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Physiological and biochemical responses of almond rootstocks to drought stress

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Abstract: Water availability is a very important factor for the growth and development of plants, which limits the plant production capacity. Rootstocks are widely utilized to improve plants tolerance to various biotic and abiotic stresses. In this study, physiological and biochemical responses of fifteen almond rootstock candidates to drought stress were investigated under in vitro conditions. The shoot tips from fifteen almond rootstock candidates were cultured in MS medium containing 1.0 mg/L BAP, 0.01 mg/L IBA, 30 g/L sucrose and 7 g/L agar. Plantlets were exposed to 0%, 1% and 2% polyethyleneglycol (PEG) as drought stress levels during four weeks. At the end of the stress period, the genotypes were evaluated in terms of total number of shoots per explant, the proline, chlorophyll, total phenolics, total flavonoids and total protein contents, and the superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) enzyme activities of the shoots were determined. The results showed that the drought stress increased total flavanoids, phenolic compounds and proline contents, while it reduced the number of shoots, chlorphyll a, b and total chlorophyll contents. Moreover, the drought stress increased the activities of APX, CAT and SOD enzymes in all genotypes. On the other hand, it decreased the protein content in six genotypes but increased the protein content in the nine genotypes. Based on the results, it was observed that the almond genotypes were generally tolerant to the drought. However, it was determined that the genotypes numbered 9, 29 and 185 showed more tolerant to the drought as compared to the other genotypes. These results suggest that improving the antioxidant system can enhance the drought tolerance of rootstocks.

Key words: *Prunus amygdalus*, almond, seedling, total phenolic substance, total flavonoid, proline content

1. Introduction

Fruit species are widely distributed in temperate, subtropical and tropical belts and can be potentially exposed to numerous abiotic and biotic stresses during cultivation, handling, storage and distribution (Serce et al., 2010; Engin and Mert, 2020; Kaskoniene et al., 2020).

Stress can be defined as the factors negatively affecting the plant growth. Therefore, it causes a decline in plant growth functions, respiration and photosynthesis and negatively affects the chlorophyll and protein syntheses (Özen and Onay, 2007). The stress in plants is examined under two groups as the abiotic stress caused by factors such as drought, salinity and cold, and the biotic stress caused by bacteria, viruses and fungi (Ma et al., 2016; Orhan et al., 2020).

In today's world where water resources are scarce, drought has been the biggest threat and the main cause of famines that occurred in the past years. As the water capacity of the world is limited, rapidly increasing population, uncontrolled urbanization and wrong agricultural practices lead to the depletion of existing water resources (Somerville and Briscoe, 2001). The causes such as global warming, the depletion of water resources, and climate change will continue to be among the major threats affecting agricultural production in the future (Zhao and Running, 2010). The severity of the drought may vary depending on many factors such as the precipitation formation and the distribution, evaporation and soil moisture storage capacity (Wery et al., 1994). Water shortages and the drought in agricultural ecosystems have caused the yield losses in many crops and major problems worldwide. Therefore, saving water and growing drought-tolerant products have become today's primary goals to ensure world food security. In the last decade, great progress has been acomplished in the plant drought tolerance, with new findings and the rapid development of many new techniques and methodologies (Luo et al., 2019).

During drought stress, the water balance in the plant tissues deteriorates, the cells remain small due to the loss of turgor and the peripheral synthesis is negatively affected (Lichtenthaler, 1996; Zengin, 2007; Amira,

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2011). Moreover, under stress conditions, the synthesis of reactive oxygen species and free radicals increase in the plant, causing oxidative stress in the cell (Tsugane et al., 1999). Plants develop antioxidant defense mechanisms in order to be protected from the negative effects of free radicals and to survive (Apel and Hirt, 2004; Perezperez et al., 2012; Zhou et al., 2013; Li et al., 2016; Shangguan et al., 2018). Nonenzymatic antioxidant substances such as total flavonoids, total phenolics and proline, and the enzymatic antioxidants such as SOD, CAT and APX play an important role in the stress defense systems of plants.

In recent years, drought has become one of the most important problems in many parts of the world with the effects of global warming. In this respect, the drought tolerance has become one of the most important criteria in rootstock breeding. Almond is generally a drought tolerant species. However, there is a wide variation in drought tolerance within the same species. Therefore, determining the more drought tolerant almond genotypes will have important advantages in terms of breeding and yield. The drought tolerant genotype selection requires a lot of tasks under field conditions. Therefore, screening and selecting drought tolerant genotypes from selected elite clones via plant tissue culture is an effective and economical process. In this study, it was aimed to determine the drought tolerance levels of some promising almond rootstocks and the realtionship between the drought tolerance and the biochemical contents and enzyme activities of the roothstocks under in vitro conditions.

2. Materials and methods

In the study, 15 superior rootstock candidates selected (according to the characteristics of regular fruiting and healthy development) from Isparta region by Yıldırım (2007) were used as material. For this purpose, a few drops of tween 20 was added to the shoot tips taken at the beginning of vegetation, and it was disinfected by shaking for 20 min in an 18% commercial sodium hypochlorite solution. Then, the shoot tips were washed 3 times for 5 min with sterile distilled water. In the study, shoot tips were cultured in the MS medium containing 1.0 mg/L BAP and 0.01 mg/L IBA (Channuntapipat et al., 2003). A total of 30 g/L sucrose, 7 g/L agar were added to the nutrients medium and its pH was adjusted to 5.7. In order to be used in the drought stress tolerance studies, they were subcultured 4 times in MS medium containing the same growth regulator combination until a sufficient number of shoots were obtained.

In the drought stress experiments, 0%, 1% and 2% polyethyleneglycol (PEG) was additionally added to the reproduction medium (Ahmad et al., 2020). After the cultures were grown in the climate chamber for 4 weeks, the genotypes were examined in terms of their responses to the stress conditions. The condition of climate chamber was 25 ± 1 °C with a 16-h photoperiod under cool white fluorescent light and the light intensity of inside was set to 140 ± 10 mmol/m^2s. After the incubation, the genotypes were evaluated in terms of total number of shoots per explant. In addition, the biochemical analyzes including the proline, chlorophyll, total phenolics, total flavonoids and total protein contents and the SOD, CAT and APX enzyme activities of the shoots were performed.

2.1. Analysis performed in stress applications

2.1.1. Determination of proline content

Proline content of the samples obtained from in vitro conditions were determined as described by Liu et al. (2012a). A total of 0.1 g of sample was taken and homogenized with 2 mL of 3% sulfosalicylic acid. A total of 200 µL of the prepared plant extraction was added to the same amount of ninhydrin and glacial acetic acid and was incubated in a water bath for 1 h at 100 °C. Then its activation was stopped on ice. After this mixture was extracted with 1 mL of toluene, the absorbance at 520 nm of the toluene fraction aspirated from the liquid phase was read in the spectrophotometer. The proline concentration was determined as µmol proline g^{-1} fresh weight with the help of calibration curve.

2.1.2. Determination of chlorophyll content

Zhang and Huang's (2013) method were used to determine the chlorophyll content. A total of 0.1 g of sample was weighed and homogeneously fractured in 100% DMF. The homogenate obtained was centrifuged at 10,000 g for 10 min. The absorbance of the upper phase (liquid part) taken after centrifugation was measured at 664 and 647 nm wavelengths and the amount of chlorophyll a, chlorophyll b and chlorophyll a + b was calculated with the help of the following formula (Inskeep and Bloom, 1985; Aono et al., 1993; Sibley et al., 1996).

[chlorophyll a] = 12.7 × A664 – 2.79 × A647

[chlorophyll b] = 20.7 × A647 – 4.62 × A664

[chlorophyll a + chlorophyll b] = 17.90 × A647 + 8.08 × A664.

2.1.3. Determination of the total phenolic content

A total of 0.2 g leaf sample was added to 10 mL of 80% methanol and homogenized with a homogenizer and then mixed in a shaking incubator for 15 min at room temperature. Then, after being centrifuged at 4000 × rpm for 10 min, the liquid part was separated, and after adding 80% methanol on the solid part again, the same processes were repeated. The final volume was adjusted to 25 mL with 80% methanol. Total amounts of phenolic compounds in leaf samples extracted with methanol were determined using Folin Ciocalteu colorimetric method as described by Singleton and Rossi (1965). The total amount of phenolic compounds was determined in terms of gallic

acid in the form of mg g^{-1} wet weight by the measurements at a wavelength of 765 nm in the spectrophotometer and using the curves prepared with a standard gallic acid solution.

2.1.4. Total amount of flavonoid substance

The total amount of flovanoid substances were determined using the method as described by Zhisken et al. (1999). A total of 0.2 g of sample was homogenized with a homogenizer in 10 mL of 80% methanol and mixed for 15 min at room temperature in a shaking incubator. Afterwards, it was centrifuged at 4000 × rpm for 10 min, the supernatant was separated, and 80% methanol was added to the pellet again and the same process was repeated. The final volume was adjusted to 25 mL with 80% methanol. A total of 1.5 mL of distilled water and 75 µL of 5% sodium nitrite solution were added onto 0.25 mL of methanol extract and incubated for 6 min at room temperature. After incubation, 0.15 mL 10% AlCl3 was added to the mixture and it was incubated again for 5 min. After incubation, 0.5 mL of 1 M NaOH was added and the absorbance was read at 510 nm wavelength. The results were calculated according to the catechin standard and expressed as mg/g.

2.1.5. Total soluble protein content

The total protein content was determined according to the method described by Hartree-Lowry (1972). A total of 5 mL of cold EtOH and 1 g sample were mixed and homogenized with a homogenizer. Subsequently, the samples were centrifuged at 10,000 g for 20 min at 4 °C and the liquid part was removed. A total of 8.333 mL of 80% cold EtOH was added on the remaining solid part and the solid part was dissolved thoroughly. Then the liquid part was removed by centrifugation under the same conditions. A total of 5 mL of protein extraction buffer (50 mM Tris + 1.2 M NaCl Ph: 7.0) was added to the solid part and the pellet was thawed and left to incubate on ice for 30 min. Then it was centrifuged under the same conditions and the supernatant was filtered with a miracloth. A total of 1 mL was taken from the protein extract and 0.9 mL of reagent A was added to it and vortexed. It was incubated at 50 °C for 10 min and cooled to room temperature. Then 0.1 mL reagent B was added to the tubes and mixed. It was again incubated at room temperature for 10 min. After incubation, 3 mL reagent C was quickly added to the tubes and mixed. After incubation at 50 °C for 10 min, it was cooled to room temperature. Finally, the absorbance values of the samples were read at 650 nm wavelength. Results were calculated according to standardization (BSA) and expressed as mg/mL.

2.1.6. Determination of SOD enzyme activity

SOD enzyme activity was determined according to the method reported by Constantine and Stanley (1977). For this purpose, 3 mL of reaction mixture containing 50 mM potassium phosphate solution (pH: 7.3), 13 mM L-methionine, 75 µM Nitroblue Tetrazolium (NBT), 0.1 mM EDTA, 4 µM riboflavin and 0.25 mL enzyme extract was incubated under 48 µmol photons m^{-2} s^{-1} light intensity for 10 min and the absorbance values were measured at 560 nm in the spectrophotometer. Since 1 unit of SOD activity was defined as the amount of enzyme required to inhibit 50% of the photoreduction of NBT in the presence of riboflavin and light, SOD activity was determined accordingly and the unit was evaluated as mg $protein^{-1}$.

2.1.7. Determination of CAT enzyme activity

CAT enzyme activity was determined as described by Beers and Sizer (1952). The enzyme activity was determined by the method of determining the decrease in H_20_2 absorbance at 240 nm by the spectrophotometer. For this purpose, 3 mL of the reaction mixture was prepared which contained 50 mM potassium phosphate solution (pH: 7.0), 15 mM H_2O_2 and 50 µL enzyme extract. The reaction was started by the addition of the enzyme. The CAT activity was calculated using the extinction coefficient (ε: 39.4 mM^{-1} cm^{-1}) and expressed as µm min^{-1} mg $protein^{-1}$.

2.1.8. APX enzyme activity

APX enzyme activity was determined using the procedure as described by Nakano and Asada (1981). For this purpose 4 g sample was homogenized in 12 mL 50 mM potassium phosphate buffer (1 mM EDTA, 2 mM DTT and 1 mM ascorbic acid) (Ph: 7.3) at 10,000 g, and centrifuged at 4 °C for 15 min. After centrifugation, the supernatant was used for the analysis. A total of 0.1 mL of enzyme extract was added to 0.9 mL 0.05 M sodium phosphate buffer (0.5 mM ascorbate, 0.1 mM Na_2EDTA and 1.2 mM H_2O_2, pH: 7.0), and readed at a spectrophotometer at 470 nm wavelength. After holding for 3 min and the absorbance was read again at the same wavelength. Results were expressed as mol/min/g protein.

2.2. Statistical analysis

Completely randomized design was used in in vitro drought stress experiment with 6 replications per treatment and five plants per replication. The data were subjected to the analyses of variance (ANOVA) at $p \leq 0.05$ using MINITAB statistical software (MINITAB Inc., Coventry, UK). Means were separated by Tukey's multiple range test at $p \leq 0.05$.

3. Results and discussion

A total of 0%, 1% and 2% polyethylene glycol (PEG) was applied to the genotypes in the MS medium. After the shoots were grown in the climate chamber for 4 weeks, their responses to the stress conditions were examined. The effects of drought stress treatments on the number of shoots and chlorophyll contents in the 15 different genotypes were presented in Table 1. In the study, it was

Table 1. Shoot number and chlorophyll content of almond genotypes under different levels of drought stress conditions.

Genotype number	Total number of shoots (number/explant)			Chlorophyll a (mg/g)			Chlorophyll b (mg/g)			Chlorophyll a + b (mg/g)		
	Control	1% PEG	2% PEG	Control	1% PEG	2% PEG	Control	1% PEG	2% PEG	Control	1% PEG	2% PEG
9	3.80 A*a**	1.90 Bab	1.50 Babc	4.20A1	3.13Ag	1.69Bf	32.71Af	15.48A1	15.94Bd	36.89Ag	18.60Ah	17.61Be
29	2.20 Aabc	1.45 Aab	1.95 Aab	6.55Ae	3.73Bf	2.36Ce	38.31Ab	34.23Bb	20.15Cabc	44.84Ab	37.95Bb	22.49Cbc
40	2.30 Aabc	1.35 ABab	1.15 Bbc	3.27Aj	1.77Bj	1.55Cf	27.69Ah	16.16B1	13.28Cef	30.95Aj	17.92Bh	14.82Cf
54	2.55 Aabc	2.10 ABab	1.55 Babc	6.31Cf	4.26Ad	3.03Bd	37.88Ac	44.16Ba	21.32Ca	44.17Ac	48.40Ba	24.34Ca
120	2.35 Aabc	1.25 Bab	1.35 Babc	8.31Ab	2.46Bh	2.30Be	38.93Aa	22.55Be	21.36Ca	47.22Aa	25.00Be	23.65Cab
121	1.80Abc	0.85ABb	0.65Bbc	5.20Ah	3.60Bf	3.05Cd	32.48Af	30.31BCc	20.34Cab	37.66Af	33.89Bc	23.39Cab
129	1.75 Abc	1.45 Aab	1.15 Abc	6.36Af	5.39Bc	3.15Cd	33.10Ae	28.76BCd	18.45Cc	39.44Ae	34.13Bc	21.60Ccd
134	2.80 Aabc	2.00 ABab	1.00 Bbc	4.25A1	2.33Bh1	1.72Cf	39.02Aa	18.39Bh	13.59Cef	43.26Ad	20.70Bg	15.30Cf
163	2.55 Aabc	1.30 Bab	1.10 Bbc	2.56Al	3.08Bg	2.37Ce	22.26Aj	15.89B1	12.21Cf	24.80Ak	18.96Bh	14.57Cf
176	1.45Ac	0.85Ab	1.00Abc	9.98Aa	3.98Be	3.50Cc	36.98Ad	29.97Bcd	20.02Cabc	46.94Aa	33.93Bc	23.52Cab
183	3.25 Aab	2.75 Aa	2.65 Aa	7.94Bd	7.23Ab	6.50Ba	25.87A1	20.74Bf	13.90Cef	33.80A1	27.95Bd	20.39Cd
185	2.35 Aabc	1.65 Aab	1.65 Aabc	2.54Al	1.27Bk	1.24Cg	21.32Ak	19.49Bgh	16.10Cd	23.85Al	20.75Bg	17.34Ce
196	2.70 Aabc	0.60 Bb	0.90 Bbc	8.07Ac	7.68Ba	6.09Cb	27.48Ah	18.27Bh	14.38Cde	35.53Ah	25.94Be	20.46Cd
228	2.05 Abc	1.45 ABab	0.50 Bc	5.36Ag	2.50Bh	2.18Be	31.46Ag	20.59Bfg	15.78Cd	36.80Ag	23.08Bf	17.95Ce
241	2.35 Aabc	1.85 ABab	1.00 Bbc	3.06Ak	2.17B1	1.46Cfg	27.79Ah	22.73Be	19.06Cbc	30.84Aj	24.89Be	20.51Cd

* The difference between the averages shown in different capital letters on the same row for each feature is statistically significant. ** The difference between the averages in different lowercase letters in the same column is statistically significant ($p < 0.05$).

determined that the number of shoots in all genotypes decreased significantly as compared to the control treatment in response to the increase in the level of drought stress. When evaluated in terms of shoot number, it was determined that the genotypes numbered 183, 29, 54, 9, 120 and 185 demonstrated higher drought tolerance in 2% PEG application as compared to the other genotypes. In the study, it was determined that the chlorophyll a, chlorophyll b and total chlorophyll (a + b) contents varied significantly according to the genotypes (Table 1). It was determined that the chlorophyll contents were significantly decreased in 1% and 2% PEG applications as compared to the control. As parallel to the increase in the drought level, the total chlorophyll contents of the genotypes decreased by 27.33% to 64.63%.

The effects of drought stress applications on the total flovonoid, proline, total phenolic and total protein contents in 15 different genotypes were presented in Table 2. In all genotypes, it was determined that the total flovonoid content increased with increasing drought severity. It was determined that the increase in total flovonoid content with increasing drought severity was very high in the genotypes numbered 9, 163, 40 and 121 (147%, 126%, 86% and 77%, respectively). In the study, it was observed that the proline content of the genotypes increased significantly with the increase in the drought stress. Proline contents varied between 2.64 µmol/g and 8.38 µmol/g in the control treatment and between 6.23 µg/mL and 16.24 µmol/g 2% PEG application according to the genotypes. However, it was determined that the increase in proline content was higher in genotypes 29, 185, 40 and 134 (405%, 390%, 379%, 360%, respectively) as compared to the other genotypes with increasing drought severity. In the study, it was determined that the total amount of phenolic compounds in all genotypes increased with the increase in drought severity. In some genotypes (9, 121, 134 and 241), this increase was determined to be up to 3 folds as compared to the control. The protein contents of the genotypes varied between 0.28 mg/mL and 0.89 mg/mL in the control treatment. In parallel with the increase in the drought severity, it was determined that there were increases from 50% to 165% in the protein content of the genotypes numbered 9, 29, 54, 176, 183, 185, 196 and 241. However, it was determined that 2% PEG application decreased the protein content as compared to the control in the other genotypes.

The effects of drought stress applications on APX, CAT and SOD enzyme activities performed in 15 different genotypes were presented in Table 3. In the research, it was determined that all 3 enzymes increased with the increasing drought severity. Especially, APX enzyme activity in genotypes numbered 9, 40, 134, 163, 183, 185 and 196 increased more than 5 times in 2% PEG application as compared to the control. Moreover, CAT activity demonstrated increases more than 5 times in the genotypes 29, 40, 129, 134, 163, 185 and 196 in 2% PEG treatment as compared to the control. On the other hand, SOD enzyme activity increased more than 3 folds as compared to the control in 2% PEG application in genotypes numbered 9, 29, 120, 129 and 241.

Drought stress in plants occurs when the water coming to the roots is insufficient or the transpiration rate is too high (Lisar et al., 2012). The mechanism of tolerance to drought stress in plants can be examined under two main headings: tolerance and avoidance. Drought tolerant plants operate their protective mechanisms under stress conditions (total phenolic substance synthesis, protein, proline, increase in enzyme activity, etc.) to maintain their osmotic balance and maintain their turgority during stress conditions. The drought avoidance mechanism is that plants stop their growth and development completely in arid conditions, as in desert plants, and maintain their vitality when they become dormant (Mundree et al., 2002; Kuşvuran et al., 2011).

In general, in the study, it was determined that the number of shoots decreased as the PEG concentration added to the nutrient medium to create drought stress increased. In the study, it was determined that the genotypes numbered 9, 29, 54, 120, 183 and 185 in MS environments containing 2% PEG came forward in terms of the number of exiles as compared to the others. Therefore, these genotypes were more drought tolerant than the others. Sivritepe et al. (2008) cultured GiselA 5 cherry rootstock in the MS medium containing 0%, 1%, 2% and 4% PEG to determine their response to the drought stress. The researchers stated that the shoot length and chlorophyll content decreased in parallel with the drought severity. Similarly, in a study conducted on vines, it was reported that the number and length of shoots decreased in parallel with the severity of drought (Babalık et al., 2016). In two different citrus rootstocks, the number of shoots decreased by 5 times and the length of the shoots decreased by almost 50% with the increase in drought severity in the mediums containing 0%, 1%, 2%, 4%, and 6% PEG (Şimşek et al., 2018). Moreover, it has also been reported by other researchers that the number of shoots, shoot length and leaf chlorophyll contents decreased with the increases in drought severity (Kaynaş and Eriş, 1998; Sakalauskaite et al., 2006; Liu et al., 2012b; Bolat et al., 2014).

Phenolic compounds (such as flavonoid and phenolics), which have strong antioxidant properties by binding the reactive oxygen radicals to themselves, constitute the most important secondary metabolite products of plant metabolism (Halliwell, 2008; Berli et al., 2010; Babalık, 2012). It has been stated that these

Table 2. Flovonoid, proline, total phenolic and total protein contents of almond genotypes under different levels of drought stress conditions.

Genotype number	Flavonoids (mg/g)			Total phenolics (mg/g)			Proline (µmol/g)			Total protein (mg/mL)		
	Control	1% PEG	2% PEG	Control	1% PEG	2% PEG	Control	1% PEG	2% PEG	Control	1% PEG	2% PEG
9	0.34Cf	0.58Bde	0.84Ae	0.47Cgh	0.75Bıj	1.48Aefg	3.07Cef	6.05Bghı	9.02Af	0.28Be	0.32ABg	0.42Ac
29	0.70Cbc	0.80Bc	0.88Ade	1.09Cde	1.37Bcd	1.80Acd	2.64Cf	5.15Bı	13.34Acde	0.38Cde	0.83Bbc	0.96Aa
40	1.00Ca	1.25Ba	1.86Aa	2.72Ca	3.14Ba	3.75Aa	2.89Cef	5.18Bı	13.85Acd	0.54Bcd	0.97Aa	0.48Bbc
54	0.72Cbc	0.85Bc	0.96Ad	0.58Cfg	0.77Bhı	1.18Ahıj	8.38Ca	9.31Bcd	12.70Ade	0.45Ccd	0.66Bcd	0.86Aa
120	0.70Cbc	0.79Bc	0.90Ade	1.14Bcd	1.20Bdef	1.41Afgh	3.51Cdef	7.52Befg	9.80Af	0.56Abc	0.32Cg	0.36Bc
121	0.44Cef	0.52Bef	0.78Ae	0.59Bfg	1.12Aefg	1.28Aghı	4.47Ccde	10.03Bbc	12.33Ade	0.89Aa	0.51Bef	0.59Bb
129	0.58Ccd	0.78Bc	0.88Ade	1.27Bc	1.39Bcd	1.55Adef	8.27Ca	9.81Bbc	12.12Ae	0.57Abc	0.46Bef	0.42Bc
134	0.42Bef	0.44Bf	0.56Af	0.33Ch	0.50Bj	1.09Aj	3.53Cdef	5.57Bhı	16.24Aa	0.41Bde	0.54Aef	0.42Bc
163	0.46Cef	0.80Bc	1.04Ad	0.57Cfg	0.86Bghı	1.10Aj	6.13Cbc	11.16Bab	16.16Aa	0.58Abc	0.55Ade	0.48Bbc
176	0.98Ba	1.05Bb	1.17Ac	1.09Cde	1.59Bbc	1.93Ac	2.94Cef	4.50Bı	6.23Ag	0.72Bb	0.92Aab	1.09Aa
183	0.82Cb	1.05Bb	1.37Ab	1.59Bb	1.73Bb	2.23Ab	5.28Cbc	7.77Bdef	9.09Af	0.68Cb	0.80Bbc	1.03Aa
185	0.40Cef	0.50Bef	0.63Af	0.75Cef	1.07Bfgh	1.38Afgh	3.28Cdef	12.64Ba	16.09Aab	0.40Bde	0.42Bfg	1.06Aa
196	0.67Cbc	0.77Bc	0.88Ade	1.07Cde	1.29Bde	1.67Acde	6.31Cb	7.74Bdef	11.82Ae	0.46Ccd	0.60Bde	0.86Aa
228	0.62Ccd	0.72Bcd	0.98Ad	0.99Bde	1.21Bdef	1.52Aefg	6.15Cb	8.98Bcde	14.75Aabc	0.60Abc	0.53Aef	0.56Ab
241	0.47Cde	0.61Bde	0.83Ae	0.77Cef	0.95Bfgh	2.26Ab	4.84Cbcd	6.86Bfgh	14.55Abc	0.54Ccd	0.74Bcd	0.98Aa

* The difference between the averages shown in different capital letters on the same row for each feature is statistically significant.
** The difference between the averages in different lowercase letters in the same column is statistically significant ($p < 0.05$).

Table 3. Enzyme activities of almond genotypes under different levels of drought stress conditions.

Genotype number	CAT (U/mg protein)			SOD (U/mg protein)			APX (mol/min/g protein)		
	Control	1% PEG	2% PEG	Control	1% PEG	2% PEG	Control	1 %PEG	2% PEG
9	8.87Cab	12.01Bb	13.31Ad	6.63Cg	15.69Bfg	22.11Af	0.12Cd	2.24Ba	4.00Aa
29	5.71Cef	12.21Bb	13.97Ad	5.45Bg	25.94Abcd	28.14Acde	0.58Cab	1.03Bcd	2.12Ad
40	7.29Cbcde	8.81Bdef	19.47Ab	7.95Cg	14.30Bg	21.92Af	0.12Cd	0.78Bde	1.40Aef
54	8.35Cabc	10.15Bcd	12.46Adef	22.64Cabc	27.70Abc	30.64Bbcd	0.58Bab	0.74Bdef	1.41Aef
120	7.85Cbcd	9.62Bdef	11.97Aefg	5.07Cg	9.87Bh	25.08Aef	0.53Cabc	0.93Bde	1.27Afg
121	7.23Ccde	9.11Bdef	11.60Afgh	12.23Cf	18.98Bef	26.98Ade	0.60Bab	0.73ABdef	0.93Ahı
129	4.08Cg	6.98Bgh	10.06Ahı	5.30Cg	15.94Bfg	27.25Acde	0.63Bab	1.37Ab	1.67Ade
134	7.49Cbcd	11.72Bbc	17.17Ac	20.83Cbc	28.94Bab	41.67Aa	0.20Bcd	0.41Bfg	1.01Aghı
163	4.78Cfg	8.49Befg	10.50Aghı	26.10Ca	32.39Ba	41.76Aa	0.07Bd	0.20Bg	0.70Aı
176	7.14Ccde	9.97Bde	13.38Ade	16.83Cde	19.22Bef	30.60Abcd	0.29Cbcd	0.61Bef	1.24Afgh
183	5.42Cfg	6.44Bh	8.95Aı	13.89Cef	22.67Bde	28.38Acde	0.53Cabc	1.26Bbc	3.08Ab
185	9.87Ca	19.66Ba	23.23Aa	18.85Ccd	23.78Bcd	26.78Ade	0.10Cd	0.37Bfg	1.75Ad
196	6.40Cdef	7.95Bfgh	23.23Afgh	23.52Cab	25.33Bbcd	34.17Ab	0.09Cd	0.55Bef	1.11Afgh
228	6.34Cdef	7.99Bfgh	9.95Ahı	15.58Cdef	24.30Bcd	31.07Abc	0.74Ba	0.94Bde	1.75Ad
241	8.74Cabc	9.83Bde	10.80Agh	4.50Cg	16.20Bfg	27.17Ade	0.69Ca	1.53Bb	2.59Ac

* The difference between the averages shown in different capital letters on the same row for each feature is statistically significant.
** The difference between the averages in different lowercase letters in the same column is statistically significant ($p < 0.05$).

substances played the roles in the removal of free oxygen radicals. However, similar to our study, it has been reported by many researchers that there was an increase in the amount of substances such as total flavonoids and total phenolics synthesized in plant tissues under stress conditions (Rezazadeh et al., 2012; Valifard et al., 2014; Bolat et al., 2014; Krol et al., 2014; Rebey et al., 2017). It has been reported that the proline synthesis showed variations under drought stress conditions depending on the genotypes and severity of drought (İpek, 2015). The increase in proline synthesis, which is an osmotic regulator, caused a decrease in the water potential within the cell and facilitated their tolerance to high evaporation by preventing the water loss between cells. In addition to being an osmotic regulator, proline also has biological functions such as energy source and antioxidant properties (Ahmed et al., 2008; Deluc et al., 2009; Chookhampaeng, 2011). Ashraf et al. (2005) reported that proline was the first osmolyte that accumulated in almost all plants during stress and protected the plants against stress conditions. Moreover, Babalık (2012) reported that proline synthesis increased in the plant tissues under stress conditions, and the increased proline level might be an indicator of tolerance to stress conditions. In our study, it was found that there was an increase in proline accumulation with the increase in drought severity. Moreover, it has been reported by other researchers that the proline synthesis increased under stress conditions (Çetin et al., 2011; Lum et al., 2014; Per et al., 2017; Ghaffari et al., 2019; Fiasconaro et al., 2019; Furlana et al., 2020).

In the study, it was determined that the total protein contents decreased in 6 genotypes (genotypes 40, 120, 121, 129, 163 and 228) with increasing drought severity, but increased in the other genotypes. It has been reported in previous studies that the protein synthesis decreased with the increase in drought severity in some genotypes (Gong et al., 2005; Bertamini et al., 2006) but increased in the others (Bray, 1997; Campalans et al., 1999). The reactions of plants to the stress conditions are quite complex. In some studies, it has been reported that the amino acid content increased in parallel with the decrease in protein content under stress conditions. Some researchers have reported that the increase in amino acids was due to the breakdown of proteins. Some researchers have reported that stress conditions increase the synthesis of proteins involved in the synthesis of abscisic acid and heat shock proteins, and thus increasing the adaptability of plants to drought stress (Campalans et al., 1999). Heat shock proteins belong to a larger group of molecules called chaperones. They function in the stabilization of the structure of other proteins. These proteins have low molecular weights and are synthesized in the cell during environmental stress (Wahid et al., 2007). Moreover, Coca et al. (1994) reported that the heat shock proteins were also stimulated by different stresses such as drought, anaerobic conditions and low temperatures. In addition, the increase in the amount of proteins that have a protective effect against stress conditions in the drought-tolerant plants also limits some biochemical degradations that occur under stress conditions and play a role in the elimination of toxic substances (Bray, 1997). The increase in the synthesis of these stress proteins is a response to deal with all stress conditions, including water deficiency. Most stress proteins are water soluble. Therefore, they contribute to the stress tolerance by the hydration of cellular structures (Wahid et al., 2007). Plants can protect their vitality by developing some enzymatic antioxidant defense mechanisms against reactive oxygen species under stress conditions. SOD, CAT and APX are among the most important of these enzymatic antioxidants. It has been reported by many researchers that these antioxidant enzyme activities increases under stress conditions and thus plants can survive (Gong et al., 2005; Doupis et al., 2011; Babalık, 2012).

In the study, it was determined that the rates of increases in CAT enzyme activities showed variations according to the genotypes. In some studies, it was reported that CAT enzyme activity increased with the increase in drought severity (Sivritepe et al., 2008; Yang et al., 2009; Bolat et al., 2014; Babalık et al., 2016), while some studies reported that it decreased or did not change (Ünyayar and Çekiç, 2005; İpek, 2015). Ünyayar and Çekiç (2005) stated that the response of plants to drought stress might vary according to the species and even varieties. Similarly, in parallel with the increase in PEG ratios, significant increases were observed in the amount of SOD in all genotypes, and the increases of 5 to 7 fold as compared to the control were observed in the 29, 120, 129 and 241 genotypes. In a study conducted by Wang et al. (2012), it was stated that the more drought-resistant *Malus prunifolia* species had higher SOD enzyme activities than the more sensitive Malus hupehensis species. Similarly, other researchers reported that SOD enzyme activity increased with the increase in drought severity (Jung, 2004; Yang et al., 2009; İpek, 2015). However, Li et al. (2008) reported that in some Pyrus species, SOD enzyme activity increased under mild drought stress but decreased in severe drought. In the study, an increase in APX enzyme activity was determined in all genotypes with the increase in drought severity. It has been reported by other researchers that the increase in APX enzyme activity under stress conditions was higher especially in the drought tolerant plants (Yaşar et al., 2008; Ersöz, 2009, Babalık, 2012; Kuşvuran and Abak, 2012). Zrig et al. (2015) reported in their study that the increase in APX enzyme activity in Mazzetto almond variety showed a protective effect against environmental

stresses. Studies have reported that APX enzyme activity is responsible for the regulation of reactive oxygen species as a signal and CAT enzyme activity eliminates ROSes. In addition, it have been reported that the increase in APX enzyme activity in the plants under stress conditions plays an important role in maintaining the free radical level under the control (Mittler, 2002; Kuşvuran, 2012).

4. Conclusion

The breeding rootstocks tolerant to abiotic and biotic stress factors increase its importance day by day, so that very large areas can be made suitable for production. At this point, the seedling rootstocks constitute an important advantage against the climate changes (drought, sudden floods, etc.) and the increasing environmental stress (salinization, chemical pollution, etc.) conditions, which are considered certain to occur in the future in the world. Janick and Moore (1996) have reported that almond rootstocks are widely used in the arid and unfavorable soil conditions, they do not show incompatibility with almond varieties, and their adaptation ability to arid and calcareous soils are better. Moreover, Sousa and Pereira (1994) stated that the most important feature of rootstocks used in almond cultivation in the Mediterranean region is the ability to adapt to arid conditions. As a result, it was determined that the almond genotypes were generally tolerant to the drought. However, it was determined that the genotypes numbered 9, 29 and 185 showed more tolerance to the drought as compared to the other genotypes.

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