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Effect of long-term salinity on growth, chemical composition and mineral elements of pistachio (*Pistacia vera* cv. Badami-Zarand) rootstock seedlings

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ABSTRACT

In recent years, one of the most important problems of pistachio growing is increasing the salinity of soil and water which has decreased the quality and quantity of this crop. In this study, the effect of different levels of salinity on growth of pistachio rootstock cv. Badami Zarand has been evaluated. Pistachio seeds were planted in pots containing loamy sand soil and the salinity treatments were performed four weeks after germination. Seedlings were treated with 25, 50, 100 and 150 Mm of NaCl, $CaCl_2$ and $MgCl_2$ with SAR: 13 that were conducted in 8 steps gently. After 50 days, at the end of salinity duration, seedlings were harvested and morphological traits (length and diameter of shoots and roots as well as fresh and dry weight of shoots and roots) were recorded. Biochemical factors (proline, reduced sugar, starch) and mineral elements (Na^+, K^+, CL^- and Na^+:K^+) were also measured. Results showed that growth characteristics (fresh and dry weight of shoots and roots and shoot height) were decreased under salinity stress especially in 150 Mm treatment but the length of root was not significantly affected. Although the concentration of reduced sugars and proline in the leaves were decreased in150 mM salinity, there was no significant difference among other treatments. Results also showed that the tissue concentrations of Na^+, Cl^- and Na^+: K^+ ratio were increased with increasing the salinity level, while the increasing of Na concentration and Na^+:K^+ ratio was significant only in 150 Mm salinity. Lower Na^+:K^+ ratio in shoots suggests the possible better K^+-Na^+ discrimination by carrier in cell root or K^+ versus Na discrimination at the sites of xylem loading.

Keywords: Salinity, Proline, Reduced sugar, Starch and Na^+:K^+ ratio.

INTRODUCTION

Increasing salinization of arable lands is an important problem to crop production in many parts of arid and semi-arid regions of the world [11]. Approximately 20% of cultivated area and half of the irrigated area of world is affected by increasing salinity [42]. Pistachio is one of the most important commercial trees grown in Iran, USA, Turkey and Syria. Pistachio plantations encompass about 440000 ha in Iran recently. Most pistachio plantations in all over the world are on saline soils (EC>6 dS/m) and irrigated with low quality and saline water. Low quality of soil and water has reduced the yield of pistachio in recent years. Pistachio plant is known to be tolerant to salts [24, 8]. There are two main negative effects of high salt concentrations that influence the plant growth and development.

Water deficit and ion toxicity are associated with them [19]. Najmabadi (1969) reported that pistachio can grow on lands that are too saline for other crops, however symptoms of toxicity in pistachio in susceptibility to salinity have been reported previously [4, 31, 24, 8].Salinity have been reported to cause an inhibition of growth and development, reduction in photosynthesis, respiration and protein synthesis [6, 23]. Adverse effects of salinity on gro wth, photosynthetic rates and morphological change in the leaves of pistachio have been shown [21, 25, 5]. Accumulation of metabolites that act as compatible solutes is one of the usual responses of plants to changes in the external osmotic potential. Complex Sugars, sugar alcohols and charged metabolites are important osmolytes in plants under abiotic stress [30]. Proline accumulation is a common metabolic response of plants to salinity stress[35, 7, 37, 21]. Proline and other osmolytes protect the macromolecules and also scavenge reactive oxygen species under stress condition [14]. Soluble sugars accumulate in plant under salinity stress. This accumulation may be due to transformation of starch to sugar or less consumption of carbohydrates by the tissue [16]. It may act in the first case as osmotic adjustment factor, in the second case as a factor to maintain the stability of membranes and proteins [29]. Although there are some reports of evaluating the pistachio seedlings under salt stress, there are few studies regarding to the details of seedling responses under salt stress. Therefore, the aim of present study was to evaluate the vegetative characteristics, biochemical parameters and some mineral elements as the response of pistachio seedlings to salinity.

MATERIALS AND METHODS

2.1. Plant material, growth conditions and stress treatments

The experiment was conducted during 2010-2011 in a controlled greenhouse at pistachio research Institute, Rafsanjan, Iran. Germinated pistachio seeds (*Pistacia vera* cv. Badami-Zarand) were sowed in the plastic pots. The characteristics of the soil have been shown in Table 1. Seedlings were grown in the greenhouse at day/night temperature: 30/25±4˚C; relative humidity: 45% and photoperiod: 16 h. In order to maintain the vegetative growth of seedlings, nitrogen and phosphorous were applied uniformly to all pots at the rate of 100 mgL^{-1} NH_4NO_3 and 100 mgL^{-1} KH_2PO_4. The seedlings were allowed to grow in the pots for 35 days without salt treatments. During this period, pots were irrigated with deionized water. Then they were exposed to constant levels of salt stress. This was accomplished by irrigation with salt solutions (treatments: 25 (Control), 50, 100 and 150 mM salt concentrations). Artificial soil salinities were simulated according to the salinity components of the region's soil as a combination of salts (NaCl (70.5%), $CaCl_2$ (20%) and $MgCl_2$ (9.5%)). Salinity treatments were conducted in eight steps gently.

Growth characteristics

After 50 days of starting salinity treatments, pistachio seedlings were cut at soil surface and the roots washed free of soil. After measuring the length of shoot and root, shoot and root fresh weights were also recorded. Plant materials were washed thoroughly with tap water, then twice with distilled water. In order to estimate the dry weight, the samples were being oven-dried at 75°C for 48 h, reaching to a constant weight, thereafter, weighed and finely ground.

Biochemical analysis

Reducing sugars analysis

Fresh leaf samples (0.02 g) were ground in 15 ml of deionized water and then boiled for 0.5 min. After extraction, concentration of reducing sugars was measured by the method described by Smogyi (1952). The absorbance was measured at 600 nm. Glucose was used as standard solution.

Starch analysis

0.1 g of leaf was homogenized in 80% ethanol. After extraction, the concentration of starch was measured by the method of Hedge and Hofreiter (1962). The absorbance was measured at 630 nm. Glucose was used as standard solution.

Proline analysis

Proline content was extracted from the fresh leaf samples according to Bates et al. (1973). 0.02 g of fresh leaf sample was homogenized in 10 ml 3% aqueous sulfosalisylic acid. The homogenates were centrifuged at 10000 $\times g$ for 5 min. 2 ml of supernatants was mixed with 2 ml of acid-ninhydrin and 2 ml of glacial acetic acid in a test tube. The reaction mixture was extracted with 4 ml toluene and the chromophore containing toluene was aspirated and the absorbance was measured at 250 nm. L- proline was used as standard solution.

Mineral nutrient analysis
Mineral nutrient analysis was performed for the shoot samples. Fresh shoot samples were washed with deionized water, ground and ashed at 550˚C for 8 h. The ash dissolved in HCl and the concentrations of Na^+ and K^+ in the digest solution were measured with a flame photometer [39]. Cl^- concentration was determined by the coulometric-amperometric titration with $AgNO_3$ [26].

Ca^{2+} and Mg^{2+} were determined by the complexometric titrations with EDTA [26].

Statistical analysis
This experiment was analyzed as completely randomized design. Analyses of variance were performed using the General Linear Models procedure of SAS (SAS Institute Inc., Cary, NC, USA). Duncan's multiple range test was applied to compare the treatments.

RESULTS

Growth parameters
Effect of salinity on shoot height of pistachio
Results showed that the maximum height of shoot was observed for control and the minimum was for 150 Mm salinity. Salinity increasing from 25 to 150 mM significantly decreased the shoot height but there was no significant difference between 50 and 100 mM salinity (Table 1).

Effect of salinity on fresh and dry weights of pistachio shoot
Fresh weight of shoots was decreased by increasing the salt level but there were no significant difference between 50 and 100 mM salinity. Results also indicated that 150 mM salinity significantly decreased the dry weight of shoots and there were no significant difference between 50 and 100 mM compared to the control (Table 1).

Effect of salinity on pistachio leaf number
It was found that different levels of salinity significantly decreased the leaf number compared to the control but there were no significant difference between 25mM and 50 mM and also 100mM and 150 mM salinity (Table 1).

Effect of salinity on root length of pistachio
It was shown that although the root length of salt treated pistachio seedlings was decreased by increasing the salt level, this difference was not significant (Table 1).

Effect of salinity on fresh and dry weight of pistachio root
Control seedlings showed the maximum root fresh and dry weights. The minimum root fresh and dry weights were observed in150 mM salinity (Table 1).

Table 1. The effect of different salinity and polyamine treatments on some morphological traits of pistachio seedlings (*Pistacia vera* cv. Badami-Zarand).

Salinity	Shoot height (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Leaf No.	Shoot diameter (mm)	Root lenght	Root diameter (mm)	Root fresh weight (g)	Root dry weight (g)
25 (mM)	22.8±2.1 a*	2.3±0.1 a	0.9±0.08 a	16.8±0.5 a	0.25±0.01 bc	30.6±2.6 a	0.20±0.01 c	0.71±0.03 a	0.36±0.05 a
50 (mM)	21.9±1.3 ab	2.1±0.2 b	1.06±0.1 a	16.4±1.4 a	0.26±0.05 ab	29.9±2.1 a	0.27±0.02 a	0.76±0.1 a	0.36±0.03 a
100 (mM)	21.4±1.6 b	2.1±0.4 b	0.9±0.12 a	14.5±2.1 b	0.27±0.02 a	29.9±1.8 a	0.27±0.01 a	0.76±0.02 a	0.34±0.06 ab
150 (mM)	18.0±3.2 c	1.6±0.2 c	0.7±0.09 b	14.5±1.8 b	0.23±0.01 c	28.9±3.8 a	0.22±0.02 b	0.65±0.05 b	0.24±0.01 c

** Values are means±SE*
*** Different letters within a column indicate significant differences by Duncan's multiple range test at P < 0.05.*

Effect of salinity on root and shoot diameter
It was observed that exposure of pistachio to salinity led to a significant increase in root diameter compared to the control but it was decreased in 150 mM salinity. The minimum and maximum shoot diameter was observed in 150

mM and 100 mM salinity respectively. There were no significant difference between 100 and 50 mM salinity (Table 1).

Biochemical parameters

3.2.1. Proline content

Increasing the concentration of salt stress resulted in higher leaf proline content, although there was no significant difference among 25, 50 and 100 mM salinity (Fig. 1).

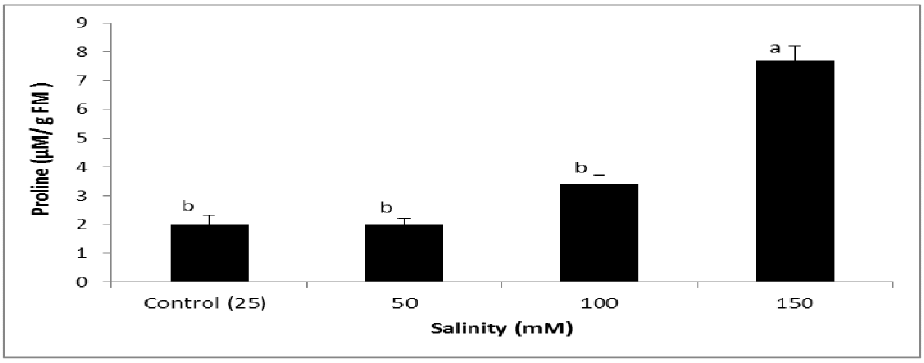

Fig 1. The effect of different salinity treatments on proline concentration of pistachio seedlings (*Pistacia vera* cv. Badami-Zarand). Vertical bar represents SE.

Reduced sugars concentration

Pistachio seedlings responded to elevated salt concentration by increasing the accumulation of leaf reducing sugars but the difference among 50, 100 and 150 mM salinity was not significant (Fig. 2).

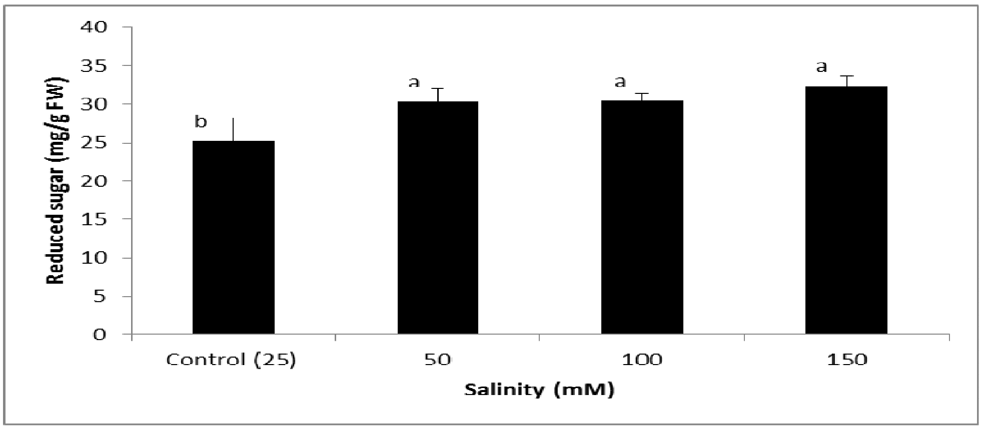

Fig 2. The effect of different salinity treatments on proline concentration of pistachio seedlings (*Pistacia vera* cv. Badami-Zarand).

Starch concentration

Results showed that the minimum accumulation of starch was observed for the highest salinity level (150 mM), although the effect of different salinity treatments was not significant (Data are not shown).

Mineral nutrients parameters

Na^+ concentration

Increasing the concentration of salt stress resulted in higher leaf Na^+ concentration, although there was no significant difference among 25, 50 and 100 mM salinity (Table 2).

K^+ concentration

K^+ concentration was reduced in 150 Mm salinity, although there was no significant difference among 25, 50 and 100 mM salinity (Table 2).

Na^+: K^+ ratio

The maximum Na^+: K^+ ratio was observed in 150 mM salinity but the difference of 25, 50 and 100 mM salinity was not significant (Table 2).

Cl^- concentration

It was found that by increasing the salinity level, the concentration of Cl^- was increased significantly (Table 2).

Table 2. The effect of different salinity treatments on some mineral element concentrations of pistachio seedlings (*Pistacia vera* cv. Badami-Zarand).

Salinity	Na^+ (g/100g DW) *	K^+(g/100gDW)	Na^+: K^+	Cl^- (g/100g DW)
25 mM	0.32±0.01 c **	2.3±0.2 a	0.18±0.01 b	1.7±0.12 d
50 mM	0.34±0.04 c	2.4±0.1 a	0.17±0.01 b	2.4±0.24 c
100 mM	0.37±0.03 c	2.1±0.6 ab	0.16±0.03 b	3.3±0.81 b
150 mM	0.95±0.09 a	1.75±0.06 b	0.38±0.09 a	3.6±0.74 a

* *Values are means±SE*

** *Different letters within a column indicate significant differences by Duncan's multiple range test at $P < 0.05$.*

DISCUSSION

Our results showed that increasing salt stress reduced the growth parameters of pistachio seedlings which was expected and in agreement with previous findings [17, 15] but significant reduction of growth (dry weight of shoot and root) was only found in 150 mM salinity. Dry weight of shoot and root were reduced significantly which was in agreement with Spigel-Roy et al. (1997) that suggested that the growth of pistachio trees under stress surpass that of all other fruit trees species. effect of Ion toxic in plant tissues, the nutritional imbalances by such ions, osmotic stress [38] and the decrease of photosynthetic activity [17, 15] can cause the reduction of pistachio seedlings growth. Our data showed that shoot growth of seedlings were affected more than root growth under salinity stress. Thus, the salinity stress influence the shoots more than roots and the reduction of shoot dry and fresh weights was more attributed to the lower shoot height and leaf number and the development of smaller leaves that is in agreement with the previous findings by Behboudian et al. (1986). Visible stress injury such as chlorosis, necrosis and defoliation was observed by increasing the salt level and it was more severe in 150 Mm salinity.

Plant accumulate compatible osmolytes such as proline and sugars when they are subjected to salinity stress and they appear to protect plants from such stresses [42]. In this study, pistachio responded to elevated salt concentrations by increasing the accumulation of leaf reducing sugars. However, there were no significant difference among 50, 100 and 150 mM salinity. Reducing sugars act as an osmolyte and can increase the osmotic pressure of the cell [41]. In addition, the increase of soluble sugars in leaves could be associated with decreasing the growth under salinity. Lower rates of carbon assimilation and decrease of yield accumulation are associated with carbohydrate accumulation in plants [2] which confirms our result. The specific inhibitory effect of sugars on photosynthesis or the expression gene of photosynthesis has been reported in several studies [10, 40]. Moreover, proline is an organic molecule that acts as a stabilizer of sub-cellular structures, protector proteins during dehydration, enzymatic regulator and a sink for energy during stress condition [27]. Our data showed that the proline content of leaves was increased significantly in 150 Mm salinity and this is an evidence for efficient role of this metabolite as osmo-protectant in salinity stress in pistachio seedlings. Extensive research has demonstrated that proline accumulation is a consistent response of plants under stress, including salt stress [35, 12, 7, 37]. Kheder et al. (2003) also suggested that proline improved the salt resistance by protecting the protein turnover machinery against stress damage and up regulating stress – protective proteins. This research indicated that proline has an important role in pistachio tolerance to salinity stress which was emphasized by Hokmabadi et al. (2005). Accumulation of proline during salt stress might due to the higher rates of proline synthesis and lower magnitude of proline oxidation in tolerant genotype.

Starch concentration was significantly decreased in150 mM salinity. The reduction of photosynthesis in salt stress could be the cause of starch decrease in pistachio leaves. The reduction of photosynthesis in pistachio under salt stress has been reported in some studies [5, 17, 15]. There are much attention about advers effects of Cl^- and Na^+ on physiological and biochemical process and how these ions effect on plant growth in habition [20, 28, 36]. Hokmabadi et al., (2005) reported that the accumulation of Na^+ and Cl^- in leaves increased with increasing concentrations these ions in the irrigation water. On the other hand, it was shown that nutritional imbalances by such ions cause to be the reduction of growth. Thus, in addition to the toxic effects of high Cl^- and Na^+ concentrations in plant, the salts changes the uptake of other nutrients. In this study, Na^+ concentration of leaves were increased by increasing the salinity level. The concentration of K^+ and Na^+:K^+ ratio were not significantly affected in 25, 50 and 100 mM salinity. These results might be attributed to the exchange between Na^+ and K^+ by carrier root in cell membrane or K^+ versus Na^+ discrimination at the sites of xylem loading. Also, the result is in agreement with Hokmabadi et al. (2005) on 'Badami' pistachio cultivar and Sepaskhah and Maftoun (1981) that showed 'Fandoghi' pistachio cultivar was more sensitive to salinity than 'Badami' cultivar because of greater uptake of Cl^- and Na^+. But this results is disagreement with Lolaei et al. (2012) on olive plant under salinity stress that showed significan decrease of K^+ and K^+:Na^+ in leaves this plant. This characteristic of 'Badami' pistachio could be introduced as a mechanism for increasing to salt tolerance. In other research, Picchioni et al. (1990) reported that pistachio is a plant with the ability to save Na^+ in the roots which can be considered as the other mechanism of pistachio seedlings to salinity stress.

CONCLUSION

In conclusion, the findings of this study showed that salt stress negatively impact on pistachio growth. However, this negative influence was significant at concentrations exceeding 100 Mm salinity. The data also showed that the mechanism of tolerance to salinity in this pistachio rootstock might be attributed to the better K^+- Na^+ discrimination and accumulation of osmolytes (proline and reduced sugars) under salt stress condition .

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