



The Effect of Different Water Potentials on Seed Germination and Growth of some Persian Walnut Populations

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ABSTRACT

In this study, seven walnut populations (Rabor, Hanza, Sardoueih, Dalfard, Bidkhan, Dehbakri, and Baft) were selected from Kerman province and compared for drought stress tolerance. Water potential between 0 and -1.5 MPa was obtained by polyethylene glycol 6000 solutions. Germination percentage, leaf relative water content (LRWC), and growth parameter of seedlings diminished with increasing drought stress, but it varied across the genotypes. The Dehbakri genotype was the most tolerant to osmotic stress. In contrast, Dalfard and Bidkhan were the most sensitive to osmotic stress in which no germination occurred at -1.5 MPa. Additionally, drought treatment increased proline and soluble sugar content in the shoot and root in the tolerant genotypes. These results suggest that the accumulated proline and soluble sugar promote drought stress. Overall, the concentration of phosphorus, potassium, calcium, magnesium, and manganese increased with a reduction in the water potential while nitrogen, copper, zinc, and iron elements dropped with the decrease in water potential. Using cluster analysis, Dehbakri was classified as the most tolerant genotype. Baft, Sardoueih, Rabor, and Hanza were semi-sensitive genotypes; finally Dalfard and Bidkhan were classified as the most sensitive genotypes. For future production of drought tolerant rootstock, Dehbakri genotype is suggested to be used in breeding programs.

Introduction

Water deficit is one of the severe limitations of crop growth especially in arid and semiarid regions of the world as it plays a fundamental role in plant growth, development, and productivity (Chaves and Oliveira 2004; Shamim et al., 2013). The effect of water deficit on the plants depends on the growth stage. For example, in the seedling stage, water deficit prevents the

formation of leaf and root development (Shivhare and Lata, 2016). Persian or English walnut (*Juglans regia* L.) is one of the high economically valuable tree species of northeast, northwest, and central regions of Iran (Vahdati and Lotfi, 2013). Walnut trees require large amounts of water for the best growth and productivity and are more sensitive to drought stress among similar

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plants (Fulton and Buchner, 2006). The majority of walnut trees across the world are propagated either through seed or grafting onto seedling rootstocks. Detection of genomic resources tolerant to drought stress at different growth stages is vitally important for such arid and semiarid regions throughout the world (Shivhare and Lata, 2016; Arab et al., 2019). According to FAO (2017), Persian walnut production in Iran is 349,192 tons in shell which is the second large producer in the world. Kerman province is the leading area for walnut production in Iran, with nearly 17,095 ha under cultivation. With varied eco-geographical regions, it is one of the main centers for Persian Walnut diversity, with walnut populations being widely distributed in this province (Vahdati et al., 2009; Lotfi et al., 2009; Lotfi et al., 2010; Behrooz et al., 2019; Vahdati et al, 2015). The current study aimed at comparing different responses to drought stress in seven walnut populations adapting to local conditions and finding the best genetic resources that are tolerant to drought stress. Further, for the first

time we evaluate the effect of water stress on the status of nine micro and macro elements in Persian walnut. We hypothesized that Dehbakri genotypes would be tolerant to drought stress and could be used in breeding programs for rootstock production.

Material and Methods

Plant material

The experiments were conducted in 2013 and 2014 in the laboratory of the Soil Science Department at University of Jiroft, Kerman Province, Iran. Half-Sib seeds from seven open pollinated walnut populations were selected from geographical (some genotype) areas in Kerman province including Rabor, Hanza, Sardoueih, Dalfard, Bidkhan, Dehbakri, and Baft (Fig. 1). According to experimental results, Bidkhan and Dalfard genotypes were not used in 2014 experiment. Table 1 presents the geographical coordinates and altitudes corresponding to each surveyed area

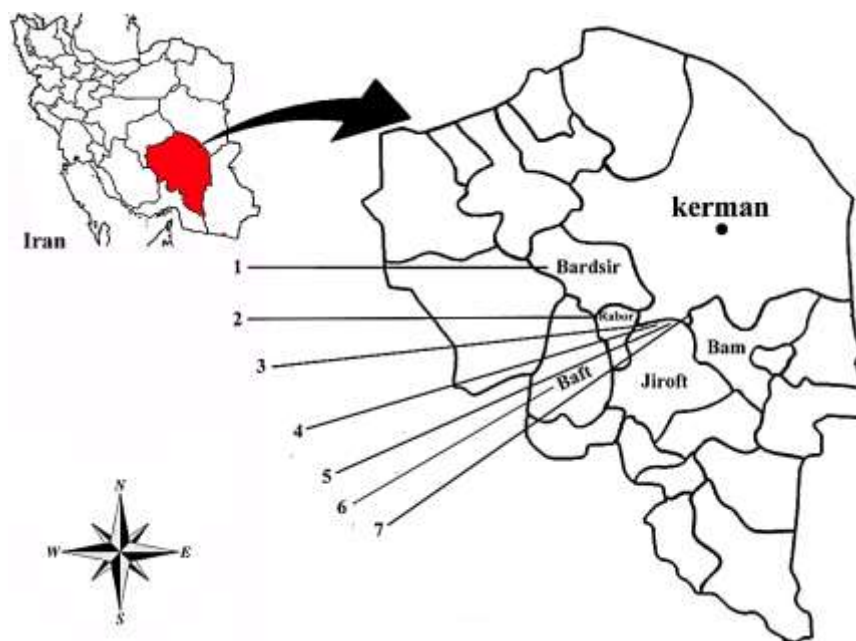


Fig. 1. Location of the researched walnut populations in Kerman province. 1) Bidkhan, 2) Rabor, 3) Hanza, 4) Sardoueih, 5) Dalfard, 6) Baft and 7) Dehbakri

Table 1. Ecological and geographical data of the studied localities of the walnuts studied.

No	Region	Longitude (E)	Latitude (N)	Altitude (M)	Average annual temperature (°C)	Annual rainfall (mm/year)
1	Rabor	57° 03' 49"	29° 14' 59"	2300	13	250
2	Hanza	57° 11' 09"	29° 17' 43"	2200	12	325
3	Sardoueih	57° 20' 32"	29° 14' 15"	2000	12	300
4	Dalfard	57° 38' 39"	28° 58' 21"	1500	15.8	310
5	Bidkhan	56° 30' 25"	29° 36' 40"	1600	14.7	260
6	Dehbakri	57° 54' 39"	29° 03' 12"	2200	15	315
7	Baft	56° 36' 08"	29° 13' 59"	2280	14.87	320

Experimental conditions

Water stress was applied through incubation at five different concentrations of PEG 6000 providing solutions with water potentials including -0.25, -0.5, -0.75, -1, and -1.5 MPa (Michel and Kaufmann 1973). The entire process of seed preparation for germination, chilling requirement, and growth conditions was performed according to the procedure by Vahdati et al. (2009). The experiment was conducted in Plant Science Laboratory, University of Jiroft, Jiroft, Iran.

Determination of water content and growth parameters

The water content of the leaves was expressed as relative water content (RWC) according to the following equation: $RWC = (FW-DW) \times 100 / (SW-DW)$, where FW represents the fresh weight, SW denotes the water-saturated weight, and DW indicates the dry weight after a 12 h of drying at 105°C (Gigon et al., 2004).

Once the primary root lengths of the control seedlings reached an average of ≈ 4 cm, the shoot and root length, shoot and root fresh and dry weight, shoot to root length ratio, shoot to root weight ratios as well as plant fresh and dry weight were measured. Furthermore, the final germination percentage (FGP) and tissue water content (TWC) were calculated (Vahdati et al., 2009).

Proline measurement

Proline content was quantified using the procedure by Bates et al. (1973). The root and shoot samples (0.5 g) were homogenized with 5 ml of 3% sulfosalicylic acid and then centrifuged at 3000g for 20 min. The filtrate (2ml) was combined with acid-ninhydrin (2ml) and glacial acetic acid (2ml) in a test tube and boiled at 90°C for 55 min. Once the reaction mixture was cooled down, 5 ml toluene was added. The chromophore-containing toluene was separated and the absorbance was measured at 520 nm using a UV-visible spectrophotometer (PG instruments LTD.).

Soluble sugar and starch analysis

Starch and soluble sugar were analyzed from leaves and roots at the end of the experiment according to the procedure of Dubois et al. (1956) using glucose as standard. Specifically, 0.5 g fresh weight of roots and shoots was homogenized with deionized water; the extract was filtered and treated with 5% phenol and 98% sulfuric acid; the mixture remained still for 55 min and then absorbance was calculated at 485 nm through a spectrophotometer (PG instruments LTD.).

Measurement of macro and micro elements

In the end of the experiment, the potassium concentration in the extract (Thomas et al., 1982) was

measured by a flame photometer (JENWAY version 7 PFP) (Hemke and Sparks, 1996) while iron, zinc, manganese and copper via atomic absorption a spectrophotometer (GBC model AVANTA). Also, phosphorus was measured by a spectrophotometer (V-1100 model) at a wavelength of 880 MAPADA (Wahing *et al*, 1989). The nitrogen content was measured using micro-Kjeldal digestion method (Bremner and Mulvaney, 1982). The calcium and magnesium concentrations were measured by complex meter method with EDTA (Loeppert and Suarez, 1996). All experiments were conducted in the Soil Science Laboratory, University of Jiroft, Jiroft, Iran.

Statistical analysis

Data were subjected to variance analysis using SAS software (SAS Institute Inc.) with seven seedlings genotypes \times six water deficient treatments. The first factor included seven seedling genotypes (Rabor, Hanza, Sardoueih, Dalfard, Bidkhan, Dehbakri, and Baft) while the second factor had six osmotic levels (0, -0.25, -0.5, -0.75, -1, and -1.5 MPa). The experimental design was factorial with a completely randomized design with three replications and 8 seeds per replicate. Treatment means were separated by the Tukey Test ($P <$

0.05). To find the tolerant genotypes, we classified them through cluster analysis via SPSS. A linear regression analysis was also performed.

Results

Seed germination

Reduction of the water potential by PEG caused a remarkable decline in the seed germination in all genotypes. In the control, the maximum germination occurred in Dehbakri genotype (87%) and the minimum in Baft genotype (67%) in 2013 (Fig. 2). The fall of the water potential to -0.75 MPa caused a reduction in the germination by less than 45% in all genotypes. Furthermore, the interaction effect of water stress and genotype on the final germination percentage ($P \leq 0.01\%$) was significant. Meanwhile, the final germination percentage depended on the genotype and drought stress. For instance, at -1.5 MPa the maximum germination was obtained with Dehbakri genotype (36%) while no germination occurred with Dalfard and Bidkhan genotypes at this water potential (Fig. 1). In 2014, the highest germination was found in Dehbakri, Hanza, and Rabor by as much as 85%, 81%, and 78%, respectively (data not shown).

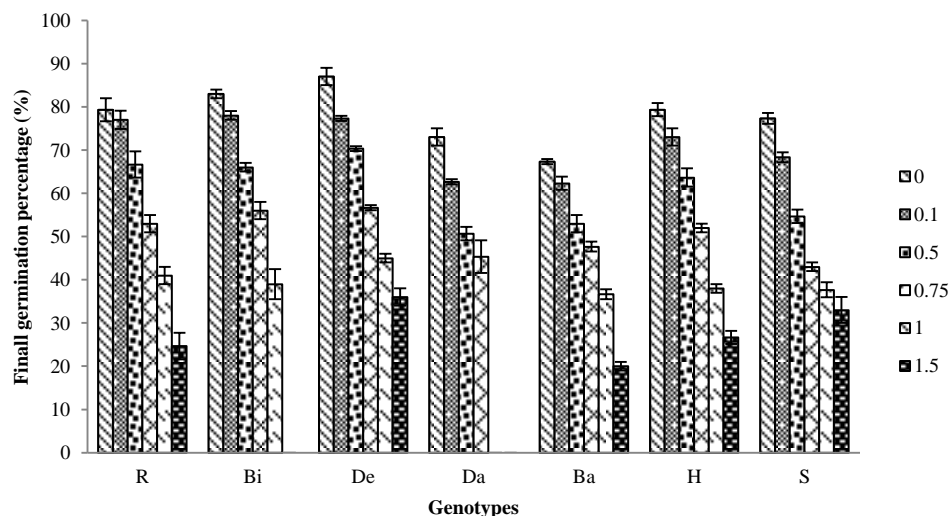


Fig. 2. Impacts of drought stress and genotype interaction on the final germination percentage of seven walnut genotypes; each bar represents the mean (\pm SE) of the three evaluation for three plants per treatment ($P \leq 0.05$). R= Rabor, Bi=Bidkhan, De=Dehbakri, Da=Dalfard, Ba=Baft, H=Hanza and S=Sardoueih.

Effect of drought stress on seedling growth

The exposure of walnut genotype to water deficit caused a significant impairment in both shoot and root growth, as compared with the control treatment. For example, the maximum shoot length was recorded in Dehbakri (32mm), Sardoueih (14mm), Hanza and Baft (14mm) at -1.5 MPa (Fig. 3a). Likewise, at this water potential for the root trait, the highest lengths were found in Dehbakri (32mm) and Sardoueih (14mm) seedlings (Fig. 3b). The shoot water content in seedlings of Baft, Dehbakri, and Sardoueih genotypes was the maximum in the control

treatment (unstressed water), but the same genotypes' seedlings had the minimum root water content under severe drought stress (-1.5 MPa) (Fig. 3c). At -1.5 MPa, seedlings of Dehbakri, Sardoueih and Hanza had a maximum root fresh weight (data not presented). In parallel to the increasing water deficit conditions, there was a significant decline in the root relative water content (RWC) with differences being recorded between the genotypes at different water potentials (Fig. 3d).

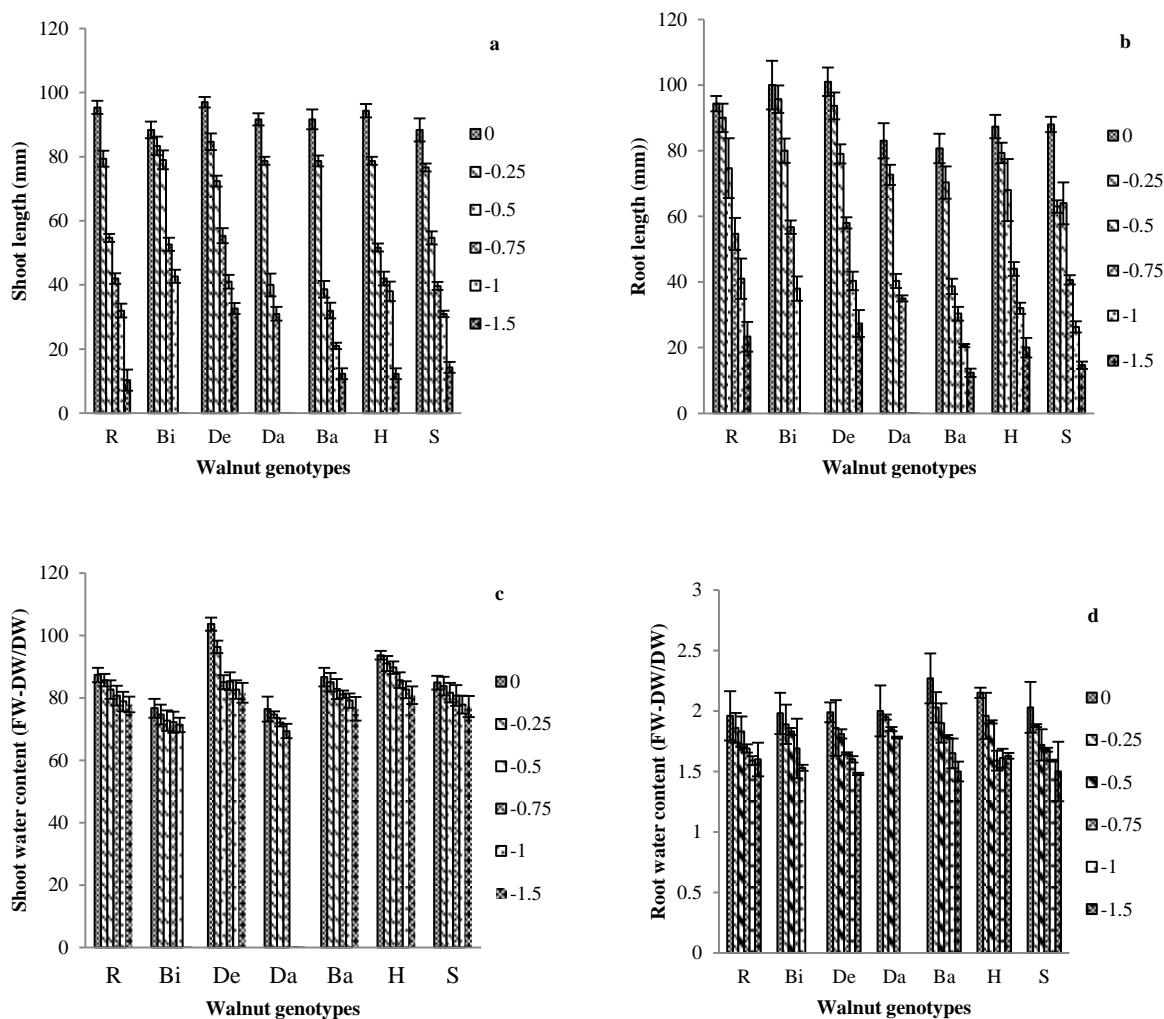


Fig. 3. Impact of drought stress and genotype interaction on shoot length (a), root length (b), shoot water content (c) and root water content (d) of seven walnut genotypes; each bar represents the mean (\pm SE) of the four evaluation for three plants per treatment ($P \leq 0.05$). R=Rabor, Bi=Bidkhan, De=Dehbakri, Da=Dalfard, Ba=Baft, H=Hanza and S=Sardoueih.

Effect of water stress on proline accumulation

Drought stress induced a marked increase in the shoot and root proline in the tolerant genotypes compared with control. In general, Dehbakri, Rabor, and Hanza genotypes contained a higher proline content in shoots as compared to Baft and Sardoueih. At the water potential of -1.5 MPa, the shoot proline content increased by 3.4-fold in Dehbakri and 2.2-fold in Hanza (Table 2). The root proline content increased by 4.03-fold in Dehbakri and 3.06-fold in Rabor (Table 2). In the

drought susceptible genotypes, no significant difference was observed in proline. For example, in Dalfard genotype, the shoot proline content was not affected by drought stress. Such results indicated that proline might lead to increased proline content as a response to drought stress, especially in tolerant walnut genotypes. Eventually, drought increased the proline concentrations more considerably in shoots than in roots. Proline accumulation per unit increased linearly with reduction

of water potential in the tolerant walnuts (Fig.4; a and b).

Effect of water stress on soluble sugar content

Compared to control, drought stress generally increased the total soluble sugar in shoots and roots in the drought-tolerant seedlings. The shoot soluble sugar content increased by 1.35-fold in Dehbakri and 1.45-

fold in Baft, and the root soluble sugar content rose by 1.38-fold in Dehbakri and 1.47-fold in Baft compared to control plants. In the susceptible Dalfard genotype, no significant difference was observed in the soluble sugar content in root and shoots (Table 3). The content of soluble sugars in shoot and root rose linearly with reduced water potential in the tolerant walnuts (Dehbakri genotype) (Fig. 4 c and d).

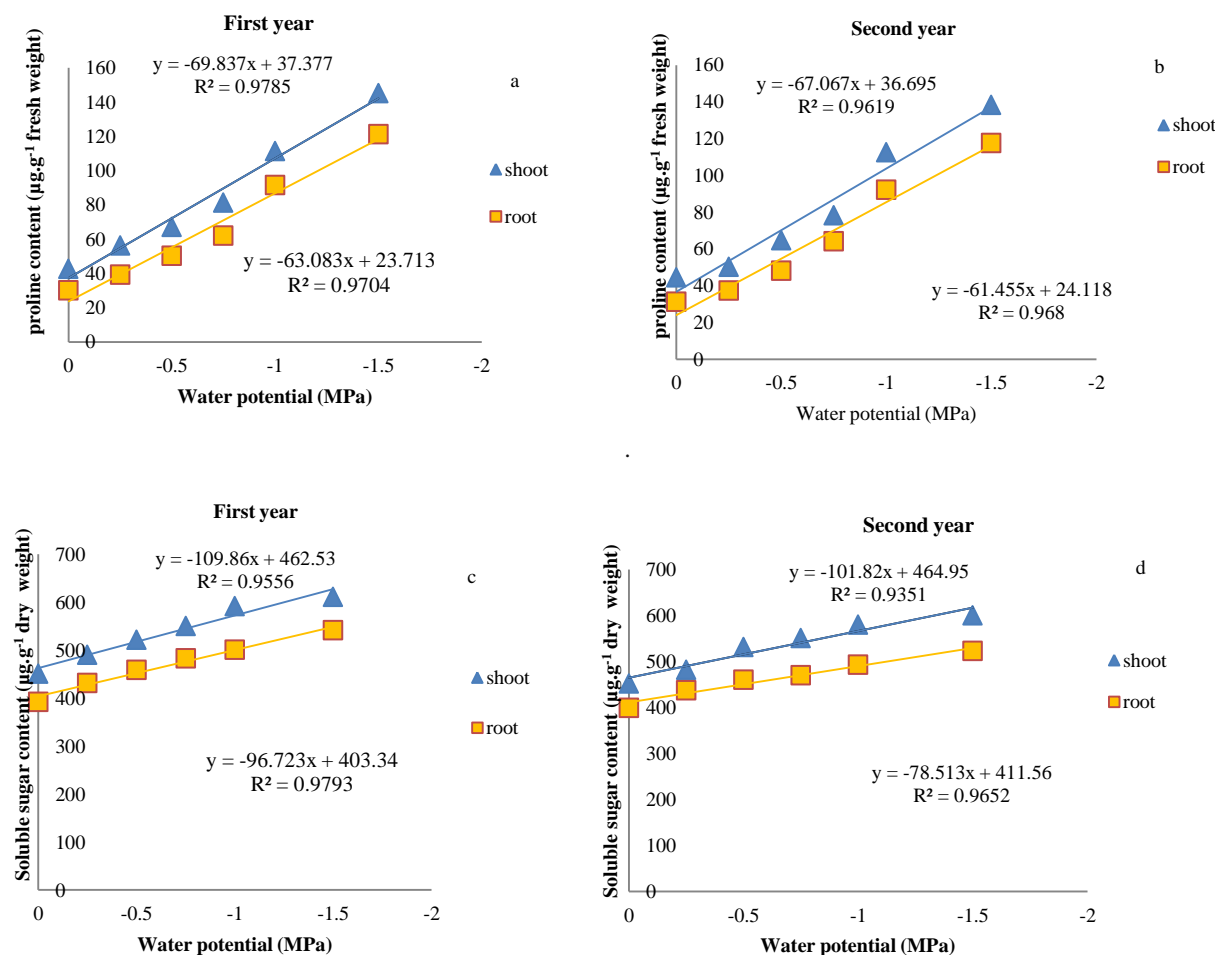


Fig. 4. Correlation between drought stress and proline content (a and b) and soluble sugar content (c and d) in shoots and roots in Dehbakri genotype in 2013 and 2014.

Effect of water stress on micro and macro elements

At the end of the study, the concentration of micro (Fe, Cu, Mn and Zn) and macro (P, N, K, Ca and Mg) elements was measured in the leaf of genotypes. Overall, the concentration of phosphorus, potassium, calcium, magnesium and manganese increased with reduction of water potential (Table. 4). For example, potassium concentration increased sharply in relation to in the severity of drought stress (-1.5 MPa). The leaf potassium concentration increased by 1.44 times in Dehbakri genotype as compared to the control plants. Nitrogen, copper, zinc and iron elements diminished with the decrease in the water potential. However,

copper had the minimum variation among the levels of water potential (Table 4).

Cluster analysis

Euclidean distance was calculated based on all traits related to drought tolerance at the osmotic level of -1.5 MPa. Based on the results from the cluster analysis, the seedlings of the tested genotypes were classified into three groups: Bidkhan and Dalfard as the first group with minimum tolerance; Baft, Sardoueih, Rabor, and Hanza as the second; and Dehbakri as the third and greatest drought-tolerant group (Fig. 5). Finally, Dehbakri was the least affected seedling by water stress. In contrast, Bidkhan genotypes were severely affected.

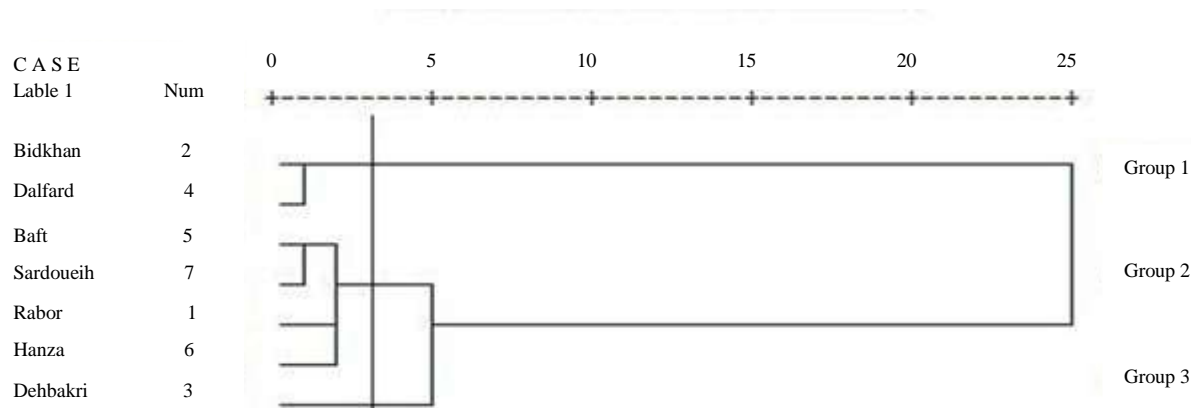


Fig. 5. Dendrogram of the UPGMA clustering algorithm using square Euclidean distance based on all trait means related to drought tolerance at the -1.5 MPa. Groups 1 and 3 include sensitive and tolerant seedlings

Table 2. Changes in proline content of shoots and roots in the presence of polyethylene glycol 6000 at various concentrations for seven walnut genotypes seedlings. Means followed by the same letter are not significantly different at $P \leq 5\%$, according to the Tukey test.

Genotypes	Shoot proline content ($\mu\text{g}\cdot\text{g}^{-1}$ fresh weight)						Root proline content ($\mu\text{g}\cdot\text{g}^{-1}$ fresh weight)					
	Water Potential (MPa)						Water Potential (MPa)					
	0	-0.25	-0.5	-0.75	-1	-1.5	0	-0.25	-0.5	-0.75	-1	-1.5
Rabor	52.31k-m	60.35j-l	77.32fg	99.2cd	105.35cd	122.31b	35.01p-s	44.65l-o	54.61kl	79e-g	90.61cd	107.31b
Bidkhan	49.01lm	59.31j-l	76.1fg	91.29de	121.12b	-	31.64rs	40.65n-r	63i-k	71.62g-j	89.31c-e	-
Dehbakri	42.61m	56.27j-m	67.02g-j	81.31ef	111.3bc	145.2a	30.03s	39.31o-s	50.34l-n	62.3jk	91.6cd	121.3a
Dalfard	52.01lm	52.12lm	53.62j-m	51.31lm	-	-	32.67q-s	47.62l-o	42.63m-q	48.31l-o	-	-
Baft	58j-l	48.31lm	61.01h-l	61.62h-l	67.3g-j	67gh	40.65n-r	43.31m-p	50.61l-n	49.1l-o	54kl	56.34kl
Hanza	56.01j-m	66.31g-j	81.65gh	98.31cd	121.1b	124b	32.61q-s	42.61m-q	51.31lm	73.37f-i	83.61d-f	97.60bc
Sarduoeh	72.1f-i	73.29f-h	77.6e-g	79.3e-g	82.61ef	78.2e-g	42.59m-q	54.01kl	63i-k	69.61g-j	76.3f-h	67.1h-j

Table 3. Changes in soluble sugar content of shoots and roots in the presence of polyethylene glycol 6000 at various concentrations for seven walnut genotypes seedlings.

Genotypes	Shoot soluble sugar content ($\mu\text{g}\cdot\text{g}^{-1}$ fresh weight)						Root soluble sugar content ($\mu\text{g}\cdot\text{g}^{-1}$ fresh weight)					
	Water Potential (MPa)						Water Potential (MPa)					
	0	-0.25	-0.5	-0.75	-1	-1.5	0	-0.25	-0.5	-0.75	-1	-1.5
Rabor	419.66gh	439.78gh	457.31f	467.95ef	490.31de	521bc	346.31jk	360ij	371.07hi	390.64gh	411.46ef	440.12cd
Bidkhan	398.1h	443.21fg	515.65cd	531.62bc	535.67bc	-	290.32p-r	320.66lm	341.62jk	371.5hi	391.45g	-
Dehbakri	450.78f	490.32de	521.29bc	550.09b	591.43a	610.71a	391.9fg	431.25de	458.66c	482.78b	500.81b	541.54a
Dalfard	198.05o	200.78o	208.52o	212.24o	-	-	201.48v	204.91v	206.18v	212.39v	-	-
Baft	282.31n	295.1mn	308.61l-n	340.54i-k	385.5i	410.4h	240.89u	255.31tu	292.01p-r	320lm	332.46kl	356ij
Hanza	296.49l-n	305.62l-n	305.52l-n	325.86j-l	355.58ij	366.26i	279.34rs	297.1o-r	301.2m-q	304.41m-o	312.73l-o	320.54lm
Sarduoeh	300.28l-n	301l-n	305.63l-n	300.67l-n	312.32l-n	313l-m	270.32st	282.02rs	296.1o-r	293.3o-r	299.04o-r	300.71n-q

Mean followed by the same letter are not significantly different at $P \leq 5\%$, according to the Tukey test.

Table 4. Concentrations of micro and macro elements in shoot of different drought stress groups of the studied walnut genotypes.

	N (%)						K (%)					
	0	-0.25	-0.5	-0.75	-1	-1.5	0	-0.25	-0.5	-0.75	-1	-1.5
R	5.2a	4.6a-e	3.5i-m	3.69i-h	3.13l-n	4.8a-d	6.00m-o	6.56j-m	6.51j-n	6.60j-m	6.70i-l	6.76h-l
Bi	5.12ab	4.2c-h	3.8f-l	3.42i-n	3.9e-l	-	7.53b-f	7.73b-d	7.46b-g	7.56b-f	7.64b-e	-
De	4.95a-c	4.27c-h	3.2m-l	3.31m-l	3.18m-l	4.61a-e	6.6j-m	7.13d-j	7.13d-j	7.40b-h	6.9f-k	7.53b-f
Da	4.2c-h	4.34c-f	4.35c-f	3.79f-l	-	-	4.4q	5.5op	6.13m-o	6.6j-m	-	-
Ba	4.03e-j	3.34m-l	3.55j-l	3.4i-n	3.62h-m	3.4i-n	5.2p	5.63op	5.64op	5.86o-n	7.8a-c	7.9ab
H	4.27c-h	4.16c-h	3.88f-l	2.76n	3.2m-l	-	5.6op	6.6j-m	6.6j-m	7.00e-j	7.3b-i	-
S	4.53a-f	4.03e-j	3.66h-m	3.21m-l	2.87mn	4.17e-i	6.16l-o	6.3k-n	8.4a	7.16d-j	7.5b-f	7.46b-d
	Ca (%)						Mg (%)					
R	0.66l-n	1.04i-l	1.00i-m	1.92a-d	1.97a-c	1.96a-c	0.63ab	0.47d-h	0.45e-h	0.46g-h	0.47d-h	0.48c-h
Bi	0.54n	0.73k-n	1.2h-j	1.85a-d	1.9a-d	-	0.61bc	0.52b-g	0.50b-g	0.51b-g	0.25i	-
De	0.91i-n	0.85j-n	0.79j-n	1.80a-d	1.9a-d	1.73a-d	0.48c-g	0.50b-g	0.50b-g	0.75a	0.60b-d	0.45g-h
Da	1.70b-g	1.78a-d	1.74a-d	1.72b-e	-	-	0.49c-h	0.55b-f	0.57c-e	0.61bc	-	-
Ba	0.76k-n	0.85j-n	0.74k-n	1.69c-g	2.13a	2.10ab	0.44e-h	0.45e-h	0.45e-h	0.46e-h	0.43e-h	0.52b-g
H	0.95i-n	1.32e-i	1.56f-i	1.70b-f	1.31f-i	-	0.54b-g	0.46g-h	0.51b-g	0.52b-g	0.42f-h	-
S	0.60mn	0.80j-n	1.12i-k	1.29g-i	1.81a-d	2.01a-c	0.50b-g	0.41gh	0.53b-g	0.54b-g	0.46d-h	0.36hi
	Fe (Mg.g ⁻¹)						Mn (Mg.g ⁻¹)					
R	0.14b-e	0.17b-d	0.13d-f	0.13d-f	0.13d-f	0.13d-f	0.10b-g	0.10b-g	0.10b-g	0.08fg	0.10b-g	0.10b-g
Bi	0.13d-f	0.13d-f	0.13d-f	0.14b-e	0.13d-f	-	0.12bc	0.11b-e	0.08fg	0.10b-g	0.10b-g	-
De	0.09ef	0.15b-e	0.16b-d	0.15b-e	0.14b-e	0.13d-f	0.10b-g	0.10b-g	0.10b-g	0.09c-g	0.10b-g	0.10b-g
Da	0.13d-f	0.16b-d	0.15b-e	0.16b-d	-	-	0.09c-g	0.10b-g	0.11b-f	-	-	-
Ba	0.11d-f	0.11d-f	0.12d-f	0.11d-f	0.14b-e	0.16b-d	0.18a	0.09c-g	0.10b-g	0.09c-g	0.11b-e	0.12bc
H	0.17b-d	0.12d-f	0.18bc	0.19b	0.12d-f	-	0.10b-g	0.10b-g	0.12bc	0.08fg	0.10b-g	-
S	0.13d-f	0.13d-f	0.16b-d	0.12d-f	0.27a	0.13d-f	0.12bc	0.08fg	0.12bc	0.10b-g	0.13b	0.10b-g

Table 4. Continued.

	Zn (Mg.g ⁻¹)						Cu (Mg.g ⁻¹)						
	0	-0.25	-0.5	-0.75	-1	-1.5	0	-0.25	-0.5	-0.75	-1	-1.5	
R	0.62bc	0.46f-m	0.45i-m	0.44j-m	0.44j-m	0.45i-m	0.13e-k	0.16a-d	0.15b-h	0.14b-j	0.13e-k	0.14b-j	
Bi	0.60cd	0.52e-h	0.51e-j	0.50e-j	0.25n	-	0.12f-l	0.12f-l	0.13e-k	0.14b-j	0.12f-l	-	
De	0.48f-m	0.50e-j	0.50e-j	0.71a	0.69ab	0.46f-m	0.09kl	0.14b-j	0.12h-k	0.14b-j	0.15b-h	0.12f-l	
Da	0.45i-m	0.54de	0.43k-m	0.60cd	-	-	0.14b-j	0.16a-f	0.15b-h	0.16a-f	-	-	
Ba	0.42lm	0.44j-m	0.43lm	0.42m	0.50e-j	0.53ef	0.12f-l	0.11i-l	0.12h-k	0.12h-k	0.12h-k	0.12h-k	
H	0.53ef	0.45i-m	0.52e-h	0.52e-h	0.42m	-	0.12h-k	0.15b-h	0.17a-c	0.16a-f	0.18a	-	
S	0.50e-j	0.42m	0.55de	0.55de	0.46f-m	0.31n	0.13e-k	0.09kl	0.16a-f	0.12h-k	0.11i-l	0.12h-k	
	P (%)												
R	0.45ac	0.45ac	0.35a-h	0.39a-f	0.25i-j	0.47a							
Bi	0.44a-d	0.40a-f	0.34a-h	0.25i-j	0.19j	-							
De	0.48a	0.46ab	0.38a-f	0.27e-j	0.34a-h	0.44a-d							
Da	0.33a-h	0.39a-f	0.29e-j	0.25f-j	-	-							
Ba	0.35a-h	0.41a-e	0.32a-h	0.33a-h	0.28e-j	0.38a-f							
H	0.33a-h	0.34a-h	0.28e-j	0.34a-h	0.20ij	-							
S	0.31a-h	0.29e-j	0.30a-h	0.28e-j	0.21ij	0.20ij							

R= Rabor, Bi=Bidkhan, De=Dehbakri, Da=Dalfard, Ba=Baft, H=Hanza and S=Sardoueih. Those with the same letter in each row do not differ significantly by Tukey's test ($P < 0.05$).

Discussion

The current study found that germination rate was affected by drought stress. However, there were variations in the degree across the populations. Dehbakri, Sardoueih, and Hanza genotypes had the greatest germination at -1.5 MPa, at which Bidkhan and Dalfard genotypes had no germinated seed, however. Harris *et al.*, (2002) reported that the first and foremost impact of water stress is impaired germination. Water deficit has been known to severely reduce seedling stands and germination (Kaya *et al.*, 2006) Our results of reduced germination percentage with PEG are in agreement with a number of studies, e.g. on walnut (Vahdati *et al.*, 2009; Lotfi *et al.*, 2009; Lotfi *et al.*, 2010); citrus (Zaher-Ara *et al.*, 2016); and mango (Zhang *et al.*, 2019). The plant growth rate was affected by water stress, genetic, ecological, morphological and physiological parameters as well as their complicated interactions (Farooq *et al.*, 2009). For example, an early morphological response of plants to water deficit was delay in shoot and root fresh and dry weights (Zeid and Shedeed, 2006) as well as shoot and root length (Lotfi *et al.*, 2010a). These accord with our results since plant growth was significantly inhibited in the seven walnut genotypes under water stress. In addition, a marked difference was observed between these seven genotypes in terms of shoot and root traits under drought stress. We can infer from these data that difference in genetic background may be the main cause; alternatively, climatic conditions such as water stress and altitude would allow the trees to get used to new environmental conditions (Close and Wilson, 2002; Negash, 2003; Zhu *et al.*, 2006). Compared with control treatments, proline accumulation increased significantly in line with the severity of water stress (-1.5 MPa). It was higher in Dehbakri genotype than in other tolerant genotypes, i.e. Hanza and Rabor. Increased proline during stress has multiple protective roles. Under long-term abiotic stress,

proline was viewed as a neutral osmolyte protecting cellular structures and stabilizing enzymes (Kishor *et al.*, 2005; Mishra and Dubey, 2006; Sharma and Dubey, 2005; Lotfi *et al.*, 2019). Furthermore, as an osmoprotectant, proline proved to have the following advantages: possessing an antioxidant function, activating detoxification pathways, participating in cellular homeostasis by protecting the redox balance, functioning as a protein precursor, providing an energy source for the stress-recovery process and even a signaling molecule (Hoque *et al.*, 2008; Islam *et al.*, 2009; Székely *et al.*, 2008). In plants under stress conditions, it is of key importance to conserve PSII and PSI activity together with electron flux via the photosynthetic electron transport chain. For synthesis of proline in chloroplast, the cycle of calvin, pentose phosphate, and glutamate must be stopped. Thus, proline synthesis in the chloroplast may result in an efficient oxidation of photosynthetically produced NADPH, thereby providing the required NADP⁺ for electron acceptor and avoiding the use of O₂, causing ROS generation (Hare *et al.*, 1997; Szabados and Savouré, 2010). The important role of proline accumulation in abiotic stress such as drought has been affirmed by a variety of studies: *J. regia* (Lotfi *et al.*, 2010; Karimi *et al.*, 2018), *Malus domestica* (Zhang *et al.*, 2015), *Pistacia vera* (Esmaeilpour *et al.*, 2016). Under drought stress, sugars can be effective on physiological responses and osmotic adjustment, as they are involved in metabolic signaling (Liu *et al.*, 2011). In our study, soluble sugars increased in shoots and roots in the tolerant genotypes. This accumulation, especially in shoots, may cause a significant change in the osmotic pressure. It should also be a result of starch hydrolysis and conversion into soluble sugars (Irigoyen *et al.*, 1992; Lotfi *et al.*, 2010a). Based on a report by García-Sánchez *et al.* (2007), under drought stress the soluble

sugars increased in leaves and while starch diminished in Citrange and Cleopatra Mandarin. In Satsuma, under drought stress conditions, sugar accumulation increased in fruits (Yakushiji *et al.*, 1998). The high coefficient of determination (R^2) observed in the tolerant genotypes confirmed that proline and soluble sugar content have a dramatic effect on walnut tolerance to drought stress. The same effect has been observed in a couple of studies on olive (Sofo *et al.*, 2004), grapevine (Schultz and Matthews, 1993), apple (Wang *et al.*, 1995), citrus (Zaher-Ara *et al.*, 2016), and cherry trees (Ranney *et al.*, 1991). In this research, some elements including phosphorus, potassium, calcium, magnesium, and manganese increased in the leaf under drought stress conditions. K plays a significant role in the regulation of water status in plants (Mengel *et al.*, 2001). Other micro and macro elements have important roles in plants against water stress such as protein stability (N, Mg), cell division, and improving the response to drought (P) (da Silva *et al.*, 2011). In conclusion, our results support the hypothesis that mineral content, proline, and soluble sugar content in the shoot and root during water stress would be a part of the physiological response of Persian walnut genotypes to intense water deficit. Overall, this osmolyte could be used as a biochemical marker for finding the tolerant genetic resource of walnut genotype under drought stress. Nevertheless, studies are required to advance our findings of the effect of drought stress on 2-year-old walnut genotypes including Dehbakri, Hanza, and Rabor.

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