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Effects of UV-B radiation on some physiological and biochemical aspects in two cultivars of barley (*Hordeum vulgare* L.)

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ABSTRACT

Barely cultivars (*Hordeum vulgare* L. cvs Giza 121 and Sahrawy) were grown under controlled conditions in growth chamber with and without UV-B radiation. After 40 days, the growth parameters such as plant length, fresh and dry weights of plants as well as leaf area had decreased in both varieties. Furthermore, plants treated with UV-B radiation exhibited reduced contents of chlorophylls, carotenoids and other photosynthetic pigments, photosynthetic rate, Rubisco N, total leaf N, total soluble protein and flavonoids concentrations with a great decrease in Giza 121 indicating that there was a difference between Giza 121, and Sahrawy in the resistance to the effect of UV-B radiation. Superoxide anion (O_2) generation rate, hydrogen peroxide (H_2O_2), malondialdehyde (MDA) contents and relative electrolyte conductivity (EC) and activity of some antioxidant enzymes (SOD, CAT and AP) in both cultivars were also measured. Significant increase in these parameters was found indicating the drastic effect of UV-B radiation on the membrane system.

KEYWORDS: *Hordeum vulgare,* UV-B radiation, morphology, physiology

INTRODUCTION

It is well known that due to stratospheric ozone depletion, the influx of UV-B radiation (280-315 nm) to the surface of the Earth has increased significantly (Hader *et al.* 1995 and Banerjee & Hader 1996). However, the increase in UV-B level adversely affects a number of physiological and metabolic processes in higher plants such as reduction in plant height, leaf area, increased respiration, decrease in the photosynthetic capacity, stomatal closure(Dai *et al.*1992), DNA and protein damage in the living cells (Vincent & Roy 1993) and other biological systems. Furthermore, it has been reported that the supplementation of visible radiation with UV-B radiation causes a reduction in amounts of Chl and carotenoid contents, soluble protein and Rubisco in leaves of C_3 plants. (Strid *et al.* 1990).

Furthermore, when plants are under stress, the production of active oxygen can increase and the defence mechanisms must be activated to improve the active oxygen scavenging capacity of the plants. In these defence processes, both enzyme and nonenzyme mechanisms are involved. Enzymatic defence includes superoxide dismutase, catalase and peroxidase. It is thought that UV-B causes oxidative stress in plants although the mechanism of generation of active oxygen species in UV-B irradiated plants is not known (Strid 1993; Rao *et al.*1996). Several studies on the animal and plant cells showed that UV-B radiation altered the structure of membranes due to lipid peroxidation (Kramer *et al.* 1991) as shown by increases in malondialdehyde (MDA) concentration. Also oxidation of membrane-bound proteins, may occur after irradiation with UV-B radiation.

In order to understand the causes of such physiological shifts, further investigations at the cell structure level are needed. Therefore, the present study assessed some physiological and biochemical responses of *Hordeum vulgare* L.cvs Giza 121 and Sahrawy plants to enhance level of UV-B radiation. The author investigated : 1) the growth, photosynthetic rate, transpiration, stomatal resistance, and the amounts of total leaf nitrogen, Chl and

carotenoids contents, soluble protein, and ribulose biphosphate (Rubisco). 2) determine whether or not the oxidative stress represented by $O_2^{\bullet-}$ generation rate, H_2O_2 and MDA content as well as electric conductivity (EC) was induced by UV-B radiation. (3) evaluate the response of oxidative enzymes (SOD, CAT and AP) as defense system to UV-B radiation and finally to confirm the difference between the two varieties in the sensitivity to damage by UV-B radiation.

MATERIALS AND METHODS

Plant material and culture conditions: Seeds of barley (*Hordeum vulgare* L.) cvs Giza 121 and Sahrawy were germinated at 30°C for 48 h and then sown in 1-1 plastic pots containing a vermiculite – quartz sand mixture (2:1, v/v) to which a nutrient solution (1/5 strength Hoagland nutrient solution) was applied. All pots were maintained in a growth chamber (2m × 1.0m × 1.5m) under 30:20°C day/night temperature, 60-70% relative humidity and a photoperiod of 14 h of 800 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR) for 7 days, then plants from each variety were divided into two sets, one used as control and receiving visible radiation (PAR) and the other set was subjected to visible radiations plus UV-B radiation for 40 days.

UV-B setup and measurement: Supplemental UV-B radiation was provided by UV-B emitting fluorescent lamps (Q Panel Icn., Cleveland, Ohio) following the method of Ambasht & Agrawal (1997). Each lamp was covered with 0.13 mm thick cellulose diacetate (CA) film, which absorbed radiation emitted by lamps below 290 nm. For the controls, lamps were covered with 0.13 mm thick polyester film (Cadillac Plastics, Baltimore, Md.), which absorbed radiation emitted by lamps below 320 nm The CA film was replaced weekly and Mylar-D film replaced biweekly. Plants were artificially irradiated for 5 h per day in the middle of the photoperiod (10:00- 21:00) from the date of sowing until maturity. UV lamps were kept 40 cm above the canopy throughout the experiment. Leaf samples for all analyses were collected from the fully expanded third leaf below the tip of the stem from both control and treated plants.

Measurement of photosynthetic and, transpiration rates and stomatal resistance: Photosynthesis and transpiration rates and stomatal resistance were measured with a LI-6200 Portable photosynthesis system (Li-Cor-Lincoln, NE, USA)

Determianation of chlorophyll content, carotenoids and Flavonoids concentrations: Total chlorophylls and carotenoids were extracted from leaf discs with 80% acetone and determined according to Jensen (1978). Estimation of flavonoid level was done by using the methods of Flint & Caldwell (1984).

Determination of Rubisco. N, soluble protein N and total N: Determinations of amounts of Rubisco N, soluble protein N and total leaf nitrogen (N) were done according to the method of Hidema *et al.* (1996).

Determination of superoxide generation rate and hydrogen peroxide concentration: Superoxide ($O_2^{\bullet-}$) generation rate was estimated based on the method of Schneider and Schlegel (1981). The $O_2^{\bullet-}$ concentration, was calculated by the methods of Bors *et al.* (1982). Hydrogen peroxide (H_2O_2) was extracted from leaves as described by Patterson *et al.* (1984). Standardization of H_2O_2 was performed to minimize the interference of catalase (Patterson *et al.* 1984).

Lipid peroxidation determination: Lipid peroxidation represented by the amount of malondialdehyde (MDA) concentration was estimated by the method of Heath & Packer (1968). Concentration was calculated using the extinction coefficient, 155 mM^{-1} cm^{-1}.

Membrane permeability measurement: Membrane permeability of a leaf was measured by

electrolyte conductivity (EC) according to Yan *et al.* (1996). The percentage of electrolyte leakage was calculated as follows,

$EC=\frac{C_1}{C_2}\times 100(\%)$, where C_1 & C_2 are the electrolyte conductivities measured before and after boiling, respectively.

Enzyme activity assays: Fresh leaves (0.5 g) were homogenized under ice-cold conditions in 8 ml of 50mM phosphate buffer (pH 7.0), 10 mM ascorbic acid (ASA) and 1.0% (w/v) polyvinylpyrrolidone. The homogenate was centrifuged at 15000 g for 20 min. The supernatant was used for enzyme assays. Superoxide dismutase (SOD, EC 1.15.1.1) was assayed by the method of Stewart & Bewley (1980). One unit of SOD activity was defined as the amount of enzyme which caused 50% inhibition of the initial rate of reaction in the absence of enzyme (SOD). Catalase (CAT, EC 1.11.1.6) activity was assayed as the decrease in H_2O_2 in the reaction mixture containing 75 mM sodium phosphate (pH 7.0), 45 mM H_2O_2, and 0.05 ml enzyme extract. The absorption decrease at 240 nm during 1 min was recorded (Chance and Maehly 1995). Ascorbate peroxidase (AP, EC 1.11.1.7) measurement was based on the method of Nakano & Asada (1981). Change in absorption at 290 nm was recorded 1 min after addition of H_2O_2. In all enzyme analyses, protein concentration was measured by Bradford method (1976). All spectrophotometric analyses were conducted on a 360 UV/visible light spectrophotometer (Shimadzu, Kyoto, Japan).

Statistical analysis: The experimental layout was a randomized block design. All values are means of 3-6 replicates per treatment. Results were subjected to *t* – test for comparison between two means.

RESULTS

Growth, photosynthetic and transpiration rates and stomatal resistance: In comparison with the control, it was found that UV-B radiation significantly affected the growth parameters of the above ground parts of Sahrawy and Giza 121 plants such as plant length (PL), tiller number (TN), leaf area (LA), fresh and dry weight of plants (FW; DW). Fig. 1 shows a remarkable reduction in all of these parameters as percentages of the control values, but the degree of reduction was significantly greater in Giza 121 than that in sahrawy. In this connection, the rate of photosynthesis was reduced significantly and amounted to 13 and 26 % respectively in Sahrawy and Giza 121 plants grown with supplemental UV-B radiation (Table 1). Transpiration rate was also declined whereas stomatal resistance increased significantly in response to UV-B radiation relative to controls particularly (Table 1).

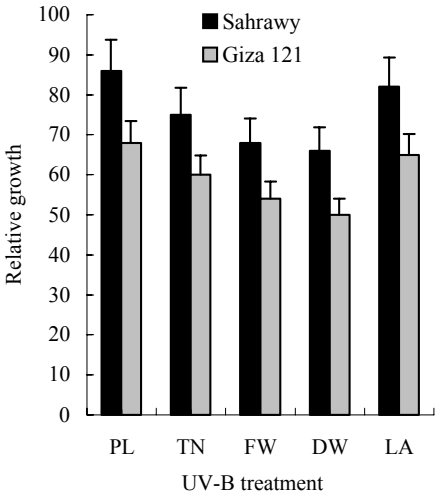

Fig.1. Effect of UV-B radiation on plant length (PL), tiller number (TN), fresh weight (FW), dry weight (DW) and leaf area (LA) of the above ground parts for Giza 121 and sahrawy plants. Relative values (%) are the ratios of the values for the growth para

Plants	Treatment	Photosynthesis (μmol CO_2 $m^{-2}S^{-1}$)	Transpiration (mol H_2O $m^{-2}S^{-1}$)	Stomatal resistance (S cm^{-1})
Giza 121	Control	26.86 ± 1.12 a	0.024 ± 0.001 a	0.38 ± 0.002 a
	UV-B	19.88 ± 0.12 b	0.016 ± 0.004 b	0.63 ± 0.004 b
Sahrawy	Contol	29.86 ± 1.22 a	0.034 ± 0.008 a	0.72 ± 0.008 a
	UV-B	25.98 ± 1.81 a,b	0.031 ± 0.003 b	0.88 ± 0.006 b

Table 1. Effect of UV-B radiation on photosynthesis, transpiration and stomatal resistance in Sahrawy and Giza 121 plants. Values are mean ± SE (n=5).Values for treated plants followed by the same letters as control are not significantly different at $p < 0.05$.

Chlorophyll and carotenoids concentrations: Total chlorophyll content of control plants tended to be higher than UV- B treated plants (data not shown), indicating that supplementation of UV-B radiation reduced the increases in the total Chl content, the percentage reduction being 16 and 29 % in Sahrawy and Giza 121, respectively (Table 2). Moreover, Chl *a* and Chl *b* contents were also reduced compared to controls, whereas the ratio of Chl *a* to Chl *b* was similar in both varieties grown under UV-B radiation (Table 2). A similar pattern of changes was obtained in carotenoid content.

Table 2. Effect of UV-B radiation on total Chl *a*, Chl *b*, Chl *a/b* ratio and carotenoids concentrations in Sahrawy and Giza 121 plants. Values are mean ± SE. Values for treated plants followed by the same letters as control are not significantly different at $p < 0.05$.

Plants	Treatment	Chl *a*	Chl *b*	Total Chl	Chl *a/b*	Carotenoids
Giza 121	Control	10.2 ± 0.1 a	2.8 ± 0.06a	13.0 ± 0.8a	3.64 a	1.94 ± 0.089a
	UV-B	7.2 ± 0.1 b	2.0 ± 0.04b	9.2 ± 0.9b	3.40 b	1.31 ± 0.086b
Sahrawy	Control	9.6 ± 0.1 a	2.4 ± 0.04	12.0 ± 0.8 a	4.0 a	1.72 ± 0.08 a
	UV-B	8.1 ± 0.09 b	2.0 ± 0.03	10.1 ± 0.6b	4.0 a	1.48 ± 0.05 b

Rubisco N, soluble protein N and total N: It was evident from the results given in Table 3 that supplementation of UV-B radiation reduced the total N, soluble protein and Rubisco N in the leaves of Sahrawy and Giza 121 plants. The greatest reduction in the level of each kind of nitrogen was observed in Giza 121 than in Sahrawy grown under UV-B radiation

Table 3. Effect of UV-B radiation on the partitioning of nitrogen (N) into soluble protein N and Rubisco N in the leaves of Sahrawy and Giza 121 at 40 days. Values are mean ±SE (n=5).Values for treated plants followed by the same letters as control are not significantly different at $p< 0.05$.Values in parentheses are the percentages showing the ratio of soluble protein N and Rubisco N to the total leaf N.

Plant	Treatment	Total N	Sol. Protein N Nmol N m^{-2}	Rubisco N
Giza 121	Control	179 ± 4.8 a	107.4 ± 9.0a (59.8)	67.2 ± 8.4a (37.4)
	UV-B	132 ± 3.9 b	63 ± 6.2 b (45.9)	31.4 ± 7.2 b (23.5)
Sahrawy	Control	182 ± 6.2 a	109 ± 9.6 a (59.9)	68.6 ± 5.3 a (43.2)
	UV-B	158 ± 4.9 b	80 ± 5.2 b (50.6)	53.7 ± 2.9 b (37.2)

Superoxide generation rate, hydrogen peroxide, and MDAconcentrations and EC: Under injurious UV-B stress, the rate of O_2 generation, and MDA (malondi aldehyde) and H_2O_2 concentrations as well as EC (electric condrictivity) as oxidative responses were increased significantly in both varieties compared to control as shown in Table (4).

Table 4. Effect of UV-B radiation on superoxide (O_2) generation rate, malondialdehyde (MDA) and H_2O_2 concentration, and electric conductivity (EC) of Giza 121 and Sahrawy leaves at 40 day. Values are mean ± SE (n=5). Values in parentheses are the percentages of UV-B treatment values relative to control. Means within a column followed by the same letters are not significantly different at $P < 0.05$.

Plants	Treatment	O_2^{-} Nmol (g fwt)$^{-1}$m^{-1}	MDA Nmol (g fwt)$^{-1}$	H_2O_2 nmol (gfwt)$^{-1}$ min^{-1}	EC (%) mmhos S cm^{-1}
Giza 121	Control	10.11 ± 0.8 a (100)	18.17 ± 1.1a (100)	31.4 ± 1.2 a (100)	15.22 ± 1.2 a (100)
	UV-B	14.82 ± 0.5 b (146)	26.54 ± 2.4 b (146)	37.2 ± 2.1 b (116)	23.12 ± 1.1 b (152)
Sahrawy	Contol	9.29 ± 0.9 a (100)	14.48 ± 0.6 a (100)	31.2 ± 1.1 a (100)	14.42 ± 0.8 a (100)
	UV-B	11.86 ± 0.8 b (128)	19.26 ± 0.5 b (133)	33.9 ± 0.2 b (105)	18.42 ± 0.7 b (128)

Enzyme activity: Antioxidant enzyme activities in the leaves treated with UV-B radiation are presented in Fig 2. In terms of the time course it was found that behaviour of superoxide dismutase (SOD), catalase (CAT) and ascorbic peroxidase (AP) did not show the same pattern of variation in all treated plants. SOD activity was enhanced at 20 days in both varieties, then declined only in Giza 121. At 40 days there was no difference in SOD activity between Giza 121 which had been treated with UV-B and control. By contrast, the maximum increase in the activity of SOD in Sabrawy plants was observed at 40 days, being 26% in UV-B treated plants compared with controls. A similar trend was observed in catalase activity. However, UV-B exposure caused a reduction in AP levels for treated plants, at the same time control plants maintained the same levels of AP activity during the experimental period.

Flavonoids: Flavonoids in leaves of both varieties appeared to be affected by UV-B radiation as indicated by their concentrations in treated leaves as compared with control. The levels of flavonoids were significantly greater with UV-B exposure (Table 5).

Table 5. Effect of UV-B radiation on the concentration of flavonoid in barley plants. Values are mean ± SE (n = 8). Within a sampling day, values for treated plants followed by the same letter as control are not significantly different at $P < 0.05$.

Time (days)	Giza 121		Sahrawy	
	Control	UV-B	Control	UV-B
	A_{300} mg^{-1} fresh leaf			
10	0.06 ± 0.001 a	0.094 ± 0.001 b	0.08 ± 0.002 a	0.18 ± 0.001 b
20	0.52 ± 0.002 a	0.70 ± 0.004 b	0.66 ± 0.006 a	0.84 ± 0.004 b
30	0.60 ± 0.005 a	0.86 ± 0.003 b	0.76 ± 0.003 a	1.34 ± 0.006 b
40	0.72 ± 0.004 a	0.99 ± 0.002 b	0.96 ± 0.005 a	1.52 ± 0.002 b

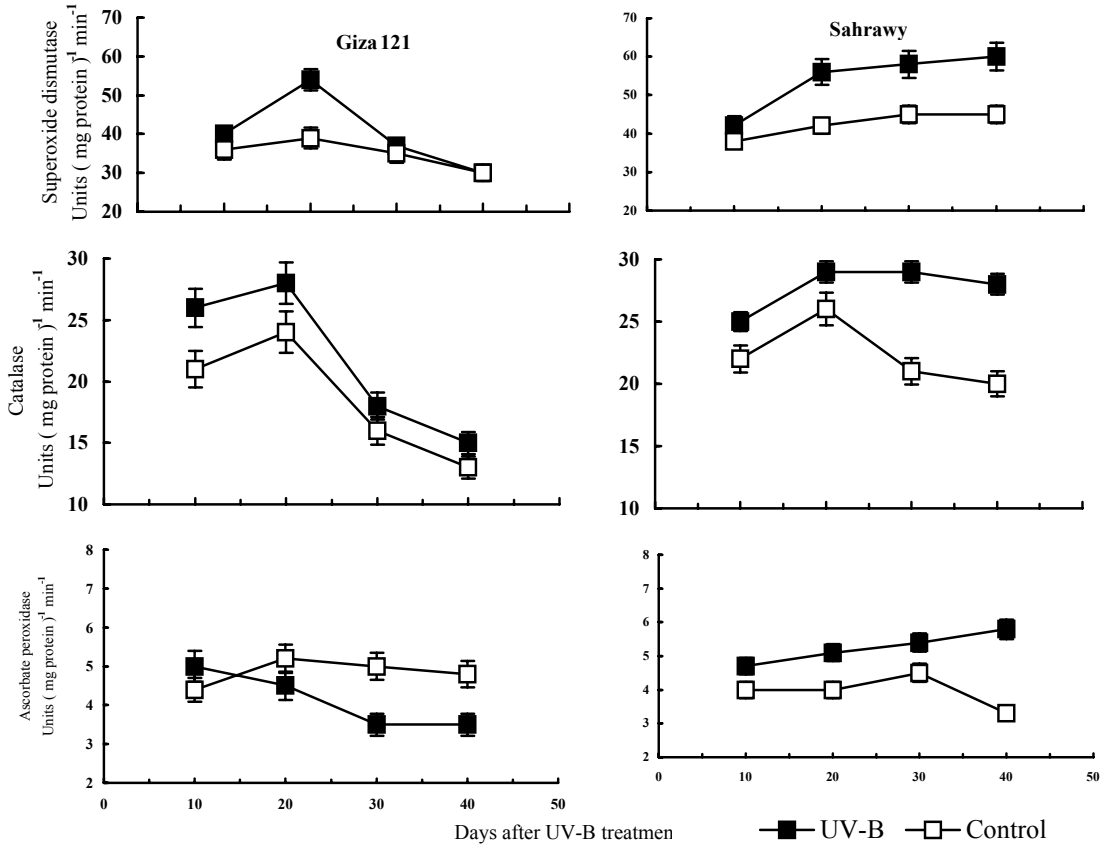

Fig.2. Effect of UV-B radiation on the activities of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (AP) in Giza 121 and sahrawy leaves. Values are mean + SE (n=5). One unit of enzyme activity is define as the absorbance change per min at the wave length of 560 nm for SOD, 240 nm for CAT and 290 nm for AP

DISCUSSION

Several studies have indicated that UV-B radiation can deleteriously affect the physiological processes and overall growth in a number of plants species (Ambash & Agrawal 1994). However, this study provides supporting evidence for the damage effects of UV-B radiation on two varieties of barley plant (*Hordeum vulgare* L. cvs Giza 121 and Sahrawy) and confirms the difference between these varieties in the sensitivity to the damage by UV-B radiation. In this study, it was found that UV-B radiation reduced some growth parameters of the above ground parts of both varieties such as plant length, tiller number, plant fresh and dry weights and leaf area (Fig. 1), as reported in other studies (Dai *et al.* 1992). Since plant growth is an integrated result of all biochemical, physiological and metabolic processes in plants, the significant reduction in Giza 121 and Sahrawy exposed to UV-B radiation strongly implies that UV-B interferes with one or more functional processes resulting in reduction of plant growth.

Enhanced UV-B radiation resulted in a significant reduction of photosynthetic activity in both varieties as shown by the gas exchange measurements. The reduction in the rate of photosynthesis was accompanied by a decrease in transpiration rate and simultaneous increase in stomatal resistance (Table 1). In this connection, Ambash & Agrwal (1998) suggested that

one reason for the reduction in photosyntheis may be due to stomatal closure under enhanced levels of UV-B. The decrease in photosynthesis could also have been the result of a corresponding decrease in the density of photosynthetic units which might have been associated with the reduction in Chl content and carotenoid concentration of UV-B exposed plants (Table 2). This finding is consistent with the results obtained by Strid *et al.* (1990).

It is often assumed that UV-B radiation may affect the photosynthetic pigments, either because of inhibition of their synthesis or increased destruction. In this connection, Strid (1993) suggested that UV-B radiation has no specific effects on the enzymes of the chlorophyll biosynthetic pathway but, rather, influences the genetic regulation of chlorophyll binding protein, leading to destruction of chlorophyll. Furthermore, since carotenoids are reputed to protect photosynthetic membranes against internally and externally generated photooxidative products, a reduction in carotenoids could thus lead to a reduction in the photosynthetic process. Therefore, it appears reasonable to postulate that a decrease in photosynthetic rate was responsible for the combination of stomatal limitation and reduction in photosynthetic pigments.

Moreover, UV-B radiation greatly reduced the amounts of total leaf N, soluble protein N and Rubisco N. The greatest reduction in each kind of nitrogen particularly Rubisco N, which is considered as a key enzyme in photosynthesis, was observed in Giza 121 than Sahrawy (Table 3). These results are in accordance with the finding of Hidema *et al*. (1996) who suggested that the amount of Rubisco plays a very important role in overcoming the inhibitory effects of supplemental UV-B radiation. It is therefore, suggested that the difference between the amounts of Rubisco in Giza 121 and Sahrawy plants might be partially responsible for the difference between varieties in resistance to UV-B radiation.

Likely, the most important effect of UV-B radiation on barley leaves in the present study was the increase in the levels of oxidative stress factors such as active superoxide radical (O_2^{-}) and its derivatives such as hydroxyl radical(OH^{-})and hydrogen peroxide (H_2O_2) which have been proposed as lipid peroxidation initiators (Predieri *et al.*1995) and caused significant increases in lipidperoxidation (as indicated by MDA accumulation) followed by membrane leakage (as evidenced by increase in EC) and aggravation of cellular damage (Table 5).

It has already been well documented that in plants, the amount of superoxide dismutase (SOD) as a key antioxidant enzyme of plants, is influenced by both development and environmental stimuli (Casano *et al.* 1994). However, the behaviour of the SOD enzyme in plants exposed to UV-B stress is scarcely documented and only in dicotyledonous species (Strid *et al.* 1996). As shown in Fig. 2, SOD activity was enhanced especially in Giza 121 at 20 days of UV-B exposure then declined. This behaviour contrasts with that shown by Sahrawy when treated with UV-B radiation. Where the maximum activity of SOD was at 40 days. Dai et al (1997) have reported that there are two reasons that may explain the induction of SOD activity: (1) SOD can induced by substrate $O_2^{\cdot-}$ and (2) the plants have developed some protective mechanisms for acclimating to elevated UV-B radiation. In the present study, it was found that the increase in $O_2^{\bullet-}$ generation due to UV-B exposure could have stimulated SOD and promoted the activity of other antioxidant enzymes. Accordingly the difference between the two varieties may be due to the mechanisms by which the active oxygen species are generated and very likely correlated with the degree of tolerance of these plants to UV-B stress. However, tolerance of plants to UV-B is certainly due to the enhancement of SOD activity and is also due to the capacity of other antioxidant enzymes such as catalase (CAT) and ascorbic peroxidase (AP) as well as non enzymatic antioxidant substances.

Catalase (CAT) and ascorbate peroixdase (AP) enzymes are likely important components of the antioxidant defence system for scavenging H_2O_2. Fig 2 shows that catalase activity was enhanced simultaneously by UV-B radiation especially at 20 days in Giza 121

and up to 40 days in Sahrawy, whereas peroxidase activity decreased in both varieties. In other words, catalase and peroxidase activities showed an inverse relationship similar to that which have been seen in other plants treated with UV-B radiation (Pangopoulos et al. 1990).

In addition to scavenging enzymes, both varieties have evolved some protective mechanisms to keep the deleterious reactions of UV-B radiation to a minimum. Flavonoids have been shown to be active in scavenging reactive oxygen radicals in leaves treated with UV-B exposure, indicating that flavonoids may play a role in oxidative stress as a defence mechanism in higher plants to provide protection against UV-B radiation (Caldwell & Flint 1994). In the present study, flavonoids showed an increase in both varieties particulary in Sahrawy plants, suggesting that these varieties may activate a defence mechanism against UV-B damage by increasing flavonoids.

In conclusion, the present study confirms that Sahrawy was more resistant to the growth-inhibitory effects of UV-B than Giza 121 and the difference between varieties in the resistance to UV-B radiation became very clear when plants were grown under UV-B radiation for a long time.

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الملخص العربى

تأثير الأشعة فوق البنفسجية علي بعض الدالات الفسيولوجية والبيوكيميائية في نباتين من الشعير

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إستخدم في هذا البحث نوعين من الشعير أحدهما متداول زراعته في الصحراء الغربية وهو الشعير الصحراوي والآخر في وادي الدلتا وهـو جيزة 121 وتم استزراع النوعين في وجود وغياب الأشعة الفوق بنفسجية لمدة 40 يوما وذلك بهدف دراسة تأثير تلك الأشعة علـي بعـض الدالات الفسيولوجية والبيوكيميائية التى يمكن أن تحدث في النباتين من جهة ومن جهة أخري يمكن المقارنة بين النباتين لمعرفة أيهمـا أكثـر مقاومة أو أكثر حساسية لتلك الأشعة 0 وقد أوضحت النتائج أن دالات النمو المستخدمة في القياس قد نقصت بشكل ملحوظ تحت تأثير الأشـعة فوق البنفسجية وما ترتب عليه من نقص في المحتوي الكلوروفيلي والكاروتيني وكذلك النقص في معدل عملية البناء الضوئي وأيضا النقص في المحتوي النيتروجيني والبروتين الذائب وبروتين انزيم الربيسكو Rubisco وكان التباين في التأثير واضـحا وجليـا بـين نـوعي الشـعير المستخدمان في البحث حيث كان الشعير الصحراوي أقل تأثرا بالأشعة فوق البنفسجية من الشعير جيزة 121 0 وأظهرت نتائج هـذا البحـث أيضا أن للأشعة فوق البنفسجية تأثرا ضار علي الأغشية البلازمية للخلايا من خلال الأكسدة الدهنية لمكون الغشاء والمتمثلة في زيادة كل مـن معدل تكوين الأوكسجين النشط وتركيز أيدروكسيد الايدروجين ومركب المالون داي الدهايد MDA والزيادة الملحوظة فـي نشـاط بعـض الإنزيمات المضادة لتلك الأكسدة وخاصة إنزيمي سوبر أوكسيد ديسميوتيز SOD والكتاليزCAT وقد نوقش ذلك في البحث 0