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Drought-induced oxidative damage and antioxidant responses in peanut (*Arachis hypogaea* L.) seedlings

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Abstract Two cultivars of peanut (*Arachis hypogaea* L.) which were designated as resistant (Florispan) and sensitive (Gazipasa) according to their growth retardation under drought stress conditions were compared for their oxidative damage and antioxidant responses. Sixteen days-old peanut seedlings were subjected to PEG-6000 solutions of two different osmotic potentials; −0.4 and −0.8 MPa, and various growth parameters, photosystem II activity, changes in malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and proline levels, activities of ascorbate peroxidase (APX), catalase (CAT), peroxidase (POX) and gluthatione reductase (GR) enzymes were determined. Both cultivars exhibited water deficit at −0.8 MPa osmotic potential of PEG-6000 and H_2O_2 levels significantly increased during exposure to −0.4 MPa osmotic potential. However, H_2O_2 levels were under control in both cultivars at exposure to −0.8 MPa osmotic potential. Significant proline accumulation was observed in the tissues of cv. Florispan at −0.8 MPa osmotic potential, whereas proline accumulation did not appear to be an essential part of the protection mechanism against drought in cv. Gazipasa. No significant variation in chlorophyll fluorescence values were detected in neither of the cultivars. Enzyme activity measurements revealed that Gazipasa copes well with lesser magnitudes of drought stress by increasing the activity of mainly APX, and during harsh stress conditions, only APX maintains its activity in the tissues. In cultivar Florispan, GR activity appears to take role in lesser magnitudes of drought stress, whereas CAT and APX activities appear to be very crucial antioxidative defenses during intense stress conditions. The results indicate that, the level of proline and activities of the enzymes CAT and APX are important mechanisms for the maintenance of drought tolerance in peanut plants.

Keywords *Arachis hypogaea* · Drought stress · Antioxidant enzymes · Proline · Lipid peroxidation · Oxidative stress

Abbreviations

PEG	Polyethylene glycol
MDA	Malondialdehyde
APX	Ascorbate peroxidase
CAT	Catalase
POX	Peroxidase
GR	Gluthatione reductase
SOD	Superoxide dismutase
RWC	Relative water content

Introduction

Peanut (*Arachis hypogaea* L.), which is cultivated on 23 million ha area with an annual production of about 35 million tons, is one of the most important legume crops grown worldwide. It is produced mainly for its high quality edible oil and proteins. In the semi-arid tropics, where about 70% of the peanuts are grown, drought is a major constraint to peanut production. In fact, water shortage is the main constraint to plant growth and productivity over

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much of the land surface (Kramer 1983). The change in water content and subsequently in cell volume are decisive factors explaining the effects of desiccation, since osmotic adjustment is a mechanism to maintain cell volume during drought in natural conditions (Kaiser 1987).

The first symptom of drought is rapid inhibition of shoot and, to a lesser extent, root growth. This is closely followed by stomatal closure with associated reductions in transpiration and CO_2 uptake for photosynthesis. The generation rate of activated oxygen species (AOS) such as H_2O_2 (hydrogen peroxide), O_2^- (superoxide), O_2 (singlet oxygen) and OH (hydroxyl) radicals increase in these hostile conditions, enhancing leakage of electrons to molecular oxygen. These cytotoxic AOS can destroy normal metabolism through oxidative damage to lipids, proteins and nucleic acids (Fridovich 1986). The peroxidation of lipid membranes in vivo further ensures a steady supply of free radicals (Jain et al. 2001). Extended drought condition leads to interrupted reproductive development, premature leaf senescence, wilting, desiccation and death (Schulze 1986).

Plants have evolved oxygen-scavenging system consisting of non-enzyme antioxidant metabolites, such as ascorbate (AsA), reduced glutathione (GSH), proline and various antioxidant enzymes including SOD, APX, CAT, POX and GR (Bowler et al. 1992). One of the best-characterized biochemical responses of plant cells to osmotic stress is the accumulation of organic osmolytes like proline and betaines (McCue and Hanson 1990). Proline is known to form long-lived adducts with free radicals, thus mitigating their damaging potential (Floyd and Zs-Nagy 1984). It also stabilizes membranes and maintains protein conformation at low leaf water potentials (Reddy et al. 2004). Proline was also found to be effective hydroxyl radical scavenger in vitro (Smirnoff and Cumbes 1989). The enzyme SOD dismutate O_2^- to H_2O_2 and is present in the cytosol and different organelles (Ushimaru et al. 1995). Catalase eliminates H_2O_2 by breaking it down to H_2O and O_2. Unlike APX, which is dependent on the presence of NADPH as a reducing agent, catalase does not require any reducing equivalents (Mallick and Mohn 2000). Peroxidases which are located in cytosol, vacuole as well as in extracellular space scavenge H_2O_2 by oxidation of various substrates. In higher plants, several distinct isozymes of APX which are located in cytosol and various organelles convert H_2O_2 to H_2O using ascorbate as an electron donor (Madhusudhan et al. 2003). Since ascorbate is oxidized to monodehydro ascorbate in APX catalyzed H_2O_2 decomposition, a system for regeneration of ascorbate is necessary. In plant cells, a regenerating cycle involving AsA, GSH and specific enzymes monodehydroascorbate reductase, dehydroascorbate reductase and GR is present which is designated as ascorbate-gluthatione cycle or Halliwell Asada pathway (Foyer and Halliwell 1976). As part of this cycle, the reduction of oxidized gluthatione to GSH is carried out by GR in a NADPH dependant reaction (Noctor and Foyer 1998).

There is controversy on the responses of CAT, APX and GR activities to osmotic stress (Bai et al. 2009). The effects of water stress reported in the literature on enzyme activities are disputable, depending both on the degree of tolerance of the plant, and on the way of water stress (Contour-Ansel et al. 2006). In any case, these systems in overall, help plants growing in adverse environments alleviate the deleterious effect of active oxygen species (AOS) which are produced at increased rates (Jebara et al. 2005).

Water deficit is one of the most important abiotic stresses and strategies to sustainable use of water and extensive screening for drought resistant peanut cultivars are of extreme importance. In addition, improvement of plant drought resistance are urgent and should integrate conventional breeding and biotechnological approaches. Identification of the physiological and biochemical components of antioxidative defense system, which has a potential to confer drought tolerance, is essential for the characterization of tolerant cultivars and improve drought stress tolerance in crops. The studies on peanut that investigate the biochemical basis of stress responses are scarce and to our knowledge, none of them studied the involvement of antioxidative defense mechanisms in drought tolerance. In the present work, two different concentrations of polyethylene glycol (PEG) were used to imitate different levels of water stress in four different cultivars of peanut. Changes in growth parameters, relative water content (RWC), lipid peroxidation, proline content and activities of antioxidant enzymes CAT, APX, GR, and POX were examined and compared in two peanut cultivars which physiologically differ in their sensitivity to drought stress.

Materials and methods

Plant materials, growth conditions and stress treatments

Seeds of peanut (*Arachis hypogaea* L.) cultivars; Florispan (Spanish type) and Gazipasa (Turkish type) were kindly provided by West Mediterranian Agricultural Research Institute (Turkey). The seeds were surface sterilized with 2% sodium hypochloride solution and then rinsed three times with sterile distilled water. Plants were grown in pots filled with perlite in a controlled growth chamber at 23 ± 2°C with 16 h light (400 μmol m^{-2} s^{-1}) and 8 h dark photo-cycle. Perlite provided a controlled medium, content of which depends only on the nutrient

solution used. Seeds were germinated by using half strength Hoagland's solution (Hoagland and Arnon 1950). On 16th day of germination, drought stress treatments were initiated by applying half strength Hoagland's solution containing polyethylene glycol-6000 (PEG 6000) of −0.4 and −0.8 MPa osmotic potential, for 12 days. Control experiments were also carried out with the seedlings, which were given half strength Hoagland's solution without PEG. Plants were harvested on the 28th day of growth. Each set of experiment was performed at least three times.

Growth parameters

Shoot and root parts of peanut cultivars Florispan and Gazipasa were removed after 28 days of growth and lengths/fresh masses were measured. The dry masses of tissues were recorded after drying in an oven at 70°C for 48 h. Two cultivars were designated as resistant and sensitive according to length, fresh mass and dry mass of their shoot and roots.

Relative water content

Six leaf discs from cv. Florispan and cv. Gazipasa, which were collected after 12 days of stress treatments, were used for relative water content (RWC) determination. After wet mass determination, the tissues were floated on distilled water for 24 h at room temperature. The hydrated shoot tissues were weighed to determine the turgid mass (TM). The tissues were subsequently dried in an oven at 60°C for 48 h and weighed to determine the dry masses. Relative water content was calculated according to the Smart and Bingham (1974) by using the formula; $RWC(\%) = \{WM-DM\}/\{TM-DM\} \times 100$.

Chlorophyll fluorescence

In dark-adapted leaf tissues, "Fv/Fm" value indicating the maximum photochemical yield of PSII was determined by the use of OS5-FL Modulated Fluorometer.

Determination of membrane damage

Membrane electrolyte leakage and lipid peroxidation in terms of malondialdehyde (MDA) content were determined to assess the membrane damage after drought stress treatments. Membrane leakage was estimated by the measurement of electrolyte leaked from leaves according to the method of Nanjo et al. (1999). 6 leaves per plant were put into separate 15 ml falcon tubes and immersed in 5 ml of 0.4 M mannitol at room temperature with gentle shaking for 3 h. Electrical conductance were measured and recorded by using Mettler Toledo MPC 227 conductivity meter as C1, indicating initial conductivity. The tubes containing the samples were placed in boiling water for 10 min and cooled down to the room temperature. The conductances were measured and recorded as C2. The conductivity due to leakage is expressed as the percentage of the initial conductivity over the total conductivity [(C1/C2) × 100].

Lipid peroxidation was determined by estimating the malondialdehyde (MDA) content according to the method of Ohkawa et al. (1979). Fresh shoot tissues were weighed as 0.2 g and homogenized with liquid nitrogen by the addition of 1 ml of 5% trichloroacetic acid (TCA). The homogenates were transferred to tubes and centrifuged at 12000 rpm for 15 min at room temperature. Freshly prepared 0.5% thiobarbituric acid (TBA) in 20% TCA and supernatant in equal volumes were put into eppendorf tubes and incubated for 25 min at 96°C. The tubes were placed in ice bath and then centrifuged at 10000 rpm 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. MDA contents were calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}.

H_2O_2 content determination

H_2O_2 content was estimated according to the method of Bernt and Bergmeyer (1974). About 0.5 g shoot tissue from both control and treatment groups were homogenized with liquid nitrogen and the powders were suspended in 1.5 ml of 100 mM potassium phosphate buffer at pH 6.8. The suspensions were then centrifuged at 18000 g for 20 min at 4°C. Enzymatic reaction was started with 0.25 ml supernatant and 1.25 ml peroxidase reagent consisting of 83 mM potassium phosphate buffer at pH 7.0, 0.005% (w/v) o-dianizidine, 40 μg peroxidase/ml at 30°C. The reaction was stopped after 10 min by adding 0.25 ml of 1 N perchloric acid and the reaction mixture was centrifuged at 5000 g for 5 min. The absorbance of the supernatant was measured at 436 nm and the amount of hydrogen peroxide determined by using an extinction coefficient of 39.4 mM^{-1} cm^{-1}.

Proline content determination

Proline contents were calculated according to the method of Bates et al. (1973). About 0.3 g shoot tissue from both control and treatment groups were homogenized with liquid nitrogen and the tissue powders were suspended in 1 ml of 3% sulphosalicilic acid. Following centrifugation at 1000 g for 5 min at 4°C, 0.1 ml of supernatants were mixed with 0.2 ml acid ninhydrin, 0.2 ml 96% acetic acid

and 0.1 ml 3% sulphosalicilic acid. The mixtures were incubated at 96°C for 1 h, mixed with 1 ml toluene and further centrifuged at 1000 g for 5 min at 4°C. Upper phases were collected and the absorbencies were read at 520 nm. The amounts of proline were determined by using an extinction coefficient of 0.9986 mM^{-1} cm^{-1} that was derived from proline standard curve.

Determination of enzyme activities

Leaf samples from control and drought treated groups were homogenized with liquid nitrogen and suspended in specific buffers for enzyme activity analysis. The suspensions were centrifuged at 12000 g during 20 min at 4°C and the supernatants were used for activity analysis by considering protein amounts. The protein amounts in shoot extracts were determined by Bradford method (Bradford 1976) using bovine serum albumin as a standard.

Ascorbate peroxidase activity determination was done according to the method of Wang et al. (1991). Samples containing 100 μg protein 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. Assay medium consisted of 50 mM potassium phosphate buffer (pH 6.6), 0.25 mM ascorbate and 1 mM H_2O_2 which initiated the reaction. The decrease in the absorbance of ascorbate was monitored for 90 s at 290 nm at room temperature. Nanomole ascorbate consumed per min was defined as one unit of APX.

Catalase activity determination was done according to the method of Chance and Maehly (1995). Samples containing 100 μg protein were suspended in 1 ml of 50 mM Tris–HCl suspension solution at pH 7.8. Assay medium was consist of 50 mM potassium phosphate buffer at pH 7 and 10 mM H_2O_2. The decrease of the H_2O_2 absorbance was followed for 90 s at 240 nm at room temperature. Nanomole hydrogen peroxide consumed per min was defined as one unit of CAT.

POX activity was determined according to Shannon et al. (1966). The reaction mixture consisted of 3 ml, 0.1 M phosphate buffer (pH 7.0), 0.04 ml, 0.1 M H_2O_2, 0.04 ml, 0.2% *O*-dianisidine and sample containing 25 μg protein. The change in absorbance was recorded at 470 nm for 90 s.

GR activity was determined according to the method of Sgherri et al. (1994). Samples containing 100 μg protein were suspended in 1 ml of suspension solution containing 100 mM K-PO_4 buffer (pH 7.8), 1% PVP, 0.1 mM EDTA and 0.5 (v/v) Triton X-100. 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 monitored continuously for 90 s at 340 nm at room temperature.

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 Tukey test, which is one of the Post Hoc multiple comparisons of one-way ANOVA of SPSS 11.0 (Statistical Package for Social Sciences, SPSS Inc., Illinois).

Results

Growth parameters

Shoot and root dry masses of Gazipasa cultivar were affected considerably by PEG treatments while no significant change was observed in Florispan tissues (Table 1). Root dry mass of Gazipasa significantly increased when it was subjected to PEG solution with −0.4 MPa osmotic potential. However, osmotic potential of −0.8 MPa significantly decreased root dry mass. Although insignificant, root dry mass of Florispan increased with drought stress intensity. Growth retardation was more clear in Gazipasa cv. when compared to Florispan.

Shoot and root water contents decreased steadily on increasing concentrations of PEG for both cultivars except for the roots of Gazipasa that decreased with −0.4 MPa osmotic potential and increased at −0.8 MPa osmotic potential. Similarly shoot and root lengths decreased with increasing osmotic stress. However, Gazipasa roots and shoots showed an insignificant growth upon exposure to −0.8 MPa osmotic potential when compared to −0.4 MPa osmotic potential.

Membrane integrity parameters, PSII activity, H_2O_2 and proline content

Relative water content which is an expression of water retaining capacity decreased with increasing PEG concentrations and showed significant ($P < 0.05$) decrease for both cultivars at −0.8 MPa osmotic potential (Table 2). Malondialdehyde level, which is an indicator of lipid peroxidation increased significantly for the cultivar Florispan at both −0.4 and −0.8 MPa osmotic potentials. However, increase in MDA level for Gazipasa was insignificant for both osmotic potentials.

Ion leakage showed an insignificant increase at −0.4 MPa osmotic potential in Florispan and remained steady at −0.8 MPa. Although insignificant, ion leakage decreased with increasing concentrations of PEG for Gazipasa. Both cultivars showed a similar pattern for H_2O_2 content upon stress exposure. In both cultivars, H_2O_2 amount increased significantly at −0.4 MPa and reduced to

Table 1 The effect of 12 days-PEG treatment on growth parameters

Osmotic potential of PEG solution (MPa)	Shoot dry mass (mg)	Root dry mass (mg)	SWC (%)	RtWC (%)	Shoot lenght (cm)	Root lenght (cm)
	Response of cultivar Florispan					
0	0.39 ± 0.08	0.26 ± 0.03	81.43	90.07	21.67 ± 0.44	18.50 ± 0.29
−0.4	0.42 ± 0.03	0.28 ± 0.01	79.81	88.03	17.33 ± 0.33[a]	16.33 ± 0.17[a]
−0.8	0.22 ± 0.02	0.33 ± 0.02	74.71[a]	76.60[a]	15.00 ± 0.29[a]	15.83 ± 0.17[a]
	Response of cultivar Gazipasa					
0	0.38 ± 0.07	0.14 ± 0.02	85.39	91.03	23.83 ± 0.17	17.17 ± 0.17
−0.4	0.29 ± 0.05	0.24 ± 0.02[a]	82.21	86.52[a]	16.33 ± 0.67[a]	13.50 ± 0.29[a]
−0.8	0.14 ± 0.03[a]	0.08 ± 0.00[a]	80.56[a]	89.33[a]	18.50 ± 0.29[a]	13.83 ± 0.17[a]

SWC and RtWC stands for shoot and root water content, respectively

[a] The values are significantly different on 5% significance level when compared to control treatment

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

Osmotic potential of PEG solution (MPa)	RWC (%)	MDA (nmole/gFW)	Ion leakage (%)	H_2O_2 (nmole/gFW)	Proline (μmole/gFW)	Fv/Fm
	Response of cultivar Florispan					
0	44.37 ± 1.47	13.38 ± 0.60	49.25 ± 3.29	6.31 ± 1.54	0.73 ± 0.38	0.69 ± 0.00
−0.4	41.20 ± 3.49	19.05 ± 1.83[a]	55.55 ± 9.08	12.86 ± 1.76[a]	2.00 ± 1.22	0.67 ± 0.01
−0.8	31.31 ± 1.39[a]	20.08 ± 0.56[a]	49.41 ± 2.85	9.54 ± 1.70	2.89 ± 1.18[a]	0.69 ± 0.00
	Response of cultivar Gazipasa					
0	51.51 ± 4.23	16.04 ± 1.60	44.41 ± 2.53	4.20 ± 2.06	0.91 ± 0.48	0.71 ± 0.01
−0.4	50.23 ± 4.29	18.18 ± 1.85	38.29 ± 7.78	13.05 ± 2.17[a]	1.53 ± 0.75	0.70 ± 0.00
−0.8	35.19 ± 0.97[a]	20.10 ± 0.35	32.37 ± 3.86	6.05 ± 2.05	2.10 ± 1.02	0.70 ± 0.01

RWC and MDA stands for relative water content and malonedialdehyde, respectively

[a] The values are significantly different on 5% significance level when compared to control treatment

harmless levels at −0.8 MPa osmotic potential. Although insignificant, amount of proline in the tissues increased steadily with increasing concentrations of PEG. Fv/Fm value which shows the maximum photochemical yield of PSII in dark-adapted leaves was not significantly affected in both cultivars under PEG-mediated drought stress.

Enzymatic activity

For the cultivar Gazipasa, APX activity increased twofold and fourfold, respectively for −0.4 and −0.8 MPa osmotic potential (Table 3). For the cultivar Florispan, the enzyme activity remained unchanged during −0.4 MPa and increased only twofold at −0.8 MPa osmotic potential.

CAT activity significantly increased only in Florispan at −0.8 MPa osmotic potential. For the cultivar Gazipasa, although insignificant, the activity increased at −0.4 MPa osmotic potential and decreased under the level possessed by control at −0.8 MPa osmotic potential. There is no significant change in the activity of POX in both cultivars and GR activity increased significantly only on Florispan tissues upon drought stress treatment with −0.4 MPa osmotic potential.

Discussion

In this study, water status of the plants was significantly affected by harsh osmotic treatment. Although no significant RWC decrease was observed at −0.4 MPa osmotic potential, both cultivars exhibited water deficit at −0.8 MPa. Despite the presence of water deficit, neither of the stress parameters leaded to significant growth retardation in the cultivar Florispan as assessed by shoot and root dry masses (Table 1). However, shoot dry mass and root dry mass significantly reduced at −0.8 MPa osmotic potential in Gazipasa, although the production of an extensive fibrous root system, which resulted in a significantly high root dry mass, was one of the most distinctive anatomical responses given by that cultivar at −0.4 MPa osmotic potential (Table 1). It is known that plants may escape drought stress by cutting short their growth

Table 3 The effect of PEG treatment on the activities of APX, CAT, GR and POX enzymes in shoot tissues

Osmotic potential of PEG solution (MPa)	APX (nmole/ Asc/min/mg)	CAT (nmole/ H_2O_2/min/mg)	GR (nmole/ NADPH/min/mg)	POX (nmole/o-dianizidine/min/mg)
	Response of cultivar Florispan			
0	268.3 ± 36.9	3.21 ± 0.07	16.10 ± 1.57	10249 ± 2681
−0.4	255.3 ± 11.4	3.73 ± 0.13	41.2 ± 10.8[a]	8747 ± 1874
−0.8	497.0 ± 10.4[a]	6.43 ± 0.25[a]	36.8 ± 12.9	12218 ± 1138
	Response of cultivar Gazipasa			
0	237.7 ± 24.0	7.09 ± 0.98	38.7 ± 18.1	9156 ± 1837
−0.4	479.3 ± 12.0[a]	9.44 ± 0.42	22.58 ± 4.55	14472 ± 2081
−0.8	966.0 ± 109[a]	4.52 ± 0.22	55.1 ± 28.0	13509 ± 1827

[a] The values are significantly different on 5% significance level when compared to control treatment

duration, and avoid the stress with the maintenance of high tissue water potential either by reducing water loss or improved water uptake, or both (Farooq et al. 2009). Since roots are the only source to acquire water from soil, the root growth, its density, proliferation and size are key responses of plants to drought stress (Kavar et al. 2007). In breeding studies, selection for a deep and extensive root system has been advocated to increase productivity of food legumes under moisture-deficit conditions as it can optimize the capacity to acquire water (Subbarao et al. 1995). In this study, although root lengths significantly decreased, both cultivars exhibited increased fibrous root production, which resulted in increased root dry masses upon exposure to drought stress of −0.4 MPa osmotic potential. Although this adaptation was still valid in Florispan tissues during −0.8 MPa osmotic potential, the same potential completely inhibited root growth in Gazipasa.

Lipid membranes are vulnerable targets for stress-induced cellular damage and the extent of damage is commonly used as a measure of tolerance to the imposed stress (Zhao and Harris 1992; Gadallah 1999). In this study, higher lipid peroxidation was observed in Florispan cultivar, although this cultivar was anatomically less affected (Table 2). However, no significant ion lekage was observed for either cultivar. This observation could be explained by the presence of a rapid phospholipid replacement mechanism for the protection of membrane integrity particularly in cultivar Florispan.

H_2O_2 levels significantly increased for both cultivars at −0.4 MPa osmotic potential (Table 2). However, H_2O_2 levels were under control in both cultivars at −0.8 MPa osmotic potential that may be attributed to the efficient removal of H_2O_2 by the increased activity of APX in both cultivars and CAT particularly in Florispan. In addition, the non-enzymatic breakdown of H_2O_2 into $OH^{\cdot}$ and OH^{-} possibly took place by the way of Haber–Weiss reaction under stressed tissues.

Significant proline accumulation (4-times that of control) was observed in Florispan tissues at −0.8 MPa osmotic potential whereas, proline accumulation does not appear to be an essential part of the protection mechanism against drought in Gazipasa (Table 2).

Environmental stresses that affect PSII efficiency are known to provoke a characteristic decrease in the Fv/Fm ratio (Krause and Weis 1991). However, remarkable resistance of the photosynthetic apparatus to water shortage was reported and 30% leaf water deficit has been estimated as the limit above which the photosynthetic biochemistry is significantly affected (Cornic et al. 1992). In this study, no significant variation in the Fv/Fm ratio was detected which suggest that the efficiency of PSII did not decline (Table 2). Stable Fv/Fm ratios also confirmed the previous observations that the photosynthetic machinery is resistant to a certain level of water deficit as discussed by other authors (Chaves et al. 2002; Cornic and Fresneau 2002; Kocheva et al. 2005).

Antioxidative enzyme activities constitute the major part of the plant antioxidative defense system. Enzyme activity measurements revealed that Gazipasa copes well with lesser magnitudes of drought stress by increasing the activities of mainly APX, and although insignificant, CAT and POX to some extent, whereas, during harsh stress conditions only APX maintained its activity (Table 3). As deduced from the morphological and physiological responses of Gazipasa tissues on −0.8 MPa osmotic potential, it can be concluded that the activity of APX alone could not provide the necessary protection for harsh levels of osmotic stress. Decline in the activity of the enzyme in Gazipasa under high level of drought stress might have been resulted from enhanced production of ROS and its interaction with the enzyme leading to its possible oxidation and inactivation.

However, in cultivar Florispan, GR activity, which increased 2.5-fold at −0.4 MPa osmotic potential appears

to take role in lesser magnitudes of drought stress. In addition to removing H_2O_2, increase in GR activity result in availability of NADP that can accept electrons from ferrodoxin, thereby minimizing chances of superoxide formation (Arora et al. 2002). In spite of its importance in stressed tissues, the activity of the enzyme was not significantly high at −0.8 MPa osmotic potential that was possibly caused by the subsequent oxidation of the enzyme.

CAT and APX activities appear to be very crucial antioxidative defenses in water-stressed Florispan tissues. Since the considerably unaffected growth parameters and anatomical integrity of Florispan after drought treatment were signs for a better protection mechanism, coordinated CAT and APX activities, which are dominant in Florispan tissues, can be considered as effective antioxidative defenses in the protection of peanut tissues against the effects of H_2O_2 during harsh drought conditions. It is known that APX and CAT belong to two different classes of H_2O_2 scavenging enzymes where APX is responsible for the fine modulation, whereas CAT is responsible for removal of the excess ROIs during stress. Since CAT does not require a supply of reducing equivalents for its function, it might be insensitive to the redox status of cells and its function might not be affected during prolonged stress, unlike other mechanisms (Mittler 2002). Therefore, increase in the activities of both enzymes might have provided better protection when compared to the increase in APX activity alone.

High levels of POX activity was reported in several studies including tepary bean (Türkan et al. 2005), *Picea aspertata* (Yang et al. 2008) and caper (Ozkur et al. 2009) under drought stress. However, in the present study, POX activity did not significantly increase in either cultivars of peanut upon PEG-mediated drought stress.

In conclusion, the experimental data showed that, two different cultivars respond in slightly different ways to the imposed stress in terms of individual components, while observations reflect also a general feature in the stress tolerance. Although PEG caused rapid dehydration, PSII retains its efficiency in both cultivars, which is in accordance with the hypothesis of Kocheva et al. (2005) considering the 30% limit of leaf water deficit in the functioning of the photosynthetic machinery under various stress conditions. In both cultivars, POX activity appeared not to be directly responsible from remarkable protection against oxidative injury, whereas APX activity might be important for Gazipasa, which is possibly equipped with mechanisms that only provide protection during mild drought conditions. Florispan appeared to be more resistant in terms of physiological and anatomical parameters and can be considered as a more tolerant cultivar. Unlike Gazipasa, it revealed significant increases in proline, CAT and APX levels on higher magnitudes of stress. Therefore, strategies for the improvement of proline, CAT and APX levels in peanut tissues might possibly increase drought tolerance in this plant and should be considered for the genetic improvement of the plant for its adaptation in poorly irrigated environments. Currently, research on the salt tolerance mechanisms for the same cultivars is being conducted, which could further help in understanding the mechanisms of oxidative stress tolerance in peanut.

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