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Investigation of Climate Changes on Metabolic Response of Plants; Interactive Effects of Drought Stress and Excess UV-B

Hassan IA1,2*, Basahi JM3, Haiba NS2 and Kadi MW4

1Faculty of Science, Alexandria University, 21526 El Shatby, Alexandria, Egypt
2Centre of Excellence in Environmental Studies, King Abdulaziz University, P. O. Box 80216, Jeddah 21589, KSA
3Department of Chemistry, Faculty of Education, Alexandria University, Egypt
4Department of Chemistry, Faculty of Science, King Abdulaziz University, Jeddah, KSA

Abstract

Physiological and biochemical responses of bean (Vicia faba L.) plants to either supplementary ultraviolet (sUV-B) radiation, and/or water (WS) stress were investigated. Both stresses caused significant increases in H$_2$O$_2$ content and lipid peroxidation, indicating oxidative damage. Furthermore, increases in activities of stress markers indicated that sUV-B has a stronger stress effect than WS, and it caused greater membrane damage, as assessed by lipid peroxidation and osmolyte leakage.

The activities of ascorbate peroxidase (APX) and superoxide dismutase (SOD) were increased under both stresses when applied alone and in combination, while catalase (CAT) activity decreased under water stress as compared to the control. The combination of drought and UV-B, were more than additive, caused more severe damage than stress applied separately.

WS induced accumulation of UV-B absorbing secondary pigments (anthocyanin and flavonoids) which is likely to offer some protection from UV-B irradiation.

Keywords: sUV-B radiation; Water stress; Stress markers; Antioxidant enzymes; Proline accumulation; Secondary metabolites

Introduction

Anthropogenic activities have resulted in the reduction of stratospheric ozone [1,2], which led to a significant increase in ultraviolet-B (UV-B) radiation (290-320 nm) reaching the surface of the Earth [3,4]. Elevated levels of UV-B radiation can be stressful to plants [5]. Plants are exposed to a multitude of natural biotic and abiotic stressors. Most of a biotic stresses are connected to anthropogenic activities which are clearly causing major changes in atmospheric chemistry [6].

Wide inter- and intraspecific differences have been reported in response to UV-B with respect to growth and plant morphogenetic response [7-9] and physiological [10,11]. Some species show varied degrees of tolerance [12] while others are sensitive to present levels of UV-B radiation [13-15]. UV-B induced growth inhibition is usually associated with damage to the photosynthetic apparatus and reduction of photosystem II (PSII) efficiency [11,16].

Plants have evolved a variety of biochemical adjustments as mechanisms to protect and prevent damage caused by environmental stress(s) including UV-B radiation and water stress. The most widely observed mechanisms are the accumulation of UV-absorbing compounds in the epidermal cells such as flavonoids [17] and activation of antioxidant enzymes such as POD and SOD [1,15]. These enzymes scavenge free radicals from oxygen, and offer protections to lipids, proteins and nucleic [13,18].

UV-B is species specific, as other environmental stresses [1-19,20]. The different sensitivities of plants are partially explained by their abilities to respond to UV-B through the induction of defensive pathways [15-21].

Various stress factors competing with the supplemental UV radiation were shown to modify the UV radiation effects [22]. Water stress, is an important restricting factor that always affects agricultural productivity, particularly in arid and semi-arid regions. Feng et al. [23,24] showed that co-stresses of supplementary UV radiation and drought functioned synergically and one of them could alleviate the inhibitory effects of another under conditions of arid and semi-arid soils.

Although responses of crop physiology, growth, and yield to either water stress or UV-B radiation have been extensively studied in Northern Europe and the USA [24], knowledge of their interactive effects on crops, especially in developing countries, is extremely limited [10]. Moreover, there is paucity on the knowledge concerning the antioxidant response of plants to UV-B [25-26]. Furthermore, the mechanisms involved in the response of plants to both waters stress and UV-B are yet to be identified.

The aims of the present study were to understand the physiological and biochemical characteristics of broad bean (Vicia faba L.) under supplementary UV-B radiation and/or water stress, and to estimate its sensitivity and defense mechanisms under both stresses

Materials and Methods

Seeds of an Egyptian cultivar of bean (Vicia faba L.), obtained from a commercial source, were sown 20 cm apart at The Botanical Garden on 13/7/2011. Ten days after placing the plants half the plants

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*Corresponding author: I. A. Hassan, Faculty of Science, Alexandria University, 21526 El Shatby, Alexandria, Egypt. E-mail: iahassan_eg@yahoo.com

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were subjected to progressive drought by withholding water, while well watered plants were irrigated once a week. Well-watered and water-stressed (WS) plants were divided equally between the two sections in a split-plot design. Consequently, four treatments were distributed in each plot in a randomized Latin square design: (a) control, i.e. without UV-B radiation and well-watered (b) Plots supplied with supplementary UV (sUV-B), (c) Plants subjected to drought stress (WS) without UV-B radiation and (d) plants were subjected to both sUV-B and WS. Twenty plants were used in each treatment.

No fertilisers or other fungicides were applied at either location to avoid interference with the fungicides.

Supplemental UV-B radiation was supplied by filtered Westinghouse FS-40 sunlamps oriented perpendicular to the planted rows and suspended above the plants. Lamps were filtered either with 0.13 mm thick cellulose acetate (transmission down to 290 nm) for supplemental UV-B radiation or 0.13 mm Mylar Type S plastic films (absorbs all radiation below 320 nm) as a control. The radiation filtered through the cellulose acetate supplied a weighted daily supplemental irradiance of either 3.0 or 5.1 effective kJ m⁻² UV-BBE using the generalized plant response action spectrum [27] normalized to 300 nm. Plants beneath these cellulose acetate filtered lamps received supplemental doses in addition to ambient levels of UV-B radiation. These increased levels of UV-B radiation (supplemental+ambient) [28]. The weighted irradiance of Mylar filtered lamps was 0, so plants beneath these lamps received only ambient levels of UV-B (8.5 effective kJ m⁻² UV-BBE on the summer solstice). Spectral irradiance beneath the lamps was measured with an Optronics Spectroradiometer (Model 742) equipped with a double monochromator with dual holographic grating and interfaced with a Hewlett Packard 85 printing calculator. The Spectroradiometer was calibrated using a National Bureau of Standards traceable 1000 W tungsten halogen lamp and wavelength alignment checked with known mercury emission lines using an Hg Arc lamp.

Non Destructive Harvests

Net photosynthetic rate \( (P_n) \) and total stomatal conductance for \( CO_2 \) (g) were measured on the youngest fully expanded leaf of the main stem. Gas exchange measurements were carried out seven times at 5 d intervals to cover all growth stages (10 days after sowing) using a LI-6200 portable IRGA (LI-COR, Lincoln, USA) between 10:00 and 14:00 h (Local time). All plants were measured on each day [29] (Table 1).

Measurements of hydrogen peroxide

At the end of the drought and/or sUV-B treatment(s) (45 days after sowing), plants were harvested destructively. Fifteen leaf discs (10 mm diameter) were submerged in 750 µL reagent mixture containing 0.05% guaiacol and horseradish peroxidase (350 µL L⁻¹, 250 Um L⁻¹) in 25 mM sodium phosphate buffer (pH 7.0) and incubated for 2 h at 20°C in the dark [30]. Then, a volume of 250 µL was transferred into 96-well microtitre plates and the absorbance was immediately measured at 445 nm in a plate reader photometer (SLT, Spectra, Dixons Ltd, Pure Chemicals for Laboratories, Switzerland). Commercial H₂O₂, which was used for standard curves, was calibrated by titration with KMnO₄.

Antioxidant enzymes assays

Leaves were cut from each treatment (control, WW+sUV-B, WS-sUV-B and WS+sUV-B) and immersed in liquid nitrogen and kept in a deep freezer at -80°C until the analyses were performed at Laboratories of Center of Excellence in Environmental Studies, King Abdulaziz University, KSA.

Samples were weighed and ground at about 0°C in 25 m Tris–HCl buffer containing 3 mM MgCl₂, and then the homogenates were centrifuged at 20,000 for 15 min (Centrifuge 17 S/R, Heraeus Sepatech). The supernatants were used for the enzyme assays and the results were expressed on protein basis [31].

All assays were performed using a final volume of 1 mL, with at least duplicate assays undertaken on each sample. Moreover, the assays were end-point determinations [29].

SOD (EC 1.15.1.1) activity was monitored [32]. The extraction mixture contained 50 mM phosphate buffer solution (pH 7.8), 13 mM L-methionine, 63 mM nitro blue tetraozalium and 2 mM riboflavin. The ability of the extract to inhibit the photochemical reduction of nitro blue tetrazolium was determined at 560 nm (Schimadzu UV-1201 spectrophotometer).

The amount of the extract resulting in 50% inhibition of nitro blue tetrazolium reaction is defined as one unit of SOD activity.

Catalase (EC, 1.11.1.6) activity was assayed in enzyme extract reaction mixture containing 50 mM phosphate buffer (pH 7.4). The reaction was started by adding 10 mM H₂O₂, and the reduction in absorbance was determined at 240 nm [33].

APX (EC, 1.11.1.11) activity was determined according to Maehly and Chance [33]. The reaction mixture contained 50 mM potassium phosphate, 0.5 mM ascorbate, 0.1 mM Methyleneedimethyl tartaric acid (EDTA) and 0.1 mM H₂O₂, and the absorbance was determined at 290 nm.

Protein concentrations of leaf extracts were determined as described earlier [31].

Pigment analysis

Chlorophyll was extracted in acetone from all leaves in the main stems of three plants per treatment, and determined [34].

Water-soluble pigments (flavonoids and anthocyanins) were
extracted from leaves at the end of the experiment. Leaves were ground to a powder in liquid nitrogen before extraction in 10 cm³ of acidified methanol (HCl: methanol, 1: 99, v/v). Absorption spectra of the extracts were determined using a Cary 210 spectrophotometer (Varian, Palo Alto, CA, USA), and the flavonoid and anthocyanin contents were estimated from absorbances at 300 and 530 nm, respectively [35].

Measurements of free proline concentration

Leaves (0.2 g) were homogenized in 5 ml of 3% sulphosalicyclic acid solution. After centrifugation, 2 ml supernatant, 2 ml glacial acetic acid and 2 ml 2.5% acid ninhydrin solution were added in a test tube covered with Teflon cap. The absorbance of the free proline concentration was measured at 520 nm. The proline content was expressed as μg g⁻¹ fresh weight [36].

Measurements of Lipid peroxidation

Lipid Peroxidation was measured by the amount of malondialdehyde (MDA) as end product of unsaturated fatty acid peroxidation [37].

Membrane permeability

It was measured by Electrolyte leakage [1]. Five leaves from each treatment were detached and immersed in distilled water at a room temperature and the conductivity of the solution was measured after 3 hours.

Statistical analysis

Two way ANOVA was applied to log-transformed data (Statgraphics Statistical Package 4, London, UK) to evaluate effects of WS and/or sUV-B treatments on growth and physiology of the plant. PPFD was used as a covariate in Leaf gas exchange and fluorescence data, there was no covariate used in growth measurements. The significance of difference among treatments were compared by Fisher’s least significant difference test (LSD).

Results

H₂O₂ and APX showed no significant response (P>0.05) to water stress (Figures 1a and d); while SOD and CAT activities were increased by 14 and 20%, respectively (Figures 1b and c). Exposure to sUV-B caused increases in these parameters by 18, 21, 47 and 56%, respectively (Figures 1a-d).

Exposure to both stresses was more than additive as it caused an increase by 33% in APX and H₂O₂, while it was less than additive in case of CAT and SOD, as they were decreased by 20 and 26%, respectively, (Figure 1). Furthermore, there was negative correlation between shoot fresh weight and H₂O₂ content (Data not shown).

Exposure to sUV-B caused reduction in anthocyanine by 24% (Figure 2a), while it had no significant (P>0.05) effect on total flavonoids (Figure 2b). On the other hand, WS caused increases by 75 and 46% in both pigments, respectively (Figures 2a and b). Moreover, plants exposed to both stresses simultaneously shoed increases in these pigments by 35 and 59%, respectively (Figures 2a and b).

However, chlorophyll content showed the same response as growth parameters, as it was decreased by 22, 16 and 20%, due to exposure to WS, UV and both stress together, respectively (Figure 2c).

Figure 3 showed effect of waters stress and UV-B radiation applied singly or in combination on free proline, lipid peroxidation and membrane permeability. Proline was increased by 76 and 34% after exposure to WS and sUV-B, respectively (Figure 3a), while their
interaction was less than additive (19%). On the other hand, Lipid peroxidation and membrane permeability were increased by 40 and 43%, respectively (Figures 3b and c) due to exposure to UV irradiation while water stress had no significant effect. Interaction between both stresses caused increases in these parameters by 31 and 47%, respectively (Figures 3b and c).

Discussion

Alexieva et al. [1] reported that there is an inter-relationship between drought and ultraviolet-B (UV-B) radiation in plant responses, in that both stresses provoke an oxidative burst. However, the mechanisms involved in the response of plants to both stresses are yet to be identified. Thus, elucidation of their interaction would help plants cope with changing environmental conditions [38].

The significant effect of sUV-B on the concentrations of $\text{H}_2\text{O}_2$ is in agreement with results of other researchers [39-41]. Increase in lipid production caused by stress may have occurred because of the accelerated formation of reactive oxygen species (ROS) [i.e., singlet oxygen ($^1\text{O}_2$) and $\cdot\text{OH}$]; ROS attack lipids, particularly unsaturated fatty acids, and the accelerated formation of ROS results in the formation of peroxidation products, the main one of which is MDA [15]. The reaction of such radicals with macromolecules, particularly
lipoproteins, can cause faster peroxidative damages as observed from the destruction of membrane lipids [1].

On the other hand, anthocyanins and flavonoids are affected differently by UV radiation. These pigments play an important role against UV damage in higher plants [22]. We found the highest levels of anthocyanin and flavonoids were obtained in UV-B radiation while the lowest content were observed in plants exposed to water stress (Figure 2). An increase of UV absorbing compounds caused by UV was well documented in previous studies [1,22,42]. Flavonoids compounds have effective radical scavenging capabilities and can directly contribute to enhanced photo protection against UV-B radiation. The increases in UV-B absorbing compounds, mainly in flavonoids, are recognized as a general response to UV-B stress [43]. These results suggest that the UV-B absorbing compounds are mainly synthesized in leaves and they are used to protect leaf tissue under exposure to UV. However, it seems to be produced through similar mechanisms as in the case of UV induction. Flavonoids and related compounds absorb strongly in the UV-region but not in the photosynthetically active regions of the spectrum [44], allowing photosynthesis to continue while UV wavelengths are attenuated at the epidermis.

Water stress and UV radiation lead to the increase of the contents of proline in leaves of bean in this experiment, which indicates that some wilting-induced proline accumulation occurred (Balouchi et al. [22]). It was reported that plants exposed to UV radiation accumulate proline that could protect plant cells against UV radiation-induced peroxidative processes [45]. A marked increase in proline accumulation under UV-B in the present study is in agreement with the results of Balouchi et al. [22] and this could represent adaptive responses to oxidative damage induced by UV radiation. Proline is known to be involved in alleviating cytosolic acidic associated with several stresses [46]. The removal of excess H⁺ occurring as a result of proline synthesis may have a positive effect on reduction of the UV-B induced damage. It suggests that UV radiation-induced proline accumulation protects plants against UV radiation promoted peroxidation processes.

CAT, APX, and SOD are key enzymes of the antioxidant defense system. The SOD, POD, APX, and CAT activities are also associated with UV-B exposure and other stresses such as water stress, as these enzymes act as antioxidant compounds to help reduce photooxidative damage in plant leaves. SOD accelerates the conversion of superoxide to H₂O₂, whereas CAT and APX catalyze H₂O₂ breakdown [47,48]. Flavonoids compounds have effective radical scavenging capabilities and can directly contribute to enhanced photo protection against UV-B radiation. The increases in UV-B absorbing compounds, mainly in flavonoids, are recognized as a general response to UV-B stress [43]. These results suggest that the UV-B absorbing compounds are mainly synthesized in leaves and they are used to protect leaf tissue under exposure to UV. However, it seems to be produced through similar mechanisms as in the case of UV induction. Flavonoids and related compounds absorb strongly in the UV-region but not in the photosynthetically active regions of the spectrum [44], allowing photosynthesis to continue while UV wavelengths are attenuated at the epidermis.

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**Conclusion**

In conclusion, we found that UV-B radiation and water stress increased UV screen pigments, MDA and antioxidant enzymes although water stress decreased pigment production. There was an interaction between sUV-B and WS, where the first delayed and reduced the severity of the latter. Our understanding of the relationships between crop growth and the atmospheric environment was developed substantially in the past few decades. Still, the factor of climate change and its impact on crops and food production will be further explored in future studies because global change climate might be critical event in future centuries; available data may not adequately characterize the potential effect of future, such as simultaneous changes in climate change and UV-B radiation.

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**References**


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