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Effects of calcium on eliminating the negative effects of salinity in pistachio (*Pistacia vera* L.) seedlings

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Abstract

The effects of four salinity levels (0, 30, 60, and 90 mM NaCl) and three Ca levels (0, 0.5, and 1 mM Ca as $Ca(NO_3)_2.4H_2O$) on chemical composition of pistachio seedlings Cv. *Badami* were studied in a completely randomized design (CRD) with four replications under greenhouse conditions. After 170 days, shoot and root Na, Ca, Fe, Mn, Cu, and Zn concentrations were measured. Results showed Ca applications increased shoot and root Ca concentrations. Salinity stress decreased shoot Ca concentration, while increased shoot and root total sodium uptake. It was found that the effects of salinity on the concentrations of Fe, Mn, Cu and Zn in pistachio seedlings were complex. The changes in Fe, Mn, Cu and Zn concentrations under saline conditions depended on the levels of salinity and the plant's organs. However, Ca had a significant effect on pistachio seedlings. The Ca application increased roots Fe, shoots Zn and shoots and root Cu concentrations in pistachio seedlings. The results suggest that supplementary Ca can reduce some adverse effects of high salinity on chemical composition of pistachio seedlings.

Keywords: Cu, Mn, Sand culture, Sodium, Zn.
Abbreviations: CRD-completely randomized design.

Introduction

Salinization of arable lands is a major problem to crop production in many parts of the world and especially in the irrigated fields of arid and semi-arid regions (Grattan and Grieve, 1999). Approximately 12.5% of agricultural lands in Iran are affected by increased or natural salinity (Alkhani and Ghorbani, 1992). Pistachio (*Pistacia vera* L.) has been grown commercially in Iran for many years and, currently, pistachio plantations encompass about 450,000 ha with annual production of around 200,000 tons of pistachio nuts. Most pistachio plantations are on sodic soils and irrigated with low quality and saline waters. Poor quality of irrigation water in association with sodic soils has reduced the pistachio yield over recent years, especially in the south-east of Iran in Kerman, as well as in central Iran. Despite reduced yield upon salinity, pistachio has been described as salt tolerant (Picchioni and Miyamota, 1990; Ferguson et al., 2002; Tavallali et al., 2008) and is potentially an alternative to salt-sensitive pecan (*Carya illionsis*) or almond (*Prunus amygdalus*). Symptoms of salt toxicity and cultivar differences in susceptibility to salinity have been previously described by many authors (Sepaskhah and Maftoun, 1981; Behboudian et al., 1986; Picchioni and Miyamota, 1990; Ferguson et al., 2002; Hokmabadi et al., 2005). Amount of nutrient elements significantly decreased by salinity increasing (El-Arquan et al., 2002; Tavallali et al., 2008; Shahriaripour et al., 2010; Shahriaripour et al., 2011). There is abundant evidence that salinity alters the ion transport and contents of plants (Cramer, 1997). In general, Na uptake and concentrations increased and Ca uptake and concentrations decreased in plant cells and tissues as the external Na concentration increases (Cramer, 1997). The major ions involved in salt stress signaling include Na, potassium (K^+), H^+, and calcium (Ca^{2+}). The interplay of these ions brings a homeostasis to the cell. Sodium is toxic to cell metabolism and has deleterious effects on the functioning of some cellular enzymes. High concentration of Na^+ causes osmotic imbalance, membrane disorganization, reduction in growth, and inhibition of cell division and expansion. In saline soils, the solubility of micronutrients (e.g. Cu, Fe, Mn, Mo and Zn) is particularly low, and plants grown in these soils often experience deficiencies in these elements (Page et al., 1990), but not in all cases. Experimental evidence implicates Ca^{2+} function in salt adaptation. Externally supplied Ca^{2+} reduced the toxic effects of NaCl, apparently by facilitating a higher K^+ to Na^+ selectivity (Parida and Das, 2005, Tavallali et al., 2008). Ca^{2+} is known to act as a regulator of many physiological and biochemical processes in response to abiotic stresses in plants (Bush, 1995; Bowler and Fluhr, 2000). Abiotic stress often leads to increasing free Ca^{2+} in plant, evoking gene expression and activating a series of biochemical responses that allow plants to adapt to the stress (Monroy et al., 1993). Supplemental Ca is known to mitigate the adverse effects of salinity on plant growth and Ca can increase potassium and make a decline in sodium content of plant tissue (Dabuxilatu and Ikeda, 2005). Addition of Ca can reduce Na binding cell walls (Stassart et al., 1981) and the plasma membrane (Cramer et al., 1985). Increasing Ca^{2+} concentration in the growth medium has been reported to increase Na^+ exclusion and K^+ accumulation in root of glycophytes grown in saline environments (Ashraf and O'Leary, 1997). Unlike the vast area used to grow pistachio in Iran, its average amount of production is unfortunately lower than we expect.

Calcium plays an important role in crop production, especially in saline conditions. Genotype responses to supplemental Ca and salinity have already been reported in rice (Grieve and Fujiyama, 1987), *Brassica* species (Schmidt et al., 1993), *Hordeum* species (Suhayda et al., 1992), maize (Alberico and Cramer, 1993), and pistachio (Tavallali et al., 2008). So, this study was aimed to investigate the effects of various amounts of calcium under salinity conditions on the chemical compositions of pistachio seedlings in sand culture and to determine whether effect of NaCl on chemical composition of pistachio seedling can be minimized by addition of calcium or not.

Results

Shoot and root Ca concentrations

Analysis of variance indicated that salinity, Ca and Ca×salinity interaction had significant effect on shoot Ca concentration, and Ca and Ca×salinity interaction had a significant effect on root Ca concentration of the pistachio seedlings (P=0.01). As salinity levels increased, shoot Ca concentration was reduced (Table 2). For example, with application of 90 mM NaCl, shoot Ca concentration was reduced by 14% in comparison to control. Ca applications to 1 mM increased shoot and root Ca concentrations, whereas Ca application had no significant effect on root Ca concentration. Shoot Ca concentration was more than that of root. At all salinity levels, Ca applications significantly increased shoot Ca concentration but root Ca concentration was increased only at 30 mM NaCl.

Shoot and root total Na uptake

Salinity and Ca×salinity interaction had significant effect on shoot total sodium uptake. Nevertheless, root total sodium uptake of pistachio seedlings was significantly affected by salinity levels (P=0.01). Total sodium uptake increased in shoot and root with increasing Na concentration in the nutrient solution (Table 1 and Table 2). Application of 0.5 mM calcium decreased total sodium uptake in shoot in the presence of 60 mM of NaCl (Table 2).

Shoot and root Fe concentrations

Shoot Fe concentration of pistachio seedlings was significantly (P=0.01) influenced by salinity and Ca × salinity interaction but root Fe concentration was affected by Ca and Ca × salinity interaction treatment (P=0.01) (Table 2 and 3). Salinity application with 60 mM NaCl increased shoot Fe concentration, whereas no significant difference observed between control and 90 mM NaCl treatment. Addition of 1 mM Ca significantly increased root Fe concentration. The highest Ca level (1 mM Ca) caused a 24% increase of root Fe concentration in comparison to control. Increasing Ca application in the culture medium had a positive influence on root Fe concentration, which can be described as a synergetic relationship. In the absence of salt, application of Ca caused a decrease in shoot Fe concentration, while application of Ca in 30 mM NaCl caused higher shoot Fe concentration. In the absence of Ca, increase of salinity to 60 mM NaCl, decreased the root Fe concentration in comparison to control. However, root Fe concentration increased significantly at the same amount of salinity but under higher level of Ca (1 mM).

Shoot and root Mn concentrations

Analysis of variance indicated that salinity and Ca × salinity interaction had significant effect on shoot and root Mn concentrations of pistachio seedlings (P=0.01) (Table 2 and 3). As the salinity levels increased to 60 mM NaCl, shoot and root Mn concentrations were significantly increased, whereas by increasing salinity to 90 mM NaCl, shoot Mn concentration slightly decreased. However, the reduction in shoot Mn concentration was without statistical difference (P>0.01) in comparison to control. Application of 0.5 mM Ca in all salinity levels (except 30 mM NaCl) caused the reduction in shoot Mn concentration, although just the reduction in 60 mM NaCl was significant. Calcium application to 1 mM decreased Mn concentration in root in the presence of 90 mM NaCl.

Shoot and root Cu concentrations

Salinity, Ca and Ca×salinity interaction had a significant effect on shoot and root Cu concentrations of pistachio seedlings (P=0.01) (Table 2 and 3). Increasing salinity to 60 mM NaCl increased Cu concentration in shoot but decreased Cu concentration in root. Shoot and root Cu concentrations in the highest salinity level (90 mM NaCl) were without statistical difference in comparison to control (P>0.01). Application of 1 mM Ca in the culture medium had a positive influence on the shoot and root Cu concentrations. Shoot Cu concentration increased by application of 0.5 and 1 mM Ca in the presence of 60 and 30 mM NaCl, respectively. But application of Ca in other salinity levels was without statistical difference in comparison to control. Root Cu concentration significantly increased by application of only 1 mM Ca in non-saline condition (0 mM) and 30 mM NaCl.

Shoot and root Zn concentrations

Shoot Zn concentration of pistachio seedlings was significantly (P=0.01) influenced by salinity, Ca and Ca×salinity interaction but root Zn concentration was affected by salinity and Ca×salinity interaction treatment (P=0.01) (Table 2 and 3). Salinity application of 60 mM NaCl increased shoot Zn concentration by 43%, compared to control. The results revealed that increasing salinity to 30 mM increased Zn concentration on root, but adding more amount of salinity caused a significant decrease in Zn concentration. Addition of 1 mM Ca significantly increased the Zn concentration in shoot. In the absence of Ca, application of 60 and 90 mM NaCl did not significantly increase shoot Zn concentration (P>0.01), while adding 0.5 and 1 mM Ca, and increasing salinity to 60 mM NaCl caused higher shoot Zn concentration. However, by increasing salinity to 90 mM NaCl, application of 0.5 and 1 mM Ca caused the lowest shoot Zn concentration. Addition of 1 mM Ca in the presence of 30 mM NaCl significantly increased the Zn concentration in root.

Discussions

In this study, effects of calcium on eliminating the negative effects of salinity and concentrations of some minerals in pistachio *(Pistacia vera L.)* seedlings were investigated. The results of this work demonstrated a significant effect of Ca on the negative effects of salinity.

Table 1. Influence of salinity levels on root total sodium uptake ($mg.pot^{-1}$) of pistachio seedlings.

	NaCl levels (mM) Root			
	0	30	60	90
Mean ($mg.pot^{-1}$)	9/14B	13/66A	16/23A	16/44 A

Means followed by the same letter for each source of variation (small letters for means and capital letters for means of rows and columns) are not significantly different at **P<0.01 by Duncan's test.

Table2. Effect of salinity and Ca applications on shoot nutrient concentrations of pistachio seedlings.

Salinity (mM NaCl)	Ca (mM)	Total sodium uptake mg/pot	Ca (%)	Fe ($\mu g.g^{-1}dw$)	Mn ($\mu g.g^{-1}dw$)	Cu ($\mu g.g^{-1}dw$)	Zn ($\mu g.g^{-1}dw$)
	0	2/65 ef	3/92 bc	130.7 bc	8.4 cd	22.37 c	24.37 bc
0	0.5	2/64 ef	4/20 ab	107.82 ef	7.45 d	17.55 c	17.55 e
	1	2/00 f	4/45 a	104.2 f	8.87 cd	18.15 c	22.55 cd
	0	3/15 def	3/29 de	100.15 f	8.5 cd	18.52 c	20.95 d
30	0.5	6/39 abc	4/50 a	140.65 ab	11.47 ab	21.75 c	24.12 bc
	1	5/27 bcd	4/45 a	141.5 a	9.92 bc	52.07 a	31.35 a
	0	9/06 a	3/25 de	141.37 ab	12 a	55.52 a	26.82 b
60	0.5	4/44 cde	4/21 ab	132.75 abc	9.15 cd	55.75 a	34.2 a
	1	5/91 bc	4/48 a	116.95 de	11.82 ab	40.82 b	31.75 a
	0	5/46 bcd	3/15 e	118.95 d	8.22 cd	23.45 c	24.37 bc
90	0.5	7/13 ab	3/97 b	89.07 g	7.77 d	16.60 c	17.90 e
	1	7/22 abc	3/58 cd	122.52 cd	8.17 cd	23.50 c	16.75 e

Means followed by the same letter for each source of variation (small letters for means and capital letters for means of rows and columns) are not significantly different at **P<0.01 by Duncan's test.

Amount of some nutrient elements was significantly decreased by increasing salinity. The Ca^{2+} is involved in regulatory mechanisms in plants adjusting the adverse environmental conditions such as drought stress (Bowler and Fluhr, 2000), salt stress (Melgar et al., 2007), The Ca^{2+} is an important factor in the resistance of plants to salinity (Mozaffari and Malakouti, 2006). Results of our study showed that NaCl stress led to decrease in Ca concentration in pistachio seedlings (Table 2 and Table 3). The uptake of Ca from the substrate may be depressed because of ion interaction and/or precipitation and increases in ionic strength. Similarly, Tavallali (2008) reported that salinization reduced the total Ca concentration of pistachio seedlings. In our experiment, shoots Ca concentration was more than that of root (Table 2 and Table 3). Marschner (1995) also reported that Ca was preferentially localized in the leaf vacuoles as compared to other elements. Increasing Ca, not only increased Ca concentrations in shoot tissues, but also reduced total Na uptake (Table 1). Total sodium uptake increased in shoot and root with increasing Na concentration in the nutrient solution. The increase of root total sodium uptake was higher than that of shoot (Table 1 and Table 2). Sepaskhah and Maftoun (1982) reported that more amount of sodium is accumulated in roots, because pistachio has sodium desorption mechanism. There is a negative relationship between salt tolerance and Na in plant shoots in some plant species (Hampson and Simpson, 1990). The salt-tolerant plants generally exclude Na from their shoots to prevent Na accumulation in the leaves (Ashraf, 1994). Moreover, it is well known that one of the major mechanisms of salt tolerance in plants is the ability to accumulate salts in roots and prevent the transport of toxic ions to the aerial parts of the plant (Greenvay and Munns, 1980). Calcium application to 0.5 mM decreased total sodium uptake in shoot in the presence of 60 mM of NaCl (Table 2). Dabuxilatu and Ikeda (2005) revealed that Supplemental Ca can decrease sodium content in plant tissues. Salinity application at 60 mM NaCl increased shoots Zn, Fe, Cu and shoot and root Mn concentrations, and there was not any significant difference between control and 90 mM NaCl treatments for shoot Fe, Mn and Cu concentrations. Zn concentration has been found to be increased in shoots of salt-stressed barley (Hassan, 1970), and soybean (Mass, 1972). Salinity increased the Fe concentration in the shoots of pea (Dahiya, 1976), and soybean (Mass, 1972). Salinity influence on Cu concentration of pistachio seedlings was variable. The interaction of salinity and nutrient availability was complex and salinity either increased, decreased, or had no effect on the micronutrient concentration in pistachio seedlings. Two main reasons may be responsible for these complex patterns. First, salinity changes the available concentration of these elements in soils due to an increase in the solubility of micronutrients under saline conditions (Sharply et al., 1992). Second, it is known that genotypes of plants vary widely in their response and ability to metabolize micronutrients efficiency (Marschner, 1995). Moreover, different varieties of the same species may differ in uptake efficiency of micronutrients as well. Thus, it is not surprising that variable results are available on the micronutrients of different species. Some studies with tomato indicated that salinity either had no effect (Al-Harbi, 1995) or increased (Maas et al., 1972; Niazi and Ahmed, 1984) Mn concentration in shoot. Very little attention has been directed toward salinity effect on Cu uptake and accumulation in horticultural crops. Shoot Cu concentration was found to decrease in salt-stressed maize grown in both

Table3. Effect of salinity and Ca applications on root nutrient concentrations of pistachio seedlings.

Salinity (mM NaCl)	Ca (mM)	Ca (%)	Fe ($\mu g.g^{-1}dw$)	Mn ($\mu g.g^{-1}dw$)	Cu ($\mu g.g^{-1}dw$)	Zn ($\mu g.g^{-1}dw$)
0	0	2/90 ab	144.67 g	6.85 e	35.82 def	40.58 bc
	0.5	2/57 de	140.37 gh	7.62 de	44.12 bc	41.22 bc
	1	2/72 bcde	169.12 cd	8.15 de	45.37 b	39.20 bc
30	0	2/46 e	159.62 ef	7.85 de	33.82 ef	40.95 bc
	0.5	2/60 de	131.85 hi	8.67 cd	36.97 def	43.75 b
	1	3/11 a	175.77 c	8.05 de	53.57 a	56.88 a
60	0	2/63 cde	127.87 i	11.17 ab	31.72 f	35.58 cd
	0.5	2/63 cde	153.57 f	9.82 bc	43.27 bc	44.93 b
	1	2/87 abc	203.57 a	12.27 a	33.12 f	36.33 cd
90	0	2/76 bcd	164.1 de	11.22 ab	39.25 cde	35.2 cd
	0.5	2/50 de	134.35 hi	9 cd	43.12 bc	36.53 cd
	1	2/54 de	191.02 b	6.75 e	40.45 bcd	30.08 d

Means followed by the same letter for each source of variation (small letters for means and capital letters for means of rows and columns) are not significantly different at **P<0.01 by Duncan's test.

soil (Rahman et al., 1993) and solution cultures (Izzo et al., 1991). However, NaCl-salinity substantially increased leaf Cu in hydroponically-grown tomatoes. In this study, it was shown that by increasing salinity to 30 mM the Zn concentration in roots increased, but addition of more salinity beyond this rate significantly decreased it. The relatively high concentrations of Na and/or limited water availability in plants, caused by excess soluble salts, were probably responsible for decrease in Zn concentration in tissues under saline conditions. Khosh Kholgh Sima et al. (2009) reported that Ca application partially alleviated the negative effect of NaCl. In conclusion, the concentration of micro-nutrients, shoot Zn, root Cu and Fe concentrations were influenced positively by adding 1 mM Ca, which can be described as a synergetic relationship.

Materials and methods

Plant material and nutrient solution preparation

A factorial greenhouse experiment was carried out as a completely randomized design with four replications in sand culture (perlite) during May-November 2008 at the College of Agriculture, Vali-e-Asr University of Rafsanjan, Kerman, Iran. Treatments consisted of three levels of calcium [0, 0.5 and 1 mM Ca as $Ca(NO_3)_2.4H_2O$] and four levels of salinity (0, 30, 60 and 90 mM NaCl). Plastic pots of 20 × 16 cm size were filled with 1 kg of perlite. Perlite (r=15) used in the experiments was thoroughly washed and sterilized (autoclave-sterilized: 121 ^{0}C, 15 min, at 103 kPa) (Benton, 2003). Pistachio seeds (cv. 'Badami-zarand') (all pistachio seed were prepared in Iran's Pistachio Research Institute from controlled pollination and had a certificate) were placed in muslin sacks and pre-treated for 24 h with 2 gL^{-1} Benomil solution. Four germinated seeds were planted in each pot and irrigated with distilled water. Nine weeks after planting, calcium treatments supplements with Hoagland's solution adjusted to pH 6.5, after which seedlings were thinned to two per pot. The compositions of the nutrient solution (macronutrients) for the first Ca treatment were potassium nitrate (KNO_3: 1), ammonium nitrate (NH_4NO_3: 1), magnesium sulfate ($MgSO_4.7H_2O$: 0.5) and potassium dihydrogen phosphate (KH_2PO_4: 1), for the second Ca treatment were potassium nitrate (KNO_3: 1), potassium dihydrogen phosphate (KH_2PO_4: 1), magnesium sulfate ($MgSO_4.7H_2O$: 0.5), calcium nitrate ($Ca(NO_3)_2.4H_2O$: 0.5) and ammonium sulfate ($(NH_4)_2SO_4$: 0.5) and for the third Ca treatment were potassium hydrogen phosphate (K_2HPO_4: 1), magnesium sulfate ($MgSO_4.7H_2O$: 0.5), calcium nitrate ($Ca(NO_3)_2.4H_2O$: 1) and ammonium sulfate ($(NH_4)_2SO_4$: 0.5) mM. The compositions of the nutrient solution (Micronutrients) that added in all treatments were boric acid (H_3BO_3: 24.25), manganese sulfate ($MnSO_4.H_2O$: 11.83), zinc sulfate ($ZnSO_4.7H_2O$: 3.47), iron-ethylenediaminetetraacetic acid (EDTA-Fe: 1.54), copper sulfate ($CuSO_4.5H_2O$: 1.001) and ammonium molybdate ($(NH_4)_6 Mo_7O_{24}$: 0.04) µM. Also, 0.1 mM NaCl were added to the nutrient solution in all treatments. The volume of the nutrient solution was made up daily with de-ionized water and was replaced once a week. After thirteen weeks, salinity treatments were applied in four intervals, after which seedlings were leached with distilled water. Seedlings grew for 170 days in a greenhouse. Pots were kept at field capacity by irrigating with distilled water (before Ca treatment) and nutrient solution (after Ca treatment) every day.

Determination of concentration of ions

After 170 days, the seedlings were cut at the perlite surface and the root was separated and washed. Shoot and root were dried at 70 ^{0}C for 48 h in an oven weighted and ground. The ground plant samples were ashed at 500 ^{0}C dissolved in 2 N hydrochloric acid (HCl) and made to volume with hot distilled water. The Ca, Fe, Mn, Cu and Zn concentrations were determined by atomic absorption spectrophotometer (GBC Avanta ver.1.33, GBC Scientific, Dandenong, Australia). Plant Na concentration was determined by flame photometry methods (Jenway) (Taffouo et al., 2010).

Statistical analysis

The data was used in analysis of variance (ANOVA) by MSTAT-C (Michigan State University, East Lansing, MI, USA) and means were compared by Duncan's test ($P \leq 0.01$).

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