Journal of Nuts

Journal homepage: ijnrs.damghaniau.ac.ir

ORIGINAL ARTICLE

Evaluation of the Tolerance of Four Pistachio Rootstocks to Salinity Stress Based on Morphological, Physiological and Biochemical Parameters

Samanah Hakimnejad[1], Hamid Reza Karimi[*1], Seyyed Rasoul Sahhafi[2], Majid Esmaeilizadeh[1]

[1]*Department of Horticultural Science, Faculty of Agriculture, Vali-e-Asr University of Rafsanjan, Rafsanjan, Iran*

[2]*Department of Genetics and Crop Production, Faculty of Agriculture, Vali-e-Asr University of Rafsanjan, Rafsanjan, Iran*

A R T I C L E I N F O

Keywords:
Akbari;
Badami-e- Riz-e-Zarand;
Fv/Fm;
Pistachio rootstocks;
RWC;
Specific leaf weight

A B S T R A C T

In order to evaluation of the tolerance of four pistachio rootstocks to salinity stress an experiment was performed as factorial in framework completely randomized design with four replications. Factors included rootstocks (‘Badami-e- Riz-e-Zarand’, ‘Ghazvini’, ‘Akbari’ and UCB-1) in four levels and salinity (0, 75 and 150 mM) in three levels. Results showed that rootstocks had different responses to salinity. The highest specific leaf weight and chlorophyll fluorescence parameter (*Fv/Fm*) were observed in ‘Badami-e- Riz-e-Zarand’ and ‘Ghazvini’ rootstocks. Also, potassium and calcium concentration of shoot in ‘Badami-e- Riz-e-Zarand’, ‘Ghazvini’ and, ‘Akbari’ rootstocks were higher compared to UCB-1. The UCB-1 rootstock had higher leaf area, relative water content (RWC) and chlorophyll-a content than the other rootstocks. The results of cluster analysis showed that salinity changed the position of the ‘Akbari’ and UCB-1 rootstocks in cluster whereas the position of ‘Badami-e- Riz-e-Zarand’ and ‘Ghazvini’ rootstocks unchanged in cluster.

Introduction

The use of low-quality water for irrigation increases soil salinity, followed by a decrease in yield (Silva *et al*., 2008). Salinity damages the plant by reducing the water potential, causing ion toxicity and the next changes in physiological processes (Naeini *et al*., 2006). Understanding the physiological mechanisms that make plants adapt to conditions of salt stress can be effective in selecting genotypes that are tolerant to salinity (Zaharieva *et al*., 2001). Previous studies have indicated mechanisms such as the accumulation of toxic ions inside the vacuole, the accumulation of osmotic balancing ions within the cytoplasm, the decrease in the absorption of sodium and chloride by roots and non-transference of sodium or chloride to the shoot in resistant genotypes (Garcia-Sanchez and Syvertsen, 2006). Several studies have shown that tolerance to salinity in fruit trees is affected by rootstock (Ferguson *et al*., 2002; Matsumoto *et al*., 2006; Tavallali *et al*., 2008; Adish *et al*., 2010; Karimi and Hassanpour, 2017). There have been reports on cultivars and salt-tolerant rootstocks such as pears, olives, pomegranates, mangoes and avocados (Mickelbart and Arpaia, 2002; Naeini *et al*., 2006; Tabatabaei, 2006; Karimi and Hassanpour, 2017). The effects of salinity and drought stress on the growth and concentration of mineral leaves of pomegranate have been studied and reported that tolerance cultivars have a mechanism to limit the

[*]**Corresponding author**: Email address: hrkarimi2017@gmail.com
Received: 18 September 2021; Received in revised form: 24 February 2022; Accepted: 2 April 2022
DOI: 10.22034/jon.2022.1940695.1127

absorption and transfer of sodium and chloride ions to the shoot (Karimi and Hassanpour, 2014). In different studies, the effects of salinity stress on growth and mineral concentration of pistachio seedlings studied. (Kamiab *et al*., 2012; Karimi *et al*., 2012; Karimi and Maleki Kuhbanani, 2015). Karimi and Nasrolahpour-Moghadam (2016a) studied the effect of salinity stress on the growth of male and female pistachio seedlings and they reported that, under salinity stress, the concentrations of sodium and calcium ions in roots and the concentration of carotenoid in the leaves of male seedlings were higher than those in female seedlings. In other study, Karimi and Nasrolahpour-Moghadam (2016 b) reported that in 60 mM salinity, the male seedlings are more resistant than female seedlings, while in 120 mM salinity, there were no significant difference between male and female seedlings. In a study, Karimi *et al*., (2011) studied the effects of irrigation salinity on the growth and physiological parameters of three pistachio rootstock; 'Badami-e- Riz-e- Zarand', 'Ghazvini' and 'Sarakhs' and reported that all studied rootstocks prevent the transfer of sodium to shoots however Sarakhs' rootstock had less ability in this case. In Iran, seedlings of *Pistacia vera* L. cv. 'Badami-e- Riz Zarand', 'Ghazvini', and 'Sarakhs' are used as rootstock for pistachios (Karimi *et al*., 2011). Although these rootstocks are resistant to salinity and drought, being well adapted to Iran's environmental conditions, in the long run, they have a high degree of instability in vegetative growth, as a result of sexual propagation, and take several years before being ready for grafting. Recently, the UCB-1 rootstock has been introduced in the Iranian pistachio industry. This rootstock can be propagated through tissue culture. It grows uniformly and reaches the age of grafting at a relatively early age (Ferguson *et al*., 2002; Heydari *et al*., 2021). In a study, Sanden *et al*. (2004), studied effects of salinity on 'Kerman' cultivar which grafted on *P. atlantica*, *P. integerrima*, Pioneer Gold 1 (PG1), Pioneer Gold 2 (PG2), and UCB1 (*P. atlantica x P. integerrima*) rootstocks and reported that average yield for all rootstocks at the 12 dS/m level was 81% of the control yield except UCB1 which was 65%. They also reported that the nut yield on the PG1 rootstock was unaffected salinity. There are limited reports on the evaluation of UCB-1 rootstock to salinity and or comparison it with Iranian rootstocks. The objectives of this work were the evaluation of the effect of salt stress on four pistachio rootstocks ('Ghazvini' 'Akbari' 'Badami-e-Riz-e- Zarand' and UCB-1) through different morphological, biochemical and physiological parameters.

Materials and Methods

Plant material and growth conditions

Seedlings produced of four rootstocks including *P. vera* L. cv. 'Badami-e- Riz-e-Zarand', *P. vera* L. cv. 'Ghazvini', *P. vera* L. cv. 'Akbari', and interspecific hybrid of *P. atlantica* and *P. integerrima* (UCB-1) were used in this study. Seeds of 'Badami-e- Riz-e-Zarand', 'Ghazvini' and 'Akbari' were collected from disinfected with 5% sodium hypochlorite for 15 minutes before being cultured. After germination, they were cultured in 5 litter pots with 20 cm high and 18 cm in diameter containing cocopeat and perlite (1: 1). One month after sowing the seeds of 'Badami-e- Riz-e-Zarand', 'Ghazvini' and 'Akbari', one-month-old UCB-1 tissue culture plants were cultured in pots with the same culture medium. The pots were irrigated with Hoagland solution until the start of salt treatment. Twelve months after the culturing, salt stress was applied for 45 days with irrigation water containing sodium chloride, calcium chloride and magnesium chloride salts (5: 1: 1) at 0, 75 and 150 mM concentrations (Moeinrad, 2008). To prevent the shock and accumulation of salt in the culture medium, salinity stress was gradually applied. Pots were watered based on field capacity with 30% leaching every five days so that 800 mL of Hogland solution was used for each pot. During the period of salt stress treatment, the maximum temperature in the greenhouse was 35°C, and the minimum temperature

was 23°C. The relative humidity was 60%. At the end of the experiment, the leaves were sampled to measure biochemical parameters. For this purpose, the leaves, stems and roots were harvested separately, and used for the next stages of the experiment.

Vegetative traits

Measurements included seedling height, fresh and dry shoot weight, fresh and dry root weight and, leaf number. To measure the fresh weight, the plants were first removed from the soil and then divided into two parts of the shoot and root and then was weighed using a scale. The dry weight was measured after 48 hours of drying at 70°C in the oven.

Leaf area, Specific leaf weight and specific leaf area

The leaf area was measured using leaf area meter model C1-202, USA. Specific leaf area (SLA) and specific leaf weight (SLW) were calculated using the following formula (Cowling and Campbell 1983).

Specific leaf area (SLA) = leaf area / leaf dry weight

Specific leaf weight (SLW) = leaf dry weight / leaf area

Relative water content of leaf (RWC)

To measure the leaf relative water content, ten discs with 1cm diameter were taken from the mature leaf blade of the third node with a punching machine and, then they fresh weight was calculated (FW). After, they were placed in a Petri dish containing 10 ml distilled water for six hours at the temperature of 4ºC in the darkness to make the leaf cells completely turgor. Then, they were placed on filter paper to decrease some of their moisture. After, the turgor weight was calculated (TW) and, the samples were dried in the oven with 70ºC, and their dry weight was calculated (DW). RWC was calculated with the following formula (Bastam *et al*., 2012).

$$RWC = [(FW - DW) / (TW - DW)] \times 100$$

SPAD and pigments measurement

Measurement of the single photon avalanche diode (SPAD) index was done 40 days after the application of salinity stress. The SPAD index was measured using a manual chlorophyll meter (SPAD-502) in fully developed leaflets at fifth and sixth nodes.

To measure the amount of chlorophyll and carotenoid, about 0.25 g of fresh and mature leaves were blended with 5 ml of 80% acetone in porcelain crucibles to form a uniform mixture. The samples were then transferred to centrifuge tubes and spun for ten minutes at a rate of 3500 rpm. Then, the absorbance of dissolved light was measured by a spectrophotometer at wavelengths of 646.6 and 663.6 nm. Finally, the chlorophyll concentration was calculated using the following method (Poora, 2002).

Total chlorophyll (mg/g fresh weight) = $[(17.76\times OD_{646.6}) + (7.37\times OD_{663.6})] \times [V/W]$

Chlorophyll a = $[(12.25\times OD_{663.6}) - (2.55\times OD_{646.6})] \times [V/W]$

Chlorophyll b = $[20.31\times OD_{646.6}) - (4.91\times OD_{663.6})] \times [V/W]$

V: the amount of acetone consumed

W: sample fresh weight (g)

According to the Lichten Haller (1987) method, the following formula was used for calculating carotenoids. Accordingly, the absorbance was measured at 470 nm.

$$(1000OD_{470} - 3.27[chla] - 104[chlb]) \times [5.227] \times (0.25)$$

Chlorophyll fluorescence

The chlorophyll fluorescence index was measured 40 days after the onset of stress using a chlorophyll fluorescence machine (Hansatech Ltd Packet PEA, manufactured by the UK) on a sunny day between the hours 9:30 and 14:00 (Genty *et al*., 1989). From each pot, eight adult leaves were selected from the mid-section of the seedlings, and after being placed for 30 minutes in the special clips to create dark conditions, the chlorophyll fluorescence index was recorded.

Proline content

To extract proline, 0.5g of leaf was bubbled in 5 ml of 95% ethanol in Chinese mousse.The extraction was repeated twice and every time with 5 ml of ethanol 70%. The resulting mixture was centrifuged for 10 minutes. After separating the liquid phase from the solid, the liquid portion was used for extracting proline. To determine the concentration of proline, a dilution (1 ml) of this alcoholic extract was mixed with 10 ml of distilled water and 5 ml of Nine Hydrone. This involved mixing 25.1g of Ninhydrin in 30 ml of glycine acid and 20 ml of phosphoric acid of 6 M. Then, 5 ml of glacial acetic acid was added to it and stirred for several seconds. The solution was placed in a hot bath at 90°C for 45 minutes. After removing the samples from a bath of water and cooling, 6 ml of benzene was added and the solution was stylized for 15-20 minutes to allow proline to enter the benzene phase. Finally, the high soluble phase was isolated and the absorbance of light was measured at 515 nm using aspectrophotometer (PG Instruments1 Ltd T80 UV / VIS). Proline standards were also prepared using L-proline at concentrations of 0, 25, 31, 62.5, 125, 250 and 500 (mg g^{-1} fresh weight) (Paquin and Lechasseur, 1979).

Soluble carbohydrates content

In order to determine the soluble carbohydrates content, 0.1 ml of the ethanol extract which had resulted from the proline measurement stage, was mixed with 3 ml of freshly prepared anthrone (150 mg of anthrone plus 100 ml of 72% sulfuric acid). The solution was placed in a water bath for 10 minutes to allow the reaction to proceed. It was then colored and the absorbance was measured by a spectrophotometer (PG Instruments1 Ltd T80 UV / VIS) at a wavelength of 625 nm. To prepare the sugar standard, pure glucose was used at concentrations of 0, 250, 500, 750, 1000, 1250, 2000, 2250, and 2500 (mg L^{-1}) (Irigoyen *et al*., 1992).

Phenolic compounds content

The measurement of phenolic compounds was carried out using Singleton *et al*. (1999) method. Samples were homogenized with a mixture of methanol/water (70:30) on an ice bath. Then, 0.25 ml of the extract was mixed with 0.25 ml of a Folin-Ciocalteu solution and 2 ml of distilled water. After 3 minutes, 0.25 ml of saturated sodium carbonate solution (Na_2CO_3) was added at room temperature. Then, a water bath of 37°C was used for maintaining the resulting mixture for 30 minutes. The absorption rate was measured at 750 nm wavelength using a spectrophotometer. Gallic acid was used as the reference standard and the results were calculated by measuring its weight (in mg) per gram of fresh weight.

Elements analysis of shoot and root

In this study potassium, calcium, magnesium, sodium and chloride were measured separately in the roots and shoots. Samples of shoot and root were ashed in a muffle oven at 550 ± 25° C. The resulting white ash was then dissolved in 5 ml of 2N HCl- and adjusted to volume of 50 ml for determination of Na, K and Mg concentration. Potassium and sodium were measured by a Flame Photometer (Model PFP7, JENVY, England) (Kalra and Maynard, 1991). Calcium, magnesium and chloride were measured by the titration method (Estefan *et al*., 2013).

Data analysis

This study was conducted as factorial experiment in framework completely randomized design (CRD) with two factors including, rootstocks at four levels (*P. vera* L. cv. 'Badami-e- Riz-e-Zarand', *P. vera* L. cv. 'Ghazvini', *P. vera* L. cv. 'Akbari', and interspecific hybrid of *P. atlantica* and *P. integerrima* (UCB-1)) and water irrigation salinity in three levels (i.e. control, 75 and 150 mM) with three replications. The analysis of variance (ANOVA) was done using SAS software version 9.4 and drawing charts by Excel

software. Then, the mean values of the measurements were analyzed according to Duncan's multiple range tests at 0.05% level. In addition, the rootstocks were compared in terms of salt resistance by cluster analysis using ward's method in the 75 and 150 mM salinity levels using SPSS software, version 22.

Results

Height of seedling and number of leaves

According to analysis variance, it was identified that plant height was affected by the rootstock and the interaction of rootstock and salinity was not had significantly effects on the plant height (Table 1). The highest of plant height was observed in 'Ghazvini' rootstock and the lowest it was obtained with UCB-1 rootstock; however there was no significant difference between the 'Ghazvini' and 'Badami-e- Riz-e-Zarand' rootstocks. Also, the results of variance analysis showed that the leaf number was affected by rootstock and salinity. The highest and lowest number of leaves was obtained with 'Akbari' and UCB-1 rootstocks respectively, although there was no significant difference between the 'Akbari' and Qazvini and 'Badami-e- Riz-e-Zarand' rootstocks (Table 2). Salinity decreased number of leaves of plants so that the lowest number of leaves was obtained in 75 and 150 mM salinity levels (Table 2).

Table 1. Analysis variance for growth, echo physiological and biochemical parameters of four pistachio rootstocks.

Treatment	df	Plant height	Leaf number	Leaf fresh weight	Leaf dry weight	Stem fresh weight	Stem dry weight
Rootstock	3	142.918 **	46.597 **	0.637 **	0.113 **	1.445 **	0.610 **
Salinity	2	0.822 ns	11.147 **	0.070 *	0.007 ns	0.011 ns	0.012 ns
Rootstock*Salinity	6	3.078 ns	1.651 ns	0.088 **	0.009 ns	0.218 *	0.050 ns
Error	27	6.747	1.927	0.018	0.004	0.072	0.021
CV (%)		22.73	25.90	22.96	21.19	18.29	18.17
Treatment	**df**	**Plant fresh weight**	**Plant dry weight**	**Root fresh weight**	**Root dry weight**	**LA**	**SLA**
Rootstock	3	8.535 **	2.790 **	1.631 **	0.364 **	26.675 **	4629.38 **
Salinity	2	0.409 ns	0.162 ns	0.565 *	0.088 ns	0.063 ns	116.448 **
Rootstock*Salinity	6	0.595 ns	0.176 ns	0.252 ns	0.104 *	1.443 ns	28.102 ns
Error	27	0.412	0.106	0.117	0.029	3.022	19.309
CV (%)		17.68	16.81	23.40	20.65	25.54	21.08
Treatment	**df**	**SLW**	**RWC**	**SPAD**	**Chll. a**	**Chll. b**	**Total Chl**
Rootstock	3	0.019 **	916.933 **	566.681 **	0.195 **	0.020 ns	0.076 ns
Salinity	2	0.000 ns	872.300 **	40.293 ns	0.290 **	0.012 ns	0.293 *
Rootstock*Salinity	6	0.002 *	405.857 *	62.425 ns	0.076 *	0.018 ns	0.074 ns
Error	27	0.000	117.853	52.648	0.024	0.013	0.061
CV (%)		30.14	21.76	12.34	27.75	7.14	11.33
Treatment	**df**	**Cartenoides**	**Fv/Fm**	**Pi**	**Soluble carbohydrate**	**Phenol**	**Proline**
Rootstock	3	36762.6 ns	0.145 **	37.839 **	2.673 **	0.000 ns	0.031 **
Salinity	2	114980 ns	0.068 *	49.786 **	1.160 *	0.000 ns	0.081 **
Rootstock*Salinity	6	64546.1 ns	0.037 *	13.431 *	1.361 **	0.000 ns	0.017 **
Error	27	50351.9	0.014	0.750	0.288	0.002	0.003
CV (%)		7.65	22.17	25.89	23.92	7.710	23.17

** and* significant at 1 and 5% , respectively; ns: no significant.

Fresh weight of leaf, stem, root and plant

According to the analysis of variance, leaf fresh weight and stem fresh weight were significantly affected by interaction of rootstock and salinity ($P < 0.05$) (Table 1). The results showed that salinity decreased leaf fresh weight in 'Badami-e-Riz-e-Zarand' and 'Ghazvini' rootstocks, whereas it was not affected on 'Akbari' and UCB-1 rootstocks. In 150 mM salinity, the highest and lowest leaf fresh weight was observed in 'Akbari' and UCB-1 rootstocks, respectively. The results of means comparison of rootstock and salinity interaction showed that in UCB-1 rootstock, stem fresh weight was increased with increasing salinity whereas in other rootstocks was not affected by salinity. According to the results of analysis of variance, root fresh weight was affected by the rootstock and the salinity. Root fresh weight increased with increasing salinity so that the highest root fresh weight was observed in salinity of 150 mM. Also, there was a significant difference between rootstocks in terms of root fresh weight; so that the highest root fresh weight was in the 'Badami-e- Riz-e-Zarand' and the lowest in UCB-1. Also, the results of variance analysis showed that plant fresh weight was affected by rootstock (Table 1). Rootstocks had a significant difference in terms of plant fresh weight, so that the highest plant fresh weight was observed in the 'Badami-e-Riz-e-Zarand' (4.471 g) and the lowest in UCB-1 (2/271 g) however there was no significant difference between the 'Badami-e- Riz-e-Zarand' and 'Ghazvini' rootstocks (Table 2).

Table 2. Mean comparison of growth, physiological and biochemical parameters of four pistachio rootstocks.

Treatment	Plant height (cm)	Leaf number	Plant fresh weight (g)	Leaf area (cm^2)	Root fresh weight (g)	Stem dry weight (g)
Salinity (mM)						
0	11.542 a	6.354 a	3.696 a	6.821 a	1.254 b	0.783 a
75	11.188 a	4.500 b	3.392 a	6.880 a	1.406 ab	0.765 a
150	11.517 a	5.250 ab	3.775 a	6.732 a	1.682 a	0.843 a
Rootstocks						
Badami	14.100 a	6.450 a	4.471 a	6.307 b	1.934 a	1.008 a
Ghazvini	14.325 a	5.800 a	4.074 ab	6.153 b	1.610 b	0.932 a
Akbari	10.825 b	6.875 a	3.578 b	5.623 b	1.343 b	0.792 b
UCB1	5.889 c	1.972 b	2.271 c	9.396 a	0.919 c	0.433 c
Treatment	**Leaf dry weight (g)**	**Plant dry weight (g)**	**SLA**	**SPAD**	**Chll b (mgg^{-1} fw)**	**Total Chl (mgg^{-1} fw)**
Salinity (mM)						
0	0.323 a	1.885 a	18.086 b	56.575 a	1.657 a	2.334 a
75	0.282 a	1.815 a	23.752 a	60.217 a	1.608 a	2.028 b
150	0.337 a	2.078 a	20.725 ab	59.450 a	1.674 a	2.223 a
Rootstocks						
Badami	0.346 a	2.325 a	10.581 b	62.790 ab	1.668 a	2.172 a
Ghazvini	0.397 a	2.205 a	9.361 b	62.930 a	1.702 a	2.159 a
Akbari	0.356 a	2.031 a	11.630 b	57.090 b	1.602 a	2.137 a
UCB1	0.148 b	1.107 b	55.246 a	48.300 c	1.620 a	2.334 a

Means with a common letter in each column are not significantly different (Duncan test, *P = 0.05*).

Dry weight of leaf, stem, root and plant

The results of the variance analysis showed that leaf, stem and plant dry weight were only affected by rootstock (Table 1). The highest and lowest of the leaf dry weight, were observed in the 'Ghazvini' and UCB-1 respectively, although there was, no significant difference between 'Ghazvini' with 'Badami-e- Riz-e-Zarand' and 'Akbari'. Also, results showed that 'Badami-e- Riz-e-Zarand' and 'Ghazvini' rootstocks had the highest stem dry weight. The lowest stem dry weight was observed in UCB-1 rootstock. Root dry weight was affected by interaction of rootstock and salinity ($P < 0.05$ Duncan's test) (Table 1). Results of means comparison showed that in 'Badami-e- Riz-e-Zarand' rootstock, root dry weight increased at 150 mM salinity compared to control whereas in other rootstocks it was no observed significant difference between 150 mM salinity and control (Table 3). At 150 mM salinity, the highest dry weight of root was observed in the 'Badami-e- Riz-e-Zarand' rootstock (1.261 g) and the lowest in UCB-1 rootstock (0.627 g) (Table 3).

Leaf area and SPAD index

The results of the variance analysis showed that leaf area and SPAD index were only affected by the rootstock (Table 1). Results of means comparison showed that the highest leaf area was observed in UCB-1 rootstock however there was no significant difference between the other rootstocks in terms of leaf area. The results of means comparison showed that the lowest of SPAD index was observed in the UCB-1 and the highest was observed in 'Ghazvini', however there was no significant difference between 'Ghazvini' and 'Badami-e-Riz-e-Zarand' rootstocks (Table 2).

Specific leaf weight and specific leaf area

The results of the variance analysis showed that the specific leaf weight was affected by the rootstock ($p<0.01$) and interaction of rootstock and salinity ($p<0.05$) (Table 1). The results of interaction of rootstock and salinity showed that in 'Akbari' rootstocks, specific leaf weight increased with increasing salinity whereas in other rootstocks was not affected by salinity. In 150 mM salinity, 'Akbari' and UCB-1 had the highest and lowest specific leaf weight respectively (Table 2). The results also showed that the specific leaf area was affected by rootstock and salinity ($p<0.01$) (Table 1). Results of means comparison showed that the highest specific leaf area was observed in the UCB-1, whereas there was no significant difference between other rootstocks. Salinity increased specific leaf area compared to control, however there was no significant difference with 75 mM and 150 mM salinity (Table 2).

Relative water content

The results of the variance analysis showed that RWC was affected rootstock, salinity and interaction of rootstock and salinity ($p<0.05$) (Table 1). The results of rootstock and salinity interaction showed that salinity decreased RWC in UCB_1 rootstock compared to the control, however in 150 mM salinity; there was no significant difference between rootstocks (Table 3).

Chlorophyll and Carotenoid

The results of the variance analysis showed that chlorophyll a was affected by the rootstock, salinity and interaction of rootstock and salinity (Table 1). Results showed that salinity decreased chlorophyll a in UCB-1 rootstock compared to control whereas in other rootstocks there was no significant difference between 150 mM salinity and control. The results of the variance analysis showed that the total chlorophyll content was affected by salinity, so that the total chlorophyll content decreased at 75 mM salinity compared to control (Table 2).

Quantum function of photosystem II (Pi) and (Fv/Fm)

The results of the variance analysis showed that quantum function of photosystem II (Pi) and photosynthetic efficiency index (*Fv/Fm*) were affected by rootstock, salinity and interaction of rootstock and salinity (Table 1). At 150 mM salinity, the highest photosynthesis efficiency index (*Fv/Fm*) was observed in 'Badami-e- Riz-e-Zarand' rootstock and the lowest it in 'Akbari' however, there was no significant difference between the 'Badami-e-Riz-e-Zarand' and 'Ghazvini' and also 'Akbari' and UCB-1 (Table 3). Results also showed that response of rootstocks to salinity was different related to quantum function of photosystem II (Pi) so that salinity decreased Pi in 'Badami-e- Riz-e-Zarand' and 'Akbari' whereas it was no significant effect on UCB-1 and 'Ghazvini' rootstocks. At 150 mM salinity level, the highest and lowest quantum yield of photosystem II were observed with 'Badami-e- Riz-e-Zarand' and UCB-1 respectively however, there was no significant difference between 'Badami-e-Riz-e-Zarand'and 'Ghazvini'.

Proline

According to the variance analysis, it was identified that proline of leaf was affected by rootstock, salinity and interaction of rootstock and salinity ($p \leq 0.01$) (Table 1). The results showed that salinity increased proline content of leaf in 'Akbari' and 'Badami-e-Riz-e-Zarand' rootstocks compared to the control. At 150 mM salinity, the highest of proline content of leaf was observed in the 'Akbari', and the lowest was recorded in the 'Ghazvini' rootstock however there was no significant between 'Badami-e-Riz-e-Zarand' and UCB-1 (Table 3).

Table 3. Interaction of salinity and rootstock on growth, physiological and biochemical parameters of four pistachio rootstocks.

Rootstock	Salinity (mM)	Leaf fresh Weight (g)	Stem fresh Weight (g)	Root dry Weight (g)	RWC (%)	Proline (mg/gfw)	Soluble carbohydrate (mg/gfw)	Chll a (mg/gfw)	*Fv/Fm*
Badami	0	0.828 a-c	1.764 ab	0.832 b-d	69.655 a	0.197 cd	2.542 bc	0.525 b-d	0.752 a
	75	0.585 d	2.016 a	0.809 b-d	54.410 ab	0.223 cd	4.138 a	0.439 cd	0.685 a-c
	150	0.575 d	1.748 ab	1.261 a	59.314 a	0.368 b	2.287 bc	0.533 b-d	0.613 a-c
	Mean	0.654 A	1.833 A	0.997 A	60.945 A	0.273 B	2.919A	0.502 B	0.676 A
Ghazvini	0	0.920 a	1.840 a	0.903 bc	34.064 cd	0.148 d	2.321 bc	0.520 b-d	0.618 a-c
	75	0.840 a-c	1.690 ab	0.884 b-d	39.016 b-d	0.209 cd	1.669 b-d	0.443 cd	0.644 a-c
	150	0.623 cd	1.566 a-c	0.848 b-d	52.704 a-c	0.199 cd	2.618 b	0.414 d	0.529 b-d
	Mean	0.777 A	1.685 A	0.875 A	43.006 B	0.187 C	2.244 B	0.455 B	0.590 A
Akbari	0	0.661 b-d	1.643 a-c	1.111 ab	35.390 b-d	0.212 cd	1.219 d	0.726 bc	0.705 ab
	75	0.428 de	1.166 cd	0.715 cd	37.116 b-d	0.243 cd	2.305 bc	0.388 d	0.295 e
	150	0.887 ab	1.281 b-d	0.881 b-d	50.824 a-c	0.517 a	2.054 b-d	0.556 b-d	0.372 de
	Mean	0.681 A	1.355 B	0.900 A	42.082 B	0.343 A	1.879 B	0.557 B	0.449 B
UCB1	0	0.240 ef	0.690 e	0.385 e	70.254 a	0.243 cd	1.575 cd	1.145 a	0.395 de
	75	0.200 ef	0.850 de	0.564 de	30.253 d	0.247 cd	2.027 b-d	0.401 d	0.470 c-e
	150	0.145 f	1.337 bc	0.627 c-e	61.033 a	0.301 bc	2.081 b-d	0.808 b	0.382 de
	Mean	0.195 B	0.959 C	0.525 B	53.846 A	0.264 B	1.895 B	0.784 A	0.417 B

Means with a common letter in each column are not significantly different (Duncan test, *P = 0.05*).

Soluble carbohydrates of leaf

According to the variance analysis, Soluble carbohydrates of leaf were affected by rootstock, salinity and interaction of rootstock and salinity ($p \leq 0.01$) (Table 1). In 'Akbari' and 'Badami-e- Riz-e-Zarand' rootstocks, the soluble carbohydrates of leaf increased at 75 mM salinity compared to control

whereas in other rootstocks, there was no significant difference between the control and other levels of salinity (Table 3).

Total phenolic compounds of leaf

According to variance analysis, total phenolic compounds of leaf were not affected by rootstock, salinity and interaction of rootstock and salinity (Table 1).

Potassium content in shoots and roots

According to the variance analysis, K concentration of shoot was influenced by rootstock and interaction salinity and rootstock (Table 4). At 150 mM salinity, K concentration of shoot was significantly increased in the 'Badami-e- Riz-e-Zarand' rootstock compared to control whereas in other rootstocks there was no significant difference between control and other salinity levels (Table 5). Potassium concentration of root was only affected by the rootstock and salinity (Table 4). Results of means comparison showed that UCB-1 and 'Ghazvini' had the highest and lowest of potassium concentration in root, although there was no significant difference between the 'Badami-e- Riz-e-Zarand' and 'Ghazvini'. At 75 mM salinity, potassium concentration of root was increased compared to control, however no significant difference was observed between the of 75 and 150 mM salinity levels (Table 6).

Shoot and root calcium

According to the results of variance analysis, the calcium concentration of the shoot was affected by the rootstock and interaction of rootstock and salinity (Table 4).The results of showed that in 150 mM salinity, 'Badami-e- Riz-e-Zarand' and UCB-1 rootstocks had the highest and lowest of calcium concentration of shoot, although there was no significant difference between the UCB-1 and 'Ghazvini' and 'Akbari' rootstocks (Table 5). The calcium concentration of root was not affected by rootstock, salinity and interaction of rootstock and salinity.

Table 4. Analysis variance for nutrient elements of four pistachio rootstocks.

Treatment	df	Shoot				Root			
		K	K (Tr)	Na	Na (Tr)	K	K (Ab)	Na	Na (Ab)
Rootstock	3	0.058 **	0.142 **	0.250 **	4.045 **	0.928 **	0.425 *	0.432 **	8.517 **
Salinity	2	0.014 ns	0.023 *	0.726 **	7.348 **	0.420 *	0.553 *	0.482 **	5.043 **
Rootstock*Salinity	6	0.016 *	0.016 *	0.193 **	1.543 **	0.233 ns	0.664 **	0.163 *	2.190 *
Error	27	0.004	0.006	0.042	0.406	0.100	0.113	0.0467	0.681
CV (%)		20.64	27.79	2.040	17.99	16.08	22.46	1.430	22.29
Treatment	**df**	**Shoot**				**Root**			
		Na/K	**Ca**	**Cl**	**Mg**	**Ca**	**Cl**	**Na/K**	**Mg**
Rootstock	3	455.105 **	0.009 **	12.737 ns	0.185 **	0.29 ns	24.328 **	0.568 *	0.074 ns
Salinity	2	13.216 ns	0.002 ns	27.231 ns	0.089 *	0.074 ns	104.425 **	0.8520 **	0.145 ns
Rootstock*Salinity	6	82.518 **	0.002 *	19.309 ns	0.147 **	0.118 ns	3.310 ns	0.095 ns	0.150 *
Error	27	6.523	0.000	8.456	0.019	0.146	1.436	0.140	0.049
CV (%)		21.81	20.65	38.90	20.27	25.80	17.50	15.73	25.73

** and* significant at 1 and 5% , respectively; ns: no significant; Ab: absorption; Tr: transfer

Shoot and root magnesium

Magnesium concentration of shoot was affected by the rootstock, salinity and interaction of rootstock and salinity (Table 4). The results showed that in 'Akbari' rootstock, the magnesium concentration of shoot was decreased with increasing salinity although at 150 mM salinity there was no significant difference

between ‘Akbari’ and other rootstocks. Also magnesium concentration of root was affected by interaction of rootstock and salinity. The results showed that with increasing salinity, the magnesium concentration of root increased in ‘Akbari’ rootstock. At 150 mM salinity, the highest of magnesium concentration of root was observed in ‘Akbari’ rootstock, however, had not significant difference with other rootstocks (Table 5).

Shoot and root sodium

Sodium concentration of shoot and root were affected by the rootstock, salinity and interaction rootstock and salinity (Table 4). In all rootstocks, salinity increased the sodium concentration of shoot, so that the highest sodium concentration of shoot was observed at 150 mM salinity with UCB-1 rootstock however, there was no significant difference between UCB-1 and ‘Ghazvini’ (Table 5). Sodium concentration of root also was affected by interaction of rootstock and salinity. With increasing of salinity, sodium concentration of root was increased in all rootstocks. The highest sodium concentration of root was found in ‘Akbari’ and the lowest it was observed in ‘Badami-e- Riz-e-Zarand’, although no significant difference was observed between the ‘Badami-e- Riz-e-Zarand’ and UCB-1 (Table 5).

Ratio of sodium to potassium in shoot and root

According to the variance analysis, the ratio of sodium to potassium in shoot was affected by the rootstock and interaction of rootstock and salinity (Table 4). The results showed that at 150 mM salinity, the highest and lowest of sodium to potassium ratio was observed in UCB-1 and ‘Badami-e-Riz-e-Zarand’ respectivly however, there was no significant difference between ‘Badami-e-Riz-e-Zarand’ and ‘Ghazvini’ in this case (Table 5). According to variance analysis sodium to potassium ratio of root was significantly affected by rootstock and salinity. The lowest of sodium to potassium ratio was observed in UCB-1 although there was no significant difference between other rootstocks (data not show). Salinity increased sodium to potassium ratio in root so that the highest of sodium to potassium ratio in root was observed in 150 mm salinity (data not show).

Shoot and root chloride

According to variance analysis, chloride concentration of shoot was not affected by salinity, rootstock and interaction of salinity and rootstock whereas chloride concentration of root was affected by rootstock and salinity (Table 4). The highest chloride concentration of root was found in ‘Ghazvini’ and the lowest was observed in the UCB-1, although no significant difference was observed between the UCB-1, ‘Akbari’ and ‘Badami-e- Riz-e-Zarand’ rootstocks (data not show).

Potassium absorption and transfer

According to variance analysis, potassium adsorption and transfer was affected by rootstock salinity and interaction of rootstock and salinity (Table 4). The results showed that at 150 mM salinity, the highest rate of potassium absorption was observed in the ‘Badami-e- Riz-e-Zarand’ and the lowest in UCB-1 rootstock, although there was no significant difference between ‘Badami-e- Riz-e-Zarand’ and ‘Ghazvini’ and ‘Akbari’. The results of interactions of rootstock and salinity on rate of potassium transfer showed that potassium transfer rate increased in ‘Badami-e-Riz-e-Zarand’ with increasing salinity, whereas there was no significant difference compared to control (Table 5).

Table 5. Interaction of salinity and rootstock on nutritional minerals *of four pistachio rootstocks.*

Rootstock	Salinity (mM)	Shoot						Root			
		Na	Na (tr)	Ca	Mg	K	K (tr)	Na	Na (ab)	Mg	K (ab)
Badami	0	0.188 g	2.435 d	0.133 b-d	0.516 c	0.333 b-d	0.306 b	0.396 ef	3.340 cd	0.768 a-c	1.456 a-c
	75	0.237 d-g	3.559 a-c	0.129 b-d	0.622 c	0.323 b-d	0.367 b	0.403 d-f	3.704 b-d	0.848 a-c	1.521 a-c
	150	0.351 b-d	4.716 a	0.201 a	0.689 c	0.504 a	0.517 a	0.411 d-f	3.869 b-d	0.867 a-c	1.988 ab
	Mean	0.268 B	3.685 AB	0.159 A	0.617 B	0.398 A	0.409 A	0.404 B	3.661 B	0.831 A	1.688 A
Ghazvini	0	0.297 d-g	4.242 a	0.144 bc	0.496 c	0.361 bc	0.357 b	0.3.95 ef	3.609 cd	0.936 ab	1.384 bc
	75	0.321 d-f	4.315 a	0.141 bc	0.636 c	0.353 b-d	0.332 b	0.333 f	2.903 d	0.458 c	1.385 bc
	150	0.334 c-e	4.119 ab	0.135 b-d	0.622 c	0.339 b-d	0.301 b	0.511 bc	4.618 a-c	0.864 a-c	1.608 a-c
	Mean	0.319 B	4.214 A	0.140 A	0.588 B	0.350 A	0.327 B	0.439 B	3.800 B	0.764 A	1.474 AB
Akbari	0	0.204 fg	2.619 cd	0.178 ab	1.177 a	0.444 ab	0.320 b	0.432 c-f	5.137 ab	0.683 bc	1.676 a-c
	75	0.448 ab	3.913 ab	0.114 cd	0.933 b	0.286 cd	0.170 c	0.507 b-e	3.604 cd	0.998 ab	1.416 bc
	150	0.275 d-g	3.974 ab	0.141 bc	0.551 c	0.352 b-d	0.336 b	0.669 a	5.476 a	1.149 a	1.718 ab
	Mean	0.3.06 B	3.547 B	0.144 A	0.853 A	0.360 A	0.281 B	0.549 A	4.813 A	0.964 A	1.615 A
UCB1	0	0.229 e-g	1.091 e	0.061 e	0.544 c	0.154 e	0.069 c	0.481 b-e	0.864 e	1.184 a	0.404 d
	75	0.484 a	3.730 a-c	0.090 de	0.952 ab	0.225 de	0.108 c	0.561 ab	3.034 d	0.665 bc	2.074 a
	150	0.441 a-c	3.048 b-d	0.100 c-e	0.628 c	0.251 c-e	0.143 c	0.527 b-d	3.328 cd	0.909 ab	1.056 c
	Mean	0.385 A	2.623 C	0.084 B|	0.708 B	0.210 B	0.107 C	0.523 A	2.409 C	0.919 A	1.178 B

Means with a common letter in each column are not significantly different (Duncan test, $P = 0.05$): Ab: absorption; Tr: transfer.

Sodium absorption and transfer

According to variance the analysis, sodium absorption and transfer was affected by rootstock and interaction of rootstock and salinity (Table 4).The results showed that in all rootstocks, except to 'Badami-e- Riz-e-Zarand', the rate of sodium absorption increased with increasing salinity. At 150 mM salinity, the highest rate of sodium absorption was observed in 'Akbari' and the lowest in UCB-1, although no significant difference was observed between UCB-1 and 'Badami-e- Riz-e-Zarand' rootstocks. The results of interaction of rootstock and salinity showed that, in all rootstocks, except to 'Ghazvini', salinity increased the rate of sodium transfer to the shoot. At 150 mM salinity, the highest rate of sodium transfer was observed with 'Badami-e-Riz-e-Zarand' rootstock and the lowest it with UCB-1 rootstock (Table 5).

Cluster analysis

Cluster analysis was carried out by the Ward's method for all measured parameters in 0, 75 and 150 mM salinity separately (Fig. 1). At 0 mM salinity, in 5 Euclidean distance, pistachio rootstocks were divided in two main groups including 'Badami-e- Riz-e-Zarand', 'Ghazvini' and 'Akbari' in first group and UCB-1 in second group. 'Badami-e- Riz-e-Zarand' rootstock had the closest distance with 'Akbari'. The position of rootstocks changed at salinity treatments compared to non-stress, so that at 75 mM salinity, the 'Badami-e- Riz-e-Zarand' and 'Ghazvini' were clustered in the first group and the 'Akbari' and UCB-1 were in the second group. At 150 mM salinity, similar to the control, 'Badami-e-Riz-e-Zarand', 'Ghazvini' and 'Akbari' were clustered in one group and the UCB-1 in a separate group, although at this salinity level, 'Badami-e- Riz-e-Zarand' had closer distance to 'Ghazvini' than 'Akbari'. Variance analysis and means comparison two group of rootstocks at 150 mM salinity showed that two group had significant difference related to measured traits (Table 6).

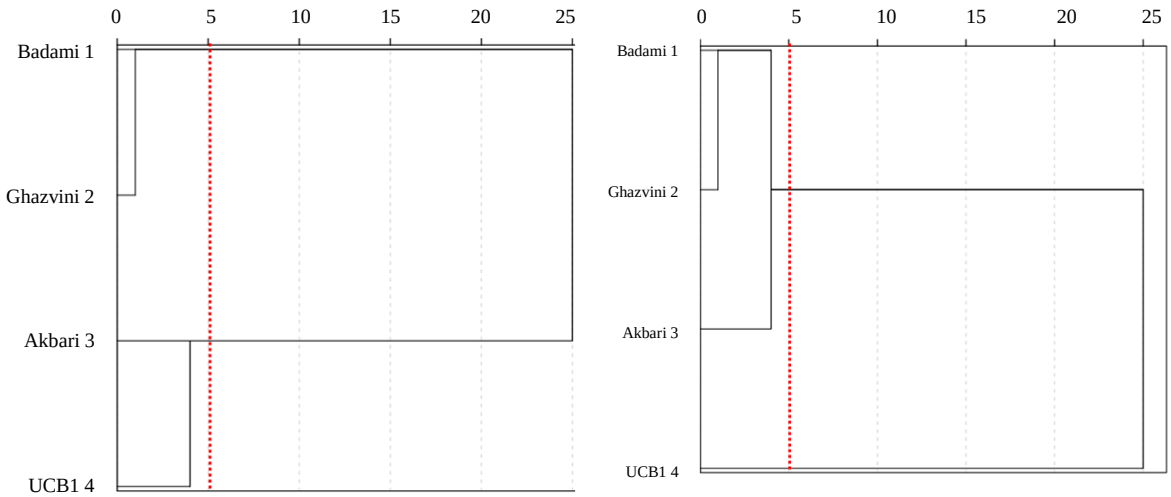

Fig. 1. Dendrogramrepresenting relationships among four pistachio rootstocks using ward's method in 75 mM (left) and 150 mM (right) salinity.

Table 6. Analysis of variance for 2 clusters of four pistachio rootstocks.

Traits	Cluster		Significant
	I	II	
Plant height	13.41	5.41	**
Leaf number	6.25	1.25	**
Leaf fresh weight	0.69	0.14	**
Leaf dry weight	0.39	0.12	**
Stem dry weight	0.91	0.55	**
Root dry weight	0.99	0.62	**
Plant fresh weight	4.01	2.80	**
Plant dry weight	2.27	1.31	**
RWC	46.36	70.25	ns
LA	5.82	10.34	**
SPAD	62.18	48.36	**
SLA	11.55	57.41	**
SLW	0.11	0.01	**
carotenoids	5.47	3.29	ns
Chl a	0.50	0.80	**
Chl b	1.69	1.57	ns
Total chl	2.18	2.38	ns
Pi	3.09	0.41	*
Na shoot	3.20	4.41	ns
Na shoot (tra)	4.26	3.04	**
Na root	0.40	0.48	ns
K shoot	0.39	0.25	**
K shoot (tra)	0.38	0.14	**
K root (ab)	1.77	1.05	**
Ca shoot	0.16	0.10	**
Ca root	1.43	1.43	ns

ns: not, significant;*, ** significant at 5 and 1 of probability levels, respectively using t test.

Discussion

Salinity is one of the most important environmental factors that are affected pistachio yield. According to present study, the leaves were more susceptible to salt stress than the stem, which can be related to accumulation of sodium and chloride ions in the leaves and their effects on the nutrients balance and water relationships of leaves (Adish *et al*. 2010; Tester and Davenport 2003; Wang and Nii 2000)). The results of present study showed that salinity increased root dry weight in 'Badami-e- Riz-e-Zarand' rootstock. The potential of production roots in salinity conditions has been reported as a mechanism for resistance of genotypes to salt stress (Munns 2002). Therefore, more tolerance to the 'Badami-e-Riz-e-Zarand' rootstock to salinity can be related to its root system. The results also showed that UCB-1 had lower specific leaf weight than 'Badami-e- Riz-e-Zarand', 'Ghazvini' and 'Akbari' although it had larger leaf area. In similar study, Flowers *et al*. (1977) reported that, in some plants, salinity decreased leaf area and increased specific leaf weight. Increasing in specific leaf weight can be considered as a mechanism to better maintain water conditions of plants under salinity stress (Levitt 1980). Results of present study showed that rootstocks had different specific leaf area under salinity stress which can be considered as indicator of salt tolerance in pistachio rootstocks (Ball 2002; Sefton *et al*. 2002). It has been reported that plants with higher specific leaf area had lower chlorophyll content and photosynthetic rate with low water use efficiency (Omamt *et al*., 2006). In present study, soluble carbohydrate of leaf increased at 75 mM salinity in 'Badami-e- Riz-e-Zarand' and 'Akbari' rootstocks, which can be postulated as one of the salt tolerance mechanisms in these rootstocks (Zhu 2001). The increase in soluble carbohydrates in 'Akbari' rootstock was with decreasing shoot fresh weight which was in line with previous studies. It has reported that salinity decreased vegetative growth and increased soluble carbohydrates in plants (Yancey *et al*. 1982). In this study, salinity, increased proline in the leaf of 'Akbari' compared to the control. In some studies have reported that proline accumulation in pistachio leaves is related to tolerance to salinity (Hokmabadi *et al*. 2005; Karimi *et al*. 2014). Results of chlorophyll index showed that 'Badami-e- Riz-e-Zarand' and 'Ghazvini' rootstocks had higher chlorophyll index than 'Akbari' and UCB-1. It may be related to higher ability of these rootstocks to maintain chlorophyll in salt stress (Orabi *et al*. 2010; Lawler 1995). In the highest salinity level (150 mM) salinity, 'Badami-e- Riz-e-Zarand' can save *Fv/Fm*. This result was in line with the findings of Behboudian *et al*. (1986) on pistachio. They reported that sodium chloride damaged to the mesophilic cells of leaves and reduced carbon dioxide assimilation and also the photosynthetic efficiency in pistachios. In this study, photosynthesis efficiency index (Pi) decreased with increasing salinity. Among the studied rootstocks, 'Badami-e- Riz-e-Zarand' had higher photosynthetic performance (Pi) than other rootstocks. It reported that photosynthesis efficiency index (Pi) is as important parameter to evaluation of genotypes to salinity. In all rootstocks, sodium concentration of the shoot and root increased with increasing salinity and 'Badami-e- Riz-e-Zarand' and 'Ghazvini' had lower sodium concentration in the shoot and root which can be related to tolerance of these rootstocks to salinity stress. The results were with line Karimi *et al*. (2011) who reported 'Badami-e- Riz-e-Zarand' and 'Ghazvini' rootstocks had more tolerance to salinity stress. They reported that transfer sodium to shoot is inhibited by root in all pistachio rootstocks, although this ability is different between rootstocks. Rootstocks had different in related to potassium concentration of shoot and transfer it from root to shoot. It has reported that transfer more potassium to the shoot is one of the tolerance mechanisms of plants to salinity stress (Mohamad-khani and Salehi 2005; Banakar and Ranjbar, 2010). Based on present study, at 150 mM salinity UCB-1 rootstock had the lowest calcium concentration of shoot which can be related to genetic

of this rootstock. In similar study Tavallali *et al*. (2009) postulated that increased sodium concentration in root reduced calcium concentration of the root and lead to an increase in the sodium concentration of shoot. One of the mechanisms of salt tolerance in plants is the low sodium/potassium ratio. It has been identified that tolerance genotypes had a lower sodium/potassium ratio compared to sensitive genotypes (Khan *et al*. 2002). The results of this study showed that the highest sodium to potassium ratio of shoot was observed in the UCB-1 and the lowest it with 'Badami-e- Riz-e-Zarand' and 'Ghazvini' rootstocks. This mechanism have previously been reported in pistachio (Ferguson and Zhang 2002; Bani-Nasab, 2005). The results of cluster analysis showed that salinity unchanged the performance of 'Badami-e- Riz-e-Zarand' and 'Ghazvini' rootstocks whereas the 'Akbari' and UCB-1 rootstocks changed position in cluster.

Conclusions

'Badami-e-Riz-e-Zarand' and 'Ghazvini' rootstocks had more effective in lower uptake of sodium and it transfer to shoot. They also had a low sodium/potassium ratio than other studied rootstocks. 'Akbari' rootstock had lower sodium uptake and also higher accumulation proline in its leaves. UCB-1 was better in terms of leaf area, RWC and chlorophyll a than other rootstocks. It is suggested that future studies be conducted to investigate the molecular mechanism of these rootstocks to salinity stress.

Conflict of interest

The authors declare that they do not have any conflict of interest.

References

Adish M, Fekri M, Hokmabadi H (2010) Response of Badami- Zarand pistachio rootstock to salinity stress. Journal of Nuts. 1, 1-11.

Ball MC (2002) Interactive effects of salinity and irradiance on growth: implications for mangrove forest structure along salinity gradients. Trees 16(2), 126-139.

Banakar MH, Ranjbar G (2010) Evaluation of salt tolerance of pistachio cultivars at seedling stage. American-Eurasian Journal of Agricultural & Environmental Sciences 9, 115-120.

Bani-Nasab B (2005) Seasonal changes carbohydrate of nutrient and effect of foliar application and nitrogen application with alternative bearing of pistachio trees. Ph.D. Thesis. Faculty of Agriculture. Shiraz University. Iran.

Barazzouk S, Kamat PV, Hotchandani S (2005) Photoinduced electron transfer between chlorophyll a and gold nanoparticles. American Journal of Physical Chemistry B. 109(2), 716-723.

Bastam N, Baninasab B, Ghobadi C (2013) Improving salt tolerance by exogenous application of salicylic acid in seedlings of pistachio. Journal of Plant Growth. Regulator. 69, 275-284.

Behboudian MH, Walker RR, Torokfaivy E (1986) Effects of water stress and salinity on photosynthesis of pistachio. Scientia Horticulturae. 29, 251-261.

Chartzoulakis KS (2005) Salinity and olive: growth, salt tolerance, photosynthesis and yield. Agricultural Water Management. 78(1-2), 108-121.

Cowling RM, Campbell BM (1983) Definition of leaf consistence categories in the fynbos biome and their distribution along an altitudinal gradient in the South Eastern Cape. South African Journal of Botany. 2,176-185.

Duran-Zuazo VH, Martinez-Raya H, Aguilar-Ruiz J (2003) Salt tolerance of mango rootstock (*Magnifera indica* L. cv.Osteen). Spanish Journal of Agricultural Research. 1(1), 67-78.

Estefan G, Sommer R, Ryan J (2013) Methods of soil, plant, and water analysis. A Manual for the Wana Region. 170-176.

Ferguson B, Zhang X C (2002) Materials for terahertz science and technology. Nature Materials. 1(1), 26-33.

Flowers TJ, Troke PF, Yeo AR (1977) The mechanism of salt tolerance in halophytes. Annual Review of Plant Physiology. 28, 89-252.

Garcia-Sanchez F, Syvertsen JP, Martinez V, Melgar JC (2006) Salinity tolerance of "Valencia" Orange trees on rootstocks with contrasting salt tolerance is not improved by moderate shade. Journal of Experimental Botany 121, 1-10.

Genty B, Briantais JM, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochimica et Biophysica Acta. 990 (1), 87-92.

Heydari M, Sharafi Y, Tabatabaei SJ, Hokmabadi H (2021) How Pistachio Hybrid "P. Integerrima × P. Vera " grows and responses to NaCl salinity. International Journal of Fruit Science. 21(1), 133-146.

Hokmabadi H, Arzani K, Grierson PF (2005) Growth, chemical composition, and carbon isotope discrimination of pistachio (*Pistacia vera* L.) rootstock seedlings in response to salinity. Crop and Pasture Science. 56(2), 135-144.

Irigoyen JJ, Einerich DW, Sanchez-Díaz M (1992) Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. Physiologia Plantarum. 84(1), 55-60.

Kalra YP, Maynard DG (1991) Methods manual for forest soil and plant analysis. Forestry Centre. Edmonton, Alberta, 125P.

Kamiab F, Talaie A, Javanshah A, Khezri M, Khalighi A (2012) Effect of long-term salinity on growth, chemical composition and mineral elements of pistachio (*Pistacia vera* cv. Badami-Zarand) rootstock seedlings. Annals of Biological Research. 3(12), 5545-5551.

Karimi HR, Ebadi A, Zamani Z, Fatahi R (2011) Effect of water salinity on growth indices physiological parameters in some pistachio rootstocks. Journal of Plant Nutrition. 34, 935-944.

Karimi HR, Zamani Z, Ebadi A, Fatahi R (2012) Effects of Water Salinity on Growth Indices and Physiological Parameters in Some Wild Pistachio. International Journal of Nuts and Related Sciences. 3(1), 41-48.

Karimi HR, Hasanpour Z (2014) Effects of salinity and water stress on growth and macro nutrients concentration of pomegranate (*Punica granatum* L.). Journal of Plant Nutrition. 37(12), 1937-1951.

Karimi HR, Maleki-Kuhbanani A, Roosta HR (2014) Evaluation of inter-specific hybrid of *P. atlantica* and *P. vera* L. cv. 'Badami - Riz-e-Zarand' as pistachio rootstock to salinity stress according to some growth indices and echo-physiological and biochemical parameters. Journal of Stress Physiology & Biochemistry. 10 (30), 1-14.

Karimi HR, Kuhbanani AM (2015) The evaluation of inter-specific hybrid of *P. atlantica*× *P. vera* cv.'Badami Zarand'as a pistachio rootstock to salinity stress. Journal of Nuts. 6(2), 113-122.

Karimi HR, Nasrolahpour-Moghadam S (2016 a) Male pistachio seedlings exhibit more efficient protective mechanisms than females under salinity stress. Scientia Horticulturae 211, 118-125.

Karimi HR, Nasrolahpour-Moghadam S (2016 b) Study of sex-related differences in growth indices and eco-physiological parameters of pistachio seedlings (*Pistacia vera* cv. Badami-Riz-e-Zarand) under salinity stress. Scientia Horticulturae. 202, 165-172.

Karimi H, Hassanpour N (2017) Effects of salinity, rootstock, and position of sampling on macro

nutrient concentration of pomegranate cv. Gabri. Journal of Plant Nutrition. 40(16), 2269-2278.

Khan MH, Singha KL, Panda SK (2002) Changes in antioxidant levels in *Oryza sativa* L. roots subjected to NaCl salinity stress. Acta Physiologiae Plantarum. 24(2), 145-148.

Lawler DW (1995) The effect of water deficit on photosynthesis, In: N. Smirnoff (Ed.). Environment and Plant Metabolism. Milton Park, England: Bios Scientific Publishers.

Levitt J (1980) Responses of plants to environmental stresses. 2nd Volume, Water, Radiation, Salt and Other Stresses, Academic Press, New York.

Levy Y, Syvertsen JP (2004) Irrigation water quality and salinity effects in citrus trees. Horticultural Reviews. 30, 37-82.

Metheney PD, Reyes HC, Ferguson L (1998) Blended drainage water irrigation of pistachios, cv Kerman, on four rootstocks in the southern San Joaquin valley of California. Acta Horticulturae 470, 493-501.

Mickelbart MV, Arpaia ML (2002) Rootstock influences in ion concentrations, growth, and photosynthesis of 'Hass' avocado trees in response to salinity. Journal of the American Society for Horticultural Science. 127(4), 649-655.

Moeinrad H (2008) The relationship between some physiological traits and salt tolerance in pistachio genotypes. Desert. 13(2), 129-136.

Mohamad-khani A, Salehi MH (2005) Effect of salinity on uptake and transport of potassium level in pistachio. 9th Iranian Soil Science Congress. Karaj. P, 311-312.

Munns R (2002) Comparative physiology of salt and water stress. Plant Cell Environmental. 25(2), 239-250.

Naeini MR, Khoshgoftarmanesh AH, Fallahi E (2006) Partitioning of chlorine, sodium and potassium and shoot growth of three pomegranate cultivars under different levels of salinity. Journal of Plant Nutrition. 29, 1835-1843.

Omamt EN, Hammes PS, Robbertse PJ (2006) Differences in salinity tolerance for growth and water-use efficiency in some amaranth (*Amaranthus* spp.) genotypes. New Zealand Journal of Crop and Horticultural Science. 34(1), 11-22.

Orabi SA, Salman SR, Shalaby MA (2010) Increasing resistance to oxidative damage in cucumber (*Cucumis sativus* L.) plants by exogenous application of salicylic acid and paclobutrazol. Journal of Agricultural Science. 6(3), 252-259.

Paquin R, Lechasseur P (1979) Observations surunemethode de dosage de la prolinelibredans les extraits de plantes. Canadian Journal of Botany. 57(18), 1851-1854. https://doi.org/10.1139/b79-233

Sanden BL, Ferguson L, Reyes HC, Grattan SR (2004) Effect of salinity on evaporation and yield of San Joaquin valley pistachios. Acta Horticulturae. 664; Dol: 10.17660 ActaHortic. 2004.664.73.

Sefton CA, Montagu K, Atwell BJ, Conroy JP (2002) Anatomical variation in juvenile eucalypt leaves accounts for differences in specific leaf area and CO_2 assimilation rates. Australian Journal of Botany. 50(3), 301-310.

Silva C, Martinez V, Carvajal M (2008) Osmotic versus toxic effects of NaCl on pepper plants. Plant Biology. 52(1), 72-79.

Singleton VL, Orthofer R, Lamuela-Raventos RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-ciocalteu reagent. In Methods in enzymology. Academic press. 299, 152-178.

Suriyan C, Chalermpol K (2009) Effect of salt stress on proline accumulation, photosynthetic ability and growth characters in two maize

cultivars, Pakistan Journal of Botany. 41, 87-98.

Tabatabaei SJ (2006) Effects of salinity and N on the growth, photosynthesis and N status of olive *(Olea europaea* L.) trees. Scientia Horticulturae. 108, 432- 438.

Tavallali V, Rahemi M, Panahi B (2008) Calcium induces salinity tolerance in pistachio rootstocks. Fruits. 63(5), 285-296.

Tavallali V, Rahemi M, Maftoun M, Panahi B, Karimi S, Ramezanian A, Vaezpour M (2009) Zinc influence and salt stress on photosynthesis, water relations, and carbonic anhydrase activity in pistachio. Scientia Horticulturae 123(2), 272-279.

Tester M, Davenport R (2003) Na^+ tolerance and Na^+ transport in higher plants. Annals of Botany. 91(5), 503-527.

Wang Y, Nii N (2000) Changes in chlorophyll, ribulosebisphosphate carboxylase-oxygenase, glycine betaine content, photosynthesis and transpiration in *Amaranthus tricolor* leaves during salt stress. Horticultural Science and Biotechnology. 75(6), 623-627.

Yancey PH, Clark ME, Hand SC, Bowlus RD, Somero GN (1982) Living with water stress: evolution of osmolyte systems. Science. 217, 1214-1222.

Zaharieva M, Gaulin E, Havaux M, Acevedo E, Monneveux P (2001) Drought and heat responses in the wild wheat relative *Aegilops geniculata* Roth. Crop Science. 41(4), 1321-1329.

Zhang Y, Xie Z, Wang Y, Su P, An L, Gao H (2011) Effect of water stress on leaf photosynthesis, chlorophyll content and growth of oriental lily, Russ. Journal of Plant Physiolology 58(5), 844–850.

Zhu JK (2001) Plant salt tolerance. Trends in Plant Science. 6, 66–71.