Zinc alleviates salt stress and increases antioxidant enzyme activity in the leaves of pistachio (*Pistacia vera* L. 'Badami') seedlings

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Abstract: The mechanism(s) by which Zn alleviates NaCl stress was investigated in *Pistacia vera* L. 'Badami'. Pistachio seedlings were treated with 0, 800, 1600, 2400, and 3200 mg of NaCl kg^{-1} of soil, along with Zn (0, 5, 10, and 20 mg kg^{-1} of soil). NaCl stress induced high oxidative stress, increasing lipid peroxidation, electrolyte leakage, and lipoxygenase (LOX, E.C. 1.13.11.12) activity to high levels. Zn supplement efficiently reduced all these adverse effects of salt stress. However, there were no significant differences among the above parameters in plants treated with Zn only. NaCl stress significantly increased the activity of antioxidant enzymes. The activity of antioxidant enzymes (superoxide dismutase [SOD, E.C. 1.15.1.1], catalase [CAT, E.C. 1.11.1.6]. and ascorbate peroxidase [APX, E.C. 1.11.1.11]) increased significantly in NaCl + Zn-treated plants, as compared with those treated with either NaCl or Zn alone. These results support the positive effects of Zn on antioxidant enzyme activity scavenging the reactive oxygen species produced in response to salt stress. Zinc treatment caused a significant increase in the amount of phenolics and in ascorbic acid content, as compared with salt treatment alone. Zinc treatment alone did not result in very high antioxidant enzyme activity in non-stressed plants.

Key words: Antioxidant enzymes, pistachio, salt stress, zinc effects

Introduction

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. It is well known that zinc is an important component of many important enzymes, and is a structural stabilizer for proteins, and membrane and DNA-binding proteins (Zn-fingers) (Vallee and Auld 1990). Salt stress is a major obstacle to the successful use of salt-affected soils for plant production. Methods for successfully using saline soils have received worldwide attention. Salt stress was reported to be mitigated by Zn. Zinc could reduce the adverse effects of NaCl (Marschner and Cakmak 1986; Parker et al. 1992). Alpaslan et al. (1999) concluded that, in salt affected areas, zinc application could alleviate Na and Cl injury in plants. In rice (Aslam et al. 2000) and barley (Abou Hossein et al. 2002) it was reported that

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zinc application repressed Na^+ transport in plants grown in salinized solutions, with concomitant improvement in plant growth. Sharma et al. (1994) reported that added zinc enhanced the growth of cabbage, and improved the chlorophyll content and photosynthetic activity in the leaves.

Aerobic organisms face a constant threat from reactive oxygen species (ROS), including the superoxide radical ($O_2^{\bullet-}$), the hydroxyl radical ($OH^{\bullet}$), singlet oxygen ($^1O_2$), and hydrogen peroxide (H_2O_2), which are inevitably generated naturally via a number of cell metabolic pathways (Becana et al. 2000; Kanazawa et al. 2000), such as photosynthesis, photorespiration (Foyer and Noctor 2000), fatty acid oxidation, and senescence (Vitoria et al. 2001). ROS have the potential to interact non-specifically with many cellular components, triggering peroxidative reactions and causing significant damage to membranes and other essential macro-molecules, including photosynthetic pigments, protein, nucleic acids, and lipids (Foyer et al. 1994; Lin and Kao 2000). Therefore, levels of ROS must be carefully monitored and controlled in cells. Plants possess several antioxidant defense systems, comprised of enzymatic and non-enzymatic components that normally keep ROS in balance within the cell. For instance, they use a diverse array of enzymes, such as superoxide dismutases (SODs), catalases (CATs), and peroxidases, as well as low molecular mass antioxidants, including ascorbate and reduced glutathione (GSH), to scavenge different types of ROS (Foyer et al. 1994). SOD catalyzes the dismutation of superoxide to hydrogen peroxide and oxygen. However, hydrogen peroxide is also toxic to cells and has to be further detoxified by CAT and/or peroxidases to water and oxygen. An unfortunate consequence of salinity stress in plants is the excessive generation of ROS (Foyer et al. 1994). Excess production of ROS during salinity stress results from impaired electron transport systems in chloroplasts and mitochondria, as well as via other pathways such as photorespiration. Most studies have shown that resistance to salt stress is usually correlated with a more efficient antioxidant system (Olmos et al. 1994; Shalata and Tal 1998).

Zinc is known to have a stabilizing and protective effect on biomembranes against oxidative and peroxidative damage, and loss of plasma membrane integrity, as well as on membrane permeability alteration (Bettger and O'Dell 1981). Zinc ions bind to ligands containing sulfur, nitrogen, and to a lesser extent oxygen, and preferentially bind to the membrane proteins (Bettger and O'Dell 1981). The balance between free radical generation and free radical defense determines the survival of the system. Therefore, Zn may have a role in modulating free radicals and their related damaging effects by enhancing plants' antioxidant systems (Zago and Oteiza 2001).

The present study focused on the influence of Zn treatment on antioxidant enzyme activity in pistachio seedlings (*Pistacia vera* L. 'Badami') under salt stress. Our objectives were to determine whether Zn is involved in regulation of antioxidant enzymes and LPO under salt stress, and to elucidate the physiological mechanisms by which salt stress is alleviated by Zn in pistachio seedlings.

Materials and methods

Plant material and treatments

The experiments were conducted between February and December 2008 in the research greenhouse of the Agricultural College of Shiraz University. The soil used in the study was loamy Chitgar series soils taken from depths of 0-30 cm (fine-loamy, carbonatic, thermic Typic Calcixerepts) located at Sarvestan township, 85 km southeast of Shiraz. Some physical and chemical properties of this soil are given in The soil samples were air-dried and crushed to pass through a 2-mm sieve, and zinc treatments were combined thoroughly with the soil and supplied at the rate of 0, 5, 10, and 20 mg kg^{-1} of soil as $ZnSO_4$, $7H_2O$. Zinc-treated soils were put in plastic pots at the rate of 7.5 kg per pot. Pistachio (*Pistacia vera* L. 'Badami') seeds were placed in muslin sacks and were soaked for 24 h in 0.4% captan solution. The seeds were then planted in sand and kept at 30 °C for 1 week. Eight germinated seeds were planted in each pot and the pots were irrigated with deionized water twice a week to keep the soil water content higher than the field capacity (20%, soil dry weight basis). Nitrogen and P were applied uniformly to all pots at the rate of 50 mg kg^{-1} of soil, and Cu and

Mn at the rate of 5 mg kg^{-1} of soil, as NH_4NO_3, KH_2PO_4, $CuSO_4$, $5H_2O$, and $MnSO_4$, H_2O, respectively. After 25 days 4-leaved seedlings were thinned to 4 per pot. Seven days later, salt treatments of 0 (control), 800, 1600, 2400 and 3200 mg of NaCl per kg of soil were added to the pots at 3-day intervals, each time using 0.5 L of irrigation water. One hundred days after salt treatment, various analyses were performed. Treatments were arranged in a factorial experiment based on a completely randomized design (CRD) with 4 replications.

Determination of the percentage electrolyte leakage

The percentage electrolyte leakage from fresh leaf tissues was determined using an electrical conductivity meter (Ohm 419) and was used to assess changes in cell membrane permeability. The procedure used was based on the method of Sairam et al. (1997). Leaf samples (fifth fully developed leaf from the top) were cut into 1-cm segments; after rinsing 3 times with distilled water to remove surface contamination they were then placed in individual stoppered vials containing 10 mL of distilled water. The vials were kept at 40 °C for 30 min. Electrical conductivity of the bathing solution (EC1) was read after this time. Samples were then placed in a boiling water bath for 10 min and after the bathing solution was cooled to room temperature a second reading (EC2) was taken. ELP was calculated as [1 – (EC1 / EC2)] × 100.

Ascorbic acid content

Ascorbic acid was isolated with 6% trichloroacetic acid from 100 mg of frozen leaf tissue. Four milliliters of the extract was mixed with 2% dinitrophenylhydrazine (2 mL), followed by the addition of 1 drop of 10% thiourea solution (in 70% ethanol). The mixture was boiled for 15 min in a water bath and after cooling at room temperature 5 mL of 80% (v/v) H_2SO_4 was added to the mixture at 0 °C. The absorbance of the solution containing the hydrazone complex was determined at 530 nm in a spectrophotometer (Shimadzu model 160 A) (Mukherjee and Choudhuri 1985).

H_2O_2 determination

Leaf hydrogen peroxide (H_2O_2) content was determined according to Velikova et al. (2000). Frozen leaf tissue (70 mg) was homogenized in an ice bath with 5 mL of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 12,000 rpm for 15 min and 0.5 mL of the supernatant was added to 0.5 mL of 10 mM potassium phosphate buffer (pH 7) and 1 mL of 1 M KI. The absorbance of the supernatant was read at 390 nm in a spectrophotometer (Shimadzu model 160 A).

Phenolics determination

Phenolic compounds in frozen leaf tissue were extracted with 80% methanol. To 200 μL of the extract were added 1 mL of Folin-Ciocalteau reagent and 800 μL of sodium carbonate solution (7.5%) reagent. Subsequently, the mixture was incubated in the dark for 45 min. Finally, the absorbance was read at 790 nm in a spectrophotometer (Shimadzu model 160 A) (AOAC, 1990). The amount of phenolic compound was reported as gallic acid equivalents (mg) using the linear equation based on the standard curve.

Lipid peroxidation

Lipid peroxidation was determined by measuring the amount of malondialdehyde (MDA) formed using the thiobarbituric acid reactive substances (TBARS) method described by Heath and Parker (1968). Frozen leaf samples (0.5 g) were homogenized in 10 mL of 0.1% trichloroacetic acid (TCA) and the homogenate was centrifuged at 15,000 rpm for 15 min. To a 1.0-mL aliquot of the supernatant, 4.0 mL of 0.5% thiobarbituric acid (TBA) in 20% TCA was added. The mixture was then heated at 95 °C for 30 min in an oven, and then cooled in an ice bath. After centrifugation at 10,000 × g for 10 min, the absorbance of the supernatant was recorded at 532 and 600 nm. The MDA content (nmol g^{-1} FW) was calculated using an extinction coefficient of 155 mM cm^{-1} after subtracting the non-specific absorbance at 600 nm.

Lipoxygenase activity (LOX, E.C. 1.13.11.12)

Plant material (300 mg of frozen leaf samples) was homogenized in 50 mM sodium phosphate buffer (pH 7.0), 1 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 2% (w/v) polyvinylpyrrolidone (PVP), 1% (v/v) glycerol, and 0.1% (v/v) Tween 20. The extract was centrifuged at 15,000 × g for 20 min and the supernatant was immediately used to assay for lipoxygenase activity, according to Ederli et al.

(1997). LOX activity was measured spectroscopically at room temperature by the addition of 1 mM linoleic acid in 0.1 M sodium acetate buffer (pH 5.6) to the extract and by reading the increase in absorbance at 234 nm. The extinction coefficient of $(25 \text{ mM L}^{-1})^{-1}$ cm^{-1} was used to convert absorbance values to micromoles of conjugated diene. One unit of activity was defined as the amount of enzyme that catalyzed the synthesis of 1 μmol of hydroperoxide (HPOD) min^{-1}. Enzyme activity was expressed in katals.

Enzyme assays

Treated plant material (1 g of frozen leaf samples) was ground in 50 mM sodium phosphate buffer; pH 7.8 for SOD, and pH 7.0 for CAT and APX. The homogenates were centrifuged at $12,000 \times g$ for 20 min at 4 °C. The supernatants were used to measure the activity of the enzymes. The protein content in the supernatant was determined according to Lowry et al. (1951).

The superoxide dismutase (SOD) activity assay was based on the method described by Giannopotitis and Ries (1977). One unit of enzyme activity was defined as the amount of enzyme required to result in 50% inhibition of the rate of nitro blue tetrazolium reduction measured at 560 nm. Enzyme activity was expressed in katals.

Catalase (CAT) activity was determined according to Cakmak and Marschner (1992). The reaction mixture in a total volume of 2 mL contained 25 mM sodium phosphate buffer (pH 7.0) and 10 mM H_2O_2. The reaction was initiated by the addition of 100 μL of enzyme extract and the activity was measured by determining the initial rate of disappearance of H_2O_2 at 240 nm ($E = 39.4 \text{ mM}^{-1} \text{ cm}^{-1}$) for 30 s. Enzyme activity was expressed in katals.

Ascorbate peroxidase (APX) activity was determined according to Nakano and Asada (1981). The reaction mixture in a total volume of 2 mL consisted of 25 mM sodium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.25 mM ascorbate, 1.0 mM H_2O_2, and 100 μL of enzyme extract. H_2O_2-dependent oxidation of ascorbate was followed by a decrease in the absorbance at 290 nm ($E = 2.8 \text{ mM}^{-1} \text{ cm}^{-1}$). Enzyme activity was expressed in katals.

Statistical analysis

Data are presented as the means for each treatment (n = 4). Means were subjected to analysis of variance using Tukey's HSD test at the 5% probability level. Analysis of variance was performed using SPSS v.13.0 software (SPSS, Inc., Chicago, IL, USA).

Results

Electrolyte leakage percentage (ELP)

NaCl treatment impaired cell membrane permeability by increasing ELP. Zn supplementation (especially at 10 mg kg^{-1} of soil) reduced the electrical conductivity of tissue external solutions in NaCl-treated plants. Treatment with Zn alone showed lower differences in ELP in non-stressed plants (Table 2).

Ascorbic acid content

Responses of pistachio seedling leaves to salinity and zinc interactions are shown in Table 2. Ascorbic acid content in the zinc-deficient seedlings was lower than in those supplied with zinc, particularly at higher NaCl levels. The amount of ascorbic acid in seedlings supplied with 10 mg Zn kg^{-1} of soil was approximately 1 mg 100 mg^{-1} of DW, followed by a slight decrease with 5 and 20 mg Zn kg^{-1} of soil.

Table 1. Some physical and chemical properties of the soil used in the experiments.

Texture	Water content (%, dry wt basis)		pH paste	EC_e (dS m^{-1})	NaHCO$_3$-extractable P (mg kg^{-1} soil)	NH$_4$OAC-extractable K (mg kg soil^{-1})	CEC (Cm$_c$ kg^{-1})	DTPA-extractable Zn (mg kg soil^{-1})
	Field capacity	Permanent wilting point						
Loam	20	10	7.8	1.2	13.5	63	12	1.7

Table 2. Effects of zinc treatments on ascorbic acid content and electrolyte leakage percentage of *Pistacia vera* L. 'Badami' seedlings under NaCl stress.

NaCl levels (mg kg^{-1})	Zn levels (mg kg^{-1})				
	0	5	10	20	Mean
Ascorbic acid [mg (100 mg D.W.)$^{-1}$]					
0	1.045 d†	1.146 bc	1.207 a	1.126 c	1.131 A
800	0.934 fgh	1.126 c	1.176 ab	1.136 bc	1.093 B
1600	0.863 jk	0.984 e	1.106 c	0.954 efg	0.977 C
2400	0.732 l	0.914 ghi	0.974 ef	0.883 ij	0.876 D
3200	0.651 m	0.833 k	0.904 hij	0.732 l	0.780 E
Mean	0.845 D	1.000 B	1.073 A	0.966 C	
ELP					
0	69.47 ijkl	68.67 jkl	65.82 l	68.21 kl	68.04 E
800	73.05 efghi	72.11 ghij	70.94 hijk	71.75 ghijk	71.96 D
1600	76.69 def	74.54 defgh	72.24 ghij	73.02 fghi	74.12 C
2400	82.16 b	77.94 cd	75.24 defg	76.89 de	78.06 B
3200	87.31 a	84.46 ab	81.24 bc	83.07 b	84.02 A
Mean	77.74 A	75.54 B	73.09 C	74.59 B	

	Ascorbic acid	ELP
NaCl level	**	**
Zn level	**	**
NaCl × Zn	**	**

† Means followed by the same letter (small letters for means and capital letters for means of rows and columns) are not significantly different at 5% level of probability using Tukey's test.
** significant at P < 0.01.

H$_2$O$_2$, MDA, and phenolics content

As expected, salt stress significantly increased the H$_2$O$_2$ content in the leaves of pistachio seedlings. Zn significantly reduced the H$_2$O$_2$ content in both salt-stressed and non-stressed leaves (Figure 1). However, in both controls and in the lowest salinity level (800 mg NaCl kg^{-1} of soil), supplemental zinc at 20 mg kg^{-1} of soil did not significantly reduce the H$_2$O$_2$ content. At higher salinity levels increasing the amount of supplemental Zn significantly reduced the H$_2$O$_2$ content.

The amount of MDA, which represents the degree of lipid peroxidation, significantly increased as salinity increased. The interaction of supplemental zinc with all NaCl levels resulted in significant decreases in the MDA content, as compared with NaCl treatments without Zn. The effects of Zn on reducing MDA levels were especially noticeable at high salinity levels (Figure 1).

Phenolic content, as an antioxidant agent, was highest in the leaves of control plants (Figure 1). As NaCl levels increased phenolic content decreased significantly, as compared to control plants. Zn treatment caused a noticeable increase in phenolic content, in contrast to salt treatment alone. The highest phenolic increase occurred at 20 mg Zn kg^{-1} of soil.

A positive correlation between the percentage of electrolyte leakage, and MDA (r^2 = 0.946) and H$_2$O$_2$ (r^2 = 0.848) was observed (Figure 2).
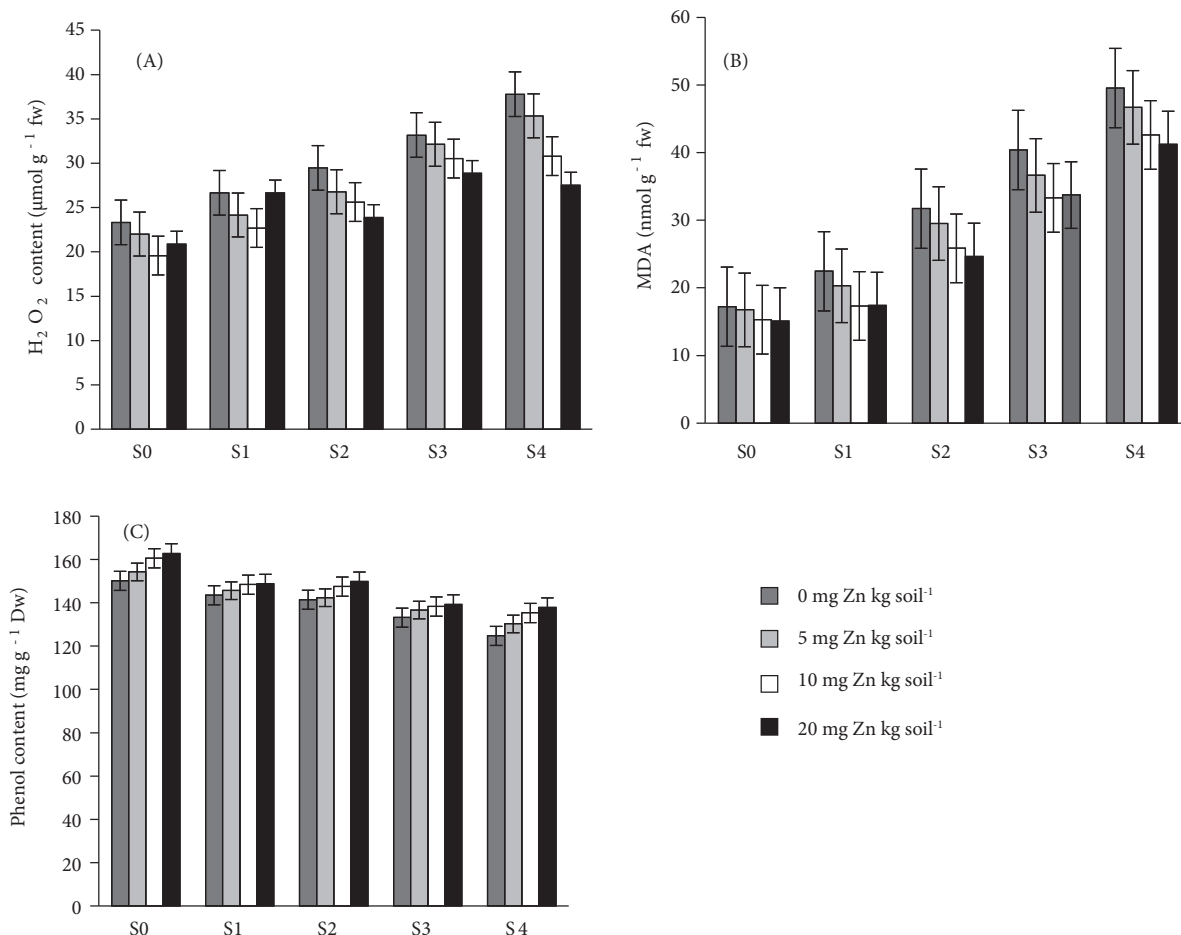
Figure 1. Effects of Zn on H_2O_2 (A) MDA (B) and phenol content (C) in seedling leaves of *Pistacia vera* L. 'Badami' under NaCl stress: S0, S1, S2, S3, and S4 refer to 0, 800, 1600, 2400, and 3200 mg NaCl kg^{-1} soil, respectively.
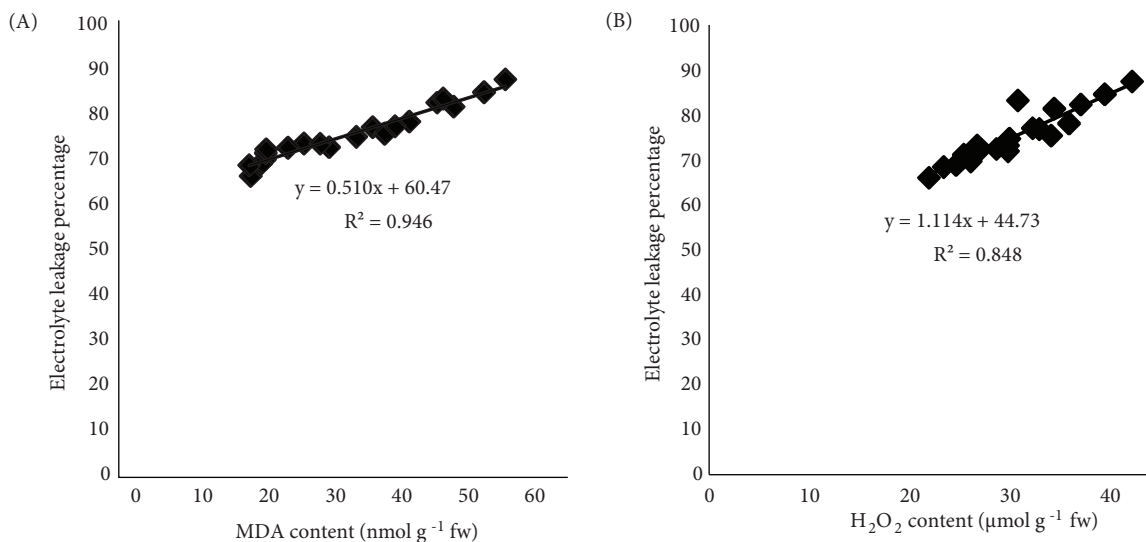

Figure 2. The relations between electrolyte leakage percentage and the concentrations of malondialdehyde (MDA) (A) and hydrogen peroxide (H_2O_2) (B) in treated leaves of pistachio.

Lipoxygenase (LOX) activity

The results obtained for LOX activity also confirmed the role of Zn in membrane stabilization. NaCl treatment resulted in a very high increase (321%) in lipolytic enzyme activity (over 3-fold). This enzyme activity was significantly decreased in NaCl-treated plants supplemented with Zn (especially at 20 mg Zn kg^{-1} of soil), supporting the inhibitory effects of Zn on this fatty acid oxidizing enzyme and on restoring membrane stability. Plants treated with Zn showed only a very small increase in LOX activity (Figure 3).

Antioxidant enzyme activity

Analysis of the antioxidant enzymes SOD, CAT, and APX revealed a higher increase in their activity in NaCl-treated plants supplemented with Zn than in plants treated with NaCl alone. Salt stress significantly increased leaf SOD activity; however, salt stress at the highest NaCl levels slightly increased SOD activity. Zn treatment caused a noticeable increase in SOD activity, in contrast to salt stress alone, with 20 mg Zn kg^{-1} of soil being more effective, indicating the higher efficacy of this antioxidant enzyme in the presence of Zn supplementation (Figure 4).

The trends for CAT and APX activity in the presence and absence of zinc in salt stressed plants were similar to those observed for SOD (Figures 4-6). Zinc treatment was more effective in increasing APX activity than CAT activity. As the levels of Zn increased, enzyme activity increased; however, there were no significant differences between 10 and 20 mg Zn kg^{-1} of soil treatments, especially at higher NaCl levels (Figures 5 and 6). Thus, these results suggest that NaCl-induced membrane damage and oxidative stress were controlled efficiently by Zn supplementation.

Discussion

This study was carried out to analyze the mechanism(s) by which Zn alleviates NaCl toxicity in *P. vera* L. The interaction between a micronutrient and a stressor may be important for understanding, analyzing, and improving plant defense strategies through various factors. The main site of attack by salinity in a plant cell is usually the cell membrane (Logani and Davies 1980). Salinity induces severe lipid peroxidation via ROS. This leads to a cascade of
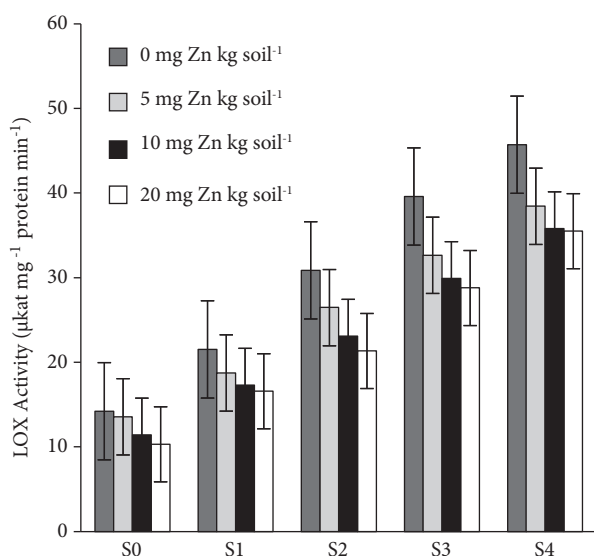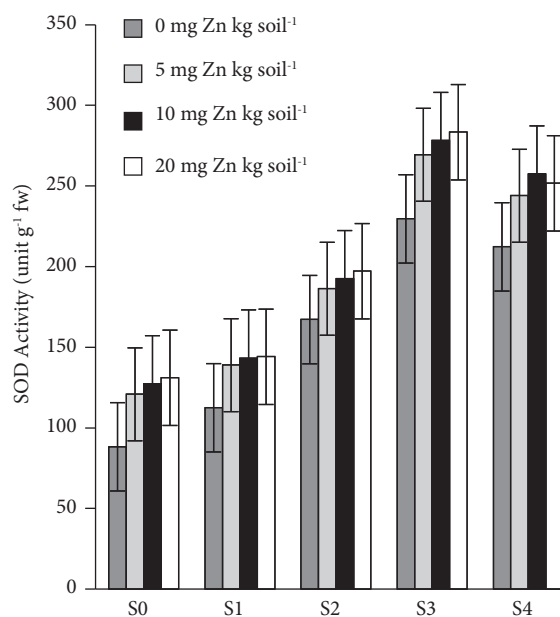

Figure 3. Effects of Zn on LOX activity in seedling leaves of *Pistacia vera* L. 'Badami' under NaCl stress: S0, S1, S2, S3, and S4 refer to 0, 800, 1600, 2400, and 3200 mg NaCl kg^{-1} soil, respectively.
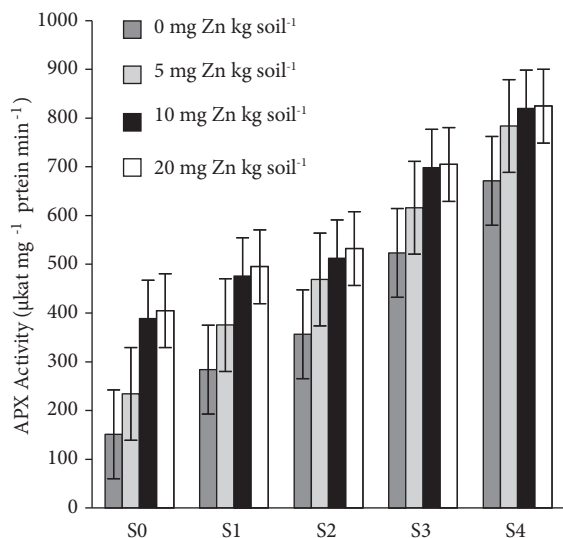

Figure 4. Effects of Zn on SOD activity in seedling leaves of *Pistacia vera* L. 'Badami' under NaCl stress: S0, S1, S2, S3, and S4 refer to 0, 800, 1600, 2400, and 3200 mg NaCl kg^{-1} soil, respectively.

Figure 5. Effects of Zn on APX activity in seedling leaves of *Pistacia vera* L. 'Badami' under NaCl stress: S0, S1, S2, S3, and S4 refer to 0, 800, 1600, 2400, and 3200 mg NaCl kg^{-1} soil, respectively.
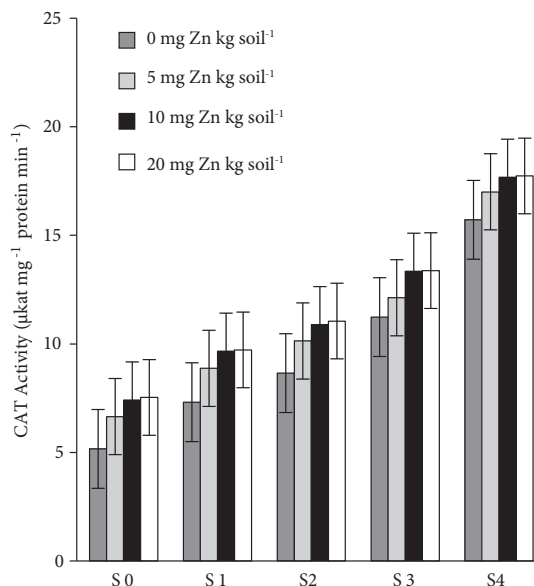

Figure 6. Effects of Zn on CAT activity in seedling leaves of *Pistacia vera* L. 'Badami' under NaCl stress: S0, S1, S2, S3, and S4 refer to 0, 800, 1600, 2400, and 3200 mg NaCl kg^{-1} soil, respectively.

cyclical reactions resulting in the repetitive formation of short chain alkanes and lipid acid aldehydes completely destroying the lipid structure. The other damaging effects of ROS are dimerization and polymerization of proteins, which again alter the cell membranes (Logani and Davies 1980). This is evident from the results of lipid peroxidation, which show that NaCl increased the MDA level (Figure 1). The increase in LOX activity due to salinity suggests higher lipolytic activity of the membrane and oxidation of membrane-bound fatty acids, which propagate lipid peroxidation (Lacan and Baccou 1998).

The present study's results also show that there were very high increases in ion leakage (Table 2), and MDA and H_2O_2 contents (Figure 1) in response to NaCl treatment, indicating that salt stress could damage the integrity of the cellular membrane, as well as cellular components, such as lipids and proteins. We observed that Zn treatment reduced ion leakage and MDA content, thereby alleviating the damage normally caused by salt stress. Fridovich (1986) reported that elevated levels of H_2O_2 and $O_2^{\bullet-}$ caused by stress facilitate the formation of highly active hydroxyl radicals (OH$^\bullet$). These hydroxyl radicals are generally considered to be the most likely ROS to initiate the peroxidation destruction of lipids that lead to membrane damage. In the present study the positive correlation between electrolyte leakage, and MDA and H_2O_2 (Figure 2) supports the hypothesis that salinity induces increases in H_2O_2 and $O_2^{\bullet-}$ levels, thus initiating lipid peroxidation via the formation of hydroxyl radicals, causing membrane damage and ion leakage in pistachio leaves.

Research has conclusively shown that the majority of antioxidant activity may be attributed to phenolic compounds (Rice-Evans et al. 1997). The evidence suggests that zinc treatment increased phenolic content in salt-stressed pistachio seedlings, thus mitigating salt toxicity and improving the growth of seedlings (Figure 1). Lower H_2O_2 content was observed as well when Zn was added under salt stress (Figure 1). These results suggest that Zn can partially prevent oxidative damage in pistachio seedlings due to salt stress. Generation of free radicals and increased lipid peroxidation under salt stress may have resulted in an increase in membrane permeability and loss of membrane integrity (Dionisio-Sesc and Tobita 1998), leading to leaky and damaged membranes, increased electrolyte leakage, and loss of ions.

It is evident from the present study's results that Zn supplementation reduced lipid peroxidation, electrical conductivity, and LOX activity (Figure 3). Zinc has a unique property of existing in a divalent state, without any redox cycling, and is thereby stable in a biological medium in which the oxidoreductive potential is subjected to continuous flux (Vallee and Falchuk 1993). As a result of these Zn properties, membrane lipid packing is protected from ROS peroxidation, which in turn prevents ion leakage from ion channels (Bray and Bettger 1990). In plants treated with only Zn (no NaCl treatment) none of the above parameters significantly changed, indicating that the plant system was affected only in the presence of toxic NaCl concentrations. Zn binds preferably to the -SH groups of the membrane protein moiety, and protects phospholipids and proteins from thiol oxidation and disulfide formation (Chvapil 1973), either by direct binding, binding to a site close to the sulfhydryl group, or by conformational change resulting in apparent stability of the enzymes, membrane proteins, and the lipid structure (Sharma et al. 1994).

Abiotic stress, such as salinity, is known to trigger oxidation of NADPH, leading to superoxide radical production ($O_2^{\bullet-}$) (Kawano et al. 2001). The protective effect of Zn has been reported to be due to its ability to inhibit NADPH oxidation and oxygen-centered free radical generation (Cakmak and Marschner 1988a, 1988b). The superoxide radical ($O_2^{\bullet-}$) produced is deleterious, which indirectly produces a potent oxidant hydroxyl radical ($OH^{\bullet}$) by reacting with H_2O_2. This reaction, known as the site-specific Haber-Weiss reaction, is catalyzed by Fe(III) reduction to Fe(II), followed by the reduction of H_2O_2 by Fe(II). As Fe (III) is strongly associated with polyanionic targets, such as DNA or cell membranes, these oxidants are produced in their immediate vicinity causing the repetition of this reaction (Fridovich 1986).

Osmotic and ionic stress due to salt stress can result in increased concentrations of ROS (Zhu 2003; Parida and Das 2005); therefore, ROS-scavenging antioxidant enzymes, such as SOD, CAT, and APX, play a vital role in removing these destructive oxidant species. By catalyzing the detoxification of $O_2^{\bullet-}$ to O_2 and H_2O_2, SOD blocks cell damage caused by $O_2^{\bullet-}$ (Cakmak 2000). CAT and peroxidases like APX break down H_2O_2 to H_2O and O_2. The results of the present study show that the plants treated with Zn had very high SOD (Figure 4), CAT (Figure 6), and APX (Figure 5) activity, indicating that there was efficient ROS scavenging activity in the system. The combined action of SOD and CAT efficiently eliminates hydrogen peroxide and superoxide, and indirectly protects plants against more toxic hydroxyl radicals. In plants treated only with NaCl (no Zn supplementation) the activity of all these enzymes increased, though insignificantly. The same trend was also observed in plants treated with Zn only, indicating that the increase in enzyme activity in response to NaCl + Zn treatment was due to extreme oxidative stress caused by NaCl and the protection against salt stress by high levels of antioxidant enzymes induced by Zn (Figures 4-6). Probably, Zn is able to facilitate the biosynthesis of antioxidant enzymes (Cakmak 2000).

In plants treated only with NaCl (at each NaCl level), excess ROS produced by salinity stress could have been scavenged by antioxidant enzymes. It has been suggested that very high levels of H_2O_2 inhibit Cu-Zn-SOD (Casano et al. 1997) via the reduction of Cu^{2+} to Cu^+, and that the formation of excess hydroxyl radicals also in turn inhibits Cu-Zn-SOD. Similarly, the CAT enzyme is also sensitive to $O_2^{\bullet-}$ and can be inactivated by increasing superoxide levels (Zago and Oteiza 2001). Aside from these antioxidant enzymes, there are alternative non-enzymatic routes for the conversion of $O_2^{\bullet-}$ to H_2O_2 using an antioxidant agent such as ascorbic acid (Noctor and Foyer 1998). However, upon exposure to high levels of NaCl, we observed a significant decrease in the total level of ascorbic acid, as compared to the control. Zn-induced enhancement of ascorbic acid content is very beneficial for plants, facilitating the detoxification of $O_2^{\bullet-}$ to O_2 and H_2O_2.

In conclusion, Zn effectively protected *Pistacia vera* L. 'Badami' from salt-induced oxidative stress by inhibiting the peroxidation of membrane lipids and lipolytic activity of LOX, and by facilitating the proper functioning of membrane proteins. The observed increase in the activity of the enzymes that scavenge ROS proves the role of Zn as an antioxidant-mediating agent and its roles in alleviating oxidative stress. Hence, it can be concluded that Zn supplementation is beneficial to living systems coping with NaCl toxicity.

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