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Arch. Biol. Sci., Belgrade, 65 (4), 1423-1433, 2013 DOI:10.2298/ABS1304423I

CUMULATIVE ABIOTIC STRESSES AND THEIR EFFECT ON THE ANTIOXIDANT DEFENSE SYSTEM IN TWO SPECIES OF WHEAT, *TRITICUM DURUM* DESF AND *TRITICUM AESTIVUM* L.

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Abstract – The combined effects of heat and UV-B on the antioxidant system and photosynthetic pigments were investigated in the leaves of *T. durum* Desf. and *Triticum aestivum* L. The photosynthetic pigment content, *in vitro* evaluation of the antioxidant system activities including DPPH radical scavenging activity, and super oxide anion radical scavenging activity were determined. In addition, the antioxidant enzyme activities, such as superoxide dismutase (SOD) and guaiacol peroxidase (GPX), were determined. Heat and UV-B irradiation alone caused a significant decrease in the photosynthetic pigment content, radical scavenging activity and super oxide radical scavenging activity in the two studied plants. The antioxidant enzymes SOD and GPX were stimulated in response to UV and/or heat stresses. The elevation of enzyme activities was higher under heat than under UV-B, especially in *T. aestivum*. According to our findings, it can be concluded that combined heat and UV-B provided cross-tolerance; otherwise, single stress was found to aggravate the responses.

Key words: Cumulative stresses, UV-B, heat, wheat, antioxidant activities

INTRODUCTION

Abiotic stresses such as heat, cold, drought, salinity, nutrient deficiency, ozone, heavy metals, UV-B radiation, visible light, chemical toxicity and oxidative stress are serious threats to agriculture. Stress-induced reactive oxygen species (ROS) accumulation is counteracted by enzymatic antioxidant systems that include a variety of enzymatic scavengers, such as superoxide dismutase (SOD), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and catalase (CAT), and non-enzymatic low molecular metabolites, such as ascorbic acid (ASH), glutathione (GSH), α-tocopherol, carotenoids and flavonoids (Mittler et al., 2004 and Gratao et al., 2005). Plant stress tolerance may therefore be improved by the enhancement of *in vivo* levels of antioxidant enzymes (Chen and Dickman, 2005). The above-mentioned antioxidants found in almost all cellular compartments demonstrate the importance of ROS detoxification for cellular survival (Gratao et al., 2005). Recently, it has also been shown that ROS influence the expression of a number of genes and signal transduction pathways, which suggests that cells have evolved strategies to use ROS as biological stimuli and signals that activate and control various genetic stress-response programs (Albert et al., 2010)

The equilibrium between the production and the scavenging of ROS may be perturbed by various biotic and abiotic stress factors such as drought, UV radiation, salinity, heavy metals, temperature extremes, nutrient deficiency, air pollution, herbicides and pathogen attacks. These disturbances in equilibrium lead to a sudden increase in the intracellular

levels of ROS, which can cause significant damage to cell structures (Gill and Tuteja, 2010).

Heat stress affects the grain yield and quality of wheat in many countries through affecting sink strength and source capacity. Wheat genotypes express a differential response to chronic heat as well as heat shock (Yang et al., 2002). Responses to UV-B stress vary among the higher plant species and their effects on many metabolic processes can be very deleterious due to its high energy (Hideg et al., 2006). It can affect DNA, proteins, and the photosynthetic machinery in plants (Surabhi et al., 2009; Albert et al., 2010) and at the same time, activates defense mechanisms such as the upregulation of UV-B-absorbing compounds such as phenolics and flavonoids biosynthesis (Kakani et al., 2003; Jansen et al., 2004) and antioxidant enzymes (Ren et al., 2007; Cakırlar et al., 2011).

The aim of the present work was to investigate the interactive effects of exposure to heat and/or UV-B irradiation on the photosynthetic pigments and the activity of antioxidant enzymes in two species of wheat plants. In addition, *in vitro* evaluation of antioxidant activities including DPPH radical scavenging and superoxide anion radical scavenging activities, were measured.

MATERIALS AND METHODS

Materials and experimental design

Two species of wheat (*Triticum durum* Desf. and *Triticum aestivum* L.) from the many species used in the breeding program of the Agriculture Research Center; Giza, Egypt, were screened for their germination response to soil moisture level and were selected for the present study.

Seeds were soaked in continuously aerated distilled water for 24 h in darkness. At the end of the soaking period, twelve seeds were sown in the greenhouse in plastic pots (15 cm diameter x 20 cm height). The pots were furnished with a hole at the bottom and this was covered with filter paper before filling with sand. Each pot contained 1500 g of sterilized sandy soil (70%) and 30% vermiculite for fifteen days under a16/8 hr day/night cycle (40 pots per species). Light intensity was 420 μmol $m^{-2}s^{-1}$ at the canopy of the plant supplied by a mixture of fluorescent and incandescent lamps at a controlled temperature of 27/23˚C and 60/65 RH. The pots were irrigated to about 90% of the water-holding capacity (WHC) with distilled water. Fifteen days after sowing, the pots were irrigated with half strength Hoagland solution for up to twenty day and then the pots for each species were divided into two sets. In the first set, half the pots were subjected to heat stress (45°C 2 h/day) and the other half as control for three days. In the second set, heat and control pots were placed in a UV-B exposure cabinet inside the greenhouse. UV-B radiation was supplied by three UV-emitting tubes (TL12/100W/01, Philips, Holland) positioned 50 cm above leaf level for 2 h daily. Rack height, lamp spacing and lamp power were adjusted to maintain a total daily flux of biologically effective UV-B radiation, normalized to 1.6 Wm^{-2} d^{-1} (Caldwell, 1971). This relatively high level of UV-B, which corresponds to the maximum mid-summer and clear sky irradiance that could be anticipated, was selected for comparison with other studies on wheat rather than to attempt a realistic simulation of field conditions. Control plants were illuminated by lamps wrapped with polyester film (Folanrm 0.1mm, Floex, Munich, Germany) which blocks both UV-B and UV-C. UV-B radiation was routinely measured with a broadband UV-B sensor (peak wavelength: 313nm; Delta-T Devices Ltd., UK). Plants were rotated under the lamp banks every day in an attempt to minimize potential effects resulting from microenvironment variation. All measurements and chemical analyses were made on the second leaf. The UV-B dosages were performed daily on the heat and control plants.

Photosynthetic pigments

Photosynthetic pigments, i.e. chlorophyll *a*, chlorophyll *b* and carotenoids, were extracted with N,N-dimethylformamide (DMF) and determined according to Inskeep and Bloom (1985). Formula and extinction coefficients used for determination of

photosynthetic pigments were calculated according to Inskeep and Bloom (1985).

In vitro evaluation of antioxidant activities; Superoxide anion radical scavenging activity

Superoxide anion scavenging activity was determined as described by Beauchamp and Fridovich (1971) with some modifications. All solutions were prepared in 0.2 M phosphate buffer (pH 7.4). A sample of wheat contained in 50 or 100 µg/mL was mixed with 3 mL of reaction buffer solution (pH 7.4) containing 1.3 µM riboflavin, 0.02 M methionine and 5.1 µM nitroblue tetrazolium. The reaction solution was exposed to two 30 W fluorescent lamps for 20 min and the absorbance was then measured at 560 nm. The chemical antioxidant butylated hydroxytoluene (BHT) was used as standard. The reaction mixture without any sample was used as control. The superoxide anion radical scavenging activity (%) was calculated as (1-absorbance of sample/absorbance of control) ×100.

Radical scavenging activity (reducing potential for DPPH)

Scavenging activity was determined for 1,1-diphenyl-2-picrylhydrazyl (DPPH) and the effect of methanolic extracts on DPPH degradation was estimated according to Hanato et al. (1988). A methanolic solution of sample (0.1 mL) was mixed with 1 mL of 0.2 Mm DPPH dissolved in methanol. The reaction mixture was incubated for 20 min at 28 °C in the dark. The control contained all the reagents without the sample and was used as blank. The DPPH radical scavenging activity was determined by measuring the absorbance at 517 nm using a spectrophotometer. The DPPH radical scavenging activity (%) of the sample was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{AB - AA}{AB} \times 100$$

where AB is the absorption of the blank sample and AA is the absorption of the tested extract solution. The antiradical activity was expressed as IC_{50} (mg ml^{-1}), the antiradical dose required to cause a 50% inhibition (three replicates per treatment). A lower IC_{50} value corresponds to a higher antioxidant activity of plant extract (Patro et al., 2005).

Antioxidant enzymes activity

Extraction of enzymes

Fresh wheat leaves (≈ 0.5 g fresh material) were ground to a fine powder at 4°C in liquid nitrogen. Frozen powder were transferred into 10 ml of ice-cold extraction buffer containing 100 mM KH_2PO_4/K_2HPO_4, pH 7.8, 5 mM ascorbate, 400 mg of insoluble polyvinylpolypyrrolidone (PVP) and 2% Triton X-100 (Schwanz et al.,1996), mixed for 1 min, and incubated on ice for 30 min. The homogenate was centrifuged at 15,000 g for 30 min at 4°C. Aliquots of 3 ml of supernatant were passed through a column filled with sephadex G-25 (PD-column-pharmacia-Germany) that had been equilibrated with elution buffer (100 mM KH_2PO_4/K_2HPO_4, pH 7.0). The elution buffer for GPX contained 1 mM guaiacol in order to maintain GPX active. The purified extracts were used immediately for the determination of SOD and GPX activities.

Enzymes assay

Standard enzymatic assays were performed at 25°C according to the following methods. Blank rates were determined in the absence of either substrate or enzymatic extracts.

Superoxide dismutase (SOD, EC 1.15.1.1)

Superoxide dismutase (SOD) activity (EC 1.15.1.1) was estimated according to Beauchamp and Fridovich (1971). Leaves (200 mg) were homogenized with 4 mL (w/v) of 50 mM Na-phosphate buffer, pH 7.0, containing 1% (w/v) polyvinylpolypyrrolidone, 1 mM EDTA-Na and 0.5 M (w/v) NaCl. The reaction mixture contained 50 mM Na-phosphate buffer, pH 7.8, 0.1 mM (w/v) EDTA-Na, 13 mM (w/v) methionine, 25 µM (w/v) nitro blue tetrazolium (NBT), 2.4

Table 1 The effects of UV-B irradiation and/or heat on the photosynthetic pigment (Chl a= Chlorophyll a; Chl b= Chlorophyll b and Car. = Carotenoids) content in the second leaves of *Triticum durum* and *Triticum aestivum* during 3 days of treatments (2h/day). Means ± SE, n = 3.

Third day			Second day			First day			Treatment	Species
mg g^{-1} FW			mg g^{-1} FW			mg g^{-1} FW				
Car.	Chl b	Chl a	Car.	Chl b	Chl a	Car.	Chl b	Chl a		
0.39 ±0.02	0.49 ±0.04	1.55 ±0.1	0.89 ±0.05	0.99 ±0.06	2.05 ±0.06	1.39 ±0.02	1.49 ±0.04	2.55 ±0.08	**C**	***Triticum durum***
0.38 ±0.04	0.47 ±0.03	1.20 ±0.04	0.88 ±0.04	0.97 ±0.06	1.71 ±0.12	1.38 ±0.03	1.47 ±0.04	2.20 ±0.06	**UV**	
0.28 ±0.03	0.37 ±0.02	1.10 ±0.04	0.78 ±0.03	0.87 ±0.05	1.60 ±0.08	1.28 ±0.03	1.37 ±0.03	2.10 ±0.05	**H**	
0.31 ±0.01	0.36 ±0.02	0.99 ±0.01	0.80 ±0.04	0.85 ±0.05	1.51 ±0.08	1.30 ±0.01	1.36 ±0.02	2.00 ±0.05	**H+UV**	
0.34 ±0.03	0.43 ±0.03	1.27 ±0.15	0.84 ±0.05	0.93 ±0.06	1.77 ±0.17	1.34 ±0.03	1.43 ±0.03	2.27 ±0.07	**C**	***Triticum aestivum***
0.40 ±0.02	0.42 ±0.02	1.10 ±0.06	0.90 ±0.06	0.91 ±0.06	1.61 ±0.05	1.42 ±0.02	1.42 ±0.02	2.10 ±0.05	**UV**	
0.27 ±0.01	0.32 ±0.02	0.96 ±0.04	0.77 ±0.03	0.82 ±0.05	1.42 ±0.07	1.32 ±0.02	1.32 ±0.02	1.95 ±0.04	**H**	
0.31 ±0.02	0.33 ±0.03	0.91 ±0.06	0.81 ±0.04	0.83 ±0.05	1.39 ±0.04	1.33 ±0.02	1.33 ±0.03	1.90 ±0.03	**H+UV**	

μM (w/v) riboflavin and 0.03 mL enzyme extract. The addition of riboflavin and the placement of tubes under fluorescent lamps providing an irradiation intensity of 185 $\mu E \cdot m^{-2} \cdot s^{-1}$ started the reaction of blue formazan accumulation. The increase in optical density was almost linear for at least 10 min. Tubes without the enzyme developed maximum color. Absorbance at 560 nm was recorded. One unit of activity was estimated as the enzyme quantity that reduced absorbance to 50% of the value compared to tubes without enzyme.

Guaiacol peroxidase (GPX; E.C. 1.11.1.7)

Measurement was based on the method of Hammerschmidt et al. (1982). Leaves were homogenized in ice-cold 50 mM K-phosphate buffer, pH 7.5, with the addition of 5% (w/v) polyvinylpolypyrrolidone (PVPP). Enzyme assays were prepared by the addition to a glass cuvette of 0.5 mL 50 mM K-phosphate buffer, pH 7.5, 0.5 mL extract, 0.5 mL 3.4 mM guaiacol and 0.5 mL 0.9 mM H_2O_2. Absorbance at 480 nm was measured and guaiacol oxidation was expressed as units per minute per mg protein. One unit of enzyme activity caused an increase of absorbance by 0.1 per min.

RESULTS

Regarding the second leaf, heat-induced changes in pigment content were accompanied by no change in leaf color that could be visually assessed. Under normal conditions, the second leaf of *T. aestivum* was richer in photosynthetic pigments than that of *T. durum*. After UV-B treatment and at the end of experimental period, it was found that photosynthetic pigments including Chl a, Chl b and total carotenoids were about 77.5%, 96% and 97%, respectively,

Table 2. The effects of UV-B irradiation and/or heat on the total chlorophyll pigment (Chl a+b= Chlorophyll a+ Chl b= Chlorophyll b), Chl a/b ratio and carotenoids/total chlorophyll ratio in the second leaves of *Triticum durum* and *Triticum aestivum* during 3 days of treatments (2h/day). Means ± SE, n = 3.

Third day			Second day			First day			Treatment	Species
mg g^{-1} FW			mg g^{-1} FW			**mg g^{-1} FW**				
Car./ Chl	**Chl a/b**	**Chl a+b**	**Car./ Chl**	**Chl a/b**	**Chl a+b**	**Car./ Chl**	**Chl a/b**	**Chl a+b**		
0.19± 0.03	3.16± 0.03	2.04± 0.09	0.29± 0.02	2.10± 0.05	3.04± 0.03	0.34± 0.03	1.71± 0.04	4.04± 0.05	**C**	***Triticum durum***
0.23± 0.04	2.55± 0.04	1.67± 0.09	0.33± 0.03	1.76± 0.08	2.68± 0.02	0.38±0.03	1.50± 0.05	3.67± 0.04	**UV**	
0.19± 0.03	2.97± 0.03	1.47± 0.06	0.32± 0.03	1.84± 0.08	2.47± 0.02	0.37±0.04	1.53± 0.05	3.47± 0.09	**H**	
0.23± 0.05	2.75± 0.02	1.35± 0.03	0.34± 0.03	1.78± 0.08	2.36± 0.01	0.39±0.03	1.47± 0.05	3.36± 0.03	**H+UV**	
0.20± 0.04	2.95± 0.03	1.70± 0.04	0.31± 0.02	1.90± 0.07	2.70± 0.03	0.36±0.03	1.59± 0.06	3.70± 0.04	**C**	***Triticum aestivum***
0.26± 0.05	2.62± 0.04	1.52± 0.07	0.36± 0.04	1.77± 0.07	2.52± 0.02	0.40±0.04	1.48± 0.03	3.52± 0.06	**UV**	
0.21± 0.04	3.00± 0.03	1.28± 0.03	0.34± 0.03	1.73± 0.06	2.24± 0.02	0.39±0.03	1.48± 0.04	3.27± 0.04	**H**	
0.25± 0.05	2.76± 0.02	1.24± 0.06	0.36± 0.03	1.67± 0.06	2.22± 0.01	0.41±0.05	1.43± 0.03	3.23± 0.03	**H+UV**	

less than the control leaves in *Triticum durum*. The corresponding values for *Triticum aestivum* were 87%, 98% and 118% (Table1). In the UV-B treated leaves (second leaves) for the two species, the Chl a/b ratio was definitely greater than that of the untreated plants. Moreover, the carotenoids/total pigment was higher relative to the control leaf (Table 2). In *Triticum durum*, UV and heat treatment were able to modify significantly the Chl a (<0.05), Chl b (<0.05) and total carotenoid (<0.05) contents in the second leaf (Tables 1 and 2). Conversely, exposure to cumulative stresses (heat +UV-B) consistently increased total chlorophylls, as well as, carotenoids in comparison with plants exposed to a single stress (Table 1). Indeed, after UV-B treatment, changes, especially in *Triticum durum*, in total chlorophylls (-18%), Chl a (-13%), Chl b (-4 %) and total carotenoids (+18%), were moderate relative to the control at the end of experimental period.

This effect of cumulative stresses on pigment accumulation affected both the Chl a/b ratio and the carotenoids/total pigment ratio. As already observed in the second leaf of our studied plants, Chl a was reduced more than Chl b, and the extent of the increment in carotenoid content was close to that registered for Chl a. The preferential degradation of Chl a relative to Chl b, was underlined by the significant fall in the Chl a/b ratio. In the same way as for the second leaf, the carotenoids/total pigment ratio determined throughout the experimental period appeared to be sensitive to the deleterious effects of stress.

The effect of UV-B and/or heat treatment on super oxide anion radical scavenging activity indicated variations between treatments and species (Figs. 2A and B). The exposure of wheat leaves to UV-B irradiation and heat treatment alone resulted in a more pronounced increase in super oxide anion radical scavenging in *T. aestivum* than in *T. durum* and the control. Prolonged exposure of the wheat plants to a single stress (heat or UV-B) resulted in a significant reduction in the rate of subsequent super oxide anion radical scavenging activity, especially in the *T.*

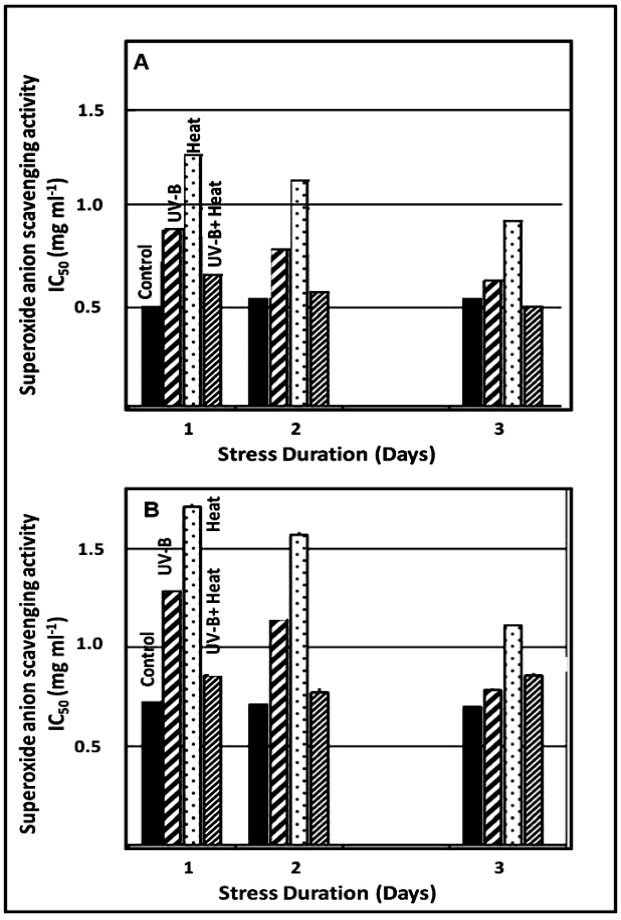

Fig. 1: The effects of UV-B irradiation and/or heat on superoxide anion scavenging activity (IC_{50}) in the second leaves of *Triticum durum*(A) and *Triticum aestivum* (B) during 3 days of treatment (2h/day). Means ± SE, n = 3.

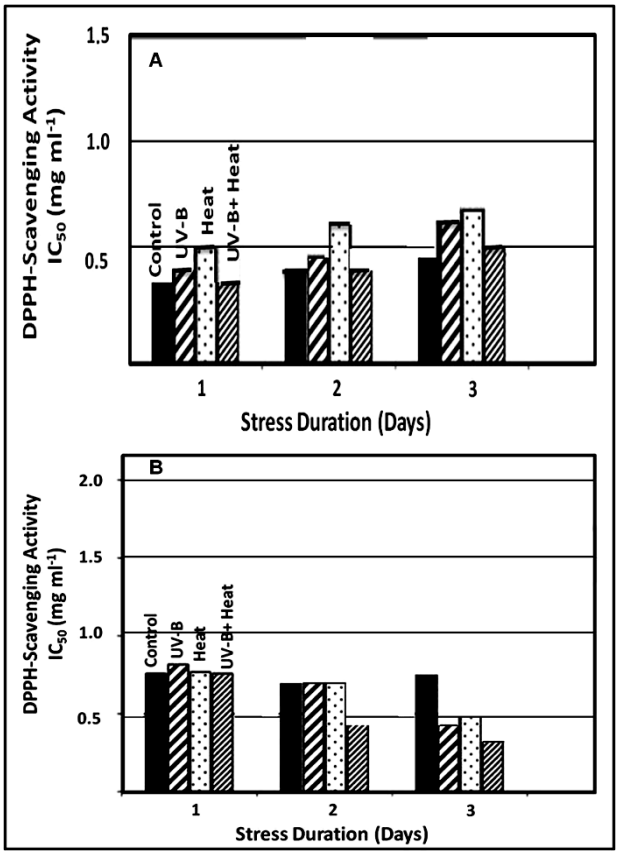

Fig. 2: The effects of UV-B irradiation and/or heat on the DPPH-scavenging activity (IC_{50}) in the second leaves of *Triticum durum* (A) and *Triticum aestivum* (B) during 3 days of treatment (2 h/day). Means ± SE, n = 3.

durum plants in comparison with the first and second days of treatment (Figs. 2 A and B). Cumulative stresses (UV-B+ heat) resulted in a steady state of increment in the superoxide anion radical scavenging capacity in *T. aestivum* throughout the experimental period, whereas in *T. durum* the scavenging capacity decreased parallel to the exposure time.

UV-B irradiation significantly increased the radical scavenging activity (lowering the IC_{50}) in *T. aestivum* (Fig. 1B). A similar pattern was observed in response to the heat treatment. UV-B administered along with treatment with high temperature caused a highly significant increase in the radical scavenging activity in *T. aestivum* more than in *T. durum* (Figs. 1A & B). On the other hand, all treatments caused a marked decrease in the radical scavenging activity (higher IC_{50}) in *T. durum*, especially at the end of experimental period. UV-B and heat alone were less effective for radical scavenging activity, especially in *T. aestivum*, than cumulative stress (UV-B+heat). By the end of experimental period, a marked decline in radical scavenging activity was observed after heat treatment and UV-B treatment alone. On the other side, the cumulative stress effect of UV-B irradiation and heat showed a highly significant increment in DPPH radical scavenging activity in both cultivars throughout the experimental time in comparison

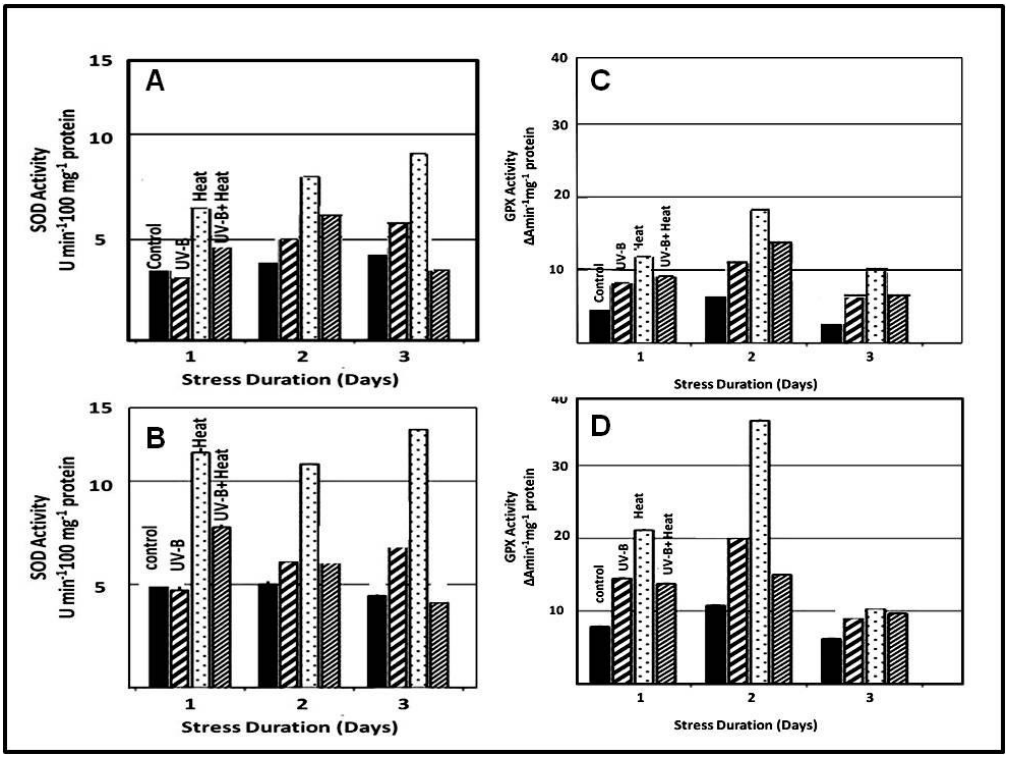

Fig. 3: The effects of UV-B irradiation and /or heat on SOD (A and B) and GPX (C and D) in the second leaves of *Triticum durum* (A & C) and *Triticum aestivum* (B&D) during 3 days of treatments (2h/day). Means ± SE, n = 3.

with the control plants (Fig. 2). *T. durum* showed greater stress response than *T. aestivum*, i.e., the DPPH activity in *T. aestivum* were 18 % and 40 % greater than in *T. durum* after 3 days of UV-B irradiation and high temperature treatment (Fig 2B).

Exposure to UV-B irradiation alone or after treatment with high temperature significantly increased the activity of SOD and GPX and the level of UV-B marker in comparison to the controls in the studied wheat species (Figs. 4A-4D). The stress marker responses of *Triticum* species to high temperature showed differences. High temperature exposure significantly increased SOD activity. GPX activity was observed from the first day of treatment and continued throughout the experimental period, especially in *T. aestivum*, but the increase was lower in *T. durum* in comparison with that in *T. aestivum* exposed only to UV-B treatments (Fig. 4A-D). High temperature significantly increased the activity of SOD from the beginning of treatment. A stress-induced alteration in the antioxidant enzymes was observed (Fig. 2): the highest (triple) increase was observed in response to heat treatment in SOD and GPX activity doubled versus the control. SOD activity (Figs. 4 A and B) greatly increased under heat stress throughout the experimental period. UV-B radiation did not cause such a significant increase on day 1. After exposure to combined stress, SOD activation was observed earlier, on day 1. In control plants, its activity was stable throughout the experiment. The highest increase in GPX activity (Figs. 4 C and D) was recorded on day 2. In plants under heat stress it reached the highest level; on that day it was intermediate under UV-B radiation and low under heat and UV-B co-treatment.

DISCUSSION

The exposure of two wheat plants to UV-B and/or heat stress resulted in a marked decrease in the pho-

tosynthetic pigment contents in our studied plants (Tables 1 and 2). It has been reported that photosynthetic pigments seem to be altered after UV-B irradiation (Agrawal and Rathore, 2007). The significant reduction in chlorophyll content as a result of heat and UV-B treatment has been ascribed to the inhibition of its biosynthesis or to the degradation of pigments and their precursors (Agrawal and Rathore, 2007). Strid and Porra (1992) suggested that the decreased photostability of chlorophyll is a direct result of UV-B and the downregulation of the gene responsible for chl *a*/*b*-binding proteins, thereby inhibiting chlorophyll biosynthesis. The decrease in chlorophyll content was also found in other recent studies (Joshi et al., 2007; Ibanez et al., 2008; Singh et al., 2008). In addition, a significant decrease in carotenoids was also determined and varies greatly after exposure to UV-B and/or heat in this study (Table 1 and 2). Carotenoids protect chlorophyll from photooxidative destruction, so a change in carotenoids could have serious consequences for the effect of UV-B radiation on chlorophyll pigments (Agrawal and Rathore, 2007; Mishra et al., 2008). Conversely, it was found that UV-B induced an increase in the carotenoid content of *Pisum sativum* Kalaycı-97 cultivar (Strid and Porra, 1992). Carletti et al. (2003) assumed that an imbalance in the photosynthetic pigment composition may be due to the effects of UV-B radiation on photosynthetic membranes and that changes in the composition of these photosynthetic pigments may be indicative of perturbations in the photosynthetic apparatus. Changes in the pigment contents reflect on the chl a/b and carotenoids/total pigments ratios as well (Table 2). The chl a/b ratio was detrimentally affected by UV-B irradiation. Similarly, the carotenoids/total pigments ratio decreased under UV-B irradiation (Table 1).

The low level of leaf damage in this investigation can be explained by the enhancement of both super oxide anion radical scavenging activity and the radical scavenging activity in response to UV-B and heat stresses together, which occur more rapidly than the response to heat stress or UV-B when imposed alone (Figs. 1 and 2). This probably indicates that super oxide anion radical scavenging activity may act synergistically with radical scavenging activity to stabilize macromolecules, thereby stabilizing the protoplasm. These results are supported by the observations of Larkin et al. (2003). *T. aestivum* was found to be more tolerant to UV-B as well as heat stress alone. The enhancement of super oxide anion radical scavenging activity and the radical scavenging activity in response to heat or UV-B treatment, especially in *T. aestivum*, revealed the occurrence of an oxidative stress that catalyzes the production of reactive oxygen species, consequently accumulating and resulting in several damages, indicating that the heat susceptibility of *T. durum* was linked to lower antioxidant enzyme activities. These different effects between the two species under the experimental treatments are judged by the criteria and behaviors of enzymatic and non-enzymatic antioxidants.

Heat, UV-B radiation and co-stresses induced changes in scavenging system enzymes (Fig. 4). SOD (Figs. 4A and B) and GPX (Figs. 4C and D) activity generally increased, but the stress-dependent alterations did not occur at the same time of stress treatment. Under heat stress, SOD activity increased earlier (day 1) especially in *T. aestivum*, than GPX activity (day 2). These results support the suggestion (Verdaguer, 2003) that SODs constitute the first line of defense against oxidative stress. After 3 days of stress treatment, a similar effect (increased SOD activity) was recorded in barley (Smirnoff, 1993), tomato (Bowler et al., 1992) and sorghum (Jagtap and Bhargava, 1995). Earlier work on barley found similar increases in GPX and GR activity (Kubiś, 2001, 2003). Some authors measured GPX activity and found neither an increase nor a decrease during water deficit (Smirnoff, 1993).

In this study, antioxidant activity was generally altered in heat-treated plants, but the effect was weaker than in UV-B-treated ones. Elevated SOD and GPX under excess UV-B radiation were noted in cucumber cotyledons (Kataria et al., 2007), winter wheat seedlings (Yang et al., 2007), mung bean cultivars (Agraval and Rathore, 2007) and spinach

chloroplasts (Lei et al., 2008). The oxidative stress conditions caused by different treatments vary; they all seem to be related to the overproduction of ROS, but they engage different pathways of the antioxidant system for their removal (Kubo et al., 1999). The combination of two stresses, UV-B and heat, elevated the activity of the investigated antioxidant enzymes. The ratios of enzyme activation were significantly lower than under heat or UV-B radiation separately. These results suggest that these co-stresses functioned synergistically, with one of them reducing the changes caused by the simultaneous application of the other stress. Alexieva et al. (2001) observed similar effects of co-stresses in pea and wheat seedlings. In contrast, other parameters were reduced by a combination of two stresses in comparison with single stresses in spring wheat, these being plant growth, photosynthetic capacity, pigment content, biomass and yield (Feng et al., 2007).

CONCLUSION

The increased defense mechanisms observed after the application of a combination of UV-B and heat stresses show that the two environmental stress acts synergistically to trigger protective mechanisms. Wheat plants activate several defense mechanisms, contributing to the maintenance of the structural integrity of the cell components and presumably alleviate (to some extent) the photooxidation damage.

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