

## Effect of drought stress on oxidative damage and antioxidant enzyme activity in melon seedlings

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**Abstract:** Drought stress is one of the most serious environmental limitations affecting the growth and productivity of plants. In the current study, oxidative damage and antioxidant responses under polyethylene glycol (PEG)-induced osmotic stress were compared in 2 melon cultivars, Kırkağaç and Galia. Melon seedlings were subjected to PEG-6000 solutions of 2 different osmotic potentials, -0.2 MPa and -0.4 MPa. Various physiological parameters, malondialdehyde (MDA), proline content, and antioxidant enzymes including catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) were measured. A reduction in fresh and dry weights of shoot and root tissues was observed. Significant proline accumulation was detected with increasing osmotic potential for both cultivars. A significant rise in MDA was detected in Kırkağaç at -0.4 MPa osmotic potential. In Galia hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) content increased significantly as PEG concentration increased. CAT showed significantly increased activity only at -0.4 MPa osmotic potential in both cultivars. PEG-induced osmotic stress altered GR activity in both cultivars. These results suggest that (i) Galia is more tolerant than Kırkağaç, and (ii) drought tolerance in both cultivars might be closely related to an increase in capacity for antioxidant enzyme activity and the osmoprotective function of proline. Comparing these responses will help to identify drought tolerance mechanisms in melon cultivars.

**Key words:** *Cucumis melo*, drought stress, antioxidant enzymes, proline, malondialdehyde

### 1. Introduction

Melon (*Cucumis melo* L.), an annual diploid plant, is an economically important horticultural crop grown in temperate, subtropical, and tropical regions worldwide. With a total production of 1.7 million tons, Turkey was the second biggest producer after China in 2009. Like many other fruits melon fruits provide a good source of potassium and vitamin C. They are also fat and cholesterol free, high in water content, and are relatively low in calories. Moreover, melons may have an anticlotting action and are also thought to lower cancer and heart disease risk (Lester, 1996).

Biotic and abiotic stresses are the most important factors that severely limit plant growth and metabolism (Makbul et al., 2011). 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). Moreover, when the usable areas on the earth are classified in view of stress factors, drought stress is one of the most widespread environmental stresses (Arora et al., 2002; Saruhan Güler et al., 2012). Although each stress factor produces its own

specific effect on plants, in general all stress conditions can cause an increase in reactive oxygen species (ROS). ROS are recognized as detrimental to biological systems because they cause the oxidation of lipids, proteins, deoxyribonucleic acid, and carbohydrates. Ultimately, toxic levels of ROS cause a chain reaction of cellular oxidation, which results in unhealthy situations and lethality. In addition to the oxidative stress related production, ROS are an inevitable outcome of normal physiological processes, such as glycolysis and photosynthesis (Mittler et al., 2004). Plant antioxidant systems, both enzymatic and nonenzymatic, play an important role in balancing and preventing oxidative damage (Foyer et al., 1994; Baysal Furtana and Tıpırdamaz, 2010). Nonenzymatic antioxidant metabolites include anthocyanin, flavonoids, carotenoids,  $\alpha$ -tocopherol, ascorbate (AsA), and reduced glutathione (GSH) (Halliwell, 1987); enzymatic antioxidants consist of superoxide dismutase (EC 1.15.1.1), ascorbate peroxidase (APX) (EC 1.11.1.11), catalase (CAT) (EC 1.11.1.6), peroxidase (POX) (EC 1.11.1.7), and glutathione reductase (GR) (EC 1.8.1.7) (Bowler et al., 1992; Çelik and

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Atak, 2012). Accumulation of ROS can destroy normal metabolism through oxidative damage to lipids, proteins, and nucleic acids (Rabinowitch et al., 1983; Fridovich, 1986). Extended drought conditions lead to interrupted reproductive development, premature leaf senescence, wilting, desiccation, and death (Schulze, 1986). In addition to an enzymatic scavenging system, accumulation of proline is one of the important adaptive strategies plants use to cope with environmental stresses, particularly low water stress. Proline is also closely related with plant drought stress as free proline can accumulate significantly in crops and other plants (Hare and Cress, 1997; Ain-Lhout et al., 2001; Kim et al., 2004; Lee et al., 2009). As an osmoprotectant in plants subjected to drought conditions, proline can accumulate to high concentrations in plant cells without disrupting cellular structure or metabolism. Therefore, proline accumulation plays an important role in osmotic adjustment, detoxification of ROS, and membrane integrity in plants under stress conditions (Smirnoff and Cumbes, 1989; Matysik et al., 2002; Demiralay et al., 2013).

Studies investigating the biochemical basis of stress responses in melon are rare, and none of them studied the performance of antioxidative defense mechanisms under drought conditions. Studies of melon and other species of the family Cucurbitaceae mostly focus on the effects of salt stress. Drought stress was rarely studied and only in a few species of Cucurbitaceae such as watermelon, pumpkin, and cucumber. Although there are many conventional melon varieties widely grown in Turkey, Kırkağaç (winter melon) and Galia (summer melon) cultivars are the most commonly cultivated in all regions of Turkey. However, they are susceptible to different abiotic stresses. The present study aimed to determine the effect of PEG-induced drought stress on proline accumulation, lipid membrane peroxidation, and antioxidant enzyme activities of stressed melon seedlings. Comparison of these responses will help identify drought tolerance mechanisms in the melon cultivars Kırkağaç and Galia, allowing for further insights into the molecular mechanisms of tolerance to drought-induced oxidative stress.

## 2. Materials and methods

### 2.1. Plant materials, growth conditions, and stress treatments

The seeds of melon (*Cucumis melo*) cultivars Kırkağaç and Galia were kindly provided by the West Mediterranean Agricultural Research Institute (Turkey). After surface sterilization, seeds were germinated in pots filled with perlite, and the plants were grown in a controlled growth chamber at  $23 \pm 2$  °C with 16 h light ( $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and 8 h dark photoperiods. Half strength Hoagland's solution (Hoagland and Arnon, 1950) was used for irrigation. On the basis of a previous experiment (data not shown), drought stress was applied to 25-day-old seedlings

with half strength Hoagland's solution containing PEG 6000 equal to  $-0.2$  MPa (5% w/v) and  $-0.4$  MPa (10% w/v) osmotic potentials for 5 days to investigate the physiological responses of melon seedlings under drought stress. PEG was used to impose low water potentials in solution. Control plants were grown in the same way and watered with half-strength Hoagland's solution without PEG. For analysis of physiological parameters including fresh and dry weights of shoots and roots, 1-month-old seedlings were harvested. Relative water content (RWC); root and shoot lengths; chlorophyll fluorescence; proline and MDA contents; and CAT, APX, and GR activities were measured. Each set of experiments was performed at least 3 times.

### 2.2. Growth parameters

Weights of shoot and root tissues were measured as fresh and dry weights. Fresh weight (FW) measurements were done at 30 days of growth with shoot and root samples taken from control and stress-treated melon cultivars. These samples were put in an oven at 60 °C for 24 h, and dry weights (DW) were measured. Shoot and root lengths were measured, and samples were photographed in order to visually show drought effects on growth.

### 2.3. Water status of leaves

Relative water content of leaves was determined by the method described by Smart and Bingham (1974). The RWC percentage was calculated using the following formula:  $\text{RWC (\%)} = (\text{FW} - \text{DW}) / (\text{TW} - \text{DW}) \times 100$ , where FW is fresh weight, DW dry weight, and TW turgid weight. In order to determine RWC, the FW of leaves collected from control and stress-applied cultivars were measured first. Then samples were put into jars filled with distilled water for 24 h at room temperature for hydration. The TW of hydrated shoot tissues was measured and they were dried in an oven at 60 °C for 24 h. Then samples were weighed to record DW.

### 2.4. Chlorophyll fluorescence

Chlorophyll fluorescence measurements were performed on randomly selected leaves (fifth leaf) of the plants. Measurements were taken in a growth room at 24 °C using a portable OS5-FL modulated fluorometer (see OS5-FL manual for additional details). Leaves were dark adapted with clips for at least 30 min before measurements. The Fv/Fm value indicating the maximum photochemical yield of PSII was determined as follows:

$$Fv/Fm = (Fm - Fo)/Fm,$$

where Fo and Fm are the minimal and maximal fluorescence yields of a dark-adapted sample, with all PSII reaction centers fully opened and closed, respectively. Fv is the variable fluorescence.

### 2.5. Determination of malondialdehyde (MDA) content

The level of lipid peroxidation in terms of MDA content was determined for the evaluation of membrane damage

generated by drought stress treatments. MDA content was determined according to Ohkawa et al. (1979). Fresh leaf tissues (0.2 g) were weighed and homogenized with liquid nitrogen by adding 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 non-specific turbidity was performed by subtracting the absorbance at 600 nm. MDA contents were calculated using an extinction coefficient of  $155 \text{ mM}^{-1} \text{ cm}^{-1}$ . The results were expressed in  $\text{nmol MDA g}^{-1} \text{ leaf FW}$ .

### 2.6. Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) content determination

$\text{H}_2\text{O}_2$  content was determined according to Bernt and Bergmeyer (1974). About 0.5 g of leaf tissues from both control and stress-treated plants 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  $18,000 \times g$  for 20 min at 4 °C. The enzymatic reaction was started with 0.25 mL of supernatant and a 1.25 mL peroxidase reagent consisting of 83 mM potassium phosphate buffer at pH 7.0, 0.005% (w/v) o-dianisidine, and 40  $\mu\text{g}$  peroxidase  $\text{mL}^{-1}$  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  $5000 \times g$  for 5 min. The absorbance of the supernatant was measured at 436 nm, and the amount of hydrogen peroxide was determined by using an extinction coefficient of  $39.4 \text{ mM}^{-1} \text{ cm}^{-1}$ .

### 2.7. Proline content determination

Assessment of free proline content was performed according to Bates et al. (1973). About 0.3 g of leaf tissues from both control and stressed plants was homogenized with liquid nitrogen, and the tissue powders were suspended in 1 mL of 3% sulfosalicylic acid. Following centrifugation at  $1000 \times g$  for 5 min at 4 °C, 0.1 mL of supernatant was mixed with 0.2 mL of acid ninhydrin, 0.2 mL of 96% acetic acid, and 0.1 mL of 3% sulfosalicylic acid. The mixtures were incubated at 96 °C for 1 h, mixed with 1 mL of toluene, and further centrifuged at  $1000 \times g$  for 5 min at 4 °C. Upper phases were collected, and the absorbances were read at 520 nm. The amounts of proline were determined by using an extinction coefficient of  $0.9986 \text{ mM}^{-1} \text{ cm}^{-1}$  that was derived from the proline standard curve. Values are expressed as  $\mu\text{mol proline g}^{-1} \text{ leaf FW}$ .

### 2.8. Determination of enzyme activities

For enzyme extracts and assays, leaf samples from each treatment were homogenized with liquid nitrogen and suspended in a buffer specific for each enzyme extraction.

The suspensions were centrifuged at  $12,000 \times g$  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.

Ascorbate peroxidase activity determination was done according to Wang et al. (1991). Samples containing 100  $\mu\text{g}$  of protein were suspended in 1 mL of suspension solution containing 50 mM of Tris-HCl (pH 7.2), 2% PVP, 1 mM of EDTA, and 2 mM of ascorbate. The assay medium consisted of 50 mM of potassium phosphate buffer (pH 6.6), 0.25 mM of ascorbate, and 1 mM of  $\text{H}_2\text{O}_2$ , 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.

Catalase activity determination was carried out according to Chance and Maehly (1995). Samples containing 100  $\mu\text{g}$  of protein were suspended in 1 mL of 50 mM Tris-HCl suspension solution at pH 7.8. The assay medium consisted of 50 mM of potassium phosphate buffer at pH 7.0 and 10 mM of  $\text{H}_2\text{O}_2$ . The decrease in  $\text{H}_2\text{O}_2$  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.

The GR activity was determined from the rate of NADPH oxidation at 25 °C according to Sgherri et al. (1994). Samples containing 100  $\mu\text{g}$  of protein were suspended in 1 mL of suspension solution containing 100 mM of  $\text{K-PO}_4$  buffer (pH 7.8), 1% PVP, 0.1 mM of EDTA, and 0.5 (v/v) Triton X-100. The assay medium consisted of 200 mM of potassium phosphate buffer (pH 7.5), 0.2 mM of EDTA, 1.5 mM of MgCl, 0.25 mM of GSSG, and 25  $\mu\text{M}$  of NADPH. Oxidation of NADPH was monitored continuously for 90 s at 340 nm at room temperature.

### 2.9. Data analysis

All analyses were done using a completely randomized design with 4 replicates ( $n = 4$ ). All data were subjected to one-way analyses of variance (ANOVA). Duncan's multiple range test was used to separate statistically different means at  $P < 0.01$  and  $P < 0.05$ .

## 3. Results and discussion

### 3.1. Growth parameters

Both dry and fresh weights of roots belonging to Kırkağaç and Galia decreased as the intensity of drought stress application increased. Likewise, it was observed that dry and fresh weights of drought-treated plant shoots decreased significantly in both cultivars as the PEG concentration increased ( $P \leq 0.05$ ) (Table 1). Drought stress caused a significant increase in the root length of stress-treated plants when compared to the corresponding control plants ( $P \leq$

**Table 1.** The effect of PEG treatments on growth parameters of 2 melon (*Cucumis melo*) cultivars.

	Osmotic potential (MPa)	Dry weight (mg)		Fresh weight (mg)		Length (cm)	
		Kırkağaç	Galia	Kırkağaç	Galia	Kırkağaç	Galia
Root	0	0.19 ± 0.046	0.21 ± 0.030	1.60 ± 0.004	3.91 ± 0.580	25.67 ± 1.45	24.75 ± 1.75
	-0.2	0.06 ± 0.007*	0.16 ± 0.035	0.74 ± 0.021**	2.38 ± 0.820	28.33 ± 1.20	23.50 ± 2.50
	-0.4	0.03 ± 0.003*	0.06 ± 0.005*	0.44 ± 0.007**	0.62 ± 0.050*	33.33 ± 6.69*	38.00 ± 1.73**
Shoot		Kırkağaç	Galia	Kırkağaç	Galia	Kırkağaç	Galia
	0	0.31 ± 0.004	0.51 ± 0.056	4.80 ± 0.043	7.69 ± 0.075	8.83 ± 0.73	19.33 ± 1.01
	-0.2	0.19 ± 0.021**	0.25 ± 0.050*	2.76 ± 0.259**	3.42 ± 0.503**	7.67 ± 0.19	18.33 ± 0.17
	-0.4	0.09 ± 0.007**	0.12 ± 0.009**	1.27 ± 0.153**	1.67 ± 0.194**	6.33 ± 0.17*	20.67 ± 0.17

Each value represents the mean of 3 replicates (n = 3) and standard error of mean (±SEM). Duncan's multiple range test showed significant differences in means (±SEM) between control and drought-treated plants at P < 0.01 (\*\*) and P < 0.05 (\*).

0.05). However, there was no significant effect on the shoot length of stress-treated plants throughout the experiment. Plants show diverse responses in terms of shoot and root length under drought stress. Many studies have shown that there are significant increases in root length and decreases in shoot length under drought stress (Turkan et al., 2004; Tuna et al., 2010). Kavar et al. (2007) also reported that the key responses to drought stress in plants are root growth, density, proliferation, and size, since roots are the only source for acquiring water from the soil. Enhancement of root growth under drought conditions allows the plant to extract more water from deeper zones.

Leaf RWC is considered a reliable and widely used indicator for defining the sensitivity of plants to dehydration (Rampino et al., 2006; Sanchez-Rodriguez et al., 2010). In our study RWC in the leaves of drought treated plants declined significantly (P ≤ 0.05). This decline was sharp under the 0.4 MPa stress application (Table 2).

### 3.2. MDA, hydrogen peroxide, and proline contents and PSII activity

When plants are subjected to environmental stresses involving drought, ROS production overcomes antioxidant

system capacity, and oxidative stress occurs, which results in cytotoxic protein damage, DNA damage, and lipid peroxidation (Yazici et al., 2007).

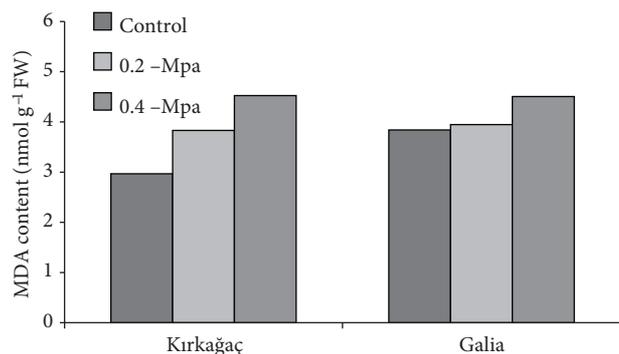
MDA is one of the end products of lipid peroxidation by free radicals, and its reaction with thiobarbituric acid to form an intensely colored chromogen makes it a convenient biomarker for determination of lipid peroxidation (Marnett, 1999). In the present study, an insignificant rise in MDA content was detected for Galia, whereas MDA concentration rose significantly in the shoot tissues of drought-stressed Kırkağaç seedlings at -0.4 MPa osmotic potential (P ≤ 0.01) compared to the control (Figure 1). The lower values of MDA in Galia indicate that at cellular levels this cultivar has an efficient free radical quenching system. These results are in a good agreement with the studies by Sairam et al. (2000), Terzi et al. (2010), and Zhang et al. (2011), which found that low concentrations of MDA are associated with drought stress tolerance in artichoke plants, common bean, and wheat, respectively.

The MDA content was significantly higher in Kırkağaç under -0.4 MPa osmotic potential over Galia. The increase

**Table 2.** Changes in H<sub>2</sub>O<sub>2</sub>, RWC content, and PSII activity in leaf tissues under -0.2 and -0.4 MPa osmotic potentials.

Osmotic potential (MPa)	H <sub>2</sub> O <sub>2</sub> (nmol g <sup>-1</sup> FW)		RWC (%)		Fv/Fm	
	Kırkağaç	Galia	Kırkağaç	Galia	Kırkağaç	Galia
0	12.79 ± 0.880	12.79 ± 0.457	85.50 ± 1.40	82.65 ± 0.77	0.855 ± 0.0034	0.854 ± 0.0052
-0.2	13.30 ± 0.868	15.26 ± 0.305*	72.81 ± 2.15*	75.84 ± 0.96*	0.857 ± 0.0092	0.866 ± 0.0035
-0.4	15.43 ± 0.667*	22.94 ± 0.457**	63.04 ± 0.74*	70.28 ± 1.84*	0.842 ± 0.0137	0.851 ± 0.0045

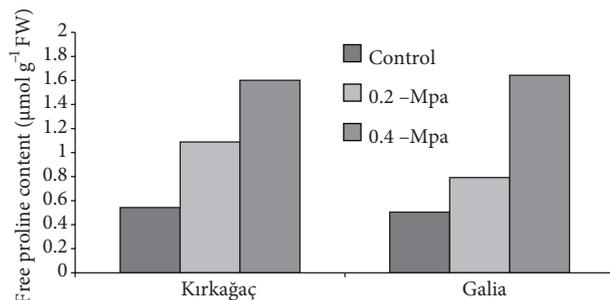
Each value represents the mean of 3 replicates (n = 4) and standard error of mean (±SEM). Duncan's multiple range test showed significant differences in means (±SEM) between control and drought-treated plants at P < 0.01 (\*\*) and P < 0.05 (\*).



**Figure 1.** Drought stress induced changes in MDA content under -0.2 and -0.4 MPa osmotic potential.

in MDA levels under drought stress suggests that water stress could cause the formation of membrane lipid peroxidation by means of ROS production (Sairam et al., 2000). The  $H_2O_2$  content grew linearly with increasing levels of drought stress in both cultivars. Accumulation of higher hydrogen peroxide content in the leaf tissues of Galia was observed under both drought stress conditions, -0.2 ( $P \leq 0.05$ ) and -0.4 MPa osmotic potentials ( $P \leq 0.01$ ). In addition,  $H_2O_2$  content increased significantly in Kırkağaç seedlings at -0.4 MPa osmotic potential ( $P \leq 0.05$ ) (Table 2). Hydrogen peroxide content was always significantly higher in Galia than in Kırkağaç.  $H_2O_2$  is a toxic compound that is produced as a result of the scavenging of superoxide radicals. Higher concentrations damage plants through lipid peroxidation and membrane injury (Nayar and Kaushal, 2002). In the present study, the lower values of  $H_2O_2$  in Kırkağaç indicate that at a cellular level this cultivar has an efficient free radical quenching system. As a result, the lower values of MDA and  $H_2O_2$  in Galia and Kırkağaç, respectively, indicate that at a cellular level these genotypes are equipped with an efficient free radical quenching system that offers protection against oxidative stress.

The accumulation of free proline under stress conditions has been correlated with stress tolerance in many plant species, and concentrations are generally higher in stress-tolerant as opposed to stress-sensitive plants (Ashraf and Foolad, 2007). In our study, the cultivars showed similar significant increment patterns for proline content in relation to severity of drought stress in shoot tissues (Figure 2). A more common explanation for the accumulation of proline is that it confers advantages by protecting membranes and proteins when RWC decreases (Reddy et al., 2004). It may be that proline accumulation increased with a significant decrease in RWC and that this accumulation can maintain membrane integrity, which is also supported by MDA results. Oxidative-stress-induced increases in free proline have been reported in many plant species including sunflower, chickpea, cucumber,



**Figure 2.** Effect of PEG treatments on free proline content.

and tomato (Behnamnia et al., 2009; Malik et al., 2010; Sun et al., 2011; Baloğlu et al., 2012; Arıkan Ceylan et al., 2012). Osmoregulation via proline molecules appears to be an essential part of the protection mechanism against drought stress in melon plants.

The ability of a plant to tolerate environmental stresses and the extent to which those stresses damage the photosynthetic apparatus can be examined by measuring chlorophyll fluorescence (Maxwell and Johnson, 2000). Chlorophyll fluorescence parameters provide a quick and accurate technique for quantifying the ability of individual species to tolerate water stress. According to previous studies, there is a strong correlation between chlorophyll fluorescence and environmental stress tolerance, and these can serve as reliable indicators of how well a plant is coping with stress (Faraloni et al., 2011; Mishra et al., 2012). However, remarkable resistance of the photosynthetic apparatus to water shortages has been reported; a 30% leaf water deficit has been estimated as the limit above which photosynthetic biochemistry is significantly affected (Cornic et al., 1992; Cornic and Fresneau, 2002). In the present study, no significant variation in the Fv/Fm ratio was observed in either cultivar at both PEG concentrations (Table 2). Stable Fv/Fm ratios also confirm previous observations that the photosynthetic machinery is resistant to a certain level of water deficit (Chaves et al., 2002; Kocheva et al., 2005).

### 3.3. Antioxidative system

Water shortages can cause oxidative damage. Therefore, plant cells need different mechanisms that will enable the detoxification of excess ROS and keep the formation and removal of ROS in balance. In order to determine the nature of antioxidant responses of melon to drought stresses, we measured the enzymatic activity of APX, GR, and CAT in the shoots of 2 cultivars subjected to PEG-mediated drought stress.

CAT is the principal enzyme that scavenges harmful oxygen species in plants (Pereira et al., 2002). In this study CAT activities in both cultivars increased markedly only at -0.4 MPa osmotic potential ( $P \leq 0.05$ ) (Table 3). The

**Table 3.** The effect of PEG treatment on the activities of APX, CAT, and GR enzymes in leaf tissues of 2 melon (*Cucumis melo*) cultivars.

Osmotic potential (MPa)	APX (nmol Asc min <sup>-1</sup> mg protein <sup>-1</sup> )		CAT (nmol H <sub>2</sub> O <sub>2</sub> min <sup>-1</sup> mg protein <sup>-1</sup> )		GR (nmol NADPH min <sup>-1</sup> mg protein <sup>-1</sup> )	
	Kırkağaç	Galia	Kırkağaç	Galia	Kırkağaç	Galia
0	297.68 ± 28.70	543.036 ± 31.20	2.94 ± 0.51	1.50 ± 0.27	33.58 ± 1.77	23.67 ± 3.56
-0.2	312.68 ± 15.90	564.107 ± 55.90	4.21 ± 0.46	1.97 ± 0.09	34.79 ± 5.56	41.29 ± 4.30*
-0.4	340.00 ± 10.40	417.143 ± 36.52*	6.18 ± 0.42**	2.57 ± 0.26*	38.52 ± 3.41*	27.62 ± 2.13

Each value represents the mean of 3 replicates (n = 4) and standard error of mean (±SEM). Duncan's multiple range test showed significant differences in means (±SEM) between control and drought-treated plants at P < 0.01 (\*\*) and P < 0.05 (\*).

increment in activity under harsh stress conditions can be explained by the very low affinity of CAT for H<sub>2</sub>O<sub>2</sub>; it becomes active at relatively high H<sub>2</sub>O<sub>2</sub> concentrations (Gechev et al., 2006). It is also suggested that higher concentrations of CAT induced by drought stress may have removed the O<sub>2</sub><sup>•-</sup> radicals and their product H<sub>2</sub>O<sub>2</sub> (Sairam et al., 2000). APX activity showed an insignificant rise in Kırkağaç as stress increased. On the other hand, APX activity in Galia seedlings cultured at -0.4 MPa osmotic potential for 5 days decreased significantly (P ≤ 0.05) (Table 3). As postulated by Celikkol Akcay et al. (2010), enhanced production of ROS under severe drought application might cause an interaction with the enzyme leading to its possible oxidation and inactivation. A decrease in APX activity under severe water stress has been previously reported (Mohammadkhani and Heidari, 2007). Drought stress treatment at -0.4 MPa osmotic potential in Kırkağaç caused a significant increase in GR activity (P ≤ 0.05), while this activity in Galia at -0.2 MPa osmotic potential was approximately 2-fold higher than in the control (P ≤ 0.05) (Table 3). It can be suggested that GR and proline are important elements fighting oxidative stress in shoot tissues of both cultivars under drought stress conditions. The investigation of various physiological and biochemical parameters showed that the responses of both melon cultivars to drought stress shared common characteristics, especially in terms of MDA, H<sub>2</sub>O<sub>2</sub>, and proline contents and CAT and GR activities, while Galia appeared to be less affected physiologically and biochemically.

The antioxidant activity of CAT in particular plays a protective role for drought tolerance in Galia and Kırkağaç (Table 3). Despite the increase in H<sub>2</sub>O<sub>2</sub> content in Galia shoots under both drought stress conditions, MDA results

did not increase, indicating lower oxidative damage to membranes. In both cultivars CAT seems to keep H<sub>2</sub>O<sub>2</sub> content at a certain level, inhibiting production of the free radicals that may lead to peroxidation of membrane lipids. In addition, slight increases in APX activity together with the observed CAT activity in Kırkağaç achieve the fine modulation of H<sub>2</sub>O<sub>2</sub> content. On the other hand, a decrease in APX activity in Galia seems to cause an increment in H<sub>2</sub>O<sub>2</sub> content, despite the activity of CAT alone. Galia showed an increase in H<sub>2</sub>O<sub>2</sub> content at lesser magnitudes of drought stress where APX and CAT had insignificant activity; however, MDA amounts were stable compared to the controls. GR activity in Galia may also protect membranes under milder drought conditions. Arora et al. (2002) found that increments in GR activity result in the availability of NADP, which can accept electrons from ferredoxin, thereby minimizing chances of superoxide formation, in addition to scavenging H<sub>2</sub>O<sub>2</sub>. Despite PEG-caused rapid dehydration, PSII maintained its efficiency in both cultivars, which is consistent with Kocheva et al. (2005), who reported the 30% leaf water deficit limit for the functioning of photosynthetic machinery under various stress conditions. According to our results, both cultivars were drought tolerant, whereas Galia appeared to be less affected physiologically and biochemically, rendering Galia more tolerant than Kırkağaç. These results are consistent with Kuşvuran et al.'s (2011) study, which employed physiological parameters and visual scales.

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