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ARTICLE

Physiological responses of wheat plant to salinity under different concentrations of Zn

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ABSTRACT In this study, the effects of different concentrations of Zn (1 and 5 µM) on wheat plants were investigated hydroponically at two levels of salinity (50 and 100 mM). High salinity, at both concentrations of Zn, significantly decreased the plant's growth and photosynthetic pigments and protein contents, while increased the membrane permeability, as well as the proline, H_2O_2 and MDH contents. The activities of antioxidant enzymes were not affected by high salinity at low concentration of Zn, but increased significantly at high concentration of Zn. Low salinity, at both levels of Zn, significantly increased the proline and H_2O_2 contents of plants and antioxidant enzymes activities, but did not significantly affect the other studied parameters. There was no significant difference in the studied parameters between plants supplied with two concentrations of Zn at 50 mM NaCl. On the contrary, at 100 mM NaCl, application of high concentration of Zn, significantly alleviated the injurious effects of salt stress by inducing antioxidant enzymes.
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KEY WORDS

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Introduction

Saline condition induces osmotic and ionic stresses in plant cells that lead to a decline in plant's growth and production by affecting physiological processes. Salt stress also induces oxidative stress by generating high levels of oxygen radicals (Chawla et al. 2013). Even if the plant species have evolved different mechanisms against these radicals that include enzymatic and non-enzymatic antioxidant systems, these systems may not act properly in high degrees of salinity or in salt sensitive plants. It has been demonstrated that tolerant plants or cultivars have more efficient antioxidant systems that help them to overcome the oxidative stress (Chawla et al. 2013; Abedini and Daie-Hassani 2015). Plant species are varying in salinity tolerance and the mechanism of tolerance. It has been confirmed that under saline condition, some chemicals could alleviate the negative effects of salt stress on plants. For example, exogenous application of osmoprotectants (Yildirim et al. 2015; Henry et al. 2015), phytohormones (Ghorbani Javid et al. 2011; Ryu and Cho 2015), minerals (Tahir et al. 2012; Wang et al. 2015), polyamines (Saleethong et al. 2013; Zhang et al. 2014) and antioxidants (Rawia Eid et al. 2011; Chenarian et al. 2015) have reduced the adverse effects of salinity on various plants.

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Zinc is an essential micronutrient for carbohydrate and protein metabolisms, membrane integrity, auxin synthesis and reproduction (Alloway 2008). Zinc deficiency is known as one of the most critical micronutrient deficiencies in plants grown in calcareous, saline, and sodic soils with high pH values (Tavallali et al. 2010). It was shown that increased soil salinity can lead to decreased zinc concentration (Genc et al. 2005). Approximately one third of the irrigated area in the world (227 million hectares) is already affected by varying degree of excess salinity/sodicity (Khan and Abdullah 2003). It has been suggested, that improving of Zn nutritional status of plants growing in saline conditions was critical for protection of plants against salt toxicity (Cakmak 2000). The protective role of Zn was ascribed to its role in maintenance of plasma membrane integrity and thus controlling the Na^+ and other toxic ions uptake. Zinc ions, are also known to be strong inhibitors of enzymes generating oxygen radicals and protect salt stressed plants from damaging attack of these compounds (Kawano et al. 2002; Weisany et al. 2012).

Wheat is the main staple crop in the world and in Iran. The proportion of arable land used for growing wheat is 72%. Due to the progressive salinization of the world arable lands, application of exogenous protectants to mitigate salt induced damages has been more important than ever. In this study, the beneficial effects of Zn application on the antioxidant system and growth of wheat plant under saline conditions was investigated.

Table 1. The composition and concentration of the nutrient solution.

Salt	Concentration (μM)
KNO_3	2.5
$Ca(NO_3)_2 \cdot 4H_2O$	2.5
$MgSO_4 \cdot 7H_2O$	1
KH_2PO_4	0.2
Fe-EDTA	50
H_3BO_3	10
$MnCl_2 \cdot 4H_2O$	2
$ZnSO_4 \cdot 7H_2O$	1
$CuSO_4 \cdot 5H_2O$	0.5
$Na_2MoO_4 \cdot 2H_2O$	0.2

Materials and Methods

The seeds of wheat, *Triticum aestivum* L. cv. Shirodi, were obtained from the Agricultural Research Center of Tabriz, Iran.

Plant growth condition

Plants were grown hydroponically in a growth chamber with a temperature of 28/20 °C, 16 h photoperiod and relative humidity of 70%. Seeds were germinated in petri-dishes and transferred to plastic containers with 2 l of 50% modified Hoagland nutrient solution (Millner and Kitt 1992) and pre-cultured for 5 days. Ten days old plants were transferred to the full strength nutrient solution (Table 1), containing 0, 50 and 100 mM NaCl and two levels of zinc (1 and 5 μM) as $ZnSO_4$. The pH of nutrient solution was adjusted to 5.8. After 20 days of treatment, the plants were used for further analyses. Fresh leaf samples were used for enzyme extraction and determination of protein content and metabolites.

Photosynthetic pigments assays

Photosynthetic pigments were extracted using 0.1 g of fresh material in 10 ml of 80% aqueous acetone. After filtering, the chlorophyll a, b and carotenoid contents were determined with spectrophotometer (Shimadzu, Japan) using wavelengths 470.0, 646.8 and 663.2 nm. Concentrations of pigments were calculated using the method of Lichtenthaler (1987).

Enzyme assays

Samples were ground at 4 °C in extraction buffer. Each enzyme assays were tested for linearity between the volume of crude extract and the measured activity. Changes in the absorbance of substrates or products were measured using a spectrophotometer.

Superoxide dismutase (SOD) activity was determined according to Giannopolitis and Ries (1977). The enzyme was extracted in 25 mM HEPES (pH 7.8) and 0.1 mM EDTA, and was centrifuged at 15000 g for 15 min. Test tubes containing 25 μl of enzyme extract, 25 μl extraction buffer and 450 μl of the reaction mixture were incubated at 22 °C and a light intensity of 400 μmol/m^2/s. The reaction mixture contained 25 mM HEPES (pH 7.6), 0.1 mM EDTA, 50 mM Na_2CO_3 (pH 10.2), 12 mM L-methionine, 75 μM NBT and 1 μM riboflavin. The reaction was started by removing a dark plastic foil from the surface of samples and continued for 10 min. One unit of SOD was defined as the amount of enzyme required to induce a 50% inhibition of NBT reduction as measured at 560 nm, compared with control samples without enzyme aliquot.

Peroxidase (POD) activity was determined using the guaiacol test (Chance and Maehly 1955). The enzyme was extracted by 10 mM phosphate buffer (pH 7.0) and assayed in a solution, contained 10 mM phosphate buffer, 5 mM H_2O_2 and 4 mM guaiacol. The reaction was started by addition of the enzyme extract at 25 °C and was followed 2 min photometrically at 470 nm. The enzyme unit was calculated as enzyme protein required for the formation of 1 μM tetraguaiacol in one min.

Catalase (CAT) activity was assayed by monitoring the decrease in absorbance of H_2O_2 at 240 nm (Chance and Maehly 1955). The enzyme was extracted in 50 mM phosphate buffer (pH 7.0). The assay solution contained 50 mM phosphate buffer and 10 mM H_2O_2. The reaction was started by addition of enzyme aliquot to the reaction mixture and the changes in absorbance were monitored for 2 min. Unit activity was taken as the amount of enzyme which decomposes 1 M of H_2O_2 in one min.

Protein and proline contents assays

Soluble proteins were determined as described by Bradford (1976) using a commercial reagent and BSA as a standard.

Proline contents of samples were assayed using the method of Bates et al. (1973). The proline was extracted with 10 ml of 3% sulphosalicylic acid solution. 2 ml of liquid was reacted with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid in 100 °C for 1 h and reaction was terminated at ice bath. The reaction mixture was extracted by 4 ml toluene. The absorbance of chromophore containing toluene was read at 520 nm. Proline concentration of samples was determined from a standard curve.

Hydrogen peroxide and malondialdehyde assays

The hydrogen peroxide content was estimated according to the Harinasut and co-workers (2003). Samples were homogenized with 0.1% (w/v) trichloroacetic acid (TCA). Mixture was centrifuged at 12000 g for 15 min. To 0.5 ml of the su-

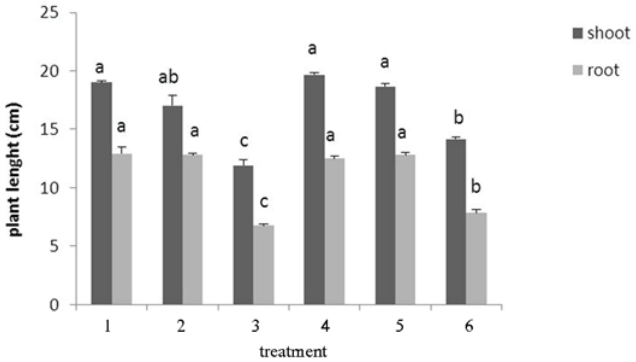

Figure 1. Effect of Zn application on shoots and roots lengths of wheat under salinity. 1: Zn 1 µM + NaCl 0 mM; 2: Zn 1 µM + NaCl 50 mM; 3: Zn 1 µM + NaCl 100 mM; 4: Zn 5 µM + NaCl 0 mM; 5: Zn 5 µM + NaCl 50 mM; 6: Zn 5 µM + NaCl 100 mM.

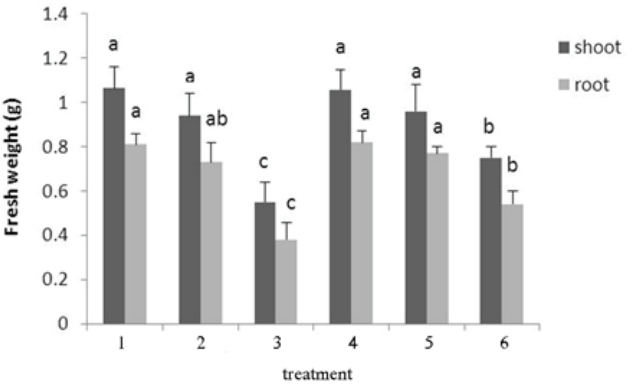

Figure 2. Effect of Zn application on shoots and roots fresh weights of wheat under salinity. 1: Zn 1 µM + NaCl 0 mM; 2: Zn 1 µM + NaCl 50 mM; 3: Zn 1 µM + NaCl 100 mM; 4: Zn 5 µM + NaCl 0 mM; 5: Zn 5 µM + NaCl 50 mM; 6: Zn 5 µM + NaCl 100 mM.

pernatant, 0.5 ml of 10 mM phosphate buffer (pH 7.0) and 1 ml of 1 M potassium iodide (KI) was added. The mixture was incubated at 25 °C for 15 min. The absorbance was measured at 390 nm. The H_2O_2 content was calculated from a standard curve prepared in a similar way.

Lipid peroxidation was estimated from the amount of malondialdehyde (MDA) formed in a reaction mixture (Heath and Packer 1968). Leaf tissues were homogenized in 0.1% (w/v) TCA. The homogenate was centrifuged at 10000 g for 5 min. To 1 ml of the supernatant, 4 ml of 20% TCA containing 0.5% thiobarbituric acid was added. The mixture was incubated at 95 °C in a water bath for 30 min, and then quickly cooled on ice. The mixture was centrifuged at 10000 g for 15 min and the absorbance was measured at 532 nm. MDA levels were calculated from 1,1,3,3-tetraethoxypropan standard curve.

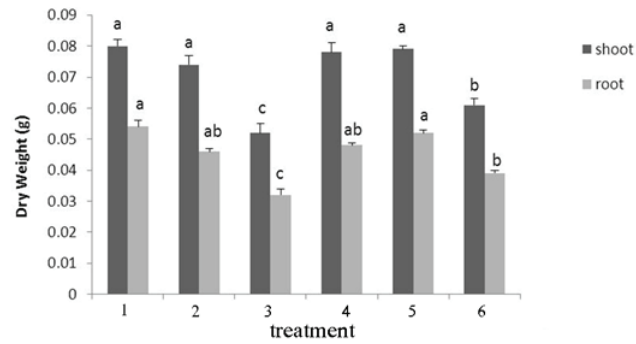

Figure 3. Effect of Zn application on shoots and roots dry weights of wheat under salinity. 1: Zn 1 µM + NaCl 0 mM; 2: Zn 1 µM + NaCl 50 mM; 3: Zn 1 µM + NaCl 100 mM; 4: Zn 5 µM + NaCl 0 mM; 5: Zn 5 µM + NaCl 50 mM; 6: Zn 5 µM + NaCl 100 mM.

Membrane permeability assay

The electrolyte leakage of membrane was assessed as the membrane permeability according to Lutts et al. (1996). Leaf discs (1 cm in diameter), were taken from the well-developed leaves and washed with deionized water, then placed in individual vials containing 10 ml of deionized water. These samples were incubated at room temperature (25 °C) on a shaker (100 rpm) for 24 h. Electrical conductivity (EC) of bathing solution (EC1) was read after incubation. The same samples were placed in an autoclave at 120 °C for 20 min, and the second reading (EC2) was determined after cooling to room temperature. The electrolyte leakage (EL) was expressed following the formula: EL = (EC1/EC2) ×100.

All chemicals and reagents used in this experiment were purchased from Sigma Aldrich, Fluka and Merck.

Statistical analysis

Experiments were conducted in complete randomized design with three replications. Analysis of variance was performed using Sigma Stat (3.02) software. The data were presented as the means ± SE for each treatment. Means were compared with Tukey's Multiple Range Test at the 5% probability level.

Results

Salinity decreased the plant growth parameters include fresh and dry weights and lengths of shoots and roots. The induced reductions in the growth parameters were insignificant at the concentration of 50 mM NaCl, but they were significant ($p<0.05$) at the concentration of 100 mM NaCl in both levels of applied Zn (Fig. 1-3).

Table 2. Effect of Zn application on photosynthetic pigments and membrane permeability of wheat under salinity.

Treatment	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Carotenoids (mg/g FW)	Membrane permeability (%)
Zn 1 µM	2.80 ± 0.034 a	3.06 ± 0.043 a	1.26 ± 0.097 ab	40 ± 1.15 c
Zn 1 µM + NaCl 50 mM	2.30 ± 0.19 ab	2.80 ± 0.2 a	1.2 ± 0.084 b	43 ± 0.577 bc
Zn 1 µM + NaCl 100 mM	1.40 ± 0.035 c	1.09 ± 0.018 b	0.58 ± 0.094 d	75.3 ± 1.2 a
Zn 5 µM	2.83 ± 0.071 a	2.94 ± .052 a	1.33 ± 0.071 a	40. 2 ± 1.31 c
Zn 5 µM + NaCl 50 mM	2.44 ± 0.16 a	2.95 ± 0.09 a	1.24 ± 0.087 ab	41.8 ± 0.64 c
Zn 5 µM + NaCl 100 mM	1.74 ± 0.1 bc	1.93 ± 0.04 ab	0.88 ± 0.072 c	68.4 ± 0.88 b

Each value represented as mean ± SE (n = 3); mean values followed by the same letter (s) are not significantly different ($p < 0.05$).

In saline conditions, application of 5 µM Zn at low salinity non-significantly, but at high salinity significantly ($p<0.05$) increased the growth parameters compared to plants supplied with 1 µM Zn (Fig. 1-3).

In non-saline conditions, there was no significant difference between plant's growths at applied levels of Zn.

Photosynthetic pigments

In plants supplied with 1 µM Zn, chlorophyll a and carotenoid contents decreased slightly and chlorophyll b content was not affected by low salinity, but these parameters decreased significantly ($p<0.05$) by high salinity compare to controls that received only 1 µM Zn. In plants supplied with 5 µM Zn, at low salinity, slight decrease in the carotenoids content and no changes in the chlorophyll a and b contents were seen. At high salinity, significant decreases in the chlorophyll a and carotenoid contents and non-significant decrease in the chlorophyll b content were seen (Table 2).

In saline condition with 50 mM NaCl, application of 5 µM Zn slightly increased the chlorophyll a and carotenoid contents of plants, but had no effect on chlorophyll b content compared to plants supplied with 1 µM Zn. In plants treated with 100 mM NaCl, application of 5 µM Zn slightly increased the chlorophyll a and b contents, and significantly ($p<0.05$) increased the carotenoid content of plants compared to those received 1 µM Zn (Table 2). In non-saline conditions, there were no significant differences between chlorophyll a and b contents of plants supplied by two levels of Zn, while carotenoids content slightly increased with 5 µM Zn (Table 2).

Membrane permeability

Plants treated with 100 mM NaCl, at both levels of applied Zn, showed significantly ($p<0.05$) higher electrolyte leakage of membranes compared to plants treated with 50 mM NaCl and controls that did not receive NaCl. Membrane permeability of 50 mM NaCl treated plants increased slightly at the concentration of 1 µM Zn and was not affected at the concentration of 5 µM Zn (Table 2).

In saline conditions, application of 5 µM Zn with 50 mM NaCl slightly, while100 mM NaCl significantly ($p<0.05$) decreased the membrane permeability of plants compared to plants supplied with 1 µM Zn. In non-saline conditions, membrane permeability showed no significant difference between two applied levels of Zn (Table 2).

Proline content

Proline content of plants significantly ($p<0.05$) increased in saline conditions. Plants treated with 50 mM NaCl had the highest amounts of proline between plants treated with 100 mM NaCl and controls at both levels of Zn (Table 3). In saline conditions, application of 5 µM Zn significantly ($p<0.05$) decreased the proline content of plants, compared to those received 1 µM Zn. In non-saline conditions, two applied levels of Zn had no noteworthy effects on proline content of plants (Table 3).

Protein content

There was a negative relation between salinity levels and protein contents of plants at two levels of Zn. In plants supplied with 1 µM Zn, induced decreases in the protein contents by both levels of salinity were significant ($p<0.05$). While in plants supplied with 5 µM Zn, only induced decrease in the protein content by 100 mM NaCl was significant (Table 3). In saline conditions, application of concentration of 5 µM Zn caused a slight increase in the protein content of plants compared to 1 µM Zn. Protein contents of plants were not affected significantly by Zn levels in non-saline conditions (Table 3).

H_2O_2 and MDA contents

In both levels of applied Zn, H_2O_2 and MDA contents of plants increased significantly by increasing salinity ($p<0.05$) (Table 3). In saline conditions, application of concentration of

Table 3. Effect of Zn application on proline, total protein, MDA and H_2O_2 contents of wheat under salinity.

Treatment	Proline (mg/g FW)	Total protein (mg/g FW)	MDA (nmol/g FW)	H_2O_2 (µmol/g FW)
Zn 1 µM	13.23 ± 1.44 d	75.73 ± 1.5 a	0.296 ± 0.04 d	0.29 ± .048 d
Zn 1 µM + NaCl 50 mM	34.13 ± 1.16 a	68.86 ± 1.79 b	1.76 ± 0.073 c	4.83 ± 0.14 b
Zn 1 µM + NaCl 100 mM	27.48 ± 1.87 b	62.04 ± 1.12 c	2.81 ± 0.06 a	5.65 ± 0.21 a
Zn 5 µM	12.94 ± 1.03 d	77.12 ± 2.1 a	0.28k4 ± 0.06 d	0.31 ± 0.17 d
Zn 5 µM + NaCl 50 mM	30.40 ± 2.40 b	71.8 ± 0.58 ab	0.67 ± .063 cd	3.57 ± 0.18 c
Zn 5 µM + NaCl 100 mM	24.73 ± 2.42 c	64.09 ± 0.53 bc	2.23 ± .166 b	4.59 ± 0.14 b

Each value represented as mean ± SE (n = 3); mean values followed by the same letter (s) are not significantly different ($p < 0.05$).

Table 4. Effect of Zn application on antioxidant enzymes activities of wheat under salinity.

Treatment	SOD activity (U/mg pro)	POD activity (U/mg pro)	CAT activity (U/mg pro)
Zn 1 µM	19.63 ± 0.66 c	1.5 ± 0.06 c	8.28 ± 0.43 bc
Zn 1 µM + NaCl 50 mM	27.39 ± 0.36 a	2.42 ± 0.2 ab	9.51 ± 0.29 ab
Zn 1 µM + NaCl 100 mM	21.17 ± 0.55 bc	1.16 ± 0.031 cd	6.17 ± 0.5 c
Zn 5 µM	20.13 ± 0.32 c	1.47 ± 0.03 c	8.18 ± 0.54 bc
Zn 5 µM + NaCl 50 mM	28.35 ± 0.43 a	2.76 ± 0.14 a	11.13 ± 0.45 a
Zn 5 µM + NaCl 100 mM	26.65 ± 0. 9 a	2.21 ± .049 b	8.87 ± 0.54 b

Each value represented as mean ± SE (n = 3); mean values followed by the same letter (s) are not significantly different ($p < 0.05$).

5 µM Zn reduced the H_2O_2 and MDA content of plants. The reduction in the H_2O_2 content were significant at both levels of salinity, but reduction in the MDA content was significant only at the concentration of 100 mM NaCl. In non-saline conditions, H_2O_2 and MDA content of plants were not affected significantly by Zn levels (Table 3).

Antioxidant enzyme activities

In plants supplied with 1 µM Zn, the activities of antioxidant enzymes SOD and POD increased significantly ($p<0.05$) in response to 50 mM NaCl, but negligible changes were seen in their activities in response to 100 mM NaCl. In plants supplied with 5 µM Zn, the activities of antioxidant enzymes SOD and POD increased significantly in both levels of NaCl. The activity of antioxidant enzyme CAT slightly affected by NaCl levels in plants supplied with 1 µM Zn, but significantly increased in plants treated with 50 mM NaCl that received 5 µM Zn (Table 4). In non-saline conditions, the activities of antioxidant enzymes were not affected significantly by Zn levels in medium. In saline conditions, concentration of 5 µM Zn increased these enzymes activities in both levels of salinity. The induced increases in the activities of antioxidant enzymes were significant for plants treated with 100 mM NaCl ($p<0.05$) (Table 4).

Discussion

The studied wheat cultivar showed sensitivity to 100 mM NaCl that had negative effects on plant growth. This result is parallel to that reported by Erdal et al. (2011). Changes in the plants metabolism in response to salinity could be responsible for the diminished growth of plants under high concentrations of NaCl (Erdal et al. 2011). Salt stress limits plant growth by adversely affecting numerous physiological and biochemical processes, including photosynthesis, antioxidant capacity and ion homeostasis (Ashraf and Harris 2009). The ameliorative effects of sufficient Zn application in improvement of detrimental effects of salinity on plants growth, that was seen obviously in 100 mM NaCl treated plants in this study, have been reported by several authors (Aktas et al. 2006; Tavallali et al. 2010; Weisany et al. 2012). It is clear that the beneficial effects of Zn application on plants growth in different environmental stresses relates to its roles in various metabolic pathways.

In this study, the content of photosynthetic pigments reduced in 100 mM NaCl treated plants. Reduction in the photosynthetic pigment content in saline conditions is reported for numerous salt sensitive plants (Weisany et al. 2011; Askari et al. 2015). It has been suggested, that the

reduction in photosynthetic pigment content may be the result of inhibitory effects of ion accumulation in chloroplasts, such as diminished stability of pigment-protein complexes and activation of enzyme chlorophyllase (Chookhampaeng 2011). Similar to results obtained from this study, the positive effect of Zn application on photosynthetic pigment content in saline conditions has been reported for soybean (Weisany et al. 2011) and tomato (Askari et al. 2015) plants. Zn contribution in chlorophyll synthesis is possibly responsible for this improvement (Balashouri 1995). Carotenoids are the one of components required for salt tolerance in plant species (Hernandez et al. 1995), because they raise the antioxidant capacity of plant, in order to protect the photosynthetic systems (Pérez-Rodríguez, 2009).

Enhancement of membrane permeability and ion leakage, which was seen at high salinity in this study, is a common phenomenon in salt stressed plants (Farhoudi et al. 2015). Sufficient Zn application could deteriorate ion leakage in salt stressed plants; this result is similar to that obtained by Eker et al. (2013). Zinc plays a critical physiological role in the structure and function of biomembranes. It is also known to be required for the maintenance of membranes through the interaction with phospholipids and sulphydryl groups of membrane proteins (Alloway 2008).

Proline accumulated in shoots of studied wheat cultivar as a response to salinity. Accumulation of proline in plants organs as an adaptation mechanism to the salinity and water deficit has been previously demonstrated (Farhoudi et al. 2015). Proline contributes to osmotic regulation of plants under saline condition. It seems that the hydrolysis of soluble proteins provides a pool of compatible osmolytes, which are important in osmotic adjustment in the presence of Na^+ (Ashraf and Harris 2009). The result obtained for the protein content of wheat cultivar is comparable to this view. In this study, proline content of salt stressed plants decreased with sufficient application of zinc. These results are comparable to that obtained by Weisany et al. (2012) in soybean. They suggested that the decline in proline content may be an outcome of the dilution effect resulted from improved plants growth. According to this study, zinc application at adequate amounts increased the protein content in salt stressed plants. Zn is necessary for the activity of the enzyme RNA polymerase and it protects the ribosomal RNA from attack by the enzyme ribonuclease. It has been proposed that the most fundamental effect of zinc on protein metabolism is through its involvement in the stability and function of genetic material (Alloway 2008).

The H_2O_2 and MDA contents of studied wheat cultivar increased in the salinity. Salt stress induces water insufficiency and increases ionic and osmotic effects leading to formation of ROS (Chawla et al. 2013). It has been shown that a plasma membrane bound, NADPH oxidase is involved in the generation of O_2^- following salt stress (Aktas et al. 2006). The increased levels of ROS in plants organs cause oxidative damage to biomolecules, such as lipids and resulting in MDA formation as the breakdown product of polyunsaturated fatty acids of membranes. The inhibitory effect of sufficient concentrations of Zn application on the production of these injurious components in saline conditions has been reported by other authors (Zago and Oteiza 2001; Tavallali et al. 2010; Weisany et al. 2012). Zinc plays a key role in controlling the generation and detoxification of free oxygen radicals and subsequent lipid membrane oxidation (Alloway 2008). It has been demonstrated that Zn ions have strong inhibitory effect on membrane bound, NADPH oxidase (Kawano et al. 2002).

In this study, significant increases in the activities of antioxidant enzymes SOD and POD and slight increase in the activity of CAT were seen at low concentration of NaCl. The increase in the activities of antioxidant enzymes could be the indicator of build-up a protective mechanism to reduce oxidative damages induced by stress (Harinasut et al. 2003; Chawla et al. 2013). It seems that the produced H_2O_2 effectively is removed by POD and CAT at low salinity. The studied cultivar of wheat could not efficiently increase SOD, POD and CAT enzyme activities at high salinity. While application of 5 µM concentration of Zn in these plants could result in increasing antioxidant enzymes activities. It has been proposed that extra amounts of H_2O_2 inhibit Cu-Zn-SOD (Casano et al. 1997) via the reduction of Cu^{2+} to Cu^+. Similarly, the CAT enzyme is too sensitive to O_2^- and can be inactivated by increasing superoxide levels (Zago and Oteiza 2001). In this study, the increased activities of SOD, POD and CAT, that were induced by Zn application at high salinity, could prevent the cell damage caused by oxygen radicals and alleviate the plant's tolerance (Chawla et al. 2013; Abedini and Daie-Hassani 2015). Zn is able to facilitate the biosynthesis of antioxidant enzymes (Cakmak 2000) and its effect on improvement of antioxidant system of salt stressed plants has been reported by several authors (Tavallali et al. 2010; Weiasany et al. 2012) for numerous plant species.

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