Effects of salinity stress on some growth, physiological, biochemical parameters and nutrients in two pistachio (*Pistacia vera* L.) rootstocks

Zahra Rahneshan, Fatemeh Nasibi & Ali Ahmadi Moghadam

To cite this article: Zahra Rahneshan, Fatemeh Nasibi & Ali Ahmadi Moghadam (2018) Effects of salinity stress on some growth, physiological, biochemical parameters and nutrients in two pistachio (*Pistacia vera* L.) rootstocks, Journal of Plant Interactions, 13:1, 73-82, DOI: 10.1080/17429145.2018.1424355

To link to this article: https://doi.org/10.1080/17429145.2018.1424355
Effects of salinity stress on some growth, physiological, biochemical parameters and nutrients in two pistachio (Pistacia vera L.) rootstocks

Zahra Rahneshana, Fatemeh Nasibib,a and Ali Ahmadi Moghadama

*Department of Biology, Faculty of Science, Shahid Bahonar University of Kerman, Kerman, Iran; bResearch and Technology Institute of Plant production (RTIPP), Shahid Bahonar University of Kerman, Kerman, Iran

ABSTRACT
In the present study, effects of salinity stress were evaluated in the leaves and roots of two pistachio cultivars (Badami-Rize-Zarand (BZ) and Badami-e-Sefid (BS)). In overall, salinity negatively affects growth of both cultivars with more pronounced effects on BS. The physiological reason of the reduction could be attributed to some extent to more depletion of photosynthetic pigment in BS. In both cultivars, salinity increased proline content. Moderate and high salinities increased the soluble sugar contents in BS. In both cultivars, Na⁺ content increased in plant organs with increasing Na⁺ in the media. Salinity treatment decreased the Fe and Pi contents in BS cultivar, while they remained unchanged in BZ. These results show that BZ cultivar exhibits more tolerance to salinity stress than BS cultivar possibly by better growth performance, accumulating more osmolytes, lesser accumulation of toxic sodium ion and lower Na⁺/K⁺ in the shoots as well as maintaining nutrient contents.

Introduction
The extent of agricultural land that is affected by high salinity is increasing worldwide, due to both natural phenomena and agricultural practices such as irrigation systems (Munns and Tester 2008). Salinity poses two major threats to plant growth: osmotic stress and ionic stress (Flower and Colmer 2008). In addition, it also manifested an oxidative stress. The deleterious effects of salinity affect different physiological and metabolic processes of plants. The responses to these changes are often accompanied by a variety of symptoms, such as the decrease in leaf area, increase of leaf thickness and succulence, abscission of leaves, necrosis of root and shoot, and decrease of internode lengths (Gucci and Tattini 1997; Kozlowski 1997; Parida and Das 2005). How plants cope with salinity and how successful their strategies are vary widely among species (Flower and Colmer 2008). The uptake of high amounts of salt by the plant leads to the increase of the osmotic pressure in the cytosol. Under these conditions, cell homeostasis is maintained by an osmotic adjustment mechanism which consists of the sequestration of large amounts of salt ions in the vacuole and/or synthesis of organic osmolytes (Munns, 2002). In many species, compatible osmoprotectant, such as proline and soluble sugars, is produced to protect the cells against the adverse effects from salt stress. High accumulation of proline is associated with tolerance to stress (Sairam and Tyagi 2004; Hokmabadi et al. 2005). Consequently, the ability to accumulate proline has often been suggested as a valuable criterion for the selection of salt tolerant genotypes (Ashraf and Harris 2004).

Na⁺ and K⁺ homeostasis plays a vital role in the growth and development of higher plants under salt conditions owing to potassium–sodium (K⁺–Na⁺) interaction and is often associated with K⁺ deficiency (Cramer et al. 1987; Parida and Das 2005). The potassium macronutrient is indispensable for several physiological processes, including the maintenance of membrane potential and turgor, enzyme activation, stomatal movement, regulation of osmotic pressure, and tropisms (Golldack et al. 2003). A high K⁺/Na⁺ ratio in the leaves is often considered as a salt tolerant marker (Maathuis and Amtmann, 1999). The calcium (Ca²⁺) content under salt stress conditions is depending on the specific physiology of the plant, the organs and its duration. It has been reported that the role of Ca²⁺ in the alleviation of salt toxicity is related to influence K⁺/Na⁺ selectivity through controlling the Na⁺ influx via nonselective ion channels (Cramer et al. 1987; Gucci and Tattini 1997; Melgar et al. 2006).

The roots are often reported to play a key role in the salt tolerance of plants as they represent the first organs that control the uptake and translocation of nutrients and salts throughout the plant. In spite of the direct exposure of these organs to saline environment, their growth is less vulnerable to salt than that of the shoots (Munns, 2002). In addition, the accumulation of Na⁺ in the roots is an adaptive response used by various woody species to avoid its toxicity in the shoots (Picchioni et al. 1990; Gucci and Tattini 1997). Consequently, the control of the root-to-shoot transport of salt can serve as a criterion for tolerance.

Considering the adverse effects of salt stress on crop growth and productivity, the identification and conservation of plant genetic resources have received considerable attention in the last few decades. The application of genetic resources for the breeding of salt tolerant genotypes is considered as a valuable tool to enhance productivity and develop sustainable agriculture in salt affected lands. Among the mostly known salt-sensitive fruit trees, pistachio (Pistacia vera L.) is considered as relatively tolerant. Despite the extensive publications on the high salinity tolerance in various pistachio cultivars, the detailed physiological mechanisms have not been addressed.
A few works have so far addressed certain aspects of salinity effects in pistachio (Ferguson et al. 2002; Chelli-Chaabouni et al. 2010; Nooghi and Mozafari 2012; Bastam et al. 2013; Hajiboland et al. 2014; Karimi et al. 2014; Karimi and Kuhbandani 2015). In this study, we used Badami-e-Sefid (BS) as a local, less known pistachio rootstocks and Badami-Rize-Zarand (BZ) as the most populated ones to evaluate and compare them based on morphological, physiological, and biochemical parameters under salinity stress.

Materials and methods

Materials and culture conditions

Seeds of pistachios (Pistacia vera L. cv. Badami-Rize-Zarand (the main pistachio rootstock in Iran’s pistachio plantation area) and Badami-e-Sefid) were surface sterilized using sodium hypochlorite (10%) for 10 min and then washed with distilled water. In the next step, seeds were treated with 0.1% (w/v) benomyl [methyl-1-(butylcarbamoyl)-benzimidazol-2yl carbamate] for 5 min. After washing with distilled water, they were placed on sterilized and moisturized filter paper in petri dish and stratified at 4°C for 4 days. After stratification, seed germination occurred at 20–25°C in dark. After emergence of roots, they were transferred to the hydroponic system and were grown on distilled water for 1 week. One-week-old seedlings were divided into 4 groups of 12 plants in each container with 3 replicates. Each group started to receive Hoagland solution (Control) or Hoagland with 50, 100, and 150 mM NaCl. After 15 days of starting different salinity treatments, plants were harvested.

Growth parameters

Shoots and roots were washed with distilled water, thereafter, they were blotted dry gently on a paper towel and dried for 48 h at 70°C for determination of dry weight (DW).

Relative growth rate (RGR) was calculated as RGR (day⁻¹): ln (DW15)−ln (DW0)/15−10, where DW0 and DW15 are the dry mass of shoot and root tissues of the plants at transplanting (t0) and harvesting times (t15), respectively (Pitman 1988). Shoot height and tap root length were measured using a ruler. 1-mm-squared graph paper was used for the determination of leaf area.

Relative water content

Relative water content (RWC) was measured according to Wheaterley (1973) and calculated as follows: RWC (%)=[(FW−DW)/(TW−DW)]×100. For the determination of turgid weight (TW), leaf samples were submerged for 24 h in distilled water, then, they were blotted dry on a paper towel and weighed.

Measurement of photosynthetic pigments

Chlorophylls and carotenoids were extracted by 80% acetone and assessed according to Lichtenthaler and Wellburn (1983).

Proline content

Free proline amount was measured according to Bates et al. (1973). A portion (0.5 g) of tissues was homogenized in 10 ml of 3% aqueous sulfosalicylic acid, and the homogenate was centrifuged at 2000g for 5 min. The extract (2 ml) was treated with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid and incubated for 1 h at 100°C in a bath. The reaction mixture was extracted with adding 4 ml toluene. The absorbance of chromophore containing toluene was recorded at 520 nm.

Sugar content

For the determination of saccharide contents, 100 mg of dry powder was extracted using 10 ml of ethanol: distilled water (8:2, v/v), and supernatant was collected after twice centrifugation. The residue from ethanol extraction was subsequently used for polysaccharide extraction by boiling water (Niknam et al. 2004). The contents of saccharides were estimated by the method of Dubois et al. (1956).

Ion content

100 mg of dried sample was digested by using 2 ml of nitric acid. The K⁺ and Na⁺ contents were determined by flame photometer (Model Jenway PFP7, UK). Ca²⁺, Mg²⁺, Fe²⁺, Pi were determined by ICP method (PerkinElmer Optima 7000 DV, US).

Experimental design

This experiment was arranged as a factorial in the framework of a completely randomized design with two factors: salinity (0, 50, 100, and 150 mM) and cultivars (Badami-Rize-Zarand (BZ), Badami-e-Sefid (BS)) with three replicates. Analysis of variance (ANOVA) was performed using SAS program version 9.1 (SAS Institute, Cary, NC). Significant differences of the mean values (P < .05 for F-test) were determined by LSD’s Multiple Range Test.

Results

Effects of NaCl on plant growth parameters and RWC

After 14 days of growing under different NaCl concentrations (control (0 mM), low (50 mM), moderate (100 mM), and high (150 mM)), the two pistachio cultivars exhibited some differences in terms of growth measurements.

The toxic effects observed on the leaves were mainly necrosis and losing chlorophyll mostly in leaves at high NaCl concentration. It was severe in lower leaves than that of upper leaves of BS cultivar plants. In this cultivar, the death of seedlings was observed to be preceded by reduction leaf area, necrosis and fall of leaves.

In our study, based on ANOVA results (Table 1), cultivar and salinity significantly affects RGR while the interaction between them was insignificant. While the RGR of BS significantly decreased at salt concentration above 50 mM, those of BZ showed no significant change except a significant decrease at 150 mM NaCl (Figure 1d).

Moderate and high salinities negatively affected shoot length in both cultivars (Table 1). Root length significantly decreased at high salinity in BS while it was unchanged in...
In term of ANOVA result for the ratios of root length to shoot length, significant changes were observed between cultivars, salinity treatments and their interaction (P < .05). The ratios of root length to shoot length significantly increased in BZ at moderate and high salinities (Figure 1c).

A significant decrease in leaf number was observed in 100 and 150 mM NaCl treatments in both cultivars, respectively (Table 1, Figure 1e). Salinity had no significant effect on the leaf area of BZ while treatment of plants with 100 and 150 mM NaCl induced significant reduction of leaf area in BS cultivar.

In the present study based on ANOVA result, salinity significantly affects RWC (P < .001). The RWC did not significantly decrease at low salinity in both cultivars (Figure 2a) and a maximum of 93% and 92% was obtained for BS and BZ, respectively. A drastic reduction in RWC was observed at high salinity for both cultivars.

### Effects of salinity on photosynthetic pigments

According to the ANOVA results, chlorophylls a and b, total chlorophyll and carotenoids contents were significantly affected by cultivar, salinity, and their interactions (Table 2). As shown in Figure 2, the contents of total chlorophyll and carotenoids negatively affected in BS cultivar by moderate and high salinity treatments while they were unaffected in BZ cultivar except a reduction in total chlorophyll at high salinity concentration.

---

**Table 1.** ANOVA results of the effect of salt stress on root and shoot lengths (RL, SL), RL to SL ratio, leaf number, and area RGR of pistachio genotypes.

| Source of variation | df | RL Mean squares | SL Mean squares | R/S ratio Mean squares | Leaf number Mean squares | Leaf area Mean squares | RGR Mean squares |
|---------------------|----|----------------|----------------|-----------------------|-------------------------|-----------------------|------------------|
| Genotype (G)        | 1  | 7.15 ns        | 76.68 ***      | 0.86*                 | 2.67***                 | 152.51***             | 0.003096*** |
| Salinity (S)        | 3  | 268.19**       | 98.08***       | 0.57*                 | 13.44***                | 31.07***              | 0.000334*** |
| G×S                 | 3  | 223.83**       | 3.67 ns        | 0.65*                 | 0.33 ns                 | 6.20 ns               | 0.000023 ns |
| Error               | 16 | 34.74          | 3.17           | 0.14                  | 0.875                   | 4.83                  | 0.000033     |

ns, not significant.

* P < .05; ** P < .01; *** P < .001.

---

**Figure 1.** Effect of salinity on growth of two pistachio cultivars. Values are mean values ± SE of three replicates. Bars with different letters show significant differences at P ≤ .05 (LSD).
Effects of salinity proline and sugar contents

In our study based on ANOVA results (Table 3), significant differences were found between cultivars, salinity treatments, and their interactions in the proline content in leaves and roots. Moderate and high salinities induced a significant increase in proline contents in the leaves and roots of BZ cultivar (Figure 3b,c).

Salinity stress influenced significantly soluble saccharides and polysaccharides in leaf and root of both cultivars (Table 3). Soluble saccharides significantly decreased at high salinity concentration in leaves and roots of BS cultivar. While it increased at 50 and 100 mM NaCl in its leaves and roots, respectively. NaCl decreased the contents of polysaccharides at moderate and high salinities in both leaves and roots of BZ cultivar (Figure 4).

Effects of salinity on mineral contents

In both pistachio cultivars, the Na⁺ accumulation in different organs was proportional to the exogenous NaCl at moderate and high salinities.

Table 2. ANOVA results of the effect of salinity stress on Chl a, Chl b, TChl, carotenoid, leaf RWC of pistachio genotypes.

| Source of variation | df | Mean squares |
|---------------------|----|-------------|
|                     |    | RWC | Chl a | Chl b | Total Chl | Carotenoids |
| Genotype (G)        | 1  | 2.67*** | 0.83*** | 0.28*** | 2.05*** | 0.035*** |
| Salinity (S)        | 3  | 132.94*** | 1.01*** | 0.19*** | 2.05*** | 0.017*** |
| GxS                 | 3  | 13.00* | 0.24*** | 0.05* | 0.52** | 0.020** |
| Error               | 16 | 2.375 | 0.039 | 0.011 | 0.072 | 0.003 |

ns, not significant.

Table 3. ANOVA results of the effect of salinity stress on leaf and root MDA, proline, soluble saccharides, and polysaccharides of pistachio genotypes.

| Source of variation | df | Mean squares |
|---------------------|----|-------------|
|                     |    | Proline | Soluble saccharides | Polysaccharides |
|                     |    | Leaf | Root | Leaf | Root | Leaf | Root |
| Genotype (G)        | 1  | 3.80*** | 5.68*** | 22.43* | 475.26*** | 6.30* | 10.01*** |
| Salinity (S)        | 3  | 2.36*** | 1.97*** | 63.54*** | 69.86*** | 45.32*** | 12.42*** |
| GxS                 | 3  | 3.96*** | 0.35** | 270.91*** | 102.59*** | 7.78** | 4.87** |
| Error               | 16 | 0.03 | 0.04 | 2.97 | 2.70 | 1.15 | 0.62 |

ns, not significant.

Figure 2. Effect of salinity on RWC, Chl a, Chl b, TChl, carotenoid of pistachio cultivars. Values are mean values ± SE of three replicates. Bars with different letters show significant differences at P ≤ .05 (LSD).
concentration in the medium ($P < .001$) (Table 4). All salinity treatments significantly increased the Na$^+$ levels in the shoot and roots of both cultivars. This increase was more pronounced in the shoot for BS than that for BZ cultivar. The highest levels of Na$^+$ were found in the roots of BZ at 150 mM NaCl treatment (Figure 5a, b).

Salt treatments induced noticeable variation in K$^+$ content in all parts of both cultivars ($P < .001$) (Table 4). Moderate and high salinity significantly decreased K$^+$ contents in shoots of the both cultivars when it was compared to that of the control plants (Figure 5c). A significant decrease in K$^+$ content was observed in the roots of both cultivars in all NaCl concentrations (Figure 5d).

The cultivar, salinity, and interaction between them strikingly affect the Na$^+$/K$^+$ ratio of the shoots (Table 4). For both cultivars, salinity dramatically increased the Na$^+$/K$^+$ ratio in

**Figure 3.** Effect of salinity stress on leaf and root proline contents of pistachio cultivars. Columns with different letters are mean values ± SE of three replicates. Bars with different letters show significant differences at $P \leq .05$ (LSD).
all tissues with a more remarkable effect on shoots of BS cultivar (Figure 5e,f).

Ca²⁺ content in roots of both pistachio cultivars was significantly affected by salinity (Table 5, \( P < .001 \)). Significant decreases were recorded in the Ca²⁺ levels of BS cultivar roots. Moreover, the Ca²⁺ level in the roots of BZ showed no significant variations with the control, except a significant decrease at 150 mM (Figure 6a).

Based on ANOVA results (Table 5), the root Mg²⁺ contents of both cultivars were influenced negatively by salt stress. In general, Mg²⁺ content of BS cultivar was higher than BZ regardless of the salt stress level (Figure 6c).

Salinity treatment also significantly decreased the Fe²⁺ and Pi levels in root of BS whereas no significant variations were observed at all NaCl concentrations in BZ cultivar (Table 5, Figure 6b,d).

Discussion

In plants, salt stress is a critical factor that severely affects plant growth and metabolism. Salinity stress involves complex and variable mechanisms that related to different metabolic pathways of various organs. In the present study, several parameters have been developed to evaluate the salt stress tolerance in two pistachio cultivars.

Growth has been considered as the result of different physiological mechanisms and its reduction after salt treatment has been widely described in different literature (Munns 2002). In both cultivars at low and moderate salinities showed no plant death after 15 days of culture, the plant death was observed for BS cultivar at 150 mM NaCl in contrast to BZ. Such response could be attributed to higher degree of salinity tolerance in BZ cultivar.

Sign of leaf necrosis has often been correlated with high sodium (Karakas et al. 2000) or chloride accumulation (Picchioni and Graham, 2001) in leaves. This symptom was more severe in lower and upper leaves of BS cultivar.

The results showed a negative relationship between salinity stress and vegetative growth parameters such as RGR, leaf number, leaf area, and shoot length. However, the responses of cultivars were different. These findings are in agreement with the results which have been reported for pistachio rootstocks (Karimi et al. 2014).

In a previous study, growth inhibition under severe salinity in pistachio has been attributed to a decrease in carbon assimilation due to stomatal limitation and/or metabolic

| Table 4. ANOVA results of the effect of salinity stress on shoot and root Na⁺, K⁺, Na⁺/K⁺ ratio of two pistachio genotypes. |
|---------------------------------------------------------------------------------------------------------------|
| Source of variation | df | Na⁺ | K⁺ | Na⁺/K⁺ ratio |
|---------------------|----|-----|-----|---------------|
|                      |    | Shoot | Root | Shoot | Root | Shoot | Root |
| Genotype (G)        | 1  | 16.8*** | 0.38** | 2.21*** | 17.00**** | 0.049*** | 0.0096*** |
| Salinity (S)        | 3  | 129.8*** | 202.57*** | 27.63*** | 113.93*** | 0.362*** | 0.8508*** |
| G×S                 | 3  | 3.4* | 3.46*** | 0.22*** | 2.28* | 0.013*** | 0.0042*** |
| Error               | 16 | 0.72 | 0.23 | 0.86 | 0.57 | 0.001 | 0.0027 |

ns, not significant.

\(^* P < .05; ** P < .01; *** P < .001.\)
impairment (Hajiboland et al. 2014). Moreover, the reduction in plant growth under saline conditions has also been attributed to a result of direct inhibition of cell division and expansion (Zhu, 2001; Munns, 2002).

The root length to shoot length ratio in BZ cultivar showed a significant increase in moderate and high salt concentrations but they showed no variations in BS cultivar. These findings suggest a higher water uptake capacity in BZ cultivar. As a consequence, increase in water uptake capacity allowed the ion dilution to prevent toxic level in cytosol (Chelli-Chaabouni et al. 2010).

RWC was significantly decreased at moderate and high salinities in both cultivars. This decline was more pronounced for BS cultivar compared to BZ cultivar. RWC directly reflects the water status of plants and its reduction indicates that salinity resulted in water deficit in plants. The negative effect on plant water relations was induced by an increase in soluble salts which decelerate the uptake of water and nutrients causing osmotic effects and toxicity (Yang et al. 2009; Jiang et al. 2014).

In the present work, the photosynthesis pigments, including chl a, chl b, Tchl, significantly reduced by moderate and high salinity treatments in BS cultivar while they remained unchanged at least at moderate salinity in BZ. Several studies suggest chlorophyll content as a biochemical marker of salt tolerance in plants. It is known that salt tolerant plants show increased or unchanged chlorophyll levels under salinity conditions whereas chlorophyll contents decreased in salt-sensitive plants (Stepien and Johonson, 2009; Ashraf and Harris, 2013). In general, decrease of these pigments under salt stress is considered to be a result of slow synthesis or fast breakdown of the pigments in cells (Ashraf, 2003).

Moreover, the carotenoid contents as auxiliary pigments were unchanged under salinity treatments in BZ. Several studies have shown the role of carotenoids as an effective antioxidant which protect and stabilize photochemical processes of photosynthesis under stress condition. Carotenoids play a vital role in preventing the chlorophyll-photosensitized formation of $^1$O$_2$ by intercepting chlorophyll triplet states (Demmig-Adams and Adams, 1996). Therefore, it can be

Table 5. ANOVA results of the effect of salinity stress on root Ca$^{2+}$, Mg$^{2+}$, Fe$^{2+}$, and Pi of two pistachio genotypes.

| Source of variation | df | Mean squares |
|---------------------|----|--------------|
|                     |    | Ca Mg Fe P   |
| Genotype (G)        | 1  | 53.58*** 7.22*** 2.95*** 1.66*** |
| Salinity (S)        | 3  | 32.43*** 1.46*** 0.23*** 4.21*** |
| GxS                 | 3  | 12.96*** 0.13*** 0.10* 2.09*   |
| Error               | 16 | 0.7216 0.06 0.02 0.57      |

ns, not significant.

*P < .05; **P < .01; ***P < .001.
suggested that the seedlings of BZ cultivar by maintaining total chlorophyll and carotenoid contents are more tolerant to salinity stress.

Under salinity stress plants accumulate compatible solutes such as proline and soluble sugars which are known for their osmoprotection activity (Chelli-Chaabouni et al. 2010). The accumulation of metabolites that act as compatible solutes is one of the common responses of plants to changes in the external osmotic potential (Hasegawa et al. 2000; Munns and Tester 2008). Proline can be considered as a biochemical marker of salt stress level in pistachio (Shamshiri and Fattahi, 2014). In the present study, moderate and high salinity induced a significant increase in the free proline content in the leaves and roots for both cultivars. This increase was more remarkable for BZ cultivar. Our results are in agreement with those previously reported for pistacia species (Chelli-Chaabouni et al. 2010), P. vera variety (Hokmabadi et al. 2005; Karimi et al. 2014). In addition to acts as a compatible osmolyte, proline plays a protective role against salt stress in plants. It can act as an enzyme protectant, free radical scavenger, cytosolic pH buffer stabilizer for subcellular structures and cell redox balancer (Verbruggen and Hermans, 2008). Therefore, BZ cultivar with more proline accumulation under high salinity could be more resistant when compared with BS cultivar.

The salt treatments induced noticeable variations in the soluble sugar contents in both parts of each of pistachio cultivars under investigation. The accumulation of soluble sugars in response to salinity was observed in leaves and roots of BZ. It is believed that under salinity stress allocation of assimilates for osmotic homeostasis as well as partitioning into roots along with other compatible solutes contribute to osmotic adjustment (Parida and Das, 2005; Hajiboland et al. 2014). Polysaccharide may act primarily as energy reserves (Franz, 1979). The high water-binding capacity of hydroxyl groups in the polysaccharide allows to hydrate and thus store huge reserves of water, which may offer plants the ability to resist physiological drought (Clarke et al. 1979). Pectic polysaccharides, rich in anionic groups, may act not only as a water capacitor but also as a Na⁺ sequestering compound in order to protect photosynthetically active tissues (Edmond Ghanem et al. 2010).

Under salinity treatments, the Na⁺ contents of leaves, stem, and roots of both cultivars were dramatically increased. For BS cultivar, the highest amount of NaCl was observed in the leaves, suggesting an important accumulation in the leaves, while for BZ cultivar the most amount of NaCl was found in the roots at 150 mM NaCl. BZ plants seemed to control Na⁺ accumulation in the aerial parts by reducing its translocation from roots and by its accumulation in roots. Moreover, because of higher ratio of root to shoot DW of BZ, it’s favoring the accumulation of toxic ions in roots and thus minimizing its negative effects on the shoot growth. This response also reported in other woody plants (Liu et al. 2012; Acosta-Motos et al. 2015, Martínez-Alcántara et al. 2015).

A significant decrease in the K⁺ content was observed in root and stem of both cultivars with increasing salt concentration. It is known that K⁺ can play an important role in plant growth and development as well as in the maintenance of osmotic adjustment and cell turgor (Marschner, 1995). In addition, it is the major cation in plants which counterbalances the negative charge of anions, and plays a crucial role in the activation of the enzymes involved in the metabolism and synthesis of proteins and carbohydrates, as well as in the regulation of stomata movement (Marschner, 1995).

The Na⁺/K⁺ ratio, another indicator of the plant response to high salinity, is reported to increase in many plants after treatment (Shabala and Cuin 2008). Roots of the both cultivars had higher Na⁺/K⁺ ratio than that of stem and leaves.
At the high salinity concentration, Na\(^+\)/K\(^+\) ratio in the leaves of BS was significantly more than that of BZ. In the other hand, leaves of BZ had less Na\(^+\)/K\(^+\) ratio compared to that of BS. Altogether these data showed that Na\(^+\) was sequestered in the roots of BS plants.

Calcium is known to play a crucial role in maintaining the integrity of the plasma membrane in the root cells, thus restricts the toxic effect of Na\(^+\) (Rengel, 1992; Gucci and Tattini, 1997). It acts as a secondary messenger in the regulation of signal transduction pathways for the response to abiotic stress and in the promotion of K\(^+\)/Na\(^+\) selectivity (Rengel, 1992; Maathuis and Amtmann, 1999; Shabala et al. 2006). In addition, calcium has been shown to ameliorate salt stress by enhancing the selective absorption of potassium in plants under higher concentrations of sodium and allow plants to perform osmotic adjustment by enhancing ion uptake (Epstein, 1998; Schachtman, 1999) and control of aquaporins (Carvajal et al. 2000). The replacement of calcium from membranes and cell walls by sodium was often suggested to be one of the primary responses to salinity stress (Cramer et al. 1985; Rengel 1992). In the present study, all salt treatments strongly decreased calcium content in BS roots, while it remained unchanged at low and moderate salinities in BZ cultivar. In overall, these findings suggest that tolerant cultivar has the ability to maintain constant levels of Ca\(^{2+}\) level under moderate salinity stress. In addition in a previous study by Karimi and Maleki Kuhbanani (2015) showed that salinity treatment increases Ca\(^{2+}\) in the shoot of BS plants.

Moreover, it has been shown that salinity affects the plant growth by Mg\(^{2+}\) deficiency (Khan et al. 2000). Our results revealed a decreased Mg\(^{2+}\) content in response to NaCl stress in the roots of both cultivars. This decrease may influence the activity of some enzymes, which requires Mg\(^{2+}\) for catalysis as well as chlorophyll synthesis. Moreover, salinity treatment decreased significantly the Fe\(^{3+}\), Pi contents in BS cultivar while they were unchanged in BZ cultivar. Although phosphorus is not typically considered the most limiting nutrient of plant growth, negatively affected the growth of BS seedlings. With regarding these data, it seems that the reduction of growth and chlorophyll content in BS cultivar under salinity stress might be a consequence of disturbance in nutrient uptake.

**Conclusion**

This work integrates morphological, physiological, and biochemical responses of two pistachio cultivars to NaCl stress. These two cultivars showed some variation in response to salinity. It is interesting to note that the salinity stress differently modulated the nutrient levels and these responses were cultivar dependent. Growth parameters such as RGR, leaf number, and shoot length were decreased in both cultivars while root to shoot length ratio increased in BZ cultivar. While BS showed some extent of mortality in high level of salinity, there was no sign of death in BZ cultivar. Higher amount of chlorophyll, maintenance of carotenoids, and increase in proline and soluble sugar contents in both leaves and roots, BZ cultivar make it more tolerant to salinity stress. Moreover, in high salinity treatment BS seedlings showed higher Na\(^+\)/K\(^+\) ratio in the roots than that of the leaves. High accumulation of toxic ion in root leads to avoid leaf from deleterious effects of ion toxicity and protection of photosynthesis process. Less reduction in key nutrients (K\(^+\), Ca\(^{2+}\)) or maintenance constant levels of nutrient (Fe\(^{2+}\) and Pi) was observed in BZ. In term of growth and ion accumulation, different organs showed some extent of variation. Finally, high interspecific variability was observed in growth, physiological, and biochemical parameters. These variations can be useful in early screening for salt tolerance between pistachio rootstock.

**Acknowledgment**

The authors thank Dr. Khezieri for providing seeds of the two pistachio cultivars.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**References**

Acosta-Motoso JR, Diaz-Vivancos P, Álvareza S, Fernández-García N, Sánchez-Blancoa MJ, Hernández JA. 2015. NaCl-induced physiological and biochemical adaptive mechanisms in the ornamental Myrtus communis L. plants. J Plant Physiol. 183:41–51.

Ashraf M. 2003. Relationships between leaf gas exchange characteristics and growth of differently adapted populations of blue panic grass (Panicum antidotale Retz.) under salinity or waterlogging. Plant Sci. 165:69–75.

Ashraf M, Harris PJC. 2004. Potential biochemical indicators of salinity tolerance in plants. Plant Sci. 166:3–16.

Ashraf M, Harris PJC. 2013. Photosynthesis under stressful environments: an overview. Photosynthetica. 51:163–190.

Baastam N, Baninassab B, Gholabzadeh C. 2013. Improving salt tolerance by exogenous application of salicylic acid in seedlings of pistachio. Plant Growth Regul. 69:275–284.

Bates LS, Walderd RP, Teare ID. 1973. Rapid determination of free proline for water stress studies. Plant Soil. 39:205–208.

Carvajal M, Cerda A, Martínez V. 2000. Does calcium ameliorate the negative effect of NaCl on melon root water transport by regulating aquaporin activity? New Phyol. 145:439–447.

Chelli-Chaabouni A, Ben Mosbah A, Maalej M, Gargouri K, Gargouri-Bouzid R, Driira N. 2010. In vitro salinity tolerance of two pistachio rootstocks: Pistacia vera L. and P. atlantica Desf. Environ Exp Bot. 69:302–312.

Clarke AE, Anderson RL, Stone BA. 1979. Form and function of arabinogalactans and arabinogalactan-proteins. Phytochemistry. 18:521–540.

Cramer GR, Läuchli A, Polito VS. 1985. Displacement of Ca\(^{2+}\) by Na\(^+\) from the plasmalemma of root cells a primary response to salt stress? Plant Physiol. 79:207–211.

Cramer GR, Lynch J, Läuchli A, Epstein E. 1987. Influx of Na\(^+\), K\(^+\), and Ca\(^{2+}\) into roots of salt-stressed cotton seedlings: effects of supplemental Ca\(^{2+}\). Plant Physiol. 83:510–516.

Demmig-Adams B, Adams WW. 1996. The role of xanthophylls cycle carotenoids in the protection of photosynthesis. Trends Plant Sci. 1:21–26.

Dubois M, Gille KA, Hamilton JK, Rebers PA, Smith F. 1956. Colorimetric method for determination of sugars and related substances. Anal Chem. 28:350–356.

Edmond Ghanem M, Han RM, Classen B, Quin-Leclerc I, Mahy G, Ruan JC, Qin P, Pérez-Alfoce F, Lutts S (2010) Mucilage and polysaccharides in the halophyte plant species Kosteletzkya virginica: localization and composition in relation to salt stress. J Plant Physiol. 167:382–392.

Epstein E. 1998. How calcium enhances plant salt tolerance. Science. 280:1906–1907.

Ferguson L, Poss JA, Grattan SR, Grieve CM, Wang D, Wilson C, Donowon TJ, Chao CT. 2002. Pistachio rootstocks influence scion growth and ion relations under salinity and boron stress. J Am Soc Hortic Sci. 127:194–199.

Flowers TJ, Colmer TD. 2008. Salinity tolerance in halophytes. New Phyol. 179:945–963.
Franz G. 1979. Metabolism of reserve polysaccharides in tubers of *Orchis morio* L. Planta Med. 36:68–73.

Golldack D, Quigley F, Michalowski CB, Kamasani UR, Bohnert HJ. 2003. Salinity stress-tolerant and-sensitive rice (*Oryza sativa*) regulate AKT1-type potassium channel transcripts differently. Plant Mol Biol. 51:71–81.

Gucci R, Tattini M (1997). Salinity tolerance in olive. In: Janik. J. (Ed.), Horticultural reviews, John Wiley & sons, Inc., USA: 177–214.

Hajiboland R, Norouzi F, Poschenrieder C. 2014. Growth, physiological, biochemical and ionic responses of pistachio seedlings to mild and high salinity. Trees. 28:1065–1078.

Hasegawa PM, Bressan RA, Zhu J-K, Bohnert HJ. 2000. Plant cellular and molecular responses to high salinity. Annu Rev Plant Physiol Plant Mol Biol. 51:463–499.

Hoagland DR, Arnon DI. 1950. The water culture method for growing plants without soil. Calif Agric Exp Stat Circular. 347:25–32.

Hoagland DR, Arnon DI. 1950. The water culture method for growing plants without soil. Calif Agric Exp Stat Circular. 347:25–32.

Hokmahadi H, Arzani K, Grierson PF. 2005. Growth, chemical composition, and carbon isotope discrimination of pistachio (*Pistacia vera*) rootstock seedlings in response to salinity. Aust J Agric Res. 56:135–144.

Jiang X, Qi W, Xu X, Li Y, Liao Y, Wang B. 2014. Higher soil salinity causes more physiological stress in female of *Populus cathayana* cuttings. Acta Ecol Sin. 34:225–231.

Karacas B, Bianco RL, Rieger M. 2000. Association of leaf marginal scorch with sodium accumulation in salt-stressed peach. Hort Sci. 35:83–88.

Karimi HR, Maleki Kuhbanani A. 2015. The evaluation of inter-specific hybrid of *P. atlantica* × *P. vera* cv. 'Badami Zaranad' as a pistachio rootstock to salinity stress. J Nuts. 6(2):113–122.

Karimi HR, Maleki Kuhbanani R. 2014. Evaluation of inter-specific hybrid of *P. atlantica* × *P. vera* cv. 'Badami-Rize-Zaranad' as pistachio rootstock to salinity stress according to some growth indices, eco-physiological and biochemical parameter. J Stress Physiol Biochem. 10(3):5–17.

Khan MA, Ungar IA, Showalter AM. 2000. The effect of salinity on growth, water status, and ion content of a leaf succulent perennial halophyte, *Suaeda fruticosa* (L.) Forsk. J Arid Environ. 45:72–85.

Kozlowski TT. 1997. Responses of woody plants to flooding and salinity. Tree physiology monograph no. 1. Victoria, Canada: Heron Publishing. pp. 1–29

Lichtenhaler H, Wellburn AR. 1983. Determination of total carotenoids and chlorophyll a and b of leaf extracts in different solvents. Biochem Soc Trans. 603:591–593.

Liu C, Li C, Liang D, Wei Z, Zhou S, Wang R, Ma F. 2012. Differential expression of ion transporters and aquaporins in leaves may contribute to different salt tolerance in *Malus* species. Plant Physiol Biochem. 58:159–165.

Maathuis FJM, Amtmann A. 1999. K+ nutrition and Na+ toxicity: the basis of cellular K+/Na+ ratios. Ann Bot. 84:123–133.

Marschner H. 1995. Mineral nutrition of higher plants, Second Edition. London: Academic Press.

Martinez-Alcantara B, Martinez-Cuenca MR, Quiñones A, Iglesias DJ, Primo-Millo E, Forner-Giner MA. 2015. Comparative expression of candidate genes involved in sodium transport and compartmentation in *citrus*. Environ Exp Bot. 111:52–62.

Melgar JC, Benloch M, Fernandez-Escobar R. 2006. Calcium increases sodium exclusion in olive plants. Sci Hort. 109:303–305.

Munns R. 2002. Comparative physiology of salt and water stress. Plant Cell Environ. 25:239–250.

Munns R, Tester M. 2008. Mechanisms of salinity tolerance. Annu Rev Plant Biol. 59:563–581.

Niknam V, Bagherzadeh M, Ebrahimzadeh H, Sokhansanj A. 2004. Effect of NaCl on biomass and content of sugars, proline and proteins in seedlings and leaf explants of *Nicotiana tabacum* grown in vitro. Plant Biol. 48:613–615.

Nooghi FH, Mozafari V. 2012. Effects of calcium on eliminating the negative effects of salinity in pistachio (*Pistacia vera*) L. seedling. Aust J Crop Sci. 6(4):711–716.

Parida AK, Das AB. 2005. Salt tolerance and salinity effects on plant: a review. Ecotoxicol Environ Safe. 60:324–349.

Picchioni GA, Graham CJ. 2001. Salinity, growth and ion uptake selectivity of container-grown *Crataegus opaca*. Sci Hort. 90(1):151–166.

Picchioni GA, Miyamoto S, Storey JB. 1990. Salt effects on growth and ion uptake of pistachio rootstock seedlings. J Am Soc Hort Sci. 115:647–653.

Pitman MG. 1988. Whole plant. In: Baker D.A., Hall J.L., editor. Ion transport in plant cells and tissues. Longman Scientific & Technical Harlow U.K. p. 346–391.

Rengel Z. 1992. The role of calcium in salt toxicity. Plant Cell Environ. 15:625–632.

Sairam RK, Tyagi A. 2004. Physiology and molecular biology of salinity stress tolerance in plants.Curr Sci. 86 (3):407–421.

Schachtman D, Liu W. 1999. Molecular pieces to the puzzle of the interaction between potassium and sodium uptake in plants. Trends Plant Sci. 4:281–287.

Shabala S, Cuin TA. 2008. Potassium transport and plant salt tolerance. Physiol Plant. 133:651–669.

Shabala S, Demidchik V, Shabala L, Cuin TA, Smith SJ, Miller AJ, Davies JM, Newman IA. 2006. Extracellular Ca2+ ameliorates NaCl-induced K+ loss from Arabidopsis root and leaf cells by controlling plasma membrane K+-permeable channels. Plant Physiol. 141:1653–1665.

Shamshiri MH, Fattahi M. 2014. Evaluation of two biochemical markers for salt stress in three pistachio rootstocks inoculated with arbuscular mycorrhiza (*Glomus mosseae*). J Stress Physiol Biochem. 10(1):335–346.

Stepien P, Johnson GN. 2009. Contrasting responses of photosynthesis to salt stress in the glycophyte Arabidopsis and the halophyte thallungiella: role of the plastid terminal oxidase as an alternative electron sink. Plant Physiol. 149:1154–1165.

Verbruggen N, Hermans C. 2008. Proline accumulation in plants: a review. Amino Acids. 35:753–759.

Wheatherley PE. 1973. Studies in the water relations of cotton plants. New Phytol. 78:293–305.

Yang F, Xiao X, Zhang S, Korpelainen H, Li C. 2009. Salt stress responses in *Populus cathayana* Rheder. Plant Sci. 176:669–677.

Zhu JK. 2001. Plant salt tolerance. Trends Plant Sci. 6:66–72.