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ANTIOXIDANT RESPONSES OF PEANUT (*ARACHIS HYPOGAEA* L.) SEEDLINGS TO PROLONGED SALT-INDUCED STRESS

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Abstract: In this study, the effects of long-term NaCl treatment were investigated in two cultivars of peanut designated as drought-resistant and drought-sensitive. Growth parameters, changes in the concentrations of MDA, H_2O_2 and proline, and the activities of antioxidant enzymes were determined under salinity stress. Growth parameters indicated the superiority of cv. Florispan to cv. Gazipaşa under milder salinity stress treatment. However, comparative analysis of the two cultivars showed that MDA, H_2O_2, ion leakage levels and photosystem II activities were not significantly different, except for the proline activity, which increased only in Florispan leaf tissues under 100 mM salt treatment. Among the processes that govern the tolerance in peanut tissues, proline level and the activity of glutathione reductase (GR) appeared to be only components that play an important part in salt stress protection.

Key words: Antioxidant enzymes; lipid peroxidation; oxidative stress; proline; salt

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INTRODUCTION

Peanut (*Arachis hypogaea* L.) is one of the most important legume crops grown worldwide as a source of edible oil and vegetable protein. Arid and semi-arid regions constitute more than half of the peanut production area and 15% of these regions are salt-affected (Jefferies, 1981; Shannon, 1984; Reddy et al., 2003). Salinity is one of the main environmental stress factors that limit legume productivity and plays a major role in determining legume distribution across different environments (Melchiorre et al., 2009; Giannakoula et al., 2012). Legumes are known to be either sensitive or moderately resistant to salinity.

Salt sensitivity can be attributed to toxic ion accumulations in different tissues, which disturb some enzyme activities (Zahran, 1991). High concentrations of Na^+ in the soil also cause the disruption of intracellular ion homeostasis, membrane dysfunction and inhibition of metabolic activity, resulting in inhibition of growth and yield reduction (Hasegawa et al., 2000). Plant growth can be expressed as biomass production, which it is a measure of net photosynthesis. Therefore, environmental stresses affecting growth also affect photosynthesis (Parida and Das, 2005). Photosynthesis may decrease due to stomatal closure or by the direct effect of salt on the photosynthetic apparatus (Eyidoğan and Öz, 2007). Salinity imposes two stresses on plant tissues, one of them being water-deficit stress that results from the relatively high solute concentrations in the soil, and the other is ion-specific stress resulting from altered K^+/Na^+ ratios and Na^+ and Cl^- concentrations (Apse and Blumwald, 2002). Salt stress causes water deficit as a result of osmotic effects on a wide variety of meta-

bolic activities of plants, and this water deficit results in oxidative stress because of the formation of reactive oxygen species (ROS), which include singlet oxygen, superoxide anion radicals, hydroxyl radicals and hydrogen peroxide (Parida and Das, 2005).

Plants have a multi-level antioxidant system consisting of both non-enzymatic and enzymatic antioxidants such as catalase (CAT; EC 1.11.1.6), ascorbate peroxidase (APX; EC 1.11.1.11), superoxide dismutase (SOD; EC 1.15.1.1) and glutathione reductase (GR; 1.8.5.1). Biochemical studies have shown that accumulation of compatible solutes in plants upon exposure to salt stress contribute to turgor maintenance in plant cells. Proline accumulation is one of the most frequently reported modifications induced by salt stress in plants, and it is often thought to be involved in stress-resistance mechanisms (Misra and Saxena, 2009). Proline also alleviates salt stress-induced enhancement in oxygenase and carboxylase activities of RuBisCO (Sivakumar et al., 2000). It was found to be an effective hydroxyl radical scavenger *in vitro* (Smirnoff and Cumbes, 1989). It is known that the peroxidation of lipid membranes by free radicals is both a reflection and a measure of stress-induced damage at the cellular level (Jain et al., 2001). Therefore, measuring the end-products of lipid peroxidation such as malondialdehyde, a good marker for stress-induced damage, is one of the most widely used assays for the determination of oxidative damage (Shulaev and Oliver, 2006). The studies on peanut of the physiological basis of abiotic stress responses are few and to our knowledge, none of them has examined the detailed antioxidative defense mechanisms in salinity tolerance *in vivo*. In the present study, two different growth conditions possessing different concentrations of sodium chloride were used to mediate salinity stress in two peanut cultivars. The effects of salt stress were investigated by considering the biochemical and physiological changes in growth parameters, shoot and root water contents (SWC and RtWC), lipid peroxidation, proline content and chlorophyll fluorescence together with the enzymatic antioxidant responses. Drought-induced oxidative damage and antioxidant responses in drought-resistant and drought-sensitive peanut cultivars were investigated in our previous studies (Celikkol et al., 2010). Comparison of the effects of drought and salinity on the same peanut cultivars may provide a broader view of peanut tolerance mechanisms against abiotic stress conditions.

MATERIALS AND METHODS

Plant materials, growth conditions and stress treatments

Two different peanut (*Arachis hypogaea* L.) cultivars, Florispan (Spanish type) and Gazipaşa (Turkish type), were used in the study. The seeds were provided by the West Mediterranean Agricultural Research Institute (Turkey). Seed surface sterilization was carried out with 2% sodium hypochloride by continuous shaking for 1 min. In order to eliminate sodium hypochloride, the seeds were rinsed with sterile distilled water at least 4 times. Plant growth and germination were achieved in pots filled with perlite and watering with half-strength Hoagland solution for 16 days (Hoagland and Arnon, 1950). Half-strength Hoagland solution was used as a nutrient solution in, since plants showed vigorous growth such as more and healthier leaves under saline and non-saline conditions with this solution (Rubio et al., 2011). Salt stress was applied to these plants with half-strength Hoagland solution containing 0, 100 mM and 300 mM NaCl for 16 days. On the 28th day of growth, plants were harvested at the same time, approximately 2 h after initiation of application during daylight. Each set of experiments was performed at least three times.

Growth parameters

After stress application, the shoots and roots of the two peanut cultivars were collected. In addition to the length of shoot and root, the fresh weights of these parts were also recorded. In order to determine the dry weight, the tissues were dried in an oven at 70^{o}C for 2 days.

Chlorophyll fluorescence

Fv/Fm value indicating the maximum photochemical yield of PSII was determined in dark-adapted leaf tissues that had been subjected to the dark for 30 min, by the use of an OS5-FL Modulated Fluorometer (Tyngsboro, MA).

Determination of membrane damage

Lipid peroxidation in terms of malondialdehyde (MDA) content and membrane electrolyte leakage were determined to evaluate the membrane damage with salt stress. Lipid peroxidation assay was carried out following the method of Ohkawa et al. (1979). For these assays, 0.2 g of fresh shoot tissues was homogenized with liquid nitrogen after addition of 1 mL of 5% trichloroacetic acid (TCA). The homogenates were transferred to fresh tubes and centrifuged at 11200 × *g* for 15 min at room temperature (Sigma, St. Louis, MO). Equal volumes of supernatant and 0.5% thiobarbituric acid (TBA) in 20% TCA (freshly prepared) were put into Eppendorf tubes and incubated for 25 min at 96°C. The tubes were placed in an ice-bath after incubation and then centrifuged at 7800 × *g* for 5 min. Absorbance of the supernatant was determined at 532 nm and the correction for non-specific turbidity was performed by subtracting the absorbance at 600 nm using a Shimadzu UV-Vis spectrophotometer. MDA contents were calculated using an extinction coefficient of 155 $mM^{-1}cm^{-1}$.

To assess membrane leakage, electrolyte leaked from leaves subjected to salt stress as well as control were measured by following the protocol of Nanjo et al. (1999). For electrolyte measurement, six leaves per plant were incubated in 5 mL of 0.4 M mannitol at room temperature by gentle shaking for 3 h. Two conductivity measurements were performed for each sample using an MPC 227 conductivity meter. Data obtained from fresh samples were assigned as C1. Second measurements were carried out using samples boiled for 10 min and cooled down to room temperature. The conductivity recorded from these samples was designated as C2. The conductivity due to leakage is expressed as the percentage of the initial conductivity over the total conductivity [(C1/C2)* 100].

H_2O_2 content determination

The H_2O_2 content of tissues was measured according to the method of Bernt and Bergmeyer (1974). For this analysis, homogenizations were carried out with liquid nitrogen using 0.5 g shoot tissue from both control and salt-treated plants. Homogenized samples were suspended in 1.5 mL of 100 mM potassium phosphate buffer at pH 6.8 and then centrifuged at 18000 × *g* for 20 min at 4°C (Sigma, St. Louis, MO). The determination of H_2O_2 content was performed via an enzymatic reaction with peroxidase. Enzymatic reaction was initiated at 30°C with 0.25 mL of supernatant and 1.25 mL of peroxidase reagent consisting of 83 mM of potassium phosphate buffer at pH 7.0, 0.005% (w/v) o-dianisidine, 40 µg peroxidase/mL. The reaction was stopped by adding 0.25 mL of 1 N perchloric acid after 10 min. The supernatant was obtained by centrifugation at 5000 × *g* for 5 min. The absorbance of the supernatant was measured at 436 nm with a UV-Vis spectrophotometer (Shimadzu, Japan), and the amount of hydrogen peroxide was determined using an extinction coefficient of 39.4 $mM^{-1}cm^{-1}$.

Proline content determination

The protocol of Bates et al. (1973) was used for the estimation of proline content. For this analysis, homogenizations were carried out with liquid nitrogen using 0.3 g shoot tissue from both control and salt-treated plants. Extracts were suspended in 1 mL of 3% sulphosalicylic acid. Centrifugation was carried out at 15 000 × *g* for 5 min at 4°C to settle the extracts. One hundred µl of supernatants were pipetted out and mixed with 200 µL of acid ninhydrin, 200-µL 96% acetic acid and 100 µl 3% sulphosalicylic acid. The reaction mixture was incubated at 96°C for 1 h and then toluene was added. After centrifugation, the upper phases were collected and absorbances were read at 520nm with the UV-Vis spectrophotometer (Shimadzu, Japan). The amounts of proline were determined using an extinction coefficient of 0.9986 $mM^{-1}cm^{-1}$ that was acquired from a proline standard curve.

Determination of enzyme activities

In order to determine enzyme activities, leaf samples from control and salt-treated plants were homogenized with liquid nitrogen. Powders were suspended in specific buffers for enzyme activity analysis. The suspensions were centrifuged at 12 000 × *g* for 20 min at 4°C and the supernatants were used for activity analysis. The Bradford protocol was followed to determine the protein amounts in shoot extracts by using bovine serum albumin as a standard (Bradford, 1976).

The ascorbate peroxidase activity assay was carried out following the method of Wang et al. (1991). Homogenates were suspended in 1 mL of suspension solution containing 50 mM Tris-HCl (pH 7.2), 2% PVP, 1 mM EDTA and 2 mM ascorbate. The required amount of suspension containing 100 µg of protein was added to the assay medium consisting of the 50 mM potassium phosphate buffer (pH 6.6), 0.25 mM ascorbate and 1 mM H_2O_2. APX activity was assayed by measuring the oxidation of ascorbate at 290 nm and at room temperature for 90 s. One unit of APX was defined as the amount of enzyme required to consume 1 nanomole ascorbate min^{-1}·mg^{-1}protein.

Catalase activity determination was done according to the method developed by Chance et al. (1995). Homogenates were suspended in 1 mL of 50 mM Tris-HCl suspension solution at pH 7.8. The assay medium consisted of 50 mM potassium phosphate buffer at pH 7 and 10 mM H_2O_2. Addition of the required amount of suspension containing 100 µg of protein initiated the decrease in the H_2O_2 absorbance. Reaction was followed for 90 s at 240 nm at room temperature. One unit of CAT was defined as the amount of enzyme required to consume 1 nmol hydrogen peroxide 1 nanomole ascorbate min^{-1}·mg^{-1}protein.

Glutathione reductase (GR) activity was determined according to the method of Sgherri et al. (1994). Homogenates were suspended in 1 mL of suspension solution containing 100 mM potassium phosphate buffer (pH 7.8), 1% PVP, 0.1 mM EDTA and 0.5 (v/v) Triton X-100. The assay medium consisted of 200 mM potassium phosphate buffer (pH 7.5), 0.2 mM EDTA, 1.5 mM $MgCl_2$, 0.25 mM GSSG and 25 µM NADPH. Oxidation of NADPH was started with the addition of suspension from the samples containing 100-µg protein and the reaction was monitored continuously for 90 s at 340 nm at room temperature.

POX activity was determined according to Shannon et al. (1996). The reaction mixture consisted of 3 mL of 0.1 M phosphate buffer (pH 7.0), 0.1 M H_2O_2 (0.04 mL), 0.2 % o-dianisidine (0.04 mL) and sample containing 25 µg of protein. The change in absorbance was recorded at 470 nm for 90 s.

Data analysis

Experiments were performed with three to five replicates per analysis. The significance of the treatment effects was determined at 5% probability level by using one-way ANOVA with MINITAB 15.0.

RESULTS

Growth parameters

Shoot biomasses of either cultivar were not significantly affected by the NaCl treatments, while root biomasses decreased 50% in both cultivars under the 300-mM salt treatment (Table 1). Total length reduction in shoots of Gazipaşa was significant with 32% and 84% under 100 mM and 300 mM salt treatments, respectively, whereas the reduction in shoot length of Florispan was significant with 50% only under the 300-mM salt treatment. Similarly, while root lengths of Gazipaşa significantly decreased by 37% and 53% upon 100-mM and 300-mM NaCl treatments, respectively, a significant 32% reduction in root length of the cultivar Florispan only took place under the 300-mM salt treatment. In both cultivars, root/shoot ratios showed 2.4-fold increases on average only under the 300-mM salt treatment. The decrease in SWC was significant in the 100- and 300-mM salt-treated shoots of Gazipaşa. On the other hand, the cultivar Florispan and root tissue of Gazipaşa did not lose water under the two different intensities of salt stress.

Table 1. The effect of 12 days NaCl treatment on growth parameters.

Concentration of NaCl (mM)	Shoot dry mass (mg)	Root dry mass (mg)	SWC (%)	RtWC (%)	Shoot Length (cm)	Root Length (cm)	Root/shoot ratio
	Response of cultivar Florispan						
0	0.23±0.02	0.12±0.01	84.34	91.42	17.50± 1.91	17.67±1.67	2.59±0.39
100	0.26±0.03	0.11±0.02	75.77	90.60	14.17± 2.20	12.17±2.05	2.28±0.49
300	0.19±0.01	0.06*±0.01	77.55	90.79	8.75*±0.44	12.00*±0.50	
	Response of cultivar Gazipaşa						
0	0.22±0.01	0.12±0.01	91.72	90.93	25.83±1.67	15.50±1.04	1.52±0.19
100	0.24±0.02	0.11±0.00	74.06*	91.28	17.50*±0.72	9.83*±0.44	1.41±0.10
300	0.22±0.01	0.06*±0.01	58.63*	92.28	4.17*±1.44	7.33*±0.88	4.50*±0.76

SWC – shoot water content; RtWC – root water content. *The values are significantly different on 5% significance level when compared to the control treatment

Table 2. The effect of 12 days NaCl treatment on membrane integrity parameters, PSII activity (Fv/Fm), H_2O_2 and proline content in shoot tissues

Concentration of NaCl (mM)	MDA (nmol/gFW)	Ion Leakage (%)	H_2O_2 (nmol/gFW)	Proline (µmol/gFW)	Fv/Fm
	Response of cultivar Florispan				
0	9.60±1.39	11.20±0.42	6.29±1.54	0.79±0.19	0.69±0.01
100	10.17±1.51	15.90±0.12	11.12±1.12	11.69*±4.79	0.69±0.02
300	18.02*±3.11	87.06*±2.90	13.98*±0.63	37.31*±1.32	0.63±0.04
	Response of cultivar Gazipaşa				
0	11.08±2.53	13.02±1.46	7.27±0.33	0.89±0.30	0.71±0.01
100	12.17±2.39	16.80±0.30	11.59±0.99	0.51±0.18	0.72±0.01
300	25.27*±3.74	96.5*±4.46	15.13*±0.85	27.88*±8.71	0.69±0.01

MDA stands for malondialdehyde. *The values are significantly different on 5% significance level when compared to the control treatment.

Membrane integrity parameters, PSII activity, H_2O_2 and proline content

Malondialdehyde levels, H_2O_2 levels and ion leakage percentage exhibited similar significant increases only under 300-mM salt stress in both cultivars (Table 2). The increase in ion leakage was severe, on average 7.5-fold as compared to the non-treated peanut plants. The photosystem II activities of both cultivars remained unaffected under both intensities of salt stress. A difference in the responses of the two cultivars was only apparent in terms of proline levels, with the cultivar Florispan showing a 15-fold increase under the 100-mM NaCl treatment, while the proline level in the cultivar Gazipaşa remain unaffected at the same NaCl concentration. Proline levels in both Florispan and Gazipaşa cultivars increased 31-fold and 47-fold under the 300-mM salt treatment, respectively.

Enzymatic activity

Responses of the antioxidative enzymes were also very similar in the two cultivars in terms of activities. APX activity remained unchanged in both cultivars under the two levels of salinity stress, while CAT activity significantly decreased in both cultivars (Table 3). POX activities noticeably but insignificantly increased under salt treatment. GR activity exhibited a significant increase under 100-mM salt stress.

DISCUSSION

Under intense salt treatment, the shoot and root dry masses were affected similarly for both cultivars that had given different responses under drought stress in our previous study (Celikkol et al., 2010). The reduction in dry mass for both cultivars was especially

Table 3. The effect of 12 days NaCl treatment on the activities of APX, CAT, GR and POX enzymes in shoot tissues

Concentration of NaCl (mM)	APX (nmol Asc min^{-1} mg^{-1}prot)	CAT (nmolH_2O_2 min^{-1} mg^{-1}prot)	GR (nmol NADPH min^{-1} mg^{-1}prot)	POX (nmol o-dianisidine min^{-1} mg^{-1}prot)
	Response of cultivar Florispan			
0	395.67±58.9	3.74±0.67	13.15±1.32	10249±2681
100	265.60±50.4	2.83*±0.08	25.08*±2.02	15536.27±1083
300	309.33±74.7	2.49*±0.59	18.80*±1.33	16277.53±284
	Response of cultivar Gazipaşa			
0	381.57±52.8	7.11±0.67	13.30±1.35	9156±1837
100	517.33±29.3	3.86*±0.25	26.88*±0.91	11372.83±1770
300	316.63±53.3	4.90*±0.09	22.75*±2.75	18859.17±4661

*The values are significantly different on 5% significance level when compared to the control treatment.

intense with the 300-mM NaCl-treated roots. On the contrary, under mild drought stress, both cultivars responded with the production of an extensive root system, which resulted in increased root dry mass (Celikkol et al., 2010; Zhang et al., 2012). It could be proposed that specific adaptations for osmotic stress disappear when Na^+ and Cl^- ions accumulate in toxic concentrations in addition to water deprivation under salt stress. Gazipaşa responded with reduced SWC, shoot and root lengths upon 100-mM salt exposure, while Florispan did not exhibit any significant change for this intensity of stress treatment. Comparison of the physiological parameters of the drought-resistant cultivar Florispan with the drought-sensitive cultivar Gazipaşa under salt-mediated stress points to the superiority of Florispan in salt and drought tolerance.

Shoot growth is known to be more sensitive than root growth under drought and salt stress conditions. It was proposed that a reduction in leaf area development relative to root growth would decrease the water use by a plant, thus allowing it to conserve soil moisture (Munns and Tester 2008). In this study, root/shoot ratios as physiological response determinants significantly increased for both cultivars upon treatment with 300 mM NaCl, while no increment was detected for the 100-mM NaCl treatment. Singh and Chatrath (2001) also observed similar results by stating that salt stress reduces dry matter content, increases root:shoot ratio, and diminishes leaf size.

In spite of the physiological superiority of the cultivar Florispan under salt stress, membrane integrity parameters, H_2O_2 level, Fv/Fm ratio and activities of most of the antioxidative enzymes exhibited very similar patterns in two cultivars. Levels of MDA, which is the end product of membrane fatty acid peroxidation, increased with the intensity of salt stress and exhibited nearly 2-fold increases under 300-mM salt treatment in both cultivars. The increase in H_2O_2 levels also showed a similar pattern to MDA levels in both cultivars. Under drought stress, increased activities of APX in both cultivars and CAT activity particularly in Florispan were possibly effective in controlling H_2O_2 levels in the tissues (Celikkol et al., 2010). However, under salt stress, no significant activity change was observed for APX, and the activity of CAT significantly decreased under 100- and 300-mM NaCl treatment in both cultivars. This may correspond to the disruption of intracellular ion homeostasis and inhibition of metabolic activity by the toxic effects of Na^+ and Cl^- ions (Hasegawa et al., 2000) at concentrations approaching 100 mM, which was also determined in previous *in vitro* studies (Greenway and Osmond, 1972; Flowers and Dalmond, 1992; El-Danasoury, 2010). It was also indicated by Zhu (2007) that excess amounts of Na ions at the root surface interfere with plant K nutrition. Because of the similar chemical nature of Na and K ions, Na has a powerful inhibitory effect on K uptake by the root. Potassium deficiency inevitably prompts growth inhibition because K, as the most abundant cellular cation, plays a critical role in maintaining cell turgor, membrane potential and enzyme activities. Deterioration in membrane integrity was similar in both cultivars, not being affected

by the 100-mM salt treatment and exhibiting nearly a 7.5-fold increase in the leakage of solutes with the 300-mM salt treatment.

Accumulation of various amino acids, especially proline is a common plant response under salt stress (Girija et al., 2002). It is known that proline has protective functions as an osmolyte, contributes to the maintenance of the redox balance and is a component of stress relief and development (Szabados and Savouré, 2010). Many studies also reported that salinity resulted in substantial proline accumulation in leguminous plants, including alfalfa, soybean, pea, peanut, green gram and chickpea (Tramontano and Jouve, 1997; Jain et al., 2001; Girija et al., 2002; Eyidogan and Oz, 2007; Panda and Khan, 2009), the amount depending on the species, cultivar, tissue type and age. The cultivar Florispan was more efficient in the production of proline under intense drought stress when compared to the drought-sensitive Gazipaşa (Celikkol et al., 2010), and gave a similar response under salt stress. Significant proline accumulation was observed in Florispan upon exposure to 100-mM NaCl stress, while the decrease in proline levels observed in Gazipaşa under 100-mM NaCl stress was statistically insignificant. For the intense level of salt treatment, responses were severe with 47- and 31-fold increases in proline content for Florispan and Gazipaşa, respectively. The availability of water to plants is limited by the redundant quantities of soluble salts in the growth environment. A decrease in plant water potential must be balanced immediately by a decrease in osmotic potential through increased solute content in order to maintain the turgor potential (Mudgal et al., 2010) and this response is claimed to be an effective stress-tolerance mechanism. Under mild stress conditions, our results confirm these findings since the increments in proline concentration were higher in Florispan, which has stable growth measurements when compared to Gazipaşa.

In this study, distinctive reductions in growth parameters also show the variation of energy expenditure between the cultivars during osmotic adjustment to abiotic stress. This kind of energy consumption was previously stated by Greenway and Gibbs (2003) as one of the main factors for reduced growth. Although proline accumulation possibly has an important role in osmoregulation and antioxidative protection against the deleterious effects of salinity, especially in Florispan, the level of proline accumulation in both cultivars under intense salt stress conditions might also result from the breakdown of proline-rich proteins as previously proposed by Tewari and Singh (1991).

As in the case of drought stress, the photosynthetic apparatus of neither of the peanut cultivars was negatively affected by the two different levels of salt stress. Cornic et al. (1992) reported that a remarkable resistance of the photosynthetic apparatus to water shortage exists and 30% leaf-water deficit has been estimated as the limit above which the photosynthetic process is significantly affected. In this study, in spite of the observation of a 36% decrease in the SWC of Gazipaşa, the Fv/Fm ratio, which is an indicator of the efficiency of photosystem II, was not affected, demonstrating the high resistance of peanut against oxidative stress and ion toxicity. Oxidative stress resistance in peanut was also previously stated by Reddy et al. (2003) and Govind et al. (2009), whereas much lower concentrations of NaCl adversely affected the photosynthetic machinery in different genotypes of the legume *Lotus* (Melchiorre et al., 2009), common bean (Gama et al., 2009) and barnyard grass (Abogadallah et al., 2010).

Various enzymes are known to take roles in defense against ROS under stress conditions. They catalyze the synthesis, degradation and recycling of antioxidant molecules and can directly sustain the removal of ROS from the cells. Some controversy exists regarding the patterns of antioxidative enzyme activities under oxidative stress. It is known that, in some cases, salt-sensitive cultivars respond by increasing the levels of antioxidative enzymes (Munns and Tester, 2008). Contradictory results were also obtained in relation to the effect of salt and drought on the activity and level of various isozymes of SOD and APX (Gueta-Dahan et al., 1997). It was reported that in pea the response of salt-tolerant and salt-sensitive cultivars in terms of enzymatic and non-enzymatic metabolite production under salt-induced oxidative

damage was not consistent, with CAT being the only reliable marker of salt tolerance in a set of pea cultivars (Noreen and Ashraf, 2009). It is usually difficult to presume an expected pattern for enzyme activities under stress conditions since they are highly variable depending on the plant species, cultivar, type and age of tissue as well as the type and intensity of the oxidative stress. In the present study, the activity of APX remained unaffected under the salt treatments, whereas the activity of CAT showed a sharp decrease in both cultivars, explaining the uncontrolled increase in H_2O_2 levels and subsequent increases in MDA and ion leakage in peanut shoots. It was reported that CAT and APX activities showed no significant change in green gram leaves under short-term salinity stress of 150 mM (Panda and Khan, 2009). The activities of the two enzymes showed a steep decline in common bean after 10 days of salinity treatment at an NaCl concentration of 100 mM (Gama et al., 2009). Noreen and Ashraf (2009) also reported a decreased activity of CAT in pea shoots under 120-mM salinity stress after 20 days of treatment. Swaraj and Bishnoi (1999) also proposed that salinity decreases the activity of H_2O_2-scavenging enzymes like CAT and APX in legumes. However, both CAT and APX appeared to take a role in oxidative stress tolerance in peanut under PEG-mediated drought stress (Celikkol et al., 2010), indicating the adverse effect of the ion toxicity component of salinity stress on the activity of particular antioxidative enzymes.

On the other hand, Harinasut et al. (2003) indicated that the decrease in the activity of CAT in some plants is the consequence of the change in peroxidase activity as well as of the SOD/ascorbate-glutathione cycle as oxygen reactive scavenging systems. In this study, it was observed that although not significant, POX activity increased with the intensity of stress in both cultivars. In the ascorbate-glutathione pathway, GR catalyzes the rate limiting last step, thus the increase in GR activity in plants results in the accumulation of glutathione (GSH) levels and ultimately confers tolerance to plants. Higher GSH levels were observed in tobacco transgenics overexpressing the dehydroascorbate gene, and the increased GR levels effectively protected these plants from membrane damage when subjected NaCl (Lee et al., 2007). We observed that salt stress markedly increased only the activity of GR in both cultivars. A two-fold increase in the activities was obtained in the presence of 100 mM NaCl, while although significantly higher when compared to the control, lower activities was detected in the presence of 300 mM NaCl, possibly due to ion accumulation. In this study, no sign of membrane damage was observed in terms of MDA content and ion leakage under milder NaCl stress conditions, which might be the result of increments in GR activities. Therefore, our results confirmed that GR is important for plant protection against oxidative stress. Further investigations in GR mRNA levels of salt-stressed peanut tissues may reveal whether the decrease in enzyme activity results from a decrease in transcription levels or from enzyme inactivation.

CONCLUSIONS

Although differing in terms of physiological features, especially under 100-mM salt treatment, the two cultivars responded in a very similar manner for every parameter investigated, except proline content, which showed a 21-fold increase in Florispan shoots. Salinity tolerance appears to have more components than drought tolerance and among the processes that govern tolerance, proline level and the activity of GR appear to play an important part in the protection of peanut tissues under salt stress.

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