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Research Article

The Effects of Ultraviolet Radiation on Some Antioxidant Compounds and Enzymes in *Capsicum annuum* L.

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Abstract: Plants have evolved mechanisms to avoid and repair UV radiation damage; it is not surprising that photomorphogenic responses to UV-B and UV-C are often assumed to be adaptations to harmful radiation. Free radicals generated by UV are likely to be involved in the induction of antioxidant defence system. In this study plants were grown for 5 weeks in controlled conditions. Plants were grown in vermiculite medium before applying the UV treatments, and irrigated daily by Hoagland solution for 5 weeks. After 5 weeks, plants were exposed to UV-A (320-390 nm), UV-B (312 nm), and UV-C (254 nm) irradiation with a density of 6.1 (Wm-2), 5.8 (Wm-2) and 5.7 (Wm-2) for 2 weeks. Each plant was exposed to UV in their light period for 27 min per day for 14 days. In each experiment 4 replicates were used. Data were analyzed using SPSS software, and averages were compared by Duncan's test. Activity of antioxidant enzymes could be found in the plant antioxidant defence system. The activity of antioxidant enzymes (namely, peroxidase, polyphenol oxidase, ascorbate peroxidase, catalase, and glutathione reductase) enhanced in leaves and roots of pepper plants in response to UV-B and UV-C radiation. The increase in the activity of antioxidant enzymes could minimise the effects of ultraviolet radiation. UV treatments induced significant increase in ascorbic acid in leaf and root of exposed plants. The present study was conducted to determine the role of antioxidant defence mechanism in UV-A, UV-B and UV-C treated plants of *Capsicum annuum* L.

Key Words: UV radiation, Antioxidant enzyme, *Capsicum annuum*

Introduction

The spectrum of UV radiation reaching the earth's surface is divided into lower energy (UV-A, 320-400 nm), higher energy (UV-B, 280-320 nm), and UV-C (254-280 nm) regions. Plant responses to any given dose of radiation are different, UV-A is less effective than UV-B (Barta et al., 2004) and UV-C.

Global stratospheric ozone of solar ultraviolet radiation has significantly decreased during the last decade (Frederick, 1990). Chlorofluorocarbons are the main culprits for the depletion of the stratospheric ozone layer (Frederick, 1990). Free radicals are fundamental for a biochemical process. They are produced by respiration, some cell-mediated immune functions, and environmental stress (Tiwari, 2004). Ultraviolet radiation increases the production of reactive oxygen species. These reactive oxygen species (ROS) are reactive and cytotoxic (Bowler et al., 1992). ROS, produced as a result of various abiotic stresses, need to be scavenged for maintenance of normal growth. Plants have protective mechanisms such as antioxidative enzymatic detoxification mechanism. These enzymes can mitigate the UV-induced damage. Plants have a number of enzymatic and nonenzymatic detoxification mechanisms to scavenge either the active oxygen species (AOS) or secondary reaction products (Arora et al., 2002; Bartling et al., 1993; Rao et al., 1996). Synthesis of phenolic substances such as anthocyanin and flavonoids have been observed in UV-B treated *Arabidopsis thaliana* (L.) Heynh. seedlings (Ravindran et al., 2001). However, antioxidants such as ascorbic acid and glutathione (GSH) are involved in the neutralization of secondary products of AOS reactions (Conklin et al., 2000). Many plants have

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scavenging systems for protection from destructive oxidative reactions (Arora et al., 2002). However, little information is available on the effects of UV radiation on the activated oxygen species metabolism and antioxidant enzymes activity in *Capsicum annuum* L. This knowledge can provide information on the possible involvement of antioxidants as a defence against ROS generated by UV radiation, thus allowing an insight into the molecular mechanism of plant tolerance to UV induced oxidative stress. Hence, the objective of the present investigation was to study the effect of UV radiation on changes in some antioxidant enzymes activity and antioxidant compounds in pepper plant.

Materials and Methods

Plant growth and treatments

Seeds of *Capsicum annuum* L. were germinated at 27 °C for 24 h, and then sown in plastic pots containing 1 kg of coarse sand and vermiculite (2:1, V/V). Plants were grown in the greenhouse at 25/20 °C (day/night), with a 16 h light / 8 h dark photoperiod for 35 days. After that, selected plants were subjected to ultraviolet radiation with UV lamps. UV-A (320-390 nm), UV-B (312 nm), and UV-C (254 nm) irradiation with a density of 6.1 (Wm^{-2}), 5.8 (Wm^{-2}), and 5.7 (Wm^{-2}), respectively (measured with UV sensor model: LEYBOLD DIDACTIC). UV-A, UV-B, and UV-C lamps were purchased from the Philips Company. Each plant treated with UV in their light period for 27 min daily for 14 days. Four replicates were used for each treatment.

Measurements of antioxidant enzymes activity

Catalase activity was assayed by measuring the rate of disappearance of H_2O_2 following the procedure of Dhindsa et al., (1981). In 5 cm^3 of 50 mM potassium phosphate buffer pH 7, 0.5 g of leaf sample was homogenised and 1% PVP centrifuged at 4 °C for 10 min at 15,000 g. An aliquot of 1 cm^3 of the supernatant of the enzyme extract was added to the reaction mixture containing 1 cm^3 of 1.5 M H_2O_2. Decrease in H_2O_2 is considered as an activity of enzyme and measured in 240 nm during 30 s.

GR activity was assayed in 2 cm^3 of 100 mM TRIS-HCl buffer (pH 7.2) containing 0.2 mM NADPH, 5 mM glutathione disulphide (GSSG), and 100 µ dm^3 of plant extract (Anderson, 1996). The change in absorbance at 340 nm was recorded at 25 °C in a spectrophotometer. Enzyme activity was based on the oxidation rate of NADPH using an extinction coefficient of 6.2 mM^{-1} cm^{-1}.

Peroxidase and polyphenol oxidase activities were determined by the methods described by Kara and Mishra (1976).

APX activity was measured by the method of Nakano and Asada (1981). Enzymes activity was expressed as g^{-1} FW^{-1}. Protein concentration in enzyme extracts was determined according to Bradford (1976).

Measurements of the content of ascorbic acid, dehydroascorbic acid and total ascorbate

ASA and DHAS were determined as described by Mc de Pinto & Gara (1999). Simply 0.5 g of tissue were homogenized in 10 ml metaphosphoric acid 5% and centrifuged for 15 min at 10,000 g. For ASA assay, 300 µl of supernatant was used and these solutions were added to the extract in the following order: 750 µl potassium phosphate buffer and 300 µl distilled water. For the DHAS assay, 300 µl of supernatant was also used and the following solutions were added: 750 µl potassium phosphate buffer (pH = 7.2) and 150 µl of 10 mM dithiothreitol, the mixture was incubated at room temperature for 10 min, and then 150 µl of 0.5% N-ethylmaleimide was added. Both samples were vortexed and incubated at room temperature for 10 min. To each sample, 600 µl of 10% (w/v) TCA, 600 µl of 44% (v/v) H_3PO_4 , 600 µl of 4% (w/v) bipyridyl in 70% (v/v) ethanol, and 10 µl of 3% $Fecl_3$ were added. After vortex-mixing, samples were incubated at 40 °C water bath for 20 min, and then samples brought up and vortexed again and incubated at 40 °C water bath for 20 min. Absorbance of samples at 525 nm was recorded. A standard curve of ASA and DHAS was used for the calculation of ASA and DHAS concentration.

Statistics. Quantitative changes of different parameters were analyzed through analysis of variance (ANOVA) and Duncan's multiple range test was used to determine the significant differences among treatments. Standard error of mean was also calculated for presentation in figures.

Results

Capsicum annuum plants grown under the UV-A, UV-B, and UV-C treatment showed different enzymatic activities. Peroxidase activity was significantly increased by artificial UV-B and UV-C radiation in both leaf and root. Peroxidase activity was significantly increased in UV-B and UV-C treated pepper leaves by 35 and 48%, respectively, but in UV-A treated plants there was no significant increase (1%) in comparison with the control. Peroxidase activity was significantly increased in UV-B and UV-C treated pepper roots by 17% and 22%, respectively, in comparison with the control. Although UV-B and UV-C enhanced the activity of peroxidase in both leaf and root, the magnitude of increase in this enzyme activity was much higher in leaf of exposed plants to UV-B and UV-C when compared with the roots of the same plants (Figure 1). Similarly, a significant increase in polyphenol oxidase activity was observed in UV-B and UV-C treated plants. Polyphenol oxidase activity was significantly increased in UV-B and UV-C treated pepper leaves by 27% and 31%, respectively, but in UV-A treated plants there was no significant increase (6%) in comparison with the control. Polyphenol oxidase activity was significantly increased in UV-B and UV-C treated pepper roots by 19% and 27%, respectively, but in UV-A treated plants there was no significant increase (2%) in comparison with the control (Figure 2). UV-B and UV-C treatments also increased catalase activities in both leaf and root of the treated plants. Catalase activity was significantly increased in UV-B and UV-C treated pepper leaves by 23% and 24%, respectively, but in UV-A treated plants there was no significant increase (5%) in comparison with the control. Catalase activity was significantly increased in UV-B and UV-C treated pepper roots by 35% and 37% respectively, but in UV-A treated plants there was no significant increase (4%) in comparison with the control (Figure 3). In addition, ascorbate peroxidase activity slightly increased under UV-A treatment but a significant increase in activity was observed in both roots and leaves of UV-B and UV-C treated plants. Ascorbate peroxidase activity was significantly increased in UV-B and UV-C treated pepper leaves by 24% and 31%, respectively, but in UV-A treated plants there was no significant increase (5%) in comparison with the control. Ascorbate peroxidase activity was significantly increased, in UV-B and UV-C treated pepper roots by 17% and 22%, respectively, but in UV-A treated plants there was no significant increase (5%) in comparison with the control (Figure 4). The same trend in the activity was observed for glutathione reductase. Glutathione reductase activity was significantly increased in UV-B and UV-C treated pepper leaves by 17% and 37%, respectively, but in UV-A treated plants there was no significant increase (2%) in comparison with the control. Glutathione reductase activity was significantly increased in UV-B and UV-C treated pepper roots by 22% and 29% respectively but in UV-A treated plants there was no significant increase (1%) in comparison with the control (Figure 5). In this study, a significant increase in

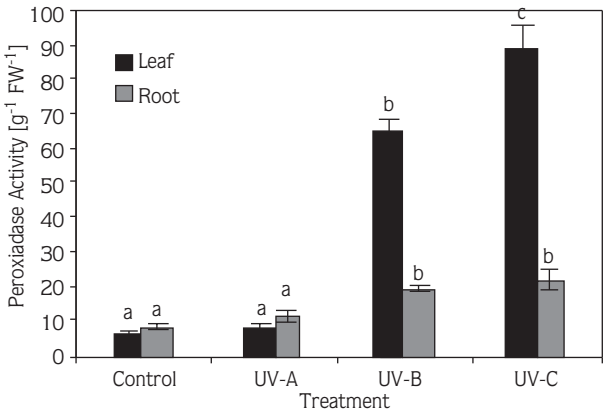

Figure 1. Effect of UV-A, UV-B, and UV-C treatment on peroxidase activity. The values, significant at $P < 0.05$ according to Duncan's test, are the means of 4 replicates and vertical bars indicate standard error of mean for leaf, root, and UV radiation. The comparison between the mean for leaf and root content of these enzymes is not shown.

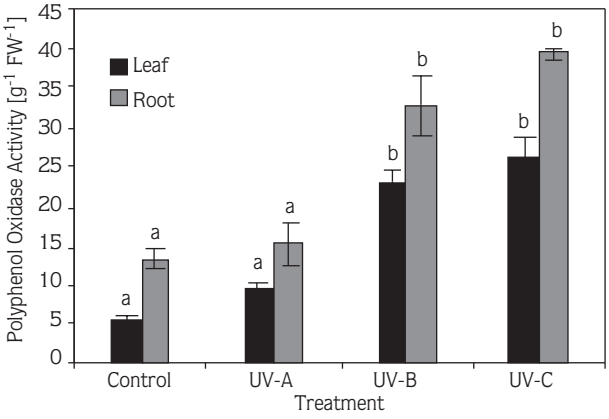

Figure 2. Effect of UV-A, UV-B, and UV-C treatment on polyphenol oxidase activity. Values, significant at $P < 0.05$ according to Duncan's test, are the means of 4 replicates, and vertical bars indicate standard error of mean for leaf, root and UV radiation The comparison between the mean for leaf and root content of these enzymes is not shown.

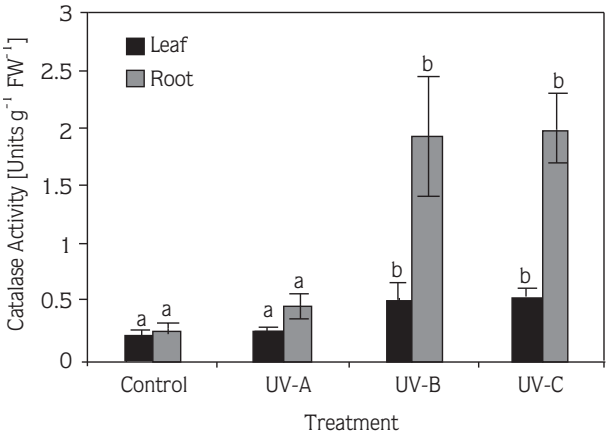

Figure 3. Effect of UV-A, UV-B, and UV-C treatment on catalase activity. Values, significant at $P < 0.05$ according to Duncan's test, are the means of four replicates, and vertical bars indicate standard error of mean for leaf, root and UV radiation. The comparison between the mean for leaf and root content of these enzymes is not shown.

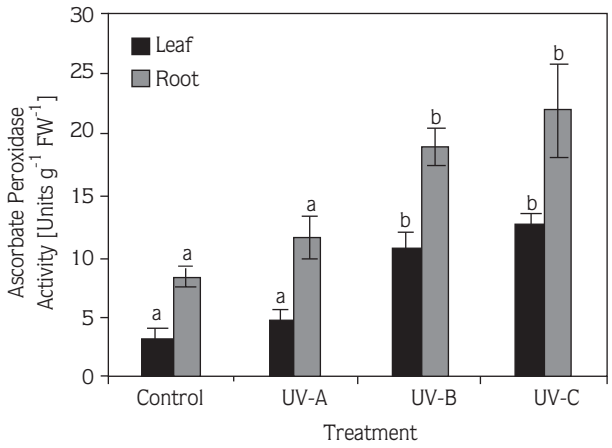

Figure 4. Effect of UV-A, UV-B, and UV-C treatment on ascorbate peroxidase activity. Values, significant at $P < 0.05$ according to Duncan's test, are the means of 4 replicates, and vertical bars indicate standard error of mean for leaf, root and UV radiation. The comparison between the mean for leaf and root content of these enzymes were not shown.

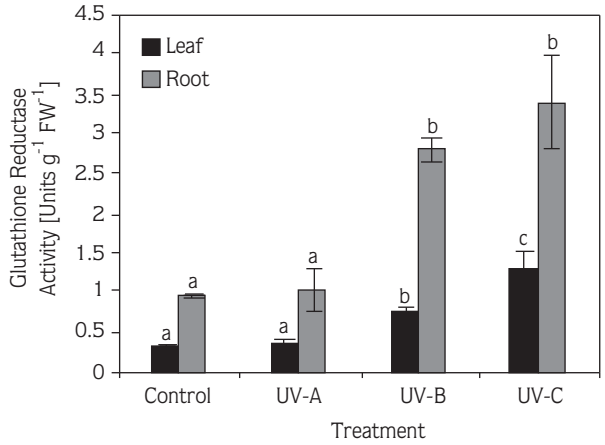

Figure 5. Effect of UV-A, UV-B, and UV-C treatment on glutathione reductase activity. Values, significant at $P < 0.05$ according to Duncan's test, are the means of four replicates, and vertical bars indicate standard error of mean for leaf, root and UV radiation. The comparison between the mean for leaf and root content of these enzymes is not shown.

ASA, DHAS, and total ASA content was recorded in UV-A, UV-B, and UV-C treatments but the magnitude of increase was much higher in leaf when compared with root. Ascorbic acid content was significantly increased in UV-A, UV-B, and UV-C treated pepper leaves by 6%, 13%, and 17%, respectively, in comparison with the control. Ascorbic acid content was also significantly increased in UV-A, UV-B, and UV-C treated pepper roots by 3%, 9%, and 12%, respectively, in comparison with the control (Figure 6). Dehydroascorbic acid content was significantly increased in UV-A, UV-B, and UV-C treated pepper leaves by 2%, 4%, and 10%, respectively, in comparison with the control. Dehydroascorbic acid content was significantly increased in UV-B and UV-C treated pepper roots by 7% and 10%, respectively, but in UV-A treated plants there was no significant increase (3%) in comparison with the control (Figure 6). Total ascorbate content was significantly increased in UV-A, UV-B, and UV-C treated pepper leaves by 5%, 9%, and 14%, respectively, in comparison with the control. Total ascorbate content was significantly increased in UV-A, UV-B, and UV-C treated pepper roots by 3%, 8%, and 9%, respectively, in comparison with the control (Figure 6).

Discussion

Oxidative stress in plants is mitigated by the activation of antioxidant defences, including antioxidant enzymes such as peroxidase, polyphenol oxidase, catalase (CAT), ascorbate peroxidase (AP), and glutathione reductase (GR). Oxidative stress is accompanied by the synthesis of hydrogen peroxide, which is normally detoxified by CAT activity in the peroxisomes and by AP in the cytosol, mitochondria, and chloroplasts (Foyer et al., 1997; Asada, 1999). In the present study, catalase activity was increased by UV-B and UV-C treatment. Peroxidase

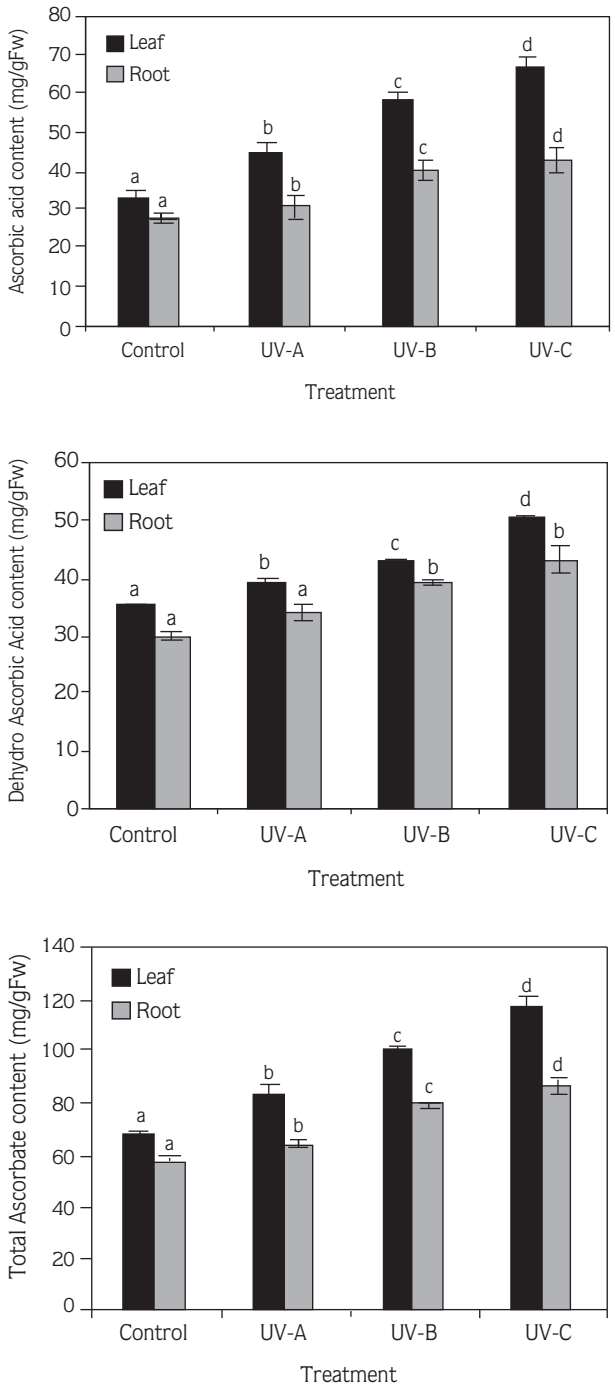

Figure 6. Levels of ASA, DHAS, and total ASA in leaves and roots of *C. annuum* L. treated with supplementary UV-A, UV-B, and UV-C. Values, significant at $P < 0.05$ according to Duncan's test, are the means of 4 replicates, and vertical bars indicate standard error of mean for leaf, root, and UV radiation. The comparison between the mean for leaf and root content of the ascorbates is not shown.

activity is also an important component of the antioxidant stress system for scavenging H_2O_2. However, catalase changes H_2O_2 into O_2, whereas peroxidase decomposes H_2O_2 by oxidation of co-substances (Gaspar et al., 1991). Further, peroxidase has been reported to promote the utilisation of phenolic compounds as co-substrates (Otter & Polle, 1994). Gaspar et al. (1985) stated that increased basic peroxidase activity in response to stress decreases the indole acetic acid concentration and promotes acidic peroxidase synthesis.

The increased trend in polyphenol oxidase activity in UV-B and UV-C treated plants was observed in our studies. Activation of antioxidant enzymes by UV-B has earlier been observed in *Arabidopsis thaliana* (Rao et al., 1996), wheat (Sharma et al., 1998), and cucumber (Takeuchi et al., 1996). Ascorbate peroxidase activity was also increased in UV-B and UV-C treated plants after 14 days of treatment. Varying responses in antioxidants under UV-B exposure have been reported, depending on species and intensity of radiation (Rao et al., 1996; Dai et al., 1997). 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). Enzymes such as peroxidase, polyphenol oxidase, ascorbate peroxidase, catalase, and glutathione reductase showed enhanced activity in UV-B and UV-C treated plants and these enzymes might serve as acclimatisation mechanisms to scavenge the toxic free radicals of oxygen produced under stress condition. The results of the present work illustrate that in *Capsicum annuum*, UV-B and UV-C radiation generates antioxidant substances that provide protection against UV-B and UV-C radiation.

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.

In the present study, a marked increase in ASA, DHAS, and total ASA in UV-B and UV-C treatment (Figure 6) represents adaptive responses against oxidative damage induced by UV radiation. An increase in both ASA and DHAS that leads to an increase in total ascorbate showed that the ascorbate-glutathione cycle in this condition is active and has a key role in the detoxification of AOS. In the ascorbate-glutathion cycle, APX catalysis is the first step of the H_2O_2 scavenging pathway by oxidizing ASA and producing MDHAS, which can dismutate

spontaneously to ASA and DHAS or enzymatically reduced to ASA by GSH-dependent DHAR (Rao et al., 1996). Ascorbic acid is postulated to maintain the stability of plant cell membranes against oxidant damage by scavenging cytotoxic free radicals (Halliwell, 1982).

Therefore it could be concluded that in *Capsicum annuum* L. plants UV radiation induced both enzymatic and nonenzymatic activities that protect plants against UV radiation in a given dose.

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