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An enhancing effect of visible light and UV radiation on phenolic compounds and various antioxidants in broad bean seedlings

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Exposure of dark- or ambient visible light-grown broad bean seedlings to low (LL) and high (HL) visible light intensities, UV-A or UV-C, either alone or in combination, induced significant increases in total phenolic compounds as well as in anthocyanins content, throughout the germination period, as compared with the respective levels in control seedlings. In general, as compared with control levels, exposure of both dark- or light-grown broad bean seedlings to LL, HL, UV-A or UV-C, induced significant increases in the contents of non-enzymatic antioxidants (total ascorbate; ASA-DASA and total glutathione; GSSG-GSH) and enzymatic antioxidant activities (superoxide dismutase; SOD, catalase; CAT, ascorbate peroxidase; APO and glutathione reductase; GR). The obtained results are discussed in relation to induced mechanisms of protection and repair from the inevitable exposure to damaging visible light and UV radiation.

Introduction

It is now well authenticated that plants employ strategies of photoprotection to counter the detrimental effects of excessive visible and UV radiation absorption. Thus, plant responses to increased UV radiation include increased xanthophyll-cycle pool size[1] as well as the increase of contents of gallotannin, stilbene and flavonol phenolic compounds.[2] Flavonols have been implicated to provide photoprotection against UV irradiation through a screening function.[3] Furthermore, Balakumar and Selvakumar[4] reported that the total phenol content in leaves of cowpea has increased proportionately with the dose of UV radiation. Generally, in plants showing higher level of phenol synthesis under stress conditions, higher level of polyphenol oxidase (PPO) activity also is noted.[5]

Anthocyanins accumulate in young, expanding foliage of various plant species in response to UV radiation exposure and it has been hypothesized that anthocyanin provides a UV sunscreen.[6] UV responsive anthocyanin production in a rice cultivar was associated with a specific phase of phenylalanine ammonia lyase (PAL) biosynthesis.[7] They found that the anthocyanin induction in rice seedlings is mediated exclusively by the UV component of sunlight.

Santos et al.[8] have emphasized that UV radiation interferes with SOD similarly as do other stresses. The tolerance of *Cassia auriculata* L. seedlings to UV is certainly due to the enhancement of SOD activity and other antioxidative enzymes which is consistent with the results of Santos et al.[9] in potato, wheat and maize, Prasad and Zeeshan[10] in *Plectonema boryanum* and Agarwal[11] in Cassia seedlings. The UV enhancement of CAT and peroxidase (POD) activities, which are both responsible for detoxification of H_2O_2 are probably equally important in the detoxification of H_2O_2, generated by SOD in Cassia seedlings. Increases in activities of peroxidases by UV radiation have been observed in several species including Cassia species[12] and potato.[9]

The present study was carried out in an attempt to substantiate previous results and to obtain better understanding towards the induced radiation stress effects and the induced mechanisms involved in protection and repair from damaging UV-C radiation or highlight intensity either alone or in combination in *Vicia faba* seedlings. The approach applied was to follow the effects of visible light and UV irradiations on UV-absorbing compounds, non-enzymic and enzymic antioxidant system.

Results and Discussion

Changes in UV-absorbing compounds. The available results (**Fig. 1A and B**) indicate that the contents of total phenolic compounds of the control as well as of the variously treated broad bean seedlings showed a progressive decrease with progress in duration of the germination period, whereas a reversible situation was observed for anthocyanins. Exposure of dark- or light-grown seedlings to UV-A, UV-C, LL and HL intensities led to significant variable increases in both anthocyanins and in contents of total phenolic compounds as compared with the respective control levels. The calculated percentages of positive change in these

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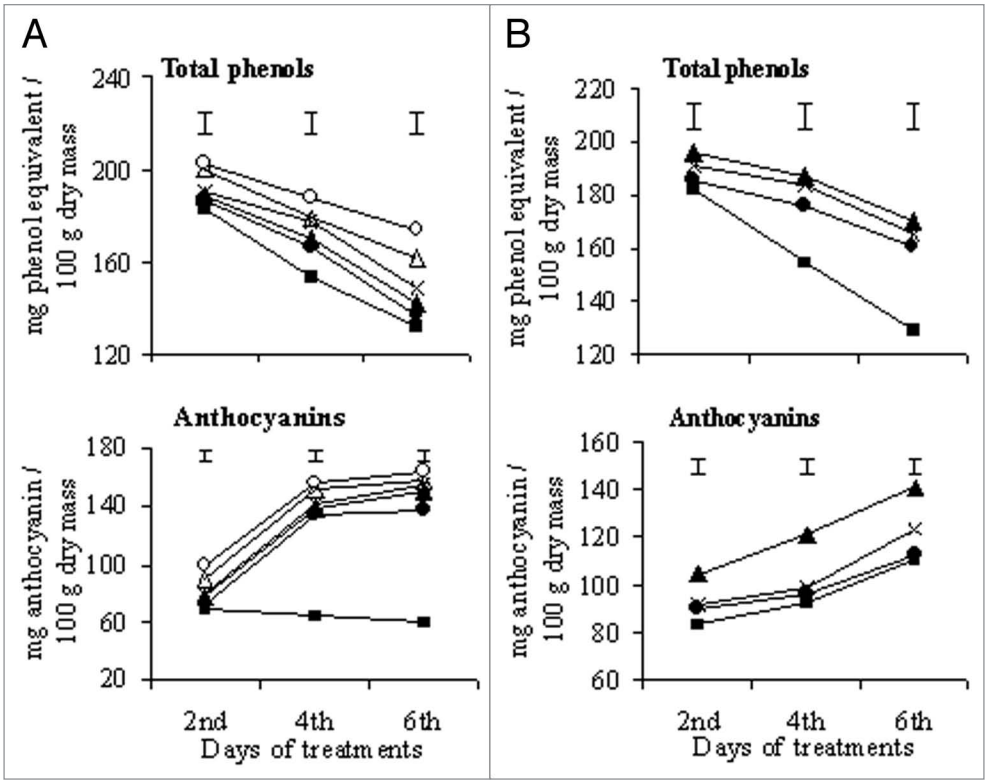

Figure 1. (A) The effects of low visible light (LL) or UV radiations (UV-A and UV-C) on UV-absorbing compounds in *Vicia faba* seedlings germinated in darkness. (-■- Control; -▲- LL; -●- UV-C; -x- LL + UV-C; -△- UV-A; -○- LL + UV-A). Vertical bars represent the standard error (±S.E.). (B) The effects of high visible light (HL) or UV-C radiations on UV-absorbing compounds in *Vicia faba* seedlings germinated in ambient light. (-■- Control; -▲- HL; -●- UV-C; -x- HL + UV-C). Vertical bars represent the standard error (± S.E.).

named compounds appeared in the following order: (LL + UV-A > UV-A > LL + UV-C > LL > UV-C) and (HL > HL + UV-C > UV-C) for dark- and light-grown seedlings respectively (**Table 1**).

In support of the present results, Santos et al.[9] stated that flavonoids reduce damage from UV radiation because they act as UV-filters reducing the penetration of potentially damaging UV-radiation. Jansen et al.[13] reported that UV induces accumulation of a range of secondary metabolites in higher plants, including flavonoids and sinapic esters, which in turn affect numerous physiological functions. Furthermore, flavonoids including anthocyanins have been shown to provide protection against solar radiation-induced DNA damage and to possess free-radical scavenging activity which might offer additional protection to cells accumulating these compounds.[14] Thus, Steyn et al.[15] have suggested that the association between anthocyanins and oxidative stress appears to relate to the ability of anthocyanins to reduce excitation pressure and hence the potential for oxidative damage.

Depending on the species and developmental stage, red, blue or UV wavelengths may play a role in anthocyanin synthesis, through mediation of phytochrome, or putative UV receptors.[16] The UV inducibility of anthocyanins and the ability of anthocyanins to absorb UV-radiation have led investigators to postulate a UV-protective role for anthocyanins, but there are still many questions that need to be answered before a general UV-protective function can be ascribed to these compounds.[14]

Changes in antioxidant system. In order to explain the different patterns of tolerance in the test bean seedlings in response to various irradiations, the possible interference of antioxidant systems must be taken into consideration. Thus, Chinnusamy et al.[17] and Close et al.[2] stated that when plants are subjected to abiotic stresses, high concentrations of reactive oxygen species (ROS), which are detrimental to cells, are formed. These cause oxidative damage to membrane lipids, proteins and nucleic acids. Plants with high levels of antioxidants both enzymatic and non-enzymatic, either constitutive or induced, have been reported to produce greater resistance to this oxidative damage in plant cells.[18,19] The main non-enzymatic antioxidants are ASA + DASA and GSSG + GSH which react with singlet oxygen, superoxide and hydroxyl radicals.

Total ascorbate and total glutathione contents appeared to show a progressive increase throughout the duration of the germination period; this being apparent in the control as well as in the variously treated beans (**Fig. 2A and B**). The highest content was achieved at the end of the germination period. As compared with control seedlings, treatment of seedlings with HL, LL, UV-C and UV-A, either alone or in combination, induced significant increases in the ascorbate and glutathione contents of the variously treated seedlings. The following sequences of treatments: LL + UV-A > UV-A > LL + UV-C > LL > UV-C and HL > HL + UV-C > UV-C for the dark- and light-germinated seeds were displayed with respect to the percentages of increase

in total ascorbate and total glutathione contents; calculated as percentage of control (**Table 1**).

Of interest in this connection, Agarwal[11] suggested that the two UV-A and UV-B doses induced the antioxidant defenses protecting the plant against major deleterious effects of ROS. The damage due to these radicals as indicated by increase in thiobarbituric acid reactive substances (TBARS) and H_2O_2 was limited indicating that the water soluble non-enzymatic antioxidants ASA + DASA and GSSG + GSH and enzymatic antioxidant defense system represented by SOD, CAT, POD and PPO is functional in Cassia seedlings. Thus, Agarwal[11] concluded that Cassia seedlings are able to cope with UV-stress.

A possible explanation for the involvement of ASA + DASA and GSSG + GSH in the UV- or light-stress response might be taken from the data of exposure of broad bean seedlings to LL, HL, UV-A and UV-C, either alone or in combination, throughout the experimental period, which display reductions in photosynthetic capacity (pigments and PSII) and therefore has a similar effect as drought, salinity, temperature, cold, pollutants, i.e., the production of ROS due to the electron leakage from electron transport chains in chloroplast and mitochondria. Because different environmental stress conditions are likely to affect all physiological processes, it is really not surprising to find that light- or UV-stress triggers the expression of antioxidative defense systems.[20,21]

UV-radiations can have negative effects on plant growth, photosynthesis and pigmentation (Younis et al., submitted). However, due to damage repair and stimulation of UV-radiation protective mechanisms, like increased UV absorbance by flavonoids, many plants are able to tolerate UV-damage effectively, especially in field situations.[22] In similar plants, UV-radiation has been shown to inhibit photosynthetic electron transport, with PSII as the major site of damage.[23]

The changes in the activities of SOD, CAT, APO and GR in the control as well as in the variously treated broad bean seedlings are shown up in **Figure 3A and B**. Careful examination revealed the following main points:

(a) Antioxidant enzyme activities of SOD, CAT, APO and GR of the control as well as of the differently treated broad bean seedlings showed a significant increase throughout the germination period.

(b) Broad bean seedlings germinated under dark and ambient light conditions and irradiated with UV-A, UV-C, HL and LL, either alone or in combination, induced significant increases in antioxidant enzyme activities throughout the germination period, as compared with the control enzyme activities.

(c) The sequence of the calculated percentages of increase in the enzyme activities of both SOD and APO were as follows: UV-C > LL > LL + UV-C > UV-A > LL + UV-A, for the dark experiment, but the sequence of increase maintained for the activities of both CAT and GR was as follows: (LL + UV-A > UV-A > LL + UV-C > LL > UV-C) and (UV-C > LL + UV-C > LL > LL + UV-A > UV-A), respectively (**Table 1**). On the other hand, in the light experiment, the sequence of the calculated percentages of increase in the activities of SOD, CAT, APO and GR were as follows (HL > HL + UV-C > UV-C).

Table 1. The percentage change (increase) for UV-absorbing compounds, antioxidant compounds and antioxidant enzymes in response to treatment of broad bean seedlings with visible light and UV on the sixth day after treatment

UV-absorbing compounds			
Parameters / Treatments		Total phenols	Anthocyanins
Darkness	Control	-	-
	LL	+8.13	+150.0
	UV-C	+3.49	+128.33
	LL + UV-C	+12.69	+156.67
	UV-A	+23.35	+161.67
	LL + UV-A	+31.72	+173.33
Light	Control	-	-
	HL	+31.96	+27.03
	UV-C	+24.98	+1.80
	HL + UV-C	+28.08	+11.71
Antioxidant compounds			
Parameters / Treatments		Total glutathione	Total ascorbate
Darkness	Control	-	-
	LL	+14.78	+7.58
	UV-C	+7.88	+3.79
	LL + UV-C	+21.18	+13.27
	UV-A	+26.11	+14.22
	LL + UV-A	+29.56	+26.54
Light	Control	-	-
	HL	+44.32	+40.63
	UV-C	+16.84	+11.72
	HL + UV-C	+34.02	+21.09
Antioxidant enzymes			
Parameters / Treatments		SOD	APO	CAT	GR
Darkness	Control	-	-	-	-
	LL	+71.52	+78.26	+51.26	+6.78
	UV-C	+77.85	+93.48	+36.68	+11.86
	LL + UV-C	+65.82	+50.0	+77.39	+8.47
	UV-A	+52.53	+41.30	+81.41	+1.67
	LL + UV-A	+34.81	+36.96	+101.51	+5.08
Light	Control	-	-	-	-
	HL	+50.98	+77.55	+73.08	+43.28
	UV-C	+28.43	+55.10	+35.10	+11.94
	HL + UV-C	+40.20	+75.51	+53.85	+25.37

In accord with the present results, Jain et al.[24] stated that the activity of antioxidant enzymes SOD, APO, guaiacol peroxidase and GR are enhanced in cucumber cotyledons in response to UV radiation. Activation of antioxidant enzymes by UV-radiation has earlier been observed in *Arabidopsis thaliana*,[25] wheat[26] and cucumber.[24] Activation of antioxidant enzymes by UV-radiation

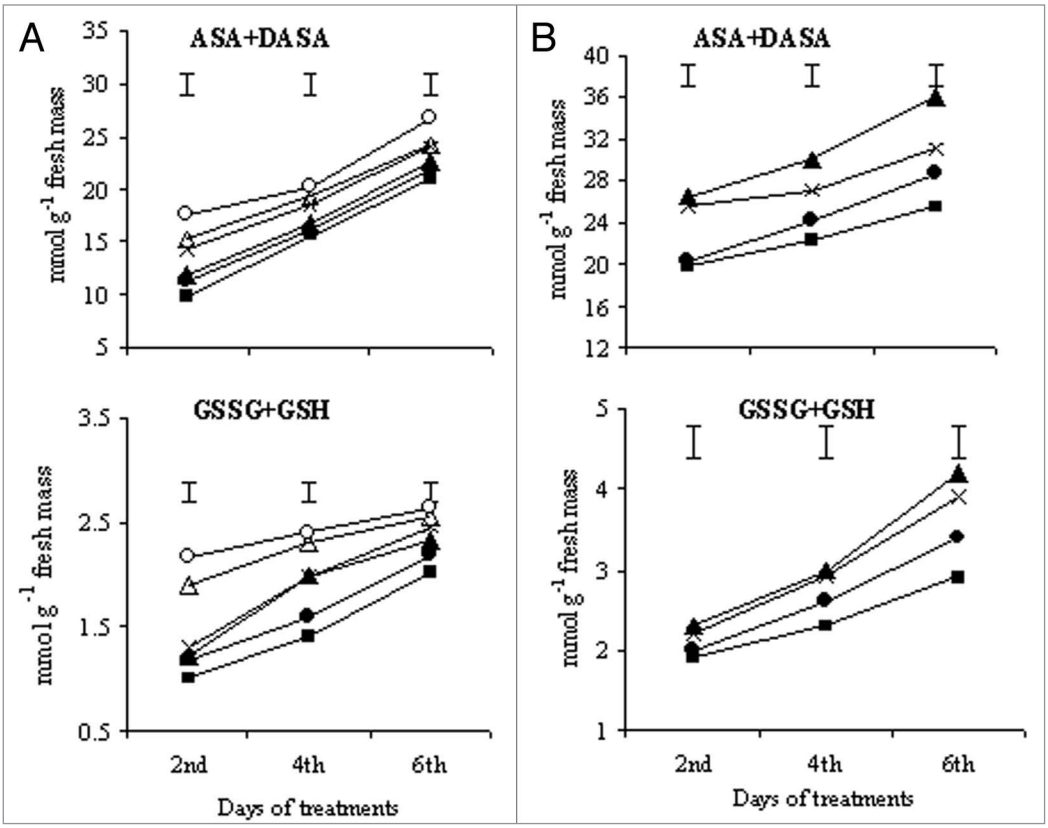

Figure 2. (A) The effects of low visible light (LL) or UV radiations (UV-A and UV-C) on antioxidant compounds; total ascorbate (ASA + DASA) and total glutathione (GSSG + GSH) in *Vicia faba* seedlings germinated in darkness. (-■- Control; -▲- LL; -●- UV-C; -x- LL + UV-C; -△- UV-A; -○- LL + UV-A). Vertical bars represent the standard error (±S.E.). (B) The effects of high visible light (HL) or UV-C radiation on antioxidant compounds; total ascorbate (ASA + DASA) and total glutathione (GSSG + GSH) in *Vicia faba* seedlings germinated in ambient light. (-■- Control; -▲- HL; -●- UV-C; -x- HL + UV-C). Vertical bars represent the standard error (±S.E.).

in the presence of externally supplied antioxidants was not observed in earlier studies.[24]

Furthermore, Alexieva et al.[27] reported that an increase in hydrogen peroxide was present in both wheat and pea species due to drought and UV-radiation. However, the increase was more evident in wheat seedlings. The hydrogen peroxide presence is an index of the reactive oxygen that is generated and utilized by enzyme systems such as SOD and CAT. In wheat and pea, an increase of the specific activities of antioxidant enzymes was evident after UV-irradiation. Thus, Beggs et al.[28] have proposed that when growth is restricted by some stress factor, other repair mechanisms such as photoreactivation, excision repair, quenching and free radical scavenging could be activated in order to alleviate the stress and prevent the damage before it becomes lethal.

In support of the above statements, Watanabe et al.[21] stated that during normal cellular metabolism, plants continuously produce ROS but they also simultaneously scavenge these ROS with the help of antioxidant enzymes and/or metabolites.[29] Although only a few investigators have reported to have measured the amounts of ROS in plant tissues exposed to HL and/or UV light,[31] it is generally accepted that high doses of visible- or UV-light induce oxidative stress in plant cells.[21]

In conclusion, the observed changes in amino acids, proteins and in nucleic acids (Yonis et al., submitted) as well as the present changes showing enhancement of accumulation of phenolic compounds and various antioxidant components, as influenced by visible light and UV-radiation, appeared to coincide with the changes maintained in growth and photosynthetic components as well as in the total amount and in the relative composition of the carbohydrate pool (Yonis et al., submitted) of broad bean seedlings. Thus, it is apparent that light responses are very diverse and any explanation of light signaling must take into account this diversity.

Materials and Methods

Plant material and growth conditions. Broad bean (*Vicia faba* cv. Egypt1) seeds were selected for homogenity and sterilized for 3 min in 10^{-3} M mercuric chloride solution, rinsed several times with sterile distilled water and then soaked for 24 h in sterile distilled water at 25 ± 1°C, with aeration to avoid anaerobiosis as a complicating factor. The seeds were then divided into a number of sets; each of 25 seeds that were allowed to germinate in 22 x 14 x 10 cm plastic boxes furnished with Whatman No.1 paper moistened with 20 cm^3 of sterile distilled water. As

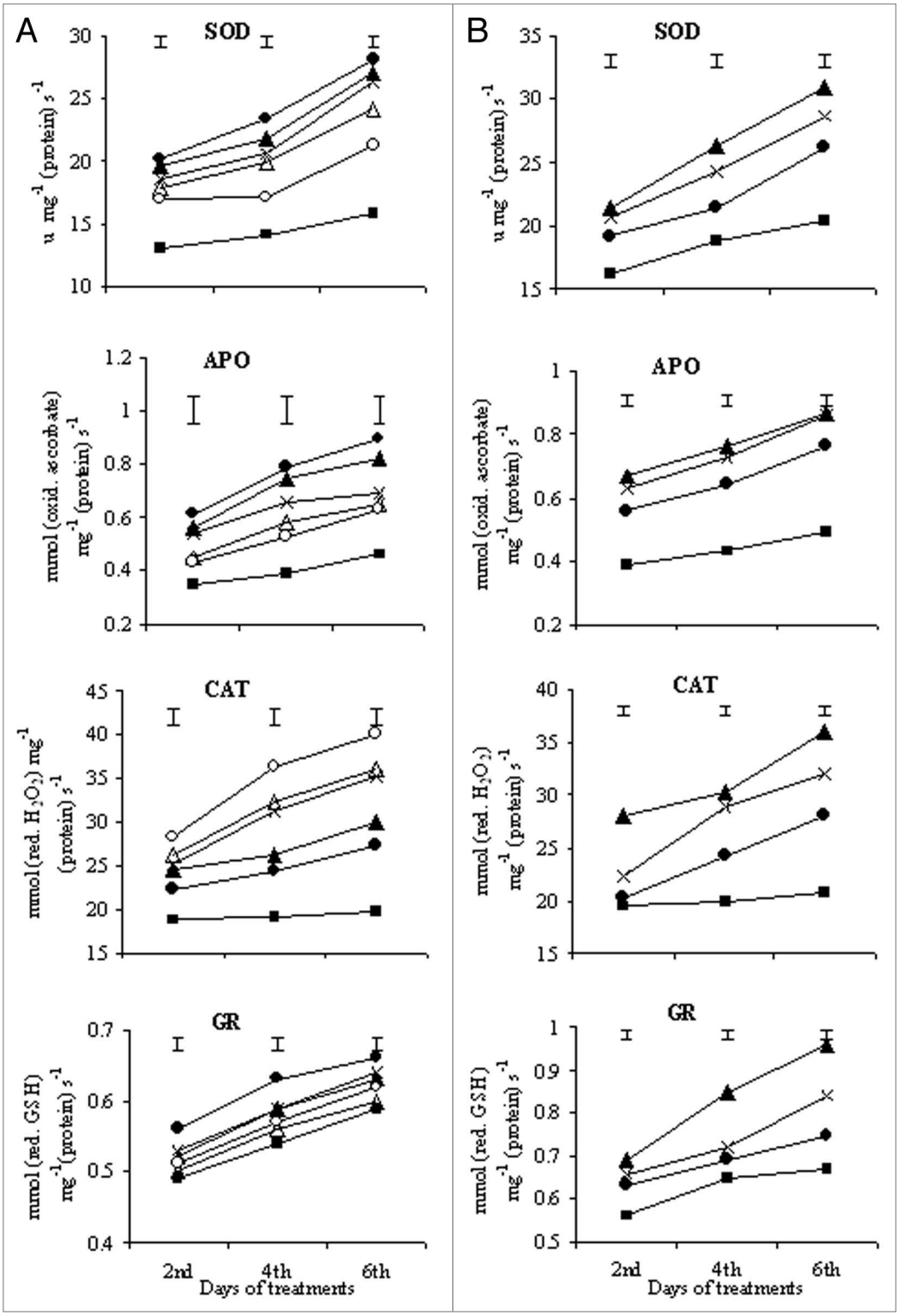

Figure 3. (A) The effects of low visible light (LL) or UV radiations (UV-A and UV-C) on antioxidant enzyme activities; superoxide dismutase, ascorbate peroxidase, catalase and glutathione reductase in *Vicia faba* seedlings germinated in darkness. (-■- Control; -▲- LL; -●- UV-C; -x- LL + UV-C; -△- UV-A; -○- LL + UV-A). Vertical bars represent the standard error (± S.E.). (B) The effects of high visible light (HL) or UV-C radiation on antioxidant enzyme activities; superoxide dismutase, ascorbate peroxidase, catalase and glutathione reductase in *Vicia faba* seedlings germinated in ambient light. (-■- Control; -▲- HL; -●- UV-C; -x- HL + UV-C). Vertical bars represent the standard error (± S.E.).

required, each box was supplied with additional 20 cm^3 of sterile distilled water.

The experimental plan, treatment and the sampling procedures were essentially the same as adopted by Younis et al. (submitted). Thus the boxes containing the seeds were grouped into two sections. In section A, the specified germination boxes were incubated in the dark whereas those boxes of section B were incubated in ambient light (12 h day and 12 h night, photosynthetically active radiation, PAR = 280 μmol m^{-2} s^{-1}). After 14 days from the start of incubation at 25 ± 0.1°C, the allotted boxes of each section were subdivided into a number of subgroups; one being left without treatment and the other subgroups were irradiated, one hour daily, for 6 days, then quickly returned back to the original germination conditions according to the following:

Dark growth conditions (Section A):

(1) Control.

(2) Exposure of seedlings for 1 h to low visible light level (LL) (40 W m^{-2}).

(3) Exposure of seedlings for 1 h to UV-C radiation (UV-C) (254 nm).

(4) Exposure of seedlings for 1 h to low visible light level (40 W m^{-2}) in combination with UV-C radiation (254 nm) (LL + UV-C).

(5) Exposure of seedlings for 1 h to UV-A radiation (UV-A) (365 nm).

(6) Exposure of seedlings for 1 h to low visible light level (40 W m^{-2}) in combination with UV-A radiation (365 nm) (LL + UV-A).

Ambient light growth conditions (Section B):

(1) Control.

(2) Exposure of seedlings for 1 h to high visible light level (HL) (160 W m^{-2}).

(3) Exposure of seedlings for 1 h to UV-C radiation (UV-C) (254 nm).

(4) Exposure of seedlings for 1 h to high visible light level (160 W m^{-2}) in combination with UV-C radiation (254 nm) (HL + UV-C).

All the exposure treatments were performed in UV-light boxes (70 x 50 x 70 cm) in which UV radiation was supplied from a compact UV lamp, 365 nm and 254 nm (8 W m^{-2}) (UVP factory, USA) which was suspended above and perpendicular to the germination boxes at a distance of 50 cm. Low visible light level was supplied from filament lamps with soft white bulbs (40 W m^{-2}) (Express factory, China) and high visible light level was supplied from blended-light mercury lamps (160 W m^{-2}) (Simlux factory, Egypt) fixed at the same distance.

Triplicate samples, immediately taken on the sixth day after the last treatment with light and UV radiation were subjected to analyses for total phenols and anthocyanin contents as well as for determination of the changes in the antioxidant system. The obtained data were statistically analyzed using one-way analysis of variance (ANOVA) and comparison among means was carried out by calculating the post hoc L.S.D. with a significant level at *$p < 0.05$. All the analyses were made using the SPSS 13.0 for Windows software package (SPSS Inc., Chicago, IL).

For better quantitative comparison among the different treatments, the percentage of change (increase or decrease) in response to each treatment, in relation to control level, was calculated throughout this investigation as follows:

Percentage change (increase or decrease) immediately after each specific treatment: [(level after treatment - control level)/ control level] x 100.

Estimation of UV-absorbing compounds. As described by Malik and Singh,[31] total phenols were estimated using Folin-Ciocalteau reagent and the optical density of the color developed was measured at 650 nm using Spekol spectrocolorimeter against a reagent blank. Anthocyanins were extracted and determined using the method of Mirecki and Teramura.[32]

Estimation of non-enzymic antioxidants. Total glutathione was determined by the method of Gossett et al.[33] Over ice, 1 g of frozen tissue was ground with sterilized sand and 5 cm^3 of ice-cold 6% (v/v) phosphoric acid (pH 2.8) containing 1 mM EDTA in a mortar. The homogenate was centrifuged at 22,000 g for 15 min and the supernatant was removed and then filtered through ultrafilter. Total glutathione was measured in a reaction mixture consisting of 0.4 cm^3 of solution A [1 mM Na_2HPO_4, 4 mM NaH_2PO_4, 0.15 mM EDTA, 0.14 mM 5,5-dithiobis-(2-nitrobenzoic acid) and 0.05 cm^3 of bovine-serum albumin (BSA)] and 0.32 cm^3 of solution B [0.05 mM EDTA, 12.5 mM imidazole, 0.05 cm^3 BSA, 5 U mg^{-1} (protein) GR, 0.4 cm^3 of the plant extract diluted 1:50 in 5% (w/v) Na_2HPO_4 (pH 7.5) and 0.8 mM NADPH]. The reaction was measured spectrophotometrically following the change in absorbance at 412 nm for 10 min.

Total ascorbate was determined by a modified method of Gossett et al.[35] Over ice, 1 g of frozen tissue was ground with sterilized sand and 10 cm^3 5% (v/v) phosphoric acid (pH 2.8) in a mortar. The homogenate was centrifuged at 22,000 g for 15 min. Total ascorbate was determined in a reaction mixture consisting of 0.2 cm^3 of supernatant, 7.5 mM KH_2PO_4 buffer (pH 7.4) containing 5 mM EDTA and 0.1 mM dithiothreitol (DTT) to reduce DASA to ASA. After 10 min at room temperature, 0.05% (w/v) N-ethylmaleimide was added to remove excess DTT. Color was developed in the reaction mixture with the addition of 0.4 cm^3 of 10% (w/v) trichloroacetic acid, 0.4 cm^3 of 4.4% (v/v) o-phosphoric acid, 0.4 cm^3 of dipyridyl in 70% (v/v) ethanol and 0.2 cm^3 of 3% $FeCl_3$. The reaction mixture was incubated at 40°C for 1 h and quantified spectrophotometrically at 525 nm.

Extraction and assay of antioxidant enzymes. Plant tissues (5 g) were homogenized in a mortar under temperature of -5°C. Subsequently soluble proteins were extracted by grinding the macerate with a small amount of sterilized sand on ice, in 5 cm^3 of 50 mM Tris-HCl, pH 7.5, containing 20% (v/v) glycerol, 1 mM ascorbate, 1 mM EDTA, 1 mM GSH, 5 mM $MgCl_2$ and 1 mM DTT. After two centrifugation steps (6 min at 12,000 g and 16 min at 22,000 g), the supernatant was stored in liquid nitrogen for determination of the activities of SOD, APO, CAT and GR.[36]

Superoxide dismutase activity was assayed by measuring the ability of the enzyme extract to inhibit the photochemical reduction of nitrobluetetrazolium (NBT);[34,35] one unit of SOD activity

being defined as the amount of enzyme required to cause 50% inhibition of the rate of NBT reduction at 650 nm. The reaction mixture contained 0.1 cm^3 of 1.3 μM riboflavin, 0.1 cm^3 of 13 mM methionine, 0.1 cm^3 of 63 μM NBT in 0.1 M phosphate buffer (pH 7.8), and 0.05–0.1 cm^3 of enzyme extract in a final volume of 3 cm^3.

Ascorbate peroxidase was assayed, as the decrease in absorbance at 290 nm due to ascorbate oxidation, by the method of Nakano and Asada.[36] The reaction mixture contained 0.1 cm^3 of 50 mM KH_2PO_4, pH 7.0, 0.1 cm^3 of 1 mM sodium ascorbate, 0.1 cm^3 of 2.5 mM H_2O_2 and 0.1 cm^3 of enzyme extract in a final volume of 1 cm^3 at 25°C.

Catalase activity was determined by measuring changes in absorbance at 240 nm corresponding to the decomposition of H_2O_2 in a reaction mixture containing 0.1 cm^3 of 50 mM KH_2PO_4, pH 7.0, 0.1 cm^3 of 10 mM H_2O_2 and 0.1 cm^3 of enzyme extract in a final volume of 1 cm^3 at 25°C.[37]

Glutathione reductase activity was assayed as the increase of absorbance at 340 nm due to the connection of GSSG to 1-chloro-2, 4-dinitrobenzene (CDNB) as described by Drotar et al.[38] The reaction mixture contained 0.1 cm^3 of 100 mM KH_2PO_4, pH 7.0, 0.1 cm^3 of 2 mM CDNB, 0.1 cm^3 of 2 mM GSSG and 0.5 cm^3 of enzyme extract in a final volume of 2 cm^3.

References

1. Kirchgeßner H-D, Reichert K, Hauff K, Steinbrecher R, Schnitzler J-P, Pfündel EE. Light and temperature, but not UV radiation, affect chlorophylls and carotenoids in Norway spruce needles (*Picea abies* (L.) Karst.). Plant Cell Environ 2003; 26:1169-79.
2. Close DC, McArthur C, Hagerman AE, Davies NW, Beadle CL. Phenolic acclimation to ultraviolet-A irradiation in *Eucalyptus nitens* seedlings raised across a nutrient environment gradient. Photosynthetica 2007; 45:36-42.
3. Landry LG, Chapple CCS, Last RL. Arabidopsis mutants lacking phenolic sunscreens exhibit enhanced ultraviolet-B injury and oxidative damage. Plant Physiol 1995; 109:1159-66.
4. Balakumar T, Selvakumar V. Phenol metabolism as a potential protective mechanism against UV-B radiation-induced oxidative stress in plants. 2nd International electronic conference on synthetic organic chemistry. Switzerland 1998; 229.
5. Mayer AM, Harel E. Polyphenol oxidases in plants. Phytochemistry 1979; 18:193-215.
6. Close DC, Beadle CL. The ecophysiology of foliar anthocyanins. Bot Rev 2003; 69:149-61.
7. Reddy VS, Goud KV, Sharma R, Reddy AR. Ultraviolet-B-responsive anthocyanin production in a rice cultivar is associated with a specific phase of phenylalanine ammonia lyase biosynthesis. Plant Physiol 1994; 105:1059-66.
8. Santos I, Almeida J, Salema R. The influence of UV-B radiation on the superoxide dismutase of maize, potato, sorghum and wheat leaves. Can J Bot 1999; 77:70-6.
9. Santos I, Fidalgo F, Almeida JM, Salema R. Biochemical and ultrastructural changes in leaves of potato plants grown under supplementary UV-B radiation. Plant Sci 2004; 167:925-35.
10. Prasad SM, Zeeshan M. UV-B radiation and cadmium induced changes in growth, photosynthesis and antioxidant enzymes of cyanobacterium *Plectonema boryanum*. Biol Plant 2005; 49:229-36.
11. Agarwal S. Increased antioxidant activity in Cassia seedlings under UV-B radiation. Biol Plant 2007; 51:157-60.
12. Agarwal S, Pandey V. Stimulation of stress-related antioxidative enzymes in combating oxidative stress in Cassia seedlings. Indian J Plant Physiol 2003; 8:264-9.
13. Jansen MAK, Gaba V, Greenberg BM. Higher plants and UV-B radiation: balancing damage, repair and acclimation. Trends Plant Sci 1998; 3:131-5.
14. Krizek DT. Influence of PAR and UV-A in determining plant sensitivity and photomorphogenic responses to UV-B radiation. Photochem Photobiol 2004; 79:307-15.
15. Steyn WJ, Wand SJE, Holcroft DM, Jacobs G. Anthocyanins in vegetative tissues: a proposed unified function in photoprotection. New Phytol 2002; 155:349-61.
16. Beckwith AG, Zhang Y, Seeram NP, Cameron AC, Nair MG. Relationship of light quantity and anthocyanin production in *Pennisetum setaceum* Cvs. Rubrum and Red Riding Hood. J Agric Food Chem 2004; 52:456-61.
17. Chinnusamy V, Jagendorf A, Zhu JK. Understanding and improving salt tolerance in plants. Crop Sci 2005; 45:437-48.
18. Younis ME, Hasaneen MNA, Tourky SMN. Plant growth, metabolism and adaptation in relation to stress conditions XXIV. Salinity-biofertility interactive effects on proline, glycine and various antioxidant compounds in *Lactuca sativa.* Plant Omics J 2009; 2:197-205.
19. Younis ME, Hasaneen MNA, Kazamel AMS. Plant growth, metabolism and adaptation in relation to stress conditions XXVI. Exogenously applied ascorbic acid ameliorates detrimental effects of NaCl and mannitol stress in *Vicia faba* seedlings. Protoplasma 2010a; 239:39-48.
20. Lopez F, Vansuyt G, Casse-Delbart F, Fourcroy P. Ascorbate peroxidase activity, not the mRNA level, is enhanced in salt-stressed *Raphanus sativus* plants. Physiol Plant 1996; 97:13-20.
21. Watanabe K, Yamada N, Takeuchi Y. Oxidative DNA damage in cucumber cotyledons irradiated with ultraviolet light. J Plant Res 2006; 119:239-46.
22. Rozema J, van de Staaij J, Björn LO, Caldwell MM. UV-B as an environmental factor in plant life: stress and regulation. Trends Ecol Evol 1997; 12:22-8.
23. Bornman JF. New trends in photobiology: Target sites of UV-B radiation in photosynthesis of higher plants. J Photochem Photobiol B: Biol 1989; 4:145-58.
24. Jain K, Kataria S, Guruprasad KN. Effect of UV-B radiation on antioxidant enzymes and its modulation by benzoquinone and α-tocopherol in cucumber cotyledons. Curr Sci 2004; 87:87-90.
25. Rao MV, Paliyath G, Ormrod DP. Ultraviolet-B- and ozone-induced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana*. Plant Physiol 1996; 110:125-36.
26. Sharma PK, Anand P, Sankhalkar S. Oxidative damage and changes in activities of antioxidant enzymes in wheat seedlings exposed to ultraviolet-B radiation. Curr Sci 1998; 75:359-66.
27. Alexieva V, Sergiev I, Mapelli S, Karanov E. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant Cell Environ 2001; 24:1337-44.
28. Beggs CJ, Schneider-Ziebert U, Wellmann E. UV-B radiation and adaptive mechanisms in plants. In: Stratospheric ozone reduction, solar ultraviolet radiation and plant life, (eds. Worrest RC, Caldwell MM) Springer-Verlag Heidelberg Berlin 1986; 8:235-50.
29. Asada K. The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. Ann Rev Plant Physiol Plant Mol Biol 1999; 50:601-39.
30. Dai Q, Yan B, Huang S, Liu X, Peng S, Miranda MLL, Chavez AQ, Vergara BS, Olszyk DM. Response of oxidative stress defense systems in rice (*Oryza sativa*) leaves with supplemental UV-B radiation. Physiol Plant 1997; 101:301-8.
31. Malik CP, Singh MB. Plant enzymology and histoenzymology. Kalyani Publishers, New Delhi 1980; 286.
32. Mirecki RM, Teramura AH. Effects of Ultraviolet-B irradiance on soybean V. The dependence of plant sensitivity on the photosynthetic photon flux density during and after leaf expansion. Plant Physiol 1984; 74:475-80.
33. Gossett DR, Millhollon EP, Lucas MC. Antioxidant response to NaCl stress in salt-tolerant and salt-sensitive cultivars of cotton. Crop Sci 1994; 34:706-14.
34. El-Saht HM. Response to chilling stress in french bean seedlings: antioxidant compounds. Biol Plant 1998; 41:395-402.
35. Giannopolitis CN, Ries SK. Superoxide dismutases I. Occurrence in higher plants. Plant Physiol 1977; 59:309-14.
36. Nakano Y, Asada K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol 1981; 22:867-80.
37. Aebi HE. Catalase. In: Methods of enzymatic analysis, (ed. Bergmeyer HU) Verlag. Chem. Weinheim Germany 1983; 3:273-86.
38. Drotar A, Phelps P, Fall R. Evidence for glutathione peroxidase activities in cultured plant cells. Plant Sci 1985; 42:35-40.