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Research Article

Assessment of Some Selected Cultivars of Almond on GF677 Rootstock in Drought Stress Conditions

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Abstract: Drought stress is the most important factor limiting the successful production of agricultural crops worldwide. The response of some almond cultivars grafted on GF677 rootstock was studied to drought stress at a research greenhouse in the Horticultural Science Research Institute of Karaj, Iran, in the 2016-2017 growing season. The plant materials included 12 almond (*Prunus dulcis* Mill.) genotypes/cultivars, including ‘Sh10’, ‘Saba’, ‘A1-16’, ‘Shokofeh’, ‘Kh1’, ‘A230’, ‘Mamaie’, ‘A13-40’, ‘A9-7’, ‘A8-24’, ‘Fragiolu’, and ‘Sh17’, which were grafted onto Gf677 rootstock. The results showed that the treated almonds differed significantly. When all studied traits are considered, it can be concluded that genotypes ‘Kh1’ and ‘A13-40’ outperformed all other genotypes and cultivars in terms of the studied morphological and physiological traits and exhibited far more tolerance to drought stress. In normal conditions, cultivars Kh1 and Mamaei had maximum proline (2.35 µmole/g), but A8-24 still showed the lowest content of proline (1.20 µmole/g). The highest K content under drought stress was obtained in A1-16 (2.8 %) and Mamaei (2.71 %) cultivars.

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1. Introduction

Almond (*Prunus dulcis* Mill.) is one of the oldest nuts with a high production rate in the world. The crop plays a significant role in the agricultural economy of the arid and semi-arid regions (Rouhi et al., 2007; Egea et al., 2009). On the other hand, drought is the most limiting factor of viable crop production in the world. It happens when a mix of physical and environmental factors creates stress inside plants and impairs their production. This impairment results from delayed or no plant establishment, debilitation or killing of the established plants, plant exposure to diseases and pests, and physiological and biochemical changes in plant metabolism (Scholz et al., 2008).

Although almond is a drought-resistant crop, it requires irrigation over the growing season to guarantee their economic crop production (Romero et al., 2004). The loss of water potential of almonds induced by water stress entails the reduction of tree growth, high leaf shedding, lower kernel weight, and the change in pericarp color. In addition, stomatal conductance and carbon metabolism are reduced (Isaakidis et al., 2004). In a study, rootstocks were exposed to daily irrigation treatments up to field capacity and drought stress for five days in July and August. The results showed that the GF677 rootstock was the most adapted to different soil moistures. This rootstock could maintain water availability for photosynthesis even in the extended droughts by gradual stomatal closure (Yadollahi et al., 2011).

A study of the response of some *Prunus* species to drought stress indicated that specific leaf area could be a morphological indicator for the assessment of drought resistance of the species. Also, the ratio of root dry weight to leaf area and the ratio of root length to leaf area exhibited high correlations with drought resistance (Rieger et al., 2003). Genotypes vary in their drought resistance. It has been documented that Masbovera is more appropriate for rain-fed farming (De Herralde et al., 2001). De Herralde et al. (2003) reported that the cultivars ‘Nonpareil’, ‘Marcona’, and ‘Giarrigues’ had a strong drought resistance trait.

Genotypes and ecotypes of almonds that show more root development are more tolerant of water stresses. The roots of *Amygdalus scoparia* ecotypes are not influenced by water stress extensively when compared to cultivated almonds, but when the seedlings of *Prunus dulcis* genotypes are irrigated adequately, their roots show more extension than the seedlings of *A. scoparia* (Sardabi et al., 2003). According to Camposeo et al. (2011), environmental conditions, especially temperature, and seasonal variations, can change leaf development and stomatal characteristics. They focused on the effect of water reduction on stomatal and leaf parameters in cultivars of almond and wild almonds (*Amygdalus webbii*) in field conditions. The results revealed broad differences in leaf area between the wild and cultivated almonds. The wild species lost its leaf area to a greater extent compared to cultivated species (% 31 vs. % 14). Almond cultivars have shown diverse responses in their leaf anatomic and physiological traits to water stresses. Some cultivars like ‘Masbovera’ have highly sensitive stomatal aperture whilst some varieties like ‘Guara’ produce thick cuticles. Accordingly, the leaf area is a genetic trait that can tolerate slight changes in environmental conditions (Gispert et al., 2011).

Zamani et al. (2002) studied the response of some almond seedlings selected from Iranian almond populations to drought stress. The results showed that the leaf area, stem length, root dry weight, and leaf water potential were lost with an increase in the irrigation interval, whereas proline content and stomatal resistance were increased. At lower levels of drought stress, drought adaptation was observed with a decrease in stomatal resistance after an initial increase. Leaf area was also decreased under the drought stress, and this reduced net photosynthesis. Likewise, the drought stress adversely influenced mesophyll conductance which is a non-stomatal factor underpinning the photosynthesis rate. Furthermore, chlorophyll a and b contents exhibited a decline in the stressful plants. However, these changes were a function of the cultivar (Javadi et al., 2006). The present study aimed to explore the tolerance of different cultivars of almonds to drought stress using physiological and morphological indicators in order to estimate the drought tolerance of resistant cultivars.

2. Material and Methods

2.1. Experimental site and plant material

The study was carried out in the Horticulture Research Station of Karaj, Iran, in 2016 on 12 almond cultivars and genotypes based on their main morphological and physiological traits related to drought resistance. The almond cultivars were obtained at the experimental orchard of the Plant and Seed Research Organization (PSRO) in Kamalabad of Karaj (Iran). The trees were assessed in drought stress and normal conditions. The cultivars were subject to the measurement of all vegetative traits, including growth and physiological parameters, so that data analysis could reveal the best combination of scion- rootstock and tolerant cultivar. After the planting of the trees in pots and their exposure to drought stress, the following morphological and physiological traits that had been shown to have close relationships with drought resistance/tolerance were examined.

2.2. Growth parameters

Growth was measured with a caliper. Also, the relative water content (RWC) of the leaves was assessed. To measure leaf RWC, four full leaves were detached from the upper part of the branch and four from the lower part. Then, after their fresh weight (FW) was recorded, they were placed in distilled water in the darkness at 4°C for 24 hours to swell. Then, they were taken out of the distilled water and were drained, and their turgidity weight (TW) was recorded. Then, the samples were oven-dried at

105°C for 24 hours to find out their dry weight (DW). Leaf RWC was calculated by the following equation:

RWC (%) = (FW- DW/ TW- DW) ×100

2.3. Proline measurement

First, 0.5 g of fresh plant material was crushed in a mortar, poured into 15-mL tubes, added with 10 mL of sulfosalicylic acid 3%, and placed in an ice-water solution for 10 minutes. Then, the tubes were centrifuged at 15000 rpm at 4°C for 10 minutes. After that, 2 mL of the supernatant was poured into 15-mL tubes and was mixed with 2 mL of ninhydrin acid and 2 mL of pure acetic acid. At the same time, 2 mL of standard 0, 4, 8, 12, 16, and 20 mg L^{-1} proline was added to new tubes and was well mixed with 2 mL of ninhydrin acid and 2 mL of glacier acetic acid. The main and standard samples were first placed in a hot water bath at 100°C for 1 hour and then in an iced water bath for 10 minutes to cool down and have all the reactions stop. Next, 4 mL of toluene was added to the solution and was mixed with a vortex for 20 seconds. The absorption of the samples was read at 528 nm with a spectrophotometer (BT600 Plus, Canada). Finally, proline content (in μmol g^{-1} FW) was found by the absorption rate of the samples and its comparison with a standard curve according to the following equation ((Bates et al., 1973):

μmole proline/g FW = (μg proline/ml × ml toluene) /[115.5μg/μmole) / (g sample/5])

2.4. K^+ measurement

To determine leaf K content, a sample of 15-20 leaves was taken from the branches grown from the middle part of the stems for leaf analysis in the plants exposed to drought. After they were washed and dried, they were placed at 70°C for 48 hours. Then, they were prepared for digestion by fresh oxidation method using sulfuric acid 96%, salicylic acid, hydrogen peroxide, and selenium (Jaiswal, 2014). An amount of 0.3 g of the plant sample was poured into a digestion tube and was mixed and shaken with 2.5 mL of the acid mixture so that all particles were soaked. After two hours, the digestion tubes were placed on a heater at 100°C for 2 hours. Then, they were cooled down and were added with 1 mL of hydrogen peroxide three times. Each time, the tube was shaken thoroughly for the reaction with hydrogen peroxide to complete. Then, they were placed on the heater again, but this time at 330°C. The digestion was conceived to be complete when the extract was bleached or turned into light yellow (2 h). The tubes were then cooled down, were added with 48.3 mL of distilled water, and were infiltrated after shaking. The K content was measured by flame photometry.

2.5. Statistical analysis

The experiment was analyzed as a randomized complete block design with three replications, each replication with two trees. 10-12 years old trees were evaluated under two conditions drought and normal stress (control). The cultivars and genotypes included A8-24, Sh17, Kh1, Shokufeh, Saba, A1-16, Sh10, A230, Fragiolu, Mamaei, A9-7, and A13-40 on a GF677 (*Prunus amygdalus* × *Prunus persica*) rootstock. Finally, the collected data were statistically analyzed by the SAS-9.1 software package. Means comparison was performed for the effect of treatments by Duncan's Multiple Range Test to select the best tolerant cultivar.

3. Results

3.1. Growth parameters

The results of the analysis of variance are presented in Table 1. Accordingly, almond cultivars exposed to drought stress and control plants (normal condition or without stress) differed significantly ($P < 0.01$ and $P < 0.05$). Means comparison for branch growth between the drought-exposed plants showed that water shortage in arid regions is a major factor limiting the growth of the trees considerably. This response was examined by measuring branch growth. All studied genotypes and cultivars exhibited significant differences between normal irrigation and drought stress conditions. The trees exposed to drought stress had lower average growth than those grown under normal irrigation conditions. This response may arise from the high demand for transpiration and/or the shortage of water that is required to build the compounds for growth. Although water deficiency during the drought stress period affected the growth of the branches, some cultivars and genotypes were influenced by this stress to a lesser extent.

The means comparison revealed significant differences in growth. The highest growth was observed in ‘Kh1’ and the lowest in ‘K7-9’. The results showed that in similar drought stress conditions, some genotypes of almonds outperformed others and showed better growth responses whilst other genotypes were severely influenced by the drought stress so that their growth was slowed down or even stopped (Fig 1 and 2). According to the comparison of the means for leaf yellowing in different irrigation treatments (Fig 3), this response was observed in all cultivars and genotypes exposed to severe drought stress, but it was stronger in some cultivars. As can be seen, ‘A9-7’ and ‘A13-40’ exhibited the highest and lowest leaf shedding, respectively. It is likely to reflect the capability of these cultivars in keeping their leaves, which is crucially important for their vital activities such as assimilation and the supply of nutrients for different parts of the plant, including fruits.

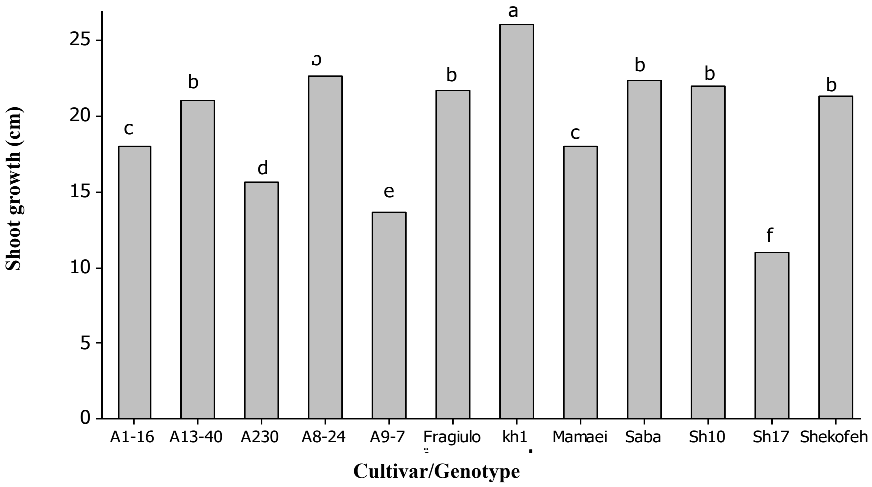

Figure 1. Means comparison for branch growth under drought stress.
Means followed by the similar letter(s) are not significantly different by Duncan test (P < 0.01).

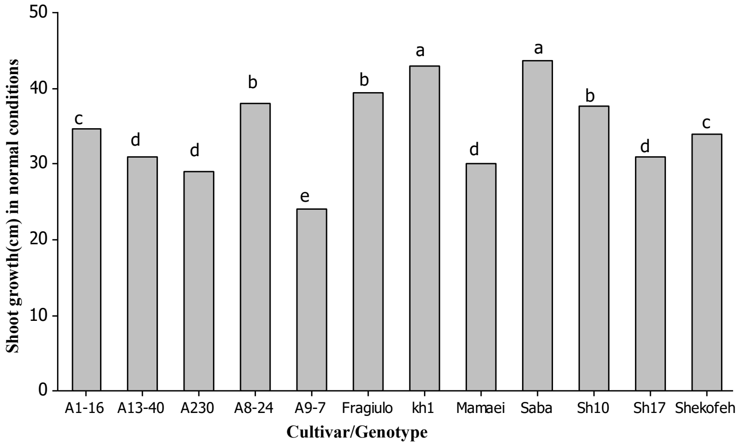

Figure 2. Means comparison for branch growth under normal conditions.
Similar letter on the bars show insignificant differences at the P < 0.01.

Table 1. The analysis of variance of morphological and physiological traits of almond cultivars in drought stress and normal conditions

Sources of variations	df	Mean of squares								
		Leaf yellowing	Growth under stress	Growth under normal conditions	RWC under stress	RWC under normal conditions	Proline under stress	Proline under normal conditions	Potassium under stress	Potassium under normal conditions
Cultivars	11	787.54^{**}	55.17^{**}	105.62^{**}	38.87^{*}	43.75*	0.33^{**}	2.04^{**}	1.11^{**}	0.138^{**}
Replication	2	3.69^{ns}	0.69^{ns}	6.36^{ns}	4.11^{ns}	3.17ns	0.007^{ns}	0.003^{ns}	0.004^{ns}	0.0001^{ns}
Error	2	5.02	2.30	4.81	3.50	2.86	0.01	0.02	0.003	0.02
C.V. (%)	4	5.97	7.80	6.34	2.49	3.30	5.45	3.52	2.23	1.67

*: Significance at the $P < 0.05$; **: Significance at the $P < 0.01$; ns: non-significance.

3.2. Physiological traits

The results of the comparison of the means revealed significant differences in the leaf relative water content (RWC) between the plants exposed to drought stress and those grown in normal conditions (Fig 4 and 5). Leaf RWC was one of the most important parameters that varied among the cultivars under similar drought stress conditions. This parameter represents the variations in the water content of leaf cells under stressful conditions. The plants exposed to the drought stress had lower leaf RWC, but the extent to which the leaf RWC was lower was different among different cultivars. In addition, the difference in the leaf RWC between normal irrigation and water deficit conditions was slighter in some genotypes, whereas it was much greater and even statistically significant in other genotypes. Similarly, some cultivars or genotypes had higher leaf RWC in their cells, and others had lower. The variation trend of leaf water showed that the leaf water content was different between the stressful conditions and the normal conditions, so it was much lower in the stressful conditions.

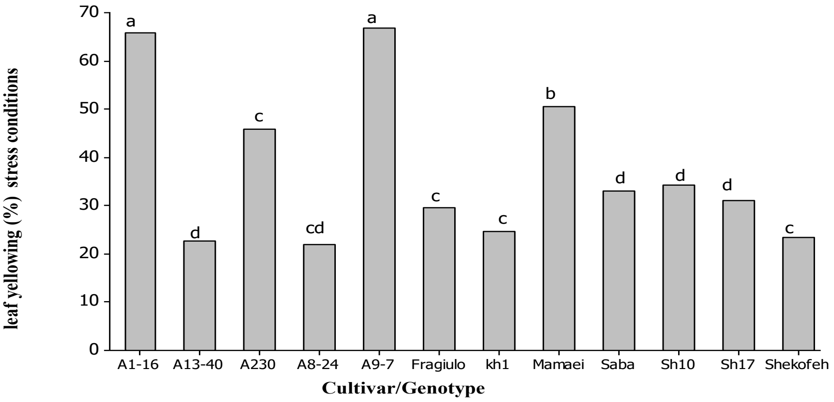

Figure 3. Leaf yellowing of almond cultivars under drought stress.
Similar letter on the bars show insignificant differences at the $P < 0.01$ level according to Duncan’s test.

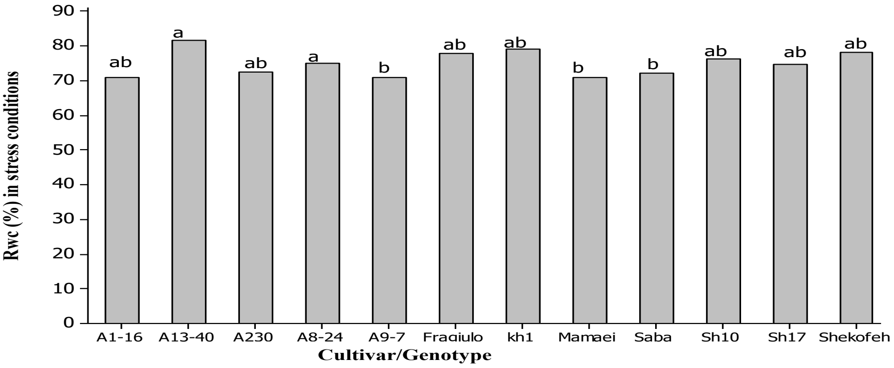

Figure 4. Leaf relative water content of almond cultivars under drought stress.
Similar letter on the bars show insignificant differences at the P < 0.01 level according to Duncan's test.

3.3. Leaf proline

Table 1 revealed that the proline content of experimental cultivars differs in both stress and normal conditions significantly. The results of the means comparison for proline content (μmol g^{-1} FW) of different almond cultivars exposed to drought stress or normal conditions are depicted in Figures 6 and 7. The cultivars that were exposed to the drought stress differed significantly in this trait. In stress conditions, the highest amount of proline was obtained in cultivars A1-16 and then Mamaei. While cultivar A8-24 showed the minimum proline content among all cultivars (Figure 6). In normal conditions, cultivars Kh1 and Mamaei had maximum proline, but A8-24 still showed the lowest content of proline (Figure 7).

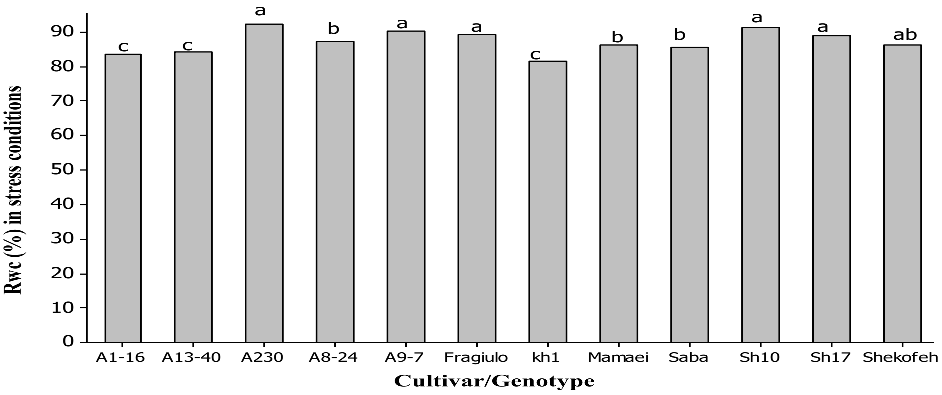

Figure 5. Leaf relative water content of almond cultivars under normal conditions.
Similar letter on the bars show insignificant differences at the P < 0.01 level according to Duncan's test.

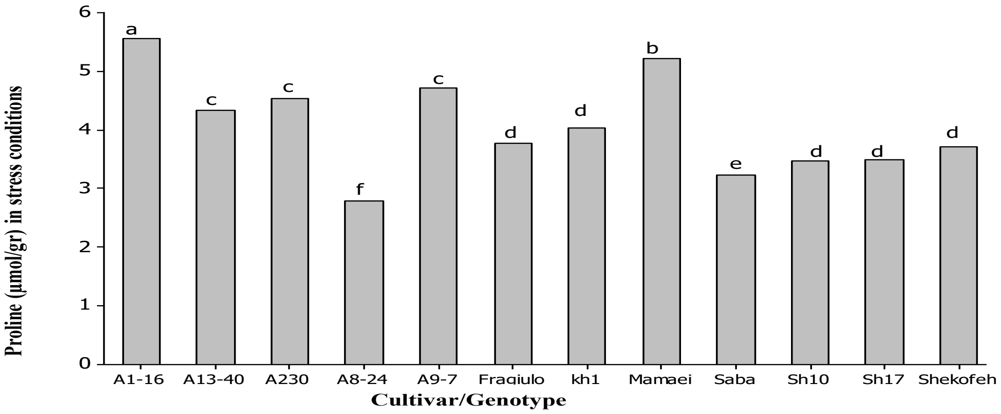

Figure 6. Proline content of almond cultivars under drought stress.
Similar letter on the bars show insignificant differences at the P < 0.01 level according to Duncan's test.

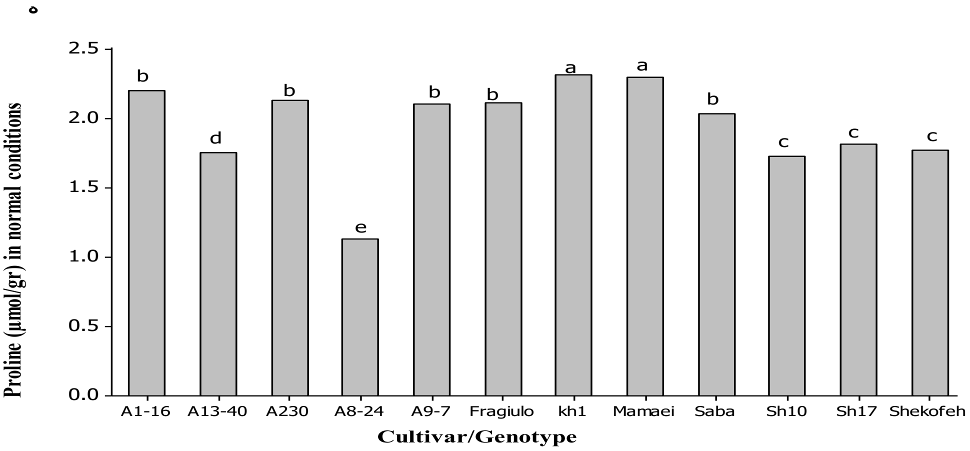

Figure 7. Proline content of almond cultivars under normal conditions.
Similar letter on the bars show insignificant differences at the P < 0.01 level according to Duncan's test.

3.4. K content

Based on the analysis of variance (Table 1), the potassium content of leaves was a significant difference under stress and normal condition. The highest K content under drought stress was obtained in A1-16 and Mamaei cultivars, while the least K content was related to Saba.

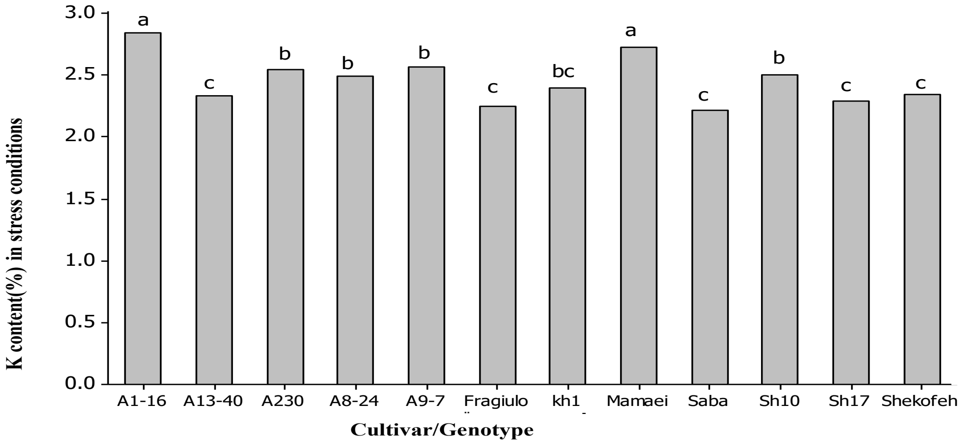

Figure 8. Leaf potassium content in studied genotypes under drought stress.
Similar letter on the bars show insignificant differences at the P < 0.01 level according to Duncan's test.

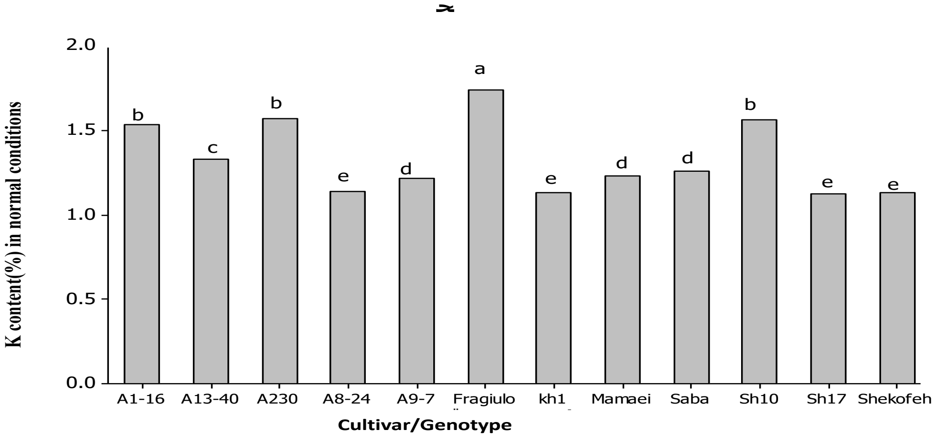

Figure 9. Leaf potassium content in studied genotypes under normal conditions.
Similar letter on the bars show insignificant differences at the P < 0.01 level according to Duncan's test.

4. Discussion and Conclusion

Plant growth under environmental stresses can be important for breeding programs of drought-tolerant cultivars. It should be noted that these findings are consistent with De Herralde et al. (2001) and Nortes et al. (2005) reported significantly different yields of almond cultivars and genotypes exposed to drought stress. Daudet et al. (2005) found that the variations in tree growth were related to the variations in water uptake and thermal expansion (Fig 1 and 2). Zokaee-Khosroshahi et al. (2014) reported that *Prunus eburnea* had the highest relative water content under drought stress compared to the other species, the least decline in the weight of roots, stems, and the whole plant, and quick abscission of leaves under stress. Based on their results, it can be concluded that *P. eburnea* has a higher level of resistance to drought stress. Akbarpour et al. (2017) studied almond cultivars under in vitro drought stress and found that drought stress caused an increase in electrolyte leakage and proline content while it reduced the RWC in almond.

The accumulation of the amino acid proline in plant tissues in response to various abiotic stresses plays a remarkable role in protecting the plants against oxidative damage of reactive oxygen species (ROS). Proline has several functions in stress adaptation, the most important ones being the osmotic adjustment and the storage of carbon, nitrogen, and energy (Liang et al., 2013). When exposed to drought stress, some cultivars synthesize a high amount of proline, and some synthesize a low amount. It has been documented that drought stress induces proline accumulation and synthesis and hinders the binding of proline to proteins and their decomposition (Pagter et al., 2005; Türkan et al., 2005). Osmotic adjustment compounds are known to include many micro-molecules, e.g. potassium, dissolved sugars, proline, and betaine. These molecules are major physiological markers for the ability of osmotic adjustment and drought resistance in plants exposed to drought stress (Wang et al., 2009). Liang et al. (2013) reported that when drought stress was applied to the roots, Abscisic acid (ABA) acted as a signal to induce proline synthesis to help the plants adapt to the environmental variations.

Studies have displayed that the amino acid proline increases in most almond genotypes or cultivars that are exposed to drought stress (Karimi et al., 2012). This is supported by our results, too. However, the extent of proline increase varies among cultivars and genotypes. Overall, as stress is intensified, more proline is synthesized. Although the cultivars in which more proline is synthesized exhibit moderate to high resistance to drought stress, the amount of proline in drought-tolerant cultivars is not necessarily more than that in other cultivars under similar stress conditions. Therefore, it seems that cultivar A8-24 has a lower proline content than other cultivars under any circumstances (drought or normal conditions). According to Barzegar et al. (2012), in almond, accumulation of proline in response to drought stress is a common trait and cannot be used as an indicator for introducing the tolerant cultivars.

Figures 8 and 9 depict that the total K content of the leaves was higher in most cultivars exposed to drought stress. This shows that potassium-containing compounds are accumulated in leaves under stressful conditions to contribute to the osmotic adjustment of the leaves. The results indicated that Saba and Fragiolu had the lowest potassium content among all the studied cultivars. Based on our results, trial almond cultivars had K content of about 1.1 and 1.8%, while the content of potassium under drought stress reached about 2,2-2.8%. According to Wang et al. (2013), plants under drought stress have a greater internal need for potassium. The supply of K can overcome the limiting effect of water stress in conditions of drought stress (Damon and Rengel, 2007; Bahrami-Rad and Hajiboland, 2017). Under water deficit stress, more potassium is required to maintain CO2 fixation of photosynthesis and protection of chloroplasts from oxidative damage (Cakmak, 2005; Bahrami-Rad and Hajiboland, 2017). Sufficient amounts of potassium can improve the biomass accumulation of plants under drought stress in comparison to minor K concentrations (Egilla et al., 2001; Wang et al., 2013). Additionally, adequate K prompts solute accumulation, thus lowering osmotic potential and assisting in maintaining plant cell turgor under osmotic stress. An adequate K status may help osmotic adjustment, which maintains higher turgor pressure, relative water content, and lower osmotic potential, thus improving the capacity of plants to tolerate drought stress (Kant and Kafkafi, 2002; Egilla et al., 2005; Wang et al., 2013)

Cultivars' genotypes differ in strategies they adopt against environmental stresses. These strategies can vary in effectiveness in different conditions and stresses depending on the conditions. The more the stress avoidance and/or confrontation methods are in a genotype, the more viable it is in adverse environments. The results revealed that no single genotype had all optimum responses to drought stress; rather, some optimal traits were observed in some of them. When all traits are considered together, it is concluded that the genotypes 'Kh1' and 'A13-40' were more drought tolerant than other genotypes.

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