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Zinc soil application enhances photosynthetic capacity and antioxidant enzyme activities in almond seedlings affected by salinity stress

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Abstract

Zinc is a critical mineral nutrient that protects plant cells from salt-induced cell damage. We tested whether the application of Zn at various concentrations [0, 5, 10, or 20 mg kg^{-1}(soil)] would protect almond (*Prunus amygdalus*) seedlings subjected to salt stress (0, 30, 60, or 90 mM NaCl). All concentrations of Zn, particularly the application of 10 and 20 mg kg^{-1}, increased the net photosynthetic rate, stomatal conductance, the maximal efficiency of PSII photochemistry, and a proline content in almond seedlings grown under salt stress; 20 mg(Zn) kg^{-1} was the most effective concentration. The activity of superoxide dismutase showed a significant increase under salinity stress and Zn application. The catalase activity decreased in the salt-treated seedlings, but recovered after the Zn treatment. Our results proved the positive effects of Zn on antioxidant enzyme activity scavenging the reactive oxygen species produced under salt stress.

Additional key words: abiotic stress; gas exchange; net assimilation rate; reactive oxygen species.

Introduction

Salt stress is one of the major environmental growth-limiting factors for most nonhalophytic plants. High concentrations of salts cannot be tolerated by most crops, a fact that severely limits the use of salt-affected soils for crop production (Tiwari *et al*. 2010). Salinity affects adversely nonhalophytes by inducing injury, inhibiting growth, altering plant morphology and anatomy, often as a prelude to tree mortality. Injury is induced not only by the osmotic effects of salts but also by specific toxic effects resulting from the accumulation of Cl^- and Na^+ (Munns and Tester 2008). Evidence has been summarized for nonosmotic effects resulting from salinity as follows: (*1*) organic solutes do not injure plants at osmolalities higher than the critical concentrations for salt injury, (*2*) individual salts have different critical concentrations for inducing injury, (*3*) certain organic solutes increase the critical salt concentration for inducing injury, and (*4*) injurious effects of salts are antagonized by Ca^{2+} (Levitt 1980, Kozlowski 1997, Tattini and Traversi 2008). Salinity reduces the rate of photosynthesis in plants (Mickelbart and Marler 1996). Although both stomatal and nonstomatal factors have been implicated in the reduction of photosynthesis following flooding with saline water, most of the reduction in photosynthetic rates is the result of nonstomatal effects. In the long term, total photosynthesis is reduced as a result of inhibited leaf development and expansion as well as early leaf abscission (Kozlowski and Pallardy 1997). Estimates suggest that about 34 million ha, including 4.1 million ha of irrigated land, are salt-affected in Iran as the consequence of naturally occurring phenomena (causing primary or fossil salinity and/or sodicity) and anthropogenic activities (Qadir *et al*. 2008). Irrigation with poor quality water is one of the main factors that leads to salt accumulation and the resulting decrease in agricultural productivity.

Almond trees (*Prunus amygdalus* Batsch) have been grown commercially in Iran for many years. At present, almond plantations cover about 170,000 ha, with an annual production of approximately 58,050 metric tons of almond nuts. Almond trees are classified as relatively sensitive to salinity (Maas 1986). Low quality irrigation water in association with the build up of salt in the soil has reduced

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Abbreviations: CAT – catalase; *E* – transpiration rate; EC – electrical conductivity; EL – electrolyte leakage; F_v/F_m – maximum quantum yield of PSII; g_s – stomatal conductance; P_N – net photosynthetic rate; ROS – reactive oxygen species; SOD – superoxide dismutase.
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the almond yields over recent years. This is especially valid for Isfahan in the center of Iran.

The development of methods for induction of stress tolerance in plants is vital and still receives considerable attention. One approach how to overcome the suppressive influence of excess soluble salts is improvement in the nutritional status of soils. Zinc deficiency is now recognized as one of the most critical micronutrient deficiencies in plants grown in calcareous, saline, and sodic soils with high pH values. Alpaslan *et al*. (1999) concluded that, in salt affected areas, Zn application could alleviate Na^+ and Cl^- injury in tomato plants. Shahriaripour *et al*. (2010) have indicated that the additional Zn can alleviate the salt injury of pistachio seedlings under NaCl stress by inhibiting Na^+ and/or Cl^- uptake or translocation. In rice (Iqbal *et al*. 2000) and barley (Abou Hossein *et al*. 2002), it was reported that Zn application repressed Na^+ transport in plants grown in salinized solutions, with concomitant improvement in plant growth. Zinc deficiency depresses plant leaf photosynthetic capacity. The reduction in chlorophyll (Chl) content and the destruction of chloroplast ultrastructure led to decline in photosynthesis in Zn-deficient plants. In cauliflower, reduction in photosynthesis induced by Zn deficiency is associated with a decrease in intercellular CO_2 concentration and stomatal conductance (Sharma *et al*. 1994). Sharma *et al*. (1995) reported a significant role of Zn in the regulation of the stomatal aperture, which is accounted for possible role of Zn in maintaining a high K^+ content in guard cells. Zinc is known to play an important role in modulating the redox balance across membranes, thereby counteracting the negative effects of reactive oxygen species (ROS) generated by oxidative stress by increasing the activity of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT) (Tavallali *et al*. 2010)

As far as we know, there are no reports on the effects of Zn soil application on enhancing the tolerance of almond seedlings to salt stress. However, the Zn rates used in the experiment were based on the soil testing and a model recommended by Iranian Soil and Water Institute (ISWI) (Milani *et al*. 1998). The objectives of this work were to determine the effect of salt stress and Zn on the photosynthetic capacity and antioxidant enzymes activity in almond seedlings and to examine whether soil application of Zn to almond seedling might be a strategy for advancing their salt tolerance.

Materials and methods

Plant materials and Zn application: The experiment was carried out in the greenhouse of the Horticulture Department of Isfahan University of Technology. Soil used in these studies was collected from 0 to 30 cm depth of Dehno village, a region in the northwest of Isfahan. The physical and chemical characteristics of the soil were as follows: the soil texture was loam; pH 7.8; electrical conductivity (EC) of 1.30 dS m^{-1}; available P and Zn of 11.3 and 0.60 mg kg^{-1}(soil), respectively. Available P content in the soil was extracted from the soil with 0.5 M $NaHCO_3$ and determined by a colorometric method (Olsen and Sommers 1990). Available Zn was extracted with diethylenetriaminepentaacetic acid (DTPA) and determined by a atomic absorption spectrophotometer (*Model 3400, Perkin Elmer*, Wellesley, MA, USA) (Chapman and Pratt 1961). The soil samples were air-dried, crushed in order to pass through a 2-mm sieve, and $ZnSO_4 \cdot 7H_2O$ was combined thoroughly with soil at the rate of 0 (Zn0), 5 (Zn5), 10 (Zn10), or 20 (Zn20) mg($ZnSO_4$) kg^{-1}(soil). Each of black polyethylene pots was first filled with a 2-cm layer of well washed sand to improve drainage and on top of this sand 7 kg of Zn-treated soil was added.

Hard shells of bitter almond (*Prunus amygdalus* var. Amara) seeds were scarified by cracking with a hammer, and then were soaked in tap water for 24 h. The nuts were then mixed with moist *Sphagnum* peat (3:1, v/v) and kept at 5 ± 1°C for 30 d in order to be stratified. After stratification period, five germinated seeds were sown directly in each polyethylene pot and all pots were irrigated with distilled water. Thirty days after sowing, seedlings at the four to five leaf stage were thinned to three uniform seedlings per pot.

Two days after thinning, each seedling was exposed to one of the following salt treatments:

Treatment	NaCl concentration [mM]	Electrical conductivity [dS m^{-1}]
S0	0	0.26
S30	30	3.28
S60	60	6.78
S90	90	9.65

Salt treatments were applied to the pots at 3-d intervals in 0.5 L of irrigation water. To avoid osmotic shock, the NaCl concentrations were increasing gradually by adding increments of 30 mM every 3 d until the desired concentration was reached. Various analyses were performed once at the end of experiment, *i.e*., 45 d after salt treatments (DAT).

The treatments were arranged in 4 × 4 factorial experiment in a completely randomized design with four replications (three seedlings per pot). The first factor was Zn at four concentrations and the second factor consisted of four concentrations of NaCl.

Gas-exchange parameters: Measurements of the net photosynthetic rate (P_N), stomatal conductance (g_s), and transpiration rate (E) were made on the youngest fully expanded leaf on each plant using a portable infrared gas

analyser (*LCI*, *ADC Bioscientific Ltd.*, Hoddesdon, UK) and a broadleaf chamber (area of 6.25 cm^2). These measurements were made between 09:00–12:00 h on a sunny day.

Electrolyte leakage (EL) was used to assess membrane permeability. This procedure was based on Lutts *et al*. (1996). Electrolyte leakage was measured using an electrical conductivity meter (*CC-501*, *Elmetron*, Zabrze, Poland). Six leaf discs were taken from the youngest fully expanded leaf on one randomly chosen plant per replicate sample (pot). After three washes with distilled water to remove any surface contamination, the six leaf discs were placed in a test tube containing 10 mL of distilled water. These samples were incubated for 24 h on a shaker at room temperature. The EL of the solution (EL_1) was read after incubation. The same samples were then placed in an autoclave at 120°C for 20 min and the second EL reading (EL_2) was taken after cooling the solution to room temperature. Electrolyte leakage was then calculated as EL_1/EL_2, and expressed as a percentage.

Maximum quantum yield of primary photochemistry in the dark-adapted state: Maximum quantum yield of PSII (F_v/F_m) is useful as an idicator of plant responses to environmental stress (Sayed 2003). The F_v/F_m ratio was determined between 09:00 and 12:00 h using a portable plant efficiency analyser (*PEA*, *Hansatech Instruments Ltd.*, King's Lynn, Norfolk, UK) on the same leaf used for gas-exchange determination. The leaves were dark-adapted for a period of 30 min using leaf clips before measurement. A saturation flash light of 3,000 μmol(photon) m^{-2} s^{-1} was applied to achiveve the maximum fluorescence. The F_v/F_m ratio was calculated as $(F_m - F_0)/F_m$, where F_m and F_0 are the maximum and basal fluorescence yields of dark-adapted leaves, respectively.

Proline content was determined according to Bates *et al*. (1973). Seedling tissue (0.5 g of fresh shoot material, FM) was homogenised in 10 mL of 3% (v/v) aqueous sulphosalicylic acid and filtered through a *Whatman No. 2* filter paper. Two mL of the filtrate were then mixed with 2 mL of acid ninhydrin reagent and 2 mL of glacial acetic acid in a test tube and the mixture was placed in a water bath at 100°C for 1 h. The reaction mixture was then extracted with 4 mL of toluene and the chromophore-containing toluene fraction was aspirated from the aqueous phase by sampler, cooled to room temperature, and its absorbance was measured at 520 nm using a *Shimadzu UV-160A* spectrometer (*Shimadzu Corp*., Kyoto, Japan). Appropriate proline standards (*Sigma Chemical Co*., St. Louis, MO, USA) were included in order to calculate the content of proline in shoot tissue sample.

Enzyme assays: Fresh leaves (0.5 g) of seedlings were ground in 8 mL of 50 mM cold phosphate buffer (pH 7.8) and centrifuged at 15,000 × *g* for 20 min at 4°C. The supernatant was used for the determination of the activities of antioxidant enzymes. The activity of SOD (EC 1.15.1.1) was assayed following the method of Giannopolitis and Ries (1977) which measures ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). The reaction solution (3 mL) contained 50 μM NBT, 1.3 μM riboflavin, 13 mM methionine, 75 nM ethylenediaminetetraacetic acid (EDTA), 50 mM phosphate buffer (pH 7.8), and 50 μL of the enzyme extract. The test tubes containing the reaction solutions were irradiated under a light (15 W fluorescent lamp, *Pars Shahab Lamp Co*., Iran) at 78 μmol(photon) m^{-2} s^{-1} for 15 min. The absorbance of the irradiated solution at 560 nm was determined with a spectrophotometer (*UV-160A*, *Shimadzu Corp*., Kyoto, Japan). One unit of SOD activity was defined as the amount of enzyme which caused 50% inhibition of photochemical reduction of NBT.

The activity of CAT (EC 1.11.1.6) was determined following the method of Chance and Maehly (1955) with some modifications. The activity of enzyme was estimated as the decline in absorbance at 240 nm due to H_2O_2 consumption. The reaction solution (3 mL) contained 50 mM phosphate buffer (pH 7.0) with 0.1 mM EDTA, 5.9 mM H_2O_2, and 0.1 mL of the enzyme extract. The reaction was initiated by adding the enzyme extract. The reduction in absorbance was recorded every 20 s for 3 min; 1 unit decomposes 1 μmol of H_2O_2 per 1 min. The activity of each enzyme was expressed on protein basis.

Statistical analysis: Data were analysed for significant differences using a factorial analysis of variance, with Zn concentration and NaCl concentration as the main factors. Statistical analysis was performed using the *SAS* software *version 8.2* (*SAS Inc*., Cary, NC, USA) and means were compared using the least significant differences (LSD) test at $P \leq 0.05$.

Results

Gas-exchange parameters: Salt stress significantly reduced the P_N; maximum reductions were observed in the S90-treated plants (Tables 1, 2). The application of Zn at the highest concentration significantly increased P_N compared with the untreated controls (Table 2). Interaction between salinity and Zn concentration showed that application of Zn20 caused a significant increase in P_N under higher salt concentrations (S60). Salt stress significantly decreased g_s and E, with maximum reduction observed in plants grown with S90 (Tables 1, 2). The application of Zn at the higher concentrations (Zn10 and Zn20) significantly increased g_s and E compared with the untreated controls (Table 2). Both g_s and E were not affected by interaction of salinity and Zn concentration.

Table 1. Analysis of variance (*ANOVA*) for NaCl salinity (NaCl), zinc concentration (Zn), and their interaction (NaCl × Zn) effects on gas-exchange parameters, physiological parameters, and enzyme activities in almond seedlings grown under salt stress. P_N – net photosynthetic rate; g_s – stomatal conductance; E – transpiration rate; EL_1/EL_2 – leaf electrolyte leakage; F_v/F_m – maximum quantum yield of PSII; PC – proline content; SOD – superoxide dismutase activity; CAT – catalase activity; ns – not significant.

Source of variance	df	*P* values							
		P_N	g_s	E	EL_1/EL_2	F_v/F_m	PC	SOD	CAT
NaCl	3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0038	0.0000
Zn	3	0.0000	0.0248	0.0002	0.0016	0.0202	0.0002	0.0076	0.0000
NaCl × Zn	9	0.0265	ns	ns	0.0000	0.0120	0.0001	ns	ns
Error	48	-	-	-	-	-	-	-	-

Table 2. Effect of Zn treatment on P_N [μmol(CO_2) m^{-2} s^{-1}], g_s [mmol(CO_2) m^{-2} s^{-1}], E [mmol m^{-2} s^{-1}], and EL_1/EL_2 [%] of almond seedlings grown under salt stress. P_N – net photosynthetic rate; g_s – stomatal conductance; E – transpiration rate; EL_1/EL_2 – leaf electrolyte leakage. Values followed by *the same letters* within a column are not significantly different at $P \leq 0.05$ in the LSD test ($n = 4$).

NaCl [mM]	Zn [mg kg^{-1}(soil)]	P_N	g_s	E	EL_1/EL_2
0	0	14.61^{b}	0.13^{ab}	6.21^{a}	18.96^{g}
	5	12.12^{bc}	0.11^{a-d}	4.56^{bc}	17.56^{gh}
	10	14.36^{b}	0.12^{ab}	5.81^{ab}	12.07^{h}
	20	18.01^{a}	0.15^{a}	6.46^{a}	19.97^{g}
30	0	12.54^{bc}	0.07^{c-e}	4.76^{bc}	36.88^{de}
	5	13.29^{bc}	0.10^{b-d}	5.22^{a-c}	51.52^{a}
	10	14.59^{b}	0.11^{a-c}	5.19^{a-c}	31.13^{f}
	20	13.36^{bc}	0.11^{a-c}	5.18^{a-c}	21.90^{g}
60	0	4.42^{g}	0.02^{fg}	2.77^{d-f}	38.81^{cd}
	5	6.12^{fg}	0.03^{e-g}	2.35^{ef}	32.61^{ef}
	10	9.06^{de}	0.06^{de}	4.03^{cd}	40.20^{cd}
	20	10.92^{cd}	0.06^{ef}	5.65^{ab}	42.84^{bc}
90	0	5.82^{fg}	0.02^{fg}	1.61^{f}	46.25^{ab}
	5	5.32^{g}	0.015^{g}	1.58^{f}	38.35^{cd}
	10	7.06^{e-g}	0.015^{g}	1.59^{f}	50.71^{a}
	20	8.08^{ef}	0.04^{e-g}	3.13^{de}	35.84^{d-f}
Means for NaCl salinity					
0		14.77^{A}	0.12^{A}	5.76^{A}	17.14^{D}
30		13.44^{B}	0.10^{B}	5.09^{A}	35.35^{C}
60		7.63^{C}	0.04^{C}	3.70^{B}	38.62^{B}
90		6.57^{C}	0.02^{C}	1.98^{C}	42.79^{A}
Means for Zn concentration					
	0	9.34^{C}	0.06^{B}	3.84^{BC}	35.22^{A}
	5	9.21^{C}	0.06^{B}	3.43^{C}	35.01^{A}
	10	11.26^{B}	0.07^{AB}	4.15^{B}	33.53^{A}
	20	12.59^{A}	0.09^{A}	5.10^{A}	30.14^{B}

Physiological parameters: Leaf EL was affected by both salt stress and the application of Zn (Table 1). Salt stress significantly increased leaf EL, with maximum increase observed in plants grown with S90 (Tables 1, 2). The application of Zn decreased EL in leaf discs, with the largest decrease in leaf EL measured when Zn20 was applied (Table 2). There was a significant interaction between salinity and Zn concentration (Table 1). However, at the highest salt concentration (S90), the greatest decrease in leaf EL occurred at Zn5 and S60 (Table 2)

Salt stress and the application of Zn significantly affected the F_v/F_m ratio (Table 1). The lowest F_v/F_m was observed in the leaves of seedlings treated with S90, which was 19.3% lower than that in the untreated control (Table 3). The Zn treatment significantly increased F_v/F_m in almond leaves. The highest F_v/F_m was observed in the leaves of seedlings treated with Zn10 and Zn20 (Table 3). An interaction between salinity and Zn concentration showed that F_v/F_m was not affected by Zn under S0 and S30 or S60. However, at the highest salt concentration (S90), the maximum F_v/F_m was observed in the leaves of Zn20-treated plants (Table 3). The F_v/F_m in Zn-treated plants was higher than that in the control plants during salt stress, indicating that Zn reduced salt-induced photoinhibition of PSII.

Table 3. Effect of Zn treatment on maximum quantum yield of PSII (F_v/F_m), proline content (PC) [μg g^{-1}(FM)], superoxide dismutase (SOD) [U mg^{-1}(protein)], and catalase (CAT) activity [U mg^{-1}(protein)] of almond seedlings grown under salt stress. Values followed by *the same letters* within a column are not significantly different at $P \leq 0.05$ in the LSD test ($n = 4$).

NaCl [mM]	Zn [mg kg^{-1}(soil)]	F_v/F_m	PC	SOD	CAT
0	0	0.82^{a}	0.33^{hi}	$14.85^{b\text{-}f}$	2.05^{ef}
	5	0.83^{a}	1.57^{g}	$15.00^{b\text{-}f}$	7.54^{a}
	10	0.83^{a}	0.60^{hi}	10.87^{ef}	8.56^{a}
	20	0.83^{a}	0.30^{i}	$16.42^{a\text{-}e}$	$4.17^{c\text{-}e}$
30	0	0.80^{ab}	0.43^{hi}	10.40^{f}	$4.01^{c\text{-}e}$
	5	$0.78^{a\text{-}c}$	0.69^{h}	$12.35^{d\text{-}f}$	4.40^{cd}
	10	$0.79^{a\text{-}c}$	0.57^{hi}	$16.47^{a\text{-}e}$	6.99^{ab}
	20	0.81^{ab}	0.50^{hi}	$16.50^{a\text{-}d}$	$2.43^{d\text{-}f}$
60	0	0.71^{cd}	1.84^{g}	$11.10^{d\text{-}f}$	2.94^{de}
	5	$0.70^{a\text{-}c}$	2.52^{f}	$14.74^{b\text{-}f}$	0.61^{f}
	10	$0.77^{a\text{-}c}$	3.86^{d}	$13.42^{c\text{-}f}$	5.27^{bc}
	20	0.70^{cd}	4.02^{d}	$18.33^{a\text{-}c}$	$3.98^{c\text{-}e}$
90	0	0.53^{e}	5.28^{c}	$15.86^{a\text{-}f}$	0.74^{f}
	5	0.65^{d}	3.04^{e}	$18.44^{a\text{-}c}$	$2.32^{d\text{-}f}$
	10	$0.73^{b\text{-}d}$	5.77^{b}	19.11^{ab}	$4.11^{c\text{-}e}$
	20	$0.76^{a\text{-}c}$	8.45^{a}	21.17^{a}	2.05^{ef}
Means for NaCl salinity					
0		0.83^{A}	0.70^{C}	13.93^{B}	5.58^{A}
30		0.79^{A}	0.55^{C}	14.28^{B}	4.46^{B}
60		0.73^{B}	3.06^{B}	14.39^{B}	3.20^{C}
90		0.67^{C}	5.63^{A}	18.64^{A}	2.30^{C}
Means for Zn concentration					
	0	0.71^{B}	1.96^{C}	13.05^{B}	2.43^{C}
	5	0.75^{AB}	1.97^{C}	15.13^{B}	3.71^{B}
	10	0.78^{A}	2.70^{B}	14.96^{B}	6.23^{A}
	20	0.78^{A}	3.32^{A}	18.10^{A}	3.16^{BC}

The leaf proline content of almond leaves was significantly influenced by salt stress and the application of Zn (Table 1). The highest proline content was observed at S90 compared with those in S0 and at the other salt concentrations (Table 3). Zn treatment increased the proline content in almond leaves. The highest proline content was found in leaves of the seedlings treated with Zn20 [3.32 μmol g^{-1}(FM)], which was 69.4% greater than that of the untreated control (Table 2). There was a significant interaction between salinity and Zn concentration (Table 1). However, the greatest increase in the leaf proline content occurred at Zn5 under S0 or S30 [1.57 and 0.69 μmol g^{-1}(FM), respectively] (Table 3).

Antioxidant enzyme activity: The salinity and Zn treatment, but not their interaction, significantly affected SOD activity (Table 1). With increasing salinity, the SOD activity increased compared with the untreated control (Table 3). Zn treatment increased SOD activity in the almond leaves. The highest SOD activity was found in the leaves of the seedlings treated with Zn20 (18.10 U mg^{-1}), which was 38.7% greater than that of the untreated control (Table 3).

The CAT activity was significantly influenced by salt stress and the application of Zn, but not by their interaction (Table 1). In contrast to SOD activity, salt stress decreased significantly the CAT activity, with maximum decrease observed in plants grown with S90 (Tables 3). The application of Zn increased the CAT activity in leaves, with the largest increase measured at Zn10 (Table 3).

Correlation between and among parameters: In this study, correlations between and among various physiological indices (EL, F_v/F_m, and leaf proline content), gas-exchange parameters, and antioxidant enzymes activity in almond seedlings subjected to salt stress were analysed (Table 4). Results showed that significant correlations occurred among these physiological indices, gas-exchange parameters, and antioxidant enzymes activity. These correlations suggested that gas-exchange parameters (P_N and g_s) were positively correlated with F_v/F_m and CAT activity, but negatively correlated with EL and proline content.

Table 4. Correlations between net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E), leaf electrolyte leakage (EL_1/EL_2), maximum quantum yield of PSII (F_v/F_m), proline content (PC), superoxide dismutase (SOD), and catalase (CAT) activity of almond seedlings grown under salt stress.Values followed by *the same letters* within a column are not significantly different at $P \leq 0.05$ in the LSD test ($n = 4$). Pearson correlation coefficient, ns – not significant, * – significant at $P \leq 0.05$, ** – significant at $P \leq 0.01$.

Parameters	P_N	g_s	E	EL_1/EL_2	F_v/F_m	PC	SOD	CAT
P_N	1	0.78^{**}	-0.03^{ns}	-0.54^{**}	0.57^{**}	0.60^{**}	-0.12^{ns}	0.45^{**}
g_s	-	1	-0.05^{ns}	-0.57^{**}	0.53^{**}	0.58^{**}	-0.11^{ns}	0.39^{**}
E	-	-	1	-0.05^{ns}	0.07^{ns}	0.28^{*}	0.15^{ns}	-0.09^{ns}
EL_1/EL_2	-	-	-	1	-0.50^{**}	0.50^{**}	0.07^{ns}	-0.36^{**}
F_v/F_m	-	-	-	-	1	-0.50^{**}	-0.13^{ns}	0.43^{**}
PC	-	-	-	-	-	1	0.44^{**}	-0.31^{**}
SOD	-	-	-	-	-	-	1	-0.18^{ns}
CAT	-	-	-	-	-	-	-	1

Discussion

In the present study, P_N, g_s, and E decreased with increasing salinity. Salt stress hinders photosynthesis at multiple levels, such as pigments concentrations, stomatal functioning, gas exchange, and enzyme activities (Sudhir and Murthy 2004). Salts may build up in the chloroplast and exert a direct toxic effect on photosynthetic processes (Munns and Tester 2008). Excessive salt concentrations can decrease partial CO_2 pressure and internal CO_2 concentrations (Bethke and Drew 1992) due to stomata closure, therefore the acceptance of electrons from PSI is decreased by lower availability of $NADP^+$ (Hernández *et al*. 1995). A decline in photosynthetic capacity under salt stress is often associated with the generation of ROS as well (Noreen *et al*. 2010). Our findings, *i.e*., the enhancement of photosynthetic parameters due to Zn under salt stress, were in agreement with that of previous works in pistachio seedlings (Tavallali *et al*. 2009). It has been reported that decrease in photosynthesis induced by Zn deficiency is correlated with a decrease in internal CO_2 concentration and stomatal conductance (Hu and Sparks 1991). Zn has an important role in stomatal regulation and its role was correlated with maintenance of a high potassium concentration in guard cells and in maintaining membrane integrity (Sharma *et al*. 1995, Tavallali *et al*. 2010). The results of the present study suggested that changes in P_N were due to stomatal limitations.

Maintaining integrity of cellular membranes under salt stress is considered an integral part of salinity tolerance mechanism (Stevens *et al*. 2006). The increase of EL in leaves under salt stress is often correlated with lipid peroxidation and membrane damage (Stevens *et al*. 2006, Bastam *et al*. 2013). Our results showed that Zn treatment decreased leaf EL under salt stress. Zn may alleviate the membrane damage induced by salt effects *via* ROS scavenging, and thus maintain membrane permeability in its proper range (Tavallali *et al*. 2009). Loss of membrane integrity under Zn deficiency may affect the uptake and accumulation of Na^+ at toxic concentrations in plants.

The F_v/F_m ratio is correlated with the efficiency of leaf photosynthesis. A decline in this ratio provides an indicator of photoinhibitory damage caused by the incident photon flux density when plants are subjected to a wide range of environmental stresses (Björkman and Demmig 1987). A fluorescence ratio of 0.8 is indicative of healthy leaf tissue capable of maximum photosynthesis (Webb and Fletcher 1996). The maintenance of F_v/F_m in Zn-treated plants under stress has been observed in previous studies (Wang *et al*. 2009).

The leaf proline content of almond seedlings under salinity conditions was higher than that of control, and the application of Zn increased it. An increase in leaf proline content has been previously reported in citrus seedlings under salinity stress (Khoshbakht *et al*. 2014). Our results are consistent with that reported by Shahriarpour *et al*. (2010), who showed that Zn application increased the proline content of pistachio seedlings grown under stress conditions. Proline has multiple functions in plants, including regulation of osmotic pressure, protection of membrane integrity, stabilization of enzymes/proteins, maintenance of appropriate $NADP^+$/NADPH ratios, and as a scavenger of free radicals (Hare and Cress 1997). Our results showed a substantial increase in the proline content following the Zn treatment, which might be attributed to strategies adapted by plants in order to cope with stressful conditions.

Salt stress led to the increase in SOD activity. The increase of SOD activity under salt stress was previously reported by Koca *et al*. (2007) and Idrees *et al*. (2011) in other plant species. Under salt stress conditions, plants were overloaded with ROS, which inhibited many plant functions and caused damage to plants in different ways (Aftab *et al*. 2011). In order to prevent ROS accumulation, various ROS-scavenging enzymes have been stimulated under salt stress. SOD is the major defense enzyme against ROS as it is the major scavenger of $O_2^{\cdot -}$ (Cakmak 2000). In agreement with the present results, seedlings of pistachio treated with Zn showed higher SOD activity compared with control seedlings under salt stress (Tavallali

et al. 2010). Cakmak (2000) reported that in a number of plant species Zn deficiency reduces CuZn-SOD activity, while a resupply of Zn rapidly restores the enzyme activity because the Zn atom is an essential structural component for the normal functioning of CuZn-SOD.

In the present experiment, the CAT activity decreased with increasing salinity. The decrease in CAT activity in response to salt stress is a phenomenon that occurs in many plant species (Shim *et al*. 2003, Hossain *et al*. 2013). In some cases, however, CAT activity increases following NaCl treatment, as observed in rice (Lin and Kao 2000) and cucumber (Lechno *et al*. 1997). This indicates that enzyme responses may vary according to the plant species. Our results showed that Zn application increased the CAT activity under salt stress and the results were consistent with that reported by Weisany *et al*. (2012). Zn is indirectly required for a high activity of the enzymes involved in H_2O_2 detoxification such as CAT. In Zn-deficient plants activity of CAT was reduced, although Zn is not a required cofactor for CAT activity. This decrease in CAT activity with Zn deficiency was assumed to be related to inhibition of CAT by $O_2^{\cdot-}$ (Cakmak and Marschner 1988).

Conclusion: In summary, the response of almond seedlings to Zn nutrition and salinity treatments outlined in this study suggests that the application of Zn could partially protect almond seedlings against injury by salt stress. Zn applied at 10 and 20 mg kg^{-1}(soil) was the most effective concentrations in providing almond seedlings with salt tolerance, especially at higher concentrations of NaCl.

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