-rich, UV-B treated leaves were less prone to photoinhibition imposed by high light and low temperature. However, it is possible that the apparent correlation between high anthocyanin and lower photoinhibitory risk found in the present study could be coincidental, and that other processes induced by UV-B could be responsible for the increase in resistance to photoinhibitory stress.

Regardless of this, the differences in the extent of photoinhibition observed in the laboratory did not result in corresponding changes in the above-ground biomass accumulation in the field, nor on dry mass of overwintering buds. In addition, the leaf and plant senescence rates measured during late season, where the slightly above zero temperatures could have enhanced the photoinhibitory risk, were the same in control and UV-B treated plants. Therefore, authors have to accept either that the increase in anthocyanins was of no adaptive significance or that the lower photoinhibitory risk counterbalanced the possible negative effects of UV-B radiation. *In situ*

*fl*uorescence measurements and photosynthetic rates of control and UV-B treated plants could help to express an opinion on the above alternatives.

Anthocyanins could also be induced by nutrient (P and N) limitation. Furthermore, the nitrogen content of the leaves was improved under UV-B supplementation. However, this can be correlated with the increased root mass under UV-B supplementation (Mendez et al., 1999). It is concluded that *P. vulgaris* is very well equipped to cope with the ongoing increase of UV-B radiation reaching the surface of the earth. In addition, the preferential increase in leaf anthocyanins may be beneficial to this plant under certain environmental conditions.

Growth and Development

At the plant level, increased UV-B radiation can result in decreases in biomass or total dry matter production and marketable yield.

A large number of experiments world-wide have addressed the impacts of enhanced UV-B radiation on plant growth (Caldwell, 1971; Krupa and Kickert, 1989; Rozema et al., 1997; Caldwell et al., 1998). Plant species (and groups) vary considerably in their response to UV-B, depending on experimental set up, treatment regimes and duration (Warner and Caldwell, 1983; Middleton and Teramura, 1994; Tevini, 1994; Weih et al., 1998). Regardless of such factors, several published (and unpublished) studies have shown evidence of plant resistance to UV-B radiation (Krupa and Kickert, 1989; Gwynn-Jones et al., 1997; Rozema et al., 1997) possibly via constitutive or induced protection against and/or repair of UV-B damage. Protection against UV-B can involve alterations in cuticle (Drilias et al., 1997) and leaf thickness (Johanson et al., 1995) and/or increased production of UV-B protective pigments (Cen and Bornman, 1990; Van de Staaij et al., 1995). In the event of protective mechanisms failing to shield the genome and photosynthetic machinery against UV-B, repair mechanisms are relied upon (Takeuchi et al., 1993). Most plant species are thought to have adequate repair capacities (photoreactivation–photorepair) to deal with projected increases in UV-B (Taulavuori et al., 1998). Nevertheless, one crucial factor to such tolerance is the duration of exposure, as longer-term studies show evidence of cumulative plant damage.

The experiment of Gwynn-Jones (2001) on *C. purpurea* contrasts with previous indoor study on the same species, as plant dry weight was not inhibited by enhanced UV-B radiation. This species is therefore tolerant to short-term exposure to enhanced UV-B under more realistic outdoor conditions. Measurements of leaf UV-B absorbing pigments and leaf respiration rates (young and mature) suggest induced leaf protection and metabolism at enhanced UV-B.

The growth reduction can be the result of a changed allocation of biomass, increasing amounts of secondary compounds or morphological alterations which lead to lower photosynthetic productivity (Teramura et al. 1990; Fiscus and Booker, 1995; Caldwell et al., 2003; Kakani et al., 2003; Liu et al., 2005;). Responses to UV-B include morphological alterations such as reduced leaf size, thicker leaves (Adamse and Britz, 1996), reduced hypocotyl length (Kim et al., 1998) and curling and bronzing of leaves (Teramura et al., 1984; Allen et al., 1998). These effects are more pronounced at lower PAR levels (Teramura, 1983; Musil, 1996). Morphological UV-B effects could either be interpreted as damaging effects when they are caused by photodestructive processes or as photomorphogenic responses mediated via photoreceptors (Barnes et al., 1990; Kim et al., 1998).

UV-B induces changes of in leaf and plant morphology (Jansen et al., 1998), but the mechanism underlying these alterations is not clear. Leaf curling is a photomorphogenic response, observable at low fluencies of UV-B that helps diminish the leaf area exposed to UV radiation. UV-B increases SLW, but it is not clear whether they represent damage or an adaptive response to elevated UV-B.

Some photomorphogenic effects and the production of flavonoids give mesophyll cells protection against UV-B radiation and thus have a role in adaptation to UV-B radiation (Ballaré et al., 1992; Mpoloka, 2008). When leaves become thicker, UV-B as well as PAR are absorbed in higher amounts in the leaves implying that leaf tissue is exposed to reduced levels of both UV-B and PAR (Ballaré et al., 1992; Adamse and Britz, 1996).

Negative impact of enhanced UV-B radiation on cotton growth included reduction in height, leaf area, total biomass and fiber quality (Gao et al., 2004). Growth reduction is mediated through leaf expansion (Pinto et al., 1999), which is a consequence of the UV-B radiation effects on the rate and duration of both cell division and elongation (Hopkins et al., 2002). In general UV radiation deleteriously affects plant growth, reducing leaf size and limiting the area available for solar energy capture (Zuk-Golaszewska et al., 2003). On the other hand the results of Zancan et al.

(2006) showed that UV-B radiation had no significant effect on plant growth. In addition, exposure of plant to UV-B radiation increased both chlorophyll content and root and leaf iron content. These findings have been achieved mainly through studies in greenhouses and exposure to artificial sources of ultraviolet radiation; extrapolation to changes on crop yield as a result of increases in terrestrial solar UV radiation is difficult (Yao et al., 2007). Salama et al. (2011) suggested that the significant increase in proline content was an important factor for providing higher tolerance to UV radiation treated plant species. In addition, increasing proline content is referred to as protective mechanism due to the generation of reactive oxygen species by UV radiation.

For instance, changes seen after supplemental UV-B radiation include biomass reductions (Lydon et al., 1986; Gwynn-Jones, 2001)**,** decreases in the percentage of pollen germination (Flint and Caldwell, 1984), changes in the ability of crop plants to compete with weeds (Barnes et al., 1990), epidermal deformation and changes in cuticular wax composition (Tevini and Steinmuller, 1987), and increased flavonoid levels (Tevini et al., 1991; Beggs and Wellman, 1985).

Photomorphogenesis is a radiation-induced change in plant form. UV-B enhancement alters the growth of several plant species but does not reduce shoot dry weight (Barnes et al., 1990). An action spectrum of the first positive phototropism (curvature) of the alfalfa hypocotyl has demonstrated that UV-B contributes to the response; plants were kept in red light to isolate this response from the similar response through phytochrome (Baskin and Lino, 1987). A cucumber mutant that lacks light-stable phytochrome (López-Juez et al., 1992) has also been used to measure photomorphogenesis after UV-B treatment. UV-B also inhibits hypocotyl growth (Ballaré et al., 1992).

However, because this mutant has some residual phytochrome function (Whitelam and Smith, 1991), the action of phytochrome in this UV-B response cannot be excluded. In the experiments with cucumber, shielding the actively growing tissues from UV radiation did not affect the magnitude of the decrease in hypocotyl length, so direct effects on cell division or elongation would not explain the UV-B-induced growth inhibition. Recovery after return to uninducing conditions was rapid, again suggesting a true photomorphogenic response to UV-B.

Interactions with other environmental factors

Water stress

Evidence of interaction between UV-B exposure and drought stress in plants has emerged in recent years, but the mechanisms of sensitivity or tolerance to combined stress have received little attention and still remain unknown. Some investigations have been carried out on agricultural or model plants, despite the fact that crops account for only 6% of the plant productivity world-wide (Tevini et al., 1983; Sullivan and Teramura, 1990; Schmidt et al., 2000). Elucidation of the interaction between drought and UV-B stresses would help in understanding the potential impact of partial stratospheric ozone depletion on plant adaptation to changing environmental condition.

Under drought stress plants become less sensitive to UV-B as the applied water stress increases. Several experiments have served to elucidate some of the water stress/UV-B interactions. Well-watered soybean plants grown in the field under enhanced UV-B radiation had reductions in growth, dry weight, and net photosynthesis compared with ambient UV-B, while no UV-B effect could be detected in water-stressed plants (Murali and Teramura, 1986). Photosynthesis recovery after water stress was greater and more rapid in UV-B treated soybeans and associated with UV-B effects on stomatal conductance rather than with internal water relations. Drought-stressed cucumber plants lost their capacity to close stomata at midday with increasing UV-B (Tevini et al., 1983). Radish seedlings were less sensitive to UV-B under water stress than cucumber. Radish had higher leaf flavonoid contents, possibly protecting seedlings by absorbing UV-B in the leaf epidermis.

Plants that endure water deficit stress effectively are also likely to be tolerant of high UV-B flux. Nevertheless the research of the interactions between UV-B and drought led to contradictory results. In field-grown soybean, a decrease in productivity following by UV-B exposure was moderated by soil water deficit (Sullivan and Teramura, 1990). The interaction between soil water deficit and UV-B stresses in cowpeas resulted in benefits from the combined stresses in terms of greater growth and development as compared with exposure to single stresses (Balakumar et al., 1993). It seems that under multiple stresses, each of the stress factors may bring out some adaptive effects to reduce the damage experienced by plants and caused by the other stress. UV-B irradiation could alleviate the negative effects of water stress on

plants or exert an additional inhibitory effect on the functional processes in plants. For example, exposure to both UV-B and water stress led to decreased growth in cucumber and radish, but protein content was increased by the combined stresses (Tevini et al., 1983). Teramura et al. (1984) have obtained similar additional injurious effects of UV-B on net photosynthesis of soybean under drought stress. Teramura et al. (1990) also reported that both genotypic differences and assimilate utilization were involved in the interaction between UV-B and water stress in soybeans. The growth of wheat seedlings (their fresh weight) was significantly inhibited by drought, UV-B irradiation, and the combination of stresses. The content of H_2O_2 increased significantly under stressful conditions. A common drought stress, UV-B radiation, and other environmental stresses could cause the accumulation of ROS and thus result in oxidative damage (Smirnoff, 1998; Alexieva et al., 2003). ROS are highly reactive and, in the absence of effective protective mechanism, they can compromise normal metabolism through oxidative damage to pigments, lipids, proteins, and nucleic acids.

In wheat seedlings, drought stress and UV-B irradiation resulted in the high H_2O_2 accumulation, which caused lipid peroxidation along with the reduction of growth. Moreover, UV-B treatment was found to cause a more severe damage than drought stress on wheat seedlings measured as more obvious reduction in growth and much more strong accumulation of H_2O_2 and increased lipid peroxidation (Tian and Lei, 2007). This data corresponded well to those of Alexieva et al. (2001) who also obtained similar results for pea and wheat seedlings. However, the combination of drought stress and UV-B irradiation was additive, in contrast to the other researcher data suggesting an antagonistic effect (Sullivan and Teramura, 1990; Alexieva et al., 2001). The growth of wheat seedlings under combined stress was much more retarded than when stresses were applied separately. Tian and Lei (2007) inferred that in their study the treatment time (7 days) was too short for wheat seedlings under each kind of stress to form protective responses to other stresses, that is, the interaction between stresses did not display their effects completely. The treatment time was longer in other studies, for example, it was 15 days in the case of cowpea (Balakumar et al., 1993).

Kyparissis et al. (2001) established that there were no significant interactive effects between supplemental UV-B radiation and additional watering on Mediterranean evergreen sclerophyll *Ceratonia siliqua* L. Previous field experiments with other Mediterranean plants, showed that supplementary watering during the summer abolished the negative (Drilias et al., 1997) or positive (Manetas et al., 1997).

Many contradictory results about antioxidant enzyme response to different stresses have emerged due to the fact that the levels of enzyme responses depend on the plant species, the developmental stage, the organs, as well as on the duration and severity of the stress (Rout and Shaw, 2001). In many plants, free proline accumulates in response to biotic and abiotic stresses, including UV-B irradiation (Carletti et al., 2003). In wheat seedlings, proline contents were up to 1.71 times higher under drought, UV-B, and combined stresses as compared with the control, respectively.

Tian and Lei (2007) concluded that drought stress and UV-B irradiation both could cause oxidative damage to plant through excessive ROS generation. UV-B caused more severe stress than drought stress, and the effect of drought and UV-B stress was additive in wheat seedlings. Authors suppose that the mechanism of combined effect of drought stress and UV-B irradiation need further study.

Concerning irrigation, the effects were as expected, with well-watered plants being taller and having more leaves compared to water stressed ones (Kyparissis et al., 2001). These effects were sustained throughout the experiment. Additionally, well-watered plants had significantly higher chlorophyll contents during the dry period. In fact, this was due to chlorophyll loss in water-stressed plants, which was abolished with additional watering. This type of response is considered a common photoprotective adaptation under photooxidative conditions (Kyparissis et al., 1995) and has also been found under water stress situations for several Mediterranean semi-decidual and sclerophyllous species (Stephanou and Manetas, 1998). In all other measured parameters, the effects of additional irrigation were negligible and only non-significant trends for increased total leaf area and above ground drymass were observed.

Kyparidis et al. (2001) assume that the growth of the evergreen sclerophyll, slow-growing plant *C. Siliqua* is not much affected by both UV-B radiation and additional watering, at least under the conditions used in this experiment. However, the subtle, mostly season-specific effects observed on some parameters could have a long-term impact on the fitness of this plant.

Visible light

The level of visible light (400-700 nm) to which experimental plants are exposed has been found to have a very great effect on UV-B injury (Ormrod and Hole, 1995). Growth chamber experiments have demonstrated that UV-B injury is greater with low levels of photosynthetic photon flux (PPF) (less than 200 µmol m^{-2} s^{-1}) than with high (ambient) levels (Tevini and Teramura, 1989). High levels of white light as well as UV-A radiation with blue light mediate photorepair mechanisms and ameliorate the UV-B injury. The relationship of PPF and UV-B effects is further complicated by the fact that a source of UV-B, whether simulated or natural, can exhibit not only different total output energies but also varying spectral composition within the range 280 to 320 nm (Krupa and Krickert, 1989). Growth chamber studies have been criticized because greater negative effects on the plant in response to UV-B exposure have been found in growth chambers than when a similar exposure take place under field conditions (Ormrod and Hole, 1995). It is important to study interaction of UV-B with another environmental variable at normal visible light level.

Nutritional status

Biologically available nitrogen is exceeding historical levels in many regions due to human activities. Studies show that plants well supplied with nitrogen are generally more sensitive to UV-B radiation. Both increases (Wand et al., 1996) and decreases (Dai et al., 1992) of leaf nitrogen content due to increased UV-B radiation have been reported, while in other cases UV-B radiation was ineffective (Wand et al., 1996). Levizou and Manetas (2001) reported that supplemental UV-B radiation improved growth in *Phlomis fruticosa* at high nutrient level, whereas greater growth inhibition by UV-B has been reported in nitrate-replete than nitrate-deficient crop plants (Hunt and McNeil, 1998). Tosserams et al. (2001) reported that photosynthetic rate of *Plantaago lanceolata* with high UV-B was not influenced by differential quantities of multiple mineral supply. Nitrogen supply accelerates some growth parameters of Mono Maple seedlings under ambient UV-B (Yao and Liu, 2006). This agrees well with the results of earlier studies (Deckmyn and Impens, 1997), however, some growth parameters were inhibited by nitrogen supply under enhanced UV-B. This indicated that the effects of high UV-B on growth completely overshadowed effects of nitrogen supply, whereas nitrogen supply increased for growth, morphological and physiological responses of Mono Maple to ambient UV-B. Authors conclude that nitrogen supply makes Mono Maple seedlings more sensitive to enhanced UV-B, though some antioxidant compounds increased. Obviously, nitrogen supply could not ease the harmful effects of high UV-B on plants, but aggravated the harm on plants.

The sensitivity of soybean to UV-B is dependent on phosphorus status (Murali and Teramura, 1985). Deficient plants are less sensitive to UV-B than are plants at optimum P levels, due at least in part to the accumulation flavonoids and to leaf thickening in P-deficient plants.

Conclusions

UV-B radiation effects are of increasing interest in plant physiology as questions are raised about the impact of enhanced UV-B in sunlight resulting from stratospheric ozone depletion. This increase in UV-B has been found to cause both photomorphogenic as well as genetic and physiological changes in plants. Photoreceptors acting through signal transduction pathways are responsible for sensing this ultraviolet radiation. Several components of the photosynthetic apparatus have been found to be affected by UV-B, with nuclear encoded genes being more sensitive to UV-B than chloroplast encoded genes. There have been significant advances in our understanding of the effects of UV-B radiation on terrestrial ecosystems, especially in the description of mechanisms of plant response. Many new developments in understanding the underlying mechanisms mediating plant response to UV-B radiation have emerged. This new information is helpful in understanding common responses of plants to UV-B radiation, such as diminished growth, acclimation responses of plants to UV-B radiation. The response to UV-B radiation involves both the initial stimulus by solar radiation and transmission of signals within the plants. Resulting changes in gene expression induced by these signals may have elements in common with those elicited by other environmental factors, and generate overlapping functional (including acclimation) responses. However, long-term effects of UV-B radiation in plants are still not well understood, therefore, more research need to be carried out over longer time periods and under field conditions, to provide definitive answers to questions such as cumulative effects of UV-B, effects of UV-B at ecosystem level, and interactions of elevated UV-B with other stress factors. Concurrent responses of terrestrial systems to the combination of enhanced UV-B radiation and other global change factors (water

availability, increased temperature, CO_2, available nitrogen and altered precipitation) are less well understood.

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REVIEW ARTICLE

UV-B radiation effects on terrestrial plants – A perspective

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Abstract

Both terrestrial and aquatic plants, the primary producers supporting life on earth, can be threatened by global climate change and particularly by UV-B radiation due to the depletion of the ozone layer in both Poles. The injurious effects of UV-B have been assessed mainly through in vitro studies and vary greatly according the dose received, the exposition period and the sensitivity of the species. Adaptive responses can include for example, synthesis of new compounds, increases of UV-B absorbing compounds or anti-oxidant enzymes. Morphological consequences are also documented such as reduced growth and thickening of leaves and cuticule. The main response of UV-B irradiation in indoor experiments is the formation of UV-B absorbing compounds such as phenolic compounds and flavonoids which function as protective screens, although in the natural habitat plants living at higher altitudes and latitudes are tolerant to UV-B due to the natural selection. The main conclusion derived from studies with terrestrial plants is that photosynthesis is not significantly affected by changes in UV-B radiation when plants grow under natural conditions. Moreover, due to the successful implementation of the Montreal Protocol the increase of UV-B radiation in most populated regions of the world (i.e., outside the regions affected by the Antarctic ozone hole) has been modest.

Key words: UV-B radiation, Terrestrial plants, Effects on photosynthesis, Effects on genetic material, Effects on UV-B absorbing compounds

Introduction

The stratospheric ozone layer protects life on Earth by absorbing ultraviolet light, which damages DNA in plants and animals, including humans. Prior to 1979, scientists had not observed ozone concentrations below 220 Dobson Units (DU), but the measurements made by NASA from 1979–2003 and by the Royal Netherlands Meteorological Institute from 2004 to the present, showed a continuous decline of DU values, reaching concentrations below 100, generally (NASA, http://earthobservatory.nasa.gov).

The ozone hole does not mean that the area monitored by satellite is free of ozone but rather it is an area in which ozone concentrations drop below the historical threshold of 220 Dobson Units. The amount of UV radiation reaching the Earth's surface varies widely around the globe and through time and depends mainly of cloud cover, concentrations of ozone in the stratosphere, oblique angle of sunlight reaching the surface, aerosol particles, sun elevation, reflectivity of the Earth's surface and depth in the water column in the case of aquatic environments.

Both aquatic and terrestrial ecosystems (including agricultural lands, and agro-ecosystems), could potentially be affected by increased solar UV-B radiation with consequences ranging from a decrease in biomass production, morphologic and metabolic changes, genetic damages, to a shift in species composition and diversity, although it must be recognized that some species are more vulnerable than others.

A comparison of the growth and physiological responses to various levels of solar UV-B in plant groups from marine, freshwater and terrestrial ecosystems was done by Rozema et al. (2002). Also a comparison of the induction of UV-absorbing compounds in plant groups and its chemical characterization and location, as well as a comparative assessment of the physiological functioning of UV-absorbing compounds as protective UV screens for plants, was performed.

According to the UNEP Report, in terrestrial areas where substantial ozone depletion has occurred, results from a wide range of field studies

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suggest that increased UV-B radiation reduces terrestrial plant productivity by about 6% (UNEP, 2010).

From the effects of UV radiation on plants, probably the most important one is related to genetic damages because the cell macromolecules such as DNA, RNA and proteins have strong absorption at 280-315 nm. However, in the natural habitat, plants are seldom affected by a single stress factor but by a multiplicity of interacting factors, such as water stress, increased atmospheric CO_2, mineral nutrient availability, heavy metals, temperature and the troposphere air pollutants (Caldwell et al., 1998) indicating that the effectiveness of UV-B radiation can be greatly increased or decreased by such factors. Elucidating the mechanisms that mediate plant responses to solar UV-B radiation is important for understanding the effects of radiation on the organism itself, on the whole community and lastly on the ecological interactions that may occur such as plant-herbivore interactions. Although we recognized that aquatic environment and particularly phytoplankton is crucial in the sea life and some areas of Antarctic sea are just below the ozone hole our discussion will be focused in terrestrial environments.

Crop plants – the target of UV-B study effects

Overexposure to UV-B may well reduce the productivity and quality of the main crop plant species to humans with serious economic and even demographic consequences. In that sense, several species and their varieties have been used to assess the effects of UV-B, such as corn (Zancan et al., 2006; de Britto et al., 2011, Campi et al., 2012), rice (Takeuchi et al., 2002), barley (Mazza et al., 1999; Bandurska et al., 2012), wheat (Correia et al., 1999; Agrawal and Rathore, 2007) and soybean (Galatro et al., 2001; Gitz et al. 2005; Chimphango et al., 2007).

In greenhouse experiments, the different doses of UV-B radiation applied (0 - control, 4, 8, 12 KJ m^{-2} d^{-1}) to *Avena fatua* and *Setaria viridis* induced changes in leaf and plant morphology. A decrease of plant height, fresh biomass of leaves, shoots and roots, leaf area and a leaf curling of both species was observed (Zuk-Golaszewska et al., 2003). In barley, a decrease in dry matter yield and water content of leaves and roots was observed after water deficit and UV-B plus water deficit, while no changes were found after treating barley plants with UV-B alone (Bandurska et al., 2012) indicating that other factors can be more injurious than the UV-B radiation itself.

The effects of UV-B (60 µmol m^{-2} s^{-1}) on primary leaves of wheat seedlings during different phases of leaf growth and development were assessed. UV-B induced an enhancement in accumulation of flavonoids during all phases of development while it caused a decline in anthocyanin content during senescence (Pradhan et al., 2008). UV-B exposure induced maximum damage to the photosynthetic apparatus during senescence phase of development although the damages were partially alleviated when UV-B exposure was accompanied by photosynthetically active radiation (PAR).

No beneficial interactions between the CO_2, temperature, and UV-B radiation on the reproductive processes of soybean were noted (Koti et al., 2005). Flower morphology, pollen production, pollen germination, pollen tube lengths, and pollen morphology were all negatively affected by CO_2, temperature and UV-B treatments alone or in combination compared with controls using the same parameters but at much lower doses.

Gao et al. (2004) studied the growth and yield responses of a maize crop exposed to enhanced UV-B radiation and the effects on seed quality under field conditions, concluding that enhanced UV-B radiation caused a significant reduction in the dry matter accumulation thus affecting the maize yield.

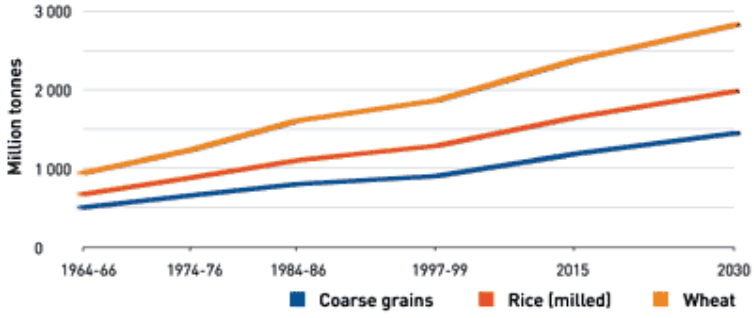

Figure 1. World demand for cereals, 1965 to 2030.
Source: FAO data and projections.

A large program research to assess the effects of UV-B on two rice cultivars was developed at the International Rice Research Institute (IRRI) located in the Philippines. The main conclusions indicated that rice yields likely will not be affected by increases in UV-B predicted from stratospheric ozone depletion under realistic tropical-field conditions based on extensive and intensive field experiments (Dai et al., 1997). This finding is extremely important since food production is a critical issue for human expansion worldwide as can be seen by the FAO demand projections for 2030 (FAO, 2002) and rice is the most important source of calories in the world (Figure 1).

Forestry species

Forestry research has focused attention on the effects of UV-B on the main species used in fast wood forestry such as poplar and eucalyptus, since decreases in productivity reduced the incomes of farmers and the efficiency of pulp and paper industry. Ren et al. (2007) studied the effects of drought and enhanced UV-B radiation and a combination of both stress factors on growth and physiology of *Populus kangdingensis* and *Populus cathayana* originating from high and low altitudes in south-west China and observed a significant reduction in plant height and total leaf area.

Solar UV-B radiation seemed to delay plant growth in all species examined (four *Acacia* and two *Eucalyptus* species) although it did not affect photosynthetic activity significantly. However, a reduced specific leaf area (SLA), and an increased leaf thickness and size of epidermis were observed in plants (Liu et al., 2005).

Other genera of particular interest in forestry are *Pinus, Salix, Betula, Picea* and *Abies.* For example*, Abies faxoniana* a key species in reforestation in the southeast of the Qinghai-Tibetan Plateau of China (Yao and Liu, 2009) when exposed to enhanced UV-B (14.33 KJ m^{-2} d^{-1}) showed a marked decline in growth parameters, net photosynthetic rate, photosynthetic pigments and the maximum quantum efficiency of PSII (Fv/Fm) compared with plants receiving ambient UV-B (11.02 KJ m^{-2} d^{-1}). The same authors (Yao and Liu, 2007) when studying the effects of similar UV-B levels on 3 and 6-year-old dragon spruce seedlings (*Picea asperata*) concluded that enhanced UV-B significantly decreased growth, needle and root nitrogen concentration, needle nitrate reductase activity and increased UV-B absorbing compounds and malondialdehyde (MDA) content in both 3 and 6-year old seedlings, while glutamine synthetase activity was not affected.

Morales et al. (2010) observed that UV-B induces the synthesis and accumulation of the flavonols myricetin-3-galactoside, quercetin-3-galactoside, quercetin-3-rhamnoside and kaempferol-3-rhamnoside in birch (*Betula pendula*) at early stages of leaf development, before the leaves are fully expanded, suggesting that individual compounds might be differentially regulated by UV-B at different stages of leaf development and that their contributions to UV protection might also vary.

Effects on photosynthesis

UV-B impairs photosynthesis in many species although the mechanisms vary greatly. For example, Greenberg et al. (1996) observed that Rubisco from *Brassica napus* exhibited changes in its large subunit. Also the biosynthesis of flavonoids and other UV-absorbing pigments occurred at UV-B levels that caused cotyledon curling.

Wheat plants (*Triticum aestivum* L.) exposed for 4 months to high UV-B levels (simulating a 20% reduction in the ozone layer) showed a decrease in total plant biomass of 18% compared to control plants (ambient UV-B). High UV-B also induces decreases in leaf area, net photosynthesis rate, transpiration rate and water use efficiency; leaf extracts showed increases in chlorophyll content and no effect on accumulation of UV-B absorbing pigments (Correia et al., 1999).

Chlorophyll content decreased but leaf soluble protein content increased in plants under solar UV-B radiation. Solar UV-B radiation apparently had a strong effect on chlorophyll degradation rather than the size of the xanthophyll cycle pool, in both acacia and eucalyptus species (Liu et al., 2005). Sangtarash et al. (2009) observed that seedlings of *Brassica napus* produced more dry matter under ambient UV-B (5 KJ m^{-2} d^{-1}) than under zero UV-B, but 10 KJ m^{-2} d^{-1} caused a decrease in dry mass, indicating that some adaptive mechanisms to the ambient UV-B exists. The highest level of UV-B irradiation also decreased stem height, leaf area, plant dry matter, water use efficiency and wax content.

Neither photosynthesis nor pigment levels of *Zea mays* leaves were affected significantly by UV-B levels (Casati and Walbot, 2004) although damage to leaf ribosomes by crosslinking three different cytosolic ribosomal proteins and chloroplast ribosomal protein L29 to RNA were

observed. Hao et al. (2000) also verified that the exposure to enhanced UV-B increased leaf chlorophyll concentration and UV-absorbing compounds in *Lycopersicon esculentum*, but decreased leaf area and root/shoot ratio.

The large spectra of plant responses are mainly due to the different sensitivity of species and cultivars and differences in experimental protocols which are responsible for the diversity of dose-effect relationships. Nevertheless, a large number of studies with terrestrial plants point out that photosynthesis (CO_2 fixation per unit leaf area) is not significantly affected by changes in UV-B radiation when plants are grown under natural conditions (Caldwell et al., 2003; Caldwell et al., 2007) although UV-B radiation may have subtle inhibitory effects on biomass accumulation, often correlated with a reduction in the rate of leaf area expansion.

Effects on genetic material

In response to UV-B exposure, plants have evolved mechanisms of protection and repair such as the accumulation of UV absorbing pigments, phosphorylation of particular ribosome proteins, or for example, histone acetylation. Due to the complete knowledge of *Arabidopsis thaliana* genome, this species is also commonly used in laboratory experiments to evaluate the effects of UV-B radiation on genes (Tong et al., 2008; Campi et al., 2012), indicating that this plant is useful as a comprehensive model.

Using microarray hybridization techniques to study the *Zea mays* acclimation responses to UV-B Casati and Walbot (2003) observed that genes encoding protein translation components were the largest functional group up-regulated by UV-B. Despite the significant ribosome damage and a decrease in translation in RNA of maize (Casati and Walbot, 2004) it has been suggested that new synthesis of ribosomes occur as a response to UV-B damage, thus indicating restoration of the capacity of protein synthesis.

Chromatin remodeling and histone acetylation are important during DNA repair by UV-B in both *Zea mays* and *Arabidopsis thaliana* (Campi et al., 2012) showing that both genetic and epigenetic effects control DNA repair in plants.

Marked differences in genetic response to UV-B of three different ecotypes of *Arabidopsis thaliana* (Kalbina and Strid, 2006) were also observed. The C24 ecotype exhibited the highest expression level of *PR-5* gene (pathogenesis-related protein gene) while the induction of hypersensitive response (HR) like spots, which resulted in necrotic lesions is rapid. Conversely, the Ws ecotype showed the lowest levels of *PR-5* transcripts and its growth rate was the lowest one.

The effects of UV-B on *Mesembryanthemum crystallinum* may be ameliorated by UV-A through the activation of DNA repair mechanisms mainly due to the action of the enzyme photolyase (Ibdah et al., 2002). However, laboratory studies with plants suggest that the effects of ozone depletion (measured by the formation of cyclobutane pyrimidine dimers in DNA) is likely to be less marked than previously thought, because UV-A (315–400 nm) may also cause significant damage by penetrating deeper into plant leaves and it is not affected by ozone shield *i.e.,* it passes almost unaltered through the atmosphere (Rousseaux et al., 1999).

Effects on UV-absorbing pigments

Increased accumulation of phenolic compounds and flavonoids is one of the main responses to UV-B radiation contributing by this way to filter out UV-B photons before they reach sensitive molecules. In *Brassica napus,* approximately 20 distinct UV-absorbing pigments were produced in response to UV-B radiation (flavonoids and other UV-absorbing pigments), their synthesis occurring mainly in the epidermal cell layer (Greenberg et al., 1996).

As a result of UV-B radiation leaves of potato plants increased constitutive flavonoids. Also, the activity of the antioxidant enzymes catalase, ascorbate peroxidase and guaiacol peroxidase increased associated with the induction of a new catalase isoform and three new guaiacol isoperoxidases (Santos et al., 2004) showing that potato plants activate several defense systems.

Xu et al. (2008) observed that solar UV-B caused oxidative stress in both isolines of soybean grown in the field (one with moderate levels of flavonoids and the other with reduced levels) and altered the antioxidant defenses mainly by decreasing superoxide dismutase activity. The greater oxidative stress was observed in the line with very low levels of flavonoids. These protective compounds (phenolic compounds and flavonoids) also influence leaf development, water relations, trophic responses (plant-herbivore interactions) and decomposition process (Xu and Sullivan, 2010).

In *Indigofera tinctoria* (L.) seedlings, the supplementary UV-B radiation significantly decreased the growth, development and changes in UV-B absorbing compounds such as anthocyanin and flavonoids. The antioxidant enzymes were unaffected and showed enhanced activities of

peroxidase, superoxide dismutase, polyphenoloxidase and phenylalanine ammonia-lyase, but not catalase (Ravindran et al., 2010), indicating in this particular case that the activation defense mechanisms were mainly based on anti-oxidant enzymes, instead of UV-B absorbing compounds.

Another important approach derived from experimental studies indicates that the enrichment of plant tissues in phenolic compounds or flavonoids as a protective response to UV-B irradiance induces a resistance to herbivores (Izaguirre et al., 2007; Kuhlmann and Muller, 2009). Moreover, the interaction of biotic and abiotic environment factors with UV-B radiation can produce cross-tolerance (*i.e.*, tolerance to one stress induced by another stress), as well as resilience to subsequent stress due to the establishment of a level of protection (Kalbin et al., 2001).

Conjugated effects of UV-B plus CO_2, UV-B plus ABA, UV-B plus drought

As previously stated, plants in their natural habitat are seldom affected by a single stress factor. In that sense several interactions of UV-B plus CO_2, UV-B plus ABA, UV-B plus drought, have been studied in laboratory conditions. The effects of UV-B radiation on tomato plants growing in a controlled environment were small even if significant alone or interacting with CO_2 or O_3, (Table 1) suggesting that substantial increases in UV-B may not have strong deleterious effects on productivity (Hao et al., 2000). *Populus kangdingensis* and *Populus cathayana* originating from high and low altitudes from south-west China, respectively (Ren et al., 2007) exhibited significant reductions in plant height and total leaf area when exposed to drought, enhanced UV-B radiation or a combination of both stress factors, indicating that the addition of another negative factor influences decisively the performance of both plants. For example in *Populus kangdingensis* plant height (in cm) decreased from 105.04 in drought-stressed regimes to 83.8 in UV-B plus drought-stressed regimes. The same occurs for the total area (dm^2), from 18.03 to 8.48. When the plant was not submitted to drought the height was 148.9 cm and the total area 32.06 dm^2. Similar results were observed for *Populus cathayana.*

Populus cathayana originating from high and low altitudes from south-west China (Lu et al., 2009) was exposed to exogenous ABA (abscisic acid), enhanced UV-B radiation or a combination of both. The results from plant height (cm), total leaf area (dm^2) and total biomass (g) were shown in Table 1.

Concluding Remarks

Despite the huge number of studies mainly in the laboratory and glasshouse conditions and the variety of responses of plants when exposed to UV-B irradiation the understanding of the complex interactions between UV-B and biota will be always limited by the incapacity to reproduce natural conditions. Even when plants are exposed to conjugated effects of UV-B plus CO_2 for example, what is seen is a partial response despite the importance of the data and the relevance of the conclusions.

Table 1. Physiological data of *Populus cathayana*[1] and *Lycopersicon esculentum*[2] exposed to UV-B plus other ambient factors.

		Plant height	Total leaf area	Total biomass
Low altitude[1]	ABA	173.2	27.59	56.33
	UV-B	164.2	20.98	40.64
	UV-B+ABA	169.0	21.56	43.97
	Control	184.0	37.14	66.13
High altitude[1]	ABA	200.8	28.77	65.33
	UV-B	174.6	24.87	58.89
	UV-B+ABA	176.2	22.74	54.69
	Control	199.6	29.50	73.79
Tomato	CO_2	73.5	2850	20.52
	UV-B	65.6	2449	14.02
	CO_2+UV-B	67.8	2729	18.55
	Ambient	66.6	2591	14.98

(1) Data from Lu et al. (2009); (2) Hao et al. (2000) – leaf area was expressed in cm2 instead dm2 in the case of *Populus*.

Moreover, several authors consider that the observed effects of UV-B on plants on indoor experiments were exaggerated and extrapolations to field conditions must questioned (Caldwell and Flint, 1997; Krizek, 2004).

In glasshouse conditions we do not have rainy days, or clouds, nor do we have aerosol particles or variations in temperature. In that sense, field studies in areas where the ozone hole is high is a priority, such as those undertaken in Antarctica. Others must be done in mountain populations since these species have natural adaptive mechanisms to tolerate to high UV-B irradiation levels. Plant data from the same Genus or Family living in different altitudes or plants from the same species along a gradient in altitude, will be extremely useful when compared with laboratory data.

The perception that UV-B radiation may trigger the synthesis of new compounds, the increase of anti-oxidant activity or the increase of known compounds such as flavonoids and phenolics, can be used to improve the quality of food although it is also suggested that the synthesis of these molecules can be used as biomarkers for the identification of stressed plants (de Britto et al., 2011).

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REVIEW ARTICLE

Harmful effects of UV radiation in Algae and aquatic macrophytes – A review

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Abstract

This study provides an overview of the available literature on the ultraviolet-B (UV-B – λ=280-315 nm) and UV-A radiation (λ=315-400 nm) effects on algae (micro and macroalgae) and aquatic macrophytes, like seagrasses and liverworts. It includes studies on prokariotic cyanobacteria, haptophytes, diatoms, dinoflagellates, red algae, brown algae and chlorophytes from freshwater (ponds, lakes) to marine littoral and Open Ocean. It also reports available studies concerning on marine and freshwater plants exposed under UV irradiation. Since the reported relationship between the human activity and the depletion of the protecting layer, the effects of ultraviolet radiation in the biological relevant wavebands on algae and on organisms in general have become an important issue over the past three decades and will be also important in the next few decades. Virtually, all aquatic organisms depend on algae and aquatic plants (submerged or near shallow line) for food, shelter, also as oxygen supplement and CO2 sequestration by photosynthetic procedure. This review reports on harmfull effects caused by ultraviolet wavebands on photosynthetic organisms in their natural habitats.

Key words: Algae, cyanobacteria, Macrophytes, UV-radiation

Introduction

Aquatic systems (freshwater, marine or brackiswater) cover about 71% of the Earth's surface, being the hydrosphere. Aquatic photosynthetic organisms are the main support of the entire life of these systems, ranging from cyanobacteria, algae, to aquatic angiosperms.

Algae are an extensive group of photosynthetic organisms distributed through a wide variety of habitats. It is a group beyond the taxonomy, as it includes several taxonomic kingdoms. Algae occur in freshwater ponds, shores and coasts attached to the bottom by more or less complex fixations of the thallus (benthic species) or live suspended in the water column, being the phytoplankton. It can be found also in the open ocean, from intertidal shores to a depth of 150 metres. There are also terrestrial forms, on soils and among bryophytes. According to individual size, and cell organization, algae can be divided into two categories: microalgae (great number of photosynthetic unicellular organisms being the smallest ones the cyanobacteria) and macroalgae (multicellular organisms characterized by a body named thallus, with no differentiation in roots, stems and leaves, although in kelp a great complexity in thallus structure can be found). Altogether, the algae probably account for more than half the world total primary production (Hoeck et al., 1995). This group is characterized by a great diversity of sizes, forms, body structure, distribution and ways of life. Virtually, all aquatic organisms depend on their production for food, shelter and oxygen supplement. They also perform CO_2 sequestration by photosynthetic procedure and play a role as pH regulators. Algae are extremely important not only ecologically but also phylogenetically (Hoeck et al., 1995) because understanding the diversity and the phylogeny of the plants relies on algae research (Hoeck et al., 1995). There is an agreement by the scientific community that life originated in the sea and that many ancient evolutionary lineages can be found there (Hoeck et al., 1995).

The interest in commercial utilization of algae (Spolaore et al., 2006; Cardozo et al., 2007; Stengel et al., 2011) or specifically of cyanobacterial secondary metabolites (Rastogi and Sinha, 2009) is another challenge and is increasing recently based

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on different fields of science (nutrition, environment, medicine, pharmacology). Many of their valuable chemical constituents exhibit multitude bioactivities with applications in the food, as pigments source, cosmetic, new natural sunscreens (Coba et al., 2009), pharmacological (Thomas and Kim, 2011), agri- and horticultural sectors, in human health (Stengel et al., 2011), carrageenans extraction (Campo et al., 2009), and in biofuels production (Chisty, 2007). Cyanobacteria, like *Anabaena* or *Nostoc* are also well documented as natural soil biofertilizerers in rice fields (Banerjee and Häder, 1996; Sinha et al., 1998, 2002).

Since publication of the 1998 UNEP Assessment, there has been continued rapid expansion of the literature on UV-B radiation, and many measurements have demonstrated the inverse relationship between column ozone amount and UV radiation (McKenzie et al., 2003), from around 1970 (20^{th} century) until the end of the 20^{th} century (Björn, 2007). There is almost complete consensus regarding the major cause of this: anthropogenic pollution of the atmosphere (Björn, 2007) due to a rapid industrialization in the past few decades related to an increase pollutants such as chlorofluorocarbons (CFCs), halocarbons, chlorocarbons (CCs), organobromides (OBS), carbon dioxide (CO_2), methyl chloroform (MCF) and dioxins (NO_x) that are responsible for the depletion of the UV-screening ozone layer in the stratosphere (references in Singh et al., 2010). The most dramatic expression of this is the Antarctic ozone hole (Björn, 2007). At northern mid-latitudes, the 1997-2000 ozone losses were around 6% relative to 1980 levels, which might result in a UV-B increase of up to 12% (McKenzie et al., 2003) as referred by Arróniz-Crespo et al. (2008). Decreased ozone levels are expected to recover to pre-1970 levels by 2050 (McKenzie et al., 2003). So, ultraviolet radiation, especially its effects on terrestrial and aquatic living organisms, became an important issue over the past three decades and will be also important in the years to come.

Artificial UV-B (λ=280-315 nm) radiation may be used as disinfectant preventing toxic algal blooms (or pathogens) on potable water (Alam et al., 2001), unfiltered surface water (Cantwell and Hofmann, 2008), lakes (Sakai et al., 2007b), wastewater treatment (Blatchley et al., 1997; Mamane et al., 2010) and ballast waters, killing potential invasive living organisms (Martínez et al., 2012). UV-C (λ=200-280 nm) radiation may be a tool to eradicate algae in caves (Borderie et al., 2011).

There are several important reviews on harmful effects of UV radiation on aquatic ecosystems: aquatic ecosystems in general (Häder et al., 1998; Häder, 2000; Hood et al., 2006); marine plankton (Davidson, 1998); marine organisms in Antarctic region (Karentz and Bosch, 2001); algae (Holzinger and Lütz, 2006); plant cells (Kovács and Keresztes, 2002); spore germination in algae (Agrawal, 2009); cyanobacteria (Sinha and Häder, 2008; Singh et al., 2010); cyanobacteria, phytoplankton and macroalgae (Sinha et al., 1998); cryptogams - cyanobacteria, algae, lichens, mosses, liverworts, pteridophytes and fungi - (Björn, 2007); macroalgae (Poll, 2003 referred by Björn, 2007); rhodophytes (Talarico and Maranzana, 2000); freshwater rhodophytes (Necchi Jr, 2005); seagrasses (Short and Neckles, 1999); corals and coral bleaching (Baker et al., 2008; Tambutté et al., 2011); molecular effects (Jenkins et al., 1995; Glatz et al., 1999); methods for DNA damage detection (Sinha and Häder, 2002); genetics (Xiong et al., 2009); cyanotoxin nodularin production (Pattanaik et al., 2010); lipids and lipid metabolism (Guschima and Harwood, 2006); lake acidification and UV penetration (references in Häder et al., 1998); carbon flux and ecosystem feedback (Wassmann et al., 2008) and ecological and environmental impact (Häder and Sinha, 2005; Carreto and Carignan, 2011). The present review concerns the major general effects that UV radiation causes to aquatic photosynthetic organisms, updating previous reviews.

Algae and UV radiation – harmful effects

Aquatic systems with high transparency of oligotrophic waters (marine and freshwaters) are exposed to the highest levels of ultraviolet radiation. Intertidal and epipelagic marine living forms also face the same situation especially those that can't move away in high light periods, like benthic macroalgae, seagrasses and other macrophytes. UV irradiation in lakes can affect photosynthesis of plankton organisms down to a depth of 10-15 m (Holzinger and Lütz, 2006). In marine waters, UV-B can penetrate down to a water depth of 20-30 m (Smith et al., 1992 referred by Dahms and Lee, 2010) and in clear Antarctic Ocean it may reach depths of 70 m (reference in Short and Neckles, 1999). In clear Antarctic oceanic waters UV-A can penetrate to a depth of between 40 and 60 m (Ban et al., 2007 referred by Dams and Lee, 2010), depending, among others, on the incidence of solar radiation, transparency of waters and wind mixed layer effects.

Tidal exposure also imposes considerable environmental stress on intertidal seaweeds such as elevated irradiance levels, temperature changes and desiccation, especially in spring low tides, which occur every month during new and full moon phases. Typically, seaweeds sensitive or intolerant to ambient stresses inhabit the lowermost intertidal zone (where emersion at low tide is brief and/or absent), while those found at higher elevations usually possess heightened tolerance to environmental fluctuations (Sampath-Wiley et al., 2008). Since UV radiation daily doses in the intertidal system are much higher than in the sublittoral zone, there is a relashionship between UV radiation tolerance and vertical distribution of intertidal macroalgae (Altamirano et al., 2003).

There are numerous studies relating harmful UV effects of radiation with decreased performances or death of the target organisms. As regard algae and aquatic plants, these studies especially concern UV-B wavebands effects on growth and development (Banaszak and Trench, 1995a; Braune and Döhler, 1996; Grobe and Murphy, 1997, 1998; Häder et al., 1998; Makarov, 1999; Cordi et al., 2001; Estevez et al., 2001; Altamirano et al., 2003; Altamirano et al., 2004; Huovinen et al., 2006; Andreasson and Wängberg, 2007; Zeeshan and Prasad, 2009; Dahms and Lee, 2010; Dahms et al., 2011), biomass, productivity and photosynthesis (Helbling et al., 2008; Sampath-Wiley et al., 2008; Zeeshan and Prasad, 2009), buoyancy (Ma and Gao, 2009), sensitivity (Banerjee and Häder, 1996; Zudaire and Roy, 2001; Marshall and Newmann, 2002; Arróniz-Crespo et al., 2008), photosynthetic pigments (Döhler and Buchmann, 1995; Döhler and Lohmann, 1995; Aráoz et al., 1998; Bhargava et al., 2005; Huovinen et al., 2006; Sampath-Wiley et al., 2008; Heo and Jeon, 2009), reactive oxygen species (Mallick and Mohn, 2000; Downs et al., 2002; He and Häder, 2002; Rastogi et al., 2011), antioxidant system (Estevez et al., 2001; Dummermuth et al., 2003, Bolige et al., 2005; Barros et al., 2006; Janknegt et al., 2007; Wang et al., 2007; Sampath-Wiley et al., 2008; Wang et al., 2008; Delgado-Molina et al., 2009; Lee and Shiu, 2009; Mogedas et al., 2009; Ryu et al., 2009; Tian and Yu, 2009; Pallela et al., 2010; Zeeshan and Prasad, 2009; Dahms and Lee, 2010; Li et al., 2010; Hupel et al., 2011, Liu et al., 2011), protein (Sass et al., 1997) and DNA damage (Buma et al., 2001; Kumar et al., 2004; Sakai et al., 2007a; Rastogi et al., 2011), nutrition quality (Leu et al., 2006; Nahon et al., 2010), lipid/fatty acid content (Skerrat et al., 1998; Khotimchenko and Yakovleva, 2005; Liang et al., 2006) enzyme activity (Lee and Shiu, 2009), C:N:P fixation (Hessen et al., 2008) and nitrogen assimilation (Döhler and Buchmann, 1995; Döhler et al., 1995; Babin et al., 1996; Braune and Döhler, 1996; Döhler, 1997, 1998; Wängberg et al., 1998; Xu and Gao, 2012), nutrient cycling (Anusha and Asaeda, 2008), P uptake (Hessen et al., 2012), system ecology (Carreto and Carignan, 2011), and synergistic effects under xenobiotics presence enhancing toxicity on target organisms (Barron and Ka'Aihue, 2001; Bhattacharyya et al., 2011).

Algae (and the organisms in general) may develop a wide strategies to cope with UV radiation like vertical migration, multiple layered cell walls, absorbing screening compounds such as carotenoids, mycosporine-like amino acids (MAAs), scytonemines (only cyanobacteria) (Banaszak and Trench, 1995b; Sinha et al., 1998; Klisch and Häder, 2008; Singh et al., 2010), protective mechanisms (e.g. Malanga et al., 1999; Marshall and Newman, 2002; Carreto and Carignan, 2011; Hupel et al., 2011), proteins and some repairing enzymes, that enable adaptation to environmental stress (Marshall and Newman, 2002; Hanelt and Roleda, 2009).

Besides the large amount of work on this subject, many of them were made under conditions with supplemental UV-B irradiance higher than would ever occur in nature (Xue et al., 2005). Most of the studies on the action of UV radiation on species cultures involve short duration photosynthesis experiments. Such studies have limited value for understanding UV-resistance in the field or adaptation of the whole organism (Holzinger and Lütz, 2006). Recent studies are conducted under experimental conditions and supplemental UV-B irradiance that tend to approach realistic UV- radiation conditions existing on Earth's surface. Some are related with a wide range of deleterious effects of UV-irradiation also describing survival mechanisms under high levels of UV-B and other environmental parameters.

The effects of UV radiation on organisms in natural conditions are complex because synergy is involved on deleterious and recovering mechanisms to face UV irradiation. The susceptibility to elevated UV-B radiation is dictated by a complex interplay between protection, repair and other factors that may lead to highly variable UV-B susceptibility among the species (Zeeshan and Prasad, 2009).

Wavebands of UV radiation: differential effects on algae

The term UV radiation (UVR) describes the UV region from 280 to 400 nm. UVR is usually divided into three spectral regions: UV-C (λmax = 200 to 280 nm), UV-B (λmax = 280 to 315 nm) and UV-A (λmax = 315 to 400 nm). Studies related with the effects of UV radiation, usually concern wavebands from 280 to 400 nm (UV-A+UV-B), compared with PAR. PAR is an abbreviation of photosynthetic active radiation, which is the spectral range of solar radiation from 400 to 700 nanometres that enables photosynthesis process by photosynthetical organisms.

The wavebands of UV radiation (UV-C, UV-B and UV-A) act differently on algae. Their modes of action are also different in other organisms, but they will not be referred here. Short UV-B wavelengths result in a higher degree of DNA damage, higher levels of oxidative stress, and greater expression of cell cycle genes, than exposure to UV-A, therefore promoting apoptosis (reference in Dahms and Lee, 2010) because longer UV-A wavebands are closer to PAR.

UV-A generally causes indirect DNA damage by the formation of chemical intermediates such as oxygen and hydroxyl radicals that interact with DNA to form strand breaks, DNA-protein cross-links and alkali labile sites (reference in Dahms and Lee, 2010). On the other hand, UV-B causes direct DNA damage by inducing the formation of cyclobutane pyrimidine dimers (CPDs) and pyrimidine-pyrimidone (6-4) photoproducts (Dahms and Lee, 2010). These products can cause mutations or have cytotoxic effects by inhibiting replication or the expression of essential genes (reference in Dahms and Lee, 2010).

UV-A is a powerful prooxidant, inducing both strand breaks and alkali labile sites (Pfeiffer et al., 2005 referred by Dahms and Lee, 2010), whereas UV-B produces mainly CPDs.

However, moderate levels of UV-A may stimulate photosynthesis and growth in both micro and macroalgae (references in Xu and Gao, 2010). *Gracilaria lemaneiformis* (Rhodophyta) shows an increase relative growth rate in the presence of UV-A, while UV-B inhibited it (Xu and Gao, 2010). The positive effect of UV-A counteracted negative effect of UV-B, resulting in an insignificant impact of UVR on growth (Xu and Gao, 2010) of this alga. Xu and Gao (2010) study, showed that during the noon period, both UV-A and UV-B resulted in the decrease of maximum quantum yield (F_v/F_m), but UV-B aided in the recovery of the yield in the late afternoon, reflecting that UV-B might be used as a signal in photorepair processes.

UV-C is the most damaging range of the spectrum (Banaszak and Trench, 2001) but it is not of biological relevance because it is totally absorbed by the atmosphere (Banaszak and Trench, 2001; Holzinger and Lütz, 2006; Basti et al., 2009).

Few studies have been carried out on the UV-C effect on established algal colonies. Borderie et al. (2011) showed that after various periods of UV-C exposure, the photosynthetic activity of algae was strongly decreased and even annihilated, which could be related to a degradation of their photosynthetic apparatus and pigment contents. After UV-C exposure, algal cells reinoculated on fresh medium were unable to proliferate (Borderie et al., 2011). UV-C radiation generates oxidative stress and genotoxicity effects. It is also known (Borderie et al., 2011) to induce programmed cell death (PCD) by a production of cyclobutane-pyrimidine dimers and DNA photoproducts, which are involved in cellular lethality, senescence and mutagenesis (references in Bordrerie et al., 2011).

Oxidative stress

Environmental stresses (high light, nutrient deficiency, drought, heavy metals, high salt concentration, extremes of temperature, UV radiation, air pollutants, water stress, herbicides, mechanical and physical stress) induce the production of reactive oxygen species (ROS) (Dummermuth, et al., 2003). ROS are always formed by the inevitable leakage of electrons onto molecular oxygen from the electron transport activities of chloroplasts, mitochondria and the plasma membrane (Mallick and Mohm, 2000). Reactive forms of oxygen include the superoxide radicals (O^{-}_{2}), singlet oxygen ($O^{\square}$) the hydroxyl radical ($OH^{\square}$) and hydrogen peroxide (H_2O_2). All these can react with certain biomolecules, altering or hampering their biochemical activities. The combined biological effect of these toxic oxygen species on organisms is termed "oxidative stress" (Mallick and Mohm, 2000).

Studies with the cyanobacterium *Arthrospira (Spirulina) platensis* by Ma and Gao (2010) show that associated accumulation of reactive oxygen species and presence of UVR resulted in the spiral breakage by oxidizing the lipids of sheath or cell membrane.

Rhodophytes like *Gelidium amansii* (Lee and Shiu, 2009) or *Corallina officinalis* (Li et al., 2010) are two examples of production of free H_2O_2 to seawater and lipid peroxidation induction when exposed to UV-B radiation. Hydrogen peroxide

itself is not particularly reactive with most biologically important molecules, but it is probably an intracellular precursor for more reactive oxidants as it passes quickly through membranes by diffusion (Apostol et al., 1989 referred by Dummermuth et al., 2003). If accumulation of ROS exceeds the capacity of enzymatic and non-enzymatic antioxidant systems, the photosynthetic apparatus is damaged due to destruction of lipids, proteins and nucleic acids, finally leading to cell death (Estevez et al., 2001; Dummermut et al., 2003). Under physiological conditions of growth, oxidative stress associated to development leads to a significant decrease on cellular antioxidant capacity (Estevez et al., 2001).

Dummermuth et al. (2003) study shows that the measurement of the in vivo fluorescence of photosystem II is a suitable tool to determine the effect of oxidative stress on macroalgae.

Growth and development

The negative effects on growth and development caused by UV-B irradiation is well documented, and usually relative growth rates (RGR) are also related to UV damage to the photosynthetic machinery, photosynthetic pigments, antioxidant enzymes and lipid peroxidation caused by increasing UV radiation.

The green macroalga *Ulva expansa* (Setch.) S. and G. grown in an indoor tank under controlled photoperiod and UV-B levels showed significantly lower growth rates on algae segments exposed to the unscreened UV-B lamps as compared to UVO-screened lamps (Grobe and Murphy, 1997). Makarov (1999) reached the same conclusion regarding the influence of UV radiation on growth rate of nine species of macroalga: Phaeophyta (*Laminaria saccharina, Alaria esculenta, Saccorhiza dermatodea, Fucus distichus, Fucus serratus, Fucus vesiculosus*), Rhodophyta (*Palmaria palmata, Porphyra umbilicalis*) and the Chlrophyta *Ulvaria obscura*. In this study the maximum growth rate was found in tests with solar radiation excluding UV radiation. *Ulvaria obscura* appeared to be the most sensitive to *in situ* levels of UV-B radiation, reducing its growth to 54%. The lower sensitivity was recorded in *Fucus vesiculosus.* Makarov (1999) also described May as a critical period for algae, which were highly affected by ultraviolet radiation during this month.

The apical segments of the intertidal macroalga *Hypnea musciformis* (Rhodophyta, Gigartinales) cultivated *in vitro* free of UV radiation showed growth rates of 9.7% day^{-1}, while algae exposed to UV-B grew only 3.2% day^{-1} (Schmidt et al., 2012).

Different growth rates were found for *Chlorella* sp cells when irradiated with 30 kJ m^{-2} UV-B as compared to unirradiated cultures: the specific growth rate immediately after the lag phase was 0.36±0.06 and 0.26±0.03 day^{-1} for unirradiated cultures and cultures irradiated with UV-B respectively (Estevez et al., 2001). Andreasson and Wängberg (2007) showed the effect of UV-B radiation on growth rate for two marine microalgae: *Dunaliella tertiolecta* (Chlorophyceae) and *Phaeodactylum tricornutum* (Bacillariophyceae). The growth rate of *D. tertiolecta* was slightly more inhibited by UV-B radiation than was the growth rate of *P. tricornutum*, with the same wavelength dependencies.

A growth-related temperature dependence of sensitivity to UV-B radiation was suggested by Altamirano et al. (2003) based on germling of three species of *Fucus* (Fucales, Phaeophyta): *F. spiralis* (eulittoral), *F. vesiculosus* (eulittoral-high sublittoral) and *F. serratus* (high sublittoral). Altamirano et al. (2003) determined the effects of different ultraviolet radiation conditions, UV radiation doses and temperatures on the relative growth rates of germlings of three species of intertidal brown macroalga. High ultraviolet-B radiation levels and low temperature, as independent factors, led to a species-specific reduction in RGR which appears to be related to the vertical distribution of the species in the intertidal zone. The inhibition of RGR ranged from 10% to even death of the germling. For the most sensitive species, high temperature in combination with a high dose of UV-B caused the death of the germlings, whereas at low temperatures germlings were able to survive.

In *Gracilaria lemaneiformis* UV radiation resulted in an insignificant impact on growth, because the presence of UV-A enhanced the relative growth rate, while UV-B inihibited it (Xu and Gao, 2010).

Sensitivity

The sensitivity to UV appears to be related with natural UV-irradiance of the environment for the same species (Marshall and Newmann, 2002). This work shows that isolates of the marine microalga *Chattonella marina* (Raphidophyte) from Australia exhibits higher tolerance to high intensities of visible light than *C. marina* collected from Japan waters. This microalga is known to cause wild and

farmed fish mortality in Japanese and South Australia waters (Marshall and Newmann, 2002).

The UV-B sensitivity is also related to life cycle stage. Cordi et al. (2001) observed that zoospores of the green intertidal macroalga *Enteromorpha intestinalis* were six fold more sensitive to UV-B exposure than mature talli. Cordi et al. (2001) also observed a greater sensitivity in the sexual reproductive phase of the life cycle of this macroalga species compared with the asexual phase. Inhibition of germination success and growth rates of settled gametes and zoospores after 1-h exposure to elevated levels of UV-B (equivalent to 27 and 31% ozone depletion) showed that damage to the reproductive cells was irreversible.

Most of the studies are concerned with high sensitivity of algae (micro and macro algae) species to UV radiation, showing the deleterious effects of these wavebands on cell integrity. Nevertheless Holzinger et al. (2009) reported that organelles like mitochondria, Golgi bodies and the nucleus of the vegetative freshwater green alga *Zygnema* remained unaffected by the radiation exposures, showing to be well adapted to ambient solar radiation and enabling the alga to cope with experimental UV exposure. According to the authors this effect is expected to persist in a scenario of enhanced UV radiation caused by stratospheric ozone depletion. *Nodularia spumigena*, a freshwater cyanobacterium, is a species that in general is not negatively affected by moderate levels of UV-B radiation (Wulff et al., 2007).

Algae from high mountains lakes, naturally exposed to high levels of UV radiation, show high UV resistance. Phytoplankton species with high resistance to increasing UV radiation have probably more adaptive capacity to survive in regions of increasing UV and tend to raise the number of individuals, reducing species biodiversity of phytoplankton communities.

So, some species are tolerant or even show stimulation when exposed to UV-B radiation, while some are highly susceptible.

Photosynthesis and photosynthetic pigments

Photosynthesis

Among various physiological processes, photosynthesis is potentially the main target of UV radiation due to a multiplicity of possible effects (Holzinger and Lütz, 2006). UVR inhibits photosynthesis, damages DNA and proteins and affect algae morphology. Jones and Kok (1966) study was the first to demonstrate the potential of UV to inhibit photosynthesis (references in Hanelt and Roleda, 2009). The UVB inhibition spectrum corresponds much more with the spectral absorption by DNA and proteins than photosynthetic pigments one (Hanelt and Roleda, 2009). Photosystem II (PSII) is a primary UV-B target (Aro et al., 1993 referred by Bouchard et al., 2008). Photosystem I (PSI) is relatively insensitive to UV-B damage (Strid et al., 1990 referred by Bouchard et al., 2008). In PSII, several possible sites of damage are associated with the D1 protein, one of the key proteins involved in a PSII repair cycle (Aro et al., 1993 referred by Bouchard et al., 2008). The primary enzyme involved in CO_2 fixation, ribulose-1,5-bisphosphatase carboxylase-oxygenase (RuBisCO), is also a suspected target of UV-B inhibition (Kumar et al., 2003; Bouchard et al., 2008). Both PSII and Rubisco have been shown to be affected by UV-B radiation (Bouchard et al., 2008). UV-B exposure may cause the loss of photosynthetic pigments (Bischof et al., 2000; Lütz et al., 2005, referred by Holzinger and Lütz, 2006), and reduce the expression of genes involved in photosynthesis (Mackerness et al., 1999 as referred by Holzinger and Lütz, 2006).

The harmful blooming raphidophyte *Chattonella subalsa* and dinoflagellate *Prorocentrum minimum*, showed a significant decline in the photochemical capacity of photosystem II (PSII), F_v/F_m (vitality indicator) in both algal species when shifted to high light, with a greater decline noted in *P. minimum*. This study also showed a rapid reduction in electron transport with an increment immediately after light exposure (Warner and Madden, 2007).

The biological weighting function (BWF) describes the effectiveness of the different wavelengths to produce biological responses, such as inhibition of photosynthesis (Andreasson and Wängberg, 2006). So, high light intensities may result in an inhibitory effect on metabolic processes. Photoinhibition will be defined as the generic outcome of the failure of photoprotection to mitigate photoinactivation, which occurs when damage of reaction centre proteins exceeds photorepair of photosystem II (Hanelt et al., 2006).

UV-B radiation may ameliorate photoinhibition in specific shallow water on tropical marine macrophytes (Hanelt and Roleda, 2009): brown algae (*Dictyota* sp., *Padina sanctae-crucis*, *Lobophora variegata*, *Sargassum polyceratium* and *Turbinaria turbinate*), the green algae (*Udotea flabellum* and *Halimeda discoidea*) and the seagrasses *Syringodium filiforme* and *Thalassia testudinum*.

Coccolitophores of *Emiliana huxleyi* species

exposed to solar UV radiation (UVR, 280-400 nm) showed a significant decrease in the rates of photosynthesis and calcification (Guan and Gao, 2010). Shorter wavelengths of UV-B led to more damages to photosynthetic apparatus than to calcifying machinery, while longer wavelengths of UV-A results more harmful to calcification. During long term exposures to solar radiation, the ratios of repair to UV—related damage increased indicating an acclimation to UV. UV—induced stress led to a protective strategy of *E. huxleyi*, sacrificing the growth by allocating energy for accumulation of UV-absorbing compounds and calcification (Guan and Gao, 2010).

Two frequently used techniques for measuring photosynthetic capacity in planktonic producers are: pulse-amplitude-modulation (PAM) fluorescence from Photo System II (PSII), and fixation of ^{14}C-labelled carbom dioxide. The first measures the function of the light-harvesting complexes, the reaction centres and the following electron transport. The second measures actual carbon fixation by Rubisco, which depends both on a functional photosynthetic electron transport and on the enzymatic reactions within the Calvin-Benson cycle. The PAM technique has considerable advantages being both cleaner and easier to perform and can be done unobtrusively (Andreasson and Wängberg, 2006). Measures indicatives of vitality are F_v/F_m and chlorophylls/phaeopigments ratio (Arróniz-Crespo et al., 2008). The rapid decreases in F_v (variable chlorophyll fluorescence) in response to increasing UV-B radiation, in addition to the fact that F_v decreased in a dose-dependent manner to UV-B, indicated that F_v may be a suitable, sensitive biomarker for UV-B exposure (Cordi et al., 2001).

Photosynthetic efficiency by the intertidal red alga *Porphyra umbilicalis* was related to immersion period and not to sun exposure (Sampath-Wiley et al., 2008). Immersion period was the greater facilitator of photoinhibitory damage and ROS generation at PSII. Authors conclude that protection via elevated antioxidant metabolism and increased PSII repair are involved in providing relief from the acute environmental stresses in the intertidal zone.

Antarctic waters indicate a reduction in photosynthesis of around 25% in the top 10-20 m due to increased UV-B radiation (reference in Malanga et al. 1999), which can compromise sustainability as a serious deregulations in trophic webs.

Photosynthetic pigments

There are basically three classes of photosynthetic pigments in algae: Chlorophylls, carotenoids and phycobilins. Chlorophylls are greenish pigments that contain a porphyrin ring. The most important is Chlorophyll *a* present in all plants, algae and cyanobacteria that photosynthesize. Chlorophyll *b* occurs only in green algae and in plants. Chlorophyll *c* is found only in the photosynthetic members of the Chromista as well as the dinoflagellates. Carotenoids are usually red, orange, or yellow pigments and include the familiar compound carotene. They are called accessory pigments because they cannot transfer energy directly to the photosynthetic pathway, but pass their absorbed energy to chlorophyll. One very visible accessory pigment is fucoxanthin, the brown pigment which colours kelps and other brown algae as well as the diatoms. Phycobilins are water-soluble pigments occurring only in cyanobacteria and Rhodophyta (red algae).

The damaging effects of UV radiation on the pigments are dependent on the UV wavebands and the exposure time (Döhler and Lohmann, 1995; Huovinen et al., 2006). UV-B induces the reduction of the number of phycobilisomes per cell in cyanobacteria (Araóz et al. 1998) and according to Häder et al. (2004) UV wavebands induce a bleaching of all pigments in rhodophyta. Contents of chlorophyll a and $c_1 + c_2$ were mainly reduced by UV-A of high intensity and by UV-B (Araóz et al. 1998). β-carotene seems to be the most sensitive pigment (Döhler and Buchmann, 1995) to UV radiation exposure. Exposing the marine haptophycean *Pavlova lutheri* and *Pavlova* spec., Döhler and Buchmann (1995) showed a reduction in fucoxanthin content and an increased in neofucoxanthin and chlorophyll c. According to these authors, the UV-induced increase in neofucoxanthin can probably be explained by a stimulation of the biosynthesis and degradation of fucoxanthin. No damaging effect on pigments was found after UV-A exposure of low intensity. Pools of glutamine, glycine, threonine, and phenylalanine were enhanced and that of glutamate reduced.

Following UV exposure, phycoerythrin (PE) fluorescence emission increases dramatically in *Nostoc* species, indicating accumulation of PE in the phycoblilisome rods (Wang et al., 2007, 2008). Nevertheless, phycoerythrin (PE) and phycocyanin (PC) in rhodophytes decrease with UV exposure (Schmidt et al., 2009) like is confirmed in *Porphyra umbilicalis* (Aguilera et al., 1999), *Gracilaria*

lemaneiformis (Xu and Gao, 2010) and *Hypnea musciformis* (Schmidt et al., 2012).

UVR did not affect the content of Chl *a* in *Gracilaria lemaneiformis* (Xu and Gao, 2010), but in the intertidal red macroalga *Porphyra umbilicalis* Chl *a* decreased by 65-67% and carotenoids showed a decrease by 75-82% (Aguilera et al., 1999).

Coralina elongata Ellis and Soland by Häder et al. (1997) study showed that Chl *a* and PE (phycoerythrin) were higher in the shade than in the sun type algae, but the two pigments did not show the same variation troughout the day. Both the depletion and recovery of this pigment were higher in the shade morphotype. The concentration of PC (phycocyanin) was very low compared with PE and only in the sun morphotype there was a significant depletion of this pigment. The concentration of SP (soluble protein) was similar in the sun and shade type algae, coinciding with the depletion of PE, PC and oxygen production. The ratio PE to SP was higher in the shade than in the sun type algae.

Studies of Huovinen et al. (2006) with the red alga *Grateloupia lanceolata* showed a decline on photosynthetic activity, phycobiliproteins and internal nitrogen content. Nevertheless these authors observed beneficial effect of UVR on recovery or photoprotective processes under enriched nitrogen conditions, but not on MAA pattern.

The brown alga *Chondrus crispus* (Phaeophyta) collected from the subtidal zone (6 m depth) increased the concentration of carotenoids with repeated exposures to UV-radiation (Yakovleva and Titlyanov, 2001). Prolonged exposures to high irradiance induced a substantial decline in the potential quantum yield of photosynthesis (F_v/F_m) and progressive pigment destruction responsible for stress damage (Yakovleva and Titlyanov, 2001). Photoinhibition of F_v/F_m exceeded 95% of control. Even after 20h under low radiance, F_v/F_m was similar to values measured immediately after stress, indicating severe photo damage (Yakovleva and Titlyanov, 2001).

Buoyancy

Buoyancy in aquatic photosynthetic organisms is essential in order to maintain them in water trophic layers for photosynthesis, reproduction and survival. In cyanobacteria, buoyancy is provided by gas vesicles that play an important role in regulating vertical distribution and nutrient acquisition (Ma and Gao, 2009). PAR (λ=400-700 nm) drives photosynthesis, but also results in photoinhibition at high levels. On the other hand, reduced levels of UVR might act as cues controlling vertical migration and enhance photosynthetic carbon fixation by phytoplankton (Ma and Gao, 2009). Ma and Gao (2009) studying *Arthrospira (=Spirulina) platensis* (important economic cyanobacterium) observed that floatation activity decreased with increased photosynthetic rates associated with increased photosynthetically active radiation (PAR), but it decreased less in the presence of UVR, which resulted in inhibitory effects (Ma and Gao, 2009). In this study, when the cells were grown under isoenergetic levels of solar PAR or UVR alone, they migrated downward under PAR but maintained buoyant under UVR. The buoyancy regulation of this photosynthetic cyanobacterium depended on the exposed levels of PAR as well as UVR, which affected photosynthesis and growth in an antagonistic way (Ma and Gao, 2009). The authors conclude that the buoyancy of *A. platensis* in water columns is likely to be dependent on diurnal photosynthetic performance regulated by solar radiation, and can hardly be considered as an active strategy to gain more energy during sunrise/sunset or to escape from harmful irradiation during the noon period.

Protein and DNA damage

Specificic studies related to DNA damage effects of UV-B exposure on cyanobacteria, micro and macroalgae were performed by Buma et al. (2001), Fabanđel et al. (2001), Sinha and Häder (2002), Kumar et al. (2004), Häder and Sinha (2005), Helbling et al. (2008), Rastogi et al. (2011) and Chen et al. (2012).

Nucleic acids absorb and are damaged by solar UV (Häder and Sinha, 2005), which attributes adverse effects on living systems (references in Rastogi et al., 2011). The two major UV-induced DNA lesions are directly by the formation of cyclobutane-pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidose photoproducts (6-4PPs, pyrimidine adducts) and their Dewar valence isomers (references in Häder and Sinha, 2005) that can alter the molecular structure of genome leading to chronic mutagenesis and cell death (references in Rastogi et al., 2011). Indirectly effects occur via the production of ROS (references in Rastogi et al., 2011). Oxidative stress, acting synergistically with UVR (Dahms and Lee, 2010) usually results in single- as well as double-strand breaks (DSBs) in the native DNA molecule, causing extensive DNA damage. UV-A waveband, in comparison to UV-B, has poor efficiency in inducing DNA damage because native DNA does not absorb them (Häder and Sinha, 2005). However, UV-A or visible light

photon (up to 670-700 nm) is still able to induce DNA damage either by producing a secondary photoreaction of existing DNA photoproducts or via indirect photosensitization reactions (references in Rastogi et al., 2011).

Methods for detecting DNA damage in algae are briefly described by Häder and Sinha (2005) but new proposals were published after them. UV-induced DNA degradation may be analysed by using radioactive methods (O'Brien et al., 1982 referred by Häder and Sinha, 2005) to determine DNA degradation in terms of a decrease in radioactivity lost from DNA. Freeman et al. (1986) (referred by Häder and Sinha, 2005) proposed a non-radioactive alkaline agarose gel method to determine single-strand breaks in nanogram quantities of DNA. UV-induced cyclobutane dimers can also be identified and quantified by using specific antibodies (Roza et al., 1988, Mitchell et al., 1991, Mori et al., 1991 as referred by Häder and Sinha, 2005). Detection of CPD may also be performed following Li and Waters (1996) method by using oligonucleotides and magnetic beads which label DNA fragments, cut at the dimers and chemical sequencing reference ladders (Häder and Sinha, 2005). Another method employs an endonuclease to cleave the DNA at the CPDs; the resulting fragments are then subjected to gel electrophoresis with subsequent image analysis to determine the length of the fragments and the frequency of CPDs per megabase pairs can then be calculated by a method designed by Quaite et al. (1992) (Häder and Sinha, 2005); Douki et al. (2000) proposed an immune-dot-blot assay technique to detect CPDs, 6-4PPs and their Dewar valence isomers after UV radiation (Häder and Sinha, 2005); Sinha et al. (2001) (as referred by Häder and Sinha, 2005) used a simple and efficient quantitative method to determine the frequency of thymine dimers in a variety of organisms such as cyanobacteria, phytoplankton and macroalgae by using thymine dimer-specific antibodies followed by blotting and chemiluminescence methods. Electrospray-mass spectrometry (Douki et al., 2000a referred by Häder and Sinha, 2005) and high performance liquid chromatography-tandem mass spectrometry (HPLC-MS) was devised to quantify thymine dimers (Douki et al., 2000b, referred by Häder and Sinha, 2005). Fanfanđel et al. (2001) proposed a specific detection of cyclobutane pyrimidine dimers in phytoplankton by a non-radioactive assay based on T_4-endonuclease V digestion; the quantification of CPDs is estimated by alkaline agarose gel electrophoresis. Kumar et al. (2004) proposed a method for detection of DNA damage in cyanobacteria by PCR assay. According to Häder and Sinha (2005) this method may not be sufficient to detect the formation of CPDs, 6-4PPs and their Dewar valence isomers in an organism after UV radiation. Chen et al. (2012) used a fluorometric analysis of DNA unwinding (FADU) as described by He and Häder (2002) and modified by Chen et al. (2009).

Phototoxicity

Direct effect of UVR exposure on biological macromolecules including DNA is called photosensitization, and generally leads to the production of singlet oxygen or other ROS that are highly damaging to biomolecules (Chen et al., 2006 referred by Dahms and Lee, 2010).

Photomodification results in the formation of new compounds that exhibits greater toxicity than the parent phototoxicant (Brack et al., 2003), being an indirect effect of UVR on biomolecules. Photomodification results in photoenhanced toxicity. Polycyclic aromatic hydrocarbons (PAHs), pesticides, herbicides or antifoulings are examples of phototoxic compounds. The toxicity of oil products, weathered oil and specific polycyclic aromatic compounds increases 2 to greater than 1000 times in the presence of UV (references in Barron and Ka'Aihue, 2001). Pesticides (Bhattacharyya, et al., 2011) and herbicides (Chen et al., 2012) are carried out to water bodies (to freshwaters and then to marine system) through run-off, drift and leaching increasing the risk of exposure in non-target organisms in which, under UV exposure, another photoenhanced toxicity may occur, disrupting the dynamics of the ecosystems. The combination of Tributil-tin (TBT) and UV-B radiation stresses also have synergistic effects affecting the first trophic level of the marine food web (Sargian et al., 2005).

Nutrients uptake

The cycling of key elements like carbon (C), nitrogen (N) and phosphorous (P) in aquatic systems depends to a large extent on productivity and fate of autotrophs. Several works demonstrated an inverse effect of UV radiation and PAR with regard to elemental ratios, notably C:P. Uptake rates of ^{15}N-ammonium of algae is affected by UV-A of high intensity and UV-B radiation. The results also show a significant reduction in total nitrate by 95.5% in the high UV-B treatment (Döhler and Buchmann, 1995; Braune and Döhler, 1996; Anusha and Asaeda, 2008). The recovery of photosynthetic activity and phycobiliproteins, was

enhanced in the algae previously incubated under PAR + UVR as compared to exposure to only PAR, suggesting a beneficial effect of UVR on recovery or photoprotective processes under enriched nitrogen conditions (Huovinen et al., 2006).

Significant increase in dissolved ammonia in water under UV-B exposure, due to photoxidation and bacterial decomposition of organic nitrogen in the system, alter the natural balance of nitrogen, oxygen and dissolved carbon in aquatic systems.

Nutritional quality

Polyunsaturated fatty acids (PUFAs) play a key role in aquatic food webs because only photosynthetic organisms synthesize them and they are essential macromolecules for heterotrophs. PUFAs are also of major importance in regulating membrane fluidity under low temperatures. Several studies have documented a negative impact of UV radiation (280-320 nm) on PUFAs (polyunsaturated fatty acids) in marine phytoplankton species: this impact has been attributed either to oxidation of previously synthesized fatty acids or to disruption of their synthesis (references in Leu et al., 2006).

Temperature is a crucial parameter, since it may have a substantial impact on fatty acid composition itself as well as on the dynamics of repair mechanisms. An initial increase in PAR intensities profoundly affected the fatty acid composition and substantially inhibited the synthesis of PUFAs, but the relative amounts of PUFAs were not reduced by UV radiation in the diatom *Thalassiossira antarctica* var. *borealis* (Leu et al., 2006). Enhanced UV radiation did cause a significant reduction in optimum quantum yield of PSII and affected some fatty acids, mainly 18:0 and 16:1 n-7. Both ambient and enhanced UV radiation caused significantly lower C:P and N:P ratios. A higher relative content of the photoprotective pigments diadinoxanthin and diatoxanthin was observed. The diatom *T. antarctica* var. *borealis* showed that brief periods with high light exposure may cause significant changes in photosynthetic activity and food quality, but the capacity for photo-acclimation seems high. The impact of UV radiation seems to be less important for food quality than that of PAR during a sudden rise in total light intensity.

Indirect UV-radiation harmful effects

Few studies are deal with indirect harmful effects of UVB. The first experimental evidence of indirect UVB effects on reproductive output through trophic response in marine plankton conducted by Kouwenberg and Lantoine (2007). In this experiment both control and UVB-stressed of a common marine diatom *Skeletonema costatum* cultures were used as food for wild pelagic copepod *Calanus helgolandicus* females collected in the NW Mediterranean. This study showed that female copepods fed on control diatoms produced three times more eggs and healthier offspring with fewer lethal naupliar deformities than those fed on UVB-exposed diatoms.

Conclusions

Photosynthetic organisms support life on Earth, and aquatic biophotosystems contribute with 50% of the global oxygen supply of all life.

A study of the effects of UV radiation is complex because the organisms face different stressors, making it difficult to identify the real magnitude of the harmful effects of ultraviolet radiation in wild communities and ecosystems.

Species with low capacity of living under UV irradiation due to their repair unability tend to disappear, unbalancing the ecosystem and reducing biodiversity.

Numerous information is available about UVR photobiology, particularly since the awareness of ozone depletion. Long term consequences of UVR exposure on organisms and its consequences in the ecosystems balance are still uncertain. High ROS formation rates are particularly important especially for organisms with early life stages in the plankton from surface waters dwelling at certain environmental conditions (cloudless sky, thin ozone layer, lack of wind, calm seas, low nutrient loading).

Ecological significance of elevated UV-B exposure in the aquatic environment may be seriously underestimated if effects on the early lifestages of algae are not considered.

Synergisms among stressors are shown to be increasingly important in the face of global environmental change and must consider both, the effects of UV-B on a sinlge species and its effects on entire communities and systems (Dahms et al., 2011).

Increasing growth rates in species resistant to UV exposure, like the forementioned raphidophyte microalga (*Cattonella* sp), which is known to cause fish mortality in Japanese waters and was also implicated in mortality of farmed finfish in South Australia, may also have important economic negative impacts on aquaculture industry

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REVIEW ARTICLE

Algae and aquatic macrophytes responses to cope to ultraviolet radiation – a Review

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Abstract

UV radiation became an important issue since the awareness of the ozone hole in Antarctida and its relationship between the human activity, the depletion of the protecting layer, and the effects of ultraviolet radiation in the biological relevant wavebands on algae and on organisms in general. All aquatic organisms are depended on algae and aquatic plants (submerged or near shallow line) for food, shelter, also as oxygen supplement and CO2 sequestration by photosynthetic procedure. So, a disturbance in this trophic layer creates a global unbalancing. Harmful effects of UV, especially UV-B were intensibly studied under laboratory and field studies, and reported in scientific reports from a large team of scientists. UV- induced repair mechanisms allowing the survival of certain species under UV irradiation is also largely documented in algae species, and in phytoplankton of the entire aquatic systems (freshwater, marine and brackiswater). This study provides an overview of the available literature on the ultraviolet-B (UV-B – λ=280-315 nm) and UV-A radiation (λ=315-400 nm) concerning the strategies of protection developed by aquatic photoauthotrophs (micro and macroalgae, and aquatic macrophytes, like seagrasses and liverworts) to fit under these wavebands of radiation. It includes studies on prokariotic cyanobacteria, haptophytes, diatoms, dinoflagellates, red algae, brown algae and chlorophytes from freshwater (ponds, lakes) to marine littoral and Open Ocean. It also reports available studies concerning marine and freshwater plants exposed to UV irradiation.

Key words: Algae, cyanobacteria, MAAs, Macrophytes, UV-radiation

Abbreviations: APX - ascorbate-peroxidase; CAT – catalase; CCs – chlorocarbons; CFCs- chlorofluorocarbons; CPDs - cyclobutane pyrimidine dimers; DAD – diode array detection; DHAR - dehydroascorbate reductase; GR - glutathione reductase; HPLC/MS - high-resolution reverse-phase liquid chromatography and mass spectrometry; hsp70 - Heat shock protein; HSPs - Heat shock proteins; Huv - high dosage of UV-B irradiation; Luv - low dosage of UV-B irradiation; M-xxx – chemical structure not identified of mycosporine-like amino acids detected at xxx nm; MAAs - mycosporine-like amino acids; MCF - methyl chloroform; MD-HAR - monodehydroascorbate reductase; MDHA – monodehydroascorbate; Muv - medium dosage of UV-B irradiation; NAC - N-acetylcysteine; NER - nucleotid excision repair; NOx – dioxins; OBS – organobromides; P334 - Porphyra-334; PER - photoenzymatic repair; PFD - photon flux densities; POX – peroxidase; PUFAs - polyunsaturated fatty acids; ROS - reactive oxygene species; SH – shinorine; SOD - superoxide dismutase; TBARS - thiobarbituric acid reacting substance; UV-A - ultraviolet-A; UV-B - ultraviolet-B; UV-C - ultraviolet-C; UVR – ultraviolet radiation.

Introduction

High increase industrialization in the past few decades resulted to an increase in anthropogenically atmospheric pollutants such as chlorofluorocarbons (CFCs), halocarbons, chlorocarbons (CCs), organobromides (OBS), carbon dioxide (CO_2), methyl chloroform (MCF) and dioxins (NO_x) is being related to the depletion of the UV-screening ozone layer in the stratosphere (references in Singh et al., 2010b).

With the shallowing ozone layer, UV radiation is increasing not only in Antarctida zone but also all over the Earth' surface, penetrating into water in depht according to multiple factors.

Algae and photosynthetic macrophytes are the support of entire life because all aquatic organisms are dependentd on their production for food, shelter, also as oxygen supplement and CO_2

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sequestration by photosynthetic procedure and as regulators of pH.

Sun radiation is crucial for photoautotrophs and is composed by UV radiation (λ_{max} = 200 to 400 nm), visible radiation (λ_{max} = 400 to 750 nm) and infrared radiation (λ_{max} >750 nm). UVR is usually divided into three spectral regions: UV-C (λmax = 200 to 280 nm), UV-B (λmax = 280 to 315 nm) and UV-A (λmax = 315 to 400 nm). Studies related with the effects of UV radiation, usually concern wavebands from 280 to 400 nm (UV-A+UV-B), compared with PAR. PAR is an abbreviation of photosynthetic active radiation, which is the spectral range of solar radiation from 400 to 700 nanometres that allows photosynthesis process by photosynthetical organisms.

The harmful effects of UV radiation on biota from marine, freshwater and terrestrial habitats are extensibly documented. Reducing primary productivity, plankton composition (Davidson et al., 1996) and denitrification inhibition (Mancinelli and White, 2000) are among deleterious effects of UV in aquatic communities. UV radiation in algae (including cyanobacteria) inhibits growth and development (Gao and Ma, 2008), biomass, productivity, photosynthesis, buoyancy (Gao and Ma, 2008; Helbling et al., 2008; Sampath-Wiley et al., 2008; Zeeshan and Prasad, 2009; Dahms and Lee, 2010; Dahms et al., 2011). As it is a stress mechanism, usually a series of reactive oxygene species (ROS) is formed that in part mediate DNA damage, mutagenesis, cellular aging, carcinogenesis and apoptosis (Downs et al., 2002; He and Häder, 2002; Häder and Sinha, 2005; Dahms and Lee, 2010; Rastogi et al., 2011).

Effects of short-wavelength solar radiation in the UVrange (λmax = 280 to 400 nm) include DNA by-products, being the most significant cyclobutane pyrimidine dimers (CPDs), which comprise 70-90% of all aberrant DNA photo-products. CPDs increase linearly with UV-B exposure, however the dose-relationship varies significantly between taxa (references in Dahms and Lee, 2010).

The effects of UV radiation on organisms in natural conditions are complex because synergy is involved on deleterious and recovering mechanisms to face UV irradiation. The susceptibility to elevated UV-B radiation is dictated by a complex interplay between protection, repair and other factors that may lead to highly variable UV-B susceptibility among the species (Zeeshan and Prasad, 2009).

Aquatic systems with high transparency of oligotrophic waters (marine and freshwaters) are exposed to the highest levels of ultraviolet radiation. Intertidal and epipelagic marine living forms also face the same situation especially those that can't move away in high light periods, like benthic macroalgae, seagrasses and other macrophytes. UV irradiation in lakes can affect photosynthesis of plankton organisms down to a depth of 10-15 m (Holzinger and Lütz, 2006). In marine waters, UV-B can penetrate down to a water depth of 20-30 m (Smith et al., 1992 referred by Dahms and Lee, 2010) and in clear Antarctic Ocean may reach to depths to 70 m (reference in Short and Neckles, 1999). In clear Antarctic oceanic waters UV-A can penetrate to a depth of between 40 and 60 m (Ban et al., 2007 referred by Dahms and Lee, 2010), depending, among others, on the incidence of solar radiation, transparency of waters and wind mixed layer effects.

Tidal exposure also imposes considerable environmental stress on intertidal seaweeds such as elevated irradiance levels, temperature changes and desiccation, especially in spring low tides, which occur every month during new and full moon phases. Typically, seaweeds sensitive or intolerant to ambient stresses inhabit the lowermost intertidal zone (where emersion at low tide is brief and/or absent), while those found at higher elevations usually possess heightened tolerance to environmental fluctuations (Sampath-Wiley et al., 2008). Since UV radiation (UVR) daily doses in the intertidal system are much higher than in the sublittoral zone, there is a relashionship between UV radiation tolerance and vertical distribution of intertidal macroalgae (Altamirano et al., 2003).

The wavebands of UV radiation (UV-C, UV-B and UV-A) act differently on algae. Their modes of action are also different in other organisms, but they will not be referred here. Short UV-B wavelengths result in a higher degree of DNA damage, higher levels of oxidative stress, and greater expression of cell cycle genes, all of which promote apoptosis, than exposure to UV-A (reference in Dahms and Lee, 2010) because longer UV-A wavebands are closer to PAR.

UV-A generally causes indirect DNA damage by the formation of chemical intermediates such as oxygen and hydroxyl radicals that interact with DNA to form strand breaks, DNA-protein cross-links and alkali labile sites (reference in Dahms and Lee, 2010). On the other hand, UV-B causes direct DNA damage by inducing the formation of cyclobutane pyrimidine dimers (CPDs) and pyrimidine-pyrimidone (6-4) photoproducts (Dahms and Lee, 2010). Moderate levels of UV-A may stimulate photosynthesis and growth in both micro and macroalgae (references in Xu and Gao,

2010). UV-C is the most damaging portion of the spectrum (Banaszak and Trench, 2001) but it is not of biological relevance because it is totally absorbed by the atmosphere (Banaszak and Trench, 2001; Holzinger and Lütz, 2006; Basti et al., 2009).

There are several important reviews on various aspects of UV radiation effects on aquatic ecosystems: aquatic ecosystems in general (Häder et al., 1998; Häder, 2000; Sinha and Häder, 2002a; Hood et al., 2006); marine plankton (Davidson, 1998); marine organisms in Antarctic region (Karentz and Bosch, 2001); algae (Holzinger and Lütz, 2006); plant cells (Kovács and Keresztes, 2002); spore germination in algae (Agrawal, 2009); cyanobacteria (Sinha and Häder, 2008; Singh et al., 2010a,b); cyanobacteria, phytoplankton and macroalgae (Sinha et al., 1998); cryptogams - cyanobacteria, algae, lichens, mosses, liverworts, pteridophytes and fungi - (Björn, 2007); macroalgae (Poll, 2003 referred by Björn, 2007); rhodophytes (Talarico and Maranzana, 2000); freshwater rhodophytes (Necchi Jr, 2005); seagrasses (Short and Neckles, 1999); corals and coral bleaching (Baker et al., 2008; Tambutté et al., 2011); molecular effects and responses (Jenkins et al., 1995; Glatz et al., 1999; Dahms and Lee, 2010; Dahms et al., 2011); ultraviolet sunscreens in dinoflagellates (Banaszak and Trench, 2001); the role of mycosporine-like amino acids in marine biota (Klisch and Häder, 2008; Pallela et al., 2010; Carreto and Carignan, 2011); methods for DNA damage detection (Sinha and Häder, 2002b); genetics (Xiong et al., 2009); cyanotoxin nodularin production (Pattanaik et al., 2010); lipids and lipid metabolism (Guschima and Harwood, 2006); lake acidification and UV penetration (Williamson, 1995, 1996 as referred by Häder et al., 1998); carbon flux and ecosystem feedback (Wassmann et al., 2008) and ecological and environmental impact (Häder and Sinha, 2005; Carreto and Carignan, 2011). The present review concerns on the main mechanisms of protection to survive under UV irradiation, updating previous reviews.

Mechanisms of protection against UV radiation

The young Earth, about $3.8x10^9$ years ago, received very high doses of UV-radiation. It is estimated that, at the time, the sun, like young T-Tauristars, emitted about 10 000 times more UV than at present (Canuto et al., 1982) referred by Rozema et al. (1997). The luminosity of the sun then was much lower than at present, resulting in temperatures below freezing (Rozema et al., 1997). Nevertheless, liquid water did occur, caused by atmospheric carbon dioxide (CO_2) levels 100 -1000 times higher than present values, which absorbed infrared radiation and created a pronounced greenhouse effect (Canuto et al., 1982, referred by Rozema et al., 1997). Release of O_2 by photosynthetic bacteria, cyanobacteria and eukaryotic algae led to a gradual increase of atmospheric O_2 and a concomitant decrease of atmospheric CO_2 (Rozema et al., 1997).

Cyanobacteria are primitive photosynthetic oxygen-evolving prokaryotes that appeared on the Earth when there was no ozone layer to protect them from damaging ultraviolet radiation (UVR). Cyanobacteria are the only known oxygenic phototrophs capable of fixing atmospheric nitrogen (reference in Giordanino et al., 2011) and probably those who have developed the more suitable mechanisms to avoid or minimize UVR stress.

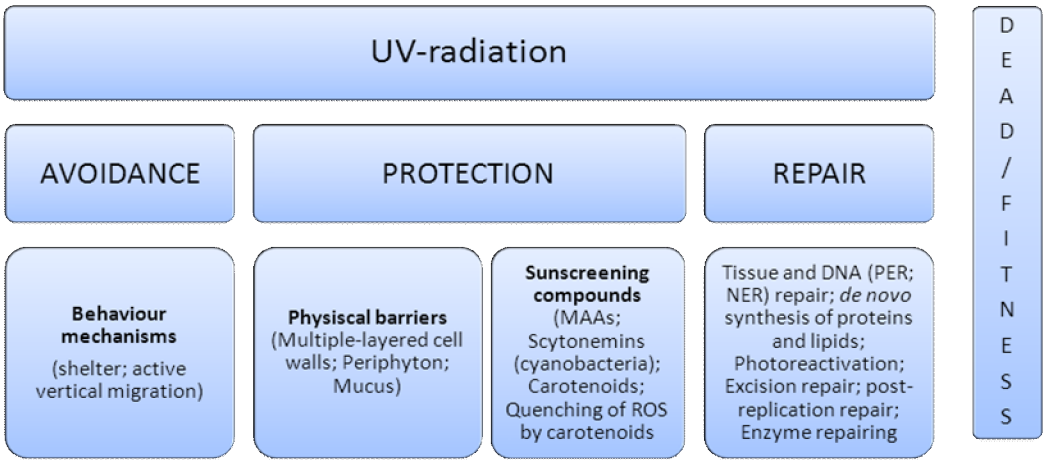

Figure 1. Mechanisms evolved by algae to cope with UV-radiation.
(based on Dahms and Lee, 2010; Dahms et al., 2011).

There are basically three different ways through which organisms have evolved to cope with UVR (Figure 1): avoiding it, protecting themselves, and repairing potential damage (reference in Dahms et al., 2011). Invertebrates avoid UVR by using sheltered and/ or deeper habitats (Dahms et al., 2011) and microalgae use self-mobility. Some develop protective coatings or make use of sunscreens while others repair damage occurred by UVR (Dahms et al., 2011). Often, these natural mechanisms are triggered by visible light intensities where they might not protect against an increase in the ratio of UVR to visible light.

Living organisms exposed to high natural UV radiation have developed a suite of mechanisms to avoid or minimize UVR stress which include vertical migration, multiple-layered cell walls, or synthesis of protecting compounds such as carotenoids, mycosporine-like amino acids (MAAs), scytonemines (only cyanobacteria), proteins and some repairing enzymes.

Behaviour mechanisms

Free living microalgae such as dinoflagellates (Banaszak and Trench, 2001) and *Euglena gracilis* (Bolige and Goto, 2007) employ positive phototaxis at low radiances and a very accurate negative gravitaxis to swim towards the surface in order to receive a sufficient amount of solar radiation for photosynthesis procedure. However, during times of excessive radiation in order to reduce damage by surface UVR and PAR, light-mediated behaviour may influence the distribution of some cellular organisms to greater depths within the water column (Banaszak and Trench, 2001), by moving down into the water column guided by negative phototaxis.

Studies of Marshall and Newman (2002) with *Chattonella marina* (a marine fitoplankton – Radiophyceae – suggests that Japanese strain (not suitable for high UV exposure) may need to vertically migrate in the turbid waters to avoid UV exposure. *C. marina* is a subsurface bloom forming, highly motile flagellate, capable of active vertical migration. The cyanobacteria *Phormidium uncinatum*, *Anabaena variabilis* and *Oscillatoria tenuis* also migrate from the water surface to lower levels in order to avoid high solar irradiance (Donkor and Häder, 1995).

Vertical mixing may aid in recovery from photo damage by transporting phytoplankton away from high UVR toward low PFD (photon flux densities) where repair processes can proceed without incurring further damage. This movement down reduces productivity as a result of being deeper in water column versus repairing damage to the photosynthetic apparatus caused by UVR or high PFD (Banaszak and Trench, 2001). However, Hernando and Ferreyra (2005), exposing cells of a bloom forming diatom *Thalassiossira* sp., to variable light conditions, during one of the field experiments when ozone was low, observed a significant reduction in photosynthesis, suggesting that vertical mixing may not be efficient enough to prevent harmful UV-B radiation effects.

Physical mechanisms (barriers)

Multiple-layered cell walls and mucilaginous sheath layer

When exposed to artificial UVR some dinoflagellates (Banaszak and Trench, 2001) may produce a physical or chemical barrier to offset the deleterious effects of this radiation. The symbiotic dinoflagellate *Symbiodinium californium* (Banaszak and Trench, 2001) develops a multiple-layered cell walls and this phenomenon disappears after the cells were returned to culture conditions in the absence of UVR (Banaszak and Trench, 1995).

Mandal et al. (2011) showed that the presence of thick mucilaginous sheath layer was among the adaptation mechanisms that allowed the intertidal cyanobacteria *Lyngbya majuscula* to withstand prolonged UV-B radiation.

Periphyton

Periphyton (algae, bacteria, mucus, sediment particles, etc) is considered detrimental to inshore seagrasses (e.g. *Zostera marina, Ruppia marina*) as it reduces the amount of light, i.e. photosynthetically available radiation (PAR), that reaches the plant surface (Brandt and Koch, 2003).

Seagrasses, such as *Zostera* and *Ruppia* spp. are a functional group of approximately 60 species of underwater marine vascular plants (Lee, 2007). They constitute habitat for a great number of animal species like fish and shellfish and they are also important nursery areas. Filtering coastal waters, dissipating wave energy and anchorage of sediments are among the important physical functions of seagrasses.

The ecological importance of periphyton on seagrass leaves has been listed as: primary producer in seagrass systems; source of food and sediment particles (calcareous algae); environmental indicator of water quality (Borowitzka and Lethbridge, 1989 referred by Brandt and Koch, 2003); UV-B filter (Brandt and Koch, 2003).

Algae as well as detritus are contributing to the reduction of light transmittance through the periphyton layer (Brandt and Koch, 2003). The strong absorption (reduced transmittance) in

wavelengths characteristic of chlorophyll *a* (430 and 663 nm) and carotenoids (401-518 nm) suggest that photosynthetic organisms are contributing to light attenuation.

Periphyton accumulation on seagrasses leaves may provide an effective UV-B filter, a factor that may be especially important in tropical marine oligotrophic waters in which UV penetrates relatively deep into the water column (Brandt and Koch, 2003). The higher transmission in the PAR than in the UV-B range allows the seagrasses to receive a higher proportion of beneficial light while reducing the detrimental radiation (Brandt and Koch, 2003). According to authors, this beneficial effect of periphyton as a UV-B filter is lost when PAR transmission reaches levels that strongly limit photosynthesis.

Production of photoprotective compounds

Sunscreening compounds protect the organism against UVR damage. Such compounds are mycosporine-like amino acids (MAAs), carotenoids, and antioxidants.

Usually UV-absorbing compounds and carotenoids increase in response to exposures with UVR, as it happens to the diatom *Skeletonema costatum* (Wu et al., 2009).

Mycosporine-like amino acids (MAAs)

MAAs are ultraviolet-absorbing molecules having absorption maxima between 320-360 nm (reference in Carreto and Carignan, 2011). They are small (<400 DA), colorless, water-soluble compounds (Sinha et al., 2007), being imine derivatives of mycosporines, which contain an amino-cyclohexenimine ring linked to an amino acid, amino alcohol or amino group (reference in Carreto and Carignan, 2011). Mycosporine-glycine and mycosporine-taurine are the only known aminocyclohexenones from marine sources (Carreto and Carignan, 2011).

Recent reports indicate that MAAs are widely distributed in marine, freshwater and terrestrial organisms taxonomically diverse (Bandaranayake, 1998), including sea anemones (Banaszak and Trench, 1995; Shick and Dunlap, 2002; Arbeloa et al., 2010), and, as referred by Carreto and Carignan (2011) they have been reported in gorgonians, corals, sponges, brine shrimp, sea urchins, starfish, holothurids, clams, ascidians and fish. MAAs may be transmitted to grazing organisms (e.g. Carefoot et al., 2000) by predatory habits from algae, or by symbiotic (corals, gelyfish) or bacterial association. The organisms have evolved the capacity to synthesize, accumulate and metabolize a variety of mycosporine-like amino acids.

Table 1 summarizes MAAs isolated by several scientific works from algae with maximum absorption ranging from 265 to 362 nm, some of them detected or partially characterized or are unknown MAAs, since no molecular formula was able to be identified. Its detection is a consequence of the development of more efficient high-resolution reverse-phase liquid chromatography and mass spectrometry (HPLC-MS) techniques. Pallela et al. (2010) and Carreto and Carignan (2011) are two examples of recent reviews concerning photoprotecting compounds in marine organisms. Pallela et al. (2010) review photoprotective compounds from algae and other marine sources for further elaborative research and their probable use in cosmeceutical and pharmaceutical industries. Carreto and Carignan (2011) is also a very usefull review since the authors describe the structure and physicochemical properties of MAAs and the modern methods used for their isolation and identification in marine biota.

A database on UV-absorbing mycosporines and mycosporine-like amino acids (MAAs) has been constructed and described by Sinha et al. (2007) providing information on various mycosporines and MAAs reported in fungi, cyanobacteria, macroalgae, phytoplankton and animals from aquatic and terrestrial habitats. It also contains information on biosynthetic routes of MAAs as well as on the absorption maxima and molecular structures of different mycosporines and MAAs, and according to authors can be found on http://www.biologie.uni-erlangen.de/botanik1/html/eng/ maa_database.htm.

MAAs have been implicated in many biochemical processes (Shick and Dunlap, 2002). Experimental evidence indicates that the major role of MAAs is to act as photo-protective UV filters and/or to act as antioxidants (Dunlap et al., 1986; Carreto et al., 1990; Dunlap and Shick, 1998; Shick and Dunlap, 2002; Carreto and Carignan, 2011). In addition, oxocarbonil-MAAs such as mycosporine-glycine (Dunlap and Yamamoto, 1995; Yakovleva and Hidaka, 2004) and mycosporine-taurine (Zhang et al., 2007) have antioxidant properties, capable of protecting against the cellular damage that high levels of reactive oxygen species (ROS) induced in organisms under different stresses (Carreto and Carignan, 2011). High concentrations of MAAs have been found in selected dinoflagellates, prymnesiophytes, cryptomonads, antarctic and artic diatoms, raphidophytes and macroalgae, especially among surface bloom forming species or bentic

macroalga exposed in intertidal shores and in dinoflagellates in symbiosis with coral communities. *In vitro* studies of various MAAs have also given support to this function by confirming the high photostability and also the release of heat to the medium as the main relaxation pathway of the photoexcited molecules (Conde et al., 2004, 2007; Carreto and Carignan, 2011).

Beyond their UV-screening properties, MAAs are described to have more characteristics, as described by Carreto and Carignan (2011), even though some of them are controversial or unsupported: they may contribute to osmotic regulation; they may act as regulatory metabolites of sporulation and germination; they may act as transducers of UV wavelengths to wavelengths utilizable for photosynthesis; they may act as "host factors", that induce release of photosynthate from endosymbiotic algae; they may play a role under desiccation or thermal stress in certain organisms; they can also act as an intracellular nitrogen reservoir; MAAs and pyrimidines may function as alarm cues in the defence secretions of the sea hare *Aplysia californica*. The discovery that MAAs can be chemical signals raises an entirely new direction for exploring their potential functions and evolution (reference in Carreto and Carignan, 2011).

MAAs production, as suggested by Marshal and Newman (2002), seems to be related to an ecophenotypic adaptation due to differing environmental conditions. The marine phytoplankton *Chattonella marina* collected from Australian and Japan exhibit differences in tolerance to high intensities of visible light: Australian strain (with high natural UV exposure) of *C. marina* produced around five times more UV-absorbing MAAs than the Japanese strain. Japanese strain was more vulnerable to UV-induced cell damage, inhibition of photosynthesis and growth, which may lead to higher cell death than in Australian strain.

Table 1. MAAs isolated from algae (macro and microalgae), including cyanobacteria, and each absorption maximum waveband.

MAA	Waveband (λ_{max}) (nm)	Reference
Unknown UV-absorbing compound	265	Xu and Gao (2010)
Unknown UV-absorbing compound	280	Guan and Gao (2010)
Mycosporine-glycine	308	Carignan et al. (2009)
Unkown MAA	310	Klisch et al. (2001)
Palytine	319	Carignan et al. (2009)
Palythine	320	Klisch et al. (2001); Carreto and Carignan (2011)
Palythine-serine	320	Carignan et al. (2009); Carreto and Carignan (2011)
Palythine-threonine	320	Carignan et al. (2009); Carreto and Carignan (2011)
Palythinol	320	Carreto and Carignan (2011)
Palythine-threonine sulphate	321	Carreto and Carignan (2011)
Palythine-serine sulphate	321	Carreto and Carignan (2011)
Unknown MAA	324	Gröniger and Häder (2002)
Mycosporine-methylamine-serine	325	Carreto and Carignan (2011)
Mycosporine-methylamine-threonie	330	Carreto and Carignan (2011)
Mycosporine-glutamic acid-glycine	330	Carreto and Carignan (2011)
Asterina-330	330	Kräbs et al. (2002); Carreto and Carignan (2011)
Unknown MAA	331	Klisch et al. (2001)
Unkown MAA	332	Gómez et al. (1998)
Palythinol	332	Kräbs et al. (2002)
Mycosporine-2-Glycine	332	Carreto and Carignan (2011)
Shinorine	333	Carignan et al. (2009); Carreto and Carignan (2011)
Shinorine	334	Klisch et al. (2001)
Porphyra-334	334	Klisch et al. (2001); Carreto and Carignan (2011)
(*E*)-palythenic acid	335	Carreto and Carignan (2011)
Mycosporine-glycine-valine	335	Carreto and Carignan (2011)
(*Z*)-palythenic acid	337	Carreto and Carignan (2011)
Unknown MAA	348	Gómez et al. (1998)
Usujirene	357	Carreto and Carignan (2011)
Palythene	360	Kräbs et al. (2002); Carreto and Carignan (2011)
Euhalothece	362	Carreto and Carignan (2011)

Recent studies reported by Coba et al. (2009) using Porphyra-334 + shinorine (P334+SH) isolated from the red alga *P. rosengurttii* showed that the topical application of P-334 + SH on the skin of the female albino hairless mice had a protective effect against UV-induced skin damage in mice and contributed to maintain the antioxidant defence system of the skin as well as expression of heat shock proteins *hsp70*, being a potential candidate for new natural sunscreens commercialization.

MAAs detection and quantification techniques:

HPLC followed by DAD identification and quantification is the commonly method used for MAA detection and quantification. A series of known masses of pure MAA standards are injected, and the resultant chromatographic peak areas are related to injected masses to yield a response factor for each MAA. The masses injected of each compound could be quantified using their specific extinction coeficients and the dilution factor but the extinction coefficient of some MAAs are not known yet (reference in Carreto and Carignan, 2011). In this case, the use of the extinction coefficient for the MAA that has the closest match in wavelength maxima, may aid in yielding a useful concentration estimate (reference in Carreto and Carignan, 2011).

Distribution of MAAs in cyanobacteria, algae and seagrasses

In most cyanobacteria able to synthesize MAAs these compounds consist of shinorine, porphyra-334 (Sinha et al., 1999, 2001, 2002, 2003; Wulff et al., 2007) and in some cases mycosporine-glycine (references in Carreto and Carignan, 2011). Sinha et al. (1999, 2001, 2002) reported that the cyanobacteria *Anabaena* sp. and *Nostoc comune* only synthesize the MAA shinorine. Unidentified MAAs were also detected in other cyanobacteria: M-315 in *Scytonema* sp (Sinha et al., 2001) and M-333 in *Nodularia spumigena* (Wulff et al., 2007). Details of the pathway and the enzymes involved in the biotransformation of primary MAAs in cyanobacteria remain to be elucidated (Carreto and Carignan, 2011).

Dinoflagellates were the earliest group in marine phytoplankton noting UV absorbance (Carreto and Carignan, 2011). *Amphidinium carterae* and *Heterocapsa triquetra* are two dinoflagellates producing only one MAA: mycosporine-glycine (Hannach and Sigleo (1998) for the first and M-335 (Wängberg et al., 1997) for the second. In *Alexandrium tamarense* Callone et al. (2006) identified eleven MAAs, being porphyra-334 and palythene the major compounds and in lower concentrations shinorine, mycosporine-glycine, palythenic acid, usujirene, palythine, palythinol, shinorine-methyl ester (M-333) and two unknown MAAs, M-320 and M335/360. MAAs in *Alexandrium escavatum* were porphyra-334, palythene, shinorine and usujirene, the last one characterisitic of surface waters phytoplankton species. *Gloeodinium viscum* contained porphyra-334, palythene and mycosporine-glycine (Banaszak et al., 2000). In *Gymnodinium linucheae* was identified mycosporine-glycine, shinorine and porphyra-334. *Gyrodinium dorsum* contained shinorine, porphyra-334, palythine, and also two more unidentified-MAAs (M-310 and M-331) (Klisch et al., 2001). In the dinoflagellate *Lingulodinium polyedra* porphyra-334, mycosporine-glycine-valine, palythine, palythinol and palythene were found (reference in Sinha et al., 1998). *Prorocentrum minimum* – a red tide dinoflagellate – synthesized shinorine and palythene (Sinha et al., 1998). *Prorocentrum micans* contained mycosporine-glycine, shinorine, porphyra-334 and asterina-330 (reference in Sinha et al., 1998). *Simbiodinium* MAA producing species contained the following MAAs, differing according species: *Simbiodinium* sp, mycosporine-glycine, shinorine, porphyra-334 and palythine (Banaszak et al., 2006); *Simbiodinium corculorum* contained shinorine, porphyra-334 and mycosporine-glycine (Banaszak et al., 2000); in *Simbiodinium microadriaticum* shinorine and porphyra-334 were detected (Banaszak and Trench, 1995), *Simbiodinium meandrinae* produced mycosporine-glycine and shinorine (Banaszak et al., 2000); in *Simbiodinium pilosum* Banaszak et al. (2000) mycosporine-glycine, shinorine and porphyra-334 were identified.

In Haptophyta (Primnesiophyceae) microalgae, Sinha et al. (1998) detected unidentified MAAs in *Phaeocystis pouchetti*. In the coccolithophor *Emiliania huxleyi* a new MAA was detected at 280 nm, but its chemical structure was not identified (Guan and Gao, 2010). The Raphydophyceae *Chattonella marina* was able to produce mycosporine-glycine, mycosporine-glycine-valine and shinorine (Marshall and Newman, 2002) after UV exposure.

The synthesis of MAAs was not part of the UVB response in several studied diatoms (references in Carreto and Carignan, 2011). In *Thalassiosira* sp. shinorine and porphyra-334 were found (Sinha et al., 1998) and other studies report

the absence of MAAs (Carreto and Carignan, 2011). Zudaire and Roy (2001) reported the synthesis of MAAs in *Thalassiosira weissflogii*. MAA signature for most diatoms consists on primary MAAs (porphyra-334, shinorine and mycosporine-2 glycine) (Carreto and Carignan, 2011). Nevertheless in *Corethron criophilum* the presence of the secondary MAAs palythine and palythene was reported (reference in Carreto and Carignan, 2011). *Pseudo-nitzschia multiseries* showed the presence of the unusual mycosporine-taurine, reported only in sea anemones (Carreto and Carignan, 2011).

In Ochrophyta microalgae, *Acetabularia mediterranea* synthesize shinorine, porphyra-334 and palythine (Sinha et al., 1998), and a reference from Sinha et al. (1998) considered asterina-330 and palythine as the only MAA produced by *Heterococcus brevicelullaris* and *Pseudococcomyxa* sp., respectively.

In microalgae chlorophyta, Xiong et al. (1999) registered *Coelastrum microsporum* as no MAA synthesizer and identified mycosporine-glycine, palythine, asterina-330, shinorine and porphyra-334 in *Ankistrodesmus spir, Chlorella minutissima, Enallax coelastroides, Pseudococcomyxa sp*. and in *Scotiella chlorelloidea. Chlorella sorokiniana* and *Scenedesmus* sp. showed M-302, M-292 as addicional MAAs (Xiong et al., 1999). Sinha et al (1998) detected shinorine only in *Enallax coelastroides, Scenedesmus* sp. and *Scotiella chlorelloidea.*

Macroalgae

Marine macroalgae are divided into three groups: green seaweeeds (Chlorophyta), red seaweeds (Rhodophyta) and brown seaweeds (Phaeophyta).

Most of the MAA-producing macroalgae belong to Rhodophyta, followed by Phaeophyta and only a few macroscopic green algae produce MAAs (Carreto and Carignan, 2011).

According to Hoyer et al. (2002), rhodophytes can be divided into three different physiological groups related to MAAs synthesis: a) species withough any traces of MAAs; b) species that contain MAAs in variable concentration dependent of environmental conditions; c) species always containing a stable and high concentration of MAAs.

Shinorine and porphyra-334 are the most common MAAs reported in macroalgae in species (tables 2, 3 and 4) collected from tropical to polar regions (references in Carreto and Carignan, 2011), but the MAA compositions of some intertidal red macroalgae may be more complex. Total MAA levels in *Palmaria palmata* (Rhodophyta) samples from shallow waters (1.5 m depths) were greater than those from deeper waters (3 m depths) (reference in Yuan et al., 2009). The same species, *Palmaria decipiens*, from references in Carreto and Carignan (2011) have more MAA compounds than *P. decipiens* analysed by a reference in Sinha et al. (1998), showing the previous more asterina-330, usujirene and the unusual M335/360. M335/360 was also identified by Callone et al. (2006) in the dinoflagellate *Alexandrium tamarense* as already forementioned.

Schmith et al. (2012) found in the intertidal rhodophyta *Hypnea musciformis* a production of MAAs and carotenoids when this alga was exposed to high levels of UVR. The phenolic compounds are also involved in protecting talus of this alga species against direct exposure to solar light radiation, especially UVR (Schmith et al., 2012). Pavia et al. (1997) had reported the same in the brown alga *Ascophyllum nodosum.*

Phaeophyta (Table 3) show shinorine and porphyra-334 as only MAA produced, and in *Fucus spiralis* only shinorine was present (Sinha et al. 1998).

Most marine macroscopic green algae investigated lack MAAs (references in Carreto and Carignan, 2011). Table 4 summarizes MAA composition in Chlorophyta macroalgae. Mycosporine-glycine and porphyra-334 are two MAA present in *Boodlea composita* and *Caulerpa racemosa* (reference in Carreto and Carignan, 2011) and also found in *Ulva lactuca* (sea lettuce) (Carefoot et al., 2000) with addicional shinorine and palythine. Gómez (1998) and coworkers found two unidentified MAA in *Dasycladus vermicularis* (M348 and M332). The algae *Prasiola crispa* subsp *antarctica* and *Prasiola stipitata* contained only M324 (reference in Carreto and Carignan, 2011; Gröniger and Häder, 2002) that was characterized as a putative MAA due to chromatographic properties (reference in Carreto and Carignan, 2011).

Table 2. MAAs identified in macroalgae *Rhodophyta* – Red seaweeds.

Algae species	MAAs detected after UV exposure	References
Acanthofora spicifera	Porphyra-334, palythine, shinorine, palythinol, asterina-330; mycosporine-glycine	Carefoot et al. (2000)
Asparagopsis taxiformis	Unidentified MAAs	Sinha et al. (1998)
Centrocerus clavulatum	Shinorine, Asterina-330, Palythine, Palythinol (trace)	Carefoot et al. (2000)
Chondrus crispus	Shinorine, palythine, palythene, palythinol	Reference in Sinha et al. (1998) Sinha et al. (1998); Karsten et al. (1998)
Condrus crispus	Shinorine, palythine, asterina-330, palythinol, palyhene	Kräbs et al. (2002)
Corallina elongata	Shinorine, palythine	Sinha et al. (1998)
Curdiea racovitzae	Palythine, shinorine, palythinol	Reference in Sinha et al. (1998)
Cystoclonium purpureum	Shinorine, porphyra-334	Sinha et al. (1998)
Dumontia incrassata	Shinorine, porphyra-334, palythine, mycosporine-gly	Sinha et al. (1998)
Gelidium sp	Shinorine	Sinha et al. (1998)
Gracilaria chinensis	Porphyra-334 (70%), shinorine (17-21%), palythine (5-10%), asterine-330 (5-10%)	Gómez et al. (2005)
Gracilaria cornea	Shinorine, porphyra-334	Sinha et al. (2000)
Gracilaria lemaneiformis	M-265 nm	Xu and Gao (2010)
Heterosigma akashiwo	M-337	Gao et al. (2007)
Hydropuntia cornea	Shinorine, porphyra-334, palythine	
Iridaea chordata	Palythine, shinorine, palythinol, palythene	References in Sinha et al. (1998)
Jania rubens	Shinorine, palythine, porphyra-334	Sinha et al. (1998)
Laurentia sp.	Porphyra-334, Palythine, Asterian-330, Shinorine, mycosporine-glycine, Palythinol	Carefoot et al. (2000)
Lithothamnion cf. antarcticum	Shinorine, porphyra-334	Reference in Sinha et al. (1998)
Palmaria decipiens	Palythine, palythene, porphyra-334, palythinol, shinorine	Reference in Sinha et al. (1998)
Palmaria decipiens	Shinorine, porphyra-334, palythine, asterina-330, palythinol, palythene, usujirene, M-335/360	References in Carreto and Carignan (2011)
Palmaria palmata	Palythine, shinorine, asterina-330, palythinol, porphyra-334, usujirene	Yuan et al. (2009)
Palmaria palmata	mycosporine-glycine, shinorine, porphyra-334, palythine, asterina-330, palythinol and palythene, M-357	Karsten and Wiencke (1999)
Phyllophora antarctica	Shinorine, palythene	Reference in Sinha et al. (1998)
Phyllophora appendiculata	Shinorine	Reference in Sinha et al. (1998)
Porphyra sp.	Porphyra-334, shinorine	Figueroa et al. (2003)
Porphyra leucosticta	Porphyra-334, palythine and asterina-330 and shinorine	Korbee et al. (2005)
Porphyra umbilicalis	Shinorine, porphyra-334	Sinha et al. (1998)
Pseudolithophyllum expansum	Shinorine, porphyra-334	Sinha et al. (1998)

Table 3. MAAs identified in macroalgae Phaeophyceae – Brown seaweeds.

Algae species	MAAs detected after UV exposure	References
	Phaeophyceae – Brown seaweeds	
Ascophyllum nodosum	Shinorine, porphyra-334	Sinha et al. (1998)
Desmarestia aculeata	Shinorine, porphyra-334	Sinha et al. (1998)
Fucus spiralis	Shinorine	Sinha et al. (1998)
Padina crassa	Shinorine, porphyra-334	Reference in Sinha et al. (1998)

Table 4. MAAs identified in macroalgae *Chlorophyta* – Green seaweeds.

Algae species	MAAs detected after UV exposure	References
Boodlea composita	Mycosporine-glycine, porphyra-334	Reference in Carreto and Carignan (2011)
Caulerpa racemosa	Mycosporine-glycine, porphyra-334	Reference in Carreto and Carignan (2011)
Cladophora rupestris	Shinorine, porphyra-334, palythine	Sinha et al. (1998)
Codium fragile	Palythine, porphyra-334	Reference in Sinha et al. (1998)
Dasycladus vermicularis	M-348, M-332	Gómez et al. (1998)
Enteromorpha intestinalis	Unknown MAAs	Sinha et al. (1998)
Prasiola crispa subsp antarctica	M-324	Reference in Carreto and Carignan (2011)
Prasiola stipitata	M-324	Gröniger and Häder (2002)
Ulva lactuca	Mycosporine-glycine, shinorine, Porphyra-334, Palythine	Carefoot et al. (2000)

Seagrasses

Seagrasses and submerged aquatic plants are subject to the influence of UV-B radiation due to the penetration of harmful UV-B wavelengths to considerable depths as forementioned.

The potential for aquatic plants to minimize UV-damage needs more investigation. However, varying degrees of response to increased UV radiation were found in seagrasses. Seagrasses *Halophila ovalis* and *Halodule uninervis* showed little UV-blocking response, exhibiting large decrease in photosynthetic efficiency and chloroplast density, showing a low resistance in UV exposure (reference in Short and Neckles, 1999). On the other hand, *Zostera capricorni*, *Cymodocea serrulata* and *Syringodium isoetifolium* were more UV tolerant due to the production of blocking pigments. Increases in UV-B radiation cause increases in plant content of phenolic and other secondary compounds (including flavonoids), which in turn, may increase the plant's resistance to herbivores and pathogens as well as decrease rates of decomposition (reference in Short and Neckles, 1999).

Scitonemin

Scitonemin is a yellow-brown lipid soluble pigment located in the extracellular polysaccharide sheath of some cyanobacteria (references in Singh et al., 2010b). Nägeli first reported it in 1849 but Proteau and coworkers (1993) identified its chemical structure in 1993. Scitonemin is a dimer composed of indolic and phenolic subunits having a molecular mass of 544 Da (Singh et al., 2010b), with a maximum wavelength of 250 nm. The linkage between two subunits in this pigment is an olefinic carbon atom that is unique among natural products (Singh et al., 2010b).

The Raman spectrum of the photoprotective pigment Scytonemin (C36H20N2O4) with the chemical name of 3,3'-bis-(4-hydroxybenzylidene)-3H,3'H-<1,1'>bi<cyclopenta<*b*>indolyl>-2,2'-dione found in cyanobacteria was obtained for the first time by Edwards et al. (1999).

Scitonemin is thought to be synthesized from metabolites of aromatic amino acids biosynthesis under high photon fluence rate (reference in Singh et al., 2010b). This pigment was isolated for the first time in an intertidal *Lyngbya* (cyanobacteria) (Edwards et al., 1999). Experimental evidence showed that both increase in temperature and oxidative stress in combination with UV-A, have a synergistic effect on high production of scytonemin (Singh et al., 2010b). This pigment is exclusively of cyanobacteria.

Antioxidant compounds

UV- radiation induces a general rise in activities of various antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POX), catalase (CAT) and glutathione reductase (GR) (Mallick and Mohm, 2000; Wang et al., 2007),

ascorbate-peroxidase (APX), dehydroascorbate reductase (DHAR) and monodehydroascorbate reductase (MD-HAR) to counteract oxidative stress (Li et al., 2010). In plants, the generated $O_2^{\cdot-}$ can beconverted into H_2O_2 and O_2 by several SOD isoenzymes: mitochondrial manganese SOD (Mn-SOD), chloroplast iron SOD (Fe-SOD) and cytosolic copper and zinc SOD (Cu/Zn SOD) (reference in Li et al., 2010). CAT and POX efficiently catalyse the breakdown of H_2O_2. APX is another powerful H_2O_2 scavenging enzyme, which utilizes AsA to eliminate the toxic product H_2O_2 by the oxidation of AsA to the monodehydroascorbate (MDHA) (reference in Li et al., 2010). APX isoenzymes are distributed in at least four distinct cellular compartments, including stromal APX (sAPX), thylakoid membrane-bound APX (tAPX) in chloroplasts, microbody membrane-bound APX (mAPX) and cytosolic APX (cAPX) (reference in Li et al., 2010). There is significant evidence showing that algae exposed to oxidative stress tend to increase the activities of ROS scavenging enzymes (references in Li et al., 2010). This indicates, as referred by Li et al. (2010), that higher and more stable antioxidant enzyme activities, either constitutive or induced, are associated with a higher stress tolerance in algae. Studies conducted by Aguilera et al. (2002), as referred by Li et al. (2010), comparing antioxidant enzyme activities among twenty-two macroalgae species (five green, seven red and ten brown) to UV radiation, showed that algal tolerance to oxidative stress was correlated with an enhancement of oxygen-reactive scavenging system. Li et al. (2010) evaluated the performance of SOD, POX, CAT and APX activities and isomorphs which catabolized $O_2^{\cdot-}$ and hydrogen peroxide in *Coralina officinalis* L. (Rhodophyta), to further identify the biochemically relevant pathways and protective mechanisms when exposed to UV-B. Results showed that superoxide dimutase (SOD) and peroxidase (POX) increased and then maintained at a relatively stable level when subjected to UV-B irradiation. Catalase (CAT) activity under medium dosage of UV-B irradiation (Muv) and high dosage of UV-B irradiation (Huv) treatments were significantly decreased. Ascorbate peroxidase (APX) activity first remained unaltered and then increased in Huv treatment. The activities of some SOD isoforms were altered by UV-B. Two new bands (POX V and POX VII) appeared upon exposure to all three UV-B dosages. CAT III activity was increased by low dosage of UV-B irradiation (Luv), whereas CAT III and CAT IV disappeared when the alga was exposed to Muv and Huv. Two bands of APX (APX VI and APX VII) were increased and a new band (APX X) was observed under Huv exposure. H_2O_2 and thiobarbituric acid reacting substance (TBARS) increased under Muv and Huv treatments. Overall, UV-B protection mechanisms are partly inducible and to a certain extent sufficient to prevent the accumulation of damage in *C. officinalis*. The antioxidant defense mechanism against ROS is pivotal for algal survival under stressful conditions (references in Li et al., 2010).

The intertidal macroalga *Hypnea musciformis*, showed a photoprotective adaptation strategy against UV-B damage, an increase of 58.9% phenolic compounds and 3.6% of carotenoids (Schmidt et al., 2012). *P. umbilicalis* (Sampath-Wiley et al., 2008) exhibited increased antioxidant metabolism, which could contribute to its success in colonizing stressful habitats like intertidal shores. In contrast, *Arthrospira (Spirulina) platensis*, showed accumulation of ROS by the presence of high levels of UVR, inhibited the activities of superoxide dismutase (SOD) and catalase (CAT) to cope with UVR (Ma and Gao, 2010).

Non-enzymatic components such as GSH (reduced glutathione), ascorbic acid, α-tocoferol, β-carotene, flavonoids, hydroquinones, among others, following exposure to UV radiation (Mallick and Mohm, 2000) have been reported and seems to be evident. Cellular thiols, especially glutathione, appear to play a key role in protection against oxidative damage arising from a number of stress conditions (Malanga et al., 1999). The role of N-acetylcysteine (NAC) as a protector against oxidative damage associated with ultraviolet in the microalga *Chlorella vulgaris* cultures was evaluated by Malanga et al. (1999). Treatments with NAC kept ascorbyl and lipid radical content in algae exposed to UV-B. Supplementation with 1mM NAC did not affect the content of lipid-soluble antioxidants (α-tocopherol, β-carotene) in algae cells (Malanga et al., 1999).

Phenolic extracts from the macroalgae *Macrocystis pyrifera* and *Porphyra columbina* exhibited high photoprotective activity, close to complete photoprotection (100%) (Guinea et al., 2012).

A comparative study with the brown seaweed *Pelvetia canaliculata* and the marine angiosperm *Salicornia ramosissima* (purple glasswort), two marine macrophytes growing in the upper intertidal zone, was conducted by Hupel et al (2011). This study showed that high doses of UV-B radiation induced few changes in carotenoid contents for

both species, suggesting efficient constitutive contents for photoprotection. This study also showed a fast acclimation of the brown seaweed, since both phenols and carotenoids related to a strong antioxidant protection. *S. ramosissima* showed a slow acclimation with a putative down regulation of phenols and the preferential involvement of carotenoids and/or other photoprotective systems.

Heat shock proteins

Heat shock proteins (HSPs) are synthesized by living cells as a response to stressful conditions, such as exposure to elevated temperatures, xenobiotics, heavy metals, free radicals, and UVR (Dahms and Lee, 2010). Among known heat shock proteins, only *hsp70* has been studied in marine ectotherms exposed to UVR. *Hsp 70* was suggested Matranga et al. (2006) (as referred by Dahms and Lee, 2010) to be a sensitive indicator of UV-B stress, as it is established by Bonaventura et al. (2006) (as referred by Dahms and Lee, 2010) a dose-dependent increase in *hsp70* protein levels in embryos of the sea urchin *Paracentrotus lividus* (Lamarck) exposed to UV-B doses. *Hsp70* had been identified in some microalgae: 46 species of cyanobacteria in six species of green algae (Chlorophyta) and in the diatoms *Thalassiosira pseudomana* and *Phaeodactylum tricornutum*. In algae, *hsp70* can help to acclimate to the environment (eg *Chlamydomonas* sp.) and adjust asymmetric divisions (*Volvox carteri*) (references in Zhang et al., 2011), or contribute to repairing photosystem II damage in *Dunaliella salina*. Fu et al. (2011) working with *Ulva pertusa* found a correlation between transcriptions and stress induction in this alga species and held that *hsp70* played an important role in the stresses. In the green seaweed *Ulva (Enteromorpha) prolifera*, the transcription of *hsp70* was up-regulated by UV irradiation, heat treatment and salinities induction, and less influenced by desiccation (Zhang et al., 2011). The authors suggest the use of *hsp70* in prediction of stress tolerance in algae and as a potential bio-indicator to monitor the stresses in seawater environments in the future.

Other UV absorbing compounds

The physiological responses of the aquatic liverwort *Jungermannia exsertifolia* sups. *cordifolia* were analyzed by Arróniz-Crespo and coworkers (2008) especially considering the UV radiation-induced responses of five hydroxycinnamic acid derivatives. This bryophyte lives in mountain streams, exposed to low temperatures and high UV levels (Arróniz-Crespo et al., 2008). This combination, high UV and low temperature, increase the adverse effects of UV. Arróniz-Crespo et al. (2008) and Fabón et al. (2012) found this liverwort species to have a dynamic protection and acclimation capacity to the irraciance and spectral characteristics of the radiation received. Studies conducted by Arróniz-Crespo et al. (2008) proposed three of the five UV-absorbing hydroxycinnamic acid derivatives as bioindicators of enhanced UV radiation: *p*-coumaroylmalic acid, 5”-(7”,8”-dihydroxycoumaroyl)-2-caffeoylmalic acid and 5”-(7”,8”-dihydroxy-7-*O*-β-glucosyl)-2-caffeoylmalic acid.

DNA repair

The most studied DNA repair process involves pyrimidine dimers repairing. The most common type of DNA damage induced by UVR is the formation of cyclobutyl pyrimidine dimmers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts, leading to mutagenic, teratogenic or lethal effects in organisms, because these lesions prevent DNA replication and transcription. Organisms use different types of DNA repair mechanisms including photoenzymatic repair (PER), nucleotid excision repair (NER) and post-replication repair (Vincent and Roy, 1993).

Photoenzymatic repair (photoreactivation) (PER), is used to reverse cyclobutane dimers in DNA. It relies on the enzymatic activity and energy of photolyase at UV and visible light wavelengths (reference in Dahms and Lee, 2010). This DNA repair mechanism is widely distributed in nature and photoreactivation has been found in members of all kingdoms, although it is apparently lacking in several species or groups such as mammals (reference in Dahms and Lee, 2010).

Nucleotic excision repair (NER) is a more complex repair process that requires damage recognition, incision of the DNA strand near the site of the lesion, the excision and resynthesis of the DNA around the damaged site, and finally, ligation of the single strand after the DNA polymerase detaches (reference in Dahms and Lee, 2010). NER appears to be universally distributed though it is not thought to be very efficient at repairing CPDs (Dahms and Lee, 2010).

UVR combined effects and ecological impacts

The cycling of key elements like carbon (C), nitrogen (N) and phosphorous (P) in aquatic systems depends to a large extent on productivity and fate of autotrophs. Several works demonstrated an inverse effect of UV radiation and PAR with regard to elemental ratios, notably C:P. Uptake

rates of ^{15}N-ammonium of algae are affected by UV-A of high intensity and UV-B radiation. The results also show a significant reduction in total nitrate by 95.5% in the high UV-B treatment (Döhler and Buchmann, 1995; Braune and Döhler, 1996; Anusha and Asaeda, 2008). The recovery of photosynthetic activity and phycobiliproteins, was enhanced in the algae previously incubated under PAR + UVR as compared to exposure to only PAR, suggesting a beneficial effect of UVR on recovery or photoprotective processes under enriched nitrogen conditions (Huovinen et al., 2006).

Significant increase in dissolved ammonia in water under UV-B exposure, due to photoxidation and bacterial decomposition of organic nitrogen in the system, alter the natural balance of nitrogen, oxygen and dissolved carbon in aquatic systems.

Several studies have documented a negative impact of UV radiation (280-320 nm) on PUFAs (polyunsaturated fatty acids) in marine phytoplankton species: this impact has been attributed either to oxidation of previously synthesized fatty acids or to disruption of their synthesis (references in Leu et al., 2006). PUFAs play a key role in aquatic food webs because only photosynthetic organisms synthesize them and they are essential macromolecules for heterotrophs. PUFAs are also of major importance in regulating membrane fluidity under low temperatures.

Photosynthetic organisms sustain life on Earth, and aquatic biophotosystems contribute with 50% of the global oxygen supply for all life.

Effects of UV radiation are complex because organisms face different stressors.

The increase of UVR absorbing pigments is a primary defence mechanism against UVR damage, since their presence reduces plankton organism's transparency in the UVR. This increases its sighting distance for predators and prey with UV vision. This presents a dilemma for transparent epipelagic zooplankton that either needs to protect itself by sunscreen or to maintain its camouflage strategy in order to prevent predation. This conflict is particularly difficult to resolve in clear, oceanic waters where UVR levels are high.

Species with low capacity of living under UV irradiation due to their incapacity of repairing systems tend to disappear unbalancing the ecosystem, and reducing biodiversity.

Increasing growth rates in species resistant to UV exposure, like the forementioned raphidophyte microalga (*Cattonella* sp) which is known to cause fish mortality in Japanese waters and was also implicated in mortality of farmed finfish in South Australia, may also have important economic negative impacts on finfish aquaculture industry.

Conclusions

A large body of information is available about UVR photobiology, particularly since the awareness of ozone depletion. Yet, long terms consequences of UVR exposure on organisms and what consequence in the ecosystems balance are uncertain. High ROS formation rates are particularly important especially for organisms with early life stages in the plankton from surface waters dwelling at certain environmental conditions (cloudless sky, thin ozone layer, lack of wind, calm seas, low nutrient loading).

The sensitivity of organisms to UVR has been shown to relate to differences in the efficiency of their protective mechanisms and repair systems (Dahms and Lee, 2010). A better understanding of such mechanisms will allow the development of technologies to monitor and address the adverse impacts of UVR (Dahms and Lee, 2010).

Details of the pathway and the enzymes involved in the biosynthesis, transference, accumulation and transformations of more than 20 fully characterized MAAs and of the recently discovered compounds, are still unknown and further studies are required. Nevertheless, there are biochemical evidences to assume that the high diversity of MAAs present in marine organisms is mainly derived from the synthesis and transformation of the so-called "primary MAAs". (Carreto and Carignan, 2011). In addition to the ecological significance of MAAs as sun-screening substances, these compounds have potential applications in cosmetics and toiletries as a UV protectors and activators of cell proliferation with therapeutic properties that may be exploited in a large amount of commercial applications (Carreto and Carignan, 2011).

Most of the scientific work available is based on laboratory tests exposing a single species and using artificial UV light to produce unrealistic environmental conditions, providing little useful information, because interspecific interactions, self-protective behaviour and chemical interactions with naturally occurring organic matter are not accounted for. It is critical to identify interactions between multiple stressors and UVR, because the combined effects are turning out to be astonishingly complex and could include abiotic interactions in addition to biodegradation and bioactivation of chemicals by UVR.

Ecological significance of elevated UV-B exposure in the aquatic environment may be

seriously underestimated if effects on the early lifestages of algae are not considered.

Synergisms among stressors are shown to be increasingly important in the context of global environmental change and must consider both, the effects of UV-B on a sinlge species and its effects on entire communities and systems (Dahms et al., 2011).

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REVIEW ARTICLE

Impact of UV-B radiation on photosynthesis – an overview

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Abstract

Ultraviolet-B (UV-B) radiation constitutes a minor part of the solar spectrum, being most of this solar radiation absorbed by the UV-screening stratospheric ozone layer. Yet, a global depletion of the ozone layer, largely due to the release of chlorofluorocarbons caused by human activities, has resulted in an increase of solar UV-B radiation at the earth's surface. Accordingly, in the temperate latitudes, such ozone decrease reached ca. 3% and 6% in the North and South hemispheres, respectively, between 2002 and 2005 (as compared to the 1970s). Despite the uncertainty of long-term predictions, it is also estimated an UV-B increase of 5-10% over temperate latitudes within the next 15 years In this context, this work aim at to present an overview of plants sensitivity/tolerance to UV-B irradiation mostly considering the key photosynthetic metabolism.

Key words: Ozone depletion, Photosynthesis, Reactive oxygen species, Ultraviolet-B radiation

Introduction

Ozone depletion and UV-B radiation on Earth's surface

Most of UV radiation does not reach Earth's surface due to its interaction to the atmospheric components. In fact, UV-C radiation might be completely absorbed by the atmospheric gases, UV-B radiation is absorbed by the stratospheric ozone layer, whereas UV-A radiation is hardly absorbed by this layer. The ozone layer can be depleted by free radical catalysts, including nitric oxide, nitrous oxide and hydroxyl, as well as atomic chlorine and bromine. Although there are natural sources for all of these species, the concentrations of chlorine and bromine have increased markedly in recent years due to the release of large quantities of man-made organohalogen compounds, especially chlorofluorocarbons, with a half-life ranging from 50 to 150 years (Madronich et al., 1998). These highly stable compounds are capable of surviving the rise to the stratosphere, where Cl and Br radicals are produced by the action of UV radiation. Each radical is then able to initiate and catalyze a chain reaction capable of breaking down over 100,000 ozone molecules. Such breakdown of ozone molecules in the stratosphere can result in a decrease of the effectiveness of UV radiation absorption and therefore more radiation reaches the Earth, with each 1% reduction in ozone causing an increase of 1.3-1.8% in UV-B radiation reaching the biosphere (Caldwell and Flint, 1994; McKenzie et al., 2003).

Nowadays ozone levels over the northern hemisphere have been dropping by 4% per decade. Much higher seasonal declines have been measured in approximately 5% of the Earth's surface, around the north and south poles, constituting the so called ozone-holes. Current stratospheric ozone levels are at the lowest point since measurements began in 1970s and global terrestrial UV-B radiation levels range between 0 and 12 kJm^{-2} on a given day, with near Equator and mid-latitudes receiving higher doses (McKenzie et al., 2011). The changes in ozone and UV-B are not uniform over the Earth's surface (Figure 1). The ozone concentrations in the high latitudes (comprising Antarctic and Arctic regions) are 40–50% lower than the pre-1980 values; in the mid-latitudes (35–60ºN and 35–60ºS) are 3–6% lower than the pre-1980 values; and, at the Equator, show minimum changes (Forster et al., 2011).

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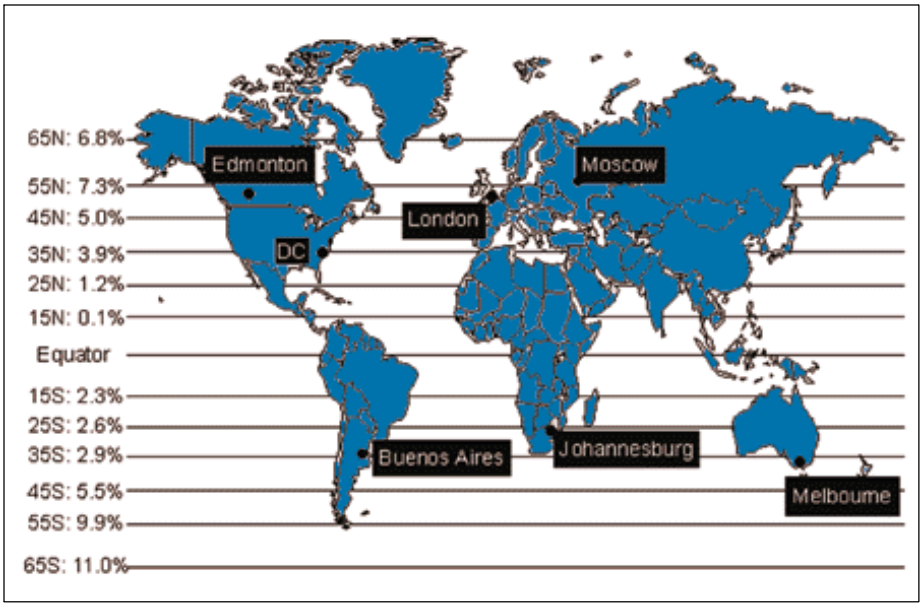

Figure 1. A 1996 study using satellite-based analyses of UV-B trends demonstrated that this radiation levels had increased at ground level. This figure shows the percent increases in average annual UV-B reaching the surface over the past 10 years. UV-B incidence is strongly dependent on latitude.
(http://www.epa.gov/airtrends/aqtrnd95/stratoz.html)

Current levels of UV-B during the cropping season are somewhere between 2-12 kJm^{-2} per day on the Earth's surface, which includes an increase of 6-14% of UV-B radiation (Forster et al., 2011) over the pre-1980 levels. A 30% increase in UV-B results in a maximum amount of 2.44 kJm^{-2} per day in UK (Forster et al., 2011), but such low levels of UV-B radiation are very uncommon during the cropping season in several parts of the world. For example, in the Cotton Belt of USA, current UV-B radiation levels are 4–11 kJm^{-2} per day during the summer season (Frederick et al., 2000), and the predicted UV-B levels based on Taalas et al. (2000) would be 4.56–12.54 kJm^{-2} per day. In China, ambient UV-B levels during soybean cropping period are in average of 8.85 kJm^{-2} per day (Li et al., 2002). A 30% increase in UV-B levels is expected to seriously affect crop production in these and other parts of the world.

Target sites of UV-B radiation in the photosynthetic pathway

UV-B radiation (280–320 nm) is a minor part of the solar spectrum, although the component that reaches Earth's surface is a promoter of a large number of responses in higher plants at the molecular, cellular and whole-organism level (Caldwell et al., 2007; Jenkins, 2009). In fact, UV-B radiation is readily absorbed by a large number of biomolecules (*e.g.*, nucleic acids, proteins, lipids), therefore leading to their photoexcitation what might promote changes on multiple biological processes, both with damaging or regulatory importance (Jenkins, 2009; Tian and Yu, 2009). Nevertheless, cconsiderable intra- and interspecific variability in sensitivity of crop plants to UV-B radiation have been reported (Teramura, 1983; Bornman and Teramura, 1993; Correia et al., 1998; Mazza et al., 2000), including at the photosynthetic level. In general, leaf photosynthesis might decrease more by enhanced UV-B radiation under growth chamber or glasshouse conditions than under field conditions due to low PAR or a low ratio of PAR to UV-B in the chambers. Despite the diversity of UV targets in plants, it seems that the photosynthetic apparatus is amongst the main action targets of UV-B, and its damage contributes significantly to the overall UV-B damage (Lidon and Ramalho, 2011; Lidon et al., 2012; Lidon, 2012).The photosynthetic pathway responses to UV-B radiation depend on experimental growth conditions and plant growth stage, UV-B dosage, flow rate and the ratio of PAR to UV-B radiation, as well as on the interaction with other environmental stresses (*e.g.*, cold, drought, mineral availability) (Bornman and Teramura, 1993; Tevini, 2004; Jenkins, 2009). The photosynthetic structures are widely impaired by UV-B radiation with impact

observed at several levels, namely through the induction of tissue chlorosis and necrosis, changes in leaf ultrastructure and anatomy (*e.g.*, in the thickness of epidermal and palisade mesophyll cell layers), degradation of photosynthetic pigments and thylakoid electron transport carriers (Bornman and Teramura, 1993; Tevini, 2004; Jenkins, 2009; Lidon and Ramalho, 2011).

Stomatal regulation and CO_2 fixation

Stomatal regulation is an important process limiting leaf photosynthesis. Although earlier studies have proposed that UV-B radiation does not significantly affect stomatal conductance (Teramura et al., 1984; Murali and Teramura, 1985, 1987; Agrawal et al., 1991; Keiller et al., 2003), several other studies demonstrated reduced stomatal conductance in response to UV-B radiation (Dai et al., 1992; Middleton and Teramura, 1993; Pal et al., 1998, 1999; Lidon and Ramalho, 2011). Also, Jansen and van-den-Noort (2000) found that high UV-B might stimulate either stomatal opening or closing in *Vicia faba*, depending on the metabolic state. However, although differences exist in genotypes tolerance to UV-B radiation (Correia et al., 1999), the induced reduction of stomatal conductance can barely be responsible for CO_2 limitation in several crops (Agrawal et al., 1991; Teramura et al., 1991; Ziska and Teramura, 1992; Zhao et al., 2003; Lidon and Ramalho, 2011). In fact, the stomatal conductance reduction is usually in much smaller extent than that of the net photosynthetic rate. Furthermore, the calculated intercellular CO_2 concentration of plants exposed to UV-B radiation presented values close or even higher than that of untreated control plants (Agrawal et al., 1991; Zhao et al., 2003; Lidon and Ramalho, 2011). All together, these results point to mesophyll imbalances that may arise from damages and/or regulatory mechanisms, both at biochemical and biophysical level (Lidon and Ramalho, 2011).

In fact, ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco) content and activity are also strongly affected by UV-B radiation in many field crops (Vu et al., 1982, 1984; Strid et al., 1990; Nedunchezhian and Kulandaivelu, 1991; Jordan et al., 1992; He et al., 1993, 1994; Huang et al., 1993; Kulandaivelu and Nedunchezhian, 1993; Mackerness et al., 1997; Correia et al., 1999; Savitch et al., 2001). That will affect V_{cmax} and, therefore, contribute to photosynthesis depression (Iwanzik et al., 1983; Teramura et al., 1991; Keiller et al., 2003; Tevini, 2004; Lidon and Ramalho, 2011; Lidon et al., 2012). Additionally, when the effect of UV-B with or without UV-A radiation on the mechanisms of UV-B reduced photosynthesis are considered, Savitch et al. (2001) found that in *Brassica napus* submitted to 200 μmol m^{-2} s^{-1} PAR the decrease in the CO_2 assimilation capacity for PAR + UV-B treated plants was associated with a decreasing capacity for sucrose biosynthesis and limited triose-P utilization. In addition, the RuBP regeneration (Allen et al., 1997; Savitch et al., 2001) and the amount of sedoheptulose 1,7-bisphosphatase (Allen et al., 1998) also decreases upon UV-B radiation exposure. On the other hand, high UV-B irradiance in combination with low PAR levels produces significant reduction in the concentration of carboxylating enzymes, whereas high PAR (higher than 1,000 μmol^{-2} s^{-1}) together with low UV-B levels do not affect Rubisco activity (Barbato et al., 1995). UV-B-induced inactivation of Rubisco could be due to modification of the peptide chain, degradation of the protein, and/or diminished transcription of the gene (Jordan et al., 1992; Caldwell, 1993). Furthermore, it is apparent that the UV-B-induced reduction in Rubisco is greater in UV-sensitive than in UV-resistant strains. These findings pose two questions: why did supplementary UV-B radiation causes a marked reduction in Rubisco; and why was this effect greater in UV-sensitive, than in UV-resistant strains? A possible explanation can be linked to the modification of proteins by photooxidation, or by reactive oxygen species (ROS) and free radicals produced during photosensitization (Caldwell, 1993; Foyer et al., 1994). These modifications would include cross-linking, aggregation, denaturation and degradation (Andley and Clark, 1989; Wilson and Greenberg, 1993; Borkman and Mclaughlin, 1995; Greenberg et al., 1996).

Photochemical reactions

As stated above, a major impact site of UV-B radiation is the chloroplast, leading to the impairment of the photosynthetic function (Bornman, 1989; Allen et al., 1998; Lidon et al., 2012). Furthermore, the integrity of the thylakoid membrane and structure seems to be even more sensitive than the activities of the photosynthetic apparatus bound within (Lidon and Ramalho, 2011; Lidon et al., 2012). Negative effects on several photosynthetic components are known, including through suppression of chlorophyll synthesis (Kulandaivelu et al., 1991), the inactivation oxygen evolution, LHCII, PSII reaction centres functionality and the thylakoid electron flux. Due to

the key role of LHCII in light absorption and energy transfer to the reaction center, as well as on thylakoid stacking, any damage to these structures can result in multiple effects on the photosynthetic functioning. Furthermore, it must be considered that UV-B radiation inhibition of LHCII (Vu et al., 1982, 1984; Strid et al., 1990; Lidon et al., 2012) is also eventually linked to a decrease in the transcription of the cab gene responsible for the synthesis of the chlorophyll a/b-binding proteins of LHCII, which may lead to the functional disconnection of LHCII from PSII (Jordan et al., 1994).

Numerous studies have also showed that in photophosphorylation processes, PSII is the most sensitive component of the thylakoid membrane on exposure to UV-B radiation (Brandle et al., 1977; Noorudeen and Kulandaivelu, 1982; Kulandaivelu et al., 1991; Melis et al., 1992; Allen et al., 1998; Chaturvedi et al., 1998; Correia et al., 1999; Bolink et al., 2001; Savitch et al., 2001; Lidon et al., 2012), what would be related to rapid degradation of the D_1 and D_2 proteins of PSII (Lidon et al., 2012). Moreover, relatively to PSII, UV-B radiation has a smaller effect on PSI (Kulandaivelu et al., 1991) and cytochrome b_6/f complex (Cen and Bornman, 1990; Lidon et al., 2012), although strong UV-B mediated effects on PSI linear electron transport (Lidon and Ramalho, 2011) and on cyclic phosphorylation (Pang and Hays, 1991) were repoted.

Within PSII, UV-B radiation acts on either the reaction center itself, producing dissipative sinks for excitation energy, which quenches the variable fluorescence and/or in the reducing site of PSII (Iwanzik et al., 1983; Lidon and Ramalho, 2011). Nevertheless other works suggest that the quinone electron acceptors (Melis et al., 1992), the redox active tyrosines (Tyr-Z, Tyr-D), or the primary step of PSII electron transport (Iwanzik et al., 1983; Kulandaivelu et al., 1991) are the primary targets of UV-B action in PSII electron transport. In this context, the water-oxidizing complex (OEC) seems to be UV-B sensitive (Lidon et al., 2012). Since the Mn cluster of water oxidation is the most fragile component of the electron transport chain, UV-B absorption by the protein matrix or by other redox components may lead to conformational changes and inactivation of the Mn cluster. Q_A and Q_B acceptors have also been frequently suggested as sensitizers of D_1 and D_2 protein damage due to the similarity of the action spectrum of D_1 protein degradation and the absorption spectrum of plastosemiquinones (Jansen et al., 1996). Thus, the importance of the donor side components of PSII primarily that of the water-oxidizing system, in sensitizing D_1 protein cleavage via hydroxyl radical formation must also be considered (Lidon et al., 2012). Indeed, a potential primary catabolism involved in UV-B induced physiological and biochemical injury has been related to the production of ROS (Caldwell, 1993; Foyer et al., 1994; Hideg et al., 1997). Through this pathway, triplet molecular oxygen continuously produced during light-driven photosynthetic electron transport, in the water splitting complex coupled to PSII, can be converted in the sequential reduction to superoxide, hydrogen peroxide and hydroxyl radical (Lidon and Henriques, 1993; Apel and Hirt, 2004).

Protection against UV-B radiation

Recovery from UV-induced dysfunction of enzymes is expected to involve protein and DNA synthesis and/or repair,, both in the chloroplast and nuclear DNA. Among proteins, the functions of D_1 and D_2 from PSII can be partially restored after UV-B damages. D_1 protein is rapidly turned over *in vivo* in a short time as 30 min (Wilson and Greenberg, 1993). On the other hand, D_2 degradation is activated by distinct photosensitizers in the UV-B and visible region of the spectrum and it has been suggested that its degradation is coupled with that of D_1, being influenced by events occurring at the quinone niche on the D_1 protein.

As defensive strategy, plants may trigger machanisms for the dissipation of excess excitation energy. Excess energy imposed to the photosynthetic apparatus may be thermally deactivated through photochemical and non-photochemical quenchers (Lidon and Ramalho, 2011). One of the main routes of heat dissipation is the xanthophylls cycle, in which violaxanthin is converted to zeaxanthin when the level of captured energy is higher than that used through photochemical events, what could happen even under moderate irradiance and depends of the species, ecological history of the plant and if environmental stresses that reduce the photochemical energy use are involved (Adams and Demmig-Adams, 1992; Ramalho et al., 2000; 2003; Batista et al., 2011). The key enzyme of this cycle is violaxanthin deeposidase, which is sensitive to UV-B radiation. Nevertheless, this process further links the catabolic action of ROS, which must also be controlled due to its damaging action over a wide number of biological molecules and structures (Lidon et al., 2012), among them prteins, lipids and nucleic acids (Öquist, 1982; Logan, 2005; Fortunato et al., 2010; Partelli et al., 2011).

The production of ROS is an inevitable consequence of photosynthetic activity (Ensminger et al., 2006), as under normal metabolic conditions, 10-30% of the thylakoid electron transport might lead to O_2 photoreduction (Bartoli et al., 1999; Logan, 2005), what further increases under stressful conditions that decrease the photochemical energy use. Oxygen itself is a strong oxidant since it possesses two unpaired electrons in its outermost π orbital. The reduction of oxygen by nonradical species needs the transfer of two electrons having parallel spins to oxygen in order to fit with parallel spins of two unpaired electrons. Oxygen, therefore, got converted to ROS by univalent reduction or by energy transfer. The more common ROS produced in plant include superoxide, hydrogen peroxide and hydroxyl radical.

The superoxide radical ($O_2^{\bullet-}$) in aqueous solution has a pKa of 4.9 and might occur in physical, chemical and biological processes (Lidon and Henriques, 1993). Moreover, hydrogen peroxide is a powerful oxidizing agent (E^O = + 1.36 V, at pH 7 for the system H_2O_2/H_2O), being the transition metals involved in its synthesis throughout the protonation of $O_2^{\bullet-}$(Lidon and Henriques, 1993), whereas hydroxyl radical has a short lifetime and a strongly positive redox potential (+2 V), being produced throughout the catalyzed Haber-Weiss cycle and therefore dependent on both H_2O_2 and $O_2^{\bullet-}$ (Lidon and Henriques, 1993).

ROS production arises in plant cells via a number of routes. Most of these highly reactive molecules of oxygen are formed in plant cells via dismutation of superoxide as a result of single electron transfer to molecular oxygen in electron transfer chains mainly during the Mehler reactions in chloroplast which is the higher source of ROS (Logan, 2005; Asada, 2006). The lack of $NADP^+$ in PSI, due to redox imbalance, causes spilling of electron on molecular oxygen, triggering the generation of superoxide. The majority of superoxide *in vivo* is thought to be produced via electron spilling from reduced ferridoxin to oxygen. Superoxide formed then undergoes dismutation, either spontaneously or facilitated by superoxide dismutases. Superoxide radicals generated by one electron reduction of molecular oxygen by Mehler reaction in PSI are rapidly converted into hydrogen peroxide by chloroplast Cu-Zn-superoxide dismutase, which is then removed by the action of ascorbate peroxidases and catalases. Yet, if such removal is compromised or insufficient much more reactive hydroxyl radicals can be formed from superoxide and hydrogen peroxide through Fe catalyzed Haber-Weiss reaction (Foyer and Harbinson, 1994; Logan, 2005; Bhattacharjee, 2010; Fortunato et al., 2010). That would further promote damages, namely at the level of the lipid matrix of chloroplast membranes (Harwood, 1998; Campos et al., 2003; Partelli et al., 2011) where the double links of polyunsaturated fatty acids (FAs) are preferential targets of ROS and free radicals (Öquist, 1982; Girotti, 1990). In addition, peroxy and alkoxy radicals formed as intermediates in membrane lipid peroxidation are also very toxic at high concentration and poses threat to several biomolecules (Lidon and Henriques, 1993). Therefore, since the production of ROS is an metabolic inevitable consequence (overexpressed under excess photochemical energy), plants have evolved efficient strategies by devising and integrating antioxidative defense mechanism that avoids the production (as the thermal dissipation mechanisms referred above) of promotes the scavenging of ROS, through a integrated network of enzyme (*e.g.*, Cu,Zn-superoxide dismutase, ascorbate peroxidase) and non-enzyme (ascorbate, glutathione) molecules acting through the ascorbate-glutathione (Haliwell-Asada) cycle to overcame the imposed oxidative stress conditions.

Although in living tissues, accumulation of ROS imposes ultimately oxidative stress and cellular damages, it seems that at low concentrations these highly reactive species may also act as signaling molecules or second messengers, therefore implicated in the modulation of normal plant development, including senescence (ageing) and many other physiological processes (Dröge, 2002; Bhattacharjee, 2012). In this context, increased levels of UV-B radiation can stimulate the generation of elevated amounts of ROS (Bhattacharjee, 2012; Lidon et al., 2012; Xie et al., 2012), thus enhancing the expression of several genes involved in natural senescence phenomena, where ROS are implicated (Hollósy, 2002).

Apart from their role in accelerated ageing and other developmental processes, ROS, including nitric oxide (Kliebenstein et al., 2002), can be seen as triggering agents of defense mechanisms against a range of stress factors, including UV-B radiation (Lidon and Henriques, 1993; Bhattacharjee, 2012; Lidon et al., 2012). In this context, Wang et al. (2009) supported that nitric oxide appeared to act in the same direction or synergistically with other ROS to induce ethyl synthesis in a defensive response under UV-B radiation in maize leaves. Also, it was reported that UV-B induced an

increase of nitric oxide that may act as a second messenger and perform antioxidant response to UV-B radiation (Zhang et al., 2003). Tossi et al. (2009) reported that when maize seedlings are UV-B-irradiated, cellular damage occurs and ROS becomes widely distributed in chloroplasts and mesophyll cells. Pretreatment with apocynin and coinciding nitric oxide accumulation prevented this damage. They also suggested that UV-B perception triggers an increase in abscisic acid concentration, which activates NADPH oxidase and H_2O_2 generation. Moreover, sensitivity to UV-B radiation also follows the rate of sequential reduction of triplet molecular oxygen produced during the photosynthetic light reactions (Lidon et al., 2012). After photoreduction of this chemical entity to superoxide and dismutation to hydrogen peroxide hydroxyl radicals are produced. Yet the rate of zeaxanthin production is stimulated in the xanthophylls cycle, limiting photodegradation of isoprenoids (Lidon et al., 2012). Moreover, UV-B radiation might become lethal if an unbalanced ascorbate peroxidation develops, as this process limits the functioning of the enzymatic antioxidant systems (i.e., the Asada Halliwell cycle), mostly because of substrate limitations for the kinetics of ascorbate peroxidase (Lidon et al., 2012). In this context, ROS can trigger an increasing lipid peroxidation (particularly of monogalactosyldiacylglycerol class) and photosystems proteolysis, as well as degradation of thylakoids structure and functioning (Lidon et al., 2012).

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REVIEW ARTICLE

Cytoskeleton-mediated signalling pathways in UV-B perception by plant cell

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Abstract

Currently, the portion of ultraviolet B (UV-B) (280–315 nm) in total solar radiation reaching the earth's surface increases steadily revealing potentially adverse effects on terrestrial organisms. Plant morphological and functional responses induced by UV-B are principally described, however, the elucidation of downstream-effectors in UV-B-triggered pathways are still of particular interest, whereas they would allow to develop protective approaches aimed to increase multiple plants' tolerance to various abiotic stresses. The main focus of this review is on the contribution of cytoskeletal proteins into UV-B signalling in plants.

Key words: Plants, UV-B, Signalling, Cytoskeleton, Reactive oxygen species, Nitric oxide

Introduction

Impacts of ultraviolet (UV, 200−400 nm) non-ionising radiation on living organisms became an important environmental issue over the past four decades since the first reports of climate and/or anthropogenic depletion of the protective stratospheric ozone layer have appeared (Ballarè et al., 2011; McKenzie et al., 2011). As UV irradiation is not photosynthetically active except a short range of UV-A waveband close to violet visible light, algae and higher plants are reluctant to counteract UV through the development of the protective mechanisms or to tolerate *via* the adaptation to UV during the process of their evolution (Holzinger and Lütz, 2006). The enhanced UV-B (280−315 nm) exposure of Earth surface reveals both a range of deleterious effects such as nucleic acids/proteins damage, alterations in photosynthesis, transpiration, growth and development, etc. (Rozema et al., 1997; Hollósy, 2002), though ambient doses of UV-B trigger adaptive morphogenic responses (for review see Jansen, 2002).

Since sessile plants are not able to respond to (a)biotic stress factors by "fight-or-flight-or-freeze" strategy as animals do, they have developed a highly branched stress signalling pathways. The finely tuned molecular net operating in plant cell in response to stress factors is not studied well, and little evidence is accumulated concerning the integrative molecules and convergent reactions in partially overlapping pathways. The starting point of UV-B signalling is UV-B sensing by the special photoreceptor that was originally identified as a regulatory protein for UV-B-triggered signal transduction (Jenkins, 2009; Rizini et al., 2011). Light perception is important for all life kingdoms, but for plants it is vital as a main energy source and photomorphogenic trigger. Specific families of photoreceptors allow plants to sense wide wavelength range of light (Jenkins and Brown, 2007; Wu et al., 2012). For instance, red/far red is perceived by phytochrome, UV-A – by phototropin and cryptochrome, and UV-B – by recently discovered UV RESISTANCE LOCUS8 (UVR8), broadly present, constitutively expressed and well-conserved among plants (Rizini et al., 2011; Wu et al., 2012). Upon UV-B exposure, both natural and simulated, UVR8 homodimer monomerizes and interacts with CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1) to relay the signal (Jenkins, 2009; Rizini et al., 2011; Wu et al., 2012).

For UV-B can evoke a generalized cellular response, information perceived by UVR8 and other putative receptors has to be transduced *via* the second messengers to the target molecules, either proteins or genes (Broschè and Strid, 2003; Frohnmeyer and Staiger, 2003; Ulm, 2006). Some of the UV-B-responsive genes encode proteins participating in DNA repair, photosynthesis, cell

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cycle regulation, biosynthesis of the protective pigments, as well as the antioxidant enzymes and other wound/defence proteins such as pathogenesis-related protein 1 (PR-1) (Hollósy, 2002; Broschè and Strid, 2003). UV-B response of plant cell also involves the reactive oxygen (ROS) and nitrogen species (RNS) formation, cytoplasmic Ca^{2+} content increase, burst-like synthesis of such phytohormones as ethylene, abscisic, salicylic and jasmonic acids, ion channels and kinase/phosphatase cascades activation and other reactions (Fraire-Velázquez et al., 2011). ROS, mainly, H_2O_2, singlet oxygen, superoxide and hydroxyl radicals could play a role of key signalling molecules under UV-B stress (Mackerness et al., 1999, 2001). Besides that, UV-B-induced plant morphological responses are assumed to be realized by the direct or indirect NO signalling (Mackerness et al., 2001; Zhang et al., 2003; Krasylenko et al., 2012). In addition, UV-B has been proposed to stimulate the expression of plant-pathogen-interaction genes *via* the octadecanoid pathway related to wound signalling (Surplus et al., 1998).

Despite the fact that numerous publications are devoted to UV signalling in plants, possible involvement of plant cytoskeleton as a highly responsive UV target is poorly studied. Therefore, in this review the effects of UV-B irradiation on plant cytoskeleton are highlighted regardless the ecological relevancy of UV-B doses as the data about the UV-B impact on plant microtubules and microfilaments is scarce.

Cytoskeleton as a common participant in responses of plant cell to environmental stimuli

Cytoskeletal network of plant cell is formed by the integrated arrays of microtubules (MTs), actin filaments, intermediate filaments, microtubule- and actin-related proteins and others (Gardiner et al., 2011, 2012; Wasteneys and Yang, 2004). Plant cytoskeleton provides the realization of such basic processes as cell division, growth and development, membrane anchorage, cell shape support and communication, polymer cross-linking, vesicle transport, cyclosis, etc. (Foster et al., 2003; Wasteneys and Yang, 2004). Its dynamic instability is one of the mechanisms of adaptive rearrangements (Baluška et al., 2001) in response to such environmental stimuli as light (Lahav et al., 2004), gravity (Kordium et al., 2008), cold (Sheremet et al., 2012), heat (Hussey and Hawkins, 2001), touch and wind (Telewski, 2006). Phytohormones (Foster et al., 2003; Wasteneys and Yang, 2004; Bright et al., 2006; Blume et al., 2012), regulatory intracellular molecules − Ca^{2+} (Lui et al., 2003), H_2O_2 **(**Bright et al., 2006), NO (Yemets et al., 2009, 2011), protein kinases and phosphatases (Yemets et al., 2008a,b; Blume et al., 2010) and many others control the organization and dynamic properties of cytoskeleton components, modulating thus the signal transduction cascades. Even more, it was postulated that the plant cytoskeleton is a major target of signalling events (Wasteneys, 2004). Multiple stress factors such as high salt (Wang et al., 2007), herbicides (Ovidi et al., 2001), and heavy/toxic metals content in soil (lithium (Bartolo and Carter, 1992), tungsten (Adamakis et al., 2010), lead (Liu et al., 2009), chromium (Eleftheriou et al., 2012) aluminium (Schwarzerovà et al., 2002), cadmium, nickel (Dovgalyuk et al., 2002) and others) as well as plant colonisation by viral, bacterial or fungal pathogens (Schmidt and Panstruga, 2007) also induce stress-responsive and/or adaptive MTs reorganization (Wasteneys and Yang, 2004).

As cytoskeleton orchestrates the important processes in plant cell listed above, and some of these basic processes are known to be affected by UV-B, we anticipate that cytoskeletal structures can be involved into intracellular UV-B signalling. Hence, tubulin in microtubules and/or actin in microfilaments as well as proteins of intermediate filaments may percept and transduce the signal from UV-B playing a role of UV-B downstream-effectors. However, both experimental design and target organisms are manifold and appreciably diverse, what makes the identification of these target molecules to be rather challenging task.

Numerous reports concerning the alterations of plant growth and morphology (root/shoot ratio and development reduction, lowered/increased rate of cell division, leaf thickening, cotyledon curling, axillary branching, increased flower number and diameter, etc.) as commonly observed responses to UV irradiation exist (Hollósy, 2002; Jansen et al., 2002; Ktitorova et al., 2006). For instance, the zygotes of *Fucus serratus* and *F. spiralis* subjected to UV-A revealed only the inhibition of cell division, though UV-B-irradiated zygotes remained spherical and could not divide, polarize and germinate to form rhizoides, what may be related to the cytoskeleton damage (Schoenwaelder et al., 2003). UV-B exposure in dose of 10.08 kJ m^{-2} d^{-1} inhibited cell division of wheat (*Triticum aestivum* L.) callus accompanied by different chromosomal aberrations such as micro- and multinuclei formation (Zhang et al., 2009) that also may be explained by the alteration of cytoskeleton organization, treadmilling and/or dynamic

instability. In leaves of pea (*Pisum sativum* L.), commelina (*Commelina communis* L.), and oilseed rape (*Brassica napus* L.) the enhanced (11−32 $kJ/m^{-2}d^{-1}$) UV-B exposure impaired the stomatal turgor and conductance, possibly, by changes in cell wall elasticity or, alternatively, by cytoskeleton reorganization in guard and neighbouring epidermal cells (Nogués et al., 1999).

In general, the underlying mechanisms of such UV-induced morphological changes remain poorly understood, and only a few articles are focused on the cytoskeleton rearrangement as one of the events highlighting UV-B-induced cellular responses in plants (Staxèn et al. 1993; Guo et al., 2010; Chen et al., 2011; Jacques et al., 2011; Krasylenko et al., 2012). Thus, the pioneer work investigated the direct effects of the enhanced UV-B (4–24 mmol photons/m^2) on *Petunia hybrida* mesophyll protoplasts that led to the reversible dose-dependent fragmentation of cortical MTs and to the inhibition of cell cycle progression in $G_1/S/G_2$ phases at 24 h after the irradiation (Staxèn et al., 1993). It has to be noted that at 72 h after the irradiation only in protoplasts exposed to 24 mmol photons/m^2 UV-B MTs were shorter than those of the non-irradiated protoplasts, while in other treatments MTs recovered their initial organization comparable to control (Staxèn et al. 1993). After the decade-long gap it was established that MTs in *T. aestivum* mesophyll protoplasts were depolymerized significantly to sticks and spots under the enhanced UV-B (10.08 $kJ \cdot m^{-2} \cdot d^{-1}$), and their fluorescence intensity decreased (Guo et al., 2010). The experiments of UV-B impacts on MTs using *gfp-map4* (*m*icrotubule-*a*ssosiated *p*rotein 4) expressed in *Arabidopsis thaliana* seedlings revealed that the UV-B exposure (13.6–68 kJ/m^2) randomize, depolymerize or/and stabilize both interphase and mitotic MTs in epidermal as well as in cortex cells of all primary root zones in dose-dependent manner (Krasylenko et al., 2012). For example, in 2 h after the 13.6 kJ/m^2 cortical MTs became randomized only in epidermal cells of the transition, elongation and partially differentiation zones as well as in epidermal cells of root tip. In 2 h after 27.2 and 34 kJ/m^2 UV-B exposure the randomization and/or fragmentation of MTs occurred not only in epidermal cells, but also in cortex cells of all root zones. The observed UV-B-induced MTs randomization is supposed to be the moving force of epidermal cell swelling and excessive root hairs formation. It was shown that the most sensitive to UV-B were cortical MTs in transition/elongation zones as they depolymerized immediately after the UV-B exposure (Krasylenko et al., 2012), what gives additional evidence that the transition zone is a signalling–response nexus in the root. In this zone the inputs from endogenous (hormonal) and exogenous (sensorial) stimuli are integrated and translated into signalling and motoric outputs as adaptive differential growth responses (Baluška et al., 2010). In details, transition zone cells are known to be sensitive to auxin, ethylene and extracellular Ca^{2+} as endogenous factors as well as mechanical pressure, aluminum, and microorganisms as exogenous factors (Baluška et al., 2001). In turn, cortical MTs in epidermal cells of the transition zone are also exceptionally sensitive to fluctuations of auxins (Takahashi et al., 2003), NO content (Yemets et al., 2009, 2011), protein kinases/phosphatase inhibitors (Sheremet et al., 2010) and cold treatment (Sheremet et al., 2012).

Furthermore, in 6 h after UV-B irradiation (27.2 and 68 kJ/m^2) of control and *gfp-map4*-expressing *A. thaliana* seedlings, whose primary roots were covered with aluminum foil in order to protect them from the direct UV-B influence (shielded roots), dose-dependent MTs randomization, depolymerization or bundling in epidermal root cells of transition and elongation zones were established by laser scanning confocal microscopy. MTs organization in epidermal cells of above- and underground *A. thaliana* organs differed in sensitivity to UV-B. The most resistant were MTs in stomatal cells of adaxial leaf surface, and, in a lesser extent, in hypocotyls, as they were oriented radially after the UV-B exposure (Krasylenko et al., 2011). In stomatal cells of non-irradiated seedlings MTs were organized in radial net of toughly adjacent bundles (Shi et al., 2009). Less resistant in comparison to stomatal cells were MTs in leaf epidermal cells that were partially depolymerized after 27,2 kJ/m^2 and completely depolymerized after 68 kJ/m^2 UV-B exposure, while in non-irradiated roots MTs oriented uniformly randomly. In cells of non-irradiated abaxial side MTs organization remains unaltered similar to control (Krasylenko et al., 2011). Relative resistance of MTs in the aboveground organs epidermal cells as compared to root cells could be explained by the presence of photoprotection and photoreparation mechanisms (Jansen, 2002). It was shown recently by other authors that UV-B inhibited the growth of *A. thaliana* leaf plates without MTs reorganization in adaxial leaf surface epidermal cells (Hectors et al., 2010, Jacques et al., 2011). The difference of

these results from our data could be explained by that other authors chronically exposed the leaves of the mature two-weeks-old *A. thaliana* plants with the elaborated defense mechanisms to low UV-B for 20 days that induced accumulative effects.

It is well-known that under chronic stresses MTs are able to be adaptively rearranged and/or reorganized because of their dynamic instability and threadmiling (Wasteneys and Yang, 2004), as well as by α- and β-tubulin posttranslational modifications, for instance, phosphorylation, acetylation and tyrosination/detyrosination cycle that define MTs stability (Blume et al., 2007; Yemets et al., 2008a; Blume et al., 2010). This can be the reason, why MTs rearrangements could not be visualized under chronic UV-B exposure by doses close to natural ones. In turn, MTs in hypocotyls' epidermal cells were more perceptible to UV-B then MTs in leaf epidermal cells, as 27.2 and 68 kJ/m^2 UV-B exposure caused partial depolymerization of MTs in hypocotyls cells (Krasylenko et al., 2011). Indeed, the indirect UV-B impacts on MTs organization in shielded *A. thaliana* root cells is an issue of special interest, since cortical MTs in epidermal cells of transition zone were the most sensitive to indirect UV-B effects. In 2 h after the UV-B exposure they become randomized or depolymerized, while in the same cells of non-irradiated roots MTs were oriented transversely. In epidermal cells of elongation and differentiation zones MTs randomization in 2 h after the UV-B irradiation also occurred, while in root apex cells there were no MTs rearrangements (Krasylenko et al., 2011). These data give extra evidence to the existence of the stress signal transduction mechanism from the irradiated aboveground organs to non-irradiated underground ones *via* such long-distance secondary messengers as ROS and RNS that were proposed to be the important secondary messengers under UV-B stress in plants *in vitro* and *in vivo* (Mackerness et al., 2001; Zhang et al., 2003; An et al., 2005; Shi et al., 2005; Krasylenko et al., 2012). It is supposed that NO could be involved into MTs reorganization under UV-B influence, because exogenous NO donors and scavenger, as well as the modulator of its endogenous content cause MTs reorganization in epidermal cells of the sensitive transition and elongation zones of *A. thaliana* primary root cells (Yemets et al., 2009, 2011).

Moreover, UV-B-induced MTs reorganization in cells of shielded *A. thaliana* roots was accompanied by the alteration of MTs-related processes of growth and differentiation. Thus, the growth inhibition of shielded *A. thaliana* primary roots was not as significant as in non-shielded ones. In 24 h after the UV-B exposure, the successive epidermal cells swelling together with the intense root hairs formation in differentiation zone of shielded roots was revealed as compared to non-irradiated roots what points out to the activation of the morphogenetic processes. In UV-B-induced plant morphogenetic response and in the formation of the respective stress phenotype may participate phytohormones (especially, auxins and ethylene) and ROS (Potters et al., 2009). Since in UV-B-exposed plant cells ROS, RNS and NO are formed (Mackerness et al., 2001; Zhang et al., 2003), we could suggest the involvement of the latter into plant morphogenic responses induced by the UV-B. Taking into account that during oxidative stress endogenous NO content in plant cells was shown to increase under UV-B exposure (Mackerness et al., 2001; Zhang et al., 2003), the combined effects of NO-modulating chemicals and UV-B irradiation on MTs organization of were studied. The seedlings of *A. thaliana* (GFP-MAP4*)* were exposed to enhanced UV-B doses (6.8–68 kJ/m^2) with or without sodium nitroprusside (SNP) as exogenous NO donor, or 2-(4-carboxyphenyl-4,4,5,5-tetramethylimidazoline-1-1oxyl-3-oxide potassium salt (c-PTIO) as its specific scavenger pretreatment. In 24 h after UV-B irradiation SNP-pretreated *A. thaliana* seedlings partially recovered MTs organization in epidermal cells of elongation zone, whereas c-PTIO-pretreated ones have not markedly improved it in cells of the same root zone (Krasylenko et al., 2012). It was also shown that SNP pretreatment of UV-B irradiated *A. thaliana* seedlings rescued the UV-B-inhibited root growth in 48 h as distinct from c-PTIO pretreatment (Krasylenko et al., 2012). Furthermore, SNP also partially recovered UV-B-altered primary root morphology and returned UV-B-disturbed MTs organization to the initial one in epidermal cells of *A. thaliana* root cells on the contrary to c-PTIO pretreatment. Hence, NO donor protects microtubule organization as well as MT-mediated root growth and development from the disrupting UV-B effects that corroborate the results of other authors (An et al., 2005; Shi et al., 2005). As MTs organization in *Arabidopsis* root cells depends on NO content under the enhanced UV-B exposure, we suppose that microtubules can be components of NO-mediated signalling cascades induced by UV-B stress.

Cytoskeletal reorganization along with DNA phototransformation, membranes and cell morphology alterations is considered to be the key hallmark of UV-induced apoptosis in mammalian

cells (Ndozangue-Touriguine et al., 2008). Nevertheless, the involvement of both MTs and microfilaments (MFs) in programmed cell death in plant cells is still unknown. To study the role of MTs and MFs in cell death, the suitable model for *in vitro* vital cytoskeleton visualization − the cells of *Nicotiana tabacum* Bright Yellow-2 (BY-2) suspension culture expressing *gfp-mbd* (*m*icrotubule-*b*inding *d*omain of MAP4) was chosen. As BY-2 cells are highly resistant to the enhanced UV-B, the doses of 34, 81 and 135 kJ/m^2 were (SNP) as used (Lytvyn et al., 2010). Dose-dependent depolymerisation of both interphase and mitotic microtubules occurred in 3 h after the exposure together with the cytoplasm shrinkage and chromatin condensation (Lytvyn et al., 2011). However, MTs were only randomized in cells that have not undergone apoptosis, as in 3 h after 81 and 135 kJ/m^2 UV-B exposure the apoptotic cells percentage was 23.8% and 29 %, respectively (Lytvyn et al., 2010). MTs depolymerization was also accompanied by micronuclei formation and cytoplasm vacuolization (Lytvyn et al., 2011).

The main finding in these experiments is the clear correspondence of MTs depolymerisation and cytoplasm shrinkage that could point out at MTs involvement in the mediation of the apoptotic cytoplasm retraction in the UV-B-irradiated plant cells. Plant mictotubular cytoskeleton is also regulated by UV-B on genetic level, since rapid transcriptome responses of maize (*Zea mays* L.) occurred in irradiated and shielded tissues, and 11 cytoskeleton genes including α- and β-tubulin 1 as well as actin 4 were downregulated (Casati and Walbot, 2003).

The organization of other plant cytoskeleton component, such as F-actin filaments, was disturbed in interphase cells of wheat root-tip under the enhanced UV-B (10.08 kJ·m^{-2}·d^{-1}) (Chen et al., 2011). The F-actin arrays disintegrated into randomized short fragments during prophase, and totally disappeared in meta-, ana- and telophases that was accompanied by such chromosome aberrations as logging, bridges formation and partition-bundle division (Chen et al., 2011).

Conclusions

The data presented here show that the cytoskeleton components could be implemented in UV-B signalling pathways, since both microtubules and microfilaments respond to this abiotic factor in dose-dependent manner either by the reorientation or by the reorganization (randomization, fragmentation or depolymerization). The paradigm shift in relation of cytoskeleton role in cell biology has been occurred recently. For a long time, it has been supposed to play a role of eukaryotic cellular "scaffolding", however, actually, the cytoskeletal proteins are the key participants in many signalling events.

In general, the identification of the cytoskeletal players in intracellular UV-B signalling cascades and the ways they cross-link in plant cell are very promising for the development of the protective approaches aimed to enhance plant tolerance to persistently pressing abiotic stress factors.

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REVIEW ARTICLE

Influence of enhanced UV-B radiation on wheat production in relation with abiotic, biotic and socioeconomics constraints

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Abstract

The light is one of the most important factors that regulate growth and development of plants. However, the increase of the ultraviolet-B radiation due to the anthropogenic action can have negative impacts on these processes, producing a decreased photosynthesis and biomass production. Zonal average ultraviolet irradiance (flux ultraviolet, FUV) reaching the Earth's surface has significantly increased since 1979 at all latitudes except the equatorial zone. Depletion of the stratospheric ozone layer leads to an increase in ultraviolet-B (UV-B: 280-320 nm) radiation reaching the Earth's surface, and the enhanced solar UV-B radiation predicted by atmospheric models will result in reduction of growth and yield of crops in the future. Over the last two decades, extensive studies of the physiological, biochemical and morphological effects of UV-B in plants, as well as the mechanisms of UV-B resistance, have been carried out. In this review we didn't obtain evidences to show that the increased UV-B radiation influences the oscillations observed in the wheat production in major producing countries in the world. The most important constraints observed on wheat production are heat (affecting up to 57% of the entire wheat area in surveyed countries), competition with weeds, and diseases (both affecting up to 55% of wheat area). Of the socioeconomic constraints listed and evaluated, the access to mechanization and availability of credit were the most often highlighted. The way to improve wheat production in the new scenarios consequence of global environmental changes is the genetic breeding. Breeding wheat cultivars with increased grain yield potential, enhanced water-use efficiency, heat tolerance, end-use quality, and durable resistance to important diseases and pests can contribute to meet at least half of the desired production increases. The remaining half must come through better agronomic and soil management practices and incentive policies.

Key words: Climate changes, Genetic breeding UV-B radiation, Yield production, Wheat

Introduction

Solar radiation is one of the major environmental factors that affect life on our planet. This radiation controls the functioning of terrestrial and aquatic ecosystems by controlling photobiological processes (photosynthesis, photoperiod, phototropism, etc.), through its action on other environmental factors (temperature, humidity, etc.) and natural cycles (cycles per day, annual, water, etc.) which ultimately affect the distribution of organisms. High radiation intensities and spectral composition changes may affect important processes in organisms, especially plants that can not move are left to adapt to such changes.

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One of the main changes that happened this last time has been increased UV-B (Blumthaler and Ambach, 1990). That is due to the destruction of the ozone layer by polluting compounds such as chlorofluorocarbons (CFCs), oxides of nitrogen, chlorine, bromine, etc. These compounds tend to form stable compounds with ozone (O_3) with a half-life of 50 to 150 years. UV-B radiation is comprised between the wavelengths 290 and 320 nm. Other components of the UV radiation are the UV-C, between 220 and 290 nm, and the UV-A between 320 and 400 nm. This last radiation is bit absorbed by O_3, so that arrives in greater quantity to the surface of the Earth and is an important photomorphogenic signal in plants and is the least harmful. By contrast, the UV-C is the most energetic and harmful to the DNA. However, since is mostly absorbed by oxygen (O_2) and O_3 in the stratosphere hardly reaches the Earth's surface.

Understanding of relationships between crop and environment has substantially improved during

the last few decades of the 20th century. Anthropogenic factors are continuously changing the environment, and projections are that atmospheric CO_2 concentrations will double and temperatures will increase by 5.5ºC by the end of current century (Houghton et al., 2001).

Current stratospheric ozone levels are at the lowest point since measurements began in 1970s (figure 1) and global terrestrial UV-B radiation levels range between 0 and 12 kJm^{-2} on a given day with near Equator and mid-latitudes receiving higher doses (Total Ozone Mapping Spectrometer, 2005, http://ozoneaq.gsfc.nasa.gov/measurements.md).

The destruction of the ozone layer has been more intense at high latitudes, particularly in Antarctica, where ozone concentrations have decreased by 40-50% compared to the values obtained in 1980 and minor changes in the area of Ecuador in 3-6% (35-60° N and 35-60° S), where UV radiation is intense in nature (UNEP, 2002). Accordingly, from 1980 to present, the flux of UV-B, mainly within the range of 290-315 nm, has increased in the troposphere on average 6-14% (Kakani et al., 2003). Therefore, since the discovery of so-called "hole" in ozone in the Antarctic, the main interest in studying the effects of ultraviolet-B radiation on plants has increased considerably.

Although UV-B comprises a small region of the electromagnetic spectrum, its effect on plants and animals is considerable. Thus, plants early in their evolution have had to adapt to their presence and develop mechanisms to reduce adverse effects. It emerged recently that there is an inter-relationship between drought and ultraviolet-B radiation in plant responses, in that both stresses provoke an oxidative burst. 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. In plants, wide inter- and intraspecific differences have been reported in response to UV-B radiation with respect to growth, production of dry matter and biochemical changes (Kramer, 1991). Some plant species are unaffected by UV-B radiation and several are apparently stimulated in their growth, but most species are sensitive and undergo damage results (Teramura, 1983). Furthermore, numerous environmental factors have also been shown to weaken or enhance the responses of plants to UV radiation.

Drought is an important environmental constraint that limits the productivity of many crops and affects both yield quality and quantity (Boyer, 1982). Drought stress reduces growth rate, stem elongation, leaf expansion and stomatal movements (Hsiao, 1973). Furthermore, it causes changes in a number of physiological and biochemical processes governing plant growth and productivity (Daie, 1988). Under field conditions, plants usually experience several stresses simultaneously, which may cause a variety of plant responses that can be additive, synergistic or antagonistic.

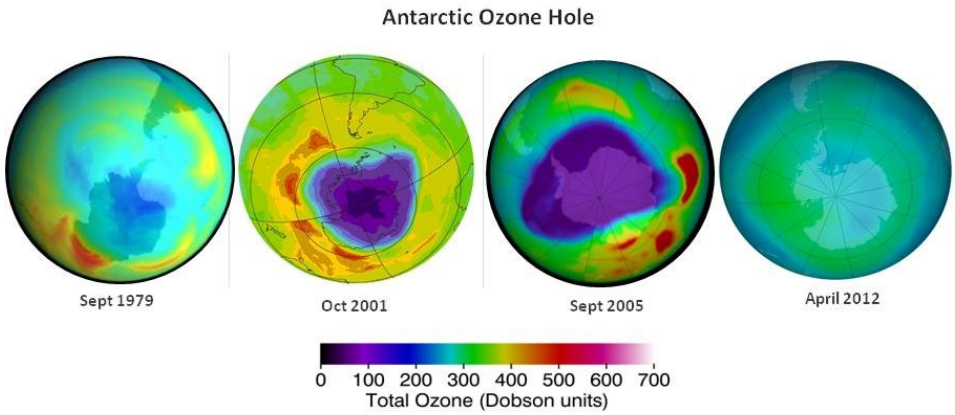

Figure 1. The false-color view of the monthly-averaged total ozone over the Antarctic pole (April 2012). The blue and purple colors are where there is the least ozone, and the yellows and reds are where there is more ozone.
Available in: http://svs.gsfc.nasa.gov/Gallery/index.html

Evidence of interaction between UV-B exposure and drought stress in plants has emerged in recent years, but the mechanisms involved have received little attention. Some investigations have been carried out on agricultural or model plants, despite the fact that crops account for only 6% of the plant productivity worldwide (Teramura et al., 1983; Tevini et al., 1983; Sullivan and Teramura 1990; Balakumar et al., 1993; Schmidt et al., 2000). Elucidation of the interaction between drought and UV-B stresses would help in understanding the potential impact of partial stratospheric ozone depletion on plant adaptation to changing environmental condition. However, the mechanisms of sensitivity or tolerance of crop plants, either in growth, yield, or combined stresses remains unknown.

Interaction of UV-B radiation with other factors of climate change

The combined effect of high UV-B radiation and water deficit has been approached in several studies, which are showing a reduction of plant growth and alteration of several physiological and biochemical processes (Alexieva et al., 2001). Both environmental factors act synergistically on plant secondary metabolism by increasing the production of flavonoids (Hofmann et al., 2003). In addition, drought and high flow UV-B radiation induce the production of cuticular waxes thus facilitating the reflection of light and water conservation (Steinmüller and Tevini, 1985). Enhanced UV-B radiation has a significant negative effect on growth and biomass production of wheat plants. Many studies agree that wheat was considered sensitive to UV-B radiation (Biggs et al., 1984, Rozema et al., 1991; van Staaij, 1994). The reduction in plant dry weight may be explained by UV-B induced changes in morphological and physiological processes. High UV-B radiation changes the structural characteristics of wheat plants, particularly by increasing Specific Leaf Area (SLA) indicated by altered leaf morphology. The trend to higher SLA indicates that UV-B radiation decreases the leaf thickness (Correia et al. 1999; Santos et al., 1993). This decrease may be important for two reasons. Primary, because it changes the light inside the leaves, which could also explain the decrease in the Chla/Chlb ratio (Deckmyn and Impens, 1995), secondly because increased SLA has been correlated with decreased photosynthetic rates, contributing to lower relative growth rates (Poorter, 1989).

Wheat yield progress worldwide

Wheat is one of the most important cultivated crops in the world with a worldwide production of 676 million tons in 2011, representing a growth of 3.4 percent from 2010 (FAO - Crop Prospects and Food Situation report, 2011). In figure 2 is represented the yield progress since 1985 to 2010. It was consider the prospects for continued yield growth, in particular that resulting from plant breeding, which it is believed is becoming a proportionally larger component of yield growth. Possible yield changes result from shifts in cropping regions or proportions irrigated changes in cropping intensity, or climate change, factors that need to be considered for a complete understanding of wheat yield changes.

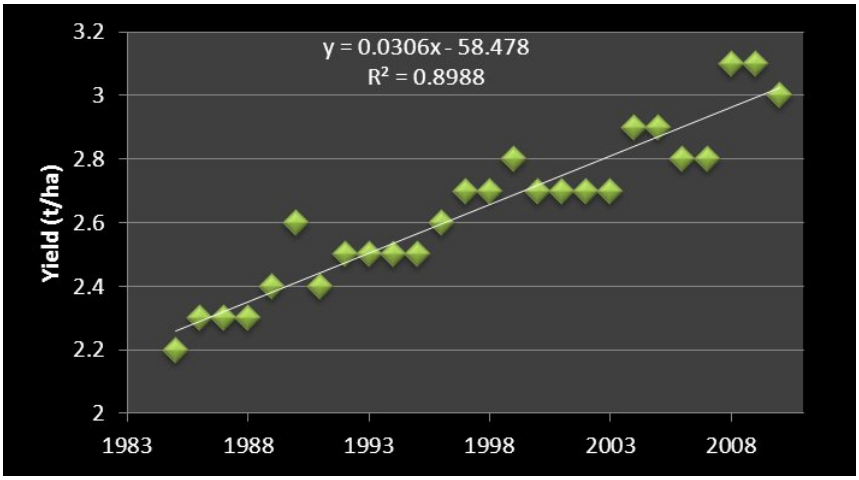

Figure 2. World yield for wheat from 1985 to 2010.
Source: FAOSTAT. 2012. (Available at http://faostat.fao.org/site/567/DesktopDefault.aspx#ancor)

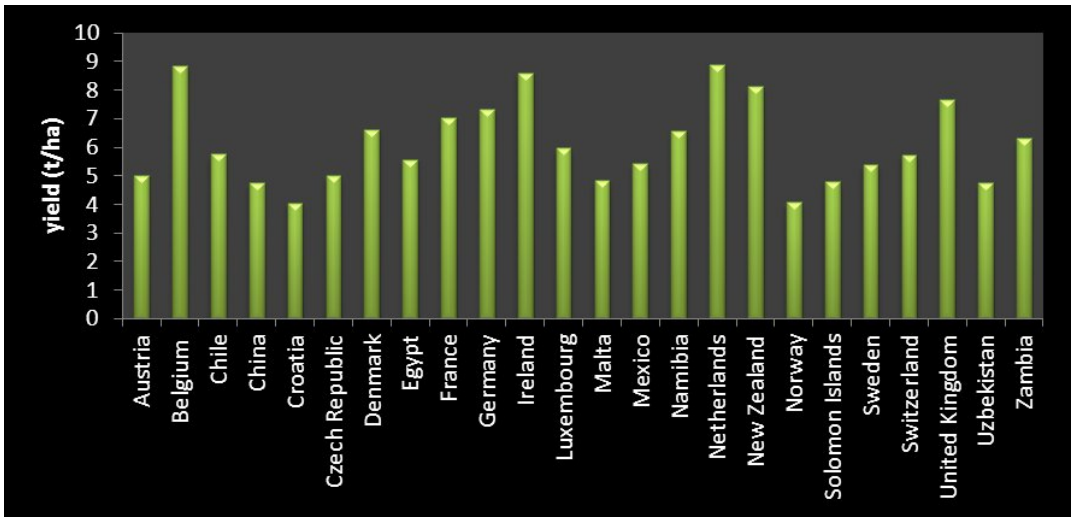

Figure 3. Wheat yields: 24 countries with more than 4 ton/ha of wheat production in 2010.
Source: FAOSTAT 2012. (Available at http://faostat.fao.org/site/567/DesktopDefault.aspx#ancor)

Figure 3 shows the highest production of wheat countries in the world, with more than 4 t/ha.

Wheat cultivated area in many countries have increased or are expected to increase in 2011 in response to strong prices, while yield recoveries are forecast in areas that were affected by drought in 2010 (FAO - Crop Prospects and Food Situation report, 2011).

The most important wheat producers in the world

When studying a period of 10 years (2000-2010), it appears that the production of wheat in major producing countries is uneven, with the exception of India and China that additionally is increasing its production in the last years**.**

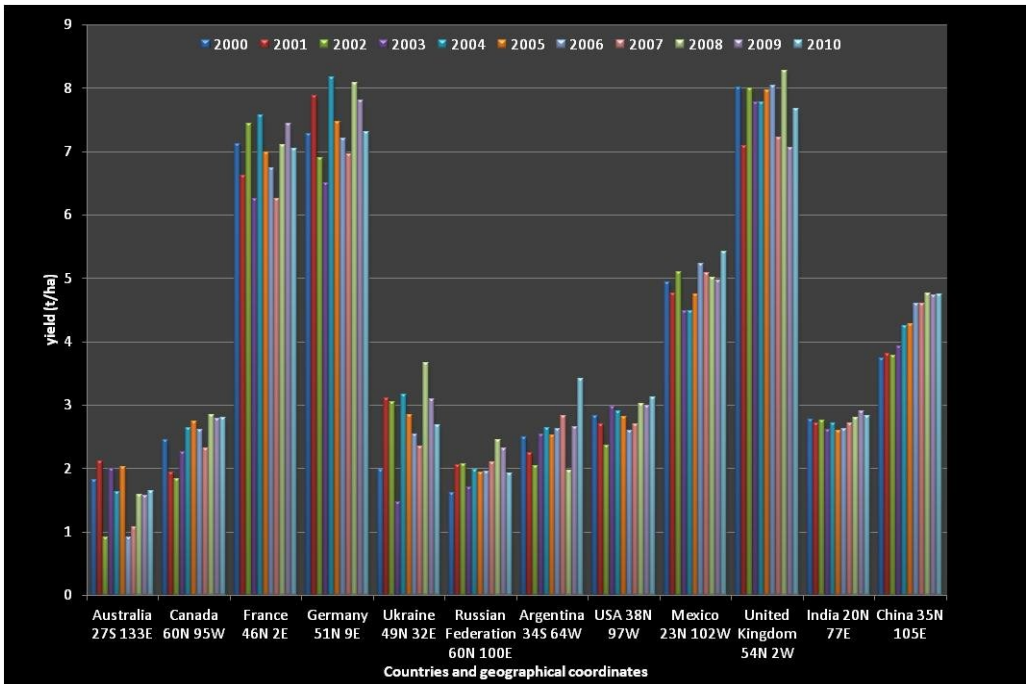

Figure 4. Average production of wheat in world major producing countries in the period 2000-2010.
Source: FAOSTAT 2012 (Available at http://faostat.fao.org/site/567/DesktopDefault.aspx#ancor)

Much of the observed fluctuations in yields in most producing countries, are primarily related to abiotic constraints, especially in Australia where the climate impact is more evident in the yield average obtained. FAO, through the report "Crop Prospects and Food Situation" (2011), estimate an increase in wheat production worldwide mainly supported by the yield of most countries shown in Figure 3 and 4, but it is interesting to highlight that there is a significant number of other constraints that can also influence the increased production in countries with lower rates of development.

Major constraints to wheat production

The actual crops productivity not only depends on the sensitivity of different species showing the effect of UV-B radiation (figure 5) but also depends on the interaction with other biotic and environmental factors (Caldwell and Flint, 1994). Accordingly, in the context of climate change experienced in recent times, has been observed, in addition to increased UV-B, an increase of CO_2 and temperature as well as significant changes in the frequency and quality of precipitation (Caldwell et al., 2007).

Figure 5. Geographical location of major wheat producing countries in the world.

The scientific consensus predicts a global temperature increase between 1.5 and 4.5°C over the next 100 years in agreement with the temperature anomalies already verified from 1992 to 2011 presented on figure 6, plus the existing increase of 0.6°C that has experienced the atmosphere since the industrial revolution (Caldwell et al., 2007).

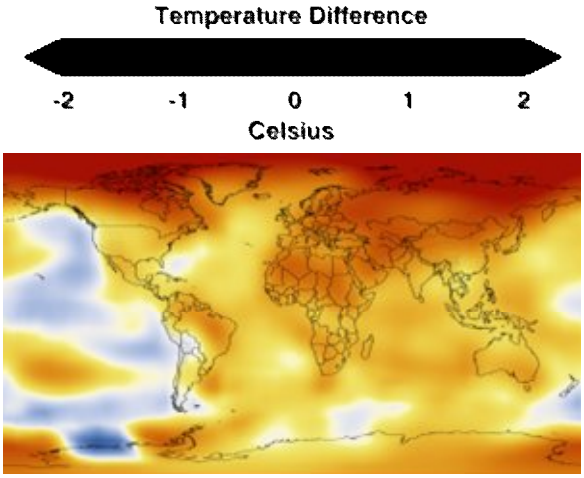

Figure 6. Global temperature anomaly data from 1992 through 2011.
Available in: http://svs.gsfc.nasa.gov/goto?3901

A survey, conduct by Kosina et al. (2007), covered nineteen developing countries, including major wheat producers, prior to the 2006 International Symposium on Increasing Wheat Yield Potential in Ciudad Obregon, Mexico. Collectively these countries represent 102 million hectares of wheat (47% of the global wheat area or 89% of the wheat area in developing countries) and 285 million tons of wheat production (45% of the global wheat production or 92% of wheat production in developing countries (FAO, 2006). The results emphasize the substantial yield losses associated with a number of critical abiotic, biotic and socioeconomic constraints, and indicate their global prevalence.

Abiotic stress

A major constraint that is estimated to affect up to 58.7 million hectares of wheat area in sample countries (57.3% of entire wheat area in surveyed countries) is heat. Average estimated yield loss caused by extreme temperatures varies between 14.7 and 31.3%, depending on the region. The total estimated loss amounts to 21 million tons. The largest areas affected by heat stress were identified in Central, South and Southeast Asia. The major threat is terminal heat stress at anthesis and during grain filling period, which accelerates maturity and reduces significantly grain size, weight, and yield (Kosina et al., 2007; Dias and Lidon, 2009).

Low rainfall (moisture stress) is the second most significant abiotic constraint to wheat production in terms of area potentially affected, concerning 42.6 million hectares (41.6% of wheat area in surveyed countries). Estimated yield loss (in average) caused by low rainfall varies between 19.3 and 50.4%, and overall is estimated to cause losses of 31 million tons. Areas potentially affected by low rainfall are present in four regions: South and Southeast Asia, Central and West Asia, Northern Africa, Sub-Saharan Africa and Latin America. The most common threat is yearly fluctuation (periodically occurring 'dry years') and irregular seasonal distribution of rainfall.

A third important constraint to wheat production, potentially affecting up to 38.4 million hectares of wheat is the declining availability of irrigation water. Average estimated yield losses caused by declining availability of irrigation water varies between 20 and 37.2% and can cause losses of up to 21.8 million tons of wheat annually. The largest proportion of potentially affected areas appears to be in South and Southeast Asia. Reasons for declining availability of irrigation water include overexploitation of ground water resources, competition with other crops (cash crops), restrictive governmental policies, and deterioration of irrigation infrastructure. Factors such as lodging, physical soil degradation and microelement deficiencies affect approximately 28-30 million hectares. Potential losses in terms of wheat production oscillate between 7.7 and 20%, which represents an aggregate loss of 6-8 million tons of wheat for each of the three constraints.

The main causes of lodging include tall varieties (weak straw), poor crop management, high yield (over 6 t/ha) in wet years (excessive irrigation), heavy rains, and windy conditions. Soil degradation is reported to occur mainly due to heavy tillage and mismanagement causing soil compaction, organic matter depletion, soil erosion, and water logging. Micronutrient deficiencies, such as an unavailability of zinc and boron, often stem from pH imbalances. Relatively smaller areas of wheat production are affected by other factors such as cold (15.8 million hectares), mainly in Central and West Asia, China and South America; salinization (11 million hectares) in Central and South Asia; and microelement toxicity (1.2 million hectares) mainly in Turkey and Brazil. These three constraints may cause annual losses of 5, 3.5, and 0.5 million tons of wheat, respectively. Cold refers to sporadic frost damage to susceptible varieties, particularly in the case of winter wheat and in mountain areas. Saline soils are a growing problem, especially in arid and semiarid areas and in fields exposed to excessive irrigation. Problems with microelement toxicity (Al, Mn, and Bo) occur mainly in areas with low pH conditions.

Biotic stress

Biotic stresses are reported to affect roughly the same area as heat stress. Estimated yield loss caused by weeds varies between 8.5 and 23.9%, depending on the region, and overall could cause up to 24 million tons in losses annually. Among the most often mentioned weeds are *Avena* spp., *Phalaris* spp., *Chenopodium* spp., *Rumex* spp., *Medicago* spp., *Amaranthus* spp., *Lolium* spp., *Polypogon* spp., *Convonvulus* spp., and *Echinochloa* spp. Likewise, diseases are rated nearly equally in importance, affecting roughly 56 million hectares. The most serious diseases cited are leaf and stripe rusts (*Puccinia triticina* and *P. striiformis*), Fusarium head blight (*Fusarium* spp.), Septoria blotch (*Septoria tritici*), powdery mildew (*Erysiphe graminis*), tan spot (*Pyrenophora tritici repentis*), spot blotch (*Bipolaris sorokiniana*), bunts (*Tilletia* spp.), and eyespot (*Cercosporella herpotrichoides*). Although pests (especially insect

pests) are usually reported as a less binding constraint in wheat, potentially affected areas cover approximately 47 million hectares. Estimated yield loss caused by pests varies between 12.2 and 22% and can overall cause up to 20 million tons of loss annually. The most often mentioned insect pests include aphids, sunn pest (*Eurygaster* spp.), Hessian Fly (*Mayetiola destructor*), weevils, termites and some other species of minor importance (Kosina et al., 2007).

Socioeconomic constraints

Many socioeconomic constraints are related to agricultural policies and institutions that potentially affect the entire wheat crop (Kosina *et al*., 2007). The first reported lack was the access to mechanization (suitable machinery) as a constraint, mainly related to high purchasing and operational costs, and unavailability of small-scale and zero tillage machinery. The second most socioeconomic constraint is availability (and level) of credit. High interest rates, insufficient credit resources, lack of timely access in rural areas, and unwillingness of financial institutions to provide credit to the agricultural sector (particularly to subsistence/staple crops) were the most frequently reported constraints. Seed availability/quality and fertilizer availability is also an important constraint (Kosina et al., 2007). Such constraints will affect more significantly the developing countries not having the same impact in developed countries; however, the impact in the global production of wheat will be important.

The importance of genetic breeding in a global environmental change

Although more food is needed for the rapidly growing human population, food quality also needs to be improved, particularly for increased nutrient content. In addition, agricultural inputs must be reduced, especially those of nitrogenous fertilizers, in order to reduce environmental degradation caused by emissions of CO_2 and nitrogenous compounds from agricultural processes. Furthermore, there are now concerns about the ability to increase or even sustain crop yield and quality in the face of dynamic environmental and biotic threats that will be particularly challenging in the face of rapid global environmental change (Tester and Langridge, 2010). This scale of sustained increase in global food production is unprecedented and requires substantial changes in methods for agronomic processes and crop improvement.

Certain aspects of global environmental change are beneficial to agriculture. Rising CO_2 acts as a fertilizer for C_3 crops and is estimated to account for approximately 0.3% of the observed 1% rise in global wheat production (Fisher and Edmeades, 2010), although this benefit is likely to diminish, because rising temperatures will increase photorespiration and nighttime respiration. A benefit of rising temperatures is the alleviation of low-temperature inhibition of growth, which is a widespread limitation at higher latitudes and altitudes. Offsetting these benefits, however, are obvious deleterious changes, such as an increased frequency of damaging high-temperature events, new pest and disease pressures, and altered patterns of drought. Negative effects of other pollutants, notably ozone, will also reduce benefits to plant growth from rising CO_2 and temperature. Particularly challenging for society will be changes in weather patterns that will require alterations in farming practices and infrastructure; for example, water storage and transport networks. Because one-third of the world's food is produced on irrigated land (Munns and Tester, 2008), the likely impacts on global food production are many. Along with agronomic- and management-based approaches to improving food production, improvements in a crop's ability to maintain yields with lower water supply and quality will be critical. Put simply, there is a need to increase the tolerance of crops to drought and salinity.

In the context of global environmental change, the efficiency of nitrogen use has also emerged as a key target. Human activity has already more than doubled the amount of atmospheric N_2 fixed annually, which has led to environmental impacts, such as increased water pollution, and the emission of greenhouse gases, such as nitrous oxide. Nitrogen inputs are increasingly being managed by legislation that limits fertilizer use in agriculture. Furthermore, rising energy costs means that fertilizers are now commonly the highest input cost for farmers. New crop varieties will need to be more efficient in their use of reduced nitrogen than current varieties (Peoples et al., 1995). Therefore, it is important that breeding programs develop strategies to select for yield and quality with lower nitrogen inputs.

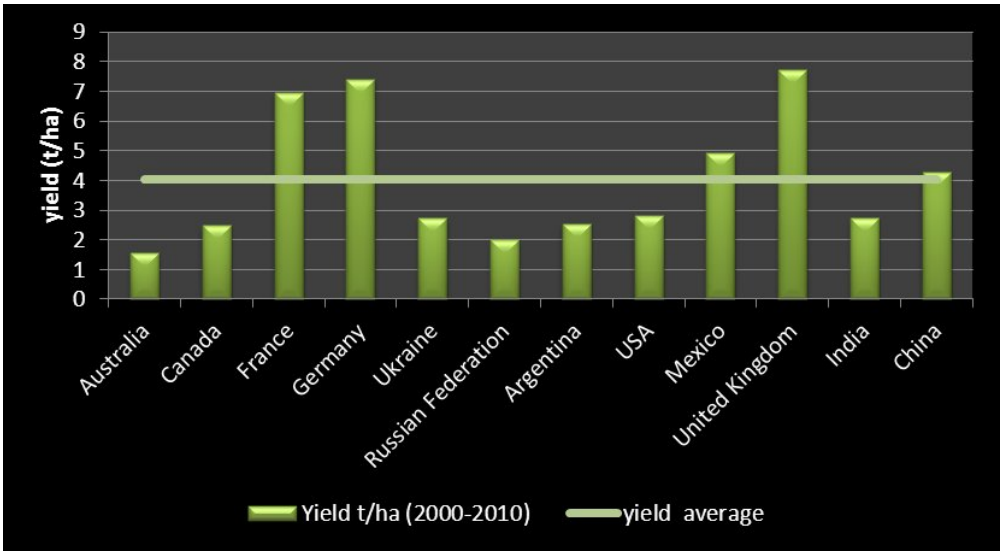

Figure 7. Yield wheat in major countries in the world.

Current approaches to crop improvement

Questionably, increased yield in conditions of abiotic stresses, such as drought and salinity, could be best achieved by selecting for increased yield under optimal production conditions: plants with higher yields in good conditions are more likely to have higher yields in stressed conditions (Richards, 1992). Such an approach will also increase yield in high-yield environments. However, it is becoming increasingly apparent that specific selection for stressed environments is efficient. Given that average global yields of wheat are more or less 4 t/ha and that there are some areas with yield as high as 7 t/ha, the majority of land cropped to wheat delivers yield below 3 t/ha (Figure 7).

Therefore, by virtue that globally low-yielding land represents much larger areas, low-yielding environments offer the greatest opportunity for substantial increases in global food production. Increasing yield by 1 t/ha in a low-yielding area delivers a much higher relative increase than does the same increase in high-yielding environments. This increase can be achieved by tackling major limitations on yield in poor environments (termed yield stability); for example, by protecting plants and yield from factors such as salinity and heat or drought periods. The local social benefits of supporting farmers on low-yielding lands would also be great. It is often thought that concentration on yield stability may come at the expense of high yield in good years; however, yield penalties in more favorable conditions do not necessarily accompany drought tolerance. Select for yield stability is harder than improved yield, because selection in breeding programs requires many years and many sites for evaluation. However, there is evidence for a genetic basis for yield stability and, hence, an opportunity for improvement (Kraakman et al., 2006). There are several clear examples where single genes have been able to substantially increase yield, notably to drive domestication (to control tiller number, branching, and seed number) and the green revolution (for dwarfing). Initial results suggest that a gene conferring increased drought tolerance may also have a widespread impact on yield (Nelson et al., 2007), which doesn't mean that efforts to maintain yield should be reduced. In particular, maintaining resistance to rapidly evolving pests and pathogens is an essential mainstay of breeding programs. Interactions between breeders, pathologists, and agronomists must be maintained to ensure that crops and cropping systems change coordinately. No-till farming, in which plowing of the soil is avoided, for example, has changed the spectrum of diseases and pests attacking crops, to the extent that a change in breeding targets was needed. The development of multiple cropping systems will also demand interactions between agronomists and breeders. However, it is clear that further is required that can be provided by traditional breeding approaches.

Expanding the germplasm base for plant breeding

The success of plant breeding over the past century has been associated with a narrowing of the available genetic diversity within elite germplasm. New sources of variation include landraces and wild relatives of crop species, and although

exploiting wild relatives as a source of novel alleles has changed, it has provided notable successes in crop improvement. A particularly important example of the introgression of genetic information from a relative was the use of the short arm of rye chromosome 1R in wheat. In the early 1990s, this wheat-rye translocation was used in 45% of 505 bread wheat cultivars in 17 countries (Rabinovich, 1998). Progressively easy gene discovery, improved enabling technologies for genetics and breeding, and a better understanding of the factors limiting practical exploitation of exotic germplasm promise to transform existing, and to accelerate the development of new strategies for efficient and directed germplasm use (Tester and Langridge, 2010).

Most crop geneticists agree that enrichment of the cultivated gene pool will be necessary to meet the challenges that lie ahead. However, to fully capitalize on the extensive reservoir of favorable alleles within wild germplasm, many advances are still needed. These include increasing our understanding of the molecular basis for key traits, expanding the phenotyping and genotyping of germplasm collections, improving molecular understanding of recombination in order to enhance rates of introgression of alien chromosome regions, and developing new breeding strategies that will allow introgression of multiple traits (Feuillet et al., 2008).

Conclusions

Different mechanisms of adaptation to UV-B radiation have been documented in plants; to date research show that, primarily, plants develop strategies to prevent the penetration of this type of light. While plants have developed early in the evolution protective mechanisms efficient enough to prevent the harmful effects of natural UV radiation, the predictions of increased UV-B radiation could have a major impact on the crops productivity. Therefore, it is necessary to deal with this problem globally, with further studies that could predict changes that cause increased UV-B radiation on the distribution of vegetation and in the biocenosis associated. Moreover, in the context of climate change experienced in recent years, which not only has resulted in increased UV-B radiation but also in increasing the atmospheric concentration of CO_2 and temperature, it is difficult to predict how it will affect the complex interactions that occur between ecological and climatic processes. For this reason, it is important to have more information that will allow predicting the impact it could have on the interactions of these factors.

Global wheat production must continue to increase 2% annually until 2020 to meet future demands imposed by population and prosperity growth. Moreover, this must be achieved under reduced water availability, a scenario of global warming, stricter end-use quality characteristics, and evolving pathogen and pest populations. Most of the production growth must occur in developing countries where wheat will be consumed.

In what wheat is concerned, the geographic locations of the main producing countries exclude the hypothesis that there is some negative influence of the UV-B radiation harmful effect in their physiological development with implications in yield. There will be different abiotic, biotic and socioeconomic constraints that will have a greater impact on global wheat production. In developing countries the complications with biotic stresses and socioeconomic factors will have more impact. Importance of genetic breeding is emphasized, as a multidisciplinary activity which has an important role in obtaining varieties more suitable to the constraints associated with environment, as well as any changes due to climate change.

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REVIEW ARTICLE

Advantages and disadvantages of UV-B radiations on Grapevine (*Vitis* sp.)

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Abstract

UV radiation, as a natural component of sunlight and frequently mentioned in relation with climatic changes, has numerous regulatory effects on grapevine physiology and biochemistry. In last decades many studies gave significant advances in the understanding of the effects of UV radiation on compounds of the primary and secondary metabolism, especially those which impact grape and wine quality. Mechanisms of plant responses to solar UV-B radiation are therefore disadvantageous: such as inhibition effect on plant growth, but also advantageous such as accumulation of phenolic compounds and improved resistance to pathogenes. UV-B affects the secondary metabolism of plants and thus indicating that solar UV-B is to be regarded as an environmental challenge rather than a damage-inducing source of stress in vitiviniculture. UV irradiation might have a positive influence on grape "healthiness" or composition and consequently a positive impact on wine quality. This review provides a synopsis of the effect of UV radiation associated variables on grapevine physiology and biochemistry as potential key factor in the future of global grape production.

Key words: Climatic changes, Grape, Quality, Radiation, UV

Introduction

Vitiviniculture has developed to one of the most important agricultural sectors globally spoken, common to all continents today. According to the data by the International Organisation of Vine and Wine, nowadays 645 mio qs of grape are produced on approx. 7.6 mio ha of vineyards worldwide (OIV, 2010).

In the last decades, climatic changes have become a "daily bread", where an increase of temperature and UV radiation, unexpected rainfalls, storms, depletion of the ozone layer etc. are all predicted and inevitable events.

UV light is an electromagnetic radiation with a wavelength shorter than that of visible light, and is commonly divided into UV-A (320-400 nm), UV-B (280-320 nm) and UV-C (<280 nm). Furthermore, much of the UV-B (~97%) and all of the UV-C are absorbed by the ozone (O_3) in the stratosphere and never reach the surface of the earth. Caldwell et al. (1989) and McKenzie et al. (1999) reported that the depletion of stratospheric ozone causes the increase of UV-B radiation which reaches the earth's surface, therefore influence of UV-B irradiation is gaining the interest of scientific community.

The vineyards receive a different "quantity" and intensity of UV-B radiation, what mainly depends on the position of the sun, the exposure and inclination of the vineyards, arrangement of the vineyard (terraces, plain etc.) and cloudiness.

The entire UV spectrum has some of the biological functions of ionizing radiation, in doing far more damage to many molecules in biological systems as those caused by simple heating effects (for example sunburn) .

Cellular components such as proteins and nucleic acids absorb this radiation, resulting in biomass reduction, impaired photosynthesis and other chloroplast functions, decreased protein synthesis, damage to DNA. Effects of UV-B radiation include oxidative stress, and reactive oxygen species (ROS) have been shown to participate directly in the damage induced by high UV-B doses (Majer and Hideg, 2012).

Plants protect themselves from this potentially harmful radiation by altering metabolic functions and a number of studies confirmed the role of UV-B in the regulation of gene expression (Surplus et al., 1998; Brosche and Strid, 2003; Ulm and Nagy,

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2005; Jenkins, 2009). Actually UV-B is to be regarded as an environmental challenge rather than a damage-inducing source of stress (Jordan, 2002; Kolb and Pfündel, 2005).

Relevant consequences to vitivinicultural are mostly altered phenolic profiles (Kolb et al., 2003), as well as susceptibility to fungal vine pathogens (Keller et al., 2003a), which tend to be more susceptible to UV-B radiation than higher plants, as well as herbivorous insects and disease vectors (Caldwell et al., 2007).

Effects on grapevine physiology

Sunlight, in the whole range of wavelengths, is recognized as the most powerful factor determining morphological and physiological variations in leaves. Many effects of UV-B radiation affect morphogenetic changes in plants (presence of leaf hairs shoot tip, young leaves (Karabourniotis et al., 1999); as well as epicuticular wax (Shepherd and Griffiths, 2006). It is a well-known fact that sun leaves display a higher leaf mass per area (LMA) and thickness (LT) than shade leaves (Groom and Lamont, 1997; Tattini et al., 2000; Evans and Poorter, 2001; Gratani et al., 2006; Temesgen and Weiskittel, 2006; Cascio et al., 2010), what was also confirmed on *Vitis vinifera* L. 'Sangiovese' (Pollastrini et al., 2011). The study confirmed that the quantity of epidermal polyphenols increased as a consequence of UV-B irradiation. Even more than the morphological effect itself it seemed an important discovery that in Mediterranean conditions the natural presence of UV is a necessary element driving morphogenetic processes that enable plants to adapt better to oxidative stresses typical of that environment (Pollastrini et al., 2011). There is also not much work to authors knowledge done on root systems: deleterious effects of UV-B radiation on mycorrhizal infection, possibly mediated by plant hormone levels, have been reported (Van de Staaij et al., 2001), but not on grapevine, and more studies on the effect of climate change associated variables on rootstocks and root systems including mycorrhiza should be conducted (Mira de Orduña, 2010).

Apart from morphogenetic changes UV irradiation plays an important role in photosynthesis. In the photosynthetic apparatus an excess of light may induce a condition of over-excitation, harmful to absorbing pigments and reaction centres (Papageorgiou and Govindjee, 2004). Unmanaged electrons lead to the formation of reactive oxygen species, thus activating mechanisms of oxidative stress (Demmig-Adams and Adams III, 2006; Cascio et al., 2010). Photosynthetic adaptations to light excesses include absorption reduction of the apparatus and increased controlled energy dissipation from the groups of pigment molecules (antennae), as well a greater amount of photosystem I (PSI), as to speed up the reduction of the final electron acceptors (NADP, Ferredoxine) (Maxwell et al., 1999; Cascio et al., 2010).

Furthermore, impacts in a wide number of photosynthetic components have been reported, including the suppression of Chlorophyll synthesis (Chl), the inactivation of oxygen synthesis, light harvesting complex of Photosystem II (LHCII), photosystem II (PSII) reaction centres and thylakoid electron flux. Furthermore, the decrease of ribulose-1,5-bisphosphate carboxylase/ oxygenase (Rubisco) content and activity, that affects maximum rate of Rubisco carboxylation, accompanied with a large reduction in the expression and abundance of both large and small subunits of Rubisco, would contribute to a lower photosynthesis activity and yield (Lidon and Ramalho, 2011).

Kolb et al. (2001) showed that epidermal UV screening of grapevine leaves (cv. 'Silvaner') after short exposure to high natural radiation is sufficient to prevent UV-B-dependent reduction of PSII activity in the vineyard. Because UV-B effects on PSII were small and transitory when compared CO_2 assimilation, they suggest that under natural light intensities UV-B inhibition of photosynthesis is not controlled by UV-B inhibition of PSII that has also been proposed by Allen et al. (1998), Xiong and Day (2001). Two years later, Pfündel (2003) developed a model to describe, how natural radiation intensities affect PSII and thereby change leaf fluorescence, concluding that PSII inhibition by natural UV could be the main factor for UV inhibition of photosynthesis.

A strong decrease in both CO_2 uptake and stomatal conductance in all leaves has as well been observed at the grapevine variety 'Chardonnay', exposed to relatively weak UV irradiation (Majer and Hideg, 2012), while on 'Sangiovese' stomatal conductances have not been affected by different light intensities in either of the UV radiation conditions (Pollastrini et al., 2011), although the differences might be due to different experimental setup and not due to cultivar.

Among the chlorophyll fluorescence parameters the quantum yield of primary photochemistry was significantly reduced in high light conditions only in the sheltered plants (-UV) (Pollastrini et al., 2011), although authors find it conflicting with their previous work, where the

reduction of primary photochemistry has been observed in mainly irradiated leaves of Fagus sylvatica (Cascio et al., 2010).

Majer and Hideg (2012) have shown different reductions of effective photochemical yields on supplemental UV-B irradiation of younger leaves (-22 %) and older leaves (-44 %). But supplemental UV-B irradiation did not affect total chlorophyll contents at 'Cabernet sauvignon', 'Malbec' and 'Chardonnay' varieties (Keller et al., 2003a; Berli et al., 2010; Majer and Hideg, 2012). On the other hand, Lafontaine et al. (2005) observed that a high UV radiation caused premature loss of total chlorophyll and a decrease in the Chl a/Chl b ratio in leaves and fruits. As already suggested, the experiments cannot be compared directly, due to the differences in plant material (variety, age etc.) and photosynthetically active radiation (PAR) conditions (Kolb et al., 2001; Pollastrini et al., 2011; Majer and Hideg, 2012). Berli et al., (2010) also showed evidence for lipid peroxidation and the activation of peroxidases by UV-B, which could be a result of increased hydroxyl radical neutralizing capacities of younger leaves (Majer and Hideg, 2012).

Tevini (1996) affirmed that higher levels of UV-radiation induce an increase in the production of protective pigments - carotenoid concentration is usually higher in shaded than in exposed berries (Bureau et al., 1998) and has previously been shown to decrease as a result of UV-exposure (Schultz et al., 1998; Schultz, 2000). This forced degradation may also indicate a weakening in the photo-protecting mechanism of the xanthophyll cycle (Demmig-Adams and Adams III, 1992; Eskling et al., 1997). Steel and Keller (2000) studied the effect of UV light reduction on carotenoid contents in leaves and berries of 'Cabernet sauvignon'. Their results showed that the reduction of UV light decreased the total carotenoid content in leaves; furthermore they witnessed a decrease of β-carotene and lutein contents in berries, which may affect the biosynthesis of aromatic compounds in grape and wine.

Núñez-Olivera et al. (2006) came across some differences between red 'Tempranillo' and white 'Viura' grapevine varieties at reduced solar UV-B radiation, where at white variety a significant decrease in contents of UV-absorbing compounds were observed. The same dynamic was observed at the variety 'Tempranillo' accompanied with a reduction of the xanthophyll cycle activity and an increase in the concentration of chlorophyll and carotenoids. Pfündel (2003) report that the carotenoid content slightly increased in the older leaves in response to UV-B radiation. Moreover, carotenoids are precursors for norisoprenoid compounds in grapes (Razungles et al., 1993), what suggested that UV-radiation may also affect grape and wine flavour. Tevini (1996) for instance reported that UV-B radiation had a positive effect on the flavour of melons.

Marais et al. (1992) found that norisoprenoid concentrations were statistically higher in sun-exposed than in shaded grape. Lee et al. (2007) reported that when leaves were removed from canopy, C13-norisoprenoid concentrations were linearly ($r > 0.90$; $p < 0.1$) and positively correlated with increasing sunlight exposure. Moreover, in contrast, in the most shaded treatments with no leaf removal there were high concentrations of norisoprenoids - β-damascenone concentrations in particular were highest when no leaves were removed. Furthermore, Hühn et al. (1999) observed UV-radiation-induced changes in indole acetic acid derivatives in grapes, which may be negative for wine quality.

Effects on grapevine biochemistry

The plants, including the grapevines, evolved a wide variety and high diversity of primary (sugar, organic acids etc.) and secondary (phenolic compounds, aromatic substances) metabolites to interact with different environmental conditions, as well as to regulate abiotic and biotic stress tolerances.

In the context of primary metabolism Tevini (1996) and Krupa et al. (1998) reported that UV radiation is likely to affect levels of the key antioxidants glutathione and ascorbate and the possible inhibition of carotenoid pigment formation and of the incorporation of nitrogen into amino acids. According to Gregan et al. (2012), UV radiation did not have a significant effect on the majority of amino acids or methoxypyrazine concentrations. The most noticeable change in amino acid and methoxypyrazine accumulation was caused by the presence of leaves over the fruiting zone, retaining these leaves maintained significantly higher concentrations in the berries at harvest.

Crippen and Morrison (1986) studied the effects of sun exposure on the compositional development of berries of the variety 'Cabernet sauvignon' and their obtained results suggested that sun-exposed berries contain significantly higher concentrations of tartrate, malate, glucose and fructose than those shaded. They also found that the

canopy-shade berries were significantly heavier than those expose to the sun, what can be ascribed to the higher water content in the berries of shaded clusters.

On the other hand, UV radiation represents an important ecological factor that leads to a cascade of reactions that ultimately result in the formation and accumulation of secondary metabolites such as phenolic compounds (Tevini, 1996), which help plants to overcome different stresses.

Apart from stress response, secondary metabolites in grape berries determine also quality of wine (aroma, astringency, colour, stability) (Ribéreau-Gayon et al., 2006), and have health benefits, such as antioxidant, anticancer, protection on cardiovasculars (Dzhambazova et al., 2011). Owing to this, many studies have focused on how to increase the levels of phenols in grape berries, including postharvest treatment (Cantos et al., 2000; Li et al., 2009).

Price et al. (1995) found that anthocyanin content in grape of 'Pinot noir' was not affected by sun exposure, which is conflicting with recent findings: Lafontaine et al. (2005) demonstrated that berries exposed to UV-B radiation increased both the concentration of total bound glycosidic secondary metabolites and phenolics. According to Doupis et al. (2011) the accumulation of the UV-B absorbing compounds under enhanced UV-B radiation and the increase in antioxidant enzymes activities constitute the main mechanisms of grapevine adaptation: they decrease UV-penetration through the epidermis, where colour formation may itself reduce UV-penetration (Kolb et al., 2001 and 2003).

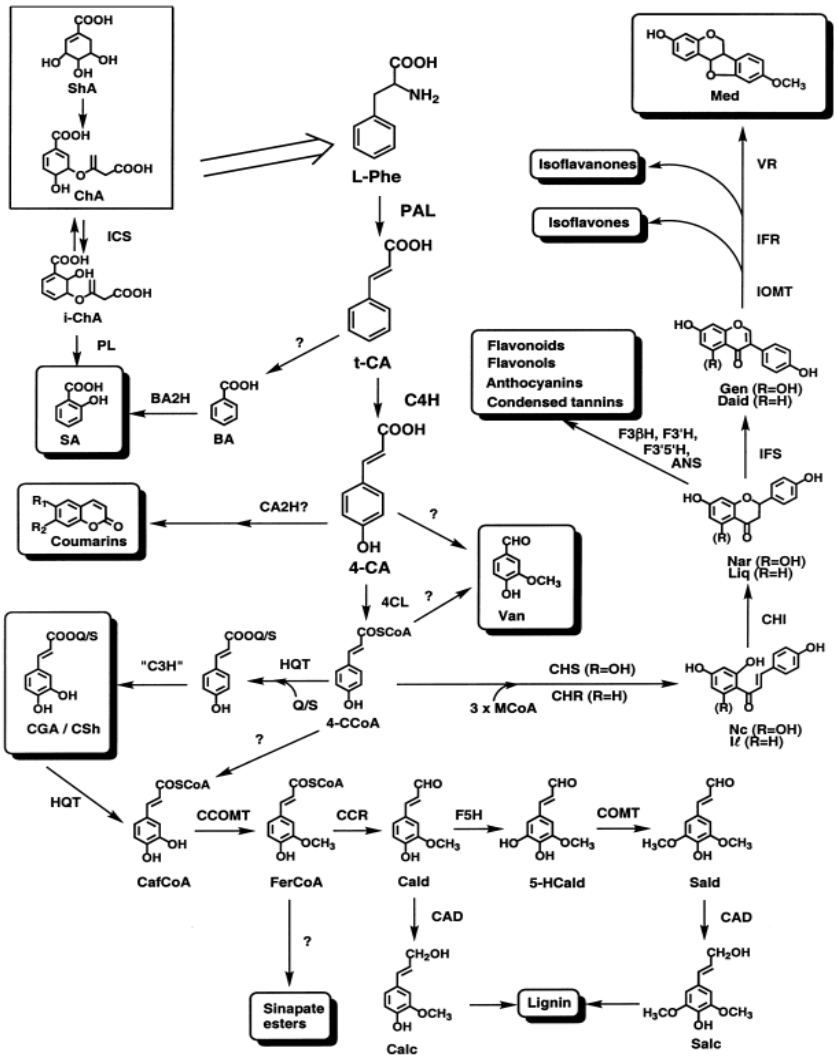

Figure 1. Phenylpropanoid pathway.
(Dixon et al. 2002)

Table 1. Main stilbenes identified in grapevines.

Stilbene	Synonym
trans- / *cis*-Resveratrol	*trans*- / *cis*-3,4',5-trihydroxystilbene
trans-Piceid	*trans*- / *cis*-Resveratrol-3-O-β-D- glucopyranosyde
Resveratoloside	Resveratrol-4'-O-β-D- glucopyranosyde
Pterostilbene	*trans*- 3,5-dimethoxy-4'hydroxystilbene
Viniferins (α,..,δ)	
Piceatannol	*trans*-3,3',4,5'-tetrahydroxystilbene
Astingine	Piceatannol-3-O-β-D- glucopyranosyde
Pallidol	*trans*-Resveratrol dimer

According to Kolb et al. (2003), Jansen et al. (2008) and Broeckling et al. (2005) the UV light stimulates the biosynthesis of secondary metabolites, where some key enzymes involved in the phenylpropanoid pathway (Figure 1) have been showed to be regulated by UV radiation Pontin et al. (2010) demonstrated that the grapevine variety 'Malbec' responded to UV radiation in a variety of general protective responses, for example the induction of pathways regulating synthesis of UV-B absorbing compounds such as the phenylpropanoid pathway, the induction of different antioxidant defence systems and the activation of pathways commonly associated with pathogen defence and abiotic stress responses. The number of literature on secondary metabolites biosynthesis regulation is increasing fast (Matus et al., 2009; Pontin et al., 2010; Berli et al., 2011; Czemmel et al., 2012; Koyama et al., 2012; Majer and Hideg, 2012; Zhang et al., 2012).

In the last decade additional attention has been given to the effect of UV-B radiation on stilbene contents in grape berries (Adrian et al., 2000; Versari et al., 2001; Cantos et al., 2003 Belhadj et al., 2008; Bavaresco et al., 2009; Pan et al., 2009; Zhang et al., 2012), leaves (Pezet et al., 2003; Vrhovšek et al., 2012) and different callus tissues (Keller et al., 2000; Keskin and Kunter, 2009). Keller et al. (2000) found that only an actively growing callus of grapevine irradiated with UV light was capable of producing stilbenes (Table 1), including *trans*-resveratrol, one of the most beneficial compounds in wine reported (Lekli et al., 2010).

A same dynamic was observed in ripening grape berries, which gradually lose their potential for synthesizing stilbenes as they reach full-ripe maturity (Pan et al., 2009). Their profile seems to be dependent upon intensity and duration of UV-B irradiation (Gil et al., 2012).

Influence on pathogens

Pathogens and pests play a major role in determining plant performance in both agricultural and natural settings. The involvement of UV radiation in the interaction between plants and their pests is of major importance and was the subject of numerous papers (Raviv and Antignus, 2004).

Powdery mildew, caused by *Uncinula necator*

U. necator is one of the most ubiquitous pathogens in winegrowing. It can develop on all green plant parts of the grapevine. The powdery mildew fungus develops on both the upper and lower surface of leaves, but thrives in shade and often develops in the interior of dense canopies.

Willocquet et al. (1996) reported that in controlled experiments at constant leaf temperature, spore germination and mycelia growth were negatively affected by the UV-B doses, irrespective of the exposition duration. In the vineyard, radiation effects increased as the time of exposition increased, indicating that both spore germination and mycelial growth activities were slowed, but not totally stopped by the different exposures.

Also Keller et al. (2003a) reported that UV irradiation plays an important role in the natural regulation of powdery mildew under field conditions, but the increase in humidity and screening of UV caused by clouds and canopy shade may contribute to favourable conditions for *U. necator* development.

Another important factor is that for example 'Chardonnay' and 'Cabernet sauvignon' differed considerably in their susceptibility to *U. necator* (Keller et al., 2003a).

Bunch rot of grapes (grey mould), caused by Botrytis cinerea

Botrytis cinerea is regarded as an important organism in viticultural and oenological as well, which may be involved in noble rot and often bunch rot. The former is a prerequisite for the

production of highly priced sweet wines in specific regions (Makra et al., 2009).

The observations indicate that grapevines of the future are likely to be more prone to infections by *B. cinerea* and that both, the development of fungal pathogen and UV-B exposure lead to enhanced activities of catalase, a ubiquitous enzyme that acts to protect tissues against oxidative damage (Steel and Greer, 2005).

The high susceptibility of grape flowers to *B. cinerea* may be related not only to their poor capacity for stilbene synthesis, but also to low levels of constitutive phenolic compounds, particularly in the receptacle area (Keller et al., 2003b). Considering its importance for secondary infections and wine quality, the effect of climate change on bunch rots requires further studies (Mira de Orduña, 2010).

Downy mildew, caused by *Plasmopara viticola*

Downy mildew represents one of the most severe infections in grapevines, as it affects both the yield and the quality of wine production. The disease is usually prevented by repeated fungicide treatments of entire vineyards which cause a high economic and environmental impact.

The results of Agati et. al. (2008) indicate that flavonoids can be significantly involved in the process responsible for the larger resistance to downy mildew in sun-exposed versus shaded grapevine leaves.

Conclusion

Just like other plants, *Vitis vinifera* L. is susceptible to increased UV-B irradiations, but negative influence (f.e. decreased photosynthesis) seems to be far less important than positive.

Núñez-Olivera et al. (2006) affirmed that grapevine varieties, typical of the Mediterranean climate zone are well adapted to the high solar radiation and their photosynthetic performance does not appear to be at risk from current levels of UV-B. With continuous global warming, grapes in other regions might adapt admittedly slowly as well, so minimising negative effects.

On the other hand, most important vitivinicultural relevant consequence of UV-B irradiation is an altered phenolic profile, which is advantageous for plant, and consequently for human health (increased levels of stilbenes and polyphenols in general). The effect seems to be even more important than recently reported by Jansen et al. (2008) and that there is a strong possibility that wine, in moderate consumption, is beneficial to human health (Yoo et al., 2010). With UV-induced polyphenol increase, health relevance should even increase, causing increased wine consumption and consequently its production.

On the other hand, extracts from 'Jacquez' (*Vitis aestivalis*; Summer grape) wine grapes, are already used for in vivo protection against UV-B-induced skin erythema, tested on healthy human volunteers (Tomaino et al., 2006) indicating that efficient protection of grapes against UV-B might have also indirect health benefits for human.

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REVIEW ARTICLE

Prospects of UV radiation for application in postharvest technology

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Abstract

UV light has been used as a germicidal agent in water treatment and surfaces disinfection because of its capacity to affect DNA of microorganisms. On the other hand, low doses of UV-C irradiation can trigger some favourable reactions in biological organs, such as fruits and vegetables, which can lead to various beneficial effects, such as improvement of their shelf-life or increase in the content of health promoting components. The objective of this work is to review the results of some recent works on the UV application on post harvested fruits and vegetables taking into account both, its direct germicidal activity and its hormectic effects. After the presentation of the hormesis concept, the application of UV to read-to-use fruit and vegetables and, specifically, to various fruits and vegetables, is discussed. The use of UV radiation strictly for hormectic purposes at commercial scale, still needs to be further investigated.

Key words: UV light, UV-B, UV-C, Postharvest technology, Fruits, Vegetables, Hormesis

Introduction

Although the use of ultra-violet light (UV) is well established for water treatment, air disinfection, and surface decontamination, its use is still limited in food treatment and in postharvest technology in particular. UV treatment has a potential for commercial use as a surface treatment of fresh-cut fruits. The ability of UV light to sanitize and retard microbial growth on the surface of fresh-cut fruits without causing undesirable quality changes has recently been recognized. Irradiation with UV light may be a more effective germicidal treatment than chlorine, hydrogen peroxide, or ozone. Recent advances in the science and engineering of UV-light irradiation have demonstrated that UV treatment holds considerable promise for shelf-life extension of fresh fruits and vegetables. Considering its importance, surprisingly little is known about the interaction of UV light with matter, especially with a complex food matrix.

The objective of this review is by no means to describe exhaustively and in detail all the work done on the effects of UV radiation on vegetables and fruits, but only to report some of the recent results that can lead to the use of UV light to postharvest treatment of fruits and vegetables.

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UV light

Light is just one portion of the electromagnetic spectrum which covers a broad range from radio waves with wavelength of a meter or more, down to x-rays with wavelength of less than a billionth of a meter. Typically, the wavelength for UV processing ranges from 100 to 400 nm (Koutchma et al., 2009). This range may be further subdivided into UV-A (315–400 nm), normally responsible for changes in human skin called tanning; UV-B (280–315 nm), which can cause skin burning and eventually lead to skin cancer; UV-C (200–280 nm), called the germicidal range, since it effectively inactivates bacteria and viruses; and the vacuum UV range (100–200 nm), which can be absorbed by almost all substances and thus can be transmitted only in a vacuum (Koutchma et al., 2009) . Short UV-C is almost completely absorbed in air within a few hundred meters. When UV-C photons collide with oxygen atoms, the energy exchange causes the formation of ozone. UV-C is almost never observed in nature, since it is absorbed so quickly.

Koutchma et al. (2009) reviewed the full range of commercially available UV sources, such as low- and medium-pressure mercury lamps, mercury-free amalgam lamps, and discussed the advantages of the pulsed UV-light sources currently under development.

The concept of hormesis

UV-C irradiation at low doses (0.25–8.0 kJ/m^2) affects the DNA of microorganisms (Terry and Joyce, 2004). For this reason UV-C treatment has

been used as a germicidal or mutagenic agent. In addition to this direct germicidal activity, UV-C irradiation can modulate induced defence in plants. So UV-C irradiation can be applied at lethal and sublethal doses. UV-C can also produce a detrimental effect on plant tissues which includes tissue structural damage, changes in cytomorphology and water permeability of inner epidermal cells (Lichtscheidl-Schultz, 1985). Nevertheless, low doses of UV-C irradiation stimulated beneficial reactions in biological organs, a phenomenon known as hormesis (Shama, 2007). Hormesis is defined as the stimulation by low doses of any potentially harmful agent (Luckey, 1980). The agents capable of bringing about these stimulatory effects may be either chemical or physical ones. Included amongst the latter are various portions of the electromagnetic spectrum. Luckey (1980) conducted an extensive survey of hormetic effects induced by both ionizing radiation and UV light.

Hormesis involves stimulation of a beneficial plant response by low or sub-lethal doses of an elicitor/agent, such as a chemical inducer or a physical stress (Terry and Joyce, 2004). Non-ionising radiation has real potential amongst physical methods for controlling postharvest diseases (Wilson et al., 1997).

According to Shama (2007), hormesis involves the use of small doses of potentially harmful agents directed against a living organism or living tissue in order to elicit a beneficial or protective response. Hormetic UV treatment is distinguished from conventional UV treatment. In conventional treatment the UV is directed at microorganisms present on the surfaces of an object, whereas in hormetic UV treatment the object itself is the target of the incident UV. The objective of the treatment is to elicit an antimicrobial response in the fruit and vegetable tissue. Both types of UV treatment employ the same wavelengths, but for hormetic treatments only low UV doses are required.

Low doses of short-wave ultra-violet light (UV-C, 190–280 nm wavelengths) can control many storage rots of fruit and vegetables. It has been reported that hormetic doses of UV-C can prolong the postharvest life and maintain the quality of fruits. These effects include delay of senescence process and fruit ripening (Gonzalez-Aguilar et al., 2007a), induction of natural defence and elicitors against fungi and bacteria (Alothman et al., 2009a).

Exposure to hermetic doses of UV radiation triggers a series of biochemical events within the plant tissue, and a number of quite distinct responses have been identified. Some responses involve the synthesis of enzymes that have activity against molds, while others result in the generation of a host of so-called phytoalexins, which are inhibitory to microorganisms. These effects are produced by the use of very low UV doses, and the time scale for the induction of such events is measured over hours or even days. Resistance to infection by pathogen is correlated with the induction of plant defence mechanism (Gonzalez- Aguilar et al. 2007b) and DNA damage (Charles et al., 2009). This is manifested through the stimulation of anti- fungal chemical species such as phytoalexins (scoparone and resveratrol), flavonoids, and degrading fungal cell-wall enzymes (chitinases, glucanases) (El-Ghaouth et al., 1998). The induction of plant defence system can also trigger the accumulation of these compounds and other phytochemicals such as carotenoids and vitamin C which exhibit antioxidant potential, improving the nutritional status of the fruit (Alothman et al., 2009a, 2009b; Gonzalez- Aguilar et al., 2007a, 2007b).

All published work on the delivery of low doses of UV to fresh produce has concerned itself with only relatively small numbers of fruit treated under laboratory conditions, and little consideration has been given to how produce may be treated on a large scale under industrial conditions. Shama (2007) considered the prospects of treating fruits with UV on a commercial scale. According to this author, any process for irradiating produce must fulfil certain essential requirements (Shama, 2007) such as: produce should not be subjected to any form of mechanical handling during irradiation that might cause it to become damaged; there should be provision for both varying the UV dose delivered and controlling the dose; UV-C treatment should not add unduly to processing costs; the design of equipment should enable high throughputs to be treated; ideally, a wide variety of different types of fruit and vegetables should be treatable.

Postharvest UV radiation: effects and possible technological applications

Ready-to-use fruit and fresh vegetables

The market sales of ready-to-use fruit and fresh vegetables have grown rapidly in recent years due to the health benefits associated with these foods. Its growth has heightened awareness about the microbiological and physiological parameters associated with quality in fresh ready-to-eat vegetables due to the relevance for industry and its economic impact. Chlorine solutions have been widely used to sanitise fruit and vegetables in the

fresh-cut industry and continues being the most commonly used sanitizer due to its efficacy, cost-effectiveness ratio and ease to use. However, chlorine has been associated with the possible formation of carcinogenic chlorinated compounds (Rico et al., 2007). This preoccupation urges fresh-cut industry to find new alternatives. These alternatives must satisfy the consumers and maintain a balance between sensory and quality. For this reason exploration and enhancement of new alternatives are essential. There is a real need to find alternatives for preservation of fresh-cut fruit and vegetables in order to improve the efficacy of washing treatments. Alternatives or modified methods have been proposed, as antioxidants, irradiation, ozone, organics acids, modified atmosphere packaging, whey permeate, etc.; however, none have yet gained widespread acceptance by the industry (Rico et al., 2007).

New techniques for maintaining quality and inhibiting undesired microbial growth are demanded in all the steps of the production and distribution chain. Allende et al. (2006) summarized and discussed some of the new techniques available in the fresh-cut industry such as the combination of sanitizers with other methods, combinations of physical and chemical treatments, the use of ultraviolet-C radiation, modified-atmospheres, heat shocks and ozone treatments, alone or in different combinations, in order to control microbial growth and maintain quality during storage of fresh-cut produce.

It has been reported that UV–C affects several physiological processes in plant tissues and, what it is more important, damages microbial DNA (Kuo et al., 1997; Lucht et al., 1998). Lado and Yousef (2002) reported that UV–C radiation from 0.5 to 20 kJ/m^2 inhibited microbial growth by inducing the formation of pyrimidine dimers which alter the DNA helix and block microbial cell replication. Therefore, cells which are unable to repair radiation-damaged DNA die and sub-lethally injured cells are often subject to mutations. A number of *in vitro* studies have demonstrated the efficiency of UV–C radiation on microbial inhibition (Gardner and Shama, 2000). Abshire and Dunton (1981) found that some species (*Pseudomonas aeruginosa*) were more sensitive than others (*Micrococcus radiodurans* and *Candida albicans*). Consistent with this, Sumner et al. (1995) found that UV–C was effective in destroying *Salmonella typhimurium* on agar plates.

Selma et al. (2008) investigated the disinfecting efficacy of ozone (O_3) and UV-C illumination, and their combination for reduction microbial flora of fresh-cut onion, escarole, carrot, and spinach wash waters collected from the industry. They achieved a maximum microbial reduction of 6,6 log CFU mL^{-1} after 60 min treatment with O_3-UV and concluded that O_3 and O_3–UV are alternatives to other sanitizers used in the fresh-cut washing processes. The use of these technologies would allow less frequent changing of spent water and the use of much lower sanitizer doses. Nevertheless, in specific applications where levels of undesirable microbial and chemical constituents are lower, UV treatment alone could be an appropriate treatment considering cost-effectiveness criteria.

Postharvest treatments of either ClO_2 or fumaric acid combined with UV-C can be useful for maintaining the quality of strawberries, including the sensory evaluations scores (Kim et al., 2010). These authors examined the combined effect of aqueous chlorine dioxide (ClO_2) or fumaric acid with ultraviolet-C (UV-C) on postharvest quality of 'Maehyang' strawberries. The strawberries were treated with distilled water, 50 mg L^{-1} ClO_2 , 0.5% fumaric acid, 5 kJ/m^2 UV-C irradiation, and a combination of 50 mg L^{-1} ClO_2/5 kJ/m^2 UV-C and 0.5% fumaric acid/5 kJ/m^2 UV-C. The combined treatment of fumaric acid/UV-C reduced the initial populations of total aerobic bacteria and yeast and molds in the strawberries by 2.25 and 2.01 log CFU g^{-1}, respectively. Sensory evaluation results indicated that the combined treatment provided better sensory scores than did the control.

Tomatoes

The use of UV-light could be employed to improve tomato nutritional qualities and lycopene content without inducing significant changes to the physical properties of tomatoes during post-harvest storage. Liu et al. (2009) treated harvested mature-green (breaker-stage) tomatoes with short bursts of UV-C, red light or sun light for up 21 days. The concentration of lycopene in tomato exocarp was significantly increased after 4 days and dramatically enhanced by UV-C or red light treatments. However, the concentration of β-carotene was not affected by UV-C or red light treatments, and decreased by sun light treatment during 21 days of storage, compared to the control samples. The colour (a* and b* values) and force required to penetrate the tomatoes were, to a small but significant extent, influenced by the light treatments. The total soluble refractive solids of all tomato samples remained the same throughout storage.

Liu et al. (2011a) studied gene expression of tomato fruit in response to postharvest UV-C irradiation (4 kJ/m^2), during 24 h after the treatment. They concluded that UV-C irradiation can induce the expression of a number of defence response genes, and suppress the expression of major genes involved in cell wall disassembly, lipid metabolism and photosynthesis. These gene changes underline the biochemical and physiological changes induced by UV-C such as increased defence ability, delayed softening, better maintenance of nutritional and sensory qualities and extension of shelf-life in tomato fruit.

UV-treatments of tomato fruits reduce the gloss of the tomato surface because those treatments affect the morphology of fruit surface wax (Charles et al., 2008a). UV-treatment may have induced biochemical modifications of the surface wax layers. The overall impact of these changes has two contrasting effects. On the one hand, changes in the physical and biochemical modifications that occur in the epidermal cell in response to UV-treatment may be conducive to an improved ability of the plant tissue to resist pathogen attack. On the other hand, altered wax layers can affect light reflectance characteristics of the fruit surface, and may also contribute to increased water loss from cuticular transpiration, both leading to changes in the appearance of the fruit.

Charles et al. (2008b) treated postharvest tomato fruit with the dose of 3.7 kJ/m2 of UV-C, which had shown optimal for inducing decay resistance, but whose treatment caused ultrastructural modifications in the pericarp. UV induced plasmolysis of the epicarp cells as well as some cell layers of the mesocarp. Collapse of these cells led to the formation of a cell wall stacking zone which restricted *Botrytis cinerea* development to the outer most part of the fruit and hindered its progression toward the inner tissues.

Charles et al. (2008c) studied the biochemical nature of cell wall modifications induced by UV-C in postharvest tomato fruit and they found that UV treatment induced the accumulation of phenolic compounds and the formation of lignin and suberin. Simple phenolic compounds induced by UV-C appear to have a fungistatic effect; and complex phenolics, lignin and suberin, play a barrier role physically impeding pathogen ingress by strengthening the cell wall stacking zone. Such a barrier would also reduce diffusion of nutrients and water from the plant tissue required to sustain fungal growth, and toxins and cell wall degrading enzymes from the fungus, which interfere with virulence of the pathogen.

UV-B irradiation appears to be a useful non-chemical way of maintaining postharvest quality and enhancing antioxidant capacity in tomato fruit. Liu et al. (2011b) applied doses between 20 and 80 kJ/m^2 to mature-green tomato of UV-B irradiation. 20 or 40 kJ/m^2 was most effective in maintaining a high level of firmness and delaying the colour development. Furthermore, 20 or 40 kJ/m^2 promoted the accumulation of total phenolic and total flavonoids, and enhanced antioxidant capacity during storage, though UV-B irradiation could reduce the ascorbic acid content. A dose of 10 kJ/m^2 had similar effects but to a lesser extent. The highest dose of 80 kJ/m^2 resulted in higher lycopene content, but showed negative effects on texture, colour, and other antioxidants. The optimum dose of UV-B for maintaining sensory qualities and enhancing antioxidant capacity was 20 or 40 kJ/m^2.

Mushrooms

UV-C radiation could potentially be used for sanitizing fresh mushrooms and may be a useful non-chemical way of maintaining mushroom quality and extending their postharvest life.

Guan et al. (2012) investigated the effects of UV-C light, applied to both sides of mushrooms, on microbial loads and product quality, during 21 days of storage at 4 ◦ C. Microflora populations, color, antioxidant activity, total phenolics, and ascorbic acid were measured at 1, 7, 14 and 21 days of storage. Additionally, the inactivation of *Escherichia coli* O157:H7 by UV-C was determined. Results showed that UV-C doses of 0.45–3.15 kJ/m^2 resulted in 0.67–1.13 log CFU g^{-1} reduction of *E. coli* O157:H7 inoculated on mushroom cap surfaces. UV-C radiation also reduced total aerobic plate counts by 0.63–0.89 log CFU g^{-1} on the surface of mushrooms. In addition, the UV-C treatments apparently inhibited lesion development on the mushroom surface. During the first 7 days, irradiated mushrooms had lower antioxidant activity, total phenolics, and ascorbic acid content than non-radiated samples.

Jiang et al. (2010) exposed shiitake mushrooms (*Lentinus edodes*) to UV-C light (4 kJ/m^2) and stored them in modified atmosphere packaging (MAP) for 15 days at 1 ± 1°C and 95% relative humidity plus 3 days at 20°C. UV-C treatment resulted in the maintenance of a high level of firmness during 15 days at low temperature and reduced the decrease in firmness during shelf-life. Furthermore, treated samples showed higher total flavonoids, ascorbic acid, and delayed the increases in both superoxide anion production rate and H_2O_2

contents. However, no clear treatment effects were seen in total phenolics contents. The treatment also increased the antioxidant enzyme activities of catalase, superoxide dismutase, ascorbate peroxidase and glutathione redutase throughout the storage period.

Baby spinaches

UV-C radiation applied at proper doses and to both sides of the baby spinaches could reduce microbial growth and extend shelf-life without adversely affecting the quality of fresh-cut baby spinach leaves. Escalona et al. (2010) applied UV-C (0, 2.4, 7.2, 12 and 24 kJ/m^2) radiation to both sides of baby spinach leaves in order to simulate a continuous production chain. The results showed effectiveness of initial microbial reductions in fresh-cut spinach at the beginning of storage using short exposure times and low radiation doses. Almost all the analysed microbial groups were reduced by UV-C radiation throughout the storage period. UV- C light significantly reduced *Listeria monocytogenes* growth in fresh-cut spinach for 14 days at 5°C. During the first 5–8 days, radiated leaves had lower *Salmonella enterica* and *Pseuomonas marginalis* counts compared to non-radiated samples. However these radiated leaves reached higher counts than control after 8 days of storage. A low UV-C dose (2.4 kJ/m^2) had a similar inhibitory influence on other microbial growth, compared to high doses such as 12 or 24 kJ/m^2. UV-C light was also effective at reducing psychrotrophic and Enterobacteria in fresh-cut spinach until 4 days at 5°C. Escalona et al. (2010) did not find the surface tissue damaged when it was inspected by electron microscopy, nevertheless they point out that it is possible that UV-C light caused some tissue damage of the spinach leaves, as measured by an increase in respiration.

Broccoli

Floret yellowing is a major limitation to shelf-life and broccoli quality. Therefore, suitable treatments are necessary to maintain quality levels until consumption. Some techniques to delay senescence have been investigated, including heat treatments, which effectively reduce yellowing among stored broccoli florets (Funamoto et al., 2002), chemical treatments such as 1-methylcyclopropene (Ku and Wills, 1999; Able et al., 2002) and ethanol vapour (Suzuki et al., 2004), low temperature (Starzynska et al., 2003) and controlled atmosphere storage (Yamauchi and Watada, 1998).

Results obtained by Costa et al. (2006) suggest that short UV-C treatments could be a useful non-chemical method to delay senescence in broccoli. Short UV-C treatments (4, 7, 10 and 14 kJ/m^2) delayed chlorophyll degradation in broccoli, with 10 and 14 kJ/m^2 doses showing the greatest delay. However, only 4, 7 and 10 kJ/m^2 doses reduced pheophytin accumulation. The UV-C treatment with a dose of 10 kJ/m^2 delayed not only chlorophyll *a* and *b* degradation but also the increase of chlorophyllase and chlorophyllperoxidase activity. In the case of Mg-dechelatase, higher activity was found immediately after the treatments, but after 4 and 6 days at 20 ◦C UV-C treated broccoli maintained lower Mg-dechelatase level than controls. The UV-C treatments also reduced tissue damage and disruption according to respiration rate and phenolic compound content. The antioxidant capacity was increased by UV-C treatments and this could be useful from the nutritional point of view.

Aiamla-or et al. (2009) reported that UV-B irradiation is effective in retaining the green colour of florets during storage. Those authors observed that, in general, broccoli florets retained more colour after UV-B irradiation than after UV-A. UV-B doses of at least 8.8 kJ/m^2 resulted in surface colour with a higher hue angle, as compared to those treated with 4.4 kJ/m^2 UV-B or without UV-B. They selected a UV-B dose of 8.8 kJ/m^2 for application to different broccoli cultivars (Pixel and Sawayutaka), harvested during the winter and early summer seasons. During storage, the 'Sawayutaka' exhibited a slower decrease in green colour of florets, when compared to the 'Pixel' cultivar. UV-B treatment delayed floret yellowing and chlorophyll degradation. Broccoli harvested in winter or early summer and irradiated with UV-B during storage at 15°C had a higher chlorophyll content and hue angle value than broccoli without UV-B treatment.

Peppers

UV-C treatment could be a useful way of reducing decay and maintaining bell pepper fruit quality, reducing chilling injury incidence and severity (Vicente et al., 2005). These authors observed that a dose of 7 kJ/m^2 avoided all symptoms of decay after 12 days at 10°C; treated fruit also kept firmer and maintained quality suggesting that the combined method (UV-C plus refrigeration at 0°C) could be a useful way of extending bell pepper postharvest life.

Strawberries

Allende et al. (2007) tested the effect of UV-C light, gaseous O_3, superatmospheric O_2 and CO_2-enriched atmospheres applied individually and in combination on the health promoting compounds and shelf-life of strawberries. The combination of different postharvest treatments had similar effects than individual treatments for 'Camarosa' strawberries. These authors concluded that all these postharvest treatments, which are commonly proposed to control microbial decay in strawberries, could have detrimental effects from a nutritional point of view, reducing phenolic and vitamin C content of 'Camarosa' strawberries.

Erkan et al. (2008), found that strawberry fruit illuminated with UV-C at different illumination durations and dosages, 1, 5 and 10 min and 0.43, 2.15 and 4.30 kJ/m^2, respectively, promoted the antioxidant capacity and enzyme activities and significantly reduced the severity of decay during storage at 10°C. UV-C illumination for 5 and 10 min showed the best results for enhancing antioxidant capacity expressed as oxygen radical absorbance capacity (ORAC) values after storage for 15 days among all the treatments. These treatments also enhanced the activities of antioxidant enzymes including glutathione peroxidase, glutathione reductase, superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase, monodehydroascorbate reductase, and dehydroascorbate reductase. The nonenzyme components such as reduced glutathione and oxidized glutathione also were increased by UV-C exposure. All UV-C dosages increased the phenolic content of strawberry fruits as well. Total anthocyanin content increased during storage in all treatments. However, UV-C illumination showed little effect on the anthocyanin accumulation. Erkan et al. (2008) also found that all UV-C dosages retarded the development of decay, but 5 and 10 min UV-C illumination gave the best decay inhibition.

Exposure to UV-C delays fruit softening, one of the main factors determining fruit postharvest life. This softening delay might be caused by changes in the activities of enzymes and proteins involved in cell wall disassembly. Expansins, polygalacturonases, endoglucanases and pectin-methylesterases are cell wall proteins or enzymes involved in fruit softening. Pombo et al. (2009) analysed the effect of a UV-C treatment on strawberry fruit softening by the activity of polygalacturonases, pectin-methylesterases and endoglucanase, and the expression of a set of genes encoding for proteins and enzymes involved in cell wall degradation. UV-C treatment delayed fruit softening, and treated fruit showed higher firmness than controls even 96 h after irradiation. The irradiation modified the expression of the genes and the activity of assayed enzymes. In general, the expression of analysed genes was reduced a few hours after irradiation, while it increased afterwards to reach similar or higher levels than the controls. Therefore, the effect of UV-C irradiation on strawberry fruit softening could be related to the decrease of the transcription of a set of genes involved in cell wall degradation, during the first hours after treatment. The same authors (Pombo et al., 2011) also studied the induction of resistance to *Botrytis cinerea* in strawberry fruit, exposed to a hormetic dose of UV-C. The results obtained showed that pre-storage treatment of fruit with UV-C results in lower losses caused by diseases and decay, and the gene expression and enzymatic activity of a set of strawberry genes that are related to plant defence against pathogens were found to be modified in the treated fruit. Therefore, the reduction in strawberry fruit decay by UV-C treatment at harvest could be related to the increase in the transcription and activity of a set of enzymes and proteins involved in the defence against pathogens.

Blueberries

Perkins-Veazie et al. (2008) found that postharvest application on Blueberries (*Vaccinium corymbosum*, cvs. Collins, Bluecrop) of UV-C radiation, prior to storage, can decrease decay caused by ripe rot (*Colletotrichum acutatum*) in blueberries and may enhance antioxidant levels as measured by total anthocyanin, total phenolics, and ferric reducing antioxidant power. Stimulation of antioxidants by UV-C radiation appears to be dependent on cultivar and that weight loss and firmness was not affected by light treatment.

The levels of flavonoids in blueberries were found to increase after illumination with UV-C (Wang et al., 2009). Phytochemicals affected included resveratrol, myricetin-3-arabinoside, quercetin-3-galactoside, quercetin-3-glucoside, kaempferol-3-glucuronide, delphinidin-3-galactoside, cyanidin-3-galactoside, delphinidin-3-arabinoside, petunidin-3-galactoside, petunidin-3-glucoside, petunidin-3-arabinoside, malvidin-3-galactoside, malvidin-3-arabinoside, and chlorogenic acid (Wang et al., 2009). Significantly higher antioxidant capacity was detected in fruit treated with 2.15, 4.30, or 6.45 kJ/m^2 compared to the control fruit. UV-C dosage of 0.43 kJ/m^2 also increased phenolics and anthocyanins, but to a

lesser extent. The optimum doses of UV-C for enhancing phytochemical content in blueberries were 2.15 and 4.30 kJ/m^2. These data suggest that proper use of UV-C illumination is capable of modifying the phytochemical content of blueberries. Time course measurements of the effects of UV-C revealed that the strongest responses of fruit to UV-C treatment occurred instantly after the illumination and the effects diminished with time.

Eichholz et al. (2011) exposed blueberry fruits to UV-B radiation with low dosage and high dosage (0.27 and 0.54 kJ/m^2, respectively) with two adaptation times (2 or 24 h). The UV-B exposure increased the total phenolic content with a maximum at the higher dose, but the adaptation times did not significantly affect it. Content of volatiles metabolites, such as terpenes and ketones, increased at high dosage and low adaptation time. Content of alcoholic compounds, as degradation products of aldehydes, decreased after low adaptation time and increased after high adaptation time.

Apples

UV-C light exposure, if applied at mild intensity, was demonstrated to be an effective non-visible technology for food surface decontamination. Manzocco et al. (2011) studied the effect of UV-C light treatments at 1.2, 6.0, 12.0 and 24.0 kJ/m^2 relative to germicidal efficiency and changes in fresh-like appearance of fresh-cut apple. Independently of UV-C light intensity, all treatments showed the same germicidal effect with 1–2 log reduction in total viable counts. Treatments at an intensity exceeding 1.2 kJ/m^2 had detrimental effects over the cells of the surface apple tissue. On contrary, those authors observed that mild treatments could extend the shelf life of fresh-cut apple slices through various induced phenomena: surface decontamination, denaturation of oxidative enzymes, prevention of browning and off-flavours, and formation of a dried protective film which inhibits microbial growth and juice leakage. Due to very low depth penetration of UV-C light, this film was very thin and would not be perceived by consumer.

Hagena et al. (2007) investigated the effect of postharvest irradiation not only on the major classes of phenolic compounds, but also on other important health and sensory related properties in the peel and flesh of red, sun-exposed and green, shade-grown 'Aroma' apples. They were irradiated with a combination of visible light and UV-B radiation or visible light alone or covered with a black cloth during the entire experiment. The results suggest that postharvest irradiation in apples can be used to improve their health benefits and colour appearance without changing important taste-related parameters or causing damage to the fruit. The antioxidant capacity, total phenols and the content of anthocyanins, quercetin glycosides, chlorogenic acid and ascorbic acid increased upon postharvest irradiation. The accumulation of flavonols started earlier and increased to a level higher than the anthocyanins. A combination of visible light and UV-B radiation was the most effective irradiation treatment and the response was greatest for the peel of the shade-grown apples. The apple flesh showed no response to any of the irradiation treatments. Postharvest irradiation improved the apple skin colour, but did not influence the level of soluble solids or titratable acidity in the apples. No visible damage or substantial weight loss was found in the apples after the irradiation treatments.

Superficial scald is an apple fruit peel storage disorder characterized by necrosis of the first hypodermal cell layers of susceptible cultivars. Because sunlight exposure reduces scald, Rudell and Mattheis (2009) hypothesized that postharvest UV–vis irradiation will, likewise, reduce scald incidence. Granny Smith fruits, that had been covered with paper bags 57 days after full bloom to limit sunlight prior to harvest, were treated, after harvest, with light from white light fluorescent bulb and from fluorescent UV lamp. They observed that postharvest irradiation treatment reduced scald development. Even on the unexposed side of irradiated apples, where light exposure was limited, scald development decreased with increased light treatment duration.

Watermelon

When properly utilized, UV-C light is a promising sanitation tool for fresh-cut watermelon, keeping its overall quality, and possibly also to other fresh-cut fruit with delicate texture (Fonseca and Rushing, 2006; Artés-Hernández, 2010). The effects seem to be dependent on the UV-C doses.

Fonseca and Rushing (2006) investigated the influence of UV-C light (254 nm) treatments with that of common sanitizing solutions used for fresh-cut produce such as chlorine and ozone on the quality and microbial populations of fresh-cut watermelon (*Citrulus lanatus*). They obtained better results with ultraviolet (UV-C) treatments; the solutions of ozone and chlorine were not effective in reducing microbial populations and give poor quality. They achieved good results with 1, 4 kJ/m^2, higher doses did not show any effect in

microbial populations or even resulted in quality deterioration. They stress out the importance of an initial not high contamination level and of a complete surface exposure.

Artés-Hernández (2010) studied the effects of four pre-packaging UV-C illumination doses (1.6, 2.8, 4.8 and 7.2 kJ/m^2) on quality changes of watermelon cubes stored up to 11 days at 5°C. UV-C did not significantly affect the final gas partial pressures within modified atmosphere packages. UV-C decreased microbial counts just after illumination, and after 11 days at 5°C, mesophilic, psycrophilic and enterobacteria populations were significantly lower in UV-C treated watermelon. Slight changes in CIE colour parameters were observed. According to sensory quality attributes, control and low UV-C treated cubes (1.6 and 2.8 kJ/m^2) can be stored for up to 11 days at 5°C while the maximum shelf-life of moderate to high UV-C treated fruit was 8 days at 5°C. Low UV-C treated watermelon cubes preserved their initial lycopene content (2.8 kJ/m^2) or slightly decreased (1.6 kJ/m^2). UV-C radiation did not significantly affect the vitamin C content while catalase activity and total polyphenols content considerably declined throughout the storage period. However, total antioxidant capacity increased, independently of UV-C doses.

Traditional thermal treatments lead to colour and dynamic viscosity changes of watermelon juice, which are mainly catalysed by its intrinsic polyphenol oxidase and pectin methylesterase (PME), respectively (Rodrigo et al., 2006). Lycopene loss has also been reported during the processing and storage of watermelon (Perkins-Veazie and Collins, 2004). Non-thermal technologies which could avoid colour and dynamic viscosity changes and lycopene loss of watermelon juice are an option for the processing of the watermelon juice. Ultraviolet-C treatments are rapid and effective to inactivate the pectin methylesterase of the watermelon juice compared to the thermal and high pressure treatments in the same time and temperature Zhang (2011).

Pomegrenates

López-Rubira (2005) obtained unclear results on the effect of the UV-C radiation on the microbial growth of minimally processed fresh arils from the sweet 'Mollar of Elche' pomegranate (*Punica granatum*, Punicaceae), that were stored under modified atmosphere packaging (MAP) at 5º C. Some of the applied UV-C treatments reduced mesophilic, psychrotrophic, lactic acid and *Enterobacteriaceae* counts. However, microbial counts were not systematically reduced throughout the shelf life. In addition, UV-C treated arils showed higher bacterial counts in a few cases. Yeasts and moulds were unaffected by the UV-C treatments. So authors concluded that no benefits were found when different UV-C radiation doses were applied, and that the use of UV-C seems to be not justified for improving the shelf life of minimally fresh processed pomegranate arils in the conditions they studied.

Grapes for winemaking

Postharvest grapes can be treated with ultraviolet-C light to produce stilbene enriched grapes to be later used in a conventional winemaking process to obtain a red wine enriched in stilbenes (Guerrero et al., 2010). These authors observed that treatments promoted a maximum concentration in trans-resveratrol and piceatannol after pressing, but with a significant loss from grape to wine. A significant increase in both piceatannol and trans-resveratrol concentration (up to 26 times and 3.2 times higher than in control, respectively) was achieved in bottled wine. Regarding the oenological parameters, the wines obtained possessed good quality.

Papaya

Cia et al. (2007) investigated the effects of gamma and UV-C irradiation on the postharvest control of papaya anthracnose, the main postharvest disease in papaya fruit, caused by *Colletotrichum gloeosporioides*. UV-C irradiation was not able to protect the fruit, and moreover, all UV-C doses caused scald on fruit.

Mango

González-Aguilar (2007b) observed that UV-C treatment maintained better overall appearance, lower decay percentage and increased shelf life of fruit. These benefits correlated positively with higher levels of total phenols and flavonoids, enzymatic activities of lipoxygenase and phenylalanine ammonia-lyase. They conclude that UV-C treatment can be a good alternative to increase the shelf life in optimal conditions of mango 'Haden'.

Citrics

UV-C light treatments for 10 min significantly reduced green mold of Satsuma mandarins, caused by Penicillium *digitatum* (Pers.) Sacc., although, this treatment caused injuries that appeared as burning and browning on the fruit surface (Kinay et al., 2005). Irradiation with UV inhibited decay in inoculated citrus fruit; it has been shown that this

treatment elicits the synthesis of the phytoalexins scoparone and scopoletin (Ben-Yehoshua, 2003).

Tahitian lime (*Citrus latifolia* Tan.), originated in South-East Asia, are usually picked and marketed while the peel is still green. Under ambient conditions, the lime fruit become yellow within a few days, which decrease their commercial value. Normally, the peel of lime fruit is green due to the presence of chlorophyll pigment (Grierson and Ting, 1978), located in the flavedo of the peel. Peel yellowing of lime fruit is attributed to the degradation of chlorophyll (Drazkiewice, 1994). In lime, the postharvest maintenance of the green colour in the peel is required to obtain premium prices. Srilaong et al. (2011), in a study, focusing on the effects of UV-B, on physical and biochemical changes in relation to chlorophyll degradation, mature green lime fruit were irradiated with UV-B light and stored them at 25º C in darkness. The authors concluded that treatment effectively suppressed chlorophyll degradation in mature green lime during storage, so they suggested that UV-B irradiation is a usable method for prolonging the postharvest life of lime fruit.

Conclusion

Water treatment based on UV radiation is a well-established technology. With few exceptions, in general, results confirm that UV radiation presents potential to become widely used through direct application on vegetables and fruits to obtain two distinct classes of beneficial effects: to reduce microbial population in these products; and, through applications of low, hormetic doses, to elicit some desirable responses in these products to improve their defence against molds, improve the content of components with beneficial effects for health, extend the shelf-life, keep or even improve the sensorial characteristics. These beneficial effects depend on the dose, application moment, fruit or vegetable species and cultivar, and exposed area. The scaling to commercial implementation needs to be evaluated.

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REGULAR ARTICLE

Existing antioxidant levels are more important in acclimation to supplemental UV-B irradiation than inducible ones: Studies with high light pretreated tobacco leaves

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Abstract

Greenhouse grown tobacco plants were exposed to supplemental ultraviolet irradiation (280-400 nm, UV-B centered) for 6 days and changes in their photosynthesis (gas exchange and electron transport) and general and specific antioxidant activities were measured. UV irradiation corresponded to 8.95 kJ m-2 d-1 biologically effective dose and was supplemented to below ambient (200 µmol m-2 s-1 photon flux density) photosynthetic photon flux density (PPFD, 400-700 nm). Two groups of plants, which were different in their leaf antioxidant capacities due to one of them having been acclimated to high irradiance (1000 µmol m-2 s-1 PPFD) before the UV treatment, responded differently. High light pretreated leaves lost approximately 25% of photosynthetic activity during the UV exposure and showed no change either in the amounts of UV-absorbing pigments or antioxidant levels. On the other hand, leaves which were exposed to UV irradiation without the preceding high light acclimation had 60% lower photosynthesis by the end of the treatment, and increased antioxidant activities. Our results emphasize the importance of base antioxidant levels over inducible pools in leaf responses to low doses of UV irradiation and may also contribute to hypotheses on acclimation under field conditions.

Key words: Ultraviolet radiation, Antioxidant capacities, UV-absorbing pigment, Photosynthesis

Introduction

High energy ultraviolet (UV, 280-400 nm) radiation, especially the UV-B region (280-315 nm) affects photosynthesis in various ways, and can lead to severe damage when applied at high doses (reviewed by Teramura and Sullivan, 1994). Under such conditions, the inhibitory effect of UV on growth and CO_2-fixation is realized through the generation of reactive oxygen species (ROS), leading to oxidative stress (Hideg and Vass, 1996; Mackerness et al., 2001). Oxidative stress is caused by pro-oxidants as a result of an imbalance between the production and the neutralizing of these compounds (Mittler, 2002; Apel and Hirt, 2004). Plants protect themselves from the harmful effects of this radiation by alterations in pigment composition, including the production of compounds reflecting or absorbing UV radiation (e.g. flavonoids). In protection against pro-oxidants, the production of enzymatic and non-enzymatic components of the antioxidant system increases (e.g. ascorbate, phenols, for reviews see Jansen et al., 2008; Zhang and Björn, 2009). It should be noted that flavonoids have a role in both types of defense mechanisms as these compounds act not only as UV screens but are good antioxidants as well (Agati and Tattini, 2010).

On the other hand, UV radiation at lower doses has recently been conceived as a more complex signal, inducing changes in morphology, gene expression and plant metabolism, through the stimulation of the antioxidant machinery of cells and finally leading to acclimation (Frohnmeyer and Staiger, 2003; for reviews see Mackerness, 2000, Jordan, 2002; Kakani et al., 2003).

Several studies report enhanced protection against oxidative stress in plants with improved antioxidant capacities, many of which include transgenic plants altered at specific points of protection against pro-oxidants. Examples include plants overexpressing different antioxidant enzymes, such as chloroplast superoxide dismutase (Sen Gupta et al., 1993), peroxisomal ascorbate peroxidase (Wang et al., 1999), or these two enzymes together with dehydroascorbate reductase (Lee et al., 2007). Tolerance against UV-B

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radiation was also increased in tobacco leaves, where reactive oxygen scavenging capacity was enhanced by preceding mild drought (Hideg et al., 2003, Kubis and Rybus-Zajac, 2008).

The aim of the present work was to test whether acclimation to high intensity visible light resulted in plants more tolerant to subsequent supplemental UV. Similarly to UV-B, strong (excess) visible light can trigger oxidative stress, although via different mechanisms: visible light mainly induces triplet chlorophyll formation and ROS (singlet oxygen) production through acceptor side modifications of the photosystem II complex (Vass, 2011). Acclimation to non-destructive (non-photoinhibitory) light intensities can induce different components of the antioxidant system (Li et al., 2009; Takahashi and Badger, 2011). Our experiments were designed to address the question whether existing antioxidants (i.e. those present at the onset of UV irradiation) or antioxidants induced by exposure to UV-B are more important in providing tolerance to UV. Although in this work these exposures are applied sequentially (first high light without UV, then lower light supplemented with UV) and under greenhouse conditions, results are expected to promote our understanding of possible interactions between responses to the UV component and the high intensity visible component of sunlight in nature.

Materials and Methods

Plant material and treatments

Tobacco (*Nicotiana tabacum* L. cv. Petite Havana SR1) seeds were sown in standard soil and plantlets were transferred into 16 cm diameter individual pots. Plants were grown in greenhouse conditions (until 5- to 6-leaves stage) at 25/20 ^{o}C, at 12 h daily irradiation with 200 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) for four weeks before the treatments started. Plants were first divided into two pretreatment groups with different light conditions. Half of them were left at the same 200 µmol m^{-2} s^{-1} PPFD (referred to as "200"), while others were exposed to 1000 µmol m^{-2} s^{-1} PPFD for 5 days (referred to as "1000"). After this period plants were further divided into 2-2 groups: one of the groups was exposed to supplemental UV-B centered radiation for 6 days (referred to as "UV"), while the other represented the untreated group (referred to as "unt"). Therefore we had four different treatment conditions: (1) 200 µmol m^{-2} s^{-1} PPFD for 12 days ("200-unt"), (2) 5 days at 1000 µmol m^{-2} s^{-1} PPFD and 6 days at 200 µmol m^{-2} s^{-1} PPFD ("1000-unt"), (3) 5 days at 200 µmol m^{-2} s^{-1} PPFD and 6 days with supplemental UV-B radiation ("200-UV") and (4) 5 days at 1000 µmol m^{-2} s^{-1} PPFD and 6 days with supplemental UV-B radiation ("1000-UV"). Supplemental UV-B light was generated from Q-Panel UVB-313EL tubes for 8 hours daily. One layer of cellulose diacetate filter (Courtaulds Chemicals, Derby, UK) was used to exclude shorter wavelength (<280 nm) UV radiation. Integrated UV-B dose was 0.84 W m^{-2} irradiance (Cole-Palmer radiometer, model 97503-00 with a broad range 312 nm centered sensor). The applied UV irradiance (280-400 nm) corresponded to 8.95 kJ m^{-2} d^{-1} biologically effective dose of which the UV-B part (280-315 nm) represented 8.04 kJ m^{-2} d^{-1}, calculated using the Biological Spectral Weighting Function developed by Flint and Caldwell (2003). This UV-B dose is close to the ambient daily biologically effective UV-B at our latitude in the northern hemisphere in summer (Bassman et al., 2001). For further details on the spectral distribution of UV irradiance from the tube panel see Majer and Hideg (2012).

Each treatment group included three plants and from each plant one fully-developed leaf was used for all the measurements, taken from the same level for excluding age effect and to ensure that the same UV and PPFD was experienced by the leaves.

Photosynthesis and electron transport measurements

Photosynthesis (CO_2 uptake µmol m^{-2} s^{-1}) was assessed on intact leaves at 200 µmol m^{-2} s^{-1} PPFD using LI-6400 Portable Photosynthesis System (LI-COR Environmental, Lincoln, Nebraska USA). Leaves were then cut off from the plants and kept in darkness for 30 min before chlorophyll fluorescence measurements with the MAXI-version of the Imaging-PAM (Heinz Walz GmbH, Effeltrich, Germany). After the dark adaptation period, minimum (F_o) and maximum (F_m) fluorescence yields were determined before and after a saturating pulse, respectively. This was followed by 30 sec long exposure to blue actinic light (160 µmol m^{-2} s^{-1} PPFD), and F and F_m' values were obtained at each illumination step. Effective PS II quantum yields were calculated as $Y(II) = (F_m' - F) / F_m'$ and relative electron transport rates were determined following the standard formula $ETR = Y(II) \cdot PAR \cdot 0.5 \cdot 0.84$ (Genty et al., 1989).

Determination of UV-B absorbing pigments

Two 0.6 cm discs were cut from each leaf and were extracted into acidified methanol and kept at 4^{o}C in darkness for 24 hours, then ground and centrifuged (3000 x g, 5 min, 4^{o}C). Supernatants were used for spectrophotometric determination of total UV-B absorption ($\sum OD_{280\text{-}315}$ g^{-1} leaf fresh

weight) (Mirecki and Teramura, 1984), using a Shimadzu UV-1601 spectrophotometer.

Leaf extraction

For total and specific antioxidant capacity measurements (total phenolics content, FRAP and hydroxyl radical scavenging) twelve 0.6 cm leaf disks were cut, weighted and were first ground in liquid nitrogen, then in 1 mL phosphate buffer (50 mM, pH 7.0, 1 mM EDTA). Cell debris was first removed by a mild centrifugation (3000 x g, 5 min, 4°C), then supernatants were re-centrifuged (30,000 x g, 25 min, 4°C) and were stored at -80°C until use. The Folin-Ciocalteu reagent was purchased from Ferak Berlin GmbH (Berlin, Germany). All other chemicals were from Sigma-Aldrich (Sigma-Aldrich Kft Budapest, Hungary).

Total phenolic content

Total phenolic content was determined with the Folin-Ciocalteu method as described by Veliglu et al. (1998). For each sample, 80 µL plant extract was mixed with 500 µL Folin-Ciocalteu reagent (previously diluted 10-times with distilled water) and allowed to stand at room temperature for 5 min, then 500 µL Na_2CO_3 (60 g L^{-1}) was added to the mixture. After 90 min incubation at room temperature, absorbance at 725 nm was measured. Gallic acid (GA) was used for calibration and total phenolic contents were expressed in µmol GA equivalents g^{-1} leaf fresh weight.

Ferric reducing antioxidant power (FRAP)

FRAP assay was carried out according to a modification of the original medicinal biochemical assay (Benzie and Strain, 1996) by Szőllősi and Szőllősi-Varga (2002). FRAP reagent was prepared by mixing sodium acetate buffer (300 mM, pH 3.6), tripyridyltriazine (TPTZ) solution (10 mM TPTZ in 40 mM HCl) and $FeCl_3$ (20 mM in water solution) in 10:1:1 ratio. For each sample, 80 µL plant extract was added to 1 mL freshly mixed FRAP reagent. After 30 min incubation time, the increase in 593 nm absorbance due to the formation of the blue-coloured ferrous form (Fe^{2+}-TPTZ complex) was measured. Ascorbic acid (AsA) was used for calibration and results were expressed as µmol AsA equivalents g^{-1} leaf fresh weight.

Hydroxyl radical scavenging capacity

Specific hydroxyl radical ($^{\bullet}OH$) scavenging was determined based on the leaf extracts` ability to inhibit the formation of the strongly fluorescent 2-hydroxyterephthalate (HTPA) generated in a reaction between terephthalate (1,4-benzenedicarboxylic acid, TPA) and $^{\bullet}OH$ (Šnyrychová and Hideg 2007). HTPA fluorescence was measured with a Quanta Master QM-1 spectrofluorometer (Photon Technology Inc., Birmingham, New Jersey, USA), using 315 nm excitation and 420 nm emission. $^{\bullet}OH$ was produced in a reaction mixture containing 500 µM TPA, 10 µM EDTA, 10 µM $FeSO_4$, 100 µM AA and 100 µM H_2O_2 in a 50 mM Na-phosphate buffer (pH 7.2). $^{\bullet}OH$ scavenging capacity of each leaf extract was characterized by its half-inhibitory concentration on HTPA formation as described earlier (Stoyanova et al., 2011). Ethanol, a strong $^{\bullet}OH$ scavenger was used for calibrating the method, and specific $^{\bullet}OH$ neutralizing capacities of leaf extracts were given as µM ethanol equivalent g^{-1} leaf fresh weight.

Ascorbate measurements

Ascorbate content of the samples was determined according to Takahama and Oniki (1992), from the absorption of ascorbate at 265nm (ε=18$mM^{-1}cm^{-1}$). Ascorbate and dehydroascorbate were measured in 50mM potassium phosphate buffer (pH 6.0), in three different assay conditions: without addition, oxidised by 0.5 units mL^{-1} ascorbate-oxidase or reduced by 2mM dithiothreitol. Samples were characterised by the amount of total ascorbate and by the ratio of oxidised to total ascorbate as described earlier (Hideg et al., 2006).

Statistics

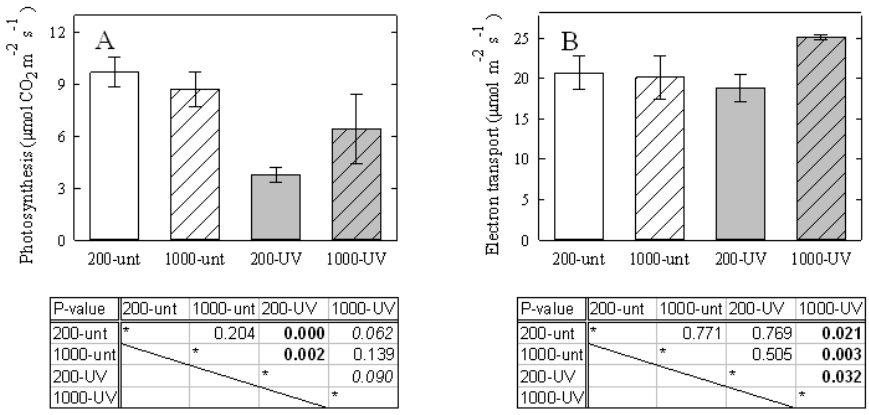
Student's t-test was used to compare means of each two groups and to calculate P-values (GraphPad, GraphPad Software Inc., La Jolla, CA, USA). SigmaPlot (Systat Software Inc., San Jose, CA, USA) was used for creating graphs.

Results and Discussion

Figure 1 illustrates the outline of the experiment and shows plant group identifiers. Data from high light pretreated leaves are labeled as "1000" and data from leaves without this pretreatment are marked with "200", referring to PPFD during the week preceding UV exposure. Plants which were not given the UV treatment and plants which were given the supplemental UV are labeled "unt" and "UV", respectively. Labels were doubled to indicate both pretreatment and UV irradiation, for example "200-UV" marks data from leaves which were exposed to supplemental UV without high-light acclimation and "1000-unt" was used for high-light acclimated leaves which were not exposed to UV afterwards (see Materials and methods section for details).

symbol				
data label	200-unt	200-UV	1000-unt	1000-UV
high light pretreatment	no	no	yes	yes
supplemental UV	no	yes	no	yes

Figure 1. Outline of the experiment and group identifiers.



P-value	200-unt	1000-unt	200-UV	1000-UV
200-unt	*	0.204	**0.000**	*0.062*
1000-unt		*	**0.002**	0.139
200-UV			*	*0.090*
1000-UV				*

P-value	200-unt	1000-unt	200-UV	1000-UV
200-unt	*	0.771	0.769	**0.021**
1000-unt		*	0.505	**0.003**
200-UV			*	**0.032**
1000-UV				*

Figure 2. (A) Photosynthesis and (B) photosynthetic electron transport of tobacco leaves belong to different treatment groups. Tables show P-values of Student's t-test in normal fonts ($p>0.1$), italics ($0.1>p>0.05$) or bold letters ($p<0.05$). For treatment group identifiers see Figure 1.

The effect of the treatments on photosynthesis is displayed on Figure 2. Figure 2A and 2B show that high light acclimation had no effect on photosynthesis: both carbon-dioxide uptake and photosystem (PS) II electron transport were the same in 200-unt and in 1000-unt leaves. UV had smaller effect on CO_2-fixation ability in high light acclimated leaves: 1000-UV leaves retained 75% of the photosynthesis of 1000-unt ones, but 200-UV photosynthesis was only 40% of 200-unt (Figure 2A). Electron transport was not lessened by UV irradiation, and was even slightly stimulated in 1000-UV leaves as compared to 1000-unt ones (Figure 2B). In this way, the observed loss in CO_2-uptake was rather due to decreased stomata conductivity than to electron transport limitation. The same, but more pronounced UV-induced decrease pattern was observed in stomata conductivity as in photosynthesis: 85% decrease in non-pre-treated tobacco leaves but only 30% decrease in 1000-UV plants, compared to their controls (data not shown). UV-B radiation has been shown to decrease photosynthetic CO_2-uptake, mainly via limiting stomata opening (Nogues et al., 1999, Jansen and van den Noort, 2000) but UV-inducible inhibition of electron transport (Renger et al., 1989, Vass et al., 1996) and Rubisco synthesis (Takeuchi et al., 2002, Choi and Roh, 2003) were also shown to be affected although the latter are usually reported in response to high UV doses.

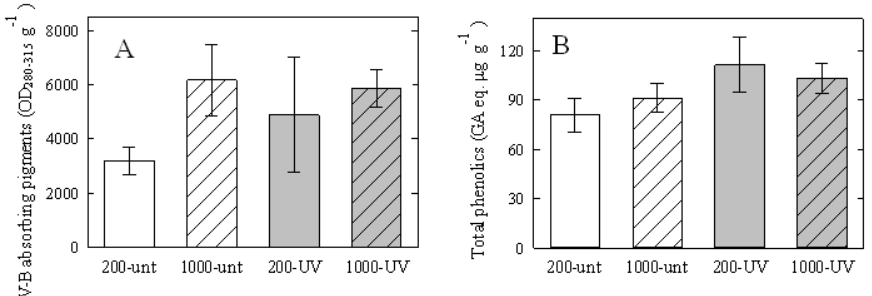
Increased epidermal UV absorption is a known component of leaf responses to UV irradiation (Caldwell et al., 1983). In our experiment the production of pigments absorbing in the UV-B region (between 280 and 315 nm) was observed in the absence of UV treatment as well: high light pretreatment almost doubled the amount of these compounds (unt-1000 and unt-200 data in Figure 3A). UV irradiation brought no significant changes in high light acclimated samples, but the amount of UV absorbing pigments increased slightly further in 200-UV leaves, although not to the amounts detected in 1000-UV ones. To interpret these results it is important to note that these are data from total leaf extracts, therefore the absorption of epidermal UV-B absorbers and of mesophyllic compounds cannot be separated. As the difference between unt-1000 and unt-200 leaves is clearly due

to the effect of high PPFD and thus cannot be expected to originate in increased epidermal UV absorption, these data show that an increase in UV absorption may reflect increased antioxidant capacity and does not necessarily refer to increased epidermal screening. Typical UV absorbing antioxidant compounds are phenolic compounds and mostly flavonoids (Winkel-Shirley, 2002, Zhang and Björn, 2009). Flavonoids are considered to act primarily as epidermal UV screening compounds, but recent evidences support the antioxidant function of flavonoids localized deeper in plant tissues in protection against excess light induced photoinhibition (Agati and Tattini, 2010).

However, analysis of total phenolic compounds did not fully confirm this (Figure 3B): unt-1000 leaves had only slightly elevated level of phenolic compounds compared to unt-200 ones. Exposure to UV irradiation increased this in 200-UV leaves to amounts characteristic to 1000-unt plants while data of 1000-UV samples were not different from their untreated pairs (Figure 3B). This shows that the increase in UV absorption in response to high PPFD was not mainly due to the increase in phenolic compounds. As Levizou and Manetas (2002) showed, although total phenol content and UV-B screening pigment contents are strongly correlated in various plant species at given circumstances, but one has to keep in mind that not all UV-B absorbing pigments are phenolics and *vice versa*. In our experiment, although high light not, but UV radiation was capable of promoting the production of a large range of phenolic compounds in which UV absorbing ones are more responsive than others.

To characterize samples further in terms of antioxidants, two antioxidant parameters, one non-specific parameter measuring total antioxidant capacity (by means of ferric reducing antioxidant power, FRAP) and a selective ROS neutralizing parameter (hydroxyl radical scavenging) were also determined.

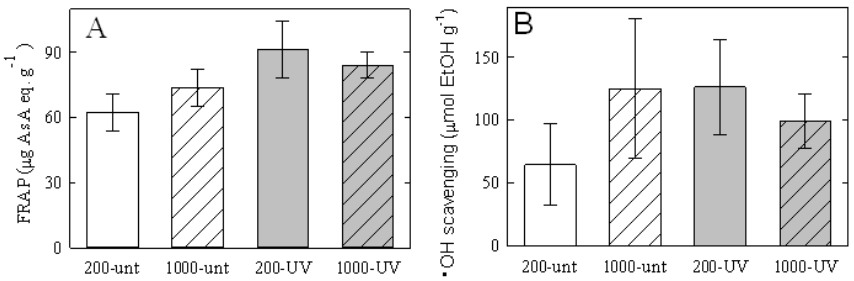
Regarding the ferric reducing ability, high light could not trigger this antioxidant power, but UV caused an almost 50% increase in 200 and a 35% increase in 1000 plants compared to 200-unt ones (Figure 4A). This parameter indicates that under supplemental UV, leaves evoke protection against hydroxyl radical ($^{\bullet}OH$) production via Fenton chemistry (Halliwell and Gutteridge, 1999) by removing free iron, should it be released from damaged iron containing proteins under more severe stress conditions. To test whether this preventive mechanism is complemented by specific antioxidant capacity, $^{\bullet}OH$ scavenging was also measured. 1000-unt leaves were 2-times richer in antioxidants that are capable of neutralizing $^{\bullet}OH$ radicals than 200-unt leaves (Figure 4B). This specific capacity were not different in 1000-UV and 1000-unt ones. UV treatment boosted the production of antioxidant responsive to $^{\bullet}OH$ radicals in the leaves without pretreatment, while there were no further increase in 1000-UV plants compared to 1000-unt ones. These suggest that the increase in $^{\bullet}OH$ radical scavenging capacity in response to high PPFD pretreatment could readily protect the leaves from additional damaging ROS effects deriving from exposure to UV.



P-value	200-unt	1000-unt	200-UV	1000-UV
200-unt	*	**0.006**	0.237	**0.002**
1000-unt		*	**0.047**	0.211
200-UV			*	*0.093*
1000-UV				*

P-value	200-unt	1000-unt	200-UV	1000-UV
200-unt	*	0.251	*0.055*	*0.051*
1000-unt		*	0.142	0.192
200-UV			*	0.490
1000-UV				*

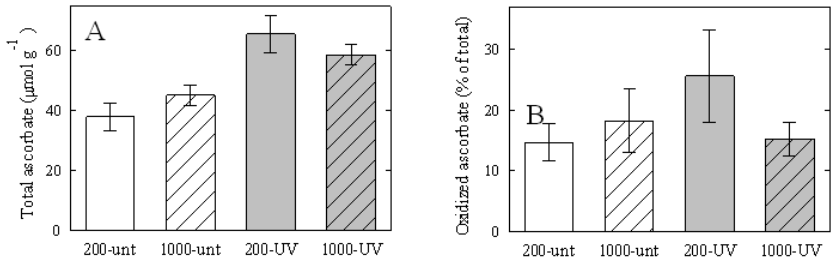
Figure 3. (A) UV-B absorbing pigments and (B) total phenolics content of tobacco leaves belong to different treatment groups.Tables show P-values of Student's t-test in normal fonts ($p>0.1$), italics ($0.1>p>0.05$) or bold letters ($p<0.05$). For treatment group identifiers see Figure 1.



P-value	200-unt	1000-unt	200-UV	1000-UV
200-unt	*	0.175	**0.032**	**0.024**
1000-unt		*	0.121	0.163
200-UV			*	0.420
1000-UV				*

P-value	200-unt	1000-unt	200-UV	1000-UV
200-unt	*	0.132	**0.049**	**0.023**
1000-unt		*	0.202	0.752
200-UV			*	0.221
1000-UV				*

Figure 4. (A) Ferric reducing antioxidant power and (B) hydroxyl radical scavenging capacity of tobacco leaves belong to different treatment groups. Tables show P-values of Student's t-test in normal fonts ($p>0.1$), italics ($0.1>p>0.05$) or bold letters ($p<0.05$). For treatment group identifiers see Figure 1.



P-value	200-unt	1000-unt	200-UV	1000-UV
200-unt	*	0.106	**0.004**	**0.004**
1000-unt		*	**0.008**	**0.009**
200-UV			*	0.166
1000-UV				*

P-value	200-unt	1000-unt	200-UV	1000-UV
200-unt	*	0.390	*0.083*	0.846
1000-unt		*	0.236	0.429
200-UV			*	*0.090*
1000-UV				*

Figure 5. (A) Total ascorbate content and (B) the ratio of oxidized ascorbate to total ascorbate content of tobacco leaves belong to different treatment groups. Tables show P-values of Student's t-test in normal fonts ($p>0.1$), italics ($0.1>p>0.05$) or bold letters ($p<0.05$). For treatment group identifiers see Figure 1.

Ascorbate is an important plant antioxidant and an increase in total ascorbate is frequently observed in leaves acclimated to stress conditions (Noctor and Foyer, 1998). Oxidation of leaf ascorbate beyond the capacity of its regeneration (i.e. an increase in concentration ratios of oxidized to reduced ascorbate) is considered as one of the many markers of oxidative stress (Heber et al. 1996, Hideg et al. 1997). While higher amounts of ROS reactive ascorbate contribute to total antioxidant capacity, ascorbate may also act as a pro-oxidant, promoting the generation of $^{\bullet}OH$ radicals through the reduction of ferric molecules (Halliwell and Gutteridge, 1999). The high FRAP value in 200-UV plants (Figure 4A) suggest an increased free iron level, which enhances the danger of ascorbate mediated ROS generation. In order to see whether UV irradiation imposed oxidative stress in our study, both amounts of total ascorbate and relative amounts of oxidized ascorbate were measured. Results in Figures 5A and 5B show that the applied supplemental UV irradiation caused oxidative stress in 200-plants only, in which the ratio of oxidized ascorbate markedly increased. 200-UV leaves had significantly higher levels of ascorbate (1.7-times) than 200-unt ones (Figure 5A), but in these samples regeneration of oxidized ascorbate was unable to keep up with oxidation and the ratio of oxidized ascorbate increased from 15% to 26% (Figure 5B). High light pretreatment, on the other hand, caused no increase in the ascorbate content (Figure 5A), or in the degree of ascorbate oxidation (Figure 5B). UV irradiation caused an increase in the ascorbate content of 1000-leaves, but these leaves were able to maintain a relatively low, 15% oxidized ascorbate ratio. Results of the above ascorbate

measurements show that an important difference between 200-UV and 1000-UV leaves is that while the former suffer mild oxidative stress the latter were rather UV-acclimated than stressed. A possible interpretation of the above data is that the increase in ascorbate content in 200-UV plants compared to 200-unt may not be all beneficial if not accompanied by efficient regeneration of oxidized ascorbate which does not contribute to the leaf's antioxidant capacity.

Conclusions

Pretreatment under high PPFD protected tobacco leaves from ROS effects derived from consecutive exposure to supplemental UV irradiation. High light pretreated leaves were rather acclimated than stressed: although lost some CO_2 incorporating capacity, these maintained a more reduced ascorbate pool and better photosynthetic electron transport. The ability to acclimate to UV appears to be due to higher levels of UV-B absorbing and $^{\bullet}OH$ radical scavenging antioxidants in these leaves, which was maintained during the UV irradiation. Leaves which did not receive the antioxidant stimulating high light treatment increased protective pathways (total phenolics, FRAP, $^{\bullet}OH$ radical scavenging) during UV irradiation to levels found in high light pretreated plants. However, these induced lines of defence could not protect tobacco leaves from UV as efficiently as high levels of defensive antioxidants already present at the onset of UV. Our data show that acclimative responses to UV overlap at several points resulting in a cross tolerance effect. Moreover, the production of UV-B absorbing components was lower in response to UV treatment than to high light pretreatment. The same phenomenon was observed by Younis et al. (2010) with overlapping antioxidant responses for high light and UV in broad bean seedlings. Bolink et al. (2001) showed the reverse: growth under UV-B radiation increased photoprotection in high light situations in both pea and bean plants based on elevated thiol and UV-absorbing compound concentrations. This suggests the possibility of a synergy in high light and UV responses in plants exposed to sunlight, with acclimation to high light helping to cope with solar UV and *vice versa*. Compounds traditionally detected as UV-absorbing pigments are an example of this, as suggested by results of the laboratory experiments presented here. Due to the application of broad band UV irradiation centered in UV-B but also containing UV-A in the present work, it would take further experiments to study whether (and to which extent) UV-A is involved in this cross tolerance.

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REGULAR ARTICLE

Differences in antioxidant mechanisms in grapevines subjected to drought and enhanced UV-B radiation

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Abstract

The differences in antioxidant properties in grapevines (*Vitis vinifera* L. cv Romeiko) exposed to either drought, enhanced levels of UV-B radiation or to the combined application of the two abiotic stressors were studied. Two-year-old grapevines grown outdoors in 25 L pots containing peat:perlite:sand (3:1:1) were used. The following treatments were applied: i) well-watered, under ambient UV-B level treatment; (ii) water-stressed, under ambient UV-B level treatment; (iii) well-watered treatment under enhanced UV-B and (iv) water-stressed treatment under enhanced UV-B. Results indicated that predawn leaf water potential (Ψ_{PD}) decreased progressively in water-stressed treatments, irrespective of the level of the UV-B radiation. All treatments exhibited a close relation between photosynthetic rate (Pn) and stomatal conductance (gs), suggesting that stomatal closure is the dominant limitation to photosynthesis. Both drought and enhanced UV-B radiation caused a significant increase in hydrogen peroxide content (H_2O_2) and lipid peroxidation (TBARS). However, the accumulation of H_2O_2 was more pronounced in plants exposed to enhanced UV-B radiation. Independent of water supply, enhanced UV-B radiation significantly increased the activities of superoxide dismutase (SOD, EC 1.15.1.1), ascorbate peroxidase (APX, EC 1.11.1.11), quaiacol peroxidase (GPX, EC 1.11.1.7) and catalase (CAT, EC 1.11.1.6) as well as carotenoids content while the expression of antioxidant enzymes was lower in plants exposed only to drought conditions.

Key words: UV-B radiation, drought, antioxidants, gas exchange, *Vitis vinifera*

Introduction

In the Mediterranean zone, high levels of ultraviolet-B (UV-B: 280-320 nm) radiation and drought are a typical combination of abiotic stresses that plants often have to cope with during the growing season. In particular, the depletion of the earth's stratospheric ozone layer that occurred in the last decades has led to an increase in levels of UV-B radiation that reach the earth's surface. Many studies have already indicated significant effects of high UV-B doses in plants at physiological, morphological, anatomical and biochemical level (Rozema et al., 1997; Jansen et al., 1998). On the other hand, drought is considered to be an important environmental constraint limiting plant growth and yield worldwide (Flexas and Medrano, 2002; Chaves et al., 2003).

Studies on interaction between high levels of UV-B and drought indicate that the two abiotic factors can induce various responses in plants that could be either additive, synergistic or antagonistic (Gwynn-Jones et al., 1999; Alexieva et al., 2001). It is suggested that the type of responses exhibited by a plant organism to the combination of these two abiotic stresses is unique, depending on the genotype, nature, intensity and duration of the stresses applied (Alexieva et al., 2003).

A common consequence of plants exposure to environmental stresses is the over-production of highly reactive oxygen species (ROS), mostly in chloroplasts, mitochondria and peroxisomes (Beis and Patakas, 2012). The accumulation of high ROS concentrations could result in RNA and DNA damage, enzyme inhibition, protein oxidation and membrane lipid peroxidation (Mittler, 2002; Scandalios, 2002). Plants have developed an efficient antioxidant system for protection against these toxic effects of ROS. In particular, the activation of certain antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and quaiacol

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peroxidase (GPX) consists a significant component of plants ROS defence network. Moreover, chlorophyll loss (due to chlorophyll degradation or chlorophyll synthesis deficiency) reduces the amount of photons absorbed by leaves under unfavourable environmental conditions, thus enhancing the photoprotective and antioxidant leaf capacity (Smirnoff, 1993). Additionally, several non-enzymatic antioxidant molecules such as carotenoids are reported to contribute in effectively dissipating the excess of excitation energy under stress conditions by quenching triplet state chlorophyll molecules and scavenging singlet oxygen and other toxic ROS that are formed within the chloroplasts (Berli et al., 2010). However, the relative contribution of these antioxidant mechanisms in the protective responses of grapevine plants exposed to drought, enhanced UV-B and/or the combination of these two stressors remained obscure.

Thus the aim of this study is to elucidate possible differences in grapevines enzymatic and non-enzymatic defense mechanisms in response to drought and enhanced UV-B radiation.

Materials and Methods

Plant material and treatments

This study was carried out in the Institute for Olive Tree and Subtropical Plants in the city of Chania, Crete, Greece (35° 32' 00" N, 24° 04' 09" E). The climate is of Mediterranean type with hot and dry summers and mild and rainy winters. A total of twenty four, two-year-old, grapevine plants of the local red variety Romeiko, (*Vitis vinifera* L.) grafted onto 110-R rootstocks were used. All plants were grown in 25 L pots containing a peat:perlite:sand potting mix (3:1:1, v/v). Canopy management practices included: winter pruning to 3 nodes per vine, removal of the extra shoots immediately after budbreak (only the most robust shoot was allowed to grow on each plant) and vertical shoot positioning. The plants were divided into four groups, each one consisting of six plants. In each group of plants, one of the following treatments was applied over a 15-day period: (i) well-watered (WW) treatment, in which the plants were irrigated daily to soil capacity while being exposed to ambient UV-B radiation (WW-ambient UV-B treatment); (ii) water-stressed (WS) treatment, in which the plants were receiving every day, 50% of the amount of irrigation water provided to well-watered plants, under ambient UV-B levels (WS-ambient UV-B treatment), (iii) well-watered treatment under supplemental UV-B, in which the well-watered plants were exposed to ambient plus 30% UV-B radiation (WW+30% UV-B treatment); and (iv) water-stressed treatment under supplemental UV-B, in which the water-stressed plants were exposed to ambient plus 30% UV-B radiation (WS+30% UV-B treatment). Irrigation water was applied with drip emitters while enhanced UV-B radiation was supplied by UV-emitting fluorescent tubes (Philips, Ultraviolet-B, TL40W/12RS, Holland), covered with preheated, for 8 hours, 0.115 mm thick cellulose acetate (CA) film (Clarifoil, Binley, Coventry, UK) which transmits essentially no UV shorter than 290 nm. The CA sheets were changed every 20 hrs of operation, to avoid ageing effects of the filter. The irradiance in the UV-B band was checked daily with a broadband radiometer (SKU 430, Skye Instruments Ltd., Powys, UK). The supplemental biological effective UV-B dose ($UV\text{-}B_{BE}$) - estimated according to the generalised plant action spectrum (Caldwell, 1971) - corresponded to 19% ozone depletion (Green et al., 1974; 1980; Green, 1983). The supplemental UV-B level, as well as Caldwell's generalised plant action spectrum were selected to correspond with other studies in areas with similar latitude (35^o) (Levizou and Manetas, 2001).

Leaf water potential determination and gas exchange measurements

Predawn leaf water potential (Ψ_{PD}) was used as a sensitive indicator of grapevine water status (Williams and Araujo, 2002). Ψ_{PD} values were determined with a pressure chamber (PMS Instrument Company, Corvallis, Oregon) according to the method of Scholander et al. (1965). The readings were taken, beginning at 4:30 a.m. and ending before sunrise, using fully expanded leaves. In particular, the fourth to sixth mature leaf from the shoot apex was used. Leaf photosynthetic rate (Pn) and stomatal conductance (Gs) were recorded with a Li-6400, portable photosynthesis system (Li-Cor Bioscience Inc, Lincoln, Nebraska, US), on fully expanded, healthy leaves in the morning (between 09:30 and 11:00) at saturated light intensities (PPFD greater than 1000 $\mu mol\ m^{-2}\ sec^{-1}$).

Enzymatic antioxidant activity, lipid peroxidation and hydrogen peroxide measurements

SOD, GPX, APX and CAT activities were determined on six leaves per treatment which were collected three times during the experimental period. The extraction medium consisted of 0.1 M K-P (potassium phosphate) buffer (pH 7.6), containing 1 mM ethylenediaminetetraacetic acid disodium salt (EDTA-Na_2), 0.5 mM ascorbate and

1% PVPP (polyvinylpolypyrrolidone). 0.3 g of leaf tissue was homogenised in 1.5 mL of the extraction buffer and the homogenate was centrifuged at 13 000 x g for 30 min. The supernatant was used for assaying the activities of the enzymes. The absorbance of the crude enzymes extract was measured with a Hitachi U-1100 spectrophotometer (Hitachi Ltd., Tokyo, Japan). SOD isoforms (EC 1.15.1.1) activity was determined using the methodology described by Becana et al. (1986) based on the capacity of the enzyme extract to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) to blue formazan. GPX (EC 1.11.1.7) assay was performed using the method described by Costa et al. (2002). GPX activity was calculated following the oxidation of guaiacol at 470 nm using an extinction coefficient of 26.6 $mMcm^{-1}$. APX (EC 1.11.1.11) activity was determined according to Nakano and Asada (1981), by measuring the change in absorbance at 290 nm ($E = 2.8\ mMcm^{-1}$). CAT (EC 1.11.1.6) activity was assayed according to Cakmak and Marschner (1992). Soluble protein content was measured according to the method of Bradford (1976), using Sigma-Aldrich, Total Protein Kit, Micro (product code TP0100, Sigma-Aldrich, Saint Louis, Michigan, USA). For the determination of hydrogen peroxide (H_2O_2) and lipid peroxidation (TBARS), leaf tissue (0.5 g) obtained from the same leaves that were used for the antioxidant enzyme assays, was homogenised with 5 mL trichloroacetic acid (TCA) 0.1% (w/v) in an ice bath. The homogenate was centrifuged at 10 000 x g for 30 min. H_2O_2 content was measured spectrophotometrically after reaction with potassium iodide (KI) (Alexieva et al., 2001). The absorbance was measured at 390 nm using a solution consisting of TCA 0.1% and pure catalase reagent as a blank, to ensure zero interference. In order to calculate the amount of hydrogen peroxide, a standard curve was prepared with known H_2O_2 concentrations. TBARS (Thiobarbituric Acid Reactive Substances) index is taken as the measure of in vivo lipid peroxidation of plant tissue. TBARS were estimated by the method of Hodges et al. (1999). The absorbance of the supernatant was read at 532 nm with the values for non-specific absorption at 600 nm and 440 nm subtracted.

Photosynthetic pigments analysis

Chlorophyll (Chl-a and Chl-b) and carotenoids (Car) were extracted from leaves with 80% acetone. Chl and Car content was determined spectrophotometrically according to the method of Lichtenthaler and Welburn (1983).

Statistical analysis

All data were subjected to a two-way ANOVA using the SPSS 15.0 (SPSS Inc., Chicago, Illinois, USA). The LSD test was used for the comparison of treatments average values. Statistical comparisons were considered significant at $P < 0.05$.

Results and Discussion

The more rapid decrease in photosynthetic rate exhibited by the plants exposed to higher UV-B intensities (Figure 1A) could be attributed to changes in stomatal conductance (Figure 1B). Indeed, stomatal conductance was significant lower in plants exposed to elevated UV-B intensities irrespective the water regime applied. Since stomatal conductance strongly depends on leaf water relations (Beis and Patakas, 2010), it was expected Ψ_{PD} values to be lower in plants under enhanced UV-B radiation (compared with WS-ambient UV-B treatment), as a consequence of the rapid stomatal closure (Figure 2). However, this was not evident in our results where WW+30% treatment exhibited higher values in predawn leaf water potential (Figure 2). The latter indicates that high levels of UV-B could probably affect stomatal function through an alternative mechanism, which is not directly related to leaf water status. This is consistent with the results obtained by Negash and Bjorn (1986) and Nogués et al. (1999).

On the other hand, the reduction of stomatal conductance is known to induce a serious decline in CO_2 concentration in chloroplasts, which in turn increases plant's susceptibility to photoinhibition (Beis and Patakas, 2012). In fact, under limiting conditions of CO_2 fixation, the rate of reducing power production could overcome the rate of its use in photosynthetic electron transport chain, thus damaging the photosynthetic apparatus. In order to prevent the production of excess reducing power plants have developed different protection mechanisms including reduction of light absorbance by adjusting chlorophyll content and/or antenna size as well as by increasing thermal dissipation (NPQ) of excess absorbed light in the light-harvesting complexes, through the xanthophyll and the lutein cycle (Müller et al., 2001; Li et al., 2009). In our results a reduction in chlorophyll photosynthetic pigments was evident in all stress treatments, being more pronounced in plants exposed to enhanced UV-B intensities irrespective the water regime applied (Fig. 3A and 3B). This is consistent with the results reported for several other species indicating a significant decrease in total chlorophyll content in plants

grown under high UV-B intensities (Liu et al., 2011). In contrast, carotenoids concentration was increased under enhanced UV-B radiation (Fig. 3C). These results are consistent with those of Majer and Hideg (2012) and Berli et al. (2010) who also indicated an increase in carotenoids in grapevine plants exposed to enhanced UV-B levels. This increase in carotenoids content could play an important photoprotective role either by dissipating excess excitation energy as heat or by singlet oxygen scavenging (Majer and Hideg, 2012). However, recent studies suggested that no singlet oxygen was produced in response to oxidative stress caused by high UV-B doses and consequently no correlation exist between total carotenoids content and singlet oxygen scavenging capacities (Majer and Hideg, 2012). Thus, the exact role of carotenoids under enhanced UV-B conditions remains to be elucidated.

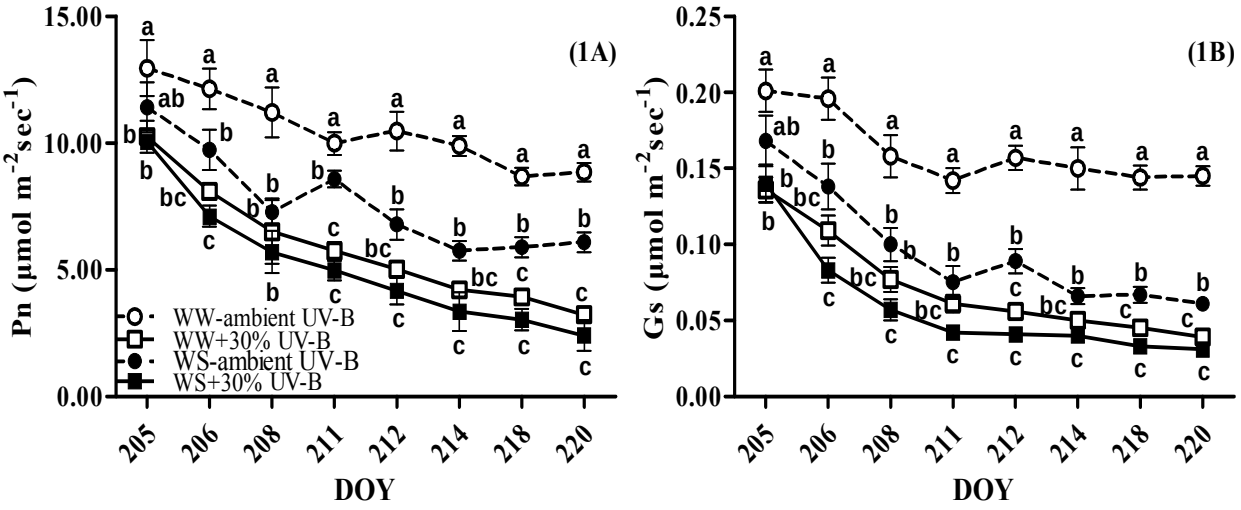

Figure 1. Changes in leaf photosynthetic rate-Pn (A) and stomatal conductance-Gs (B) throughout the experimental period (DOY-Day Of Year) in all treatments.
Each symbol represents the average ± standard error of 4 values. Different letters indicate significant difference at $P < 0.05$.

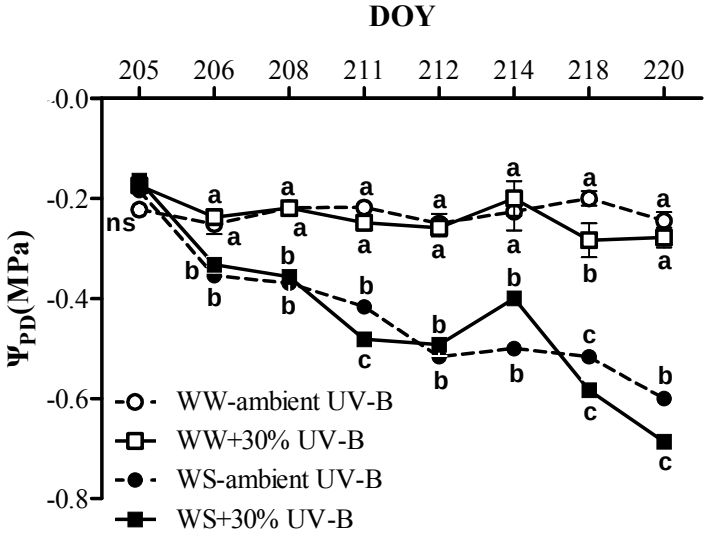

Figure 2. Changes in leaf predawn water potential (Ψ_{PD}) during the experimental period (DOY-Day Of Year) in all treatments.
Each point represents the mean value ± standard error of 4 measurements. Different letters indicate significant difference at $P < 0.05$.

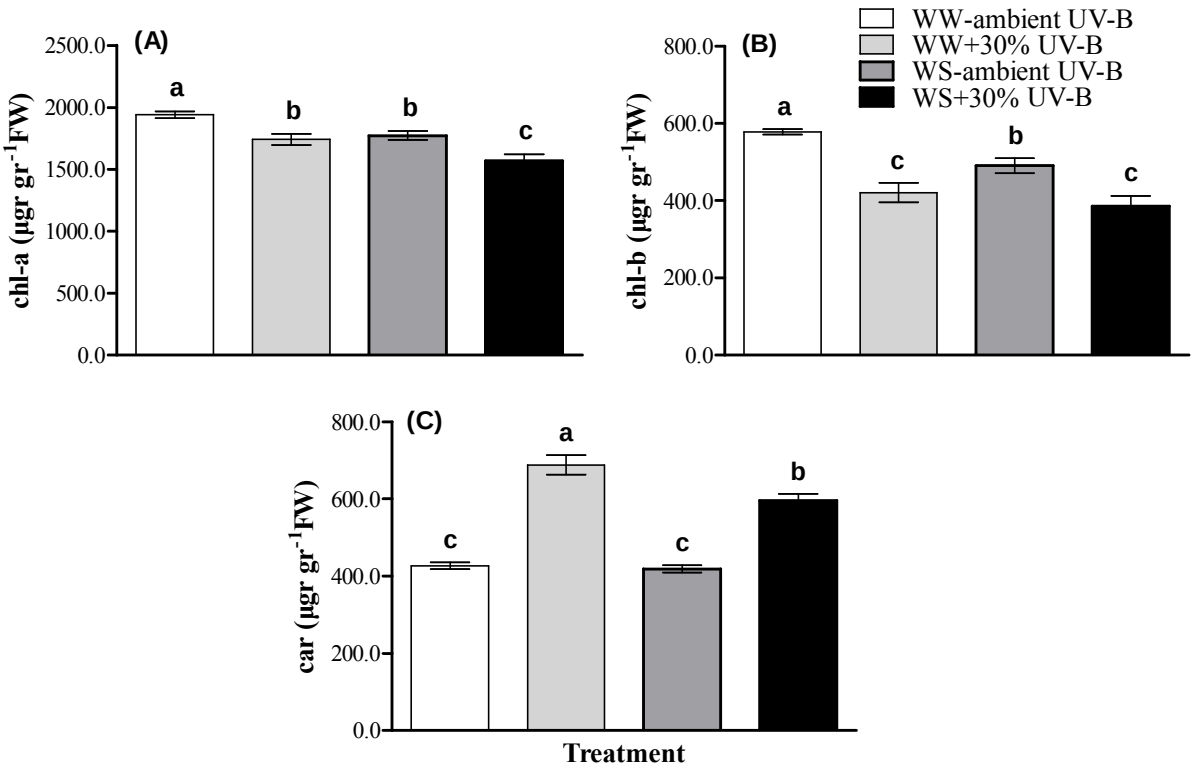

Figure 3. Average values of chlorophyll-a (chl-a), chlorophyll-b (chl-b) and total carotenoids (car) throughout the experimental period in all treatments (n=18).
Means with different letters are significantly different ($P < 0.05$).

Changes in environmental conditions result in metabolic imbalances that can induce an oxidative stress in cells by promoting the generation and accumulation of Reactive Oxygen Species (ROS). In order to cope with oxidative damage, plants have developed an antioxidant system that includes the up-regulation of different enzymes (CAT, GPX, APX, SOD) (Foyer and Noctor, 2005). SOD isoforms constitute the first line of antioxidant defence and have been identified as a family of metalloenzymes, catalyzing the scavenging of O_2^- to H_2O_2 (Noctor and Foyer, 1998). CAT isoforms are localized predominantly in peroxisomes and glycoxysomes and are heme proteins that convert H_2O_2 to water and oxygen (Dat et al., 2000), and various peroxidases (POX) such as APX and GPX are localized throughout the cell (mainly in cell walls and vacuoles) and decompose H_2O_2 by oxidation of substrates such as phenolic compounds and/or antioxidant's metabolites (Gaspar et al., 1991). In our results a significant increase in SOD activities was evident in plants exposed to enhanced UV-B radiation irrespective the water regime applied (Table 1). SOD rapidly converts O_2^- to H_2O_2 which can then be converted to water by CAT, APX and GPX. GPX and APX activities followed similar patterns to those of SOD, exhibiting higher values in plants exposed to elevated UV-B radiation (Table 1). On the other hand, CAT activity significantly increased only in plants exposed to the combination of elevated UV-B and drought (Table 1). However, the observed significant increase of all antioxidant enzymes in this treatment was proved to be ineffective to scavenge and reduce H_2O_2 concentration and consequently to protect cells from lipid peroxidation (Table 1). Considering that lipid peroxidation could be used as an abiotic stress intensity index, it can be concluded that the plants exposed to the elevated UV-B radiation and particularly the plants exposed to the combination of elevated UV-B and drought experienced more severe stress conditions compared to those exposed to drought.

Table 1. Effects of drought and enhanced UV-B radiation on the antioxidant enzymes activities as well as on H_2O_2 (μmol $gr^{-1}FW$) concentration and lipid peroxidation.
Data represent average values ± standard error (n=18). Different letters in the same column indicate significant difference at P < 0.05.

Treatments	Biochemical Parameters					
	SOD	GPX	APX	CAT	H_2O_2	TBARS
WW-ambient UV-B	49.21 ± 1.05b	0.11 ± 0.01b	0.23 ± 0.01b	8.56 ± 0.13b	1.11 ± 0.06c	12.38 ± 1.58c
WW+30% UV-B	95.25 ± 5.76a	0.30 ± 0.04a	0.38 ± 0.04a	9.34 ± 0.23b	3.74 ± 0.49ab	43.61 ± 4.82a
WS-ambient UV-B	69.38 ± 2.22b	0.15 ± 0.02b	0.27 ± 0.03b	9.34 ± 0.19b	2.66 ± 0.30b	26.63 ± 2.80b
WS+30% UV-B	110.76 ± 8.24a	0.44 ± 0.07a	0.44 ± 0.06a	10.65 ± 0.40a	4.35 ± 0.41a	49.11 ± 5.76a

SOD (U mgr-1protein); GPX (μmol quaiacol mgr-1protein min-1); APX (μmol ascorbate mgr-1protein min-1); CAT (μmol H_2O_2 mgr-1protein min-1); H_2O_2 (μmol gr-1FW); TBARS (μmol gr-1FW)

Conclusions

Exposure of grapevines to enhanced levels of UV-B radiation resulted in a more pronounced oxidative stress compared to drought. The grapevines antioxidant defence mechanism includes the activation of several antioxidant enzymes as well as changes in photosynthetic pigments and carotenoids.

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REGULAR ARTICLE

Modification of UV-B radiation effect on *Crepis capillaris* by antioxidant and environmental conditions

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Abstract

Stability of cell division and genetic structures has special significance for plant development and productivity. On the basis of literature data about the very low photolyase activity in roots, the action of UV-B radiation and its putative modifying factors were studied on the root meristematic cells of model plant *Crepis capillaris*. It is really a very sensitive system for UV-B radiation. Action of UV-B was investigated in a wide range of doses (0; 0.75;1.00; 1.13; 1.50; 2.00; 2.50; 3.00 kJ m^{-2}. The dose of 0.75 kJ m^{-2} already decreased the cell division and up to 1 kJ m^{-2} UV-B dose induced the chromosome aberrations (CAs). The supplementary B chromosome did not show any effect on CA induction, but plants with B chromosome had a more stable mitotic activity of cells. The strongest protective effect on CA induction was revealed by salicylic acid (10-4 M). Photoreactivation also showed certain decrease of the CA level, and the lowest effect was of ascorbic acid.

Key words: B-chromosome, Chromosome aberrations, Mitotic activity, Photoreactivation, Salicylic acid, UV-B radiation

Introduction

UV-B radiation, an inevitable environmental factor, has dualistic impact for plants. Stress inducing damaging effects of UV-B radiation on genome stability and physiological processes are well known, and sunlight UV-B radiation increasing the latter time is relevant not only for the human health but also for the plant productivity and production quality (Caldwell et al., 2007). Opposite to it, UV-B radiation acts also as a regulatory factor of plant life processes, even in the stress inducing conditions (Kakani et al., 2003; Ristilä et al., 2011; Robson and Aphalo, 2012).

Many stress-inducing environmental factors, including UV-B radiation, cause oxidative stress. That plant condition, induced by UV-B radiation, may be successfully changed by the exogenous anthocyanins (Woodall and Stewart, 1998; Tsoyi et al., 2008), ascorbic (Athar et al., 2008) or salicylic (Mahdavian et al., 2008) acids. In our study, ascorbic (AA) and salicylic (SA) acids decreased chromosome aberration level induced by the sunlight UV-B radiation in the meristematic root tip cells of *Crepis capillaris* (L.) Walrr. (Rančelienė et al., 2007). AA also protected *Vicia faba* plants against ozone (Turcsanyi et al., 2000). Anthocyanins from the cherry fruits revealed positive action on mitosis of onion (*Allium cepa*) and *C. capillaris* root cells and antimutagenic action against UV-B radiation in the meristematic root cells of *C. capillaris*. Concentration of cells in the prophase may be also attributed to protective action of anthocyanins as prolonging time for repair processes (Rančelienė et al., 2009).

As SA also elevates negative action of other stress-inducing factors (Horvath et al., 2007; Hayat et al., 2010) and, as demonstrated in several other studies did not included in the referred reviews, of salt stress (Gunes et al., 2007; He and Zhu, 2008; Szepesi et al., 2008), drought (Bechtold et al., 2010), metals (Ivanova et al, 2008; Popova et al., 2008), it is supposed that exogenous antioxidants may have wider application as means for the simultaneously increasing resistance not to one but to several stress-inducing factors. It may be applied in agriculture for increasing the plant production quality. The same may be attributed to AA because it activates defence-signalling genes regulating responses to ozone and pathogens (Conklin and Barth, 2004).

It was supposed that regulating action of UV-B radiation, like its interaction with modifying factors, may be effectively shown on root meristematic cells of *C. capillaris*. Information on

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the action of UV-B radiation as well as of endogenous or exogenous antioxidants on the meristematic cells is very limited (Perennes et al., 1999; Potters et al., 2000).

In the present study, we investigated the effect of UV-B radiation on meristematic root cells in a wide range of UV-B doses, compared two different *C. capillaris* karyotypes with and without supplementary B-chromosome, and action of the exogenous AA and SA on UV-B radiation effects in the meristematic root tip cells of *C. capillaris*. Effect of SA was compared with the action of photoreactivating light on the UV-B irradiated cells. Mitotic activity and induction of chromosome aberrations were studied. *C. capillaris* is very suitable for chromosome structure studies because it has only six chromosomes in diploid cells.

Meristematic root cells were chosen for two reasons. First, it allowed to carry out all treatments in the dark to escape photoreactivation and in other fully controlled environmental conditions. Second, it was believed that the root tip cells are very sensitive to UV radiation, because root cells have the lowest photolyase activity. It has been determined on different plants: rice (Hidema and Kumagai, 1998; Iwamitsu et al., 2008), *Arabidopsis* (Waterworth et al., 2002), spinach (Yoshihara et al., 2005). Maybe, other protective factors against UV action are also less expressed in root tip cells. Consequently, it was believed that in such system various modifications of UV-B radiation effects are easier to detect.

The aim of the present work was to show that meristematic root tip cells of *C. capillaris* are sensitive and useful as the test system for investigation of UV-B radiation action on plant meristematic cells as well as to investigate factors significant for UV-B radiation effect: dependence on UV-B dose, supplementary B chromosome, time of root fixation, photoreactivation, action of ascorbic and salicylic acids.

Materials and methods

A heterogeneous meristematic cell population of *C. capillaris* root tips was exposed for artificial UV-B radiation. Seed material for all experiments was grown in natural out-door conditions, typical for temperate altitude of Vilnius city (54º45´N 25º16´E) and humid continental climate. For separate experiments only fresh seeds of the same year reproduction were used. Seeds of plants with B-chromosome were obtained from J. Maluszynska through Neil R. Jones; the plants were preliminary propagated in the same conditions as the plants without B-chromosome.

Root tip irradiation

Seeds of *C. capillaris* were germinated in thermostat at 25 °C in the dark and root tips of 2-3 mm length 36 h after beginning of germination were irradiated with UV-B (312 nm, max. 2.7 mW cm^{-2}, Vilber Lourmat, France) lamp. The UV-B doses were measured with radiometer VLX-3 and sensor CX-312 (Vilber-Lourmat, France). Dose dependence was studied in a wide range – 0; 0.75;1.00; 1.13; 1.50; 2.00; 2.50; 3.00 kJ m^{-2}. Duration of irradiation from 28 sec to 1 min 50 sec, respectively UV-B dose. For examination of SA or AA (both from Sigma) effects on UV-B radiation, seeds were germinated for 36 h on distilled water or on 10^{-4} M SA or AA solutions in Petri dishes with following irradiation by UV-B lamp. Concentrations of SA and AA were chosen after preliminary studies. For photoreactivation the part of root tips were immediately irradiated with visible light.

Determination of mitotic cell activity and chromosome aberration (CA) level

Mitotic activity (MA) was determined and CAs were studied on temporary preparations stained with acetocarmine. The root tips were treated with colchicine (100 mg/l) and fixed with an acetic acid and ethanol (1:3) mixture for 3, 6, 9 hours after irradiation. The fixed root tips were stored in 70 % ethanol in a freezer until used. All cells were analyzed for MA, while CAs were determined only in the metaphase cells, as only in such cells all chromosome arrangements are clearly seen. Most of CAs are presented by chromatid and chromosome fragments.

Statistical analysis

Results were statistically evaluated as the two alternatives expressed in percentage. The mean values ± S.D. are given in Figures and Table. The significance of differences between UV-unirradiated and UV-irradiated cells were analyzed by Student′s *t*-test.

Results and discussion

UV-B dose effect

Certainly, the first question, which arises regarding the action of UV-B radiation on meristematic cells, is dose dependence of mitotic activity and chromosome stability of *C. capillaris* root cells. It was expected that the doses which are usually applied to the above-ground parts of plants are too high for the meristematic root cells. That assumption is grounded on the comparative studies of the DNA repair enzyme activity in different plant organs (Hidema and Kumagai, 1998;

Waterworth et al., 2002; Yoshihira et al., 2005). Consequently, the action of UV-B radiation on the root tip cells was studied in a wide range of UV-B doses (0; 0.75;1.00; 1.13; 1.50; 2.00; 2.50; 3.00 kJ m^{-2}). The used doses were at least three times lower than those usually used for UV-B irradiation of the above-ground plant organs. Interval between the UV-B doses was also not large, only 0.25-0.5 kJ m^{-2}. The levels of UV-B radiation on the Earth′s surface during the vegetation season are anywhere between 2 and 12 kJ m^{-2} day (UNEP, 2002; Kakani et al., 2003).

Exclusive sensitivity of *C. capillaris* root meristem cells was confirmed experimentally by UV-B radiation effects on cell division (Figure 1).

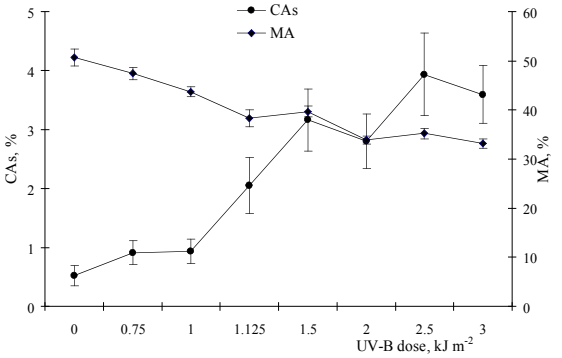

Figure 1. Mitotic activity (MA) and chromosome aberration (CA) frequency after irradiation of meristematic root cells with a wide range of UV-B doses.

Stimulation effect of the low doses on cell division may be expected. However, even the very low 0.75 kJ m^{-2} dose decreased cell division. Starting from 1 kJ m^{-2} the effect became statistically significant, and MA decrease was rather proportional to UV-B dose. CA analysis proved additionally the high sensitivity of *C. capillaris* root meristem to UV-B radiation (Figure 1), but effective doses were above 1 kJ m^{-2}. The CA doubling dose must be in the range between 1.0 and 1.13 kJ m^{-2} doses.

CAs are usually divided into two types – chromosomal and chromatid-type. Their ratio depends on the character of genotoxic factor, but increase of chromosomal type in the pool of CAs also shows the increase of the genotoxic effect. Slight prevalence of chromosomal CAs was observed even in control (UV-B unirradiated) cells, but increase of the part of chromosomal CAs proportionally to UV-B dose was also obvious (Figure 2). As it was shown with artificial UV-C radiation (Cieminis et al., 1987), CAs correlate with the level of DNA lesions.

Plants with and without B-chromosomes

The supplementary chromosomes are generally assumed to be genetically empty (Jones and Houben, 2003). However, the B chromosome in *C. capillaris* karyotype primarily discovered by Maluszynska and Schweizer (1989) is exceptional, because 45S rRNA genes are not only located in it (Jamilena et al., 1994), but also transcribed (Leach et al., 2005).

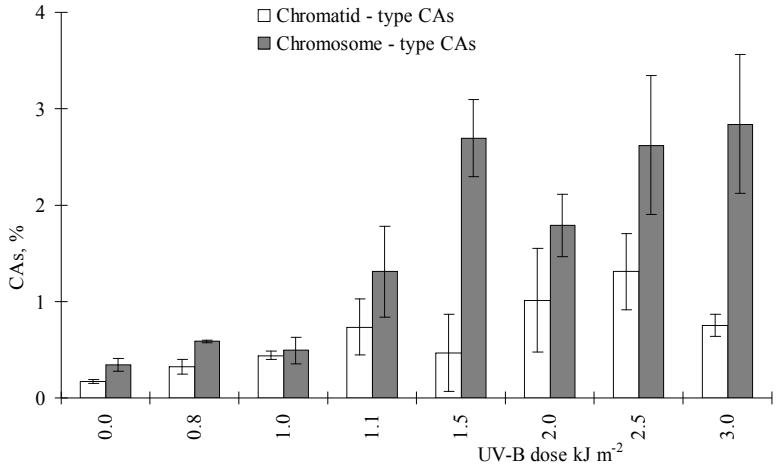

Figure 2. Relation of chromatid-type (white columns) and chromosomal-type (black columns) aberrations upon the UV-B dose.

UV radiation effects on plants with B chromosomes are not yet studied, and we expected to find differences between two groups of cells – with (B+) and without (B-) supplementary chromosome. However, results were partially disappointing, because no differences between the action of UV-B radiation on CA induction in B+ or B- cells was revealed. However, effects on mitotic activity were observed. As in the previous experiment (see Figure 1), the 1.2 kJ m^{-2} UV-B dose reduced MA in meristematic cells without B chromosome, while cells with B-chromosome were more resistant to UV-B radiation (Table). It is a noteworthy fact because cell division is coupled with the repair of DNA lesions and, in general, until DNA lesions are not eliminated, the cell division is blocked by p53-like proteins (De Veylder et al., 2007). On the other hand, the B chromosome of *C. capillaris* has 45S rRNA genes which are very important for the cell activity (Jamilena et al., 1994; Leach et al., 2005).

Therefore, it is relevant to compare mitotic activity at various time intervals after UV-B radiation (Table). Certain increase of MA after 9 h was observed even in control, i.e. UV-B unirradiated cells. We could not observe direct relation of the same effect exclusively in UV-B irradiated cells with B chromosome, but difference between the 3rd and 9th hours of fixations is much higher in the UV-B irradiated cells with B-chromosome than in cells without B-chromosome (Table).

Table. Comparison of mitotic activity (MA) and chromosome aberration (CAs) level in *Crepis capillaris* root cells with (B+) chromosome irradiated with 1.2 kJ/m².

Experimental conditions	Time after UV-B, h	Metaphases		MA	
		n	with CAs, %	n	%
UVB-/B-	3	340	0.59	638	40.0
	6	342	2.92	523	42.6
	9	268	0.37	1358	47.6
Total		950	1.37 ± 0.38	2519	44.7 ± 1.0
UVB+/B-	3	231	4.33	1115	30.6
	6	490	3.47	684	38.5
	9	151	2.65	359	38.4
Total		872	3.56 ± 0.63	2158	34.4 ± 1.1
UVB-/B+	3	233	0.43	382	40.1
	6	412	1.46	556	47.8
	9	272	1.47	1041	41.3
Total		917	1.20± 0.33	1979	42.9 ± 1.1
UVB+/B+	3	362	3.87	642	33.0
	6	413	2.66	1035	50.0
	9	274	3.28	510	54.7
Total		1049	3.24 ± 0.55	2187	46.1 ± 1.0

n – number of investigated cells.

Effects of photoreactivation, ascorbic and salicylic acids on UV-B radiation

SA about twice reduced the CA level induced by 1.2 kJ m^{-2} UV-B radiation, while effect of photoreactivation was lower but statistically significant. Combined action of SA and photoreactivation did not strengthen the protective effect against UV-B effect on chromosome stability (Figure 3).

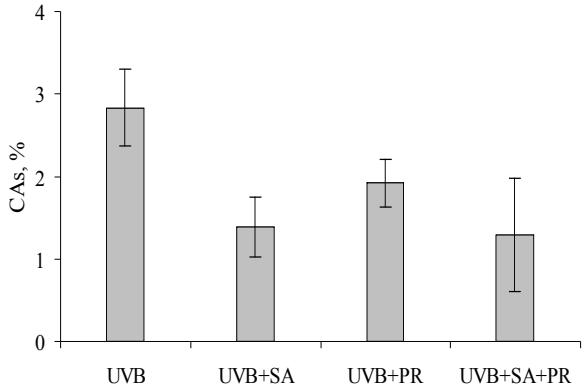

Figure 3. Action of salicylic acid (SA) and photoreactivation (PR) on chromosome aberration (CAs) induction in meristematic root cells irradiated with 1.2 kJ m^{-2} UV-B. Summarized results for three fixations: 3, 6 and 9 h after UV-B irradiation.

Observation of photoreactivation induction is an interesting fact regarding the low photolyase activity in the roots (Hidema and Kumagai, 1998). However, effect of photoreactivating light on CA level induced by the sunlight UV-B or sunlight UV-B+UV-A in *C. capillaris* root meristem was stronger (Rančelienė et al., 2004). Effect of SA on sunlight UV radiation was slightly lower (Rančelienė et al., 2007) than on artificial UV-B in the present work. The effects of photoreactivation and SA on artificial UV-B radiation were observed in our previous work (Rančelienė et al., 2007) similarly as in the present work, but the combined effect of both modifying agents was stronger than their separate actions. The seed quality of plants grown in different years may be caused by such differences.

Slight effect of AA on induction of CAs by artificial UV-B was also revealed. The effect partially depended on the time of root fixation after the UV-B irradiation. Certain effect was revealed only 6-9 h after irradiation with 1.2 kJ m^{-2} UV-B dose (Figure 4).

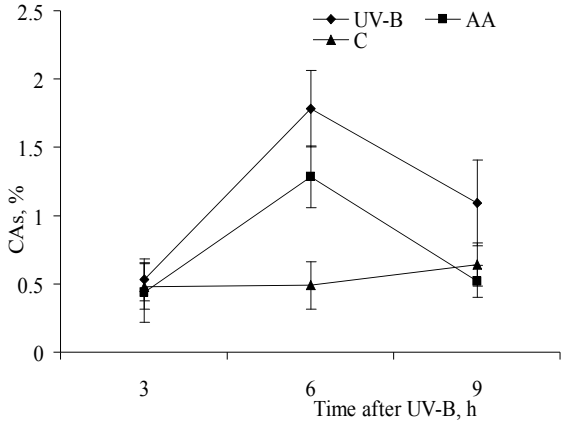

Figure 4. Action of ascorbic acid (AA) on chromosome aberration induction dependently on the root tip fixation time after irradiation with 1.2 kJ m^{-2} UV-B.
C-control, UV-B unirradiated tips

It should be noted that anthocyanin-rich extracts from cherry fruits also showed protective effect on chromosome stability and cell division in UV-B irradiated *C. capillaris* meristematic root cells (Rančelienė et al., 2009). Despite that anthocyanins-rich extracts from fruits are presented by mixture of compounds, we suggest that generally for both agents, anthocyanins and ascorbic acid, effects are due to antioxidant action. Both agents act as powerful antioxidants (Conklin and Barth, 2004; Athar et al., 2008; Tsoyi et al., 2008). Common feature of both substances is a delayed effect on mitosis (Potters et al., 2000; Rančelienė et al., 2009).

Conclusion

The root meristem of *Crepis capillaris* is a useful and very sensitive system for investigation of UV-B radiation and its various modifying agents. Low number of chromosomes in diploid karyotype allows investigating the effects on chromosome stability. Effects of UV-B action on meristematic root cells depended on the dose, presence or absence of the supplemental B chromosome or partially on salicylic or ascorbic acids, as well as photoreactivation. Modifying agents eliminate only part of chromosome lesions, and the nature of remaining chromosome aberrations needs further investigation.

Acknowledgments

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REGULAR ARTICLE

Natural variation in UV-B protection amongst *Arabidopsis thaliana* accessions

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Abstract

Pronounced altitudinal and latitudinal UV-B gradients exist across the earth. Therefore, we hypothesised that plants from different geographic origins differ in the regulation and/or magnitude of UV-protection. Eight Arabidopsis accessions with different geographic origins (altitude between 32 and 3016 m) were raised under Photosynthetic Active Radiation (PAR), PAR + UV-A or PAR + UV-A + UV-B radiation for 10 days, after which UV-B protection of photosynthesis was assessed by measuring the consequences of exposure to a pulse of acute UV-B. We found significant variation in UV-B protection among accessions exposed to PAR or PAR + UV-A. Yet, all accessions raised under PAR + UV-A + UV-B were well protected. Thus, differences between accessions are not about UV-B protection per sé, but rather about regulation of UV-B protection which varies from constitutive to inducible by UV-A and/or UV-B. Particularly striking are differential UV-A responses, whereby some high altitude accessions lack UV-A regulated accumulation of UV-absorbing pigments, but show a strong UV-A induced morphogenic response. The adaptive relevance of the differential regulation of UV-protection is discussed.

Key words: *Arabidopsis*, Carotenoid, Phenolics, Photosynthesis, UV-radiation

Introduction

Plants can adapt to local environmental conditions resulting in the evolution of ecotypes. Where plants are exposed to gradients of particular environmental effectors, adaptation may give rise to a specific pattern of phenotypic diversity. *Arabidopsis thaliana* is a variable species that is native to Europe and central Asia where it is exposed to a wide range of altitudinal, climatic, and edaphic conditions (Koornneef et al., 2004). It is likely that at least some of the phenotypic variation in *Arabidopsis* reflects local adaptation and has ecological significance (Koornneef et al., 2004). Phenotypic variation in *Arabidopsis* has been identified in traits such as disease resistance, tolerance to oxidative stress, extreme temperatures, salt and drought, flowering time and morphology, biochemical make-up, growth rate and others (Koornneef et al., 2004). Analysis of such natural diversity can contribute to the identification of gene-function, but also inform about the ecological importance of particular traits. In recent years *Arabidopsis* accessions have been used to study, among others, latitudinal clines for flowering time (Stinchcombe et al., 2004; Balasubramanian et al., 2006) , and Red / Far-Red light responses (Stenøien et al., 2002) as well as a coastal cline for sodium accumulation (Baxter et al., 2010).

In this study, we have investigated the biological effects of ultraviolet (UV) radiation on eight different *Arabidopsis* accessions that have evolved under different UV-regimes. Ultraviolet radiation (UV) penetrating the earth's biosphere consists largely of UV-A (315-400nm) with a much smaller contribution of UV-B (280-315nm). The levels of UV-B in the biosphere vary spatially and temporally depending on the ozone layer and geographic position on earth, with near equator and mid-latitudes receiving the higher doses and higher latitudes substantially less UV-B (McKenzie et al., 2001). The levels of UV radiation also increase with altitude (Piazena, 1996; McKenzie et al., 2001) and annual total levels of UV-B and UV-A have been reported to increase by 19% and 11% per 1000 m altitude respectively, in the Austrian alps (Blumthaler et al., 1992). It can be hypothesised that this UV-gradient affects plants growing at higher altitudes. Indeed, several studies have reported a positive correlation between the altitude of the growing site and the content of UV-

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protecting flavonoids and phenolic acids (Zidorn et al., 2005; Jaakola and Hohtola, 2010). An interesting question is whether the increased accumulation of flavonoids in alpine plants is regulated by UV-radiation, and if so, whether this is an inducible response, or rather a constitutively expressed trait in high altitude plants. Van de Staaij et al. (1997) compared *Silene vulgaris* ecotypes from alpine and low land origins, and found that UV-B exposure resulted in decreased flower and seed production in a low land *Silene vulgaris*, but up to 2.5-fold more seeds per plant in an alpine ecotype (Van de Staaij et al., 1997). These data implicate a degree of genetic adaptation of the UV-response in alpine plants. Similarly, an *ex situ* study showed that *Rumex acetosella* and *Plantago lanceolata* ecotypes or *Lupinus* and *Taraxacum* species originating at equatorial alpine sites were relatively UV protected compared to low land and/or higher latitude plants when grown under standardised conditions (Barnes et al., 1987). In contrast, a large scale study demonstrated a non-significant weak association between the geographic origin of *Arabidopsis* accessions and their constitutive UV-B protection of photosystem-II (Jansen et al., 2010). These seemingly contradictory reports on plant adaptation to ambient UV-B radiation levels, may well reflect experimental conditions (ratio PAR to UV-A to UV-B radiation) and the use of different proxies for UV-impact (photosynthetic damage, reproduction, biomass). UV-B radiation can potentially induce a wide range of inhibitory, effects including slower plant growth, reduced biomass, damage to photosystem II (PSII) and decrease in chlorophyll content (Jansen et al., 1998; Xiong and Day, 2001; Germ et al., 2005; Kataria and Guruprasad, 2012). UV-B also triggers morphological, physiological and metabolic acclimation responses such as accumulation of UV-B absorbing compounds, increased quenching of Reactive Oxygen Species (ROS) and DNA-repair (Rozema et al., 1997; Jansen et al., 1998; Kakani et al., 2004), and as a result many studies show none, or minimal, UV-B stress in plants raised under ambient UV-B conditions (Ballaré et al., 2011). It has been reported that exposure to UV-A can also result in direct photosynthetic damage (Turcsanyi and Vass, 2000; White and Jahnke, 2002), resulting in further ROS formation. However, there is a degree of controversy regarding UV-A and its biological effects on plants as both damaging (Nayak et al., 2003), and protective (Helsper et al., 2003; Joshi et al., 2007) UV-A responses have been reported.

In this study we have tested the hypothesis that *Arabidopsis* accessions from different geographic origins differ with respect to the regulation and/or magnitude of UV-protection of the photosynthetic machinery. To test this hypothesis we investigated the biological effects of low, chronic levels of UV-A and UV-B that facilitate acclimation, and of a high level UV-B that causes stress, on eight different *Arabidopsis* accessions. The data presented in this manuscript highlight a remarkable degree of specificity in UV-responses with UV-B protection of photosynthesis being controlled by genetic background, UV-A and UV-B radiation.

Materials and Methods

Plant material and growth conditions

Eight *Arabidopsis thaliana* accessions originating from different geographical locations were selected to study plant responses to UV radiation. These accessions (ecotypes) were selected to represent a range of latitudes and altitudes. Seeds were kindly donated by Prof. Koornneef (Wageningen University, The Netherlands and MPIZ, Cologne, Germany), and had been propagated for several generations under controlled conditions prior to use in the described experiments. Details of the selected *Arabidopsis* accessions are given in the Table 1. Following sterilization, seeds were germinated on MS plates. Seedlings that had reached the 3-4 leaf stage were transferred to individual 6 cm diameter plastic pots filled with a soil-based substrate (John Innes 2, Westland Horticulture, Winsford, UK) and perlite (John Innes 2: perlite = 4: 1 approx.). Following transplanting, plants were grown for 7 days in a growth chamber under 80 µmol m^{-2} s^{-1} PAR (Photosynthetic Active Radiation) (Philips LLD 36W/840 reflex). Growth rooms were kept at 20 ºC, under a 14/10-h light/dark cycle and a relative humidity of 75%.

Treatments and exposure conditions

After 7 days of establishment, plants were raised for a further 10 days under different PAR and UV regimes. These were:

1) PAR (35 µmol m^{-2} s^{-1}),
2) PAR (35 µmol m^{-2} s^{-1}) + UV-A (0.159 mWcm^{-2})
3) PAR (35 µmol m^{-2} s^{-1}) + UV-A (0.159 mWcm^{-2}) + UV-B (0.026 mWcm^{-2})

Table 1. *Arabidopsis* accessions and their geographical distributions.

Full Name	Abbreviated name	Country of origin	Line code	Longitude (ºE)	Latitude (ºN)	Altitude (m)
Burren	Bur-0	Ireland	CS 6643	-9.0	53.1	32
Buskerud	Bus-1	Norway	JA 46	9.9	59.9	100
Vind-1olanda	Vind-1	UK	CS 22560	-2.3	55.0	122
Martuba	Mt-0	Libya	CS 6799	38	56	137
Argentat	Ang-0	France	JA.2[b]	1.9	45.1	196
Cape Verde Islands	Cvi-1	Portugal	N 8580	-24	16	1052
Hodja-Obi-Garm	Hog	Tajikistan	CS 6179	69.7	38.7	1414
Shadara	Sha	Tajikistan	CS 929	71.3	37.3	3063

PAR was generated by Philips LLD 36W/840 reflex tubes suspended approximately 55 cm above the plants. PAR levels were kept low to minimise photoprotection and induction of antioxidative defences, i.e. to unmask UV-induced differences between accessions. UV-A radiation was generated by UV-A lamps (Philips Black light Blue TLD 36W/08). UV-B radiation was generated using Philips 36W/TL12 tubes. The small ultraviolet-C (UV-C) component that is generated by these lamps was filtered out using a cellulose acetate filter (thickness 95 µm; Kunststoff-Folien-Vertrieb GmbH, Hamburg, Germany). Radiation levels used in the present study were quantified with a spectroradiometer (USB2000, RAD, Ocean Optics). The dose of Biologically Effective UV (UV_{be}) radiation was calculated using the formula derived by Flint and Caldwell (2003). UV_{be} during growth (PAR + UV-A + UV-B condition) was 0.84 $kJm^{-2}d^{-1}$, in comparison, a typical biologically effective daily dose during clear sky summer conditions in the UK (latitude 53°N) is in excess of 24 kJ m^{-2} when calculated using Flint and Caldwell (2003) (Wargent et al., 2009). Temperatures were approximately 20°C and relative humidity ranged between 65 and 75%. The plants were maintained in the UV-B box under a similar 14h day/ 10h night cycle as used in the growth chamber.

To determine plant tolerance to UV-B, plants were exposed to a further, acute, 4 hour UV-B dose following 10 day growth under chronic UV. Detached leaves (young, fully expanded) were floated on water (adaxial site up) in open petri dishes and were exposed for 4 hour to UV-B radiation in the absence of PAR or UV-A (0.107 $mWcm^{-2}$; UV_{be} 3.46 $kJm^{-2}d^{-1}$).

Analysis photosynthetic efficiency

Young but fully expanded leaves were detached from plants raised for 10 days under one of the three different radiation regimes and the maximum photochemical efficiency of PSII (F_v/F_m) was measured following 20-25 min dark-adaptation of leaves. The maximum photochemical efficiency of leaves treated for a further four hours with acute UV-B was also determined using same procedure. The maximum photochemical efficiency of PSII (F_v/F_m) of plants was assessed using a modulated PAM (Imaging PAM, M-Series, Walz, Effeltrich, Germany) and calculated as $F_v/F_m = (F_m-F_0)/Fm$ (Krause and Jahns, 2003), where, F_m and F_0 are the maximum and minimum fluorescence, respectively. F_v represents variable fluorescence.

Analysis rosettes and extractable pigments

Levels of Chlorophyll-a (Chl-a), Chl-b, total chlorophyll (Chls), phenolics (Phe) and total carotenoids (Car) were measured following 10 days of growth under three different radiation regimes. For biochemical assays, 0.283 cm^2 of fresh leaf was used for extraction purposes. Both chlorophyll and carotenoids were extracted with methanol (MeOH: H_2O = 96: 4), while phenolics were extracted with acidified methanol [MeOH: H_2O: HCl (v/v) = 80: 19: 1] by incubating samples for 4 days in the dark at 4°C. Absorbance was determined spectrophotometrically (Genesis 10 series, Thermo Electron Scientific Instruments LLC, Madison, WI, USA) and pigments peaks were used to calculate the content of chlorophyll a, chlorophyll b and total carotenoid using the formulas of Lichtenthaler and Wellburn (1983). Absorbance at 330nm was taken as a proxy for total soluble phenolics (Mirecki and Teramura, 1984). Contents of total chlorophyll and carotenoid, and absorbance for total phenolics (i.e., 330 nm) were normalized on the basis of leaf area. Expressing pigment data on the basis of leaf weight does not substantially change results.

Following 10 days growth under PAR and UV, the rosette diameter (cm) of each plant was measured using a ruler. Two readings was taken per rosette and from opposite directions, after which the mean rosette diameter of each plant was calculated.

Statistical analysis

The experimental design consisted of three blocks each containing PAR, PAR + UV-A and PAR + UV-A + UV-B exposure treatments.

Statistical analyses of data were performed using analysis of variance (ANOVA) in the General Linear Model procedure of the SPSS package (version 18, SPSS, Chicago, IL, USA). The overall treatment effects (i.e., PAR, PAR + UV-A, PAR + UV-A + UV-B) on grouped accessions were tested using a nested ANOVA, while responses of individual accessions to different treatments as well as the responses of different accessions to each treatment were analysed separately using one-way ANOVA on the measured variables. Linear regression and Pearson's correlation of different variables with altitude were performed within the SPSS. Differences between treatments were considered significant if $P < 0.05$.

Results

Photosynthetic performance

Arabidopsis accessions were raised for 10 days under one of three distinct radiation regimes (PAR, PAR + UV-A or PAR + UV-A + UV-B) to study UV-acclimation. Following 10 days of growth, no macroscopic effects of UV-A or UV-B exposure were discernible, and the maximal quantum efficiency of photosystem II was found to vary between 0.77 and 0.80 (Figure 1a), values typically associated with healthy plants. Subsequent exposure of leaves to 4 hours acute high UV-B resulted in decreases of F_v/F_m values (Figure 1b). However, F_v/F_m values varied significant between accessions depending on the radiation regime under which plants were raised. Overall, plants raised under the PAR + UV-A + UV-B regime were least affected by acute UV-B (i.e. highest UV-B tolerance), while plants raised under a PAR-only regime displayed the largest decreases in F_v/F_m. Variations on this pattern can be observed for individual accessions. For example, Hog, Vind-1, Ang-0, Cvi-1, Bus-1, Mt-0 and Bur-0 raised under PAR + UV-A + UV-B all displayed statistically ($P < 0.001$) higher F_v/F_m values following exposure to acute UV-B than plants raised under PAR only. In contrast, Sha plants raised under PAR + UV-A + UV-B displayed a similar level of protection as plants raised under PAR-only, i.e. neither UV-A nor UV-B induced additional protection. UV-A increased protection in all tested accessions except Sha. Addition of UV-B to the PAR + UV-A mixture did not induce in additional UV-B protection in Hog and Ang-0, but increased the level of protection in Cvi-1 and Bur-0 significantly ($P < 0.001$ for both accessions).

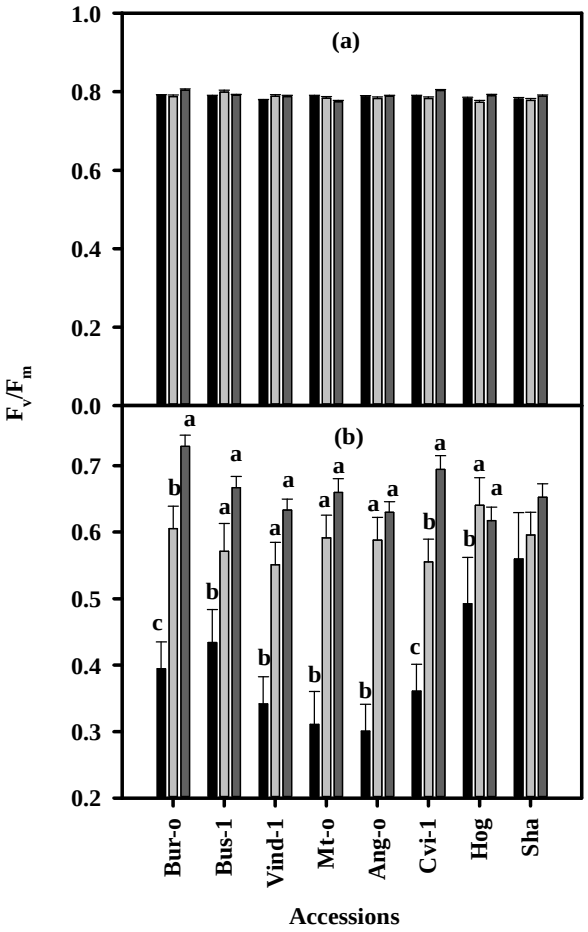

Figure 1. Photochemical efficiency (F_v/F_m) of *Arabidopsis* accessions raised under PAR, PAR + UV-A or PAR + UV-A + UV-B for 10 days (a) and subsequently exposed to acute UV-B (0.35 Wm^{-2}) for 4 hours (b).

Black, light grey and dark grey bars denote PAR, PAR + UV-A, PAR + UV-A + UV-B growth conditions, respectively. The intensities of PAR, UV-A and UV-B during growth were 35 µmol m-2 s-1, 0.159 mWcm-2 and 0.026 mWcm-2 respectively. Following exposure to acute UV-B (b), Fv/Fm was higher in plants raised under PAR + UV-A + UV-B compared to either PAR or PAR + UV-A ($P<0.01$). Variations among accessions were significant under PAR ($P<0.05$) and PAR + UV-A + UV-B ($P<0.01$) but not under PAR + UV-A. For each individual accession, different letters denote significant differences ($P<0.01$) between plants raised under the different growth conditions. Mean ± 1 SEM. n = 6-9.

Accumulation of UV-screening pigments

Levels of UV-screening pigments were determined in leaf extracts of accessions raised under different radiation conditions. Overall, plants raised under the PAR + UV-A + UV-B regime contained the highest levels of phenolics, while plants raised under a PAR-only regime displayed the lowest levels ($P < 0.001$). Variations on this pattern can be observed for individual accessions. The levels of phenolics varied significantly ($P < 0.05$) among *Arabidopsis* accessions raised under either PAR or PAR + UV-A. In contrast, all accessions were found to accumulate statistically similar levels of phenolics

under PAR + UV-A + UV-B (Figure 2). The induction of phenolics was found to be significantly ($P < 0.05$) altered by growth conditions (i.e. PAR, PAR + UV-A, PAR + UV-A + UV-B) in all accessions except Sha ($P = 0.510$) and Hog ($P = 0.216$) (Figure 2) which had similar levels of phenolics irrespective of growth conditions. In Vind-1 and Cvi-1 accumulation of phenolics was induced ($P < 0.001$) by growth under PAR + UV-A + UV-B. UV-A did not cause accumulation of phenolics in these two accessions, with statistically similar levels of phenolics in plants raised under PAR or PAR + UV-A. In contrast, substantial accumulation of phenolics was triggered by UV-A in Ang-0, and Bur-0 (both $P < 0.001$). The UV-A mediated increase in inducible total phenolics negatively correlated with altitude, i.e. the increase in phenolics content in plants raised under PAR + UV-A compared to those raised under PAR-only was negatively associated with the altitudes of origin in this small subset of *Arabidopsis* accessions (rho = 0.81, $P < 0.025$) (Figure 5b).

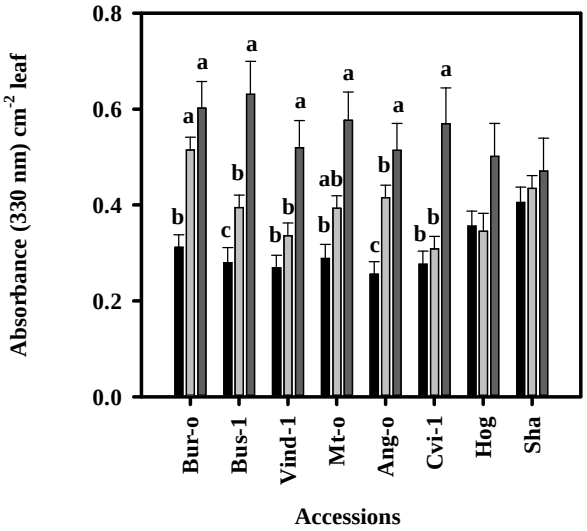

Figure 2. Induction of total phenolics (i.e., absorbance at 330 nm) in *Arabidopsis* accessions raised under PAR, PAR + UV-A or PAR + UV-A + UV-B for 10 days.
Black, light grey and dark grey bars denote PAR, PAR + UV-A, PAR + UV-A + UV-B treatments, respectively. Plants raised under PAR + UV-A + UV-B contained more phenolics than those raised under PAR or PAR + UV-A ($P<0.01$). Variations in phenolic content between accessions were significant under PAR ($P<0.05$) and PAR + UV-A ($P<0.01$) but not under PAR + UV-A + UV-B. For each individual accession, different letters denote significant differences ($P<0.01$) between plants raised under the different growth conditions. Mean ± 1 SEM. n = 6-9.

Photosynthetic pigments and carotenoids

Radiation conditions during growth had a significant ($P < 0.05$) effect on the levels of total chlorophyll and carotenoids in *Arabidopsis*. Overall, growth under PAR + UV-A increased the levels of total chlorophyll and carotenoids compared to levels in plants raised under PAR + UV-A + UV-B or just PAR, across all accessions ($P < 0.002$ and $P , < 0.001$, respectively) (Figure 3a,b). Significant ($P < 0.01$) UV-A induced increases in chlorophyll levels were noted for Sha and Hog. Levels of total carotenoids were significantly ($P < 0.05$) increased in Hog, Mt-0 and Bur-0 grown under PAR + UV-A compared to plants grown under PAR-only. Both Mt-0 and Bur-0 exhibited significantly ($P < 0.05$ and $P < 0.001$; respectively) lower levels of total carotenoids in plants exposed to PAR + UV-A + UV-B compared to plants raised under PAR + UV-A only.

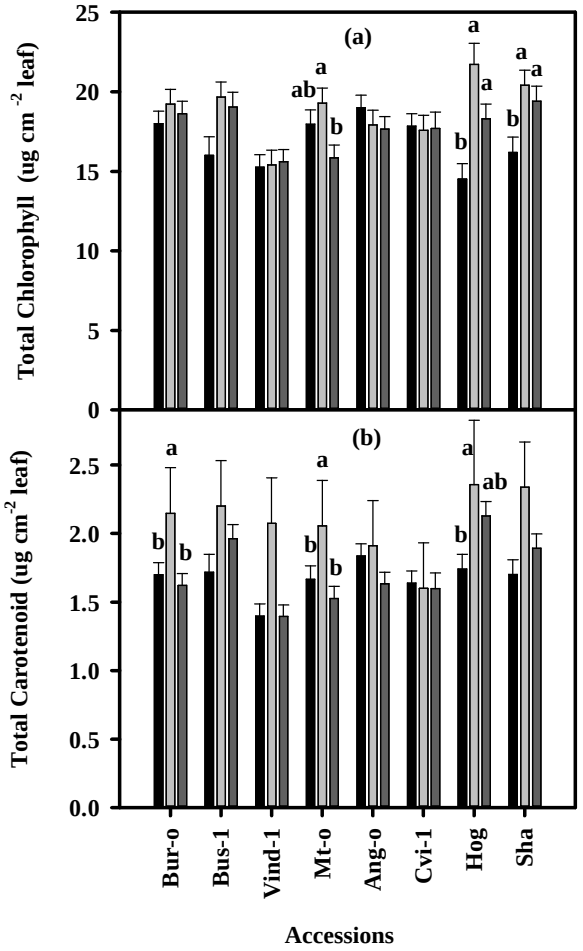

Figure 3. Levels of total chlorophyll and total carotenoids in *Arabidopsis* accessions raised under PAR, PAR + UV-A or PAR + UV-A + UV-B for 10 days.
Black, light grey and dark grey bars denote PAR, PAR + UV-A, PAR + UV-A + UV-B treatments, respectively. Plants raised under PAR + UV-A contained more chlorophyll and carotenoids than those raised under PAR or PAR + UV-A + UV-B ($P<0.05$). Variations in chlorophyll among accessions were significant under PAR ($P<0.01$) under PAR + UV-A ($P<0.01$) and under PAR + UV-A + UV-B ($P<0.05$), but for carotenoids only under PAR + UV-A + UV-B ($P < 0.01$). For each individual accession, different letters denote significant differences ($P<0.05$) between plants raised under the different growth conditions. Mean ± 1 SEM. n = 6-9.

Rosette Diameter

Rosette diameters were determined for accessions raised under different radiation conditions (Figure 4). Overall, plants raised under the PAR + UV-A regime had the greatest rosette diameter. Plants raised under PAR-only or under a PAR + UV-A + UV-B regime were considerably smaller ($P < 0.001$). Slight variations on this pattern can be observed for individual accessions. *Arabidopsis* accessions showed significant ($P < 0.001$) variation in rosette diameter when raised under PAR or PAR + UV-A + UV-B, although less under PAR + UV-A ($P = 0.726$). A significant negative association (rho = -0.67; $P < 0.025$) was noted between altitude of origin and rosette diameter of PAR-raised plants (Data not shown). Some inducible changes in rosette diameter were also associated with altitude. Thus, the change in rosette diameter of plants raised under PAR + UV-A, compared to these raised under PAR was positively associated with altitude (rho = 0.74, $P < 0.025$) (Figure 5c). There was also considerable (rho = -0.55, $P = 0.058$) negative association between the effect of UV-B and altitude (Figure 5i).

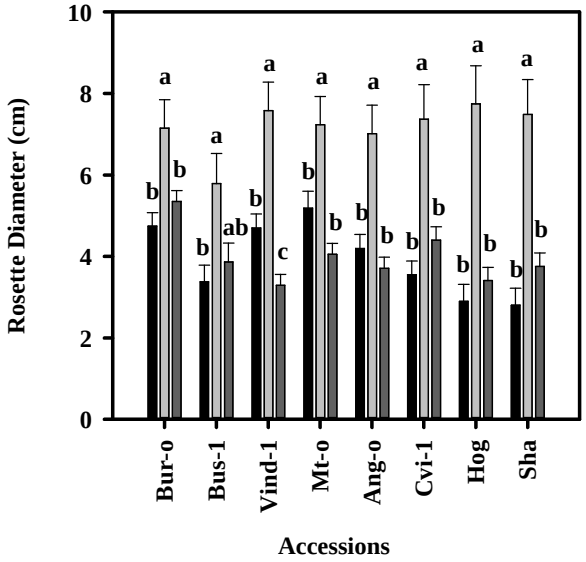

Figure 4. Rosette diameter of *Arabidopsis* accessions raised under PAR, PAR + UV-A or PAR + UV-A + UV-B for 10 days.

Black, light grey and dark grey bars denote PAR, PAR + UV-A, PAR + UV-A + UV-B treatments, respectively. Plants raised under PAR, or PAR + UV-A + UV-B were smaller than those raised under PAR + UV-A ($P<0.001$). Significant variations in rosette diameter across accessions were noted under PAR and under PAR + UV-A + UV-B ($P<0.001$) but not under PAR + UV-A. For each individual accession, different letters denote significant differences ($P<0.05$) between plants raised under the different growth conditions. Mean ± 1 SEM. n = 6-9.

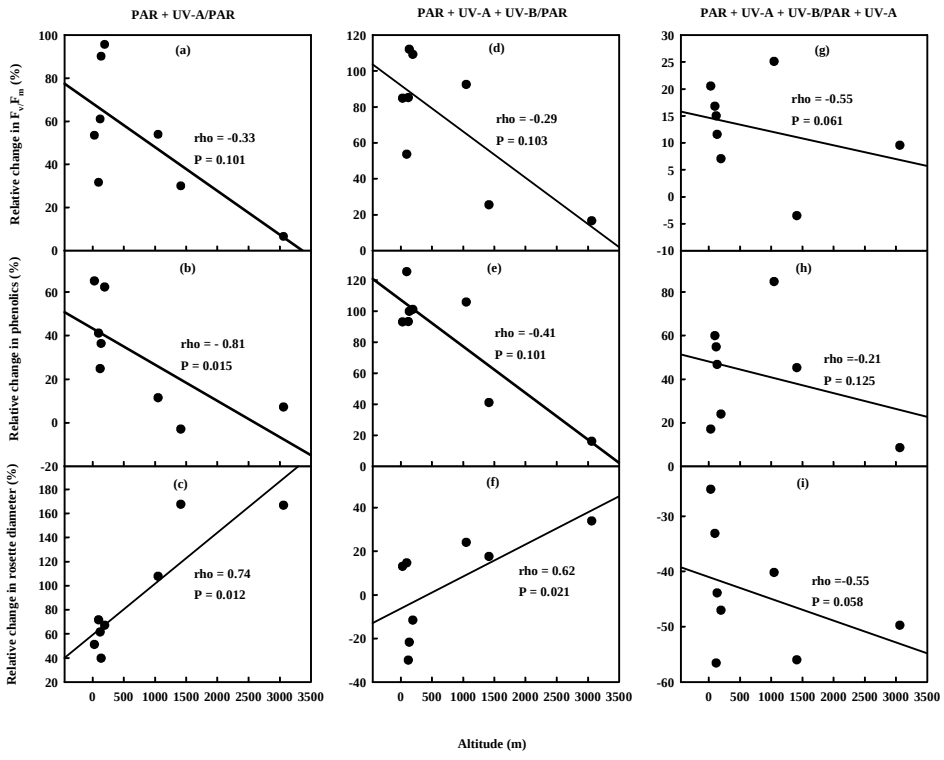

Figure 5. Relationship between altitude and changes in F_v/F_m (a, d, g), total soluble phenolics (b, e, h) and rosette diameter (c, f, i) among *Arabidopsis* accessions.

Comparisons were made between plants raised under PAR + UV-A relative to PAR (a, b, c), PAR + UV-A + UV-B relative to PAR (d, e, f) and PAR + UV-A + UV-B relative to PAR + UV-A (g, h, i). % relative changes were calculated as {(treatment – control)/control}*100. Fv/Fm was measured following exposure to acute UV-B. Mean ± 1 SEM. n = 6-9.

Table 2. Pearson's correlation between leaf physiological, biochemical and growth parameters and among different accessions raised under PAR + UV-A for 10 days.

Parameter	Phe	Rd	Chl-a	Chl-b	Chls	Car
F_v/F_m	0.36	0.28	0.81**	0.88***	0.84***	0.64*
Phe		-0.23	0.30	0.26	0.29	0.39
Rd			-0.14	0.20	-0.06	0.02
Chl-a				0.92***	0.99***	0.60
Chl-b					0.95***	0.71**
Chls						0.63*

Asterisks denote significance difference at *, P <0.1; **, P < 0.05 and ***, P < 0.01. Chlorophyll-a (Chl-a); Chlorophyll-b (Chl-b); total chlorophyll (Chls); phenolics (Phe); total carotenoids (Car); and rosette diameter (Rd).

Table 3. Pearson's correlation between leaf physiological, biochemical and growth parameters and among different accessions raised under PAR + UV-A + UV-B for 10 days.

Parameter	Phe	Rd	Chl-a	Chl-b	Chls	Car
F_v/F_m	0.66*	0.95***	0.19	0.43	0.25	-0.26
Phe		0.59	-0.04	0.07	0.01	-0.14
Rd			0.19	0.48	0.27	-0.22
Chl-a				0.93***	0.99***	0.78**
Chl-b					0.96***	0.59
Chls						0.75**

Asterisks denote significance difference at *, P <0.1; **, P < 0.05 and ***, P < 0.01.

Chlorophyll-a (Chl-a); Chlorophyll-b (Chl-b); total chlorophyll (Chls); phenolics (Phe); total carotenoids (Car); and rosette diameter (Rd).

Correlations among different parameters

Pearson's correlation coefficients were determined between UV-B protection (F_v/F_m) following acute UV-B exposure and physiological, biochemical and growth parameters across 8 accessions that had been grown for 10 days under PAR + UV-A or PAR + UV-A + UV-B (Table 2 and Table 3), respectively. The results indicate that UV-B protection of PSII (i.e. higher F_v/F_m) in plants raised under PAR + UV-A was significantly ($P < 0.1$) associated with the levels of chlorophyll a, chlorophyll b, total chlorophyll and total carotenoids, but not UV-absorbing phenolics. On the other hand, UV-B protection in plants raised under PAR + UV-A + UV-B significantly ($P < 0.1$) correlated with induction of total phenolics, but not with carotenoid levels.

Discussion

Induction of UV-B tolerance

Arabidopsis accessions raised for 10 days under various mixtures of PAR and UV radiation showed F_v/F_m values close to 0.80, which is in the range found in healthy, non-stressed plants. Many outdoor studies, using natural sunlight, also fail to show a UV effect on photosynthetic performance and growth (Ballaré et al., 2011). Generally, high levels of UV-B and/or low levels of accompanying PAR are required to impede PSII activity (Lud et al., 2003; Jansen et al., 2010). Here, despite the use of low levels of PAR, no damage to the photosynthetic machinery was measured in *Arabidopsis* grown under chronic, low UV-B (Figure 1a) indicating that damaging reactions were balanced by defence responses, i.e. the plants acclimated to the exposure conditions.

To assess the protective capacity of repair and acclimation responses we also measured F_v/F_m values in plants exposed for an additional 4 hours to acute high intensity UV-B. Under these extreme stress conditions, higher F_v/F_m values were interpreted as a greater protective capability, i.e. increased UV-B tolerance. The relative high UV-B tolerance in plants raised under PAR + UV-A + UV-B is consistent with previous reports that a key consequence of UV-exposure is the induction of UV-protection (Jansen et al., 2010; Ballaré et al., 2011). Interestingly, substantial UV-B protection is induced by UV-A radiation, emphasising the importance of solar UV-A for environmentally relevant assessments of the impacts of UV-B (Middleton and Teramura, 2003; Kotilainen et al., 2008).

The UV-B induced accumulation of phenolics is a key UV protection response that has been extensively demonstrated (de la Rosa et al., 2001). UV absorbing phenolics accumulate in vacuoles, cell walls, chloroplasts and even nuclei, and protect internal tissue of leaves and stem from UV-B radiation through their anti-oxidative capacity (Agati and Tattini, 2010). Accessions raised under PAR + UV-A + UV-B contained the highest

phenolic concentrations while the lowest levels of phenolics were noted in PAR-raised plants (Figure 2). UV-B tolerance in accessions raised under PAR + UV-A + UV-B is statistically associated with accumulation of total phenolics (Table 3). Substantial induction of total phenolics was also observed in accessions raised under PAR + UV-A (Figure 2). There seems to be considerable variation between species with respect to UV-A responses. Kotilainen et al. (2008) reported that in alder and birch specific phenolic metabolites are induced by either UV-A or UV-B, while in some cases there is even evidence for opposing effects of the two types of radiation. In contrast, in soybean (*Glycine max* L.), UV absorbing pigments are almost exclusively induced by UV-B, and not UV-A, wavelengths (Mazza et al., 2000). Levels of UV-absorbing metabolites in Scots pine (*Pinus sylvestris*) are mainly responsive to UV-A (Martz et al., 2007). It appears that some of this interspecific variation in regulation of phenolic accumulation, is present at the intraspecific level in *Arabidopsis*, thus offering scope for genetic analysis.

Notwithstanding the UV-A induced induction of phenolics in several *Arabidopsis* accessions, it appeared that there is no simple statistical association between levels of phenolics and UV-B protection of photosynthesis among the accessions raised under PAR + UV-A (Table 2). Rather, UV-B tolerance in PAR + UV-A-raised plants was significantly correlated with the levels of carotenoids, suggesting a role for these antioxidants in UV-B protection. This is consistent with work by Götz et al. (1999) who showed that the photosynthetic activities of genetically modified *Synechococcus* with higher levels of β-carotene and zeaxanthin are UV-B protected (Götz et al., 1999). It has also been shown that accumulated β-carotene in *Dunaliella bardawil* prevents UV-related photosynthetic damage through absorption of blue-light/ultraviolet-A radiation (White and Jahnke, 2002). The observed accumulation of carotenoids in plants exposed to UV-A, but not UV-B (Figure 3b), is consistent with literature reports (Jahnke, 1999; Mogedas et al., 2009). The decrease in carotenoid level in plants raised under PAR + UV-A + UV-B is less easily explained. However, Jansen et al. (2008) previously reported that UV-B mediated changes in carotenoid levels depend in a complex manner on plant species, developmental stage and UV-B dose.

UV-B mediated morphogenesis has been suggested to be a protective response with more dwarfed plants being less impacted upon by UV-B (Bogenrieder and Klein, 1982). Our data do not reveal an association between smaller rosettes and UV-B protection. In contrast, we found that a substantial increase in rosette diameter in plants raised under PAR + UV-A (Figure 4) was matched by an increase in UV-protection (Figure 1b). Furthermore, for accessions raised under PAR + UV-A + UV-B bigger rosettes were positively associated with higher F_v/F_m values after exposure to acute UV-B (Table 3) contradicting that dwarfing can simply be linked to UV-B protection.

Accession specific UV responses

We have shown substantial variation among *Arabidopsis* accessions in terms of responses to UV-A and UV-B, consistent with previous studies by Cooley et al. (2001) and Jansen et al. (2010). Protection from acute UV-B was constitutively expressed in Sha, but strongly induced in Vind-1, Ang-0, Bus-1, Mt-0, Bur-0 and Cvi-1. Analysis of individual accessions shows that the relationship between accumulation of UV-absorbing pigments and UV-B protection is complex. For example, Cvi-1 and Vind-1 raised under PAR + UV-A are considerably more UV-protected than the same accessions raised under PAR-only (Figure 1b). Yet, growth under PAR + UV-A does not result in significantly increased accumulation of UV-absorbing pigments in these two accessions (Figure 2). Vind-1 does, however, accumulate substantial amounts of carotenoids when raised under PAR + UV-A, and this may have contributed to UV-protection, although Cvi-1 displays minimal carotenoid induction under UV-A. Levels of UV-screening pigments are not significantly increased in Sha in response to growth conditions (Figure 2), matching the relatively constant expression of UV-B protection (Figure 1b). Nevertheless, Sha is capable of responding to both UV-A and UV-B as demonstrated by the accumulation of carotenoids (Figure 3) and the decrease in rosette diameter (Figure 4), respectively.

The observed phenotypic differences between accessions trigger the question of adaptive relevance. Herbivory studies have shown that the balance between constitutive and inducible protection is related to risk of attack, the extent of damage and/or cost of defence (Zangerl and Rutledge, 1996). Thus, the constitutive UV-B protection of Sha might reflect its high altitude origin and exposure to a relatively harsh climate including high levels of ambient UV. We also found that induction of total phenolics by UV-A is negatively related to altitude (Figure 5b). Thus, accessions from higher altitudes (Hog, Cvi-1 and Sha) display no, or limited, responses to UV-A.

This is surprising as these accessions would be the most UV-A exposed accessions in their high altitude habitat (Blumthaler et al., 1992).

Conclusion

Our data show that all tested *Arabidopsis* accessions achieve UV-protection when raised under PAR + UV-A + UV-B. The key finding is that the differences between accessions are not so much about protection per sé, but rather about the regulation of UV-protection. Given the complex regulatory mechanisms involved in flavonoid biochemistry (Koes et al., 2005) and anti-oxidant defences (Lidon et al., 2012), the presence of phenotypic differences in flavonoid accumulation and UV-protection should not come as a surprise.

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REGULAR ARTICLE

Adaptation strategy of barley plants to UV-B radiation

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Abstract

Elevated levels of UV-radiation can change dramatically different aspects of plant growth and development that reflects in a wide variety of morphological and physiological responses. In the present research the influence of heat pretreatment and UV-B pre-exposure on barley plant resistance to ultraviolet radiation as well as the effects of UV-B irradiation on plant vegetative and reproductive organs of two barley cultivars were analyzed. The increasing of UV-resistance of barley plants was observed in terms of seedling viability, stability of statolitic starch in embryo roots, leaf tissue structure and pollen fertility. Molecular genetic analysis using ISSR-markers showed the increasing of polymorphic level up to 80% in somatic tissue, while the decreasing of this parameter to 33% was detected in reproductive tissue under the giving conditions. Results obtained in our research suggest that UV-irradiation can cause genomic instability of barley plants and pre-treatments used in our experiment may lead to plant acclimation and adaptation to UV-B radiation.

Key words: Barley, Genome, UV-radiation, Seed heating, Xeromorphic features

Introduction

The influence of UV-B radiation on plant organisms and the impact associated with UV-B exposure have been intensively studied over the last decades. UV-B radiation, a part of the sunlight, has wavelength ranges from 280 to 320 nm. The effects caused on plants can be direct and indirect, being damages detected in many cell components including membranes, proteins, DNA, in conjunction with alterations on plant growth, morphology, physiological and biochemical processes (Jansen et al., 1998).

Two main effects are believed to be evoked by UV-B radiation in seed plants: the first is considered as a response to induced damage and the second one – as a response to the perception of UV-B by receptors and leading to UV-B induced photomorphogenesis and acclimation (Frohnmeyer and Staiger, 2003; Ulm and Nagy, 2005; Jenkins, 2009). Development of both responses depends, namely on the dose and duration of UV irradiation, wavelength and fluency rate. Activation of general stress response, implying changes in gene expression, takes place when UV-B causes damages (Ulm and Nagy, 2005). As it was identified for *Arabidopsis* that the complex of UV response locus 8 (UVR8) and the multifunctional E3 ubiquitin ligase locus (COP1) are involved in UV-B-specific responses, being their interaction the early step in a signaling pathway that ensure plant UV-B acclimation and protection (Favory et al., 2009).

Plants respond to UV-B radiation through the synthesis of protective pigments and UV-absorbing compounds, changes of gene expression that encode DNA repair proteins and enzymes responsible for scavenging reactive oxygen species (Hideg and Vass, 1996; Agrawal et al., 2009). Besides, the up-regulation of the genes of the general phenylpropanoid pathway was defined in plants under UV-B (Daugherty et al., 1994). According to (Fedina et al., 2006), the accumulation of UV-inducing compounds might act not only as protective substances but also be a consequence of stress-induced damage. It has also been suggested that selection of the most optimized

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environment to cultivate plants under UV-B radiation is one of the most important tasks of agronomy. To induce cross-acclimatization plants to UV-B exposure a various pretreatments of plant materials are usually applied. For instance, it has been shown a cross-acclimation to UV-B in four barley cultivars as a result of salt treatment (Çakırlar et al., 2008). Seed hardening by the increasing of plant heat and drought tolerance is also a useful approach to raise UV-resistance (Genkel, 1982). Plants that were grown from hardened seeds characterized by xeromorphic morpho-physiological features further correlated with their increased resistance to UV-B. However heat stress is one of the major factor limiting the productivity and adaptation of crops, especially when temperature extremes coincide with the critical stages of plant development.

The process of plant adaptation to a various environments is a complex process that includes a broad range of different morphological, physiological, biochemical and genetic changes. While the impact of UV-B radiation on plant growth, morphology, physiology and development has been studied in many researches, little is known about its influence on plant genome stability. Rearrangement of genome can be one among lots of mechanisms of plant adaptation (Kovalchuk et al., 2004). Besides, development of the plant adaptive response under stress conditions can be referred to high level of genome variability that allows plants to adapt to environments (Kunah, 2005).

Plants grow and develop under sunlight that is required for photosynthesis and under influence of UV-B and infrared radiation as the components of sunlight. In our study these two agents were chosen because they are part of natural environment and plants already have protective mechanisms against both of them that were developed during the natural selection. There were found some common features in responsive reaction to UV-B and heat stress that suggests the possible activation of the common signal transduction pathway and existence of the regulatory relationship between the plant responses to heat stress and UV damages (Jenkins et al., 1997). The aim of the present work is study the effects caused by pretreatment of barley plants with heating and UV-B irradiation on their response to UV light, in terms of morphogenetic characteristics of vegetative and reproductive tissues, and analyze mechanisms of adaptive processes that occurred in two cultivars of *Hordeum distihum* L.

Materials and Methods

Plant material and experimental setup

Two barley cultivars (*Hordeum distihum* L., 2n=14) – Ksanatu and Jergey were chosen for our study. Cultivars are of French selection, high purity and with low protein content. All seeds were divided onto three groups. The first group of seeds was treated with heating and UV-C, the second - with UV-B, caffeine and UV-C. After mentioned treatments the seeds were planted in soil. The third group of seeds was germinated on filter paper in Petri dishes for three days. Three-day seedlings irradiated with UV-B and/or UV-C and were moved to a water culture. Three days later the seedlings have been planted in soil and cultivated to study the survival of plants in natural conditions, development of vegetative and generative organs and some other morphogenetic features of development. We used different types and duration of treatments and selected the following 6 variants: 1) three days old plants were exposed to UV-B for 30 min; 2) three days old plants were UV-C irradiated for 7 min; 3) three days old plants were subjected to UV-B exposure for 30 min and they were tested with UV-C irradiation for 7 min after 4 hours; 4) soaked seeds were subjected to UV-B exposure for 30 min then pretreated with 5mM caffeine for 4 hours and tested with UV-C irradiation for 15 min; 5) seeds were pretreated with heating at 40 ^{o}C for 6 hours; 6) seeds were pretreated with heating at 40 ^{o}C for 6 hours and after three days seedlings were tested with UV-C for 7 min. Seedlings were irradiated with a 20 W Philips TL ultraviolet lamp with filter cutting off the short-wave region of the ultraviolet spectrum. Radiation dose of UV-B was 5.4 kJ/m^2 with the intensity 6 W/m^{2}s^{-1}. For UV-C a OBM-150 m was used. Radiation dose of UV-C was 3.4 kJ/m^2 and 7,2 kJ/m^2 with the intensity 8 W/m^{2}s^{-1}.

Cytological analysis

Stomata topography and leaf tissue morphology were analyzed using light microscopy. For this purpose barley leaf sections were prepared and slides were stained with acetocarmin. We determined the number of stomata per mm^2 of leaf epidermis from adaxial and abaxial sides of leaf. Material for the study was taken on the third day after test-irradiation or UV-irradiation and two weeks later. The fixation of spike was made from the differentiation stage of microsporocytes to maturation of pollen grains by using of the Navashin mixture; temporal slides were stained with acetocarmine according to the standard cytological protocol. The anthers were stained with

DAPI (0.5µg/mL) and a mixture of fluorescent dyes: fluorescein, propidium iodide and DAPI (FDA+PI+DAPI) in the following concentrations: FDA 2.5 µg/mL, PI 1.0 µg/mL, DAPI 0.5 µg/mL. Preparations were analyzed by UV microscopy (Axiostar, Carl Zeiss, Germany). After analysis in the ultraviolet spectrum, preparations were visualized using acetocarmine for studying in the visible region of the spectrum. Quantitative analysis of cell system anomalies was also performed. Anthers with microsporocytes (30) were evaluated. Additionally, 30 anthers per variant were used to estimate pollen grains. The data were statistically processed.

Determination of starch content in root cap

The resistance of statolitic starch grains of root cap was tested using hot hydrochloric acid hydrolysis (1N HCl), being the roots stained with iodine potassium iodide (IKI). The color intensity of starch was determined on a 5-point scale using light microscopy (Genkel, 1956; O'Brien and McCully, 1981). The caryopses of embryonic roots of the first plant generation were analysed.

PCR and PCR-RFLP analysis

Total plant DNA was isolated from barley leaves and anthers, using the CTAB protocol described by Doyle and Doyle (1990).

The PCR reaction mixture (25 µL) contained 10x PCR buffer (Promega, USA), 2 mM $MgSO_4$, 2 mM of each dNTPs, 15 pmol of the single primer, 2.5 U Taq-polymerase and 30 ng of the genomic DNA. PCR was performed in the thermocycler GeneAmp® PCR System 9700 (Applied Biosystems). The amplification profile considered 2 min at 94°C and 45 cycles of 30 sec at 94°C, 45 sec at 52°C and 2 min at 72°C, with the final extension of 7 min at 72°C. Totally 7 primers containing di-, tetra- and pentanucleotide repeats were chosen to analyze barley genome variability, since they had been described among polymorphic markers used in the study (Tsumura et al., 1996).

PCR-RFLP analysis was carried out using endonuclease *HpaII* (Fermentas, Lithuania). PCR-products were digested with restriction enzyme according to manufacturer's instruction.

The amplified products, restriction fragments and Gene Ruler 100 bp DNA ladder (Fermentas, Lithuania) were loaded in 1.7% (w/v) agarose gel containing 0.01% (v/v) ethidium bromide. Results were visualized in a UV chamber and processed using program GelAnalyzer.

Dendrogram of relationships between groups of barley plants, under different treatments, was constructed using the Program "PopGen32" (Yeh and Boyle, 1997).

Results

The analysis of some morphological and cytological features was carried out to define the effects in two cultivars of *Hordeum distihum* L. under the influence of UV-radiation and to assess changes of plant response to UV exposure after pretreatments.

The values of the seeds germination and the seedlings growth rate in the soil of the Ksanatu cultivar approached to the control ones (after all treatments), unless seedlings were exposed to UV-C. Moreover, plants of the Jergey cultivar displayed adaptive responses, mainly after heating of seeds (Figure 1).

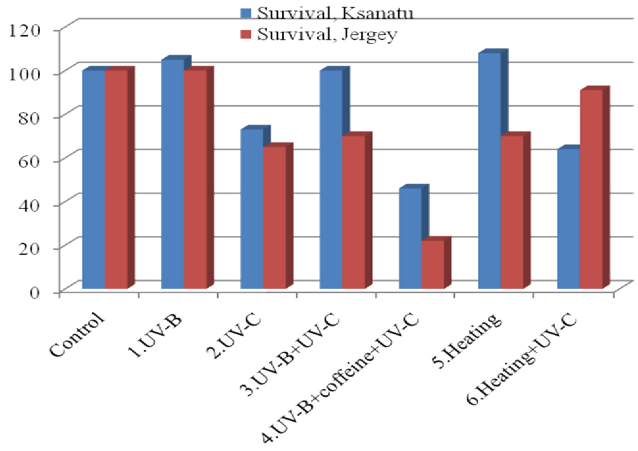

Figure 1. Effect (% relatively to the control) of a various treatments on seedlings viability and survival of plants in the soil (on the 10th days of growth).

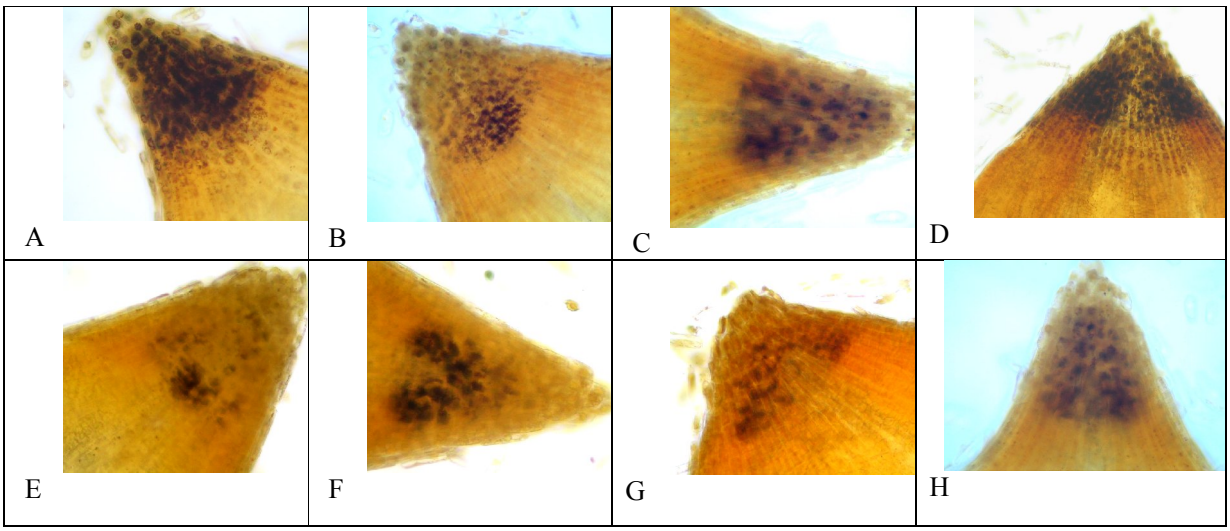

Figure 2. Effect of UV treatment and heating on the stability of starch in root cap: A - control (without hydrolysis), B - control after the hydrolysis, C - UV-B treatment (after hydrolysis); D - heat treatment (without hydrolysis), E - UV-C treatment (after hydrolysis), F- UV-B+UV-C treatment (after hydrolysis), G, H - heat treatment (after hydrolysis) (A, C, E, F,G – Ksanatu cultivar, B, D, H – Jergey cultivar).

The decreasing of seedlings viability was detected in both cultivars after the caffeine treatment, indicating the important role of the DNA repair mechanism, inducible repair particularly, to adapt to UV radiation. Eventually the increasing of seedlings viability after heat treatment was determined by alteration in cell metabolism, activation of heat shock protein synthesis specifically. It is known the concentrations of some heat shock proteins were found significantly greater at the open than shaded area, reaching their maximum in the summer, especially in plants experiencing full sunlight (Manitasevic et al., 2007).

Stability of root cap starch

Both cultivars, without any treatments, were defined as having low stability according to the results of root cap hydrolysis analysis. After treatments barley plants of Jergey cultivar were characterized as having medium resistance (3 – 4 points), while Ksanatu cultivar displayed a low resistance (2 – 3 points) (Figure 2 A - H). Thus, increasing drought resistance was observed in both cultivars, being assumed the induction of adaptive response under UV-B exposure in Ksanatu and pre-heated seeds in Jergey plants.

Structure of leaf tissues

Under the influence of UV radiation, the structure of the leaf tissues of both cultivars was altered and depended on the duration of UV exposure. The leaf palisade mesophyll of the control plants was not completely differentiated, and both types of mesophilous cells were not greatly distinguishable (Figure 3 A, C). Preferential development of palisade chlorenhimal cells was the most characteristic changes of irradiated plants (Figure 3 B, D). After UV-exposure, mesophilous tissue became more compacted and the bundle sheath cells as a layer of the large chlorenchymal (or parenchymal) cells surrounded vascular bundles (Figure 3 B, D). Reduction of the spongy tissue and developing of palisade tissue on both leaf sides are associated with the xeromorphic plants (Esau, 1965). Stomata pores on adaxial leaf side and chlorenhymal intercellular spaces were compressed. The signs of xeromorphic organization were more clearly distinguishable in the leaf of Jergey cultivar. In Ksanatu, leaf stomatal opening occurred more faster and mesophilous tissue were re-filling with air (Figure 3 E, F). The leaf stomata number and their arrangement on the both sides of the leaf were slightly different in the treated plants relatively to the control ones (Figure 4). However, changes of the number of stomata of both sides of the leaf were associated with acquired xeromorphy after UV-B exposure. The number of stomata registered on the adaxial leaf side in the Jergey cultivar increased whereas on the abaxial leaf side decreased. The rising of stomata density was accompanied with size reduction and sinking into epidermis. The number of stomata on the adaxial leaf side decreased in the Jergey cultivar after heat treatment and UV-C irradiation (Figure 4), which can be considered an adaption to a drought environment.

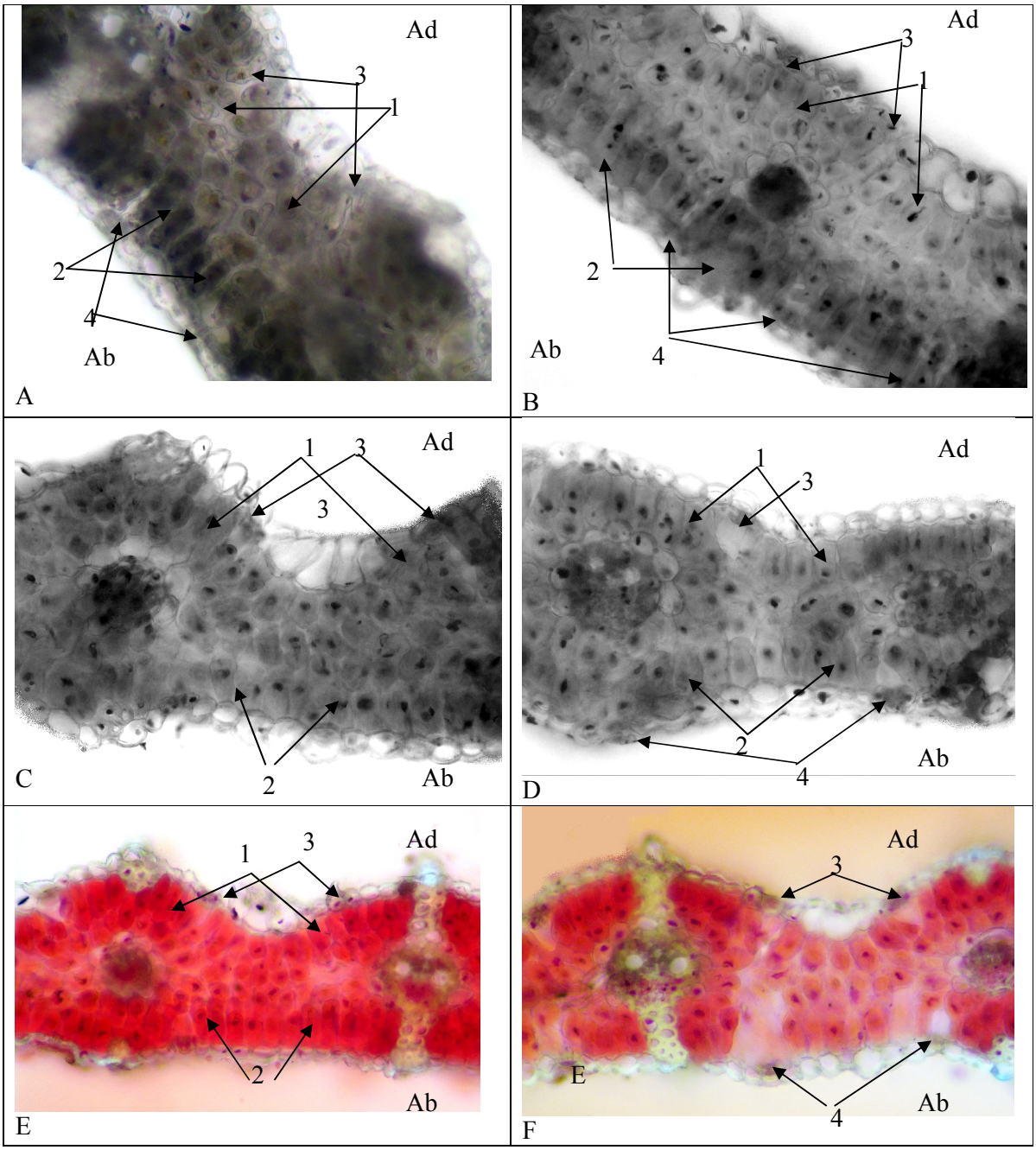

Figure 3. Changings of the leaf tissue structure under influence of UV radiation. A, C- control, B, D, E, F – UV-B exposure: the palisade chlorenhimal cells on the leaf adaxial side (1); the palisade chlorenhimal cells on abaxial side (2); stomata pore on the leaf adaxial side (3); stomata pore on the leaf abaxial side (4); (A, B, D – Jergey cultivar; C, E, F – Ksanatu cultivar) (Ad- adaxial side, Ab - abaxial side).

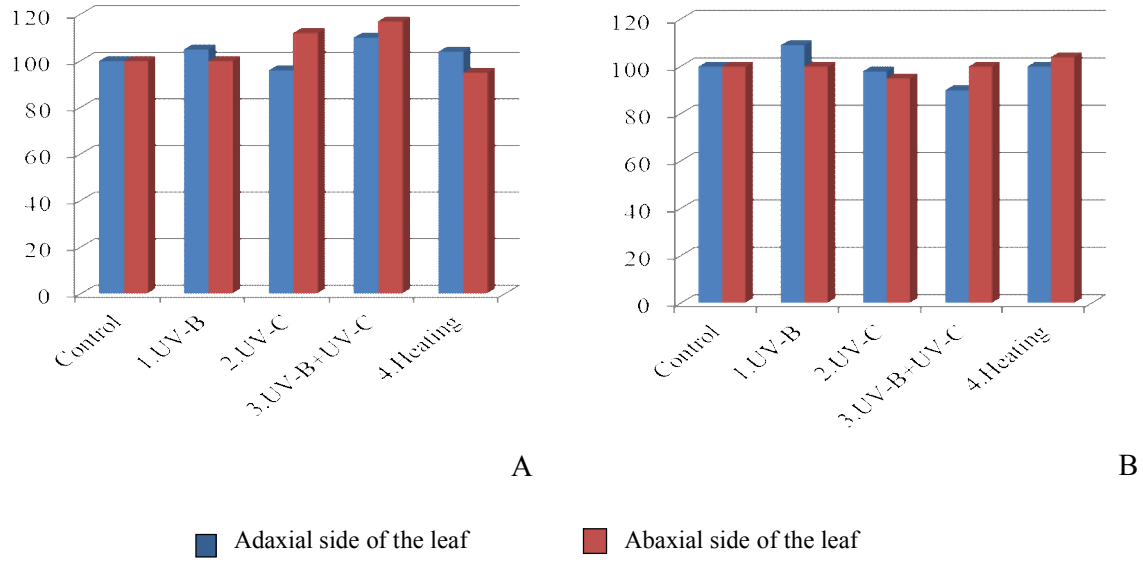

Figure 4. The number of stomata per 1mm^2 (% to control) detected on the leaf adaxial and abaxial sides of Ksanatu (A) and Jergey (B) cultivars. The number of stomata per 1 mm^2 revealed in control plants is taken as 100%.

Reproductive system microsporogenesis

Microsporogenesis is realized by the successive type with the formation of tetrads of the isobilateral structure (Figure 5 A, C). Under the influence of UV radiation, cytomixis is the main type of pathology in microsporogenesis. Accordingly, three types of cytomixis should be discriminated: weak (local), intensive and destructive (pathological). Local cytomixis seems to be a physiological norm for barley. In variant with UV-B-radiation cytomixis could cover about 20% of microsporocytes (Figure 5 A, B). In this case, stickiness and fluidity of chromatin increased in microsporocytes. We observed a kind of transitional chromatin (fragments of nuclei, chromosomes, micronuclei, bands of chromatin from cell to cell) (Figure 5 A - C). In barley the bulk of “transitional” chromatin often remained either in the composition of cynticia or in the intracellular space (Figure 5 B, C). In the case of intensive and destructive cytomixis the microsporocytes lose contact with the tapetum cells, there is a thickening of the callose of inner walls. Sometimes broken microsporocytes "sealed" in such a way and undergo apoptosis (Figure 5 C). According to Lytvyn et al. (2010) UV-B irradiation can indeed initiate apoptotic processes in plant cells.

Not all microsporangia and microsporocytes were affected by cytomixis. Most of the microsporocytes completed meiosis with the formation of normal, only rarely non-balanced, tetrads of microspores (Figure 5 D). In the variant with heating of the seeds, intensive cytomixis was rare. Moreover, destructive cytomixis was observed in reduced flowers and in immature spikes of the second growth of all variants.

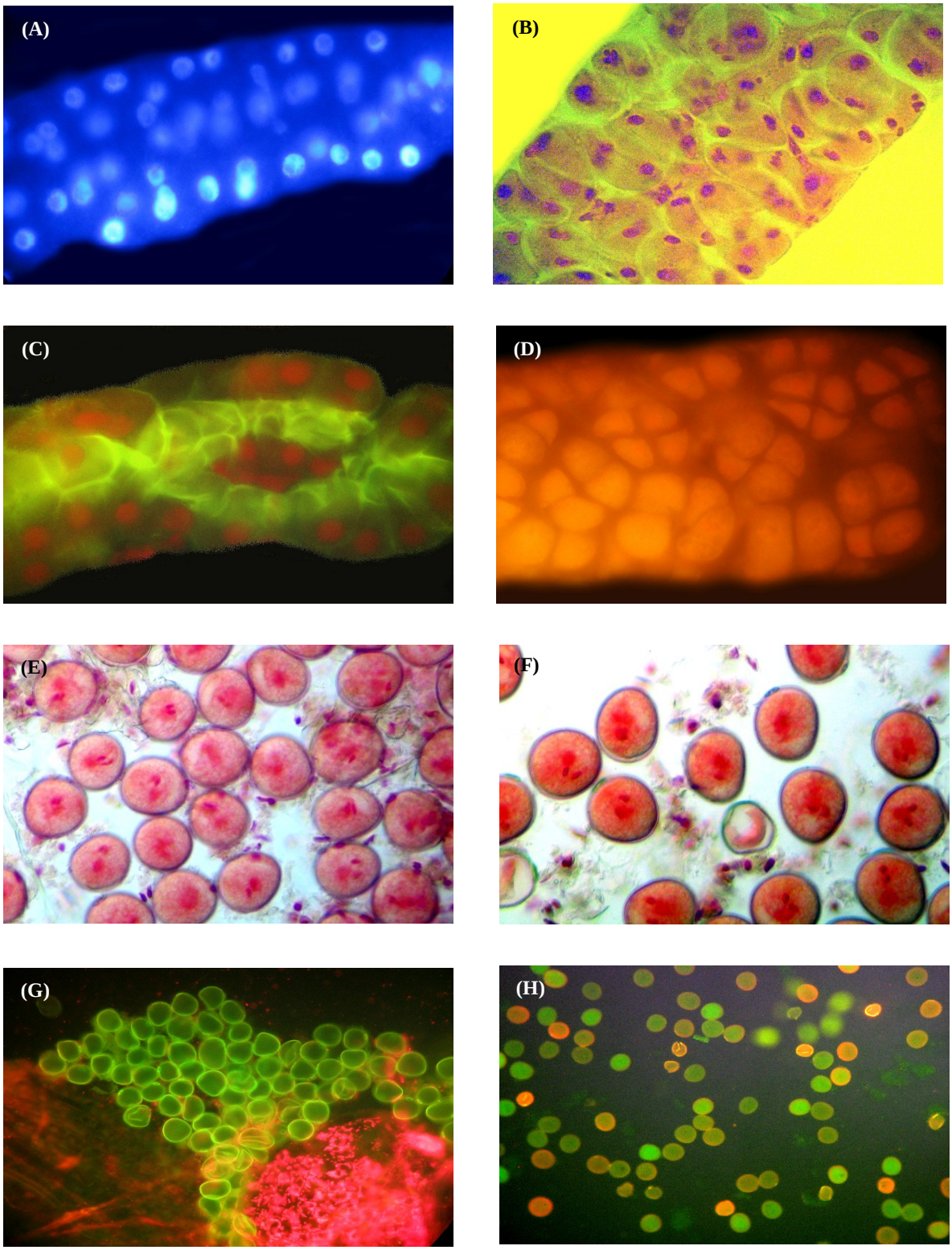

Figure 5. Microsporogenesis and pollen grains of barley plants: A - prophase of the 1st meiotic division, local and intensive cytomixis, UV-B and UV-C exposure; B - telophase of the 2nd meiotic division, intensive cytomixis, UV- C exposure; C – thickening of the callose of inner walls and "sealing" of broken microsporocytes; D – the stage of tetrads of microspors; UV exposure; E - H – three-cellular pollen grains in the control (E, G) , UV-C exposure (F) and after heat treatment (H); C, D, E, G – Ksanatu cultivar; A, B, F, H - Jergey cultivar.

Development and structure of pollen grains (PGs)

Ussually the development of male gametophyte in barley, as in most cereals, begins with releasing of microspores from the microsporocyte envelope and includes the stages that complete the formation of sporoderm, growth and polarization of microspore (Heslop-Harrison, 1979; Mascarenhas, 1989). The following steps are: the first asymmetric mitosis with polarization of two-cellular PGs, followed by the second mitotic division, linked to the synthesis of cytoplasm and deposition of reserve substances in the vegetative cell cytoplasm. The mature PGs has a pair of arrow-shaped sperms and a vegetative cell nucleus, the cytoplasm of which is filled with amyloplasts (Figure 5 E). Ultraviolet radiation led to an enhancement of polymorphism and to disturbance of polarity in microspores and two-cellular PGs, unsynchronized development, increase of the frequency in the formation of «oligoplasm» PGs (Figure 5 F). The latter evidences of nonspecific character of gametic disturbances are caused by different stress factors. The appearance of **"**oligoplasm" PGs may be associated either with mutations of specific genes of PGs, whose expression intensifies after the first mitosis or with mutation, which determines the male cytoplasmic sterility (Mascarenhas, 1990; Nirmala and Kaul, 1991) (Figure 6). However, the level of mature pollen sterility after UV-B exposure decreased (Figure 6).

In the case of UV exposure, the level of PG sterility had not a high negative correlation with cytomixis activation. For Ksanatu plants, the reduction of pollen sterility was observed under UV irradiation in all experimental variants (except the 6th variant). Nevertheless, after heat treatment cytomixis was not activated and the level of pollen sterility increased. The rising of pollen sterility level in Jergey plants was detected in all experimental variants (excepting the 2nd variant). It could be the reflection of the formation of specific responses that are connected with reduction (i.e., decreasing cell number and size) of vegetative and generative tissues and organs to develop xeromorphic phenotype. Thereby, the increasing of the pollen sterility level up to 7–8% is not a crucial, especially for self-pollinated plants.

Thus, results obtained by analysis of pollen grain sterility demonstrated differences in the response to treatments between both cultivars. Some different adaptive features of both barley cultivars due to genotype were remarkable: UV irradiation induced adaptive response in Ksanatu plants whereas seeds heating stimulated it in Jergey plants.

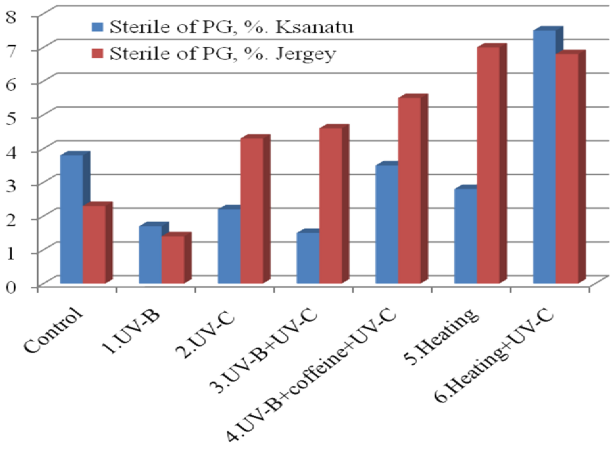

Figure 6. The percentage of sterility of pollen grains in two barley cultivars under different treatments.

Genetic analysis of barley plants

The genome variability of both barley cultivars were characterized by PCR-analysis with primers to microsatellite repeats, since these repeated sequences are widely distributed in plant genome, being considered a source of genetic polymorphism (Varshney, 2005).

The amplification with 7 primers to microsatellite repeats revealed that total pattern of PCR-fragments comprised 24 clear detectable and reproducible bands, when genetic material of vegetative organs was analyzed and 29 bands, when analysis of plant reproductive organ was conducted. The number of amplicons varied from 2 to 9 and, depending on primer content, their size range was 200–1500 bp. The high level of polymorphic products was further defined using primers to dinucleotide repeats and the low one – to pentanucleotide repeat. Comparative analysis of amplicon patterns obtained by PCR with primers to microsatellite repeats revealed differences between plants exposed to the various treatments (Figure 7).

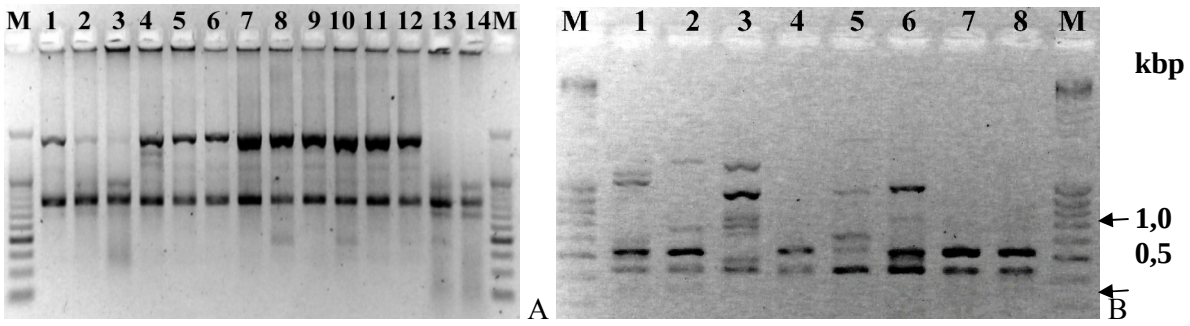

Figure 7. Electrophoregram of PCR-fragments obtained using amplification with a primer to pentanucleotide repeat and DNA isolated from leaves (A) and anther (B). M – Gene Ruler 100bp DNA ladder; A: –5 – control plants; 6–8 – UV-B irradiated plants; 9 – 12 – UV-B + UV-C irradiated plants; 13, 14 – plants grown from preheated seeds; B: 1, 2 – control plants; 3, 4 – UV-B irradiated plants; 5, 6 – UV-B + UV-C irradiated plants; 7, 8 – plants grown from preheated seeds.

PCR-analysis of barley somatic tissues

Among 7 used primers, amplification only with two oligonucleotides gave polymorphic fragments. The level of polymorphic bands detected using both primers reached to 80%. It should be noted that differences between amplicon patterns were observed also in each group of treated plants, if one of two primers was used: variability rate among control plants was 43%, exposed to UV-B and UV-B+UV-C – 57%, whereas there were no polymorphic products in amplicon pattern of plants grown from preheated seeds. The distinctive feature between the last group and the remaining plants was 1000-bp amplicon synthesized with the second primer.

Since 5 of 7 primers were found monomorphic, PCR-RFLP analysis has been carried out. Amplification products were digested with the restriction enzyme *HpaII* and differences between samples were revealed. Two patterns of restriction fragments were obtained – the first was specific for the control and UV-B exposed plants, the second one – for UV-B + UV-C irradiated plants and plants grown from preheated seeds.

PCR-analysis of barley reproductive tissues

The same 2 of 7 primers, as used to amplify DNA isolated from somatic tissues, were found to be polymorphic, if DNA extracted from reproductive tissues was amplified. But the level of polymorphic bands was low and amounted to 33%. In each group of barley plants, the value of this parameter was 65% in the control and UV-B exposed plants, 50% in the group of plants irradiated with UV-B + UV-C, although there were no polymorphic products in amplicon pattern of plants grown from preheated seeds. Results of PCR-RFLP analysis further showed no differences between the amplicon patterns of barley plants.

The relationship dendrogram between barley plants that were subjected to different treatments considered the data obtained by PCR-analysis (Figure 8).

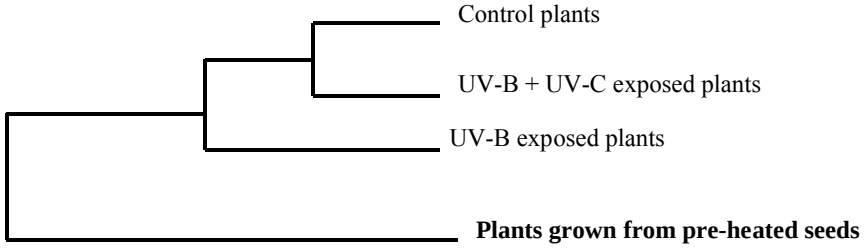

Figure 8. Dendrogram of grouping barley plants under different treatment, using the UPGMA method, on the basis of data obtained by amplification with primers to microsatellite repeats.

All samples combined into two main clusters: the first united the control and exposed to UV irradiation plants and the second was formed of plants grown from preheated seeds. It was found that, at a genome level, pre-exposure to UV promotes defense mechanisms that protect plants from action of the following stress factors. It also considers the involvement of some other genome rearrangement in response to heat treatment.

It is worth noticing that data of genetic analysis in both barley cultivars remained similar. There were slight differences between two cultivars, when the level of polymorphic bands was calculated, yet it remained in the range of 1 – 3%. The average percentage was given in this study. Moreover, differences between dendrograms of grouping barley plants of two cultivars were not defined.

Discussion

The acquired resistance to UV irradiation observed in both barley cultivars was linked to the development of complex changes at an anatomical, cytological, physiological level. These changes reflect adaptive reactions caused by genotypic potentials. The differences between both cultivars in relation to treatment used showed an adaptive response induced by UV-B exposure mostly in one cultivar and by heat pretreatment in another. Distinguishing features of xeromorphic organization after all treatments was more clearly formed in the Jergey cultivar, namely the increasing of root cap starch stability, the alteration of leaf mesophilous tissue and stoma apparatus and the increasing of pollen sterility.

Under UV influence, changes in the development of the leaf epidermal layer were detected in *Glycine max* and *Arabidopsis* plants as a result of increasing trachoma density and reduction of stomata index (Gitz et al., 2005; Lake et al., 2009). Stomata behavior depends on the concentration of ABA that varies under UV-B.

The state of the reproductive system is an important indicator of adaptation. It has been shown that additional UV-B radiation can produce genotoxic effects on the meristem, inhibit the growth and development, influence the pollination, decrease the quantity of produced pollen and the seed production of plants (Flint and Caldwell, 1984; Conner and Neumeier, 2002; Koti et al., 2004a, b, 2007). Besides the direct action on the generative organs (the main target of which is cell DNA), UV-B radiation further produces indirect effects realized by mechanisms connected with photoreception, transduction of signals and hormonal regulation (Tevini et al., 1981; Flint and Caldwell, 1984; Santos et al., 1998; Caldwell et al., 2007; Demkura et al., 2010; Keller et al., 2011; Krasylenko et al., 2011). The effect depends namely of genotype, ecotype and stage of ontogenesis (Jordan, 1996; Torabinejad et al., 1998; Caldwell et al., 2007; Li et al., 2010). The generative system might furnish increased protection against the influence of these agents (Flint and Caldwell, 1983; Barnes et al., 1988; Rozema et al., 2001; Bohne et al., 2003). However, the data on mechanisms of the effect of UV-B radiation on the generative organs are not available.

It is known that in response to a rising of the level of cytogenetic disturbances, the systems of DNA repair become more active and thereafter apoptosis induction or proliferative death of unrepaired cells via the cell selection occurs (Calendo, 2001). The role of cell selection in protective mechanisms of generative tissues against mutagenic factors remains unclear. There are two main types of cell selection in the course of the reproductive system development – premeiotic and haplontic cell selection (Gaul, 1957, 1959; Redei, 1965; Grodzinsky et al., 1996; Kravets, 2009, 2011). Cytomixis occurring in microsporocytes at the beginning of meiosis, is worthy of special attention (Heslop-Harrison, 1966a, b; Zheng et al., 1987; Bellucci et al., 2003; Guo and Zheng, 2004; Liu et al., 2007; Kravets, 2009, 2011). Via cytomixis, which is activated by exposure to UV-irradiation, the population of microsporocytes releases from the excess of genetic load, and cells with recessive lethal mutations are eliminated through haplontic cell selection.

Different treatments of seeds and seedlings could induce the emergence of mutations kept for generations and thereby extend mutagenesis. The decrease of the disturbance number in the reproductive sphere, after influence of UV radiation, is probably connected with the activation of restore processes, in particular inducible DNA-repair, cell selection and other mechanisms of unspecific responses. The increasing of sterility PG after heat treatment may be due to more significant metabolic alterations in barley plants. However, a slight increase of male sterility, promoted by recovery systems, could be associated with an adaptive response.

The increasing genetic polymorphism under influence of UV-B irradiation detected with ISSR-markers was shown in *Gnaphalium luteo-album* (Cuadra et al., 2010). It was mentioned that intraspecific variability could occur due to morphological differences between non-irradiated and irradiated plants. Similar effects (i.e., increment

of intraspecific variation under UV-B irradiation) were marked in 19 different lines of *Arabidopsis* plants (Usmanov et al., 1988) and elevated level of genome instability was revealed in tobacco plants as response to solar UV-B irradiation (Ries et al., 2000).

PCR and PCR-RFLP analyses revealed the increasing genome variability in plants that were exposed to UV exposure. Probably UV irradiation can induce recombination processes involving repeated sequences used in our research as DNA-markers. Rearrangements between homologous DNA sequences in somatic cells are strongly stimulated by DNA-damaging agents as it has been shown (Puchta and Hohn, 1996). Additionally, it should be mentioned that the polymorphic level was higher in vegetative tissues than in reproductive ones. It has been assumed that reproductive organs are generally considered to be well protected from UV-B during their development, but long-term exposure to high UV-B levels may affect the reproductive tissues of plants and cause DNA damage (Ries et al., 2000). It can be further possible that reproductive tissues have stronger or more reliable protective mechanisms that activate DNA repair processes and/or cell selection (cell death) in response to exposure to mutagenic agents.

Moreover, against UV radiation, thermal effect is not a direct mutagenic agent. Adaptative responses stimulated by heating can have another nature, likely, metabolic and hormonal. Hormonal disbalance caused by heating induces the synthesis of stress proteins including HSPs. Heat shock proteins synthesis (HSPs) protect cells against the deleterious effects of heat stress (Feder, 1999; Feder and Hofman, 1999; Iba, 2002; Krishna et al., 2004). Unfortunately, the participation of HSPs in DNA repair has received little attention. Yet, it was recently reported that some of the HSPs can reach the nucleus and it was clear that although the HSPs were not capable of repairing the DNA damages by themselves, they efficiently contribute to the different mechanisms of DNA repair, as part of their molecular chaperone capabilities, interacting with DNA repair proteins, producing their stimulation and reactivation (Nadin and Ciocca, 2012). Eventually, this could be one of the mechanisms of plant cross-acclimatization to UV radiation.

Conclusion

In the present study some morphological and cytological features were defined as physiological response to exposure to UV after pretreatment with UV-B and heating. The detected changes could be a result of nonspecific stress response whereas genome rearrangement may be implicated in specific UV-response. DNA is one of the main targets of UV-B irradiation and there is a specific DNA repair mechanisms to restore nucleotide sequences (Jansen et al., 1998), while heat stress induced mainly changes in gene expression that is specific for most stresses (Wolf et al., 2010). It is supposed that pre-heating does not activate systems to perceive UV and that is one of the reasons why plants grown from pre-heated seeds formed the separated group on the dendrogram constructed on the base of genetic analysis. To sum up genetic variability plays an important role in plant adaptive strategy. It is possible that any pretreatment of UV activates similar processes of genome rearrangement in both barley cultivars that is why we detected no significant differences of genome variability between them. The changes of morphological and cytological characteristics caused by such rearrangement and accompanied adaptation to UV are appeared to intensify xeromorphic features of both barley cultivars.

As a result of morphological and cytological researches, an adaptive response was revealed for both barley cultivars although some cultivar-specific features were defined. The increasing genome variability was detected both for vegetative and reproductive tissues. It seems that pretreatment induces a genome rearrangement that in its turn promotes activation of protective mechanisms and has reflection in changing the morphological and cytological characteristics that depends on the genetic background. Such complex changes leads to the increasing of barley plants unspecific resistance to stress factors, as a consequence of cross-acclimatization to UV radiation.

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