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VARIATION IN ANTIOXIDANTS AMONG THREE WHEAT CULTIVARS VARYING IN TOLERANCE TO NaCl

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Summary. Four-days-old seedlings of three wheat (*Triticum aestivum*) cultivars (H 168, Gimmeza 7 and Beni swif 1) were irrigated with 0 and 250 mM NaCl for the following 16 days. Growth and antioxidants of the three cultivars responded differentially to NaCl treatment. Fresh and dry weights of the three cultivars were significantly reduced, however, the least reductions were observed in H 168 while they were highest in Beni swif 1. These results indicated that the three cultivars had different relative tolerance to NaCl and this tolerance was higher in H 168 than in Beni swif 1. Moreover, the treatment caused a temporary increase in the activities of superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase. The increase was consistent in H 168, but mostly stopped in Gimmeza 7 and even inverted in Beni swif 1. Ascorbate and glutathione (GSH) were significantly increased in H 168, but they decreased in both Gimmeza 7 and Beni swif 1. Meanwhile the oxidized form of glutathione (GSSG) was higher in Beni swif 1 relative to the other two cultivars, particularly H 168 which showed the highest GSH/GSSG ratio. It can be concluded that H 168 was superior with respect to its antioxidant defense systems and should be more tolerant than the other two cultivars due to the higher antioxidant activity indicating that tolerance to NaCl seemed to be related to the endogenous levels of antioxidants.

Key words: Antioxidants; growth; salinity; tolerance; wheat.

INTRODUCTION

Salinity is one of the major environmental factors affecting plant growth and productivity (Ashraf and Harris, 2004; Mandhania et al., 2006; Azevedo Neto et al., 2006; Aghaleh and Niknam, 2009). The effects of salinity arise from osmotic and ionic toxicity. Plant metabolism should be flexible to allow plants to cope with environmental stresses. Salt stress can lead to stomatal closure, which reduces CO_2 availability in the leaves and inhibits carbon fixation, exposing chloroplasts to excessive excitation energy which in turn could increase the generation of reactive oxygen species (ROS) (Parida and Das, 2005; Parvais and Satyawati, 2008). ROS such as singlet oxygen ($^1O_2$), superoxide (O_2^-), hydrogen peroxide (H_2O_2) and

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hydroxide radical (OH^-) have potential to interact with many cellular components, causing significant damage to membranes and other cellular structures leading to oxidative damage such as enzyme inactivation, lipid peroxidation, protein degradation and DNA damage (Ashraf and Harris, 2004). As plants acclimatize to environmental stresses, potential to scavenge free radicals often increases. However, plants have enzymatic and non-enzymatic systems to protect cells from oxidative damage (Mittler, 2002; Nemat Alla and Hassan, 2007; Nemat Alla et al., 2008; Yang et al., 2009; Zushi and Matsuzoe, 2009). In the enzymatic antioxidants, superoxide dismutase (SOD) catalyzes the dismutation of O_2^- into H_2O_2 and O_2. H_2O_2 is then scavenaged by catalase (CAT) and ascorbate peroxidase (APX). CAT breaks down H_2O_2 into H_2O and O_2 whereas APX uses two molecules of ascorbate (AsA) as a reductant to reduce H_2O_2 into H_2O and meanwhile AsA is oxidized. The oxidized form is then converted back to AsA by ascorbate reductase on the expense of the oxidation of glutathione (GSH) into the oxidized form (GSSG). GSSG is reduced back to GSH by the action of glutathione reductase (GR). The present work was aimed to study the relationship between tolerance of three wheat cultivars (H 168, Gimmeza 7 and Beni swif 1) to salinity stress caused by NaCl and the responses of antioxidants in order to elucidate the relationship between the tolerance to salinity and antioxidant levels. To evaluate the degree of tolerance to NaCl, changes in growth parameters, activities of the antioxidant enzymes (SOD, CAT, APX and GR) and levels of non-enzymatic antioxidants (AsA and GSH) were monitored.

MATERIALS AND METHODS

Plant materials and growth conditions.

Grains of three wheat (*Triticum aestivum*) cultivars (H 168, Gimmeza 7 and Beni swif 1) were obtained from the Agricultural Research Center, Cairo, Egypt. The grains of the three cultivars were surface sterilized by immersing in 3% sodium hypochlorite solution for 10 min, thoroughly washed, soaked for 8 h and germinated in sand/clay soil (1:1, v/v) in plastic pots (25 cm diameter x 20 cm height). The pots were kept at 12 h photoperiod with 450-500 μmol m^{-2} s^{-1} PPFD (photosynthetic photon flux density), 75-80 % relative humidity, and 22/10°C day/night temperature regime. When seedlings were 4-days-old, irrigation water was substituted with one-fourth strength Hoagland solution and seedlings with uniform sizes were selected for treatments. At this stage, the pots containing each cultivar were divided into two groups, one was left to serve as control and the other for treatment with 250 mM NaCl. At harvest, shoots were separated from roots and collected just before NaCl treatments (zero time) and also after 4, 8, 12 and 16 days of treatments, rinsed with copious amounts of water and dried by plotting with paper towels. The fresh weights of shoots and roots were recorded and dried in an oven at 80°C for 2 days and the dry weights were then recorded.

Extraction and assay of antioxidant enzymes.

All extraction steps were carried out at 4°C. Superoxide dismutase (SOD; EC 1.15.1.1) was extracted in 50 mM phosphate, pH 7.8, 0.1% (w/v) bovine serum albumin, 5.5 mM ascorbate, and

8 mM β-mercaptoethanol. SOD activity was assayed by using the photochemical NBT method in terms of SOD ability to inhibit the reduction of NBT to form formazan by superoxide (Beyer and Fridovich, 1987). The photoreduction of NBT was measured at 560 nm. Catalase (CAT; EC 1.11.1.6) was extracted in 50 mM phosphate buffer, pH 7, and 1 mM dithiothreitol. CAT activity was assayed spectrophotometrically by determining the consumption of H_2O_2 at 240 nm in 50 mM phosphate buffer, pH 7.5 and 200 mM H_2O_2 (Aebi, 1984). Ascorbate peroxidase (APX; EC 1.11.1.7) was extracted in 0.1 M Tricine-KOH buffer, pH 8, 1 mM dithiothreitol, 10 mM $MgCl_2$, 50 mM KCl, 1 mM EDTA, 0.1% (w/v) Triton X-100, and 0.28 mM phenylmethylsulfonyl flouride. APX activity was assayed according to Nakano and Asada (1981). Glutathione reductase (GR, EC 1.6.4.2) was extracted in 100 mM phosphate, pH 7.5 and 0.5 mM EDTA. The extracts were centrifuged at 15000 x *g* for 20 min. The activity was assayed in a reaction mixture containing 100 mM phosphate, pH 7.5, 0.5 mM EDTA, 0.75 mM 5,5-dithiobis-(2-nitrobenzoic acid), 0.1 mM NADPH and 1 mM oxidized glutathione (GSSG) (Smith et al., 1988). The reaction mixture was incubated at 35°C and absorbance was measured up to 5 min at 412 nm.

Determination of non-enzymatic antioxidants.

Ascorbic acid (AsA) was extracted in 62.5 mM phosphoric acid and centrifuged at 12000 x *g* for 20 min and filtered through a 0.5 µm Millipore filter. The filtrate was loaded onto an ion exclusion column (300 x 7.8 mm) connected to analytical HPLC system, and eluted with 4.5 mM H_2SO_4 at a flow rate of 0.5 ml min^{-1}. The elution of AsA was detected at 245 nm (Ahn et al., 1999). GSH extraction was performed at 4°C in TCA (5%, w/v) and 10 mM EDTA (Anderson and Gronwald, 1991). The extracts were centrifuged at 12000 x *g* for 15 min. GSH was assayed in 100 mM phosphate buffer, pH 6.8 containing 10 mM EDTA, 1 mM 1-chloro-2,4-dinitrobenzene and 1.0 U equine glutathione-S-transferase and incubated at 35ºC for 30 min. The absorbance was recorded at 340 nm before commencing the reaction and after the reaction had run to completion. A control assay without equine GST was performed to check the possible nonenzymatic reaction. For the assay of GSSG, 2-vinylpyridine was added to mask GSH. The reaction mixture contained 100 mM potassium phosphate buffer (pH 7.5), 5 mM EDTA, 0.2 mM NADPH, 0.6 mM 5,5-dithiobis-(2-nitrobenzoic acid) and 10 U GR. The absorbance at 412 nm was immediately measured and again after 30 min of incubation at 35°C.

All values reported are means (±SD) of at least six determinations from two independent experiments. The data were firstly subjected to analysis of variance (ANOVA) followed by least significant differences (LSD) at 5% level.

RESULTS

It is evident that treatment with 250 mM NaCl resulted in significant reductions in fresh weight of shoots and roots of the three wheat cultivars (H 168, Gimmeza 7 and Beni swif 1) as compared to the respective control (Table 1). These reductions augmented with the elapse of time. The magnitude of reduction was most pronounced in Beni swif 1, reaching

Table 1. Effect of treatment with 250 mM NaCl on fresh and dry weight in shoots and roots of three cultivars (H 168, Gimmeza 7, and Beni swif 1) of wheat (*Triticum aestivum*).

	Days after treatment									
	0	4	8	12	16	0	4	8	12	16
	Shoot fresh weight [mg plant^{-1}]					Root fresh weight [mg plant^{-1}]				
H 168										
Control	155±9.1	179±11.2	210±15.2	210±19.3	204±17.2	104±6.2	140±13.2	168±13.2	174±15.2	151±11.2
Treated		166±12.0	190±16.3*	182±18.4*	186±14.1		108±11.4*	133±11.7*	145±11.7	127±6.5
Gimmeza 7										
Control	162±12.2	203±16.2	231±18.2	220±17.2	208±15.2	91±8.2	111±13.2	190±15.2	174±13.2	175±11.2
Treated		171±18.1*	196±16.9*	180±16.6*	164±13.3*		73±8.3*	128±9.5*	131±10.1*	130±13.5*
Beni swif 1										
Control	107±9.3	121±11.2	140±18.2	158±12.2	149±11.2	87±8.2	98±7.2	131±6.2	121±9.1	118±9.2
Treated		63±7.4*	77±10.4*	82±7.7*	65±6.1*		60±5.9*	82±7.2*	80±7.3*	81±7.5*
	Shoot dry weight [mg g^{-1} fresh weight]					Root dry weight [mg g^{-1} fresh weight]				
H 168										
Control	171±11.2	201±17.2	198±14.1	223±15.2	245±17.2	132±7.2	166±11.2	182±11.2	190±13.3	200±18.2
Treated		177±14.1*	172±12.6	199±13.3	223±14.2		131±12.5*	153±13.3*	165±12.7	178±13.2
Gimmeza 7										
Control	137±9.3	159±9.2	173±10.2	213±15.2	237±15.2	104±9.1	172±16.2	172±13.1	162±9.2	174±11.2
Treated		132±11.7*	147±9.1*	184±12.8*	199±13.3		132±12.9*	129±.7.3*	123±7.4*	134±12.4*
Beni swif 1										
Control	111±10.2	153±12.2	175±12.1	186±13.1	215±14.1	91±3.1	119±8.1	147±8.2	150±8.2	150±9.2
Treated		124±11.8*	143±12.4*	151±13.2*	172±13.9*		87±6.5*	110±5.1*	111±7.4*	114±7.3*

Data are means ± SD. *– Significantly different from the respective control at $p < 0.05$.

about 57% and 31% decrease in shoots and roots, respectively on the 16th day after treatment. However, fresh weight of shoots and roots of H 168 was the least, only about 9% and 16% decreases were detected in shoots and roots, respectively. The reduction in fresh weight of shoots and roots of Gimmeza 7 seemed to be moderately affected. In the same pattern, dry weight of shoots and roots of either Beni swif 1 or Gimmeza 7 was significantly decreased by NaCl during the whole experimental period. These decreases reached on the 16th day after treatment about 20% in shoots and 24% in roots of Beni swif 1 and about 16% and 23% in Gimmeza 7. On the contrary, dry weight of H 168 seemed to remain unaffected by 250 mM NaCl, shoots and roots dry weights were comparable to control values, in spite of the slight decreases detected. As shown in Fig. 1, there was a significant decline in SOD activity in the three wheat cultivars during the first 4 days of treatment with NaCl as compared to the respective control. The decline was most pronounced in Beni swif 1 than in Gimmeza 7 and H 168. By the elapse of time, a partial recovery in the activity of SOD was observed in H 168 and to a smaller extent in Gimmeza 7. As a whole, the magnitude of decline of SOD activity was lower in Gimmeza 7 than in Beni swif 1, both cultivars showed lower values of activity than H 168. On the 16th day after treatment, 250 mM NaCl increased SOD activity in H 168 by 16% but decreased the enzyme activity in both Gimmeza 7 and Beni swif 1 by about 11% and 27%, respectively as compared to the respective controls.

On the contrary, CAT activity was significantly increased by NaCl in only H 168 throughout the experimental period (Fig. 2). In Gimmeza 7 and Beni swif 1, the increase in CAT activity was restricted to the first few days following treatment but the activity declined thereafter to become on the 16th day after treatment comparable to the control levels in

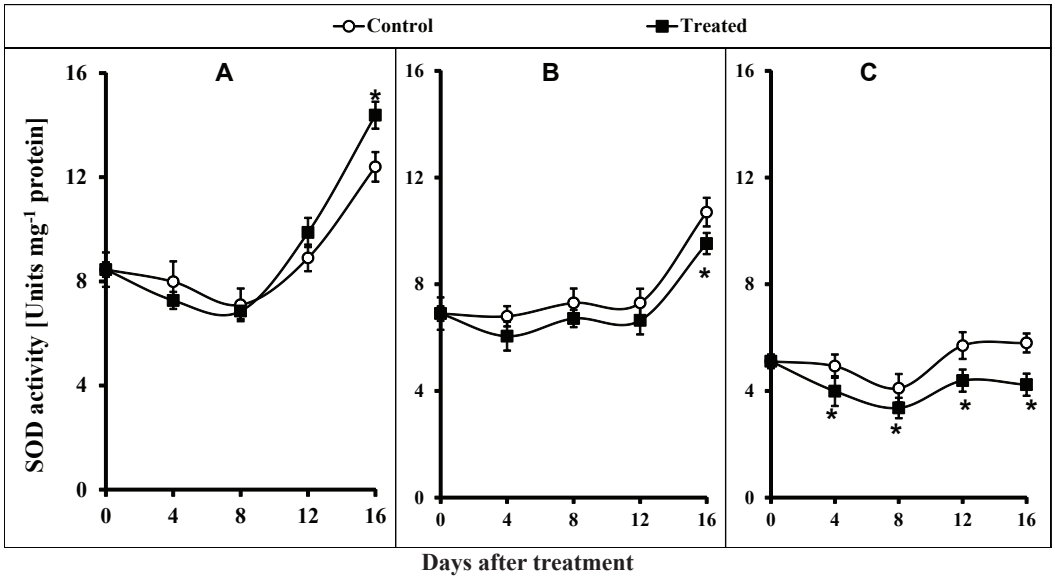

Fig. 1. Effect of treatment with 250 mM NaCl on the activity of superoxide dismutase (SOD) of three cultivars (H 168, A; Gimmeza 7, B; and Beni swif 1, C) of wheat (*Triticum aestivum*). Data are means ± SD. * – Significantly different from the respective control at $p < 0.05$.

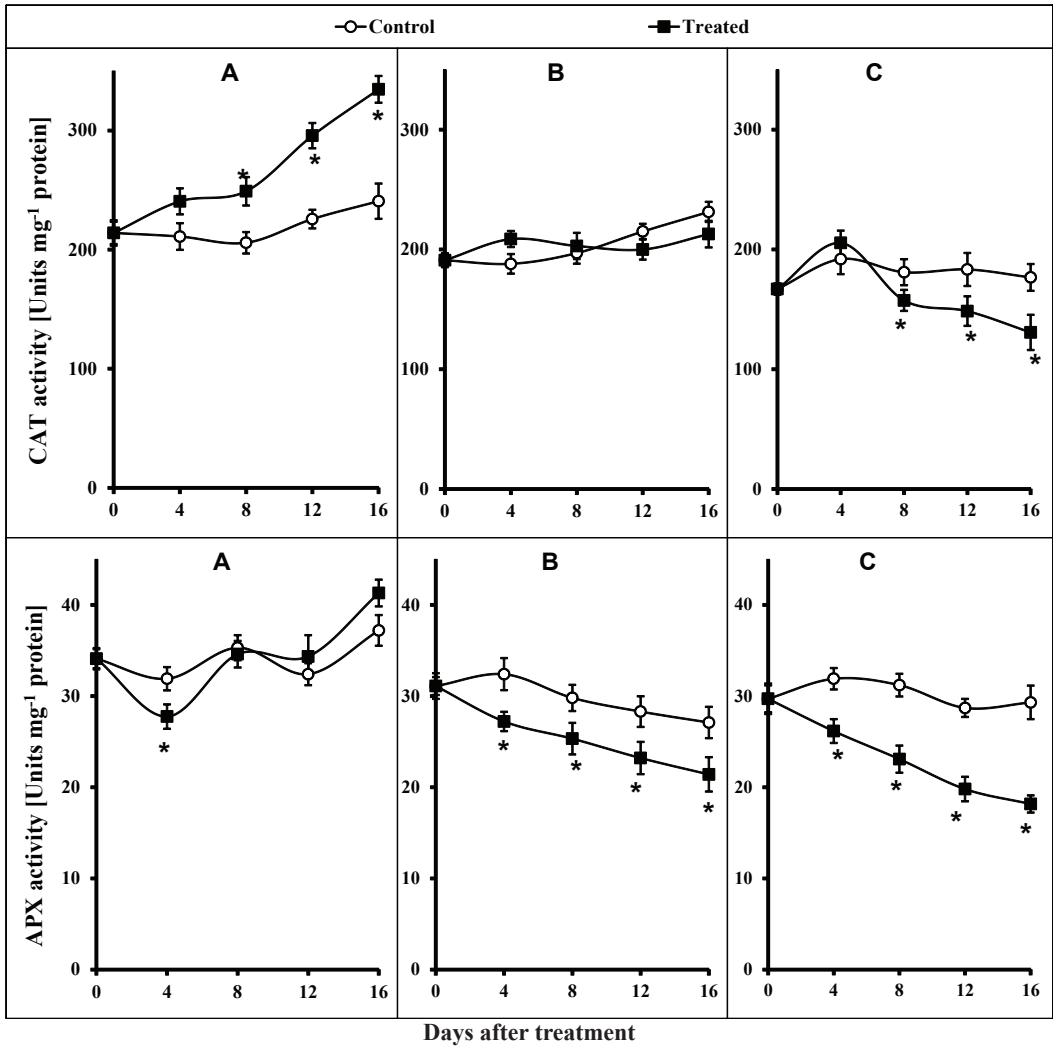

Fig. 2. Effect of treatment with 250 mM NaCl on the activity of catalase (CAT) and ascorbate peroxidase (APX) of three cultivars (H 168, A; Gimmeza 7, B; and Beni swif 1, C) of wheat (*Triticum aestivum*). Data are means ± SD. * – Significantly different from the respective control at $p < 0.05$.

Gimmeza 7 or significantly lower than the control levels in Beni swif 1. At the end of the experiment on the 16th day after treatment, the activity of CAT in the three wheat cultivars H 168, Gimmeza 7 and Beni swif 1 reached about 139%, 92% and 74% relative to controls, respectively. The same figure shows also that APX activity was significantly decreased in the three wheat cultivars by NaCl during the first 4 days. Thereafter, this decrease was nullified in H 168 and even elevated by the end of the experiment to reach about 111% of the control value. The enzyme activity in the other two cultivars remained lower than the control levels reaching in Beni swif 1 and Gimmeza 7 by the end of the experiment about 62% and 79% of the control value, respectively.

Similarly, treatment with 250 mM NaCl increased significantly GR in H 168 during the entire experimental period as compared to the control values (Fig. 3). In relation to H 168, both Beni swif 1 and Gimmeza 7

cultivars attained low levels of the enzyme activity. GR activity in both cultivars was increased by NaCl only up to the 4th day after treatment, followed thereafter by a sharp decline. On the 16th day after treatment, the activity of GR in Gimmeza 7 and Beni swif 1 were decreased by 19% and 29% compared to control, respectively relative to about 17 % increase in H 168.

In the same pattern, AsA increased significantly in H 168 as a result of treatment with NaCl (Fig. 4). This increase seemed

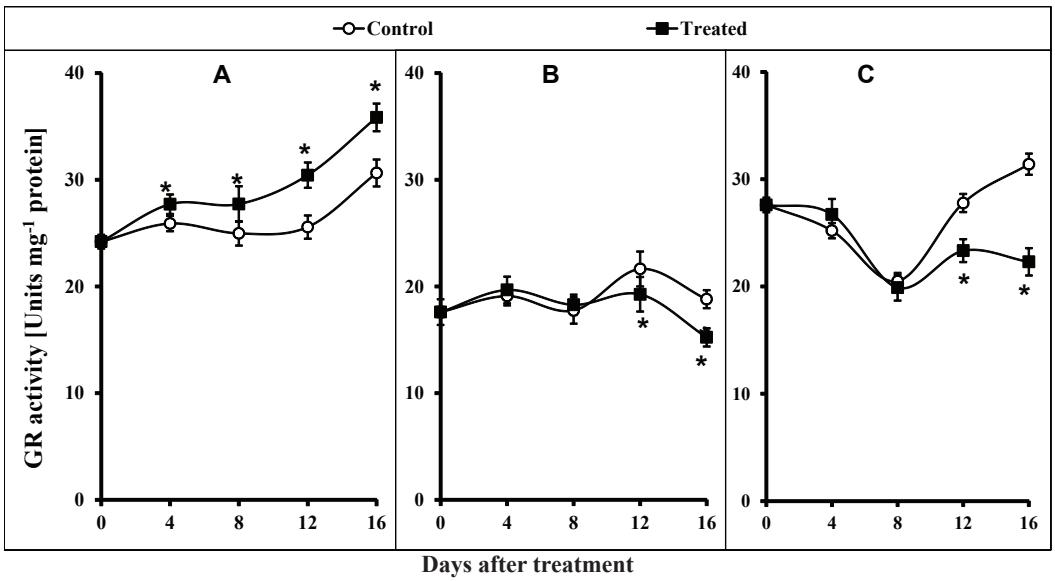

Fig. 3. Effect of treatment with 250 mM NaCl on the activity of glutathione reductase (GR) of three cultivars (H 168, A; Gimmeza 7, B; and Beni swif 1, C) of wheat (*Triticum aestivum*). Data are means ± SD. * – Significantly different from the respective control at $p < 0.05$.

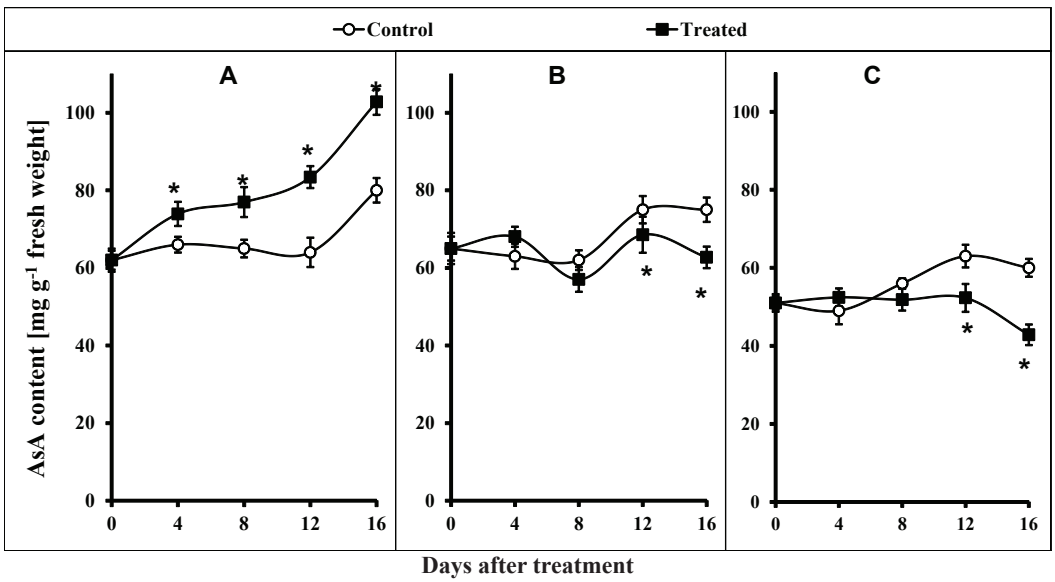

Fig. 4. Effect of treatment with 250 mM NaCl on the content of ascorbate (AsA) of three cultivars (H 168, A; Gimmeza 7, B; and Beni swif 1, C) of wheat (*Triticum aestivum*). Data are means ± SD. * – Significantly different from the respective control at $p < 0.05$.

consistent with the elapse of time. On the contrary, there were significant decreases in AsA in both Beni swif 1 and Gimmeza 7 cultivars by NaCl throughout the whole experiment as compared to the respective controls. The magnitude of decrease was greater in Beni swif 1 than in Gimmeza 7. The AsA levels in the NaCl-treated H 168, Gimmeza 7 and Beni swif 1 cultivars reached on the 16th day after treatment about 129%, 84% and 71%, respectively compared to the respective control values.

Despite decreasing AsA in Gimmeza 7, NaCl mostly seemed with no significant effect on GSH content during the whole experimental period (Fig. 5). On the other hand, NaCl significantly increased GSH in H 168 throughout the entire period as compared to control. In contrast to both cultivars, GSH of Beni swif 1 was significantly decreased by NaCl throughout the whole experiment as

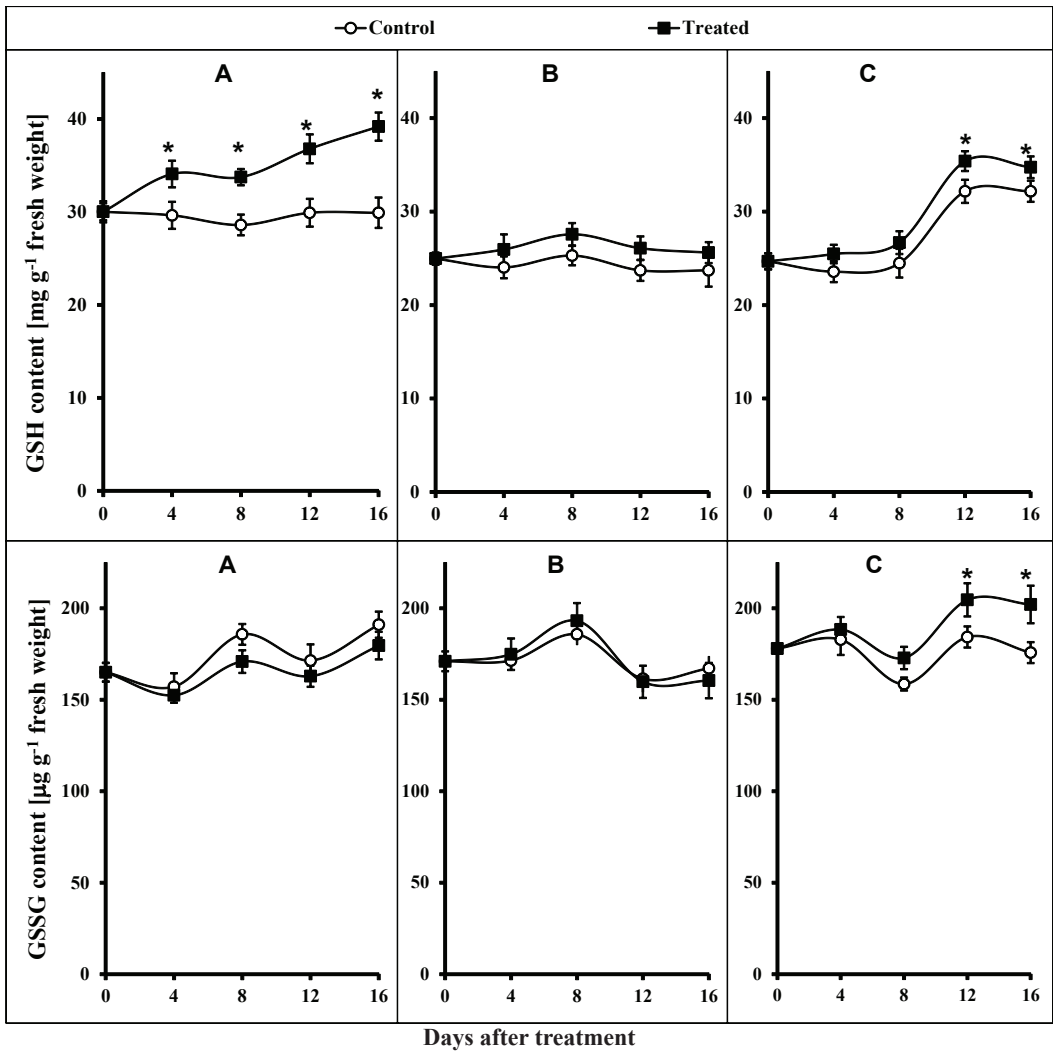

Fig. 5. Effect of treatment with 250 mM NaCl on the content of reduced glutathione (GSH) and oxidized glutathione (GSSG) of three cultivars (H 168, A; Gimmeza 7, B; and Beni swif 1, C) of wheat (*Triticum aestivum*). Data are means ± SD. * – Significantly different from the respective control at $p < 0.05$.

compared to control. GSH content in H 168, Gimmeza 7 and Beni swif 1 reached on the 16th day after treatment about 131%, 108% and 76% of the respective control, respectively. On the contrary, NaCl treatment led to significant increases in GSSG content in Beni swif 1 during the whole experiment. These increases seemed not significant on the 4th day but augmented with the elapse of time reaching on the 16th day after treatment about 115% of the control levels. GSSG levels in H 168 and Gimmeza 7 cultivars appeared to be unaffected by NaCl treatment during the experiment. These levels were most likely comparable to those of the respective controls reaching on the 16th day after treatment about 94% in H 168 and 96% in Gimmeza 7 of the control values.

The data interpolation of Fig. 5 showed that the GSH/GSSG ratio was usually higher in H 168 throughout the whole experiment than in the other two cultivars (Table 2). This ratio seemed comparable in Gimmeza 7 to that of the control during the entire period. The lowest value of the GSH/GSSG ratio was detected in Beni swif 1 during all intervals of the experiment.

DISCUSSION

Salinity reduces plant growth and lowers plant production worldwide (Shah, 2007; Gao et al., 2008; Aghaleh and Niknam, 2009; Yang et al., 2009). The physiological mechanisms involved in the reduction of plant growth resulting from salinity include turgor pressure reduction, reduction in photosynthetic activity, effects of accumulated salts on the metabolic activities, molecular responses, and oxidative stress (Duan et al., 2006; Zushi and Matsuzoe, 2009; Yang et al., 2009). The present results showed a general reduction in fresh and dry weights of the three wheat cultivars caused by 250 mM NaCl. The reduction was most pronounced in Beni swif 1 followed by Gimmeza 7 while the growth of H 168 seemed to be the least affected (Table 1). These results suggest that the three wheat cultivars have different relative tolerance to NaCl and this tolerance was higher in H 168 compared to Beni swif 1

Table 2. Changes in GSH/GSSG ratio in leaves of three cultivars (H 168, Gimmeza 7, and Beni swif 1) of wheat (*Triticum aestivum*) after treatment with 250 mM NaCl.

	Days after treatment				
	0	4	8	12	16
H 168					
Control	393	458	374	424	363
Treated		543	480	549	506
Gimmeza 7					
Control	326	340	331	357	345
Treated		360	347	396	388
Beni swif 1					
Control	350	313	375	424	445
Treated		289	316	332	294

while Gimmeza 7 seemed to moderately tolerate NaCl. The reduction in growth is considered as an evidence for stress that might be induced by different factors, among which oxidative stress. Such status of stress is a consequence of ROS generation that would react with lipids, proteins and pigments causing lipid peroxidation and membrane damage (Mittler, 2002; Ali et al., 2005; Nemat Alla et al., 2007; Nemat Alla et al., 2008). ROS initiate a variety of autooxidative chain reactions on membrane unsaturated fatty acids, producing lipid hydroperoxides and thereby cascade of reactions ultimately leading to destruction of organelles and macromolecules (Aravind and Prasad, 2005). ROS typically result from the excitation of O_2 to form $^1O_2$ or from the transfer of one, two or three electrons to O_2 to form a superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) or a hydroxyl radical (HO^-), respectively (Mittler, 2002; Nemat Alla et al., 2008). A prominent and well-characterized reaction of plants challenged with salinity is ROS generation (Mittova et al., 2004; Haung et al., 2005; Verma and Mishra., 2005; Tuteja, 2007).

Plant responses to environmental factors have often been associated with generation and/or scavenging of ROS. It is conceivable that acquisition of the stress tolerance in plants is a multi-factorial function and amelioration of ROS-scavenging systems is an important index to assess the abilities of wheat cultivars to tolerate salinity stress. The increased activities of ROS scavenging enzymes should have a greater significance as invaluable tools in the elucidation of plant metabolic regulation under stress. Antioxidants are crucial for plant defense against oxidative stress. Removal of ROS are regulated by antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) as well as various other non-enzymatic antioxidants such as ascorbate (AsA) and glutathione (GSH) (Foyer et al., 2001; Mittler, 2002; Aravind and Prasad, 2005; Nemat Alla et al., 2008).

In the present study, the activity of SOD of the three wheat cultivars declined due to NaCl treatment, however, the activity was partially recovered in H 168 by the elapse of time but not in the other two cultivars (Fig. 1). So, the activity of SOD in H 168 may suffice to withstand the amount of oxidative stress due to NaCl treatment, suggesting that this cultivar is more tolerant than the other two cultivars. On the other hand, an increase in the activity of CAT (as shown in Fig. 2) was observed in all wheat cultivars upon NaCl treatment, the magnitude of the increase being highest in H 168 and lowest in Beni swif 1. The same trend was also observed for APX activity (Fig. 2). SOD is an essential component of these defense mechanisms as it dismutates to produce H_2O_2 and O_2 (Scandalios, 1993; Allen, 1995). CAT and APX appear to play an essential protective role in the scavenging processes when coordinated with SOD activity (Massacci et al. 1995). They scavenge H_2O_2 generated primarily through SOD action. The present findings could point to an enhanced degradation of H_2O_2 formed in H 168 either directly from the oxidative stress or as a result of SOD activity. Therefore, elimination of ROS seemed faster in H 168 than in Beni swif 1 with a moderate response of Gimmeza 7. Moreover, the decreased APX activity in Beni swif 1 would result in higher

accumulation of H_2O_2 than in H 168. Such accumulation could result from a decrease in CAT activity with a consequence shortage in H_2O_2 degradation and/or a decrease in APX activity with inefficiency in H_2O_2 scavenging by AsA. In this context, Mandhania et al. (2006) found that activities of CAT and APX increased with increasing the salt stress in both salt tolerant and salt sensitive wheat cultivars.

Another factor that would contribute to countering the oxidative stress by maintaining reduced glutathione content at the cellular level is the activity of the enzyme GR. An increase in GR activity was also observed in the three wheat cultivars after NaCl treatment, however, this increase was consistent in H 168 but retracted in Gimmeza 7 and moreover inversed in Beni swif 1 to become lower than control level (Fig. 3). GR activity is believed to be an important factor limiting the degree of photo damage experienced by wheat plants upon exposure to salinity. This enzyme has been suggested to play a pivotal role in the glutathione cycle in the eukaryotic cells. Mandhania et al. (2006) indicated that GR activity increased with increasing salt stress in both salt tolerant and salt sensitive wheat cultivars. So, the present results declare that the damage which was inflicted by NaCl can be ameliorated by over-expression of antioxidant enzymes as noticed in H 168 and there are certain variations in the activity of these antioxidant enzymes among the wheat cultivars (as shown in Fig. 1-3) to counteract NaCl stress. However, the down-regulation of GR in strawberry after NaCl exposure indicates that GSH was consumed faster than its regeneration (Tanou et al., 2009). Thus, the elevated levels of GR might be able to increase the GSH/GSSG ratio, thereby ensuing the availability of GSH to scavenge ROS.

AsA and GSH play a significant role in antioxidant protection (Tausz et al., 2004). They are the most abundant low molecular weight non-enzymatic antioxidants in plant cells participating in ROS scavenging through the AsA-GSH cycle (Foyer et al., 2001). The mechanism of defense of AsA and GSH (in AsA-GSH cycle) involves scavenging H_2O_2 (Murgia et al., 2004; Nemat Alla et al., 2007; Nemat Alla et al., 2008). However, the AsA-GSH cycle may possibly not only direct ROS scavenging but it could also act as a cellular redox state rheostat (Miller et al., 2008). AsA eliminates ROS through multiple mechanisms. It maintains the membrane-bound antioxidant α-tocopherol in the reduced state, eliminates H_2O_2 through the activity of APX and has a major role in photoprotection as a cofactor in the xanthophyll cycle (Jiménez et al., 1997). Tanou et al. (2009) have indicated that NaCl treatment leads to oxidation of the AsA pool of strawberry. Oxidation of AsA results in formation of monodehydro-ascorbate (MDHA), an unstable intermediate that unless re-reduced, disproportionates to AsA and dehydro-ascorbate (DHA). Both MHDA and DHA can be reduced back to AsA through the activity of reductases.

On the other hand, GSH is a versatile antioxidant that can directly scavenge ROS. It is oxidized to GSSG during the generation of AsA by APX. Thereafter, GSSG is reduced back to GSH by GR. In accordance, Tanou et al. (2009) found that the decrease in AsA and GSH in strawberry caused by NaCl was due to the increase of DHA and to GSSG accumulation, respectively. So, the increased GSH/

GSSG ratio in H 168 (Table 2) could indicate that GR is more efficient in re-reduction of GSSG for re-reduction of DHA to form AsA. Tausz et al. (2004) have shown that when the stress becomes more intense, degradation and oxidation of GSH were observed. This conclusion could confirm the lesser damage of H 168 upon NaCl treatment compared to the other two cultivars (as indicated from growth parameters data, Table 1).

The decreases in AsA and GSH in Beni swif 1 (Figs. 4 and 5) accompanied with the great reduction in growth (Table 1) could confirm the severe state of stress imposed by NaCl to this cultivar relative to the others particularly H 168 which seemed to tolerate NaCl. The enzymatic and non-enzymatic antioxidants were mostly increased in H 168 after NaCl treatment, however, its growth was least affected. On the contrary, Beni swif 1 could be considered as the most sensitive cultivar since both growth and antioxidant levels were greatly decreased by NaCl. In confirmation, the antioxidant levels as well as growth of Gimmeza 7 responded moderately to NaCl. Therefore, wheat tolerance to NaCl might confer by differential antioxidative mechanism and so it could be concluded that it correlates with wheat tolerance to NaCl. In this context, Massacci et al. (1995) indicated that the antioxidant levels and the activities of ROS scavenging enzymes correlated with tolerance to different environmental stresses. Similar results were also detected in mulberry (Chaitanya et al., 2002). In addition, some studies have shown that decreasing activities of antioxidant enzymes were found in salt sensitive tomato under 100 mM NaCl conditions (Mittova et al., 2004). Moreover, increased SOD, CAT and APX are closely related to salt tolerance of many plants (Azevedo Neto et al., 2006; Koca et al., 2007). Also, Yang et al. (2009) reported that the increment in the activities of antioxidant enzymes during the salt stress increased the salt tolerance of *Populus cathayana*. They indicated that this seemed to be related to cell protection as a common defense mechanism rather than being a direct response. In confirmation, many researches declared that the antioxidants have been considered as a defensive team whose combined purpose is to protect cells from oxidative damage (Mittler, 2002). These findings suggest that the induction of ROS scavenging enzymes is the most common mechanism of salt tolerance for detoxifying ROS.

In conclusion, treatment with 250 mM NaCl resulted in a reduction in fresh and dry weight of three wheat cultivars, H 168, Gimmeza 7 and Beni swif 1, but to varying degrees. The observed reduction was low in H 168, high in Beni swif 1 and seemed to be moderate in Gimmeza 7. These results could imply that the three wheat cultivars have different relative tolerance to NaCl and this tolerance was higher in H 168 compared to Beni swif 1 while Gimmeza 7 seemed to moderately tolerate NaCl. Moreover, the activities of SOD, CAT, APX and GR as well as the non-enzymatic antioxidants (AsA and GSH) were mostly increased in H 168, but declined in Gimmeza 7 and particularly in Beni swif 1. Therefore, the present results clearly showed that H 168 was superior with respect to its antioxidant defense systems and should be more tolerant to NaCl than the other two cultivars due to the higher enzymatic and non-enzymatic antioxidants. So, this

tolerance appeared to be related to the endogenous levels of the enzymatic and the non-enzymatic antioxidants. This study is useful in breeding programs or transgenic researches to generate plants with elevated activities of antioxidants for improved tolerance to salinity.

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