



ORIGINAL ARTICLE

Physiological, Biochemical, and Developmental Responses of some Pistachio Genotypes under Drought Stress

Mostafa Ghasemi^{*1}, Shiva Ghasemi¹, Mehdi Mohammadi-Moghadam², Saeid Kashanizadeh¹, Mansoore Shamili³

¹Horticulture Crops Research Department, Qazvin Agricultural and Natural Resources Research and Education Center, AREEO, Qazvin, Iran

²Horticulture Crops Research Department, Semnan Agricultural and Natural Resources Research and Education Center, AREEO, Shahrood, Iran

³Associate professor, Agriculture Faculty, Hormoz Research Center, University of Hormozgan, Bandar Abbas, Iran

KEY WORDS

Ion leakage;
Leaf relative water content;
Proline;
Vegetative growth

ABSTRACT

Pistachio is one of the economic nut fruits in Iran. Water limitation is the most restrictive factor for its production. To overcome the water scarcity crisis, introducing drought-tolerant rootstocks is among the crucial breeding strategies. To investigate the drought tolerance of five Qazvin native pistachio genotypes, an experiment was carried out as a factorial experiment in a completely randomized design with four replications in the greenhouse conditions. The factors were pistachio genotypes (Madari, KalKhandan, Kalehbozi, Sefid, and Ghermez) and irrigation regime (normal conditions and drought stress). The highest relative water content under drought irrigation conditions belonged to the Sefid (59.99%), and Ghermez (59.09%) genotypes. The lowest value (54.68%) belonged to the Madari genotype. The highest electrolyte leakage under drought irrigation conditions belonged to the Madari genotype (55.75%). The lowest electrolyte leakage (42.44%) belonged to the Sefid genotype. Under drought stress, the highest amount of chlorophyll *a* (2.12 mg g⁻¹ fresh weight), total chlorophyll (3.051 mg g⁻¹ fresh weight), and carotenoid (2.38 mg g⁻¹ fresh weight) was observed in Ghermez genotype. In contrast, the highest amount of chlorophyll *b* (1.34 mg g⁻¹ fresh weight) was observed in the Sefid genotype. The lowest amounts of chlorophyll and carotenoid in the drought stress belonged to the Madari genotype. According to the results, the Ghermez and Sefid genotypes with lower electrolyte leakage and higher relative water content, chlorophyll, carotenoid, and biomass under water stress, were the more drought-tolerant genotypes. Madari and KalKhandan genotypes with higher electrolyte leakage and lower relative leaf water content, chlorophyll, and biomass were the most drought-sensitive genotypes.

Introduction

Pistachio (*Pistacia vera* L.), belonging to the Anacardiaceae, is one of the economic nut-fruit in Iran, with 420000 hectares of cultivated area, 386000

tons of produced nuts and 900 kg hectare⁻¹ yield (Anonymous, 2019; Sharifkhan *et al.*, 2020; Nazoori *et al.*, 2022). Water limitation is one of the most

*Corresponding author: Email address: mostafaghasemi1417@gmail.com

Received: 15 July 2023; Received in revised form: 5 September 2023; Accepted: 11 October 2023

DOI: 10.22034/jon.2023.1991233.1237

restrictive factors for its production therefore development of methods that can induce drought stress tolerance is vital (Shamshir and Hasani, 2015). To overcome the water scarcity crisis, introducing drought-tolerant rootstocks is among the crucial breeding strategies (Gijon *et al.*, 2010; Vahdati *et al.*, 2021). Pistachio grafting on hybrid rootstocks (UCB1× *Pistacia. terebinthus*) led to a higher growth than *Atlantica* rootstocks under drought-stress conditions (Gijon *et al.*, 2010). In apples; peaches, walnut, and cherries, the rootstock affects the scion growth and scion hydraulic conductivity (Atkinson *et al.*, 2003; Ebrahimi *et al.*, 2007; Rezaee *et al.*, 2008; Tombesi *et al.*, 2010; Zoric *et al.*, 2012; Thapa *et al.*, 2021).

In pistachio, there are different drought-tolerant mechanisms in different phenological stages, including the deep root system, the presence of wax in the leaf structure, the osmotic regulation and maintenance of cell turgor (Behzadi Rad *et al.*, 2021). Esmailpour *et al.* (2016) stated that water stress significantly increased the concentration of osmotic compounds in pistachio seedlings. The study of the effect of drought stress on several pistachio cultivars showed that the highest to the lowest physiological water consumption efficiency belonged to the cultivars Akbari, Owhadi, Kaleh-ghoochi, Ahmad Aghaei, Harati, and Rezaei-Zordars, respectively (Sajjadinia *et al.*, 2010). In grapes, it was reported that part of drought tolerance is due to their relatively large wood vessels, which led to quick recovery after a stress period (Lovisolo *et al.*, 2008). Finding drought tolerance mechanisms in local genotypes is useful for cultivar screening programs (Arab *et al.*, 2020). Considering the importance of the pistachio crop, it is necessary to find drought-tolerant rootstocks and cultivars. The main objective of this research was to investigate the drought tolerance of five native Qazvin pistachio genotypes to identify the most drought-tolerant rootstocks.

Materials and Methods

To investigate the drought tolerance of five Qazvin native pistachio genotypes and introduce the drought-tolerant genotypes, an experiment carried out at the experimental greenhouse of the Agricultural and Natural Resources Research and Education Center, Qazvin, Iran, in 2021. The seed samples were collected from the pistachio orchards of Qazvin and Buin Zahra and planted in seven-liter plastic bags with a height of 40 cm and a diameter of 30 cm (early April 2020). The seedlings were irrigated three times a week for three months up to field capacity.

The factorial experiment included as a completely randomized design with four replications (each replication as an experimental unit) and four pots in each experimental unit. The factors were five pistachio genotypes (Madari, KalKhandan, Kalehbozi, Sefid, and Ghemez), and two irrigation regimes (normal conditions, drought stress).

Three-month-old plants were affected by irrigation treatments. In normal irrigation, the soil moisture of the plants maintained at the field's capacity. For this purpose, three pots were selected and fully irrigated. Then, the surface of the pots was covered with plastic, and after 12 hours, the pots weighed at regular intervals. By fixing the weight of the pots in two consecutive weighings, the field's capacity was determined. By weighing the pots daily, the humidity maintained at the field's capacity level, and the deducted water was added to the pots (Hoseini *et al.*, 2016). However, drought exposed-plants were not irrigated for two weeks until they showed a severe decrease in leaf turgor and most of the leaves became dry and discolored (Gijon *et al.*, 2010). After the stress period (two weeks), all the plants (normal conditions, drought stress) were re-irrigated up to field capacity, for two weeks. Finally, the following characteristics were examined.

Relative water content (RWC): To measure RWC, two fully developed mature leaves were collected from the middle part of the stem (from the middle leaves of the branch), transported to the laboratory in

a plastic bag, weighed and relative leaf water content was determined by Ritchie *et al.* (1990) method.

Electrolyte leakage (EL): Two fully developed uniform mature leaves were selected from the middle part of the stem were used for examination by the method presented by Whitlow *et al.* (1992).

Proline content: To determine the contents of proline, 0.5 g of leaf sample was crushed in a mortar and mixed with 5 ml of 95% ethanol. The sediment was mixed with 5 ml of 70% ethanol. The obtained extract was centrifuged (4500 rpm, 15 min). Then 2 ml of the alcoholic extract was added to 2 ml of glacial acetic acid and 2 ml of ninhydrin reagent (a mixture of 1.25 g ninhydrin, 30 ml glacial acetic acid and 20 ml of 6 M phosphoric acid). After stirring, the mixture was heated (water bath 100°C, 60 min) and then incubated (ice water container). After cooling, 4 ml of toluene was added, shaken (vortex for 15 sec), and re-incubated under a static state (30 min). Finally, the absorption was measured at 520 nm with a spectrophotometer (Bates *et al.*, 1973).

Soluble sugars assay: To determine the soluble sugars, a leaf sample (0.5g) was crushed in a mortar and mixed with 5 ml of 95% ethanol. Then 100 µl of the alcoholic extract was mixed with 3 ml fresh anthrone (150 mg of anthrone in 100 ml of 72% sulfuric acid). The mixture was reacted in a water bath (100 °C, 10 min). After cooling, the absorbance read at 625 nm. The D-glucose (0, 62.5, 125, 250, 500, 1000, and 2000 ppm) was used to draw the standard curve (Irigoyen *et al.*, 1992).

Chlorophyll and carotenoid content: The fresh leaf sample (0.5 g) was crushed in a mortar and mixed with 20 ml of 80% acetone. The extract was centrifuged (10 min, 6000 rpm) and finally, the absorbance was read at 663 (chlorophyll a), 645 (chlorophyll b) and 470 (carotenoids) nm with a spectrophotometer (Arnon, 1967).

Fresh and dry weight: The plants were divided into root, shoot, and leaf and then organs were washed, weighed (fresh weight), and dried in an oven at 70°C for 48 hours (dry weight).

Statistical analysis

MSTATC software was used for statistical analysis, and Duncan's multiple range test was used to compare the means. Correlation analysis was calculated by Pearson coefficient. Excel 2019 software was used to draw the graphs.

Results

The results of variance analysis of the data are given in Tables 1 and 2. As can be seen, the simple effect of irrigation was significant on all the investigated traits ($p \leq 0.01$). The difference between genotypes was also significant in all parameters except proline, carbohydrates, leaf fresh weight and shoot dry weight. The interaction effect of irrigation and genotype was also significant for all parameters except soluble sugar, chlorophyll b, carotenoid and shoot fresh weight.

Table 1. Variance analysis of irrigation, genotype and their interaction on physiological and biochemical parameters of pistachio seedlings.

S.O.V.	D.F.	R.W.C.	Electrolyte leakage	Proline	Soluble sugar	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Carotenoid
Irrigation	1	2200.386 **	5684.41 **	1.86 **	544.954 **	19.922 **	8.873 **	55.385 **	9.39 **
Genotype	4	10.928 **	71.338 **	0.007 ns	1.287 ns	0.412 **	0.134 *	0.982 **	1.422 *
Irrigation × Genotype	4	9.836 *	62.399 **	0.013 *	2.154 ns	0.285 *	0.115 ns	0.739 *	0.581 ns
Error	30	2.1518	2.915	0.004	1.247	0.077	0.046	0.187	0.386

* and ** are significant at the level of 0.05 and 0.01, respectively; ns: not significant.

Table 2. Variance analysis of irrigation, genotype and their interaction on the growth parameters of pistachio seedlings.

S.O.V.	D.F.	Root fresh weight	Root dry weight	Leaf fresh weight	Leaf dry weight	Shoot fresh weight	Shoot dry weight	Shoot height	Root length	Leaf number
Irrigation	1	149.576**	38.871**	145.466**	24.194**	294.578**	120.791**	390.625**	864.900**	1050.625**
Genotype	4	9.524**	1.713**	1.187 ^{ns}	0.349 [*]	5.712 [*]	0.62 ^{ns}	50.525**	853.313**	38.313**
Irrigation × Genotype	4	9.006**	1.566**	1.575 [*]	0.711**	3.229 ^{ns}	0.75 [*]	0.38**	172.338**	26.812**
Error	30	0.359	0.102	0.549	0.114	2.09	0.253	5.625	7.483	5.708

* and ** are significant at the level of 0.05 and 0.01, respectively; ns: not significant.

The relative leaf water content

The analysis of variance showed a significant effect of irrigation regime and genotype on relative leaf water content ($p \leq 0.01$). The interaction effect of irrigation regime and genotype was significant ($p \leq 0.05$). The relative water content of leaves decreased under drought stress. The average of relative water content in normal and water stress

conditions was 72.42% and 57.59%, respectively. In, water stress conditions, the highest values belonged to Sefid (59.99%), and Ghermez (59.09%) genotypes. The lowest value (54.68%) belonged to the Madari genotype. There was no significant difference between genotypes under normal irrigation conditions (Fig. 1).

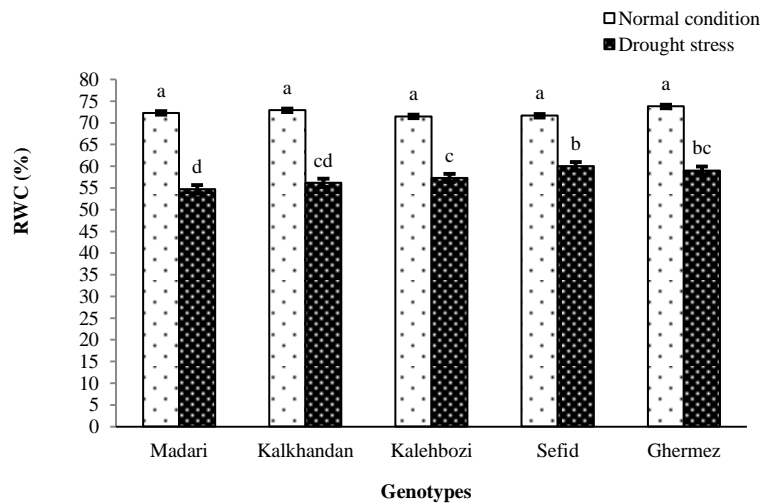


Fig. 1. The effect of irrigation regime and genotype on relative leaf water content of pistachio seedlings.

Electrolyte leakage

The variance analysis showed a significant effect ($p \leq 0.01$) of irrigation regime, genotype, and their interaction on leaf electrolyte leakage. The electrolyte leakage value under normal irrigation and drought stress conditions was 25.49% and 49.33%, respectively. The lowest electrolyte leakage (34.2%) belonged to the Sefid and Ghermez genotypes, and the highest (40.7%) belonged to the Madari genotype.

The highest electrolyte leakage under drought stress conditions belonged to the Madari genotype (55.75%). The lowest value (42.44%) belonged to the Sefid genotype. There was no significant difference under normal irrigation conditions (Fig. 2).

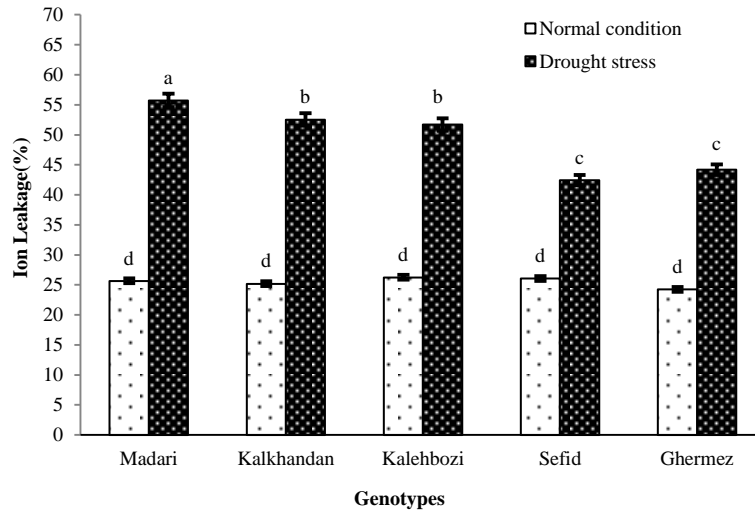


Fig. 2. The impact of irrigation regime and genotype on electrolyte leakage of pistachio seedlings.

Proline

The effect of the irrigation regime and the interaction between irrigation regime and genotype were significant on leaf proline ($p \leq 0.01$ and $p \leq 0.05$, respectively). The proline content under normal irrigation and drought stress conditions were 0.314 and $0.746 \mu\text{mol g}^{-1}$ FW, respectively. The difference

among genotypes was not significant. The highest and the lowest amount of proline under drought stress conditions belonged to the genotypes Kalkhandan (0.8232) and Sefid (0.6725), respectively. There was no significant difference under normal irrigation conditions (Fig. 3).

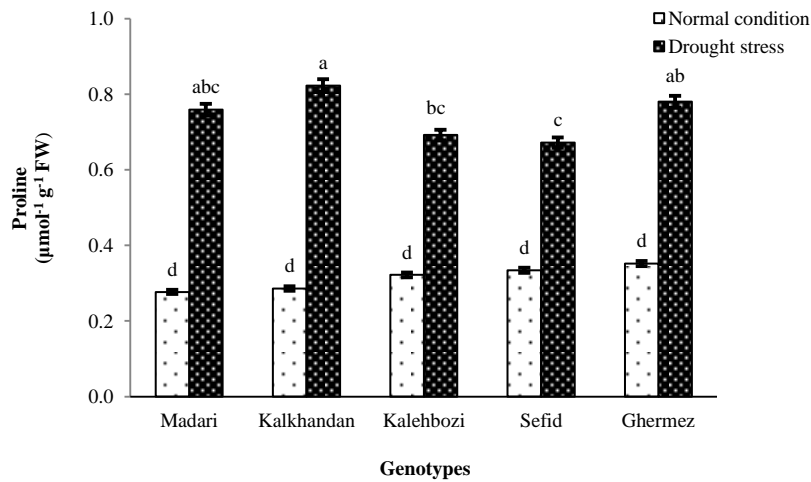


Fig. 3. The impact of irrigation regime and genotype on leaf proline of pistachio seedlings.

Soluble sugar

Based on the variance analysis, it was found that only the effect of the irrigation regime on the soluble sugar contents was significant ($p \leq 0.01$). The soluble

sugar contents of plants under normal and water stress conditions were 2.073 and 9.455 mg g^{-1} fresh weight, respectively (Fig. 4).

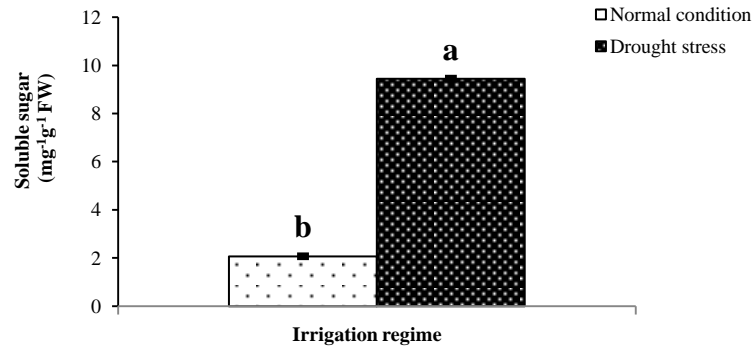


Fig. 4. The impact of irrigation regime on leaf soluble sugar in pistachio seedlings.

Chlorophyll and carotenoids

The results of variance analysis of the effects of the treatments on photosynthetic pigments are given in Table 1. The highest (2.12 mg g⁻¹ fresh weight) and the lowest (1.21 mg g⁻¹ fresh weight) contents of chlorophyll *a* under drought stress conditions were observed in the Ghermez and Madari genotypes, respectively (Fig 5). The average chlorophyll *b* under control and water stress conditions were 1.66 and 0.72 mg g⁻¹ fresh weight, respectively (Fig 6). The highest (1.34 mg g⁻¹ fresh weight) and the lowest (1.06 mg g⁻¹ fresh weight) contents of chlorophyll *b* were observed in the Sefid and Madari genotypes,

respectively (Fig 7). The highest (3.051 mg g⁻¹ of fresh weight) and the lowest (1.718mg g⁻¹ of fresh weight) contents of total chlorophyll under drought stress conditions belonged to the Ghermez and Kalkhandan genotypes, respectively (Fig 8). The carotenoid contents under control irrigation and water stress treatments were 2.25 and 1.28 mg g⁻¹ fresh weight, respectively (Fig 9). The highest (2.38 mg g⁻¹ fresh weight) and the lowest (1.32 mg g⁻¹ fresh weight) carotenoid content were found in the Ghermez and Madari genotypes, respectively (Fig 10).

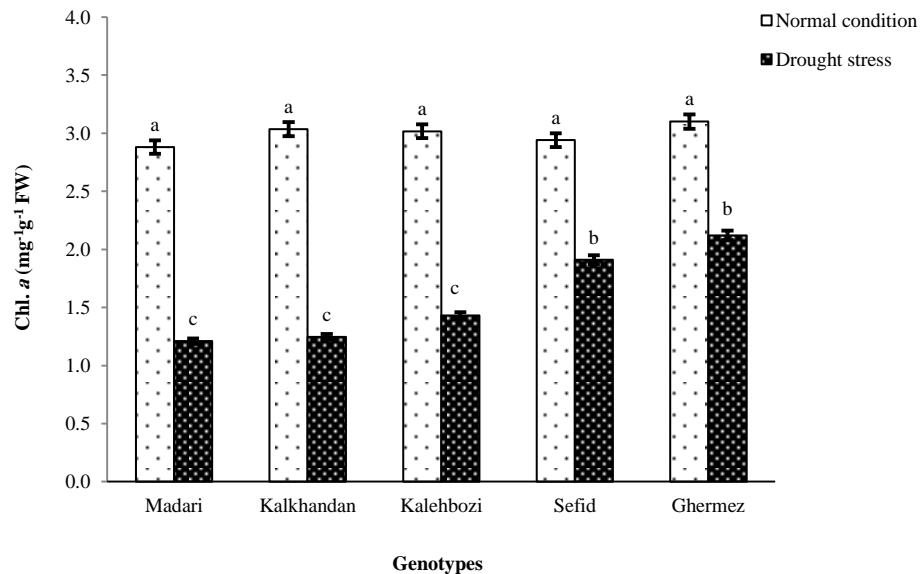


Fig. 5. The effect of irrigation regime and genotype on chlorophyll *a* in pistachio seedlings

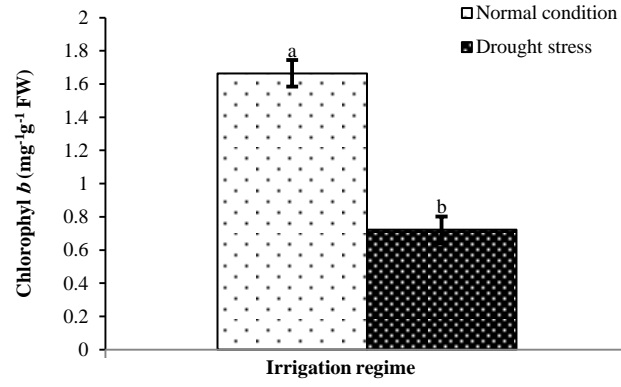


Fig. 6. The impact of irrigation regime and genotype on chlorophyll b in pistachio seedlings

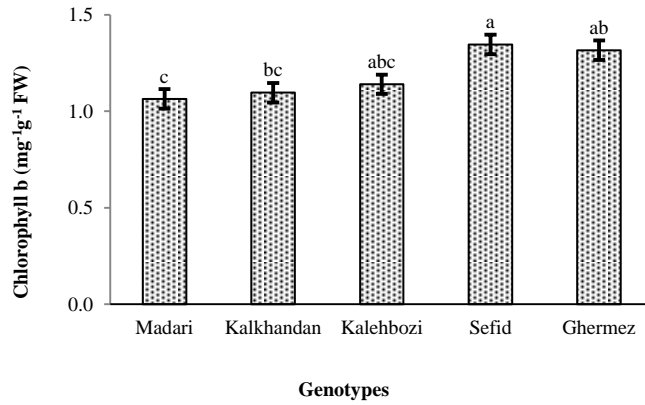


Fig. 7. The impact of irrigation regime and genotype on chlorophyll b in pistachio seedlings

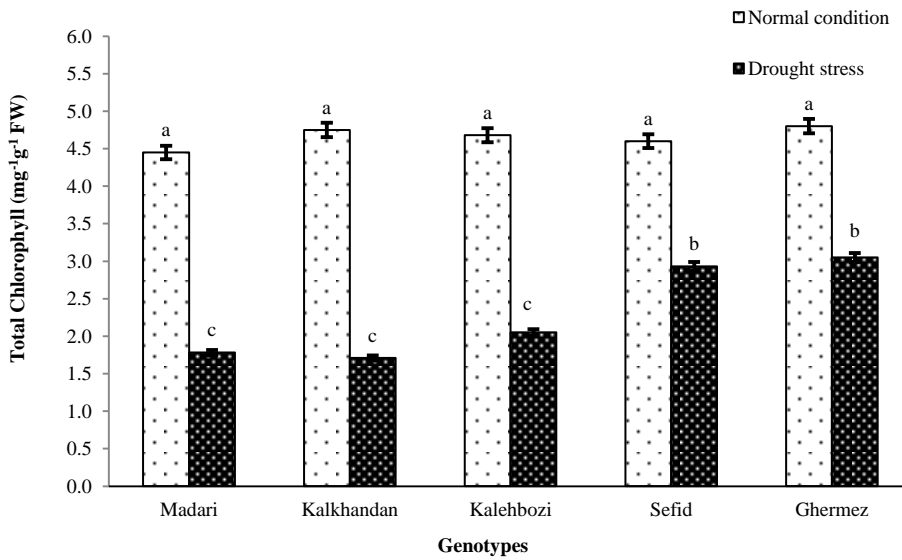


Fig. 8. The impact of irrigation regime and genotype on total chlorophyll in pistachio seedlings

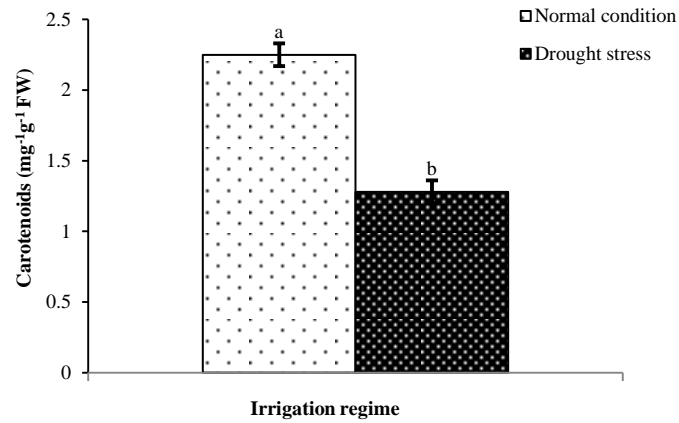


Fig. 9. The impact of irrigation regime on carotenoids content in pistachio seedlings

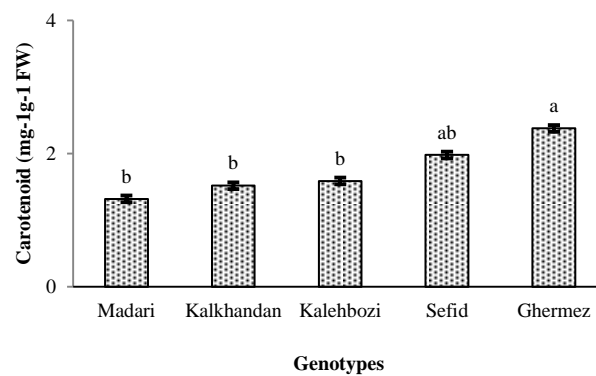


Fig. 10. The impact of genotype on carotenoids content in pistachio seedlings

Growth parameters

The root fresh weight under normal irrigation and water stress treatments were 17.7 and 3.30 g., respectively. The highest (6.3 g.) and the lowest (1.14 g.) root fresh weight under drought stress conditions were observed in the Ghermez and Madari genotypes, respectively (Fig. 11). The root dry weight under normal irrigation and water stress treatments were 3.63 and 1.68 g, respectively. The highest (2.7g) and the lowest (0.622g) root dry weight under water stress conditions belonged to the Ghermez and Madari genotypes, respectively. In normal irrigation, the difference among genotypes was insignificant (Fig. 12).

The highest (2.25 g.) and the lowest (0.66 g) leaf fresh weight under drought conditions belonged to the Sefid and Kalkhandan genotypes, respectively. There was no significant difference among plants in normal irrigation (Fig. 13). The highest (1.49 g) and the

lowest (0.366 g) leaf dry weight under drought stress conditions belonged to the Sefid and Kalkhandan genotypes, respectively (Fig. 14).

The stem fresh weight in normal irrigation and water stress treatments was 7.894 and 2.46 g, respectively (Fig. 15). Among the genotypes, the highest (6.19 g) and the lowest (3.98 g) stem fresh weight were observed in the Ghermez and Madari genotypes, respectively (Fig. 16). The highest (2.2 g) and the lowest (0.925 g) stem dry weight under drought stress conditions belonged to the Sefid and Madari genotypes, respectively (Fig. 17).

The stem length decreased under the drought stress conditions, but the root length increased. The highest stem length (32.25 and 30.75 cm) under water stress conditions belonged to the Ghermez and Sefid genotypes, respectively. There was no significant difference among the genotypes under normal

irrigation conditions (Fig. 18). The highest root length was observed in the Ghermez genotype under both normal irrigation (33.5 cm) and water stress (57.75 cm) conditions (Fig. 19). Madari genotype showed the lowest root length under normal irrigation (18.25 cm) and water stress (17.25 cm) conditions.

The mean leaf number under control irrigation and

water stress treatments were 18.25 and 8.00, respectively. The highest (12.5) and the lowest (4.5) number of leaves under water stress conditions belonged to the Ghermez and Madari genotypes, respectively (Fig 20).

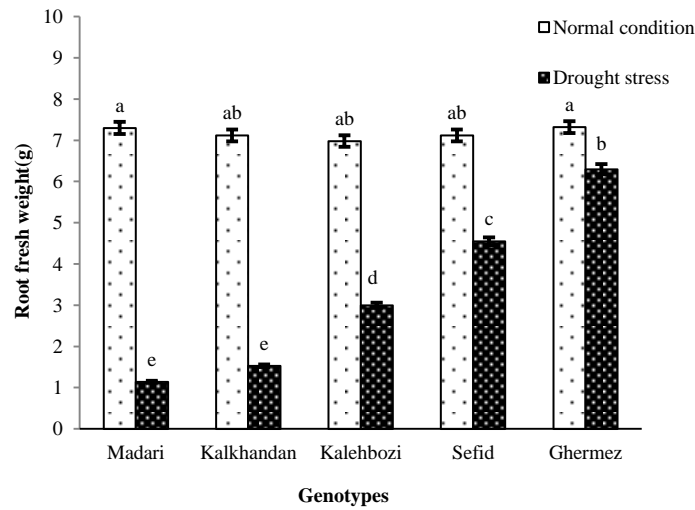


Fig. 11. The impact of irrigation regime and genotype on root fresh weight in pistachio seedlings

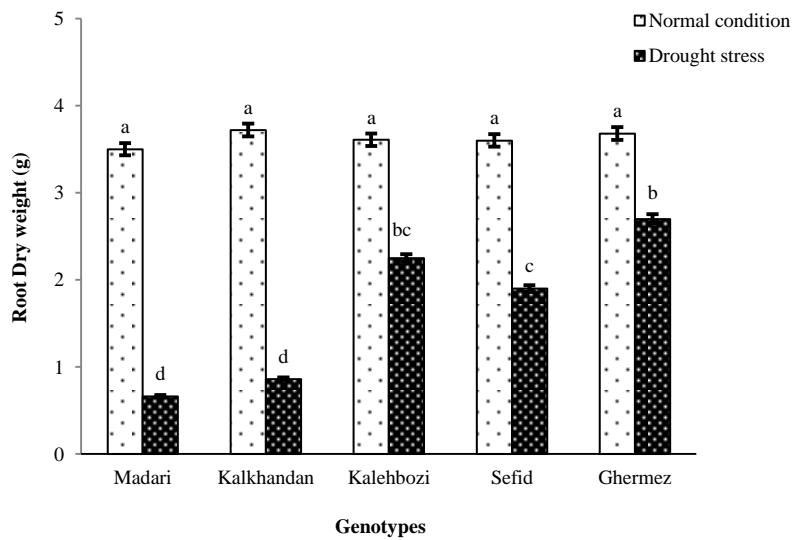


Fig. 12. The impact of irrigation regime and genotype on root dry weight in pistachio seedlings

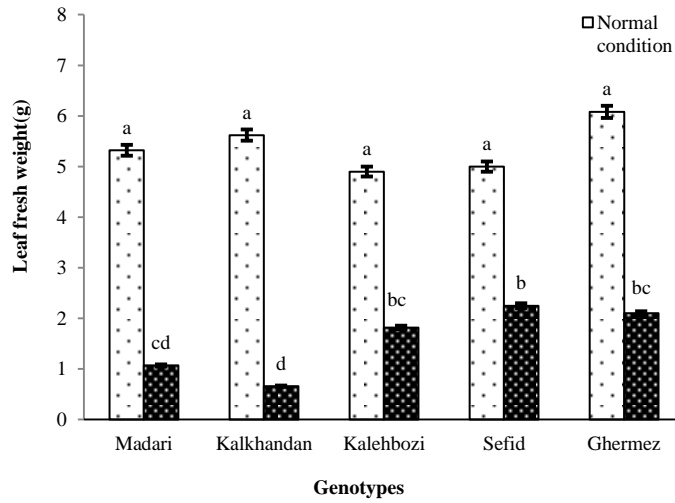


Fig. 13. The effect of irrigation regime and genotype on leaf fresh weight in pistachio seedlings

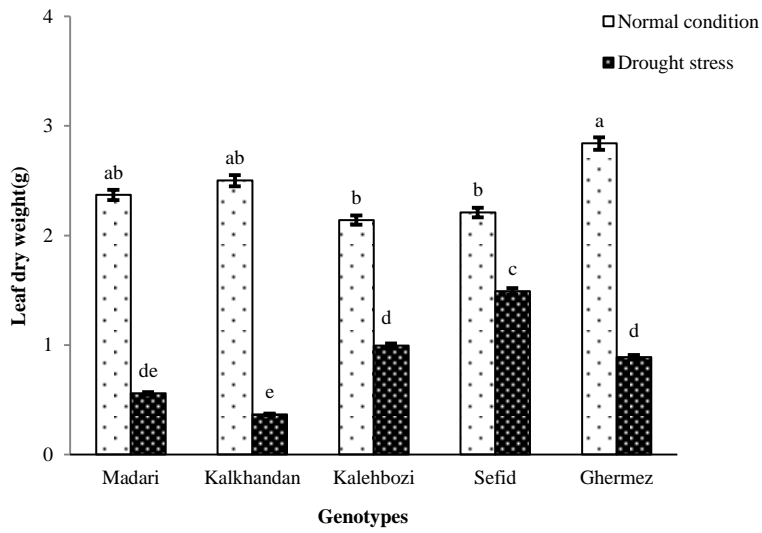


Fig. 14. The impact of irrigation regime and genotype on leaf dry weight in pistachio seedlings

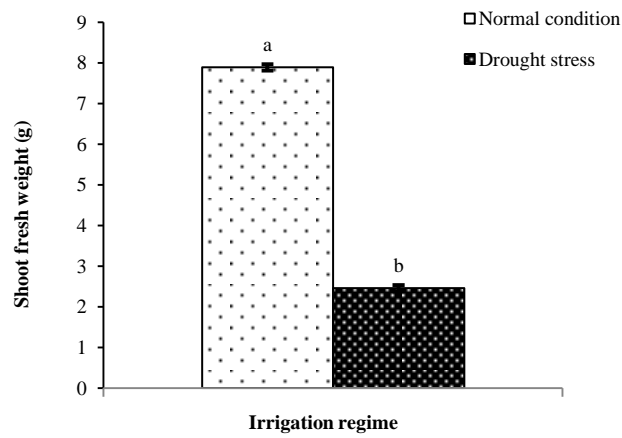


Fig. 15. The impact of irrigation regime on stem fresh weight in pistachio seedlings

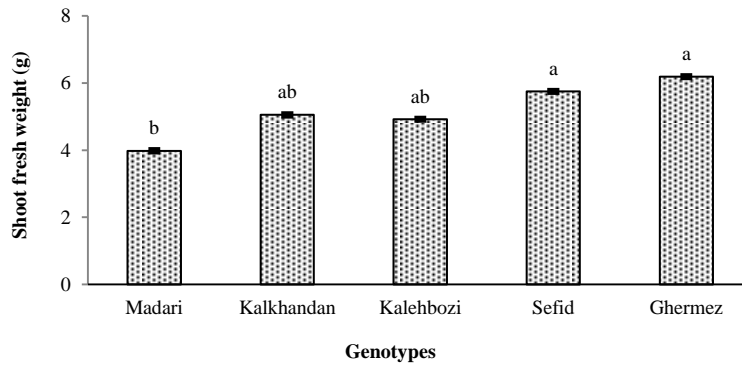


Fig. 16. The impact of genotype on stem fresh weight in pistachio seedlings



Fig. 17. The impact of irrigation regime and genotype on stem dry weight in pistachio seedlings

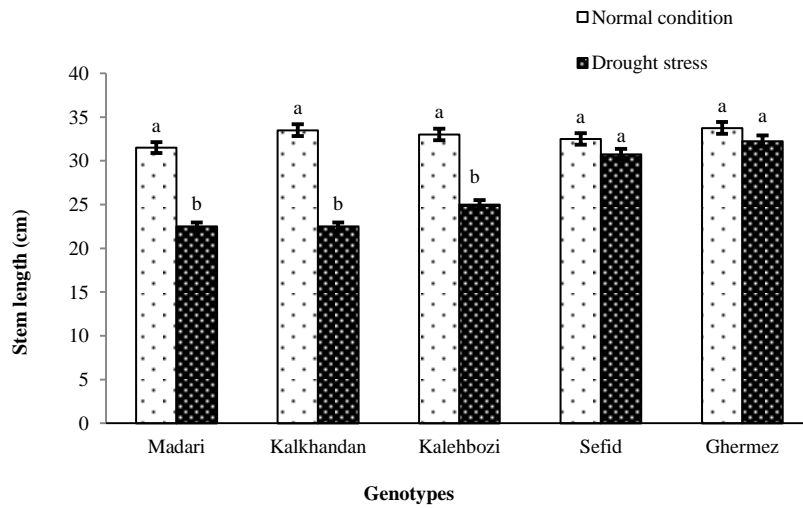


Fig. 18. The impact of irrigation regime and genotype on stem length in pistachio seedlings

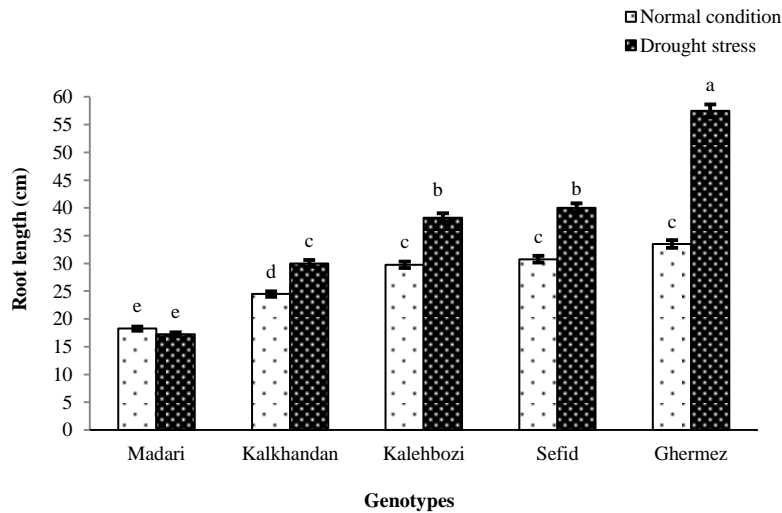


Fig. 19. The impact of irrigation regime and genotype on root length in pistachio seedlings

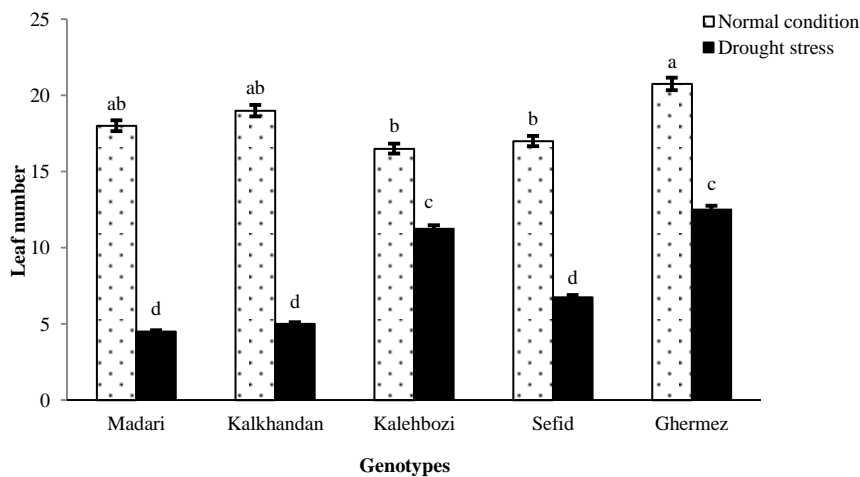


Fig. 20. The impact of irrigation regime and genotype on leaf number in pistachio seedlings

Correlation analysis between traits

Examining the correlation showed a significant positive correlation between the relative water content and chlorophyll a ($r=0.94$), chlorophyll b ($r=0.91$), total chlorophyll ($r=0.94$), carotenoid ($r=0.69$), and growth parameters (Table 3). However, a significant negative correlation was observed between ion leakage and relative water content ($r=0.98$). The genotypes tolerant to environmental stresses have lower electrolyte leakage. There was a significant negative correlation between electrolyte leakage with chlorophyll parameters and growth indices. Also, a negative relationship between the relative water

content and proline ($r=-0.93$) and carbohydrate ($r=-0.92$) was observed (Table 3). The negative relationship between relative water content, proline, and carbohydrates in plants is well-established. When plants are subjected to water stress, the production of proline and carbohydrates increases, but the relative water content decreases. This is due to proline and carbohydrate accumulation that serve as osmoprotectants, helping the plant to maintain its cellular structure and function under conditions of water stress.

Table 3. Correlation between investigated traits in pistachio genotypes under water stress conditions.

	RWC	Electrolyte leakage	Proline	Carbohydrate	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	Total chlorophyll	Carotenoid	Root fresh weight	Root dry weight	Leaf fresh weight	Leaf dry weight	Shoot fresh weight	Shoot dry weight	Shoot height	Root length	Leaf number
RWC	1																
Electrolyte leakage	-0.98**	1															
Proline	-0.93**	0.92**	1														
Carbohydrate	-0.91**	0.90**	0.95**	1													
chlorophyll <i>a</i>	0.93**	-0.949**	-0.856**	-0.83**	1												
chlorophyll <i>b</i>	0.91**	-0.929**	-0.865**	-0.859**	0.94**	1											
Total chlorophyll	0.94**	-0.955**	-0.871**	-0.856**	0.991**	0.98**	1										
Carotenoid	0.68**	-0.710**	-0.541**	-0.503**	0.841**	0.791**	0.83**	1									
Root fresh weight	0.86**	-0.911**	-0.787**	-0.746**	0.901**	0.872**	0.902**	0.74**	1								
Root dry weight	0.88**	-0.906**	-0.833**	-0.801**	0.892**	0.862**	0.893**	0.688**	0.95**	1							
Leaf fesh weight	0.96**	-0.952**	-0.892**	-0.892**	0.904**	0.892**	0.912**	0.692**	0.852**	0.87**	1						
Leaf dry weight	0.94*	-0.929**	-0.866**	-0.868**	0.867**	0.866**	0.879**	0.658**	0.825**	0.842**	0.98**	1					
Shoot fresh weight	0.9**	-0.892**	-0.831**	-0.819**	0.834**	0.807**	0.835**	0.577**	0.793**	0.795**	0.861**	0.85**	1				
Shoot dry weight	0.95**	-0.949**	-0.921**	-0.919**	0.882**	0.856**	0.884**	0.568**	0.841**	0.896**	0.916**	0.88**	0.89**	1			
Shoot height	0.74**	-0.803**	-0.650**	-0.600**	0.894**	0.853**	0.890**	0.924**	0.878**	0.829**	0.746**	0.735**	0.681**	0.69**	1		
Root length	-0.28	0.198	0.403*	0.447**	-0.101	-0.177	-0.133	0.190	0.098	0.038	-0.267	-0.248	-0.214	-0.28	0.22	1	
Leaf number	0.91**	-0.890**	-0.828**	-0.795**	0.858**	0.811**	0.851**	0.688**	0.859**	0.899**	0.943**	0.904**	0.787**	0.85**	0.73**	-0.08	1

* and ** are significant at the level of 0.05 and 0.01, respectively (each number is the average of 40 data).

Discussion

The plant that shows a lesser decrease in the relative water content under stress conditions would be a candidate for a salt or drought-tolerant plant (Balaguer *et al.*, 2002). Factors such as the plant's access to water, ability to regulate stomatal movements and osmotic regulation affect the relative water content. Also, the reduction of root growth and the increase in evapotranspiration are the factors involved reducing of the relative water content (Hall and Twidwell, 2002). In water stress conditions, the highest relative water content belonged to Sefid (59.99%), and Ghermez (59.09%) genotypes. The lowest value (54.68%) belonged to the Madari genotype.

The highest electrolyte leakage under drought stress conditions belonged to the Madari genotype (55.75%). The lowest value (42.44%) belonged to the Sefid genotype. The higher permeability of the cell membrane leads to an increase in the leakage of cell solutes (potassium, amino acids and carbohydrates) (Mandhanis *et al.*, 2006; Qinghua *et al.*, 2006). The less electrolyte leakage in the Sefid and Ghermez genotypes indicates the greater stability of their membranes than other genotypes.

Accumulation of dissolved substances and solutes or osmotic regulation is one of the particular mechanisms of cell defense against water loss or wilting (Lotfi *et al.*, 2019). Free proline accumulation due to drought stress is mainly caused by protein breakdown (Dierks-Ventling and Tonelli, 1982). Proline accumulation in response to water stress was reported in rice (Morsy *et al.*, 2007), sugar beet (Monreal *et al.*, 2007), walnut (Lotfi *et al.*, 2010) and pistachio (Ghasemi *et al.*, 2021). The highest and the lowest amount of proline under drought stress conditions belonged to the genotypes of KalKhandan (0.8232) and Sefid (0.6725), respectively. Based on correlation analysis, it was found that there was a negative relationship between proline accumulation and drought tolerance of the examined genotypes. So, there was a negative relationship between proline with

the relative content of leaf water, photosynthetic pigments and plant biomass and a positive relationship with ion leakage.

Destruction of chlorophylls due to drought stress is due to the increased production of oxygen free radicals in cells, which cause peroxidation and decomposition of pigments (Schutz and Fanmgier, 2001). The highest and the lowest contents of chlorophyll *a* and carotenoids under drought stress were observed in the Ghermez and Madari genotypes, respectively. The highest and the lowest content of chlorophyll *b* was observed in the Sefid and Madari genotypes, respectively. These results show that the photosynthetic pigments of the Ghermez and Sefid genotypes were affected by drought to a lesser extent and the Madari genotype to a greater extent.

The main effect of drought stress is the reduction of carbon fixation along with the stomatal closure, which leads to a decrease in the photosynthesis rate, carbohydrate synthesis, and plant growth (Calatayud *et al.*, 2006; Stuart *et al.*, 2011; Asayesh *et al.*, 2017). According to the results, the biomass of Ghermez, Sefid, and Kalehbozi genotypes was less affected by water stress than other genotypes. The genotypes Madari and KalKhandan were more affected by drought stress. The highest stem length under water stress conditions belonged to the Ghermez and Sefid genotypes. The highest root length was observed in the Ghermez genotype. The genotype Madari showed the lowest root length under water stress conditions. The highest and the lowest number of leaves under water stress conditions belonged to the Ghermez and Madari genotypes, respectively.

Conclusions

The results showed that the Ghermez and Sefid genotypes had lesser electrolyte leakage and more relative water content, chlorophyll, carotenoid, and biomass under water stress conditions. The genotypes Ghermez and Sefid were more drought-tolerant than other genotypes and can be considered as superior

genotypes. Madari and KalKhandan genotypes showed more sensitivity to drought with more electrolyte leakage and lower relative leaf water content, chlorophyll, and biomass. The genotype of Kalehbozi was also between these two groups.

Acknowledgements

The authors would like to thank Qazvin Agricultural and Natural Resources Research and Education Center for providing us with facilities to implement this project.

Conflict of interests

All the authors declare that there is no conflict of interest in the study.

References

- Anonymous (2019) Annual agricultural statistics. Ministry of Jihad-e-Agriculture of Iran. Available from. <http://www.maj.ir>.
- Arab MM, Marrano A, Abdollahi-Arpanahi R, Leslie CA, Cheng H, Neale DB, Vahdati K. (2020). Combining phenotype, genotype, and environment to uncover genetic components underlying water use efficiency in Persian walnut. *Journal of Experimental Botany* 23; 71(3), 1107-1127.
- Arnon AN (1967) Method of extraction of chlorophyll in the plants. *Agronomy Journal*. 23, 112-121.
- Asayesh ZM, Vahdati K, Aliniaiefard S (2017) Investigation of physiological components involved in low water conservation capacity of *in vitro* walnut plants. *Scientia Horticulturae*. 224, 1-7.
- Atkinson CJ, Else MA, Taylor L, Dover CJ (2003) Root and stem hydraulic conductivity as determinants of growth potential in grafted trees of apple (*Malus pumila* Mill.). *Journal of Experimental Botany*. 54, 1221-1229
- Balaguer L, Pugnaire FI, Martinez-Ferri E, Armas C, Valladares F, Manrique E (2002) Ecophysiological significance of chlorophyll loss and reduced photochemical efficiency under extreme aridity in *Stipa tenacissima* L. *Plant and Soil*. 240, 343-352.
- Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. *Plant and Soil*. 39, 205-207.
- Behzadi Rad P, Roozban MR, Karimi S, Ghahremani R, Vahdati K (2021) Osmolyte accumulation and sodium compartmentation has a key role in salinity tolerance of pistachios rootstocks. *Agriculture*. 11(8), 708.
- Calatayud A, Roca D, Martínez PF (2006) Spatial-temporal variations in rose leaves under water stress conditions studied by chlorophyll fluorescence imaging. *Plant Physiology and Biochemistry*. 44, 564-573.
- Dierks-Ventling C, Tonelli C (1982) Metabolism of Proline, Glutamate, and Ornithine in Proline Mutant Root Tips of *Zea mays* (L.). *Plant Physiology*. 69(1), 130-134.
- Ebrahimi A and Vahdati K (2007) Improved success of Persian walnut grafting under environmentally controlled conditions. *International Journal of Fruit Science*. 6(4), 3-12.
- Esmailpour A, Van Labeke MC, Samsone R, Boeckxa P, Van Dammea P (2016) Variation in biochemical characteristics, water status, stomatal features, leaf carbon isotope composition and its relationship to water use efficiency in pistachio (*Pistacia vera* L.) cultivars under drought stress condition. *Scientia Horticulturae*. 211, 158-166.
- Ghasemi M, Hasheminasab H, Ghasemi S, Kashanizadeh S (2021) Evaluation of Drought Tolerance of Some Native Pistachio Genotypes of Qazvin Province. *Pistachio science and technology*. 6(11), 102-122. [In Persian]
- Gijon MC, Gimenez C, Gurrero DPL, Couceiro JF, Moriana A (2010) Rootstock influences the

- response of pistachio (*Pistacia vera* L. cv. Kerman) to water stress and rehydration. *Scientia Horticulturae*. 125(4), 666-671.
- Hall RC, Twidwell EK (2002) Effects of Drought Stress on Soybean Production. *Extension Extra*. pp. 254.
- Hoseini SS, Cheniany M, Lahouti M, Ganjeali A (2016) Evaluation of resistance to drought stress in seedlings of two lines of Triticale (Triticosecale × Wittmack) with emphasis on some enzymatic and non-enzymatic antioxidants. *Iranian Journal of Plant Biology*. 8(30), 3-42. [In Persian]
- Irigoyen JH, Emerich DW, Sanchez Diaz M (1992) Water stress induced changes in concentration of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. *Physiologia Plantarum*. 84(1), 55-66.
- Lotfi N, Soleimani A, Vahdati K, Çakmakçı R. (2019) Comprehensive biochemical insights into the seed germination of walnut under drought stress. *Scientia Horticulturae* 250, 329-43.
- Lotfi N, Vahdati K, Kholdebarin B and Amiri R (2010) Drought-induced accumulation of sugars and proline in radicle and plumule of tolerant walnut varieties during germination phase. *Acta Horticulturae*. 861, 289-296.
- Lovisollo C, Perrone I, Hartung W, Schubert A (2008) An abscisic acid-related reduced transpiration promotes gradual embolism repair when grapevines are rehydrated after drought. *New Phytologist*. 180(3), 642-651.
- Mandhanis S, Madan S, Whney V (2006) Antioxidant defense mechanism under salt stress in wheat seedling. *Journal of Biologia Plantarum*. 50(2), 227-231.
- Monreal JA, Jimenez ET, Remesal E, Morillo-Velarde R, Garcia-Maurino S, Echevarria C (2007) Proline content of sugar beet storage roots: Response to water deficit and nitrogen fertilization at field conditions. *Environmental and Experimental Botany*. 60(2), 257-267.
- Morsy MR, Jouve L, Hausman JF, Hoffmann L, Stewart JMCD (2007) Alteration of oxidative and carbohydrate metabolism under abiotic stress in two rice (*Oryza sativa* L.) genotypes contrasting in chilling tolerance. *Journal of Plant Physiology*. 164(2), 157-167.
- Nazoori F, ZamaniBahramabadi E, Mirdehghan H, Yousefi M (2022) Preharvest application of sulfur as pesticide on fresh hull and kernel of pistachio (*Pistacia vera* L.). *International Journal of Horticultural Science and Technology*. 9(1), 117-129.
- Qinghua S, Zhiyi B, Zhujun Z, Quansheng Y, Qiong Q (2006) Effect of different treatment of salicylic acid on heat tolerance chlorophyll fluorescence, and antioxidant enzyme activity in seedling of *Cucumis sativa* L. *Plant Growth Regulation*. 48(2), 127-135.
- Ritchie SW, Nguyen HT, Halody AS (1990) Leaf water content and gas exchange parameters of two wheat genotypes differing in drought resistance. *Crop Sciences*. 30(1), 105-111.
- Sajjadinia A, Ershadi A, Hokmabadi H, Khayyat M, Gholami M (2010) Gas exchange activities and relative water content at different fruit growth and developmental stages of on and off cultivated pistachio trees. *Australian Journal of Agricultural Engineering*. 1(1), 1-6.
- Schutz H, Fangmier E (2001) Growth and yield responses of spring wheat (*Triticum aestivum* L. cv. Minaret) to elevated CO₂ and water limitation. *Environmental Pollution*. 114(2), 187-194.
- Shamshir MH, Hasani MR (2015) Synergistic accumulative effects between exogenous salicylic acid and arbuscular mycorrhizal fungus in pistachio (*Pistacia Vera* cv. Abareqi) seedlings under drought stress.

- International Journal of Horticultural Science and Technology. 2(2), 151-160
- Sharifkhah M, Bakhshi D, Pourghayoumi M, Abdi S, Hokmabadi H (2020) Effect of pollination time on yield and antioxidant properties of some pistachio cultivars. International Journal of Horticultural Science and Technology. 7(1), 51-58.
- Stuart L, Emel D, Bala AA, Hikmet B (2011) The drought response displayed by a DRE-binding protein from *Triticum dicoccoides*. Plant Physiology and Biochemistry. 49, 346-351.
- Thapa R, Thapa P, Ahamad K, Vahdati K (2021) Effect of grafting methods and dates on the graft take rate of Persian walnut in open field condition. International Journal of Horticultural Science and Technology. 8(2), 133-47.
- Tombesi S, Johnson RS, Day KR, DeJong TM (2010) Relationships between xylem vessel characteristics, calculated axial hydraulic conductance and size-controlling capacity of peach rootstocks. Annals of Botany. 105(2), 327-331.
- Vahdati K, Sarikhani S, Arab MM, Leslie CA, Dandekar AM, Aletà N, Bielsa B, Gradziel TM, Montesinos Á, Rubio-Cabetas MJ, Sideli GM, Serdar Ü, Akyüz B, Beccaro GL, Donno D, Rovira M, Ferguson L, Akbari M, Sheikhi A, Sestras AF, Kafkas S, Paizila A, Roozban MR, Kaur A, Panta S, Zhang L, Sestras RE, Mehlenbacher S (2021) Advances in rootstock breeding of nut trees: objectives and strategies. Plants. 10, (11), 2234.
- Whitlow TH, Bassuk NL, Ranney TG, Reichert LD (1992) An improved method for using electrolyte leakage to assess membrane competence in plant tissues. Plant Physiology. 98, 198-205.
- Zoric L, Ljubojevic M, Merkulov LJ, Lukovic J, Ognjanov V (2012) Anatomical characteristics of cherry rootstocks as possible preselecting tools for prediction of tree vigor. Journal of Plant Growth Regulation. 3(31), 320-331.

