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ORIGINAL ARTICLE

Evaluation of Inter-Spicific 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 Echophysiological and Biochemical Parameters

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Key words: Chlorophyll index, leaf area, pistachio, proline, salinity

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Pistachio (*Pistacia vera* L.) is one of the most important commercial crops in Iran. The majority of pistachio orchards is located in areas with saline soil and is irrigated with low quality and salty water. Although pistachio trees are classified as salt tolerant but previous researches have demonstrated that growth rates of pistachio trees decrease by increasing sodium chloride (NaCl) concentration in soil and water (Parsa and Karimian 1975; Sepaskhah and Maftoun 1982 and Sepaskhah *et al*., 1985). Salinity may inhibit the plant access to available soil water by increasing the osmotic potential of soil solution. Ferguson *et al*. (2002) suggested that the reason of decreasing pistachio yield at higher salinity levels is not confined to of toxicity in the plant tissue; however it appears that the osmotic potential of the soil solution impairs the ability of pistachio rootstocks to absorb water from the soil. It has been reported that salinity decreases growth and development, and protein synthesis in sensitive species (Boyer, 1982). Previous researches have identified that salinity changes photosynthetic rate, osmotic potential of leaf cells, leaf water potential, leaf relative water content of leaf, leaf temperature, transpiration rate and leaf chlorophyll and cartenoids content (Subara *et al*., 1998).

Ferguson *et al*. (2002) studied the response of *P. atlantica* Desf., *P. integrima* L. (Stewart.) and UCB_1 to salinity stress and reported that by increasing salinity, leaf area in integrima rootstock was reduced more than atlantica and UCB_1. They also reported that the B and Cl concentrations of shoot were affected by rootstocks and the highest B concentration was obtained by integrima rootstock.

Walker *et al*. (1988) reported that soluble sugar content and protein in leave were increased in treated pistachio seedling with 175 mM of water salinity. Hokmabadi *et al*. (2005) reported that by increasing salinity, the proline content of pistachio leaves was increased. Najafian *et al*. (2008) evaluated the GF_{667} (*P. amygdalus* L. × *P. persica* L.) rootstock to salinity stress and reported that chlorophyll content of leaf, leaf area, shoot fresh and dry weight, shoot length and number of node unaffected up to 60 mM of salinity compared to control. They also reported that sodium concentration of shoot was only increased at the highest salinity level (75 mM). 'Badami-Riz-e-Zarand' is the most common pistachio rootstock in Iran which is more resistant to salinity in comparison with other rootstocks as 'Sarakhs' and *P. mutica* Fisch et C. A. Mey. *P. atlantica* is another rootstock used in some countries is resistant to salinity and has high efficiency in zinc absorption compared to other rootstocks (Ferguson *et al*., 2005).

Although, the response of 'Badami- Riz-e- Zarand' and *P. atlantica* to salinity and drought has been studied but there is no report of their offsprings to salinity. Therefore, the objective of this research was to evaluate the offsprings of *P. atlantica* and *P. vera* cv. 'Badami- Riz-e- Zarand' to salinity stress.

MATERIALS AND METHODS

Plant materials and treatments

The experiment was conducted in the Research Greenhouse of Horticultural Department, Faculty of Agriculture, Vali-e-Asr University of Rafsanjan over three months. The seeds of 'Qazvini','Badami-Riz-e-Zarand' cultivars and the hybrid were obtained from Iranian Pistachio Research Institute (IPRI). Hybrid seeds were already produced during a breeding program. 'Badami-Riz-e-Zarand' cultivar and *P. atlantica* were used as female and male parent, respectively. The seeds were planted in plastic pots containing 4200 g of soil. A sandy clay soil with a pH of 7.5 and electrical conductivity of 0.85 $dS.m^{-1}$ was used for this experiment. The experiment was conducted as a factorial in the framework of a completely randomized design with two factors including rootstock ('Qazvini', 'Badami-Riz-e-Zarand' and Hybrid), and salinity levels of sodium chloride, calcium chloride and mangenesium chloride (0, 60 and 120 mM) with 3:2:1 ratio with four replications. Each replication included five seedlings. Rootstocks were exposed to salinity treatments for 45 days. The temperature of greenhouse fluctuated between 16 and 35° C during the experiment period. At the end of experiment, growth indices (root length, leaf area, plant height, leaf number, shoot fresh and dry weight and root fresh and dry weight) and eco-physiological and biochemical parameters (relative water content of leaves, water use efficiency, leaf chlorophyll index, chlorophyll fluorescence, soluble sugar content, phenolic and proline content of leaves) were measured.

Measurement of growth indices

The growth indices such as root length, leaf area, plant length, shoot and root fresh and dry weight and number of leaves were measured. Height and root length were measured by using a ruler and leaf area was measured by leaf area meter (model CI202).

Chlorophyll index and chlorophyll fluorescence

The chlorophyll index in the youngest expanded leaves was recorded with a SPAD-502 Chlorophyll Meter (Minolta Camera Co. Ltd., Japan). Four leaves from the upper parts of plants were used for the measurement of chlorophyll fluorescence by using a Plant Efficiency Analyzer, Handy PEA (Hansatech Instruments Ltd., Norfolk, UK).

Extraction and determination of chlorophyll and cartenoides

One g of fresh leaves was grinded with 20 ml of 80% acetone and was centrifuged at 5000 rpm for 5 minates and transferred the supernatant to a 100 ml volumetric flask. After extraction, the supernatant was diluted to final volume of 100 ml by 80% acetone. The absorbance was measured at 663, 645, 652 and 480 nm using a Spectronic spectrophotometer (Arnon, 1949). The chlorophyll a, b, total and caroteniod content were calculated on an exponential basis, using the equations.

Chlorophyll a (mg/g) = [12.7(D.663) - 2.69 (D645)] × [V / 1000 × W]

Chlorophyll b (mg/g) = [22.9 (D.645) - 4.68 (D.663)] × [V / 1000 × W]

Total Chlorophyll (mg/g) = [D652 × 1000 / 34.5] × [V / 1000 × W]

Carotenoids = [7.6 (D480) - 1.49 (D510)] × [V / 1000 × W]

In this equation, V and W represent the volume of used acetone and leaf sample weight, respectively and D represents the spectrophotometer's output.

Relative water content of leaf (RWC) and water use efficiency (WUE)

The relative water content of leaf was measured according to Weatherly *et al.* (1950). The relative water content was calculated on an exponential basis, using the following equation.

RWC (%) = (FW – DW / TW - DW) × 100

In this equation, FW and DW represent the fresh and dry weight of leaf discs, respectively and TW represents the weight of leaf discs after soaking in distilled water for six hours. At the end of the experiment, plants were cut at the soil level and roots were washed and cleaned from soil and were separately oven-dried at 70° C and water use efficiency (WUE) was calculated using the following equation: WUE = DW / UW

In this equation, DW and UW represent dry mass production and the amount of consumed water, respectively.

Extraction and determination of total phenol

0.1 g of developed leaves was initially extracted (Vinson *et al*., 2001) in 5 ml of ethanol and was shaken by stirring using a magnetic stirrer with 5 ml of ethanol at room temperature (25° C) for 24 hours. The supernatant was decanted and another 10 ml of solvent was added to the residue. After extraction for 1 hour, the two extracts were combined, and filtered to remove any suspended material. The filtration was diluted to final volume of 50 ml. The amount of total phenol was determined using Folin and Ciocalteu' s phenol reagent with Gallic acid as a standard. One millilitre of extract was mixed with 3 ml of 7.5 % sodium carbonate solution. After standing for 30 min at room temperature, the absorbance was measured at 765 nm using spectrophotometer T80 UV/VIS (Isfendiyaroglu and Zeker, 2002).

Extraction and determination of proline and soluble sugar

One gram of leaf sample was grounded in liquid nitrogen and mixed with 5 ml of ethanol and was centrifuged for 10 minute at 3500 rpm. Then the supernatant was decanted to new falcon. The concentration of proline was determined using reagent ninhydrin solution (1.25 gram ninhydrin mixed in 30 ml of acetic acid glacial and 20 ml phosphoric acid 6 M). One millilitre of extract was mixed with 9 ml distilled water and 5 ml reagent ninhydrin solution. Then samples were incubated at 65° C for 45 min in water bath. After cooling, the absorbance was measured at 515 nm using a Spectronic spectrophotometer (Paquin and Lechasseur, 1979). The total soluble sugar content of leaf was measured according to Irgoyen *et al*. (1992).

Extraction and determination of protein

For measurement of total protein of leaf, 0.5 g of fresh leaves was grounded in liquid nitrogen and mixed with 6.25 ml extraction buffer (12.1 g Tris-HCl, 1 lit distilled water, pH = 7.5). Then samples were decanted to Falcon (15 ml). After standing for 24 hr at 2 - 4°C, the samples were centrifuged at 16000 rpm for 30 - 40 min, then 0.1 ml of the supernatant was removed and 5 ml Bradford reagent (100 mg blue Brlyant Kumasy solved in 50 mL of ethanol 95 %, then 100 ml of 85 % phosphoric acid added and the solution diluted to final volume of 1 litter and passed of through whatman filter paper No.1) was added to it and was vortex. The absorbance was measured at 595 nm using spectrophotometer T80 UV/VIS Spectrometer PG Instruments l (Bradford, 1976).

Data analysis

Analysis of variance (ANOVA) was performed using the SAS software. If ANOVA determined that the effects of the treatments were significant ($P < 0.05$, $P < 0.01$), then the means would be compared by DMRT (Duncan's Multiple Range Test).

RESULTS

Stem height and diameter

According to ANOVA analysis, diameter of stem and stem height were affected by salinity and rootstock ($P < 0.01$) respectively (Table 1). Stem diameter was decreased by increasing salinity and the lowest stem diameter was observed in 120 mM salinity; but not significantly different compared with 60 mM salinity (Table 2). Mean comparison effects of rootstock on stem height showed the highest stem height was observed with hybrid rootstock (Table 3).

Leaf number

Leaf number was reduced by increasing salinity; however there was no significant difference between 6o mM and 120 mM salinity levels (Table 2). The results of the rootstock on the number of leaves showed that the highest number of leaves was observed in hybrid rootstock and the lowest was observed in 'Badami- Riz-e-Zarand' (Table 3).

Root length

Root length was significantly affected by all treatments ($P < 0.01$) (Table 1) and the highest and lowest root length were found in hybrid and 'Qazvini' respectively. The results of interaction between salinity and rootstock showed that in hybrid and Badami-Riz-e-Zarand rootstocks, root length was increased at 60 mM salinity and then reduced at 120 mM salinity whereas 'Qazvini' rootstock was unaffected by salinity (Figure 1).

Leaf area

Leaf area was significantly affected by all treatments ($P < 0.01$) (Table 1) and decreased by increasing salinity level so that the lowest leaf area was observed at 120 mM salinity although; there was no significant difference between 60 and 120 mM salinity levels (Table 2). The highest leaf area was obtained by hybrid rootstock and the lowest was found in Badami-Riz-e-Zarand, however there was no significant difference between 'Qazvini' and 'Badami Riz-e-Zarand' (Table 3). The results of interaction of salinity and rootstock on leaf area showed that leaf area was decreased by increasing salinity in all rootstocks however; at 120 mM salinity, hybrid rootstock had more leaf area than other ones (Figure 2).

Shoot weight

Shoot fresh weight was significantly affected by rootstock and salinity ($P < 0.05$) (Table 1) and decreased by increasing salinity. The lowest shoot fresh weight was obtained at 60 mM, however; no significant difference was found between 60 and 120 mM salinity levels (Table 2). The highest shoot fresh weight was obtained in hybrid rootstock and the lowest was found in 'Badami-Riz-e-Zarand'. Shoot dry weight was also affected by rootstocks ($P <0.05$) and the highest and lowest shoot dry weight were observed in hybrid rootstock and 'Badami-Riz-e-Zarand' respectively (Table 3).

Root weight

Root fresh weight was affected by all treatments ($P < 0.01$) (Table1). The highest root fresh weight was observed in 'Qazvini' and hybrid rootstock and the lowest was found in 'Badami-Riz-e-Zarand' (Table 2 and 3).

The results of interaction of salinity and rootstock on root fresh weight and dry weight showed that in 'Badami-Riz-e-Zarand', root fresh weight and dry weight were decreased at 120 mM salinity (Figure 3). The highest root dry weight was observed in hybrid at 120 mM salinity (Figure 4).

Chlorophyll index and chlorophyll fluorescence

Chlorophyll index was decreased with increasing salinity and the lowest rate was observed at 60 mM salinity, however there was no significant different between 60 and 120 mM salinity levels. Chlorophyll fluorescence was also affected by salinity and it was decreased in 120 mM salinity (Table 2).

Pigments of leaf

Chlorophyll b content of leaf was decreased by increasing salinity, although no significant differences were found between 60 and 120 mM salinity levels. Total chlorophyll and cartenoides were decreased by increasing salinity, so that total chlorophyll, was decreased about 56% at 120 mM salinity level compared to control (Table 2). The highest of chlorophyll b was observed in hybrid rootstock and the lowest was occurred in 'Badami-Riz-e-Zarand' (Table 3).

Relative water content of leaf (RWC) and water use efficiency (WUE)

RWC and WUE were affected by salinity and rootstock ($P < 0.05$) (Table 1). RWC was decreased by increasing salinity and the highest was observed in control, and the lowest was found at 120 mM salinity level (Table 2). The highest WUE was obtained in 'Qazvini' rootstock and the lowest in 'Badami-Riz-e-Zarand' however, there was no significant difference between hybrid and Qazvini rootstocks (Table 3).

Proline content of leaf

Proline content of leaf was increased by increasing salinity and the highest proline content of leaf was observed at 120 mM salinity (Table 2).

Soluble sugar content of leaf

Soluble sugar content of leaf was influenced by salinity and the highest soluble sugars was observed in hybrid rootstock and the lowest was occurred in 'Qazvini' however, there was no significant difference between hybrid and 'Badami- Riz-e- Zarand' (Table 3).

Protein and Phenolic content of leaf

The phenolic content of leaf was unaffected by treatments (Table 1). The results of interaction of salinity and rootstock on protein content of leaf showed that at 120 mM salinity level, the highest protein content of leaf was observed in hybrid rootstock (data not shown).

DISCUSSION

The growth of plants is affected by numerous factors, and one of them is salinity. Salinity reduces the ability of plants to absorb water that reduces the growth rates quickly (Karimi *et al.*, 2011). The present study showed that salinity led to change in some growth parameters in studied pistachio rootstocks, which is in agreement with Walker *et al.* 1987; Picchioni *et al.* 1990; Saadatmand *et al.* 2007; Karimi *et al.* 2011.

Table 1 The results of analysis variance on measured parameters of pistachio rootstocks.

Treatment	df	Stem height	Stem diameter	Leaf number	Leaf area	Root length	Shoot fresh weight	Root fresh weight	Shoot dry weight	RWC	Root dry weight
Salinity	2	12.764^{ns}	0.240**	68.004**	10.105**	50.71**	0.108 *	0.518 **	0.013^{ns}	371.6 *	0.026^{ns}
Rootstock	2	9.87**	0.126^{ns}	29/93**	7.992**	154.58**	0.132 *	0.507**	0.013*	65.71^{ns}	0.168**
salinity × Rootstock	4	4.75^{ns}	0.065^{ns}	10.72^{ns}	4.967**	19.058**	0.002^{ns}	0.460**	0.009^{ns}	85.04^{ns}	0.792**
Error	18	12.976	0.052	4.093	0.094	27/39	0.002	0.090	0.009	86.16	0.0194
CV		18.58	9.67	21.64	15/63	20/19	11.14	16.45	9.34	12.47	20.93

Treatment	df	WUE	Chll b	Total Chl	cartenoides	Ci	Cf	Proline	soluble sugars	Phenol	Protein
Salinity	2	1.216^{ns}	*0.065	**2.134	**0.163	96.17*	0.154^{ns}	**143.03	135151.79^{ns}	^{ns}1017.4	^{ns}0.163
Rootstock	2	1.899*	**0.21	^{ns}0.394	^{ns}0.026	10.16^{ns}	0.032*	75.59^{ns}	**240263.4	^{ns}1748.8	**1.025
salinity × Rootstock	4	1.43^{ns}	0.015^{ns}	^{ns}0.366	^{ns}0.029	17.15^{ns}	^{ns}0.037	132.93^{ns}	^{ns}423999.1	^{ns}1427.02	**0.494
Error	18	5.74	0.012	0.277	0.026	11.49	0.014	215.3	18.561.4	964.98	0.098
CV		23.03.14	18.8	20.01	19.34	6.25	17.56	24.5	13.39	22.8	3.1

** and* significant at 1 and 5% , respectively; ns: no significant; Ci: Chlorophyll index; Cf: chlorophyll fluorescence

Table 2 Effects of irrigation water salinity on measured parameters of pistachio rootstocks.

Salinity (mM)	Stem diameter (mm)	Leaf number	Root length (cm)	Leaf area (cm^2)	Shoot fresh weight (gr)	Root fresh weight (gr)	RWC (%)
0	2.546 a	12.37 a	25.2 b	7.40 a	1.546 a	2.106 a	80.91 a
60	2.277 b	8.66 b	29.60 a	5.90 b	1.304 b	1.7369 b	74.329 ab
120	2.25 b	7.1 b	25.64 b	5.35 b	1.324 b	1.6554 b	68.06 b

Salinity (mM)	Proline (mg/gfw)	Chlb (mg/gfw)	Total Chl (mg/gfw)	Cartnoide (mg/gfw)	Cholorophyll fluorescence	Cholorophyll index
0	48.065 b	0.668 a	3.142 a	0.984 a	0.795 a	57.909 a
60	57.96 b	0.32 ab	2.576 b	0.818 b	0.732 a	51.8 b
120	73.096	0.293 b	2.173 b	0.717 b	0.543 b	52.836 b

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

Table 3 Effects of rootstock on measured parameters of pistachio rootstocks.

Rootstock	Stem height (cm)	leaf number	Leaf area (cm^2)	Root length (cm)	Shoot fresh weight (gr)	Shoot dry weight (gr)
Qazvini	17.106 b	9.555 a	5.823 b	23.631 c	1.266 b	1.052 ab
Badami-Riz-e- Zarand	17.85 b	7.425 b	5.535 b	27.411 b	1.334 b	0.948 b
Hybrid	23.178 a	11.055 a	7.295 a	27.938 a	1.540 a	1.090 a
Rootstock	**Chl b (mg/gfw)**	**WUE (mg/ml)**	**Soluble sugar (µm/l)**	**Protein (ppm)**	**Root fresh weight (gr)**	**Root dry weight (gr)**
Qazvini	0.437 ab	0.00038 a	2626.3 b	100.9 b	1.977 a	0.793 a
Badami- Riz-e- Zarand	0.302 b	0.0002 b	3435.8 a	99.9 b	1.558 b	0.521 b
Hybrid	0.542 a	0.00034 ab	3578.6 a	106.2 a	1.962 a	0.681 a

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

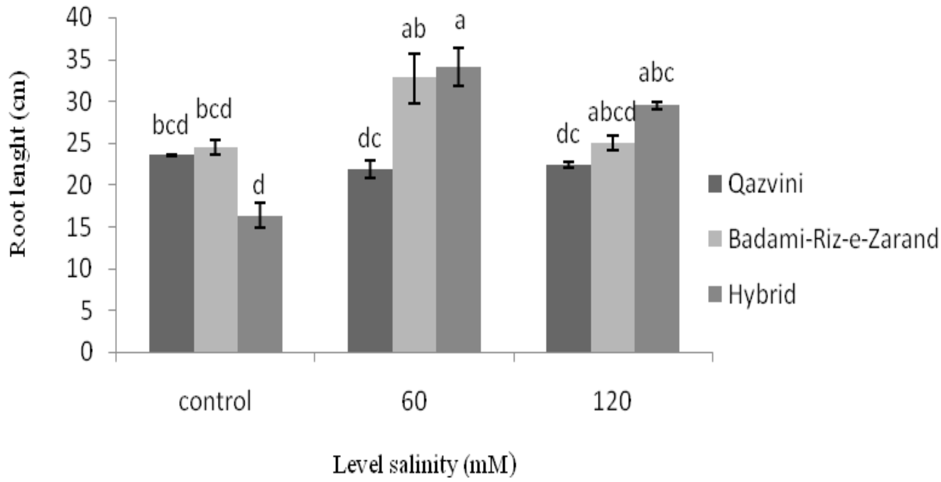

Figure 1. Interaction of salinity and rootstock on root length of pistachio.

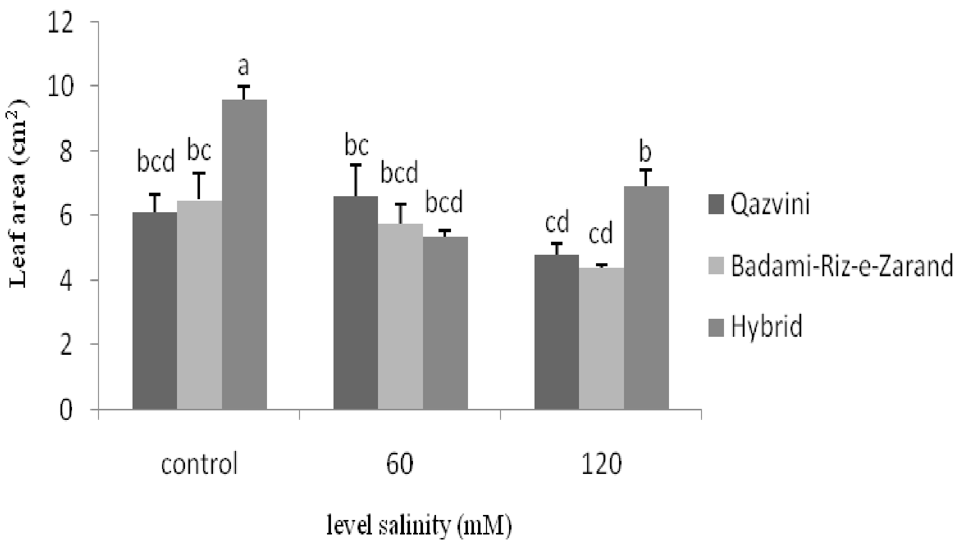

Figure 2. Interaction of salinity and rootstock on leaf area of pistachio.

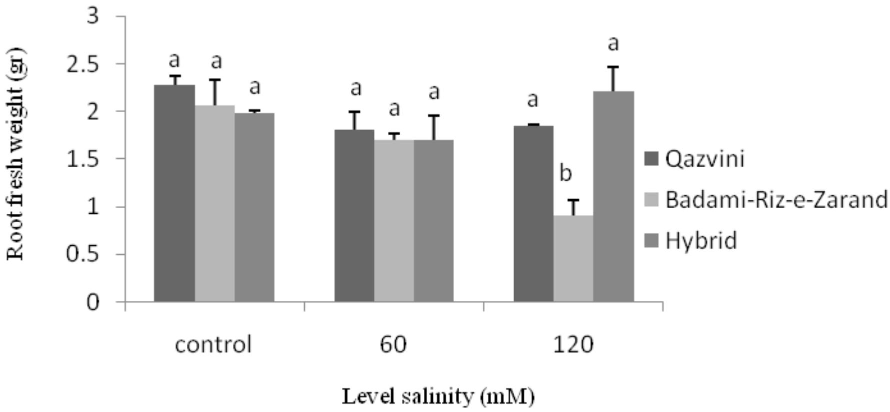

Figure 3. Interaction of salinity and rootstock on root fresh weight of pistachio.

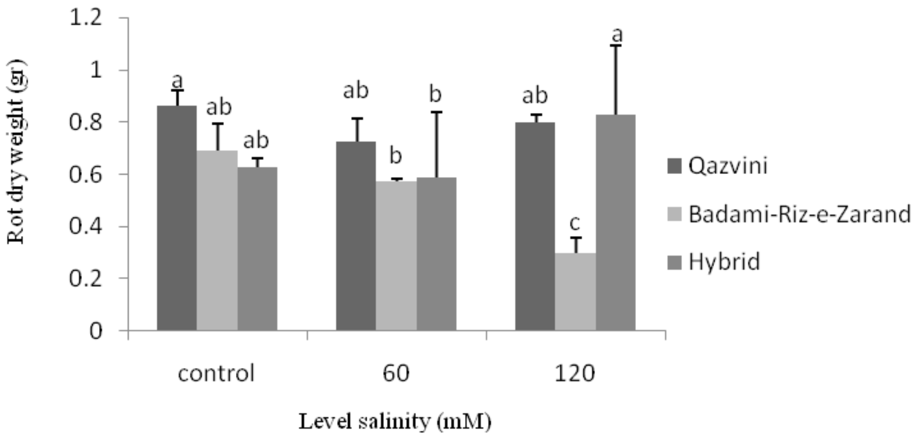

Figure 4. Interaction of salinity and rootstock on root dry weight of pistachio.

Karimi *et al.* (2011) studied effects of four salinity levels of water irrigation (0.75, 5, 10 and 15 $dS.m^{-1}$) on three pistachio rootstocks (Badami-Riz-e-Zarand, Qazvini, and Sarakhs) and reported that increased salinity level of irrigation water decreased vegetative growth of pistachio seedling. According to the present study, hybrid rootstock showed the highest vegetative growth which can be related to heterosis phenomenon. In a similar study; Ferguson *et al.* (2002), reported that UCB_1 (*P. atlantica* × *P. integrima*) was more vigorous than parents. Our findings showed that rootstocks showed different response to salinity which is in agreement with Ferguson *et al.* (2002). They studied the effect of salinity on vegetative parameters of pistachio rootstock and reported that the highest reduction in vegetative growth was observed in integerima rootstock and the lowest in UCB_1.

Leaf number and leaf area were decreased by increasing salinity. Our results are in consistent with results of Ferguson *et al.* (2002); Ranjbar *et al.* (2002); Karimi *et al.* (2011), on pistachio and may be due to reduction the accumulation of sodium and chloride ions in leaves and consequently photosynthesis. They reported that number of leaves and leaf area of salt treated pistachio seedlings were decreased by increasing salinity level.

Our finding showed that the highest shoot fresh and dry weights were obtained in hybrid rootstock. These results are correspond to the results of Parsa and Karimian 1975; Sepaskhah and Mafton 1988; Banakar and Ranjbar 2010, on pistachio. Banakar and Ranjbar (2010), reported that salt-treated pistachio rootstocks which produced drier mater is resistant to salinity.

The results of the present study confirming by the previous ones (Sepaskhah and Mafton 1988; Banakar and Ranjbar 2010; Karimi *et al.* 2011) led to decrease in root fresh and dry weight of pistachio seedlings treated by water salinity. The highest chlorophyll b content of leaf was observed in the hybrid rootstock; it may be due to lower uptake and transfer of Na^{2+} and Cl^{2+} ions to leaves. Behboudian *et al.* (1986) reported that NaCl destructed mesophyll of leaf and reduced CO_2 absorption photosynthetic efficiency of pistachio seedling treated by salinity. Ferguson *et al.* (2002) reported that chlorophyll index decreased with increasing salinity in 'Kerman' cultivar grafted on atlantica, integrima and UCB_1 rootstocks and these rootstocks can be tolerant to salinity by saving chlorophyll content and photosynthetic efficiency of leaf at higher levels of salinity.

Karimi *et al.* (2011) studied the effects of four salinity levels of water irrigation (0.75, 5, 10 and 15 $dS.m^{-1}$) on three pistachio rootstocks (Badami-Riz-e-Zarand, Qazvini, and Sarakhs) and reported that the relative water content of leaf was decreased with increasing salinity. The reduction in relative water content was rootstock-specific and similar to those found in the present study. According to our results, leaf proline content was increased by increasing salinity. These results are in agreement with those of Hokmabadi *et al.* (2005) on the pistachio and Kummar *et al.* 2003 on mulberry and may be associated with synthesis of proline or reduction in proline oxidation in salt-tolerant genotypes. The highest protein and soluble sugar content of leaves were observed in hybrid rootstock that may be due to resistance higher of hybrid rootstock compared to other rootstock to salinity. Protein and soluble sugars are osmotic compounds which are synthesized under drought and salinity stress in plant cells and increase the osmotic potential of cells (Yancey *et al.*, 1982). These results are inconsistent with the results of Karimi *et al.* (2009) on the pistachio. They reported that soluble sugar content in the 'Qazvini' rootstock was more than 'Badami- Riz-e-Zarand' and may be related to source of salt and treatment period. Total leaf protein content in hybrid rootstock was increased by increasing salinity whereas 'Qazvini' rootstock and 'Badami- Riz-e –Zarand' were not affected by salinity. Connies (1987) reported that salinity stress decreased protein synthesis, which in turn resulted in the reduction of cells plant growth; hence resistant genotypes have higher protein content in leaves.

In conclusion, the results of the present study showed that growth indices and ecophysiological parameters of pistachio seedling like other fruit trees are affected by salinity and rootstock. 'Badami- Riz-e-

Zarand' rootstock was influenced by 120 mM salinity and hybrid rootstock showed the lowest decrease in the mentioned parameters. Consequently, hybrid rootstock can be considered as more tolerant rootstock to salinity than 'Qazvini 'and 'Badami-Riz-e-Zarand' due to continuation of vegetative growth and increased osmotic compounds such as proteins and soluble sugar content in leafs.

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