



Introducing Tolerant Pistachio Female and Male Genotypes to Salinity Stress

Hamid Alipour^{*}, Hojjat Hasheminasab, Seyed Javad Hosseini

Pistachio Research Center, Horticultural Sciences Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Rafsanjan, Iran

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ABSTRACT

Pistachio is one of the most important and strategic crops in Iran which is typically cultivated under relatively saline soils and hot weather conditions. The present paper represents the results of an experiment which mainly focused on evaluating the tolerance of pistachio female and male genotypes to salinity stress. This experiment was carried out at the greenhouse of the Pistachio Research Center at Kerman, Iran, during 2016. The hybrids obtained from crosses between 17 male genotypes with a female genotype “Fandoghi” and also the combinations of 19 female genotypes after crossing with a male genotype “M15” were cultured in a completely randomized design with three replications under salt stress (4.2 dS m^{-1}) without drainage in the greenhouse condition for a period of four months. The results show an increasing of the average soil salinity in pots to 32 dS m^{-1} . The results also indicate that salinity might negatively affect growth parameters of pistachio. Therefore, selecting genotypes in this condition might lead to release tolerant genotypes. During the research, some yielded hybrids indicated the higher values for morphological parameters, the lower and higher concentration of Na^+ and K^+ under salinity stress, respectively. Meanwhile, it was found that, ‘Khanjari Damghan’ and ‘Mohseni’ among female parents, and M10, M15, and M25 among male parents are possible tolerant genotypes for further assessment in the area and also for releasing new rootstocks. The analysis of two clusters, *i.e.*, cluster 1 (‘Khanjari Damghan’, ‘Mohseni’, ‘Lak Sirizi’, ‘Ebrahimi’, ‘Sifadini’, ‘Badami Zarand’, ‘Sephid Peste Nogh’, and ‘Post Kaghazi’) and cluster 2 (M10, M15, M24, M25, and M26) for female and male genotypes, respectively, exhibits that higher morphological parameters and also higher tolerance to salinity. More importantly, the analysis showed that these two categorized sets of genotypes can be proper choices for future breeding programs or to use directly in salinity conditions.

Introduction

In arid and semi-arid areas, particularly arid zones that are irrigated with low-quality water, salinity (soil or water salinity) is considered as one of the most important environmental constraints for agriculture (Alipour, 2018; Vahdati *et al.*, 2013). About 7% of the world’s total land area is affected by salt, which is still increasing due to irrigation with saline water or other improper management (Motagh *et al.*, 2008). In many semi-arid and arid areas of the world, such as Iran, salt

evapotranspiration is common (Lotfi *et al.*, 2009; Pessarakli *et al.*, 1999; Shalhevet 1994). Accumulation of sodium in soil (*i.e.*, soil salinity) can lead to decrease yields and crop failure. Also, salt accumulation in soils may modify the growth of plant to different degrees (Akbarimoghaddam *et al.*, 2011). However, in the same saline environment, different plant species may exhibit different growth responses (Flowers, 2004).

^{*}Corresponding author: Email address: h-alipour@pri.ir

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In saline water management, it is required to introduce and identify the promising salt-tolerant lines with higher production (Minhas 1996; Rhoades *et al.*, 1992). This identification can be derived by classifications based on the relationship between yield reduction and other parameters such as soil salinity, water salinity (Ayers *et al.*, 1985; Francois *et al.*, 1994), alive seedlings, relative transpiration, genotypes, and plant water status indicators (Katerji *et al.*, 2000). In older classifications of many crops, the effects of salinity on the quality of the harvested crop have been ignored. For example, a study conducted by Akbarimoghaddam *et al.*, (2011) determined the impacts of salinity on the yield quality of pistachio cultivars. Further studies on different genotypes of pistachio (Khoyerd *et al.*, 2016; Parsa *et al.*, 1975; Parsa *et al.*, 1980; Rahnesan *et al.*, 2018a; Sepaskhah *et al.*, 1988) and for other different plant species (Ahmed *et al.*, 2016; Frota *et al.*, 1978; Khoyerd *et al.*, 2016, and Rahnesan *et al.*, 2018b), cotton (Iqbal *et al.*, 2017; Pessarakli *et al.*, 1985), tomato (Kerketta *et al.*, 2018; Pessarakli *et al.*, 1988), cereal (Aguilera *et al.*, 2017; Atak *et al.*, 2006; Livingston Iii *et al.*, 2016) and walnut (Yadollahi *et al.*, 2010) demonstrated that water uptake in plants is significantly reduced under salt or water stress conditions and genotypes, in most studies, respond to salinity in altered ways. On the other hand, it should be noted that it is difficult to compare the reported limits of salt stress survival according to the following reasons. (a) There is no standard method for measuring salt stress, (b) The limitations with the environmental condition and the stage of development, (c) Irrigation methods may decrease the salt stress and therefore raise the limits of survival, (d) Length of time exposed to the salt has not been standardized, and if not too long, growth may be inhibited without cell injury.

Pistachio (*Pistacia vera* L.) is one of the most important, widely grown commercial nut crops (Sharifkhah *et al.*, 2020) which is a high yielding tree species, appropriately adapted to extreme arid areas and to grow in almost all geographic regions where

the parental species are. Although several preliminary studies on the salt tolerance of pistachio have been conducted (*i.e.*, Bagheri *et al.*, 2019; Behboudian *et al.*, 1986; Mehdi-Tounsi *et al.*, 2017a; Picchioni *et al.*, 1990; Picchioni *et al.*, 1991; Rahnesan *et al.*, 2018a, and Sepaskhah *et al.*, 1988), introducing and releasing new and promising genotypes with high ability of salinity tolerance have not been properly examined yet. Karim *et al.*, (2005) found that pistachio cultivars give different responses to the varying NaCl.

It is worthy to be noted that, from the viewpoint of resistance to salinity and using produced seedlings under controlled pollination and tissue culture, the comparison of pistachio cultivars, female and male genotypes has been recently studied by several researchers. While effect of salt stress on seed germination has been studied in many species (Lotfi *et al.*, 2009), the relative importance of the osmotic or toxic effects of NaCl on seed germination in pistachio is not evidently determined widely. Hence, the present study has been designed to determine the effect of NaCl on the seedling growth of pistachio genotypes in greenhouse conditions.

Materials and Methods

Experimental procedure

The experiments were carried out at the greenhouse of the Pistachio Research Center in Rafsanjan, Kerman province, South-East of Iran, during 2016. Seventeen male genotypes and nineteen female genotypes, which are popular in Iranian pistachio orchards, were studied in this experiment. These genotypes are listed in Table 1. The male genotypes were crossed with a female "Fandoghi" (17 male genotypes (Table 1) × Fandoghi), while the female genotypes were crossed with one male M15, who show high salinity tolerance (19 female genotypes (Table 1) × M15). The twenty obtained hybrid seeds were planted in 7 kg pots with a loamy sand soil (ECE = 4 dS m⁻¹, pH = 7.6). Then, nine same seedlings were selected for each one and conducted in a completely randomized design (CRD) with three replications and three seedlings per

replication in greenhouse conditions. Just six weeks after planting, the pots were irrigated with NaCl-salinity condition ($ECE = 4.2 \text{ dS m}^{-1}$) without drainage for four months to find and compare their growth

parameters in these conditions. The average soil salinity in pots at the end of the experiment increased to 32 dS m^{-1} .

Table 1. Female and male genotypes used in this study.

Female genotypes		Male genotypes	
Sifadini	Sephid Peste Noogh	M23	M19
Fandoghi	Post Kaghazi	M24	M13
Badami Zarand	Seid Aliaghaee	M25	M14
Khanjari Damghan	Akbari	M26	M15
Ahmad Aghaee	Jandaghi	M22	M16
Mohseni	Sirizi	M6	M18
Sarakhs	Harati	M20	
Lak Sirizi	Gholamrezaei	M8	
Khanjari Ravar		M9	
Ebrahimi		M10	
Nish Kalaghi		M11	

Parameters' measurement

Potassium (K^+), magnesium (Mg^{+2}), calcium (Ca^{+2}), and sodium (Na^+) in root and shoot, the dry weight of root and shoot, the number of live leaves, the height of seedlings, and also the live seedlings numbers have been collected and stored for further statistical analysis. It is noted that, the sodium and potassium contents were measured via a flame photometric method, while Ca^{+2} and Mg^{+2} measurement were performed using a titration method (Johnson *et al.*, 1959).

Statistical analysis

The obtained data were statistically analyzed based on one-way ANOVA, and then mean comparisons have been made using least significant difference (LSD) at 5% level. Cluster analysis was also carried out for female and male genotypes based on Euclidian distance via the Ward method by applying Minitab 18 statistical software.

Results

The results of correlation analysis exhibit that, K^+ of root (0.39), Na^+ of stem (-0.39), the dry weight of root (0.40) and the number of live leaves (0.56) in female

genotypes, and also Na^+ of root (-0.27), dry weight of root (0.36), the dry weight of aerial parts (0.46) and the size of seedling (-0.27) in male genotypes have significant correlations with survived number of the seedlings (Table 2). Size of the seedling shows a significant correlation with the dry weight of aerial parts (0.67) in female genotypes category, and with Ca^{2+} of root (-0.58), Na^+ of root (-0.38) and the dry weight of root (-0.29) in male genotypes category. Leaf number in both male and female genotypes show a significant correlation with most of the measured parameters where its correlation with Na^+ , Mg^{2+} , and Ca^{2+} is negative. Root and shoot dry weight are positive, in most cases, correlated with other morphological traits, while they are mostly correlated in negative trend with Na^+ , Mg^{2+} , and Ca^{2+} . Separate correlations between Na^+ and K^+ in the root and shoot are positive, while the correlation of each of these parameters with other parameters is negative.

Under salinity stress, Ebrahimi and Badami Zarand genotypes as female genotypes (Table 3) and M10 and M15 as male genotypes (Table 4) show a higher number of survived seedlings. It does also show that this level of Na^+ accumulation has a toxic effect on

number of the survived seedlings. The highest seedlings length (25.83 cm) can be observed in 'Khanjari Damghan' and the lowest one (10.53 cm) has been recorded for 'Sarakh's' for female genotypes, while these values for male genotypes, have been obtained in M6 (27.33 cm) and M20 (14.67 cm), respectively.

The lowest and highest number of leaves for female genotypes have been obtained in 'Gholamrezae' and 'Harati' (1) and 'Sephid Peste Nogh' (10.33), and for

male genotypes, M13, M16 and M22 (0) and M24 and M25 (12), respectively (Table 3 and Table 4). The dry weight of root was varied between 1.05 gr ('Nish Kalaghi') to 3.35 gr ('Akbari') for female genotypes, while it was varied between 2.58 (M11) to 4.46 (M25) for male genotypes. Meanwhile, the dry weight of aerial organs was varied between 0.75 gr ('Nish Kalaghi') and 3.53 gr ('Khanjari Damghan') for female genotypes and was between 2.2 gr (M11) and 4.45 gr (M25) for male genotypes.

Table 2. Correlation between measured parameters for female genotypes (under the diagonal) and male genotypes (above diagonal).

	K ⁺ of root (%)	K ⁺ of stem (%)	Mg ⁺² of root (%)	Mg ⁺² of stem (%)	Ca ⁺² of root (%)	Ca ⁺² of stem (%)	Na ⁺ of root (%)	Na ⁺ of stem (%)	Dry weight of root (gr)	Dry weight of aerial parts (gr)	Number of alive leaf	Mean length of seedling (cm)	Number of survived seedling
K ⁺ of root (%)	1	-0.159	-0.013	-0.243	-0.107	-0.072	0.202	-0.224	-0.176	-0.132	0.348	0.212	0.024
K ⁺ of stem (%)	-0.122	1	-0.264	0.168	-0.018	-0.095	-0.052	0.247	-0.175	-0.092	0.527	-0.168	0.035
Mg ⁺² of root (%)	-0.242	-0.087	1	0.415	0.025	0.052	0.273	-0.211	-0.27	-0.258	-0.103	-0.009	-0.175
Mg ⁺² of stem (%)	0.059	-0.39	0.317	1	-0.281	-0.257	0.189	-0.044	-0.313	-0.234	0.025	0.101	-0.232
Ca ⁺² of root (%)	-0.444	-0.165	0.133	0.162	1	0.413	0.09	0	0.36	0.407	-0.274	-0.582	0.154
Ca ⁺² of stem (%)	-0.094	-0.443	-0.038	0.093	0.293	1	0.028	0.231	0.348	0.327	-0.294	-0.232	0.04
Na ⁺ of root (%)	0.555	0.08	0.032	-0.123	-0.363	-0.203	1	-0.277	-0.055	-0.136	0.062	-0.381	-0.264
Na ⁺ of stem (%)	0.051	0.085	0.111	-0.02	-0.026	0.163	0.257	1	0.025	-0.08	-0.133	-0.065	0.143
Dry weight of root (gr)	0.133	0.063	-0.258	-0.099	-0.253	-0.004	-0.099	-0.147	1	0.928	-0.267	-0.289	0.364
Dry weight of aerial parts(gr)	0.294	-0.25	-0.111	0.322	-0.119	0.08	0.08	0.057	0.412	1	-0.203	-0.214	0.463
Number of alive leaf	0.34	0.428	-0.336	-0.161	-0.273	-0.288	-0.068	-0.333	0.348	0.136	1	-0.01	0.017
Mean Length of seedling (cm)	0.256	0.032	-0.138	0.056	-0.229	-0.236	0.026	-0.133	0.294	0.668	0.17	1	-0.275
Number of survived seedling	0.392	0.075	0.002	-0.046	-0.197	-0.155	0.204	-0.388	0.398	0.237	0.557	0.148	1

Correlation higher than 0.35 and between 0.27 to 0.34 are significant at 1% and 5% levels, respectively.

Table 3. Comparison mean in female genotypes according to measured characteristics.

Genotype	Survived seedling number	Height of seedling (cm)	Number of leaf	Dry weight of aerial parts (gr)	Dry weight of root (gr)	Na ⁺ of stem (%)	Na ⁺ of root (%)	Ca ⁺² of stem (%)	Ca ⁺² of root (%)	Mg ⁺² of stem (%)	Mg ⁺² of root (%)	K ⁺ of stem (%)	K ⁺ of root (%)
Khanjari Damghan	0.33	25.83	5.66	3.53	4	3.17	0.79	1.56	3.07	3.58	1.06	1.16	0.57
Ahmad Aghaee	0.33	20.06	2	2.8	5.68	2.4	0.71	3.69	3.41	2.78	1.48	1.02	0.35
Mohseni	0.66	17.56	6.66	2.09	6.5	2.63	0.79	1.37	2.49	3.08	1.23	1.11	0.59
Lak Sirizi	0.66	23.36	5.33	2.51	5.44	2.63	0.72	1.37	3.45	2.21	3.34	1.26	0.45
Ebrahimi	1.33	19.66	5	2.72	5.76	1.83	1.05	1.75	3.23	3.86	2.17	1.29	0.43
Nish Kalaghi	0.33	14.66	2.33	0.75	3.05	1.05	0.62	1.36	3.31	3.8	5.22	0.86	0.34
Akbari	0.33	14.63	1.33	2.72	3.31	3.56	0.73	3.38	3.37	4.51	5.14	0.71	0.57
Jandaghi	0.66	17.33	4	2.56	4.05	2.96	1.01	1.62	3.07	3.29	3.56	1.23	0.47
Sifadini	1	14.96	6.66	2.01	3.75	2.49	1.21	1.89	3.83	2.16	2.75	1.49	0.53
Fandoghi	0.66	18	2.66	2.99	3.62	3.5	0.81	2.12	3.77	3.71	3.92	1.18	0.36
Badami Zarand	1.33	17.53	5.33	1.8	4.6	2.8	1.1	1.67	3.13	2.78	3.67	1.62	0.52
Sarakhs	0.66	10.53	2	0.82	3.49	3.08	0.95	1.84	3.77	2.93	4.4	1.39	0.36
Khanjari Ravar	0.23	13.33	1.33	0.95	3.9	2.8	1.04	2.72	3.47	1.56	1.74	1.23	0.48
Sephid Peste Nogh	0.66	19.5	10.33	2.18	3.95	2.21	0.99	1.78	3.09	1.43	2.5	1.58	0.47
Post Kaghazi	1	18.73	3.66	2.19	4.35	2.79	1.07	1.65	3.46	2.64	3.93	1.47	0.53
Seid Ali Aghaee	0.33	19	2	2.26	3.94	1.66	0.93	1.56	3.62	3.76	4.69	1.27	0.42
Sirizi	0.66	20.3	4	2.52	3.84	2.13	1.2	1.26	2.49	2.72	4.22	1.49	0.44
Harati	0.33	18.66	1	2.3	3.96	2.75	1.01	1.84	3.11	3.38	3.8	1.29	0.35
Gholamrezae	0.33	18.33	1	2.79	3.48	2.8	1.34	1.37	3.2	3.2	4.38	1.32	0.42
LSD 5%	0.03	1.89	0.97	0.12	0.67	0.86	0.09	0.08	0.89	0.05	1.04	0.04	0.08

Mineral analysis of root and stem samples from two categories of genotypes shows the different concentrations of measured elements. Stem accumulated higher Na^+ and K^+ than roots in both male and female genotypes. But the observed amount of Ca^{2+} in root is more than stem. Similar trends can be noticed in all genotypes. Furthermore, the mean comparison shows that all elements contents differed for genotypes. The highest portion of Na^+ uptake by root can be observed in 'Gholamrezae' (1.34%), while highest sodium accumulation in stem has been observed in 'Fandoghi' (3.5%) and 'Akbari' (3.56%). On the other hand, the lowest accumulation of Na^+ in root (0.67%) and shoot (1.05%) of male genotypes achieved for 'Nish Kalaghi'. Male genotypes show no significant difference for root and stem sodium contents (Table 4).

The result of cluster analysis for both male and female genotypes has been arranged into three main groups. Cluster 1 contains; 'Khanjari Damghan', 'Mohseni', 'Lak Sirizi', 'Ebrahimi', 'Sifadini', 'Badani Zarand', 'Sephid Peste Nogh', and 'Post Kaghazi', while 'Ahmad Aghaee', 'Nish Kalaghi', 'Akbari', 'Saraks', and 'Khanjari Ravar' are classified as cluster 2, and all the rest of the female genotypes are considered in cluster 3 (Fig. 1). For male genotypes, in a similar manner, we have grouped M6, M8 and M23 as cluster 1, M10, M15, M24, M25, and M26 as cluster 2, and cluster 3 comprises of all other genotypes (Fig. 2). The highest morphological parameters, along with higher potassium and lower sodium content have been estimated for cluster 1 for female genotypes. Similarly, cluster 2 of male genotypes exhibits the higher proper parameters with low sodium content (Table 5).

Discussion

Salinity is the main factor limiting plant growth in many pistachio orchards of Iran. Salinity tolerance is influenced by an interaction of plant, soil, and environmental conditions (Sedaghat 2011). It is found

that different and sometimes uncovered plant's factors are responsible for salinity tolerance in different roles. One of the most essential factors considered in the present study is inherited tolerance through genetic content and effective genes within a plant. Generally, specific genotypes, cultivars or rootstocks may tolerate higher levels of salt than others. On the other hand, salinity tolerance may alter during different plant growth stages. It is also worthy to be noted that pistachio rootstocks are more sensitive to high salinity during seedling stages (Hasheminasab *et al.*, 2018). Therefore, in the current study, pistachio plants with the different genetic backgrounds (as parental genotypes) crossed with each other, and then the obtained hybrids were subjected to soil salinity at the early stage of seedlings.

The results of correlation analysis indicate that the sodium content of stem and the number of survived seedlings are negatively correlated, and also the accumulation of sodium and magnesium ions in root and stem cause desiccation of leaves in seedlings and reduction of weight in root and stem.

It is verified that salinity can affect most of morphological and growth parameters in the plant as the result of nutritional and hormonal changes within plant cells. A relevantly high number of survived seedlings appear to be an essential trait to achieve facing salinity stress. These results clearly exhibit that salinity might affect on the growth parameters of pistachio in a negative trend, and therefore, selecting tolerant genotypes in this condition might lead to the release tolerant genotypes. Similar results have been obtained by different authors (Khoyerd *et al.*, 2016; Mehdi-Tounsi *et al.*, 2017; Rahnesan *et al.*, 2018c).

Table 4. Comparison mean in obtained hybrids from crosses between male genotypes with a female genotype “Fandoghi” according to measured characteristics.

Genotype	Survived seedling number	Length of seedling (cm)	Number of leaf	Dry weight of aerial parts (gr)	Dry weight of root (gr)	Na ⁺ of stem (%)	Na ⁺ of root (%)	Ca ⁺² of stem (%)	Ca ⁺² of root (%)	Mg ⁺² of stem (%)	Mg ⁺² of root (%)	K ⁺ of stem (%)	K ⁺ of root (%)
M6	0.34	27.33	7.99	2.74	2.94	1.06	1.03	1.01	0.9	1.3	0.48	1.33	0.71
M8	0.34	19	6.4	2.25	2.74	1.04	1.03	0.7	1.93	0.83	0.47	1.51	0.54
M9	0.34	19	1.2	3.05	3.03	1.05	1.04	1.88	2.28	0.93	1.16	1.39	0.34
M10	1.33	19.33	6	3.61	3.64	1.04	1.03	2.04	1.84	0.59	1.61	1.55	0.52
M11	0.34	18.67	2.8	2.2	2.58	1.08	1.01	1.72	2.34	1.5	1.81	1.03	0.41
M13	0.34	18.33	0	2.88	2.95	1.11	1.05	2.64	3.42	1.09	1.73	1.37	0.43
M14	0.34	15.67	4.8	2.3	2.6	1.08	1.08	1.39	2.81	1.4	1.69	1.48	0.56
M15	1.33	20	1.2	3.14	3.17	1.08	1.03	1.91	2.12	1.24	1.75	1.26	0.79
M16	0.34	15.33	0	3.76	4.3	1.03	1.09	1.72	2.3	1.4	0.99	1.37	0.55
M18	0.34	18.33	3.19	2.28	2.86	1.1	1.05	1.72	1.52	1.42	1.13	1.42	0.43
M19	0.34	19	6.4	3.08	2.95	1.06	1.04	1.3	3.65	1.01	1.16	1.94	0.62
M20	1	14.67	2.4	2.79	2.99	1.06	1.06	1.31	3.43	1.03	1.03	1.67	0.43
M22	0.67	21.33	0	3.25	2.92	1.11	1.02	2.37	2.4	2.4	1.39	1.78	0.31
M23	0.34	23.6	1.6	3.78	3.59	1.02	1.02	1.88	1.22	0.93	0.47	1.48	0.58
M24	1	16.33	12	3.02	3.19	1.03	1.02	1.79	1.56	0.65	0.7	1.9	0.56
M25	0.67	20	12	4.45	4.46	1.02	1.02	2.85	3.55	0.5	0.38	1.44	0.31
M26	0.67	18.67	1.2	2.53	3.12	1.02	1.01	2.67	2.19	1.05	0.7	1.97	0.36
LSD 5%	0.02	2.19	1.07	0.19	0.77	0.54	0.11	0.15	0.39	0.15	0.88	0.12	0.11

Table 5. Comparison mean of clusters in male and female genotypes.

Cluster	Number of survived seedling	Length of seedling (cm)	Number of leaf	Dry weight of aerial parts(gr)	Dry weight of root (gr)	Na+ of stem (%)	Na+ of root (%)	Ca+2 of stem (%)	Ca+2 of root (%)	Mg+2 of stem (%)	Mg+2 of root (%)	K+ of stem (%)	K+ of root (%)
Female													
1	0.87125	19.64125	6.07875	2.37875	4.79375	2.56875	0.965	1.63	3.21875	2.7175	2.58125	1.3725	0.51125
2	0.376	14.642	1.798	1.608	3.886	2.578	0.81	2.598	3.466	3.116	3.596	1.042	0.42
3	0.495	18.60333	2.443333	2.57	3.815	2.633333	1.05	1.628333	3.21	3.343333	4.095	1.296667	0.41
Male													
1	0.34	23.31	5.33	2.923333	3.09	1.04	1.026667	1.196667	1.35	1.02	0.473333	1.44	0.61
2	1	18.866	6.56	3.35	3.516	1.038	1.022	2.252	2.252	0.806	1.028	1.624	0.508
3	0.45	17.81444	2.31	2.843333	3.02	1.075556	1.048889	1.783333	2.683333	1.353333	1.343333	1.494444	0.453333

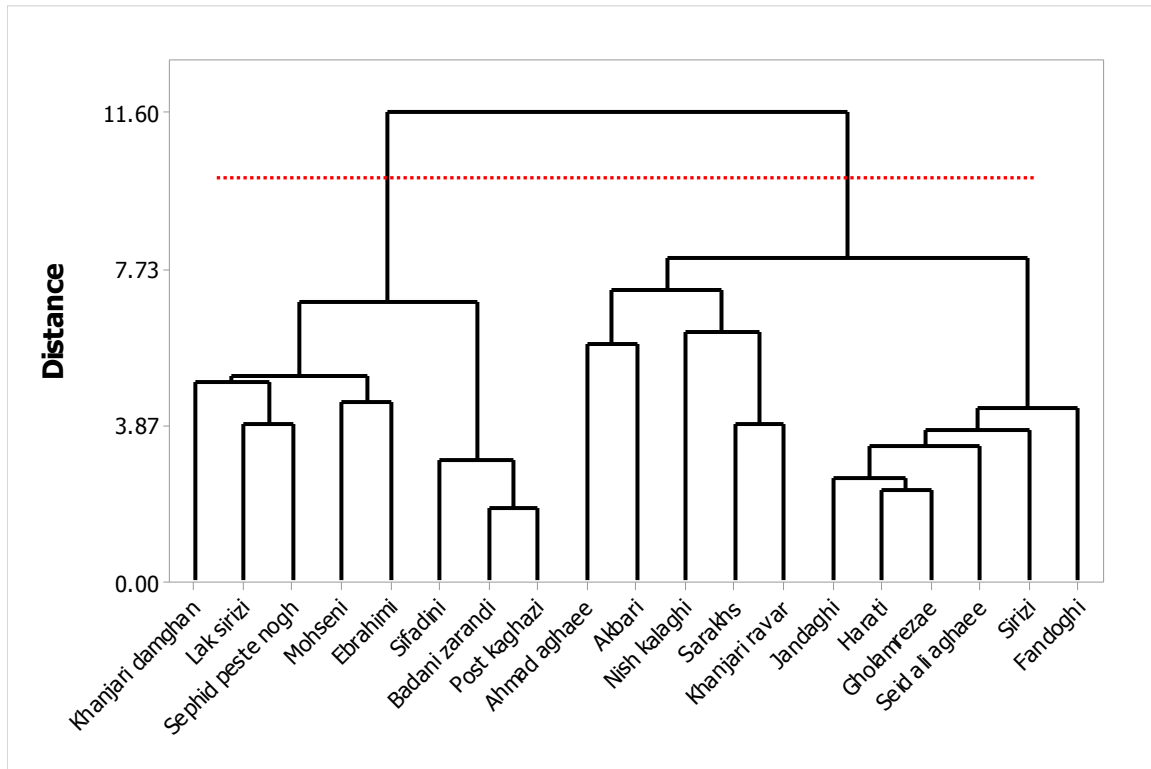


Fig. 1. Cluster dendrogram for female genotypes according to all measured parameters.

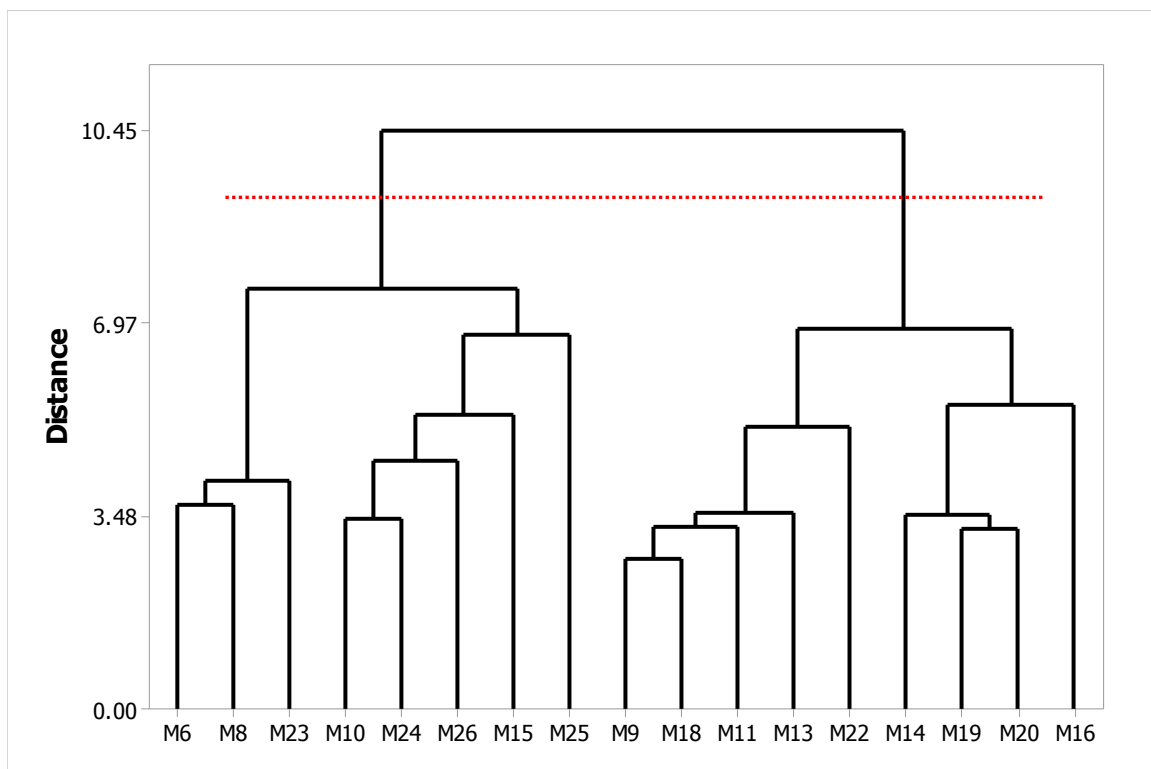


Fig. 2. Cluster dendrogram for male genotypes according to all measured parameters.

Generally, lower accumulation of sodium in plant organs provides higher resistance to saline conditions. Based on this fact, 'Nish Kalaghi' is a resistant genotype among female genotypes. On the other hand, no significant difference for sodium content in male genotypes may indicate a proper selection among generated genotypes regarding salinity tolerance. Pistachio usually does not present early senescence resulting from toxic salt effects and shows low sensitivity to salinity. Despite, the results reveal that the NaCl had a toxic effect on growing (Bybordi 2013; Karimi *et al.*, 2018; Moriana *et al.*, 2018). The underlying reasons of the sensitivity to salinity are not yet fully understood (Mirfattahi *et al.*, 2017). Salts are common and necessary components of soil. More precisely, many of them (e.g., calcium, nitrates, and potassium) are essential plant nutrients (Azarmi *et al.*, 2016; Ganjehie *et al.*, 2018; Hajlaoui *et al.*, 2010; Rahnesan *et al.*, 2018c; Tavallali 2017). Calcium, like nitrogen and potassium, is essential for plant growth; conversely excess, is also detrimental for plant survival. Plants are grouped according to their relative salinity tolerance (low, medium or high) according to their ECe ranges (Negrin *et al.*, 2017; Neocleous *et al.*, 2016; Rebey *et al.*, 2017). But it can be mentioned that, only when a relatively high amount of sodium is involved, the plant growth is adversely affected (Kong *et al.*, 2017; Levitt 1980; Machado *et al.*, 2017; Pedersen *et al.*, 2017).

One of remarkable result of the research shows that, in addition to Na⁺ concentration, another factor which significantly interferes with sensitivity to saline conditions is K⁺ concentration. In female genotypes category, 'Mohseni', 'Akbari', 'Sifadini' and 'Post Kaghazi' show the highest content of potassium in root with no significant difference, while 'Badani Zarand' has the lowest in shoot.

The lowest root's and shoot's potassium can be observed in 'Nish Kalaghi' and 'Akbari', respectively. But for male genotypes, M15 and M26 Showed the highest, while M25 and M11 Show the lowest potassium contents, respectively. It can be seen that, genotypes with higher K⁺ intended to have lower Na⁺

content. During the research, Ca⁺² and Mg⁺² were also measured in pistachio genotypes. The lowest Mg⁺² and Ca⁺² contents were observed in 'Khanjari Ravar' and 'Sirizi' and also in 'Sephid Peste Nogh' and 'Sirizi' in root and shoot sectors, respectively, while the highest content of these element were recorded for 'Nish Kalaghi' and 'Sifadini' in root, and for 'Akbrai' and 'AhmadAghae' in shoot.

And for male genotypes, the lowest Mg⁺² and Ca⁺² contents in root were recorded for M25 and M6, and in shoot were observed in M25 and M8. The highest contents of these elements were visited for M11 and M19 in root and for M22 and M25 in shoot. Saqib *et al.*, (2008) reported that salinity may increase the osmotic potential of the soil matrix and reduce plants' water uptake. Besides, Na⁺ concentrations may interfere with K⁺ uptake in soil, while Na⁺ concentration in leaf can also interfere by leaf Ca²⁺ and K⁺. Therefore, the accumulation of Na⁺ and the reduction of K⁺ and Ca²⁺ uptake and translocation in salt-stressed plants may result in severe metabolic disorder in plants.

Cluster analysis indicates that, genotypes in female cluster 1 and male cluster 2 can be more proper compared to other genotypes. Therefore, applying these two clusters for either assessing in breeding programs or directly cultivating in fields is suggested. Ferguson and Haviland (2016) stated that selecting of suitable rootstocks was one of the first and more essential strategies to reduce salinity damage in pistachio orchards. Hasheminasab *et al.*, (2018) also reported that identification of ideal parents and crossing between them are fundamental for any pistachio breeding programs to produce salt-tolerant rootstocks.

Conclusions

Examining of the number of survived seedlings, sodium contents and other measured parameters along with their correlations show that pistachio seedlings were affected by NaCl levels. The results of correlation analysis indicate that the sodium content of

stem and the survived number of seedlings are negatively correlated and also the accumulation of sodium and magnesium ions in root and stem may cause desiccation of leaves in seedlings and reduction of weight in root and stem. Also, the sodium concentration of the leaves is negatively correlated to aerial biomass. The research also shows that growth parameters of pistachio might be affected in a negative trend and the tolerant genotypes might lead to release tolerant genotypes. Some male and female genotypes showed higher values for morphological parameters under salinity stress, and a relatively lower concentration of Na⁺ and higher K⁺. For sole selection, 'Khanjari Damghan' and 'Mohseni' genotypes in the category of female genotypes, and M10, M15 and M25 in the category of male genotypes are potential tolerant genotype for further assessment in the area and for releasing new cultivar. Cluster analysis divides genotypes in three clusters for each genders, where for female cluster 1 and male cluster 2, the higher morphological parameters and tolerance to salinity can be seen. Cluster result also indicates that these two set of genotypes are potential choices for future breeding programs or directly use in saline filed.

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References

- Aguilera P, Marín C, Oehl F, Godoy R, Borie F, Cornejo P (2017) Selection of aluminum tolerant cereal genotypes strongly influences the arbuscular mycorrhizal fungal communities in an acidic Andosol. *Agriculture, Ecosystems Environment*. 246, 86-93.
- Ahmed JO, Abdulla AR, Mohammed RA (2016) Comparative on yield and its components performance and correlation in some Broad bean (*Vicia faba* L.) genotypes at Bakrajo, Sulaimani. *American-Eurasian Journal of Agriculture and Environmental Science*. 16, 635-640.
- Akbarimoghaddam H, Galavi M, Ghanbari A, Panjehkeh N (2011) Salinity effects on seed germination and seedling growth of bread wheat cultivars. *Trakia Journal of Sciences*. 9(1), 43-50.
- Alipour H (2018) Photosynthesis properties and ion homeostasis of different pistachio cultivar seedlings in response to salinity stress. *International Journal of Horticultural Science and Technology*. 5(1), 19-29.
- Atak M, Kaya MD, Kaya G, Çikili Y (2006) Effects of NaCl on the germination, seedling growth and water uptake of triticale. *Turkish Journal of Agriculture and Forestry*. 30(1), 39-47.
- Ayers RS, Westcot DW (1985) *Water quality for agriculture*, vol 29. Food and Agriculture Organization of the United Nations Rome.
- Azarmi F, Mozaffari V, Hamidpour M, Abbaszadeh-Dahaji P (2016) Interactive effect of fluorescent Pseudomonads rhizobacteria and Zn on the growth, chemical composition and water relations of Pistachio (*Pistacia vera* L.) seedlings under NaCl stress. *Communications in Soil Science Plant Analysis*. 47(8), 955-972.
- Bagheri M, Gholami M, Baninasab B (2019) Hydrogen peroxide-induced salt tolerance in relation to antioxidant systems in pistachio seedlings. *Scientia Horticulturae*. 243, 207-213.
- Behboudian M, Walker R, Törökfalvy E (1986) Effects of water stress and salinity on photosynthesis of pistachio. *Scientia Horticulturae*. 29(3), 251-261.
- Bybordi A (2013) Tolerance evaluation of late flowering almond cultivars to salinity. *Journal of Crop Production and Processing*. 3(9), 217-226.

- Ferguson L, Haviland DR (2016) Pistachio Production Manual. University of California Agriculture and Natural Resource Publication. pp. 334.
- Flowers T (2004) Improving crop salt tolerance. *Journal of Experimental botany*. 55(396), 307-319.
- Francois LE, Grieve CM, Maas EV, Lesch SM (1994) Time of salt stress affects growth and yield components of irrigated wheat. *Agronomy Journal*. 86, 100-107.
- Frota J, Tucker T (1978) Absorption Rates of Ammonium and Nitrate by Red Kidney Beans under Salt and Water Stress 1. *Soil Science Society of America Journal*. 42(5), 753-756.
- Ganjehie MG, Karimi A, Zeinadini A, Khorassani R (2018) Relationship of Soil Properties with Yield and Morphological Parameters of Pistachio in Geomorphic Surfaces of Bajestan Playa, Northeastern Iran. *Journal of Agricultural Science Technology*. 20(2), 417-232.
- Habibi G, Norouzi F, Hajiboland R (2014) Silicon alleviates salt stress in pistachio plants. *Progress in Biological Sciences*. 4(2), 189-202.
- Hajlaoui H, El Ayeb N, Garrec JP, Denden M (2010) Differential effects of salt stress on osmotic adjustment and solutes allocation on the basis of root and leaf tissue senescence of two silage maize (*Zea mays* L.) varieties. *Industrial Crops and Products*. 31(1), 122-130.
- Hasheminasab H, Esmailpour A, Tajabadipour A, Javanshah A (2018) Introduction of Pistachio (*Pistacia vera* L.) Breeding Programs for Salinity Tolerance in Iran: Capacities, Challenges and Look-Ahead Strategies. National Conference on Haloculture. National Salinity Research Center. Yazd, Iran. 15 pp.
- Iqbal M, Ul-Allah S, Naeem M, Ijaz M, Sattar A, Sher A (2017) Response of cotton genotypes to water and heat stress: from field to genes. *Euphytica*. 213(6), 131.
- Johnson C, Ulrich A (1959) Analytical methods for use in plant analysis. Californian Agricultural Experimental Station. California Agricultural Experiment Station.
- Karim A, Yuan L, Kari M (2005) Effect of NaCl Stress on Na⁺ and K⁺ Uptakes by Different Organs of Pistachio. *Acta Botanica Boreali-occidentalia Sinica* 25(9), 1805-1810.
- Karimi S, Tavallali V, Wirthensohn M (2018) Boron amendment improves water relations and performance of *Pistacia vera* under salt stress. *Scientia Horticulturae*. 241, 252-259.
- Katerji N, Van Hoorn J, Hamdy A, Mastrorilli M (2000) Salt tolerance classification of crops according to soil salinity and to water stress day index. *Agricultural water management*. 43(1), 99-109.
- Kerketta A, Bahadur V, Rajesh J (2018) Performance of different tomato genotypes (*Solanum lycopersicum* L.) for growth, yield and quality traits under Allahabad condition. *Journal of Pharmacognosy Phytochemistry*. 7(6), 1766-1769.
- Khoyerd FF, Shamshiri MH, Estaji A (2016) Changes in some physiological and osmotic parameters of several pistachio genotypes under drought stress. *Scientia Horticulturae*. 198, 44-51.
- Kong X, Luo Z, Dong H, Li W, Chen Y (2017) Non-uniform salinity in the root zone alleviates salt damage by increasing sodium, water and nutrient transport genes expression in cotton. *Scientific Reports*. 7(1), 2879.
- Levitt J (1980) Responses of Plants to Environmental Stress, Volume 1: Chilling, Freezing and High Temperature Stresses. Academic Press, INC, New York, USA, pp 23-64
- Livingston DP, Tuong TD, Isleib TG, Murphy JP (2016) Differences between wheat genotypes in damage from freezing temperatures during

- reproductive growth. European journal of agronomy. 74, 164-172.
- Lotfi N, Vahdati K, Kholdebarin B, Hassani D and Amiri R (2009) Mineral composition of some walnut cultivars (*Juglans regia* L.) for evaluation of ionome and ionomics under salt stress condition. Acta Horticulturae. 839:293-300
- Machado R, Serralheiro R (2017) Soil salinity: effect on vegetable crop growth. Management practices to prevent and mitigate soil salinization. Horticulturae. 3(2), 30.
- Mehdi-Tounsi H, Chelli-Chaabouni A, Mahjoub-Boujnah D, Boukhris M (2017) Long-term field response of pistachio to irrigation water salinity. Agricultural Water Management. 185, 1-12.
- Minhas P (1996) Saline water management for irrigation in India. Agricultural Water Management. 30, 1-24.
- Mirfattahi Z, Karimi S, Roozban MR (2017) Salinity induced changes in water relations, oxidative damage and morpho-physiological adaptations of pistachio genotypes in soilless culture. Acta Agriculturae Slovenica. 109(2), 291-302.
- Moriana A, Memmi H, Centeno A, Martín-Palomo MJ, Corell M, Torrecillas A, Pérez-López D (2018) Influence of rootstock on pistachio (*Pistacia vera* L. cv Kerman) water relations. Agricultural Water Management. 202, 263-270.
- Motagh M, Walter TR, Sharifi MA, Fielding E, Schenk A, Anderssohn J, Zschau J (2008) Land subsidence in Iran caused by widespread water reservoir overexploitation. Geophysical Research Letters. 35(16), 324-333.
- Negrin VL, Teixeira B, Godinho RM, Mendes R, Vale C (2017) Phytochelatins and monothiolis in salt marsh plants and their relation with metal tolerance. Marine pollution bulletin. 121(1-2), 78-84.
- Neocleous D, Savvas D (2016) NaCl accumulation and macronutrient uptake by a melon crop in a closed hydroponic system in relation to water uptake. Agricultural Water Management. 165, 22-32.
- Parsa A, Karemian N (1975) Effect of sodium chloride on seedling growth of two major varieties of Iranian pistachio (*Pistachia vera* L.). Journal of Horticultural Science. 50(1), 41-46 .
- Parsa A, Wallace A (1980) Differential partitioning of boron and calcium in shoots of seedlings of two pistachio cultivars. Journal of Plant Nutrition. 2(1-2), 263-266.
- Pedersen JT, Palmgren M (2017) Why do plants lack sodium pumps and would they benefit from having one? Functional Plant Biology. 44(5), 473-479.
- Pessarakli M, Szabolcs I (1999) Soil salinity and sodicity as particular plant/crop stress factors, vol 2. Marcel Decker Inc, New York, USA, 1254 pp.
- Pessarakli M, Tucker T (1985) Uptake of Nitrogen-15 by Cotton under Salt Stress 1. Soil Science Society of America Journal. 49(1), 149-152.
- Pessarakli M, Tucker TC (1988) Dry matter yield and nitrogen-15 uptake by tomatoes under sodium chloride stress. Soil Science Society of America Journal. 52(3), 698-700.
- Picchioni G, Miyamoto S, Storey J (1990) Salt effects on growth and ion uptake of pistachio rootstock seedlings. Journal of the American Society for Horticultural Science. 115(4), 647-653.
- Picchioni GA, Miyamoto S, Storey JB (1991) Boron uptake and effects on growth and carbohydrate partitioning of pistachio seedlings. Journal of the American Society for Horticultural Science. 116(4), 706-711.
- Rahneshan Z, Nasibi F, Lakehal A, Bellini C (2018a) Unravelling salt stress responses in two pistachio (*Pistacia vera* L.) genotypes. Acta physiologiae plantarum. 40(9), 172.

- Rahneshan Z, Nasibi F, Lakehal A, Bellini CJApp (2018b) Unravelling salt stress responses in two pistachio (*Pistacia vera* L.) genotypes. 40(9), 172.
- Rahneshan Z, Nasibi F, Moghadam AA (2018c) Effects of salinity stress on some growth, physiological, biochemical parameters and nutrients in two pistachio (*Pistacia vera* L.) rootstocks. Journal of Plant Interactions. 13(1), 73-82.
- Rebey IB, Bourgou S, Rahali FZ, Msaada K, Ksouri R, Marzouk B (2017) Relation between salt tolerance and biochemical changes in cumin (*Cuminum cyminum* L.) seeds. Journal of Food Drug Analysis. 25(2), 391-402.
- Rhoades J, Kandiah A, Mashali A (1992) The use of saline water for crop production-Irrigation and drainage paper 48. Food and Agriculture Organization of the United Nations. 48, 1-33.
- Saqib M, Zörb C, Schubert S (2008) Silicon-mediated improvement in the salt resistance of wheat (*Triticum aestivum*) results from increased sodium exclusion and resistance to oxidative stress. Functional Plant Biology. 35, 633-639.
- Sedaghat R (2011) Constraints in Production and Marketing of Iran's Pistachio and the Policies Concerned: An Application of the Garret Ranking Technique. International Journal of Nuts and Related Sciences. 2(1), 27-30.
- Sepaskhah A, Maftoun M (1988) Relative salt tolerance of pistachio cultivars. Journal of Horticultural Science. 63, 157-162.
- Shalhevet J (1994) Using water of marginal quality for crop production: major issues. Agricultural water management. 25(3), 233-269.
- Sharifkhan 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), 51-8.
- Tavallali V (2017) Interactive effects of zinc and boron on growth, photosynthesis and water relations in pistachio. Journal of Plant Nutrition. 40(11), 1588-1603.
- Vahdati K, Lotfi N (2013) Abiotic stress tolerance in plants with an emphasis on drought and salinity stresses in walnut. In: Vahdati K, Leslie C (eds.) Abiotic stress—plant responses and applications in agriculture. InTech, Rijeka, pp 307–365.
- Yadollahi A, Lotfi N, Vahdati K, Hassani D, Kholdebarin B and Amiri R (2010) Morphological and physiological responses to water stress for seedlings of different walnut genotypes. Acta Horticulturae 861:253-262.