

Effects of Water Salinity on Growth Indices and Physiological Parameters in Some Wild Pistachio

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Abstract: The effect of four water salinity levels (0.75, 5, 10 and 15 dS.m⁻¹) on growth indices and physiological parameters in some wild pistachio (*P. atlantica*, *P. atlantica* subsp. *kurdica*, *P. atlantica* subsp. *mutica* and *P. atlantica* subsp. *cabulica*) were investigated under greenhouse conditions. Leaf dry weight was reduced to about 30-54% at EC_w 10 dS.m⁻¹. Chemical analysis of shoot and root indicated that the concentration and distribution of Na⁺, K⁺ and Ca²⁺ in pistachio rootstocks were affected by salinity. The concentrations of Na⁺ and K⁺ increased in shoot with a rise in water salinity level. Comparison between Na⁺ concentration of shoot and root showed that all rootstocks limited the Na⁺ transportation to shoot tissue up-to 15 dS m⁻¹, and retained it in the roots. However, this ability was weaker in *Kurdica* rootstock. Leaf area was affected by salinity, expect in *Atlantica*. The maximum of leaf area and chlorophyll index were observed in *Atlantica* rootstock. In *Atlantica*, leaf area and chlorophyll index were decreased 15.76% and 12.56% at 15 dS. m⁻¹ compare to control respectively, whereas those were 19.95% and 19.08% in *Mutica*. Relative water content of leaves (RWC) was decreased in all rootstocks at solution 4 (15 dS. m⁻¹). The rootstocks were differed considering to stomata resistance of leaves response to salinity, so that; it was gradually increased in *Atlantica* and *Cabulica* rootstocks, while those were non-consistent in *Kurdica* and *Mutica*. Based on measured parameters *Atlantica* and *Kurdica* could be considered as tolerant and sensitive pistachio rootstocks to water salinity, respectively.

Keywords: *P. atlantica*, *Kurdica*, *Mutica*, *Cabulica*, Salinity, Na⁺, K⁺

INTRODUCTION

Salinity is one of the most serious environmental problems in the world and salinity stress has a negative impact on crop production, especially in irrigated fields of arid and semi-arid regions. Salinity stress adversely affects the growth of many agricultural crops through its influence on certain aspects of plant metabolism, including osmotic potential [7 & 8] specific-ion toxicities [1] and nutrient imbalance [18].

Pistachio (*P. vera* L.) is one of the important commercial crops in Iran. Majority of pistachio orchards are located in areas with saline soil and are irrigated with low quality and salty water.

Although pistachio trees are classified as salt tolerant, but researches have demonstrated that growth rates of pistachio trees are decreased with increasing in NaCl concentration in soil and there is a positive correlation between Na⁺ as well as Cl⁻ concentration in plant tissue and soil [12, 15 & 17]. Salinity stress also changed photosynthesis rate, morphology of leaves and nutrient balance in pistachio trees [4, 13 & 14]. Walker *et al.* (1987) reported that the highest chloride concentrations are in lamina and petiole of salt-treated pistachio seedlings, whereas sodium concentrations are high in root, especially the proximal end root.

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Salinity may be inhibited the plant access to available soil water by increasing the osmotic potential of soil solution. Ferguson *et al.* (2002) suggested that the main reason for decreasing in pistachio yield at higher salinity levels is decrease of water potential in plant.

Although selecting nutrient sources that do not add harmful ions to the irrigation water is a good agronomical strategy for avoiding osmotic problem, the most economical and beneficial way is using salt tolerant rootstocks. Picchioni *et al.* (1990) reported that one of the strategic tolerances in pistachio is their storage of Na⁺ in roots. 1990). Sepaskhah and Maftoun (1988) found that the average root-zone salinity (EC_e) for a 50% reduction in shoot growth was between 7.9 and 10 dS m⁻¹ depending on the seedling source.

Saadatmand *et al.* (2007) postulated that salinity stress had more negative influence than drought stress on pistachio growth. Based on their results, at lowest irrigation intervals Sarakhs variety showed a higher sensitivity to soil salinity than Qazvini, but with increasing in irrigation intervals, Sarakhs was more resistant than Qazvini to salinity.

Pistachio is usually propagated by budding or grafting selected scions onto seedling rootstocks of the same species or other *Pistacia* species. Different rootstocks are used in the different growing areas, for instance *P. atlantica*, *P. integerrima* and their hybrids are the main rootstocks in California. *P. vera* seedling are used in Turkey, while subspecies of *P. atlantica* (*kurdica*, *mutica* and *cabulica*) and *P. khinjuk* as well as *P. vera* are main rootstocks used in Iran. This comparative study was conducted to evaluate standard *P. atlantica* with subspecies of Iranian *P. atlantica* (*kurdica*, *mutica*, *cabulica*) to water irrigation salinity.

MATERIALS AND METHODS

Seeds of *P. atlantica* subsp. *kurdica*, *P. atlantica* subsp. *mutica* and *P. atlantica* subsp. *Cabulica* were gathered from different parts of Iran and standard *P. atlantica* (*P. atlantica*) provided from IRTA of Spanish. The seeds were scarified and stratificated according to the method described by Baninasab *et al.* (2002). The seeds were planted in Jiffy pots and grown in a greenhouse for 2 months. Seedlings with 3 to 4 leaves were transplanted to plastic bags, contained two kg soil. A sandy clay soil with a pH of 7.5 and electrical conductivity (EC_e) of 0.85 dS. m⁻¹ were used for this experiment. The plants were fertilized by ammonium nitrate and potassium sulphate before starting treatments. Completely randomized block experimental design was used, with three replications, and 5 pots per replication. One-year-old seedlings (*Atlantica*, *Kurdica*, *Mutica* and *Cabulica*) were exposed to salinity treatments for three months. Four salinity levels were imposed by irrigating the seedlings at electrical conductivity of 0.75 (control), 5, 10 and 15 dS. m⁻¹ (Table 1). The salt concentration of the irrigation water was increased gradually to reach determinate salt concentrations, to avoid osmotic shock to the seedlings. Afterwards, seedlings were irrigated by specified salinity levels at three-day interval. The temperature during the experiment fluctuated between 16 and 35 °C. At the end of experiment, growth indices (leaf fresh and dry weight, shoot and root fresh and dry weight and leaf area) and physiological parameters (stomata resistance, relative water content of leaves, leaf chlorophyll index and mineral elements) were measured. Leaf area of 3-5 youngest, fully expanded leaves from the terminal shoots were measured with a portable leaf area meter (LI-COR 3000, Lincoln, Neb.). The relative water content was calculated on an exponential basis, using the equation.

$$RWC = \frac{FW - DW}{TW - DW} \times 100$$

Where FW and DW represent the fresh and dry weight of leaf discs, respectively and TW represents the weight of leaf discs after soaking in distillate water for six hour. At the end of the experiment, seedlings were cut at the soil level and roots were washed free from soil and separately were dried at 70 °C,

weighed and ground to pass at 40 mesh screen. Dried samples of shoots and roots were used to measure Na⁺, K⁺ and Ca²⁺ concentrations by flame photometry and atomic absorption. Analysis of variance was carried out using a two-way ANOVA.

Table 1. Composition and properties of irrigation solutions used in this experiment and change of ECs of soil (ds.m⁻¹).

Solution	EC _w (ds.m ⁻¹)	EC _s (dS m ⁻¹)	Solute (mM/lit)			
			SAR	NaCl	CaCl ₂ .2H ₂ O	MgCl ₂ .6H ₂ O
1(control)	0.75	0.92	-	-	-	-
2	5	5.00	6	22.65	7.13	7.15
3	10	8.60	6	34.07	16.15	15.68
4	15	12.20	6	43.0	25.60	25.63

EC_w=Electrical conductivity water

EC_s=Electrical conductivity soil

RESULTS

Leaf dry weight was decreased in all four rootstocks in the salt treatments 5, 10 and 15 dS. m⁻¹ relative to the control treatment, except in Mutica. Atlantica had more leaf dry weight with solution 5, 10 and 15 dS. m⁻¹. Salinity stress resulted in Kurdica rootstock death at 15 dS. m⁻¹. Stem dry weight of the rootstocks was significantly decreased at 5 dS. m⁻¹, except in Atlantica, owing

to high vigor at low salt concentration. The salinity threshold for decreasing stem dry weight was depended on rootstock, so that the highest and lowest it was observed in Atlantica and Kurdica respectively.

Dry weight of root was decreased with increasing EC_w. The highest root dry weight was observed (Table2).

Table 2. Effects of irrigation water salinity on shoots and roots dry weight in four pistachio rootstocks.

EC _w (dS m-1)	Dry weight (g)			EC _w (dS m-1)	Dry weight (g)		
	Leaf	Stem	Root		Leaf	Stem	Root
Atlantica				Mutica			
0.75 (control)	2.00 a	1.61 a	2.00 a	0.75 (control)	0.40 a	0.40 a	0.46 a
5	1.06 b	0.97 a	1.34 b	5	0.24 ab	0.24 b	0.27 b
10	1.09 b	1.20 a	1.50 b	10	0.18 b	0.22 b	0.23 b
15	0.97 b	0.97 a	1.44 b	15	0.18 b	0.20 b	0.26 b
Kurdica				Cabulica			
0.75 (control)	0.21 a	0.25 a	0.29 a	0.75 (control)	0.50 a	0.45 a	0.43 a
5	0.15 b	0.16 b	0.15 b	5	0.23 b	0.21 b	0.27 b
10	0.11b	0.15 b	0.15 b	10	0.18 b	0.20 b	0.21 b
15	0.0 c	0.0 c	0.0 c	15	0.15 b	0.22 b	0.20 b

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

Leaf area was affected by salinity, except in Atlantica. The maximum of leaf area and chlorophyll index were observed in Atlantica

rootstock. In Atlantica, leaf area and chlorophyll index were decreased 15.76% and 12.56% at 15 dS.

m⁻¹ compare to control respectively, whereas those were 19.95% and 19.08% in Mutica.

Relative water content of leaves (RWC) was decreased in all rootstocks at solution 4 (15 dS. m⁻¹). The rootstocks were differed considering to stomata resistance of leaves response to salinity, so that; it was gradually increased in Atlantica and Cabulica rootstocks, while those were non-consistent in Kurdica and Mutica (Table 3).

Sodium content in shoot and root were increased considerably by increasing of EC_w in all rootstocks, except in Atlantica. The lowest and highest sodium concentration in shoot at solution 4 was observed with Atlantica and Kurdica, respectively. Sodium concentrations of roots were higher than the shoots. The highest sodium concentration was observed in Atlantica at solution

4 (Table 4). Sodium concentrations of shoot and root were highly correlated to salinity of irrigation water (EC_w) and growth rate of rootstocks (Table 5).

Potassium concentration was increased in roots up to 10 and then was decreased. Shoot Ca²⁺ concentration was increased in all rootstocks by increasing EC_w, whereas it was decreased in roots and then was increased.

DISCUSSION

Numerous environmental factors affect plant growth and development, which one of them is salinity. Saline stress can affect to the growth plants in two ways: Osmotic and/or toxicity effects [10]. Our finding indicated that growth reduction was due to Na⁺ toxicity rather than osmotic effect (Table3).

Table 3. Effects of irrigation water salinity on Leaf area, Chlorophyll index, Stomata resistance and RWC of four studied pistachio rootstocks.

Rootstock	Irrigation solutions			
	1	2	3	4
	Leaf area (cm ²)			
Atlantica	12.06 a	12.10 a	12.32 a	11.48 a
Kurdica	4.00 a	3.78 a	2.85 b	*
Mutica	4.57 a	4.56 b	4.22 b	4.06 b
Cabulica	4.69 a	4.09 ab	3.55 b	3.56 b
	Chlorophyll index			
Atlantica	48.52 a	49.52 a	45.48 a	42.43 a
Kurdica	47.25 a	42.02 ab	36.20 b	*
Mutica	49.38 a	44.21 ab	41.15 b	39.96 b
Cabulica	51.99 a	44.92 b	41.55 b	40.44 b
	RWC (%)			
Atlantica	62.09 a	61.53 a	49.78 ab	42.12 b
Kurdica	62.73 a	44.72 a	44.17 a	*
Mutica	64.09 a	54.25 a	53.61 a	33.00 b
Cabulica	68.37 a	57.66 a	55.50 a	33.35 b
	Stomata resistance (s/cm ²)			
Atlantica	29.07 c	95.83 b	112.90 ab	134.00 a
Kurdica	29.07 b	75.13 a	28.00 b	*
Mutica	20.93 b	82.67 a	73.63 a	44.25 b
Cabulica	28.30 b	44.60 b	80.83 a	107.80 a

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

* dried by salinity treatment.

Table 4. Effects of irrigation water salinity on concentration of mineral ions (% on dry weight.) in roots and shoots of four pistachio rootstocks.

EC _w (ds.m-1)	Root			shoot		
	Na ⁺	K ⁺	Ca ²⁺	Na ⁺	K ⁺	Ca ²⁺
Atlantica						
0.75 (control)	0.38 c	0.70 b	0.09 a	0.06 c	0.29 c	0.36 c
5	0.43 c	0.96 a	0.07 a	0.06 b	0.36 c	0.88 b
10	0.67 b	1.00 a	0.05 a	0.12 b	0.51 b	1.16 ab
15	0.92 a	0.73 b	0.08 a	0.23 a	0.78 a	1.30 a
Kurdica						
0.75 (control)	0.25 c	0.48 d	0.13 b	0.06 c	0.64 c	0.30 b
5	0.35 bc	0.62 c	0.11 b	0.48 b	0.91 b	1.09 a
10	0.51 ab	1.04 a	0.07 c	0.48 b	1.09 ab	1.24 a
15	0.75 a	0.75 b	0.21 a	0.76 a	1.25 a	1.20 a
Mutica						
0.75 (control)	0.34 b	0.62 c	0.13 a	0.06 c	0.08 c	0.39 b
5	0.72 a	0.72 c	0.11 a	0.42 b	1.08 b	1.46 a
10	0.81 a	1.15 a	0.10 a	0.48 b	1.29 a	1.20 a
15	0.81 a	0.97 b	0.12 a	0.51 a	0.97 b	1.32 a
Cabulica						
0.75 (control)	0.27 c	0.73 a	0.11 b	0.05 d	0.08 c	0.60 c
5	0.62 b	0.78 a	0.07 bc	0.26 c	0.71 b	1.00 b
10	0.72 ab	0.57 b	0.05 c	0.40 b	1.03 a	1.45 a
15	0.85 a	0.55 b	0.18 a	0.64 a	0.99 a	1.40 a

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

Table 5. Relationships between Na⁺ concentration of shoot and root (Y) and salinity of irrigation water (EC_w) (X).

Rootstock	Atlantica	Kurdica	Mutica	Cabulica	
Concentration of Na ⁺	Shoot	Y= 0.012 X + 0.0245 R ² = 0.86	Y= 0.043X + 0.1109 R ² = 0.86	Y= 0.029X + 0.1457 R ² = 0.73	Y= 0.040X + 0.0305 R ² = 0.98
	Root	Y= 0.0393X + 0.298 R ² = 0.95	Y= 0.0349X + 0.1964 R ² = 0.97	Y= 0.0307X + 0.434 R ² = 0.71	Y= 0.038X + 0.322 R ² = 0.89

The results presented here are in agreement with Saadatmand *et al.* (2007), Picchioni *et al.*, (1990), Walker *et al.* (1987), Behboudian *et al.* (1986) and Sepaskhah and Maftoun (1982) reported that salinity treatment decrease growth indices in pistachio rootstocks. The comparison of fresh with dry weight of leaves showed that fresh weight was affected by salinity more than dry weight. This may suggest that leaf fresh weight was more affected by water relationship than specific-ion-toxicity.

Root dry weight of Atlantica, Mutica and Cabulica was decreased to 34.68%, 51.06%, 54.66 respectively, as salinity increased from 0.75 to 15 dS.m⁻¹, confirming those reported by Mohammadi

et al. (2007). New evidence confirms earlier reports that root sensitivity to salinity is lower than shoot [12, 14, 18 & 20].

Leaf area was decreased by increasing salinity. It may be associated with decreasing cell elongation and cell division in leaves imposed salinity. Our findings are in agreement with the results of Munns (2002) who reported that salinity stress decreased cell elongation and cell division in leaves and caused smaller leaf size.

RWC of all rootstocks was not affected by salinity up to 10 ds.m⁻¹. It can also be related to their wild nature. Root Na⁺ concentration were one to four times higher than shoot, depending on rootstock,

which is in the same line with findings of Walker *et al.* (1987) and Puccini *et al.* (1990). The high ability of pistachio rootstocks to store Na⁺ under salinity stress was reported previously [14, 20 & 21].

The comparisons of Na⁺ concentration in the root with shoot indicated that the amount of absorption and translocation of Na⁺ among rootstocks were different. The lowest absorption of Na⁺ was observed in Mutica (0.40 % on dry weight) and the highest was in Atlantica (1.77 % on dry weight). The highest translocation of Na⁺ was observed in Cabulica (1.392 % on dry weight) followed by Mutica (0.92 % on dry weight) and the lowest was observed in Atlantica, This character can explain the mechanism of salt tolerance in pistachio rootstocks. High accumulation of the excessive salts in Cabulica and Mutica shoots was indicated that tolerance mechanisms are in the shoot cells. Localizing Na⁺ in the vacuole and balancing by compatible solutes within the cytoplasm was reported as the important salt tolerant mechanisms at cellular level.

The concentration of K⁺ in shoots was increased by salinity increment; however, concentration of K⁺ in roots was increased by increasing salinity. These results confirmed the findings of Saadatmand *et al.* (2007) on pistachio and Naeini *et al.* (2004) on pomegranate. This may be associated to concentration effect by salinity.

In conclusion it may be postulated that tolerance threshold of pistachio rootstocks to salinity stress is different, depending on species. Based on our finding can be stated that high-vigor species (Atlantica and Mutica) could be more tolerant to salinity stress than low-vigor species (Kurdica). As the mechanism of salt tolerance in pistachio species is not fully understood, further researches applying new technologies such as ultra structural studies may be proposed.

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