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Antioxidative and physiological responses of two sunflower (*Helianthus annuus*) cultivars under PEG-mediated drought stress

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Abstract: Drought stress is one of the most important yield-reducing factors in crop production. Sunflower, an oilseed crop, is severely affected by abiotic stress. In this study, 2 sunflower cultivars (Musala and Aydın) were evaluated in terms of various biochemical and physiological responses under 2 different polyethylene glycol-mediated drought stress conditions. Stress-determining parameters such as malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and proline contents were determined. Both cultivars showed similar responses at osmotic potentials of both −0.4 and −0.8 MPa. Aydın was less affected than Musala under these stress conditions. MDA, H_2O_2, and proline levels were similar at both −0.4 and −0.8 MPa osmotic potentials in the 2 different cultivars. The 2 cultivars differed significantly in ascorbate peroxidase and catalase enzyme activities, which were more prominent in Aydın for both stress levels. However, glutathione reductase activity did not appear to be an essential part of the antioxidative system in either of the cultivars. Engineering antioxidative enzyme levels might provide a potential mechanism to cope with drought stress in sunflower.

Key words: *Helianthus annuus*, drought stress, antioxidant enzymes, proline

Introduction

Sunflower (*Helianthus annuus* L.) is an important agricultural crop and the main source of unsaturated vegetable oil. Sunflower is cultivated on 25,000,000 ha with an annual production of about 35,000,000 t. Europe is the major sunflower grower and produced about 65% of the total world sunflower seed in 2010. Sunflower is one of the few crop species that originated in North America; production is now moving toward western and drier climates (Albert & Schneiter, 1997). Today, the major producers are Eastern European countries, the former Soviet Union, and Argentina (Albert & Schneiter, 1997; Liu & Baird, 2003). Because of its moderate tolerance to drought and salinity conditions, sunflower production is expanding in the arid regions of the Mediterranean area and North Africa (Miller, 1995; Connor & Hall, 1997). Sunflower seeds contain a high amount of polyunsaturated fatty acids with over 90% linoleic (18:2) and oleic (18:1) acids, which have

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potential health benefits (Lopez-Pereira et al., 2000; Leon et al., 2003; Monotti, 2004). Although genotype is the most important factor that determines fatty acid composition (Knowles, 1988), the oil percentage and unsaturated fatty acid composition of the oil are also affected by environmental factors during all stages of growth and development.

Biotic and abiotic stresses are severe limiting factors of plant growth. Abiotic stress is the primary cause of crop loss worldwide, reducing average yields for most major crop plants by more than 50% (Bray et al., 2000). Drought stress is one of the most widespread environmental stresses when the usable areas of earth are classified according to stress factors (Arora et al., 2002). Although sunflower seeds are widely used for edible oil production, meal for ruminant animals, and snacks in human diets, production and availability are not sufficient due to the increase in arid zones and adverse climatic and agronomic conditions such as drought and salinity (Camacho Barron & Gonzalez de Mejia, 1998). Drought stress directly affects growth and productivity of plants by altering plant water status. The initial effect of drought on plant development is inhibition of shoot and root growth (Celikkol Akcay et al., 2010). This is followed by stomatal closure, which limits CO_2 fixation and reduces $NADP^+$ regeneration by the Calvin cycle (Satoh & Murata, 1998). This results in reductions in transpiration and CO_2 uptake for photosynthesis. During photosynthesis and under drought stress, there is a higher leakage of electrons to O_2 through the Mehler reaction (Smirnoff, 1993). This causes an increase in the rate of activated oxygen species (AOS) such as H_2O_2 (hydrogen peroxide), O_2^- (superoxide), O_2 (singlet oxygen), and $^{\cdot}OH$ (hydroxyl) radicals (Türkan et al., 2005). The accumulation of AOS can destroy normal metabolism through oxidative damage to lipids, proteins, and nucleic acids (Rabinowitch & Fridovich, 1983; Fridovich, 1986).

To diminish the damaging effects of AOS on cellular components, plants have developed a reactive oxygen species (ROS)-scavenging system consisting of both enzymatic and nonenzymatic antioxidant mechanisms. Nonenzymatic antioxidant metabolites include β-carotenes, α-tocopherol, ascorbate (AsA), and reduced glutathione (GSH) (Halliwell, 1987); enzymatic antioxidants consist of superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), peroxidase (POX), and glutathione reductase (GR) (Bowler et al., 1992). Plant cells can produce and accumulate organic osmolytes such as proline and betaines to cope with osmotic stress, as well as benefit from the protective role of antioxidant enzymes.

Drought and salinity are becoming particularly widespread in many regions and may cause serious salinisation of more than 50% of all arable lands by the year 2050 (Wang et al., 2003). Mazahery-Laghab et al. (2003) reported that 60% yield reduction was observed in sunflower after drought application. As a result, it has become more important to elucidate the drought tolerance mechanisms of sunflower to improve its agronomic performance and obtain more resistant sunflower cultivars. In the literature, investigation of the biochemical basis of stress responses in sunflower is based on a few cultivars. In order to evaluate the physiological responses of different cultivars grown in Turkey, 2 different concentrations of polyethylene glycol (PEG) were used to imitate different levels of water stress in 2 different sunflower cultivars. Changes in growth parameters, relative water content (RWC), lipid peroxidation, proline content, and activities of the antioxidant enzymes CAT, APX, and GR were examined and compared in 2 sunflower cultivars that differ physiologically in their sensitivity to drought stress.

Materials and methods

Plant materials, growth conditions, and stress treatments

Two cultivars of sunflower (*Helianthus annuus*), Aydın and Musala, were kindly provided by the Trakya Agricultural Research Institute (Turkey). After surface sterilisation, seeds were germinated in pots filled with perlite, and the plants were grown in a controlled growth chamber at 23 ± 2 °C with a 16-h light (400 μmol m^{-2} s^{-1}) and 8-h dark photocycle. Half-strength Hoagland's solution (Hoagland & Arnon, 1950) was used for irrigation. On day 15 of germination, drought stress treatments were initiated by applying half-strength Hoagland's solution containing polyethylene glycol-6000 (PEG

6000) at −0.4 (10% w/v) and −0.8 (20% w/v) MPa osmotic potential for 5 days. Control plants were grown in the same way and were watered with half-strength Hoagland's solution without PEG. Each set of experiments was performed at least 3 times with samples collected on day 20 of growth.

Growth parameters

Shoot and root tissues of sunflower cultivars Aydın and Musala were removed after 20 days of growth, and fresh weights were measured. Dry weights were determined after the tissues were held in an oven at 70 °C for 48 h.

Determination of malondialdehyde content

Lipid peroxidation in terms of malondialdehyde (MDA) content was determined for evaluation of membrane damage generated by drought stress treatments. MDA content was determined according to the method of Ohkawa et al. (1979). Fresh root and leaf tissues were weighed to 0.2 g and homogenised with liquid nitrogen by the addition of 1 mL of 5% trichloroacetic acid (TCA). The homogenates were transferred to tubes and centrifuged at 12,000 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 an ice bath and then centrifuged at 10,000 rpm for 5 min. Absorbance of the supernatant was determined at 532 nm, and the correction for nonspecific 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 determined according to the method of Bernt and Bergmeyer (1974). About 0.5 g of leaf and root tissues from both control and treatment groups was homogenised with liquid nitrogen, and the powders were suspended in 1.5 mL of 100 mM potassium phosphate buffer (pH 6.8). The suspensions were then centrifuged at 18,000 × *g* for 20 min at 4 °C. Enzymatic reaction was started with 0.25 mL of supernatant and 1.25 mL of peroxidase reagent consisting of 83 mM potassium phosphate buffer at pH 7.0, 0.005% (w/v) o-dianisidine, and 40 μg peroxidase mL^{-1} 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. Absorbance of the supernatant was measured at 436 nm and the amount of hydrogen peroxide was determined by using an extinction coefficient of 39.4 $mM^{-1}\,cm^{-1}$.

Proline content determination

Proline contents were determined according to the method of Bates et al. (1973). About 0.3 g of leaf and root tissues from both control and treatment groups was homogenised with liquid nitrogen, and the tissue powders were suspended in 1 mL of 3% sulphosalicylic acid. Following centrifugation at 1000 × *g* for 5 min at 4 °C, 0.1 mL of the supernatants was mixed with 0.2 mL of acid ninhydrin, 0.2 mL of 96% acetic acid, and 0.1 mL of 3% sulphosalicylic 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 absorbance was read at 520 nm. The amounts of proline were determined using an extinction coefficient of 0.9986 $mM^{-1}\,cm^{-1}$ that was derived from the proline standard curve.

Determination of enzyme activities

Leaf and root samples from control and drought-treated groups were homogenised with liquid nitrogen and suspended in specific buffers for enzyme activity measurements. The suspensions were centrifuged at 12,000 × *g* for 20 min at 4 °C, and the supernatants were used for activity measurements by considering protein amounts. The protein amounts in shoot extracts were determined by the Bradford method (Bradford, 1976) using bovine serum albumin as a standard.

APX activity determination was done according to the method of Wang et al. (1991). Samples containing 100 μg of 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. The 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. Nanomoles of ascorbate consumed per minute was defined as 1 unit of APX.

CAT activity determination was performed according to the method of Chance and Maehly (1995). Samples containing 100 µg of protein were suspended in 1 mL of 50 mM Tris-HCl suspension solution (pH 7.8). The assay medium consisted of 50 mM potassium phosphate buffer at pH 7 and 10 mM H_2O_2. The decrease in H_2O_2 absorbance was followed for 90 s at 240 nm at room temperature. Nanomoles of hydrogen peroxide consumed per minute was defined as 1 unit of CAT.

GR activity was determined according to the method of Sgherri et al. (1994). Samples containing 100 µg of protein 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 monitored continuously for 90 s at 340 nm at room temperature.

Data analysis

Experiments were performed with 3-5 replicates per analysis. The significance of treatment effects was determined at a 5% probability level by one-way ANOVA and the general linear model (GLM) of Minitab 15.

Results and discussion

Growth parameters

Drought stress is known to induce different responses in plants including inhibition of growth, formation of new compounds, and changes in antioxidant enzyme activities. Shoot and root dry weights of the Musala cultivar decreased considerably under PEG treatments, while no significant change was observed in Aydın tissues. There were also significant reductions in fresh weights of shoot and root tissues of cultivar Musala under −0.4 MPa and −0.8 MPa osmotic potential, while only −0.8 MPa affected the shoot fresh weights of cultivar Aydın (Table). Plants may escape drought stress by cutting short their growth duration and avoid stress by maintaining high tissue water potential, either by reducing water loss or improving water uptake, or both (Farooq et al., 2009). In this study, cultivar Aydın was less affected by PEG-mediated drought stress than cultivar Musala with regard to vegetative growth. However, both cultivars responded with decreased fresh and dry weights in parallel with the study of Manivannan et al. (2007), which reported that drought stress decreased the whole plant fresh weight and caused a decrease in dry weight accumulation in 5 different varieties of *Helianthus annuus* to different extents.

H_2O_2 content, lipid peroxidation, and proline accumulation

Accumulation of proline in many plant species under stress has been correlated with stress tolerance, and

Table. Physiological changes under −0.4 and −0.8 MPa osmotic potential. Numbers indicate mean ± standard error (SE); percentage control values are given in parenthesis. [a]: Values are significantly different at 5% significance level when compared to control treatment.

	Osmotic potential (MPa)	Dry weight (mg)		Fresh weight (mg)	
		Aydın	Musala	Aydın	Musala
Root	0	0.031 ± 0.0002	0.018 ± 0.004	0.78 ± 0.06	0.43 ± 0.09
	−0.4	0.031 (100) ± 0.0026	0.006 (33)[a] ± 0.001	0.70 (90) ± 0.10	0.15 (35)[a] ± 0.01
	−0.8	0.028 (90) ± 0.0014	0.009 (50)[a] ± 0.001	0.54 (69) ± 0.05	0.21 (49)[a] ± 0.03
Shoot	0	0.16 ± 0.01	0.13 ± 0.008	2.53 ± 0.13	2.09 ± 0.07
	−0.4	0.14 (88) ± 0.04	0.06 (46)[a] ± 0.006	2.13 (84) ± 0.73	0.97 (46)[a] ± 0.02
	−0.8	0.12 (75) ± 0.01	0.06 (46)[a] ± 0.007	1.37 (54)[a] ± 0.08	0.91 (43)[a] ± 0.13

its concentration is generally higher in stress-tolerant than in stress-sensitive plants (Ashraf & Foolad, 2007). In this study, both cultivars showed a similar pattern for proline content under stress exposure, and it also appeared that considerable proline accumulation in both root and shoot tissues was directly related to the degree of drought stress. However, proline content in root tissues differed between the 2 cultivars, with cultivar Aydın accumulating 3.5 and 1.9 times more proline when compared to cultivar Musala at −0.4 MPa and −0.8 MPa osmotic potential, respectively (Figure 1). In the present study, considerably higher proline accumulation in Aydın roots was observed upon exposure to stress, which is also in accordance with the conserved dry and fresh weights of Aydın tissues. At the cellular level, the effects of drought stress are mainly cell disturbance, cell membrane injury, and production of ROS that cause damage to the cellular apparatus (Terbea et al., 1995; Sgherri et al., 1996; Kang & Zhang, 1997; Rauf, 2008; Terzi et al., 2010; Makbul et al., 2011). In this study, MDA contents, which are the end products of lipid peroxidation in cell membranes, increased significantly in shoot tissues of both cultivars upon exposure to drought treatments (Figure 2). Harsher drought stress conditions had a more propagative effect on Aydın shoots. Root tissues of both cultivars were not affected by drought treatments in terms of MDA level. Unaffected MDA content in the roots correspond to lower lipid peroxidation and may indicate better protection against oxidative damage in these particular tissues. In this study, shoots from

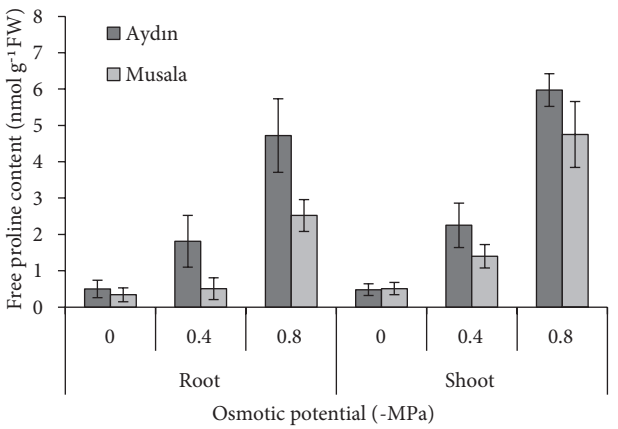

Figure 1. Effect of PEG treatments on free proline content. Vertical bars indicate ±SE.

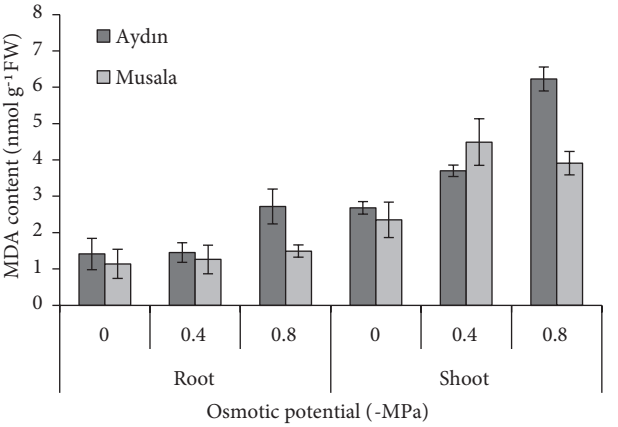

Figure 2. Drought stress-induced changes in MDA content under −0.4 and −0.8 MPa osmotic potential. Vertical bars indicate ±SE.

Aydın and Musala cultivars responded similarly in terms of H_2O_2 accumulation (Figure 3). Higher osmotic stress caused a considerable increase in H_2O_2 content in the shoot and root tissues of both cultivars. While the shoot tissues of Aydın had increasing H_2O_2 content under exposure to less osmotic stress, H_2O_2 levels were higher in Musala root tissues than Aydın root tissues at −0.8 MPa. Although there was a significant increase in the H_2O_2 content of root tissues of both cultivars, membrane integrity was not affected by severe drought conditions due to higher proline accumulation; significant proline accumulation appears to be an essential part of the protection mechanism against drought stress in the root tissues of both cultivars. In spite of the

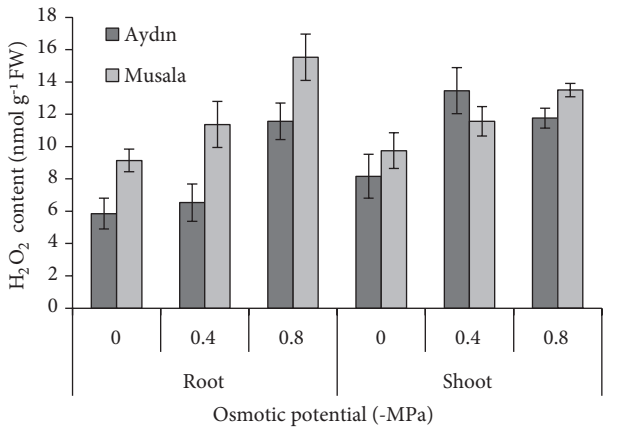

Figure 3. Drought stress-induced changes in H_2O_2 content under −0.4 and −0.8 MPa osmotic potential. Vertical bars indicate ±SE.

higher proline content in shoot tissues, there was also a significant increase in MDA content for both cultivars. This can be explained by the assumption that the protection potential of proline was not sufficient in shoot tissues to remove excess ROS. Similarly, Ghane et al. (2012) observed that drought-tolerant niger cultivars showed increased membrane damage along with higher proline accumulation under elevated water stress.

Antioxidative system

Plants have 2 major enzymatic methods for detoxifying the H_2O_2 produced by photorespiration and SOD activity. They can detoxify via CAT and/or APX (Perez-Lopez et al., 2009). The activity of APX, a H_2O_2-scavenging enzyme, increased in roots of both cultivars under −0.8 MPa osmotic potential, but it was more considerable in Musala. Similarly, a significant increase in APX activity was observed in Aydın shoot tissues under −0.8 MPa drought stress. In contrast to cultivar Aydın, in terms of APX activity there was no significant difference between control and stress-applied shoots in cultivar Musala (Figure 4). This may be due to the inhibition of APX by excess H_2O_2 (Cruz de Carvalho, 2008). Increased CAT activity in shoot tissues was observed for cultivar Aydın under −0.8 MPa osmotic potential (Figure 5). However, Musala shoots showed increased CAT activity only under −0.4 MPa drought stress. Increased CAT activity in Musala shoots under −0.4 MPa osmotic potential could not overcome the H_2O_2 accumulation alone. In addition, CAT did not show any significant H_2O_2 removal activity in the roots of either cultivar. Plants use 2 major enzymes for scavenging excess ROS: CAT and APX. We assumed that in the present

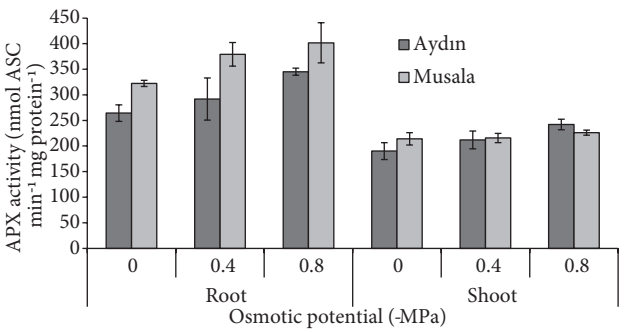

Figure 4. Effect of PEG treatments on APX activity. Vertical bars indicate ±SE.

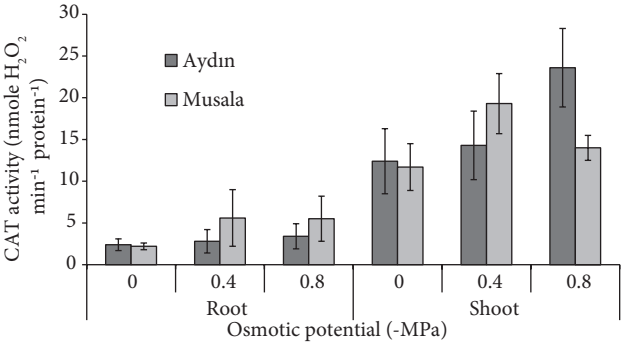

Figure 5. Drought stress-induced changes in CAT activity under −0.4 and −0.8 MPa osmotic potential. Vertical bars indicate ±SE.

study, sunflower seedlings preferred to use APX during excess ROS removal, since CAT shows less affinity to H_2O_2 under drought conditions (Mittler, 2002). In Aydın shoots, higher APX and CAT activity under −0.8 MPa osmotic potential corresponds with lower H_2O_2 values upon exposure to harsh degrees of drought treatment as opposed to the milder one. GR also plays a key role in oxidative stress by converting the oxidised glutathione (GSSG) to GSH and maintaining a high GSH-to-GSSG ratio (Alscher, 1989; Fadzilla et al., 1997). GR activity in shoots showed higher but insignificant values under drought stress treatments. GR activity in root tissues showed an insignificant increment upon exposure to −0.4 MPa in cultivar Aydın and −0.8 MPa in cultivar Musala (Figure 6). Our results were consistent with previous studies, since Zhang and Kirkham (1996) declared that GR activity in sunflower shoots was not affected by drought stress. Likewise, Chugh et al.

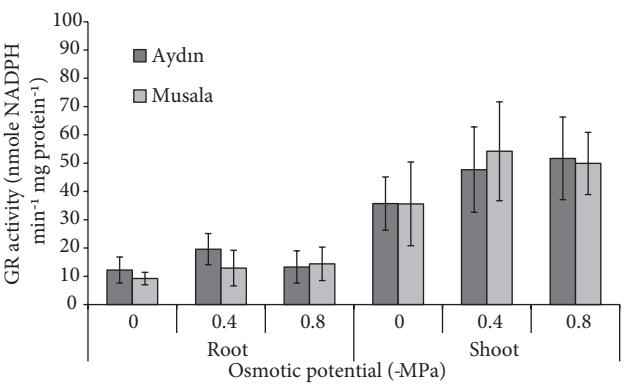

Figure 6. Alterations in GR activity under −0.4 and −0.8 MPa osmotic potential. Vertical bars indicate ±SE.

(2011) reported that GR activity in maize seedlings exposed to drought stress was insignificantly increased.

Summary and conclusions

The investigation of various physiological and biochemical parameters showed that the responses of both sunflower cultivars to drought stress shared common characteristics, especially in terms of MDA, H_2O_2, and proline levels, while cultivar Aydın appeared to be less affected physiologically. Among the parameters analysed in both cultivars, APX and proline played important roles in the protection of root tissues under harsh stress conditions, resulting in an insignificant increase in MDA. In contrast with the root tissues of cultivar Aydın, the antioxidant system was unable to prevent membrane damage and excess ROS production in shoot tissues, although the tissues showed an increase in free proline concentration and APX and CAT activities. GR activity does not seem to be an essential part of the protection mechanism against drought in either cultivar. Therefore, strategies for the improvement of APX and CAT enzyme activities in sunflower tissues could provide an effective protection system for drought stress in this important oilseed crop species. In a future study, the effect of drought stress on fatty acid composition will be determined using different sunflower cultivars.

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References

Albert A & Schneiter ED (1997). *Sunflower Technology and Production*. Madison, Wisconsin: The American Society of Agronomy.

Alscher RG (1989). Biosynthesis and antioxidant properties of glutathione in plants. *Physiologia Plantarum* 77: 457-464.

Arora A, Sairam RK & Srivastava GC (2002). Oxidative stress and antioxidative systems in plants. *Current Science* 82: 1227-1238.

Ashraf M & Foolad MR (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany* 59: 206-216.

Bates LS, Waldren RP & Teare ID (1973). Rapid determination of free proline for water-stress studies. *Plant Soil* 39: 205-207.

Bernt E & Bergmeyer HU (1974). *Methods of Enzymatic Analysis*. New York: Academic Press.

Bowler C, Montagu MV & Inze D (1992). Superoxide dismutase and stress tolerance. *Annual Review of Plant Physiology and Plant Molecular Biology* 43: 83-116.

Bradford MM (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72: 248-254.

Bray EA, Bailey-Serres J & Weretilnyk E (2000). Responses to abiotic stresses. In: Gruissem W, Buchannan B & Jones R (eds.) *Biochemistry and Molecular Biology of Plants*, pp. 1158-1249. Rockville, Maryland: American Society of Plant Physiologists.

Camacho Barron M & Gonzalez de Mejia EG (1998). Comparative study of enzymes related to proline metabolism in tepary bean (*Phaseolus acutifolius*) and common bean (*Phaseolus vulgaris*) under drought and irrigated conditions, and various urea concentrations. *Plant Foods for Human Nutrition* 52: 119-132.

Celikkol Akcay U, Ercan O, Kavas M, Yildiz L, Yilmaz C, Oktem HA & Yucel M (2010). Drought-induced oxidative damage and antioxidant responses in peanut (*Arachis hypogaea* L.) seedlings. *Plant Growth Regulation* 61: 21-28.

Chance B & Maehly AC (1995). Assay of catalases and peroxidases. *Methods in Enzymology* 2: 764-817.

Chugh V, Kaur N & Gupta AK (2011). Evaluation of oxidative stress tolerance in maize (*Zea mays* L.) seedlings in response to drought. *Indian Journal of Biochemistry & Biophysics* 48: 47-53.

Connor DJ & Hall AJ (1997). Sunflower physiology. In: Schneiter AA (ed.) *Sunflower Science and Technology*, pp. 113-182. Madison, Wisconsin: The American Society of Agronomy.

Cruz de Carvalho MH (2008). Drought stress and reactive oxygen species: production, scavenging and signaling. *Plant Signal Behavior* 3: 156-165.

Fadzilla NM, Finch RP & Burdon RH (1997). Salinity, oxidative stress and antioxidant responses in shoot cultures of rice. *Journal of Experimental Botany* 48: 325-331.

Farooq M, Wahid A, Kobayashi N, Fujita D & Basra SMA (2009). Plant drought stress: effects, mechanisms and management. *Agronomy for Sustainable Development* 29: 185-212.

Fridovich I (1986). Biological effects of superoxide radical. *Archives of Biochemistry and Biophysics* 247: 1-11.

Ghane SG, Lokhande VH & Nikam TD (2012). Differential growth, physiological and biochemical responses of niger (*Guizotia abyssinica* Cass.) cultivars to water-deficit (drought) stress. *Acta Physiologiae Plantarum* 34: 215-225.

Halliwell B (1987). Oxidative damage, lipid peroxidation, and antioxidant protection in chloroplasts. *Chemistry and Physics of Lipids* 44: 327-340.

Hoagland DR & Arnon DI (1950). The water-culture method for growing plants without soil. *California Agricultural Experiment Station Circular* 347: 1-32.

Kang SZ & Zhang JH (1997). Hydraulic conductivities in soil-root system and relative importance at different soil water potential and temperature. *Transactions of Chinese Society of Agricultural Engineers* 13: 76-81.

Knowles PF (1988). Recent advances in oil crops breeding. In: Applewhite TH (ed.) *Proceedings of the World Conference on Biotechnology for the Fats and Oil Industry*, pp. 35-38. Urbana, Illinois: American Oil Chemists Society.

Leon AJ, Andrade FH & Lee M (2003). Genetic analysis of seed-oil concentrations across generations and environments in sunflower (*Helianthus annuus* L.). *Crop Science* 43: 135-140.

Liu X & Baird WV (2003). Differential expression of genes regulated in response to drought or salinity stress in sunflower. *Crop Science* 43: 678-687.

Lopez-Pereira M, Trapani N & Sadras V (2000). Genetic improvement of sunflower in Argentina between 1930 and 1995. III. Dry matter partitioning and achene composition. *Field Crops Research* 67: 215-221.

Makbul S, Güler NS, Durmuş N & Güven S (2011). Changes in anatomical and physiological parameters of soybean under drought stress. *Turkish Journal of Botany* 35: 369-377.

Manivannan P, Abdul Jaleel C, Sankar B, Kishorekumar A, Somasundaram R, Lakshmanan GMA & Panneerselvam R (2007). Growth, biochemical modifications and proline metabolism in *Helianthus annuus* L. as induced by drought stress. *Colloids and Surfaces B: Biointerfaces* 59: 141-149.

Mazahery-Laghab H, Nouri F & Abianeh HZ (2003). Effects of the reduction of drought stress using supplementary irrigation for sunflower (*Helianthus annuus* L.) in dry farming conditions. *Pajouhesh va Sazandegi in Agronomy and Horticulture* 59: 81-86.

Miller JF (1995). Inheritance of salt tolerance in sunflower. *Helia* 18: 9-16.

Mittler R (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science* 7: 405-410.

Monotti M (2004). Growing non-food sunflower in dry land conditions. *The Italian Journal of Agronomy* 8: 3-8.

Ohkawa H, Ohishi N & Yagi Y (1979). Assay of lipid peroxides in animal tissue by thiobarbituric acid reaction. *Analytical Biochemistry* 95: 351-358.

Perez-Lopez U, Robredo A, Lacuesta M, Sgherri C, Munoz-Rueda A, Navari-Izzoc F & Mena-Petite A (2009). The oxidative stress caused by salinity in two barley cultivars is mitigated by elevated CO_2. *Physiologia Plantarum* 135: 29-42.

Rabinowitch HD & Fridovic I (1983). Superoxide radical, superoxide dismutase and oxygen toxicity in plants. *Photochemistry and Photobiology Sciences* 188: 206-213.

Rauf S (2008). Breeding sunflower (*Helianthus annuus* L.) for drought tolerance. *Communications in Biometry and Crop Science* 3: 29-44.

Satoh K & Murata N (1998). *Stress Responses of Photosynthetic Organisms*: *Molecular Mechanisms and Molecular Regulations*. Amsterdam: Elsevier.

Sgherri CLM, Ligini B, Puliga S & Navari-Izzo F (1994). Antioxidant system in *Sporobolus stapfianus*: changes in response to desiccation and rehydration. *Phytochemistry* 35: 561-565.

Sgherri CLM, Pinzino C & Navari-Izzo F (1996). Sunflower seedlings subjected to increasing stress by water deficit: changes in O_2 production related to the composition of thylakoid membranes. *Plant Physiology* 196: 446-452.

Smirnoff N (1993). The role of active oxygen in the response of plants to water deficit and desiccation. *New Phytologist* 125: 27-58.

Terbea M, Vranceanu AV, Petcu E, Craiciu DS & Micut G (1995). Physiological response of sunflower plants to drought. *Romanian Agricultural Research* 3: 61-67.

Terzi R, Sağlam A, Kutlu N, Nar H & Kadıoğlu A (2010). Impact of soil drought stress on photochemical efficiency of photosystem II and antioxidant enzyme activities of *Phaseolus vulgaris* cultivars. *Turkish Journal of Botany* 34: 1-10.

Türkan İ, Bor M, Özdemir F & Koca H (2005). Differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant *P. acutifolius* Gray and drought-sensitive *P. vulgaris* L. subjected to polyethylene glycol mediated water stress. *Plant Science* 168: 223-231.

Wang SY, Jiao H & Faust M (1991). Changes in ascorbate, glutathione and related enzyme activities during thiodiazuron-induced bud break of apple. *Physiologia Plantarum* 82: 231-236.

Wang W, Vinocur B & Altman A (2003). Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta* 218: 1-14.

Zhang J & Kirkham MB (1996). Antioxidant responses to drought in sunflower and sorghum seedlings. *New Phytologist* 132: 361-373.