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Responses of bread wheat to Zn-Glycine and Zn-Alanine fertilizers under saline soil condition

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ABSTRACT

Zinc (Zn) deficiency and salt stress are well-known soil problems and often happen parallelly in cultivated soils. In this study, Zn-amino acid complexes (Zn-AAc) were used as a source of Zn to determine their effects on salt-induced damage in wheat plants. The bread wheat (*Triticum aestivum* L. cvs. Kavir) was supplied with Zn-glycine (Zn-Gly), Zn-alanine (Zn-Ala), and $ZnSO_4$ as Zn sources at three salinity levels (EC 2, 4 and 6 dS m^{-}). Salinity caused a significant decrease in shoot dry matter and grain yield of wheat, but this negative effect was significantly improved by the application of Zn-AAc. Salt stress decreased shoot and grain Zn concentration, but this reduction was lower in plants supplied by Zn-AAc. Calcium (Ca) and potassium (K) concentrations were increased in a shoot by salinity stress while decreased in grain. Sodium (Na) concentration decreased in shoot and grain by using Zn-AAc. At all of the salinity levels, wheat supplied with Zn-AAc had lower lipid peroxidation compared to those grown under the $ZnSO_4$ source. Application of Zn-AAc increased the activities of catalase (CAT) and superoxide dismutase (SOD) in the roots of wheat plants in saline conditions. Based on the results, the adverse effects of salinity stress on wheat plants can moderately improve with Zn-AAc application.

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Amino acids; catalase; salinity; superoxide dismutase; *Triticum aestivum*; Zn nutrition

Introduction

Zinc (Zn) is one of the essential elements for the plant which plays an important role in many physiological processes such as photosynthesis, respiration, and synthesis of proteins, DNA, RNA, and plant hormones (Havlin 2014). Zn deficiency is the most common micronutrient deficiency (Sims and Johnson 1991), especially in arid and semi-arid regions (Cakmak 2000). Common Zn fertilizers such as $ZnSO_4$ and synthetic Zn chelates including Zn-EDTA and Zn-DTPA are widely used to increase the availability of Zn in the soil and to maintain suitable concentrations of Zn for plants (Khoshgoftarmanesh et al. 2010; Vadas et al. 2007). Inorganic Zn fertilizers are usually ineffective in modifying Zn deficiency because of limitations such as the conversion of soluble Zn forms into unavailable Zn-hydroxide forms in soils (Khoshgoftarmanesh et al. 2010). In contrast, synthetic Zn chelates are often more appropriate for Zn deficiency in agricultural soils, but they are usually expensive (Rodríguez-Lucena et al. 2010). The low degradability of synthetic chelates is also considered an environmental concern (Karthika et al. 2016, Alfosea-Simón et al. 2020).

Considering the several disadvantages of synthetic chelates and their side effects on plants and the environment, it is important to find a suitable alternative. Natural metal complexing agents

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such as amino acids have advantages for preserving nutrients in soils compared to synthetic chelates (Souri 2015, Kazem Souri et al. 2017, Mirbolook et al. 2020b) and can be used as an effective source of Zn in calcareous soils for various plants (Souri and Hatamian 2017; Mirbolook et al. 2020a). Amino acids are natural compounds that can bind to metals such as Zn through their amine (-NH_2) and carboxylate (-COO) groups (Ghasemi et al. 2013). The degradability of metal-amino acid complexes is also lower than that of their free amino acids (Renella et al. 2004, Ghasemi et al. 2013).

Salinity is one of the most important environmental abiotic stresses that has a significant impact on the yield and quality of crops. Reports show that about 20% of the irrigated lands (45 million hectares), which provide about a third of the world's food, are saline (Shrivastava and Kumar 2015). About 6.8 million hectares of arable land in Iran (about 40%) are affected by salinity. Salinity results in an imbalance of the cellular ions causing ion toxicity, nutrient deficiency (e.g. Ca^{+2} and K^{+}), and osmotic stress (Munns et al. 2006), thus effect on plant growth and survival (Rani et al. 2019). High Na^{+} and Cl^{-} concentrations can reduce the absorption of essential elements such as K^{+} (Khoshgoftar et al. 2006). Salinity may affect root uptake, translocation to shoot, and physiological utilization of Zn in plants (Hussein and Abou-Baker 2018). At the molecular scale, salinity increases the production of reactive oxygen species (ROS), which leads to toxicity in plants. (Jiang and Zhang 2001, Alfosea-Simón et al. 2020). ROS are reduced forms of molecular oxygen that are produced in vital processes such as photosynthesis, photorespiration, and respiration (Merwad 2020). These can destroy cell membranes and other macromolecules such as pigments, proteins, DNA, and lipids (Imlay 2003). Plant tissues produce special enzymes such as superoxide dismutase (SOD) and catalase to control ROS levels and protect cells under stress conditions (Apel and Heribert 2004). The balance between ROS production and the activity of antioxidant enzymes determines whether cell destruction has occurred (Møller et al. 2007). An increase in the activity of antioxidant enzymes under salinity can be a sign of increased ROS production under stress.

Studies have shown that Zn plays an important role in increasing the plant's defense capacity against abiotic stresses such as salinity (Alloway 2008, Hassanpouraghdam et al. 2020). The response of plants to salinity and Zn nutrition has been extensively studied (Khoshgoftarmanesh et al. 2005, Khoshgoftar et al. 2006, Yung et al. 2015, Rani et al. 2019). In addition, Zn is involved in the structure of enzymes such as CAT and SOD and Zn deficiency can stimulate oxidative stress in plants by affecting both the production and detoxification of free oxygen radicals and reducing the activity of oxidative enzymes (Cakmak 2000).

Extensive research has also shown that salinity tolerance of plants such as tomato (Mäkelä et al. 1998), wheat (Raza et al. 2006) and corn (Thakur and Rai 1982) is directly related to the concentration of amino acids in the plant. Under salinity conditions, amino acids act as osmolytes and involve in ion transport, the opening of stomata, protein synthesis, the activity of antioxidant enzymes, and the integrity of bio-membranes (Rai 2020, Alfosea-Simón et al. 2020). Significant effects of arginine application have been reported in reducing the adverse effects of NaCl salinity on rice (Lin and Kao 1995), wheat (Abdul-Qado), and bean (Zeid 2009). Also, Hoque et al. (2007) reported the positive role of other amino acids in increasing the activity of catalase and peroxidase enzymes in plants.

Recently, we synthesized complexes of several types of amino acids with Zn and evaluated their efficiency as a Zn source in the hydroponic cultivation of beans (Mirbolook et al. 2021). Amino acid complexes (especially Zn-Gly and Zn-Ala) significantly increased the Zn concentration in bean tissues compared to $ZnSO_4$. These complexes also stimulated the growth and yield of the bean plant. In the continuation of research, the application of Zn-Gly and Zn-Ala in a calcareous soil on the yield and yield components of wheat plants was investigated and it was found that the application of these complexes had an effective role in increasing wheat yield and grain enrichment by Zn (Mirbolook et al. 2020a).

According to the previous findings, we hypothesized Zn-organic complexes (including Zn-Gly and Zn-Ala) as Zn sources, are effective in alleviating salt-induced damages in wheat plants *via* improving antioxidant capacity. This hypothesis was tested by exposing bread wheat, *Tritium aestivum* L. cvs. Kavir which is a Zn-inefficient cultivar, is supplied with $ZnSO_4$, Zn-Gly, and Zn-Ala as Zn sources in three salt levels.

Materials and methods

Plant growth

This experiment was done with three different zinc sources including Zn-Gly, Zn-Ala, and $ZnSO_4$ with a concentration of 8 mg Zn kg^{-} soil and three salinity levels including ECe 2, 4, and 6 dS m^{-} in a completely randomized design with five replications under greenhouse conditions (25 °C, 30.5% w/w field capacity). The wheat plants (*Triticum aestivum* L. cvs. Kavir), most commonly cultivated in Iran and Zn-inefficient cultivar, were obtained from the breeding program of the Agricultural and Natural Resources Research Center of Mashhad, Iran. To create the artificial salinity in the soil, solutions were first prepared with different levels of salinity and various concentrations of them were added to the soil. After one week, the salinity of the soil was determined and a curve between the added saline to the soil and the salinity created in the soil was drawn and the amount of salt needed for the selected salinity was obtained (Moradi et al. 2019).

A bulk surface (0-30 cm) soil sample was sampled from a field with a pH of 7.8 in the Ferdowsi University of Mashhad in Northeastern Iran (36°18'36.2" N 59°31'48.1" E). After air-drying and passing to a 2 mm sieve, electrical conductivity (EC) in the saturated extract of the soil and the pH of 1: 5 ratios (soil: water) were investigated with an EC meter (Metrohm Ohm-644 model, Switzerland) and pH meter (Metrohm 691, Switzerland). Then, soil texture was determined using the hydrometric method (Ashworth et al. 2001) and calcium carbonate ($CaCO_3$) by the titrimetric method with HCl (Keeney and Nelson 1982). Moreover, the organic carbon was analyzed by wet digestion (Davis et al. 2017) and the amount of available Zn in the soil was also extracted with DTPA-TEA (Lindsay and Norvell 1978) and determined by atomic absorption spectrophotometer (AAS) (Perkin Elmer 3030, USA). The related data are shown in Table 1.

To prepare the pots, 4 kilograms of the soil was put into the pots with a height of 40 cm and 30 cm in diameter. Before planting the seeds, uniform rates of N and K fertilizers [100 mg kg^{-} N and K each as $(NH_4)_2SO_4$ and K_2SO_4, respectively] were added to each pot and mixed with soil. Wheat seeds were washed with distilled water and planted in the pots, thinned to five plants per pot after 10 d, and grown for 45 or 60 d. Zn fertilizers were applied with the soil application method (the best application method of Zn-amino acid complexed based Mirbolook et al. 2020a) as a solution with the concentration of 8 mg Zn kg^{-} the soil was injected at once near the seeds.

Table 1. Physical and chemical properties of the soil.

Properties	Unit
EC_e (dS m^{-1})	0.7
pH (H_2O)	7.8
SAR	3.2
Sand (%)	76
Clay (%)	12
$CaCO_3$ (%)	14
DTPA-extractable Zn (mg kg^{-1})	0.4
DTPA-extractable Fe (mg kg^{-1})	1.1
Organic C (g kg^{-1} soil)	2.1
Total N (g kg^{-1} soil)	0.6

Note. EC_e: Electrical conductivity; SAR: Sodium adsorption ratio; $CaCO_3$: Calcium carbonate; DTPA: Diethylene triamine pentaacetic acid; Zn: Zinc; Fe: Iron.

All plants were irrigated with deionized water. Salt treatments were applied 18 days after planting (at 3-4 leaf stage).

Elemental analysis

Wheat plants were sampled at maturity from each pot separately. First, the growth and yield parameters of the plants were measured. To determine element concentration in the plants, the shoot and grain were separated and after washing were dried in an oven for 48 h at 75 °C. The dried samples were ground to a fine powder to pass through a 20-mesh sieve. Next, 1 g of dry samples was placed in ceramic vessels and combusted in an electric furnace at 550 °C for 8 h. In addition, the ashed samples were dissolved in HCl 2 M and diluted to reach the range requirement. Then, based on Chapman and Pratt (1962), the concentration of Zn in the samples was performed using an AAS. The concentrations of the shoot and grain K, Ca, and Na were analyzed by a flame photometer (Model 3400, Perkin Elmer, Wellesley, MA) (Cottenie et al. 1982).

Antioxidant enzymes assay

For preparing an enzyme extract, 200 mg of root tissue was mixed with 100 mM TRIS–HCl buffer (pH 8) containing 2 mM EDTA, 5 mM dithiothreitol, 10% glycerol, 100 mM sodium borate, 4% (w/v) insoluble polyvinylpyrrolidone (PVP), and 1 mM phenylmethyl sulfonyl fluoride (PMSF). The solution was filtered and centrifuged at 12,000 *g* for 45 min at 4 °C. The supernatant was separated for enzymatic assays (Bradford 1976).

For the assay of root catalase activity, a 10 mM potassium phosphate buffer (pH 7.0) and 10 mM H_2O_2, and 0.1 mL root extract were used. The initial rate of loss of H_2O_2 was determined at 240 nm for the 70s by spectroscopy (Aebi 1984). Root superoxide dismutase activity was measured by Nishikimi et al. (1972) and stated as units of SOD g^{-} FW. The mixture was prepared with a total volume of 3 mL containing 1.2 mL sodium pyrophosphate buffer (pH 8.3, 0.052 M), 0.1 mL 186 μM phenazine methosulphate, 0.3 mL 300 μM nitroblue tetrazolium (NBT), 0.2 mL NADH (780 μM), 100 μg protein, and distilled water. With the addition of NADH, the reaction was started. The samples were incubated at 30 °C for 90 s and then 1.0 mL glacial acetic acid was added and stirred vigorously. The absorption in samples was evaluated at 560 nm. The negative control was an enzyme-free system.

Lipid peroxidation

For the determination of lipid peroxidation in the roots of wheat, plant material (0.3 g) was homogenized with 3 mL of 0.5% (w/v) the thiobarbituric acid in 20% (w/v) trichloroacetic acid (TCA), which malondialdehyde was determined as an end product of lipid peroxidation. The incubation of the solution was down at 95 °C for 30 min and the reaction was stopped in an ice bath. The centrifuging of samples was down at 10,000 *g* for 5 min and the supernatant absorbance was measured at 532 nm. The amount of nonspecific absorption at 600 nm was withdrawn. The concentration of the MDA-TBA complex was calculated using an absorbance coefficient (155 $mM^{-}cm^{-}$) (Jambunathan 2010).

Statistical analysis

Statistical analysis was performed by SPSS software in a completely randomized design; each treatment contained five replicates. Treatment effects were analyzed *via* general linear models.

Finally, the averages were compared using Duncan's Multiple Range Test at a significant level of $p < 0.05$.

Results

Analysis of variance on morphological properties and element concentration of wheat treated with different Zn sources was shown in Tables 2,3.

Shoot dry matter and grain yield

Shoot dry matter was affected by the salinity levels of the soil (Figure 1). Under low electrical conductivity (EC 2 dS m^-), shoot dry matter in the application of Zn-Gly and Zn-Ala was greater than that of $ZnSO_4$, but decreased with increasing salinity. In EC 4 dS m^-, shoot dry matter increased at 32.78% and 41.17% more than $ZnSO_4$ by Zn-Gly and Zn-Ala, respectively. In EC 6 dS m^-, Zn-AAc increased the shoot dry matter by 36.36% more than $ZnSO_4$.

The effect of salinity on the grain yield of wheat depended on the Zn source and levels of salinity (Figure 2). Wheat grain yield decreased as salinity increased. At low salinity conditions, the grain yield in plants supplied with Zn-AAc was greater than those supplied with $ZnSO_4$. The increase of salinity from 2 to 6 dS m^- reduced the grain yield by 27.6, 37.63, and 46.57% in the application of Zn-Gly, Zn-Ala, and $ZnSO_4$.

Yield and yield components of wheat

The yield and yield component of wheat plants under the various Zn sources and salinity levels was shown in Table 4. Increasing salinity significantly decreased wheat growth parameters, but the application of Zn-AAc relatively reduced the negative effects of salinity on the growth of wheat. At the low salinity, Zn-AAc increased the number of grains per spike by about 15 to 18% compared to $ZnSO_4$. Zn-Gly reduced the effect of salinity on the number of fertile spikelets but had no significant effect on reducing unfertile spikelets. 1000-grain weight was increased with the application of Zn-AAc compared to $ZnSO_4$ even in high levels of salinity.

Shoot and grain Zn concentration

Wheat plants supplied with Zn-AAc accumulated higher Zn in their shoot and grain compared with those supplied with $ZnSO_4$ (Figure 3). The effectiveness of Zn sources was different regardless of salinity level. In the application of Zn-Gly, the reduction of the Zn concentration in the

Table 2. Analysis of variance on morphological properties of wheat treated with different Zn sources.

			Mean square						
Source of variation	df	Shoot dry matter	Grain yield	Length of shoot	Length of spike	Number of grains $spike^{-1}$	Number of fertile spikelets	Number of unfertile spikelets	1000-grain weight
Zinc source source	2	0.60**	5.402**	4.267^{ns}	0.109^{ns}	2.135**	1.197**	137.504*	1.31**
Salinity levels	2	0.456**	0.770**	86.768**	7.603**	84.957**	7.803**	45.56**	0.537**
Interaction	4	0.770*	0.039**	9.187**	0.432*	4.637**	1.029**	1.280^{ns}	0.263**
Error	18	0.008	0.027	1.833	0.117	0.730	0.458	1.09	0.961

(*0.05, **0.01 significant and ^{ns} no significant correlations with Duncan's Multiple Range Test).

Table 3. Analysis of variance on element concentration in wheat treated with different Zn sources.

		Mean square							
Source of variation	df	Shoot Zn Co.	Grain Zn Co.	Shoot Ca Co.	Grain Ca Co.	Shoot K Co.	Grain K Co.	Shoot Na Co.	Grain Na Co.
Zinc source	2	7.908**	38.072**	4.308**	0.002**	365.87**	0.001 ns	25.46**	0.010**
Salinity levels	2	9.132**	56.489**	15.321**	0.009**	161.56**	0.003**	19.63**	0.047**
Interaction	4	0.13**	1.525 ns	8.976**	—	19.30**	—	0.148**	0.000
Error	18	0.484	1.164	0.113	0.000	0.726	0.000	0.082	0.000

(*0.05, **0.01 significant and ns no significant correlations with Duncan's Multiple Range Test).

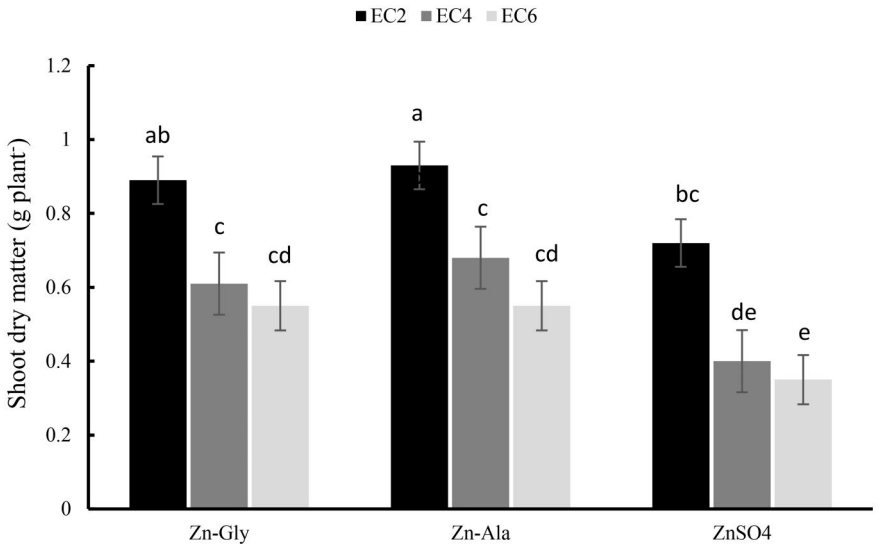

Figure 1. The effect of different Zn-amino acids complexes (i.e. Zn-Gly, Zn-ala) on the shoot dry matter of the wheat (*Triticum aestivum,* cvs. Kavir) under three salinity levels (ECs: 2, 4 and 6 dS m^-) in comparison with $ZnSO_4$.
Note Zn-Gly: Zn-Glycine; Zn-Ala: Zn-Alanine. Values are as the means of three replicates and different letters denote statistical differences (Duncan's Multiple Range Test at $p \leq 0.05$).

shoot was 17.24% with increasing salinity, however, this decline was 22% in $ZnSO_4$ application. Increasing EC from 2 to 6 dS m^- reduced the grain Zn concentration in plants supplied with Zn-Gly and Zn-Ala by 14 and 10%, respectively. This decrease was 19.63% for plants supplied with $ZnSO_4$.

Shoot and grain Ca concentration

Increasing the salinity level in soil significantly increased the Ca concentration in the plant's shoot (Figure 4). Suppling Zn-AAc resulted in a higher concentration of Ca in the shoot of wheat in comparison to $ZnSO_4$. Ca concentration in the wheat grain reduced with increasing salinity. The addition of Zn-AAc resulted in a higher concentration of Ca in the grain. At saline conditions, the Zn-Gly had a greater effect on grain Ca concentration than the other Zn source.

Shoot and grain K concentration

While salinity increased the K concentration in the shoot of wheat, the concentration of K decreased in grains (Figure 5). The application of Zn-Gly resulted in higher shoot K concentration in ECs 4 and 6 dS m^-. The addition of Zn-Gly to the soil was effective in reducing the

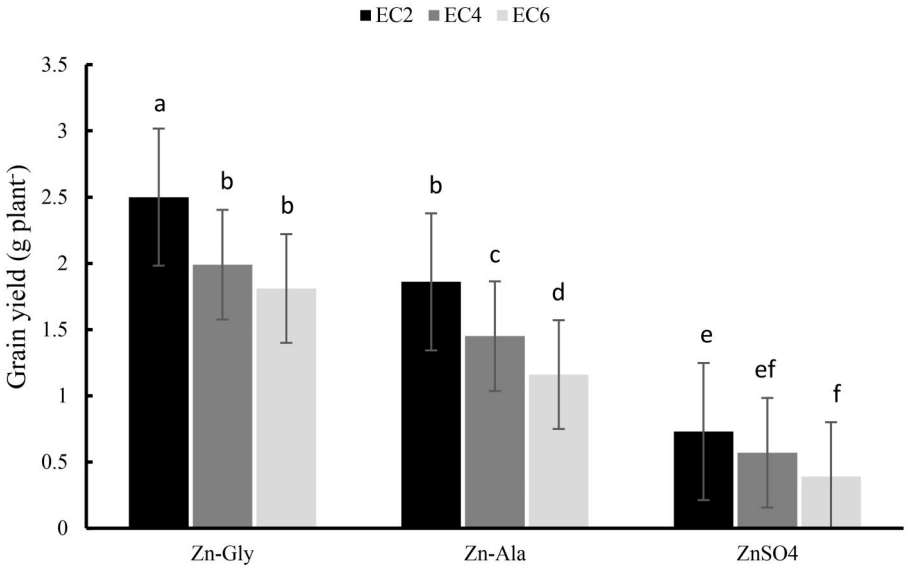

Figure 2. The effect of different Zn-amino acids complexes (i.e. Zn-Gly, Zn-Ala) on the grain yield of the wheat (*Triticum aestivum*, cvs. Kavir) under three salinity levels (ECs: 2, 4 and 6 dS m^{-}) in comparison with $ZnSO_4$.
Note Zn-Gly: Zn-Glycine; Zn-Ala: Zn-Alanine. Values are as the means of three replicates and different letters denote statistical differences (Duncan's Multiple Range Test at $p \leq 0.05$).

Table 4. The effect of different Zn fertilizers (i.e. Zn-Gly, Zn-Ala, and $ZnSO_4$) on yield and yield components of wheat *(Triticum aestivum*, cvs. Kavir*)* exposed to three levels of salinity (i.e. 2, 4 and 6 dS m^{-}).

	Length of shoot (cm)	Length of spike (cm)	Number of grains $spike^{-1}$	Number of fertile spikelets	Number of unfertile spikelets	1000-grain weight (g)
Zn-Gly						
EC = 2	34.81 ± 2.03 a	6.16 ± 0.33 a	11.33 ± 0.19 a	10.57 ± 0.25 a	3.50 ± 0.5 bc	39.31 ± 0.79 a
EC = 4	30.06 ± 1.24 b	4.24 ± 0.04 bcd	6.68 ± 0.63 cd	9.07 ± 0.23 b	5.33 ± 0.33 a	36.45 ± 0.7 b
EC = 6	27.06 ± 1.41 cd	3.76 ± 0.16 d	3.26 ± 0.27 f	8.16 ± 0.15 c	5.11 ± 0.28 a	33.86 ± 2.08 c
Zn-Ala						
EC = 2	33.53 ± 0.85 a	5.80 ± 0.26 a	10.96 ± 0.36 a	10.12 ± 0.12 a	3.37 ± 0.12 c	36.42 ± 0.41 b
EC = 4	29.47 ± 2.43 bc	4.76 ± 0.56 b	7.67 ± 1.96 c	9.07 ± 0.67 b	4.78 ± 0.77 ab	32.96 ± 0.75 cd
EC = 6	25.04 ± 0.69 d	4.10 ± 0.44 cd	4.53 ± 1.42 ef	7.58 ± 0.41 d	4.38 ± 1.7 abc	31.67 ± 0.9 de
$ZnSO_4$						
EC = 2	31.1 ± 1.15 b	5.71 ± 0.42 a	9.26 ± 0.05 b	9.01 ± 0.17 b	3.58 ± 0.08 bc	30.49 ± 1.02 e
EC = 4	28.85 ± 0.65 bc	4.42 ± 0.07 bc	5.45 ± 0.2 de	8.20 ± 0.45 c	4.29 ± 0.04 abc	28.64 ± 0.74 f
EC = 6	28.84 ± 0.82 bc	4.64 ± 0.37 bc	5.58 ± 0.08 de	8.41 ± 0.08 c	4.40 ± 0.10 abc	27.30 ± 0.17 f

Note. Zn-Gly: Zn-Glycine; Zn-Ala: Zn-Alanine. Different letters in the same column denote statistical differences (Duncan's Multiple Range Test test at $p \leq 0.05$).

negative effects of salinity on shoot K accumulation. However, at supplying Zn-Gly and Zn-Ala, no significant difference was found in the grain K concentration.

Shoot and grain Na concentration

Salinity increased Na concentration in the shoot and grain of wheat although the amount of this increase was different in the application of the type of Zn sources (Figure 6). Wheat plants supplied with the Zn-AAc had lower Na in their shoot and grain compared with those supplied with $ZnSO_4$. In EC 6 dS m^{-}, decreasing of shoot Na concentration was 32.31% and 18.67% for Zn-Gly and Zn-Ala compared with $ZnSO_4$. Also, plants supplied with Zn-Gly and Zn-Ala, decreased grain Na concentration by 19.4% and 7.14% respectively in comparison with $ZnSO_4$ in high levels

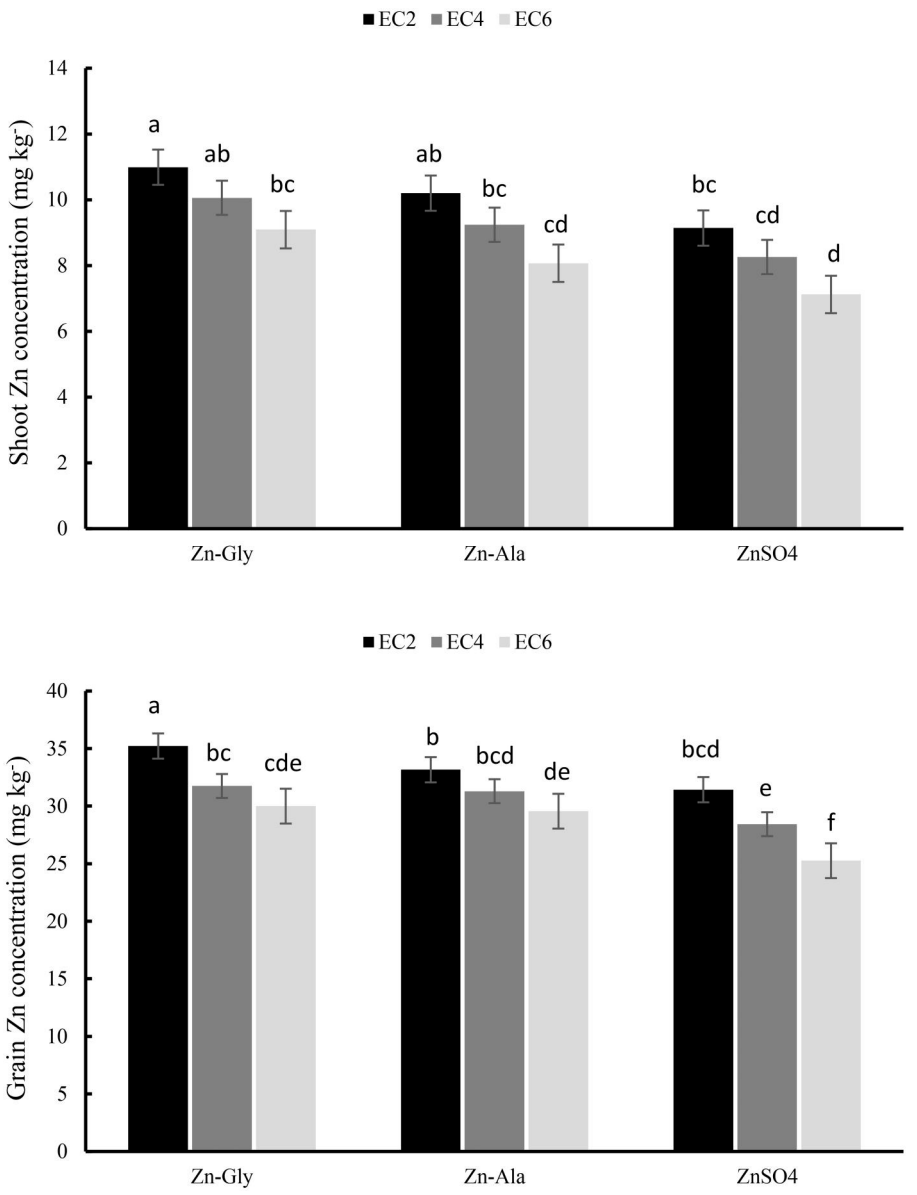

Figure 3. The effect of different Zn-amino acids complexes (i.e. Zn-Gly, Zn-Ala) on Zn concentration in shoot and grain of the wheat (*Triticum aestivum,* cvs. Kavir) under three salinity levels (ECs: 2, 4 and 6 dS m^{-}) in comparison with $ZnSO_4$.
Note Zn-Gly: Zn-Glycine; Zn-Ala: Zn-Alanine. Values are as the means of three replicates and different letters denote statistical differences (Duncan's Multiple Range Tests at $P \leq 0.05$).

of salinity. The Zn-Gly was, in general, more effective than Zn-Ala in decreasing the concentration of Na in the shoot and grain of wheat.

Shoot and grain K/Na ratio

The effect of Zn sources on the K/Na ratio in the shoot and grain of wheat was different upon salinity level (Figure 7). The highest K/Na ratio in the shoot belonging to the application of Zn-Gly at EC 4 dS m^{-}, and then decreased with increasing EC to 6 dS m^{-}. At ECs 4 and 6 dS m^{-},

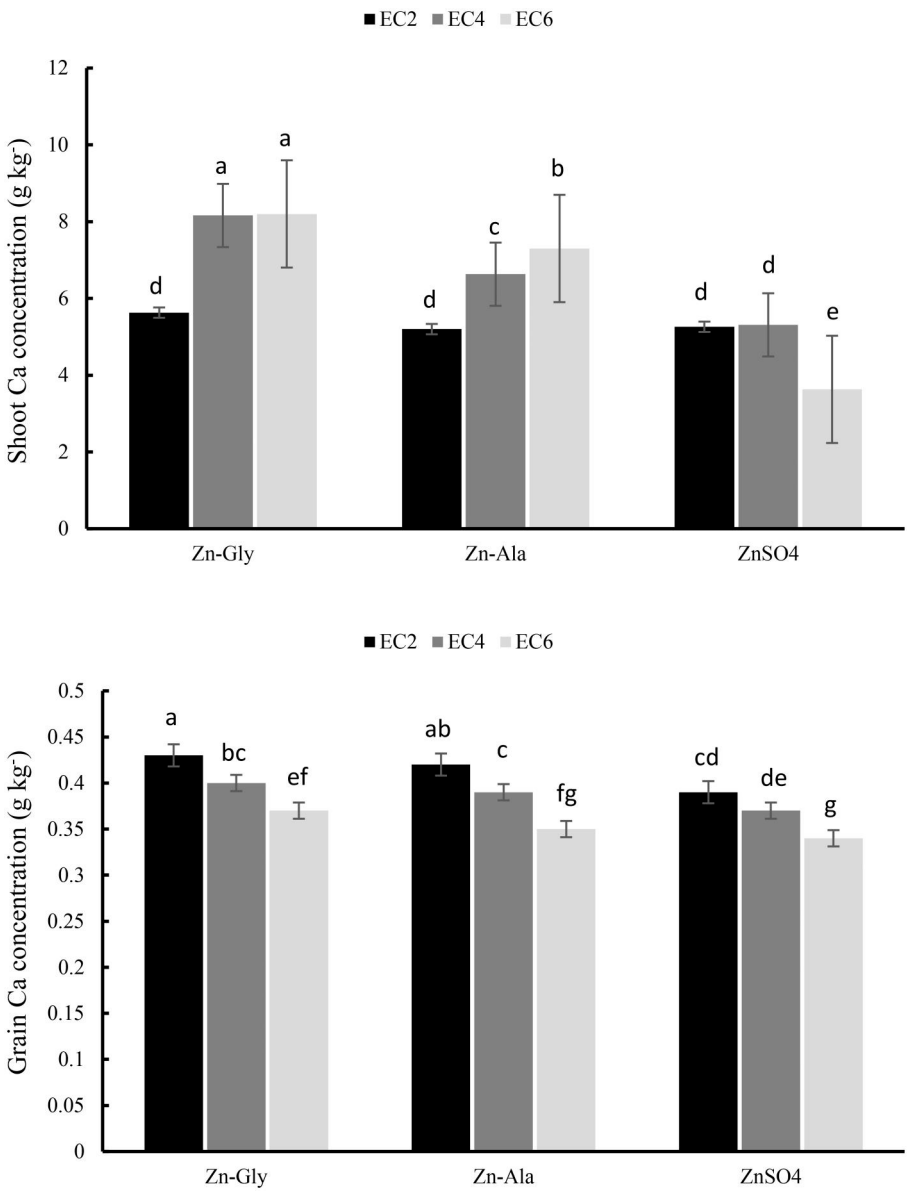

Figure 4. The effect of different Zn-amino acids complexes (i.e. Zn-Gly, Zn-ala) on Ca concentration in shoot and grain of the wheat (*Triticum aestivum,* cvs. Kavir) under three salinity levels (ECs: 2, 4 and 6 dS m^{-}) in comparison with $ZnSO_4$.
Note Zn-Gly: Zn-Glycine; Zn-Ala: Zn-Alanine. Values are as the means of three replicates and different letters denote statistical differences (Duncan's Multiple Range Test at $P \leq 0.05$).

wheat plants supplied with Zn-Gly and Zn-Ala had a higher K/Na ratio in grains than those supplied with $ZnSO_4$.

The root CAT and SOD activity

Various Zn sources caused significant changes in the activity of the CAT and SOD enzymes of the root (Figures 8, 9). The salinity stress increased the activity of CAT and SOD in the root of wheat. At all salinity levels, plants supplied with Zn-AAc had higher root CAT and SOD activity

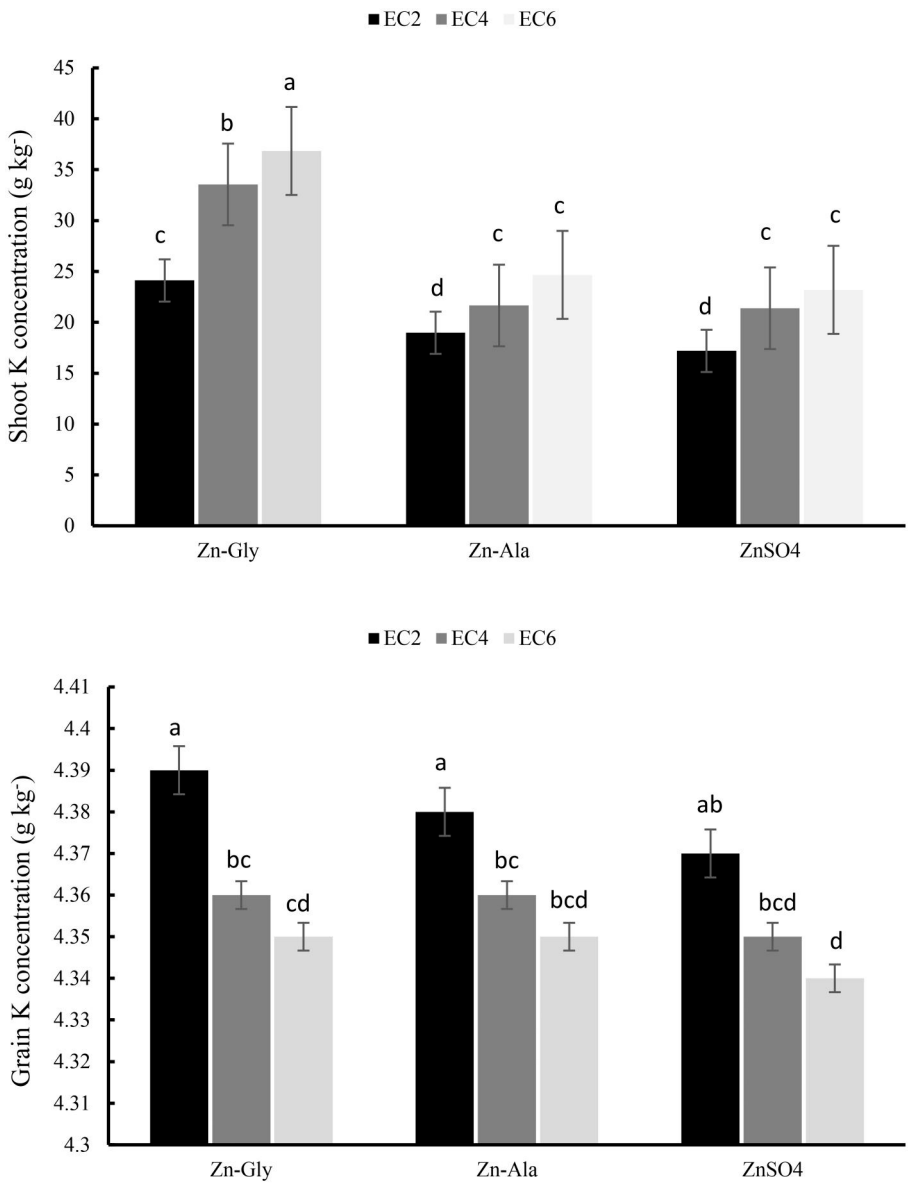

Figure 5. The effect of different Zn-amino acids complexes (i.e. Zn-Gly, Zn-ala) on K concentration in shoot and grain of the wheat (*Triticum aestivum,* cvs. Kavir) under three salinity levels (ECs: 2, 4 and 6 dS m^{-}) in comparison with $ZnSO_4$.
Note Zn-Gly: Zn-Glycine; Zn-Ala: Zn-Alanine. Values are as the means of three replicates and different letters denote statistical differences (Duncan's Multiple Range Test at $P \leq 0.05$).

in comparison to those supplied with $ZnSO_4$. Among Zn-AAc, Zn-Gly increased the CAT and SOD activity more than Zn-Ala.

Root MDA concentration (lipid peroxidation)

The root MDA concentration was dependent on salinity level and Zn source (Figure 10). Adding Zn-AAc reduced the root MDA concentration in comparison to $ZnSO_4$. The lowest root MDA

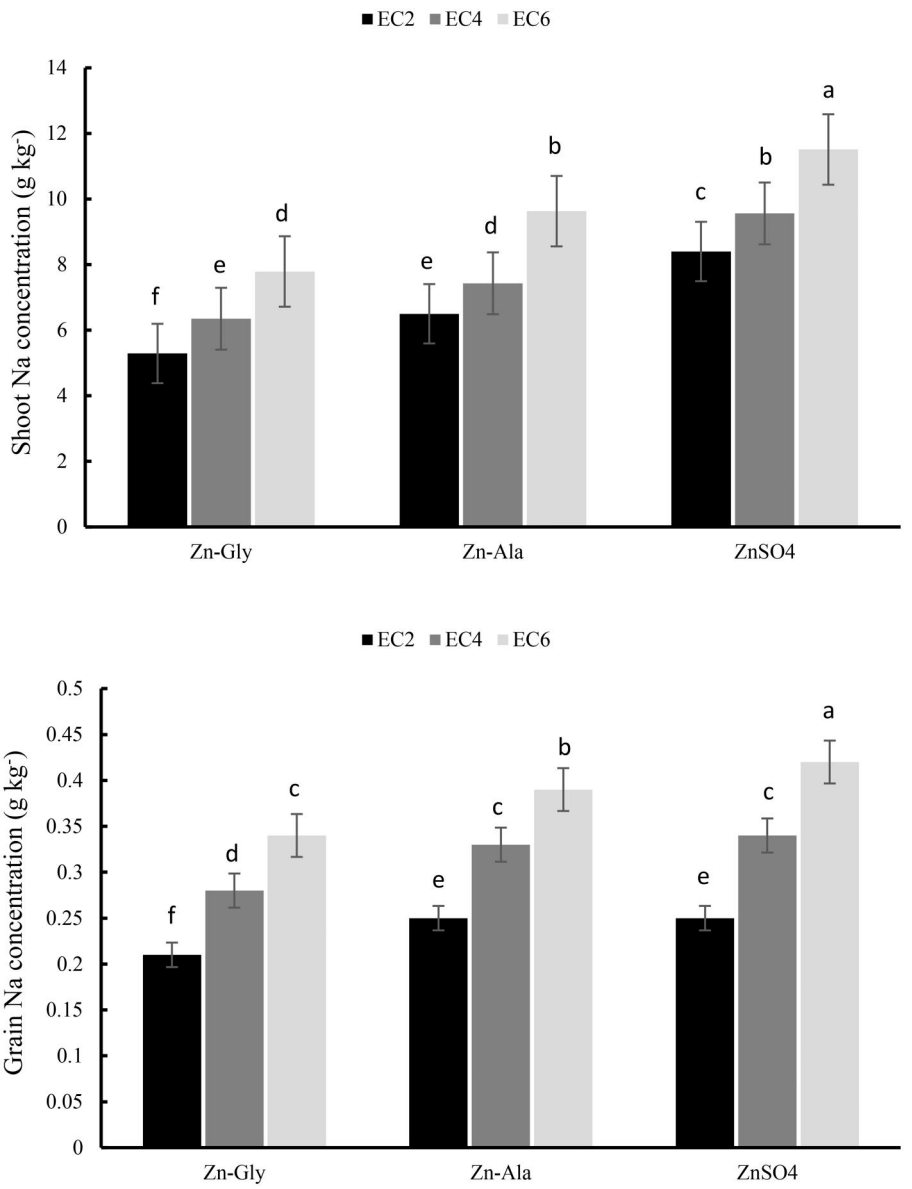

Figure 6. The effect of different Zn-amino acids complexes (i.e. Zn-Gly, Zn-Ala) on Na concentration in shoot and grain of the wheat (*Triticum aestivum,* cvs. Kavir) under three salinity levels (ECs: 2, 4 and 6 dS m^{-}) in comparison with $ZnSO_4$.
Note Zn-Gly: Zn-Glycine; Zn-Ala: Zn-Alanine. Values are as the means of three replicates and different letters denote statistical differences (Duncan's Multiple Range Test at $p \leq 0.05$).

concentration was observed in the Zn-Gly application, which decreased by about 36% compared to $ZnSO_4$, at EC 6 dS m^{-}. This decrease was 23.75% for Zn-Ala at the same EC.

Discussion

"Kavir" is a Zn-inefficient wheat cultivar (Khoshgoftarmanesh et al. 2006). As expected, by increasing salinity, the yield and yield components of wheat decreased; although the amount of

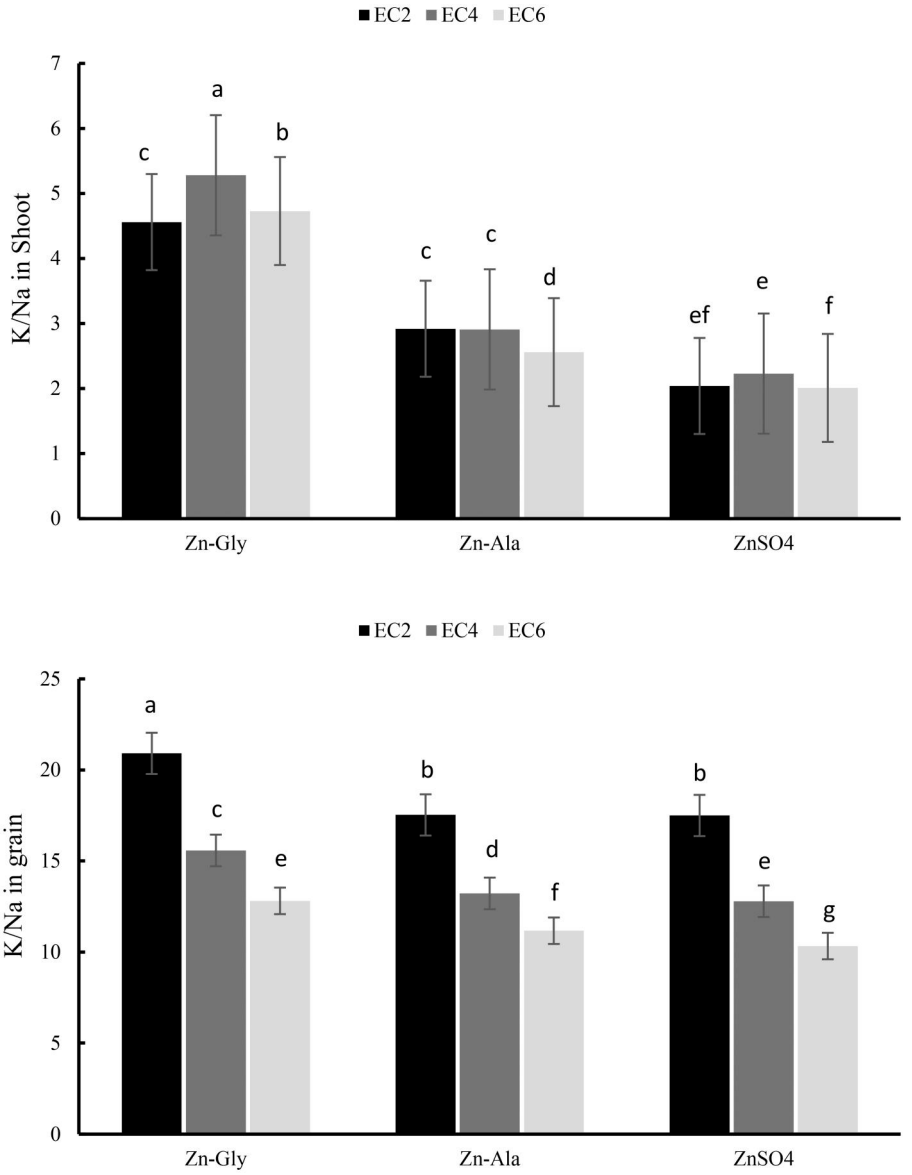

Figure 7. The effect of different Zn-amino acids (i.e. Zn-Gly, Zn-ala) on K/Na ratio in shoot and grain of the wheat (*Triticum aestivum,* cvs. Kavir) under three salinity levels (ECs: 2, 4 and 6 dS m^{-}) in comparison with $ZnSO_4$.
Note Zn-Gly: Zn-Glycine; Zn-Ala: Zn-Alanine. Values are as the means of three replicates and different letters denote statistical differences (Duncan's Multiple Range Test at $p \leq 0.05$).

this reduction was greater in $ZnSO_4$ application than Zn-amino acid complexes. The salinity levels used in this experiment were ECs 2, 4, and 6 dS m^{-}. By respecting of sensitivity of wheat to salinity, about a 50% reduction in growth and yield of wheat was predicted at the EC 6 dS m^{-}. Our results indicated 46% and 51% reductions in shoot dry weight and grain yield of wheat in the application of $ZnSO_4$. However, in plants supplied with Zn-Gly and Zn-Ala, this decrease was 38% and 40% for shoot dry weight and 27% and 37% for grain yield, respectively.

The most obvious negative effect of salinity stress on plants is growth reduction, which is caused by the reduction of osmotic potential, the imbalance of nutrients and/or the toxicity of some specific ions. (Cakmak 2000). Salinity can also decrease grain yield by shortening the grain

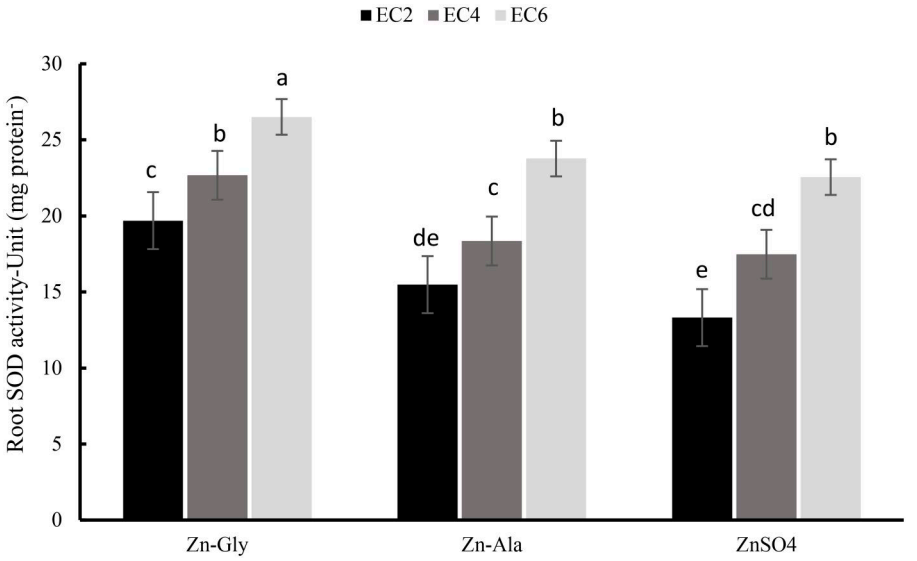

Figure 8. The effect of different Zn-amino acids (i.e. Zn-Gly, Zn-Ala) on root SOD activity of the wheat (*Triticum aestivum,* cvs. Kavir) under three salinity levels (ECs: 2, 4 and 6 dS m^{-}) in comparison with $ZnSO_4$.
Note Zn-Gly: Zn-Glycine; Zn-Ala: Zn-Alanine; SOD: super oxide dismutase. Values are as the means of three replicates and different letters denote statistical differences (Duncan's Multiple Range Test at $p \leq 0.05$).

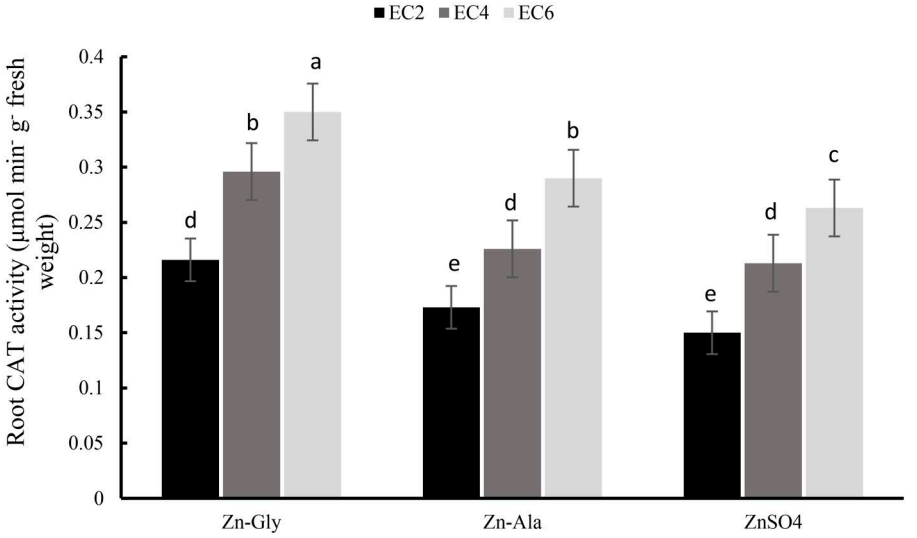

Figure 9. The effect of different Zn-amino acids complexes (i.e. Zn-Gly, Zn-ala) on root CAT activity of the wheat (*Triticum aestivum,* cvs. Kavir) under three salinity levels (ECs: 2, 4 and 6 dS m^{-}) in comparison with $ZnSO_4$.
Note Zn-Gly: Zn-Glycine; Zn-Ala: Zn-Alanine; CAT: catalase. Values are as the means of three replicates and different letters denote statistical differences (Duncan's Multiple Range Test at $p \leq 0.05$).

filling period and disrupting the translocation of assimilates to the grain (Leyva et al. 2011). Rani et al. (2019) showed that yield and yield components of wheat in saline soils could be increased by applying Zn fertilizers. They reported that with Zn application, chlorophyll and indole acetic acid were increased which led to an increase of plant photosynthesis and grain yield.

In the present study, increasing salinity reduced the Zn concentration in the plant. This could be due to the reduction of root volume and the antagonistic effect of nutrients under saline

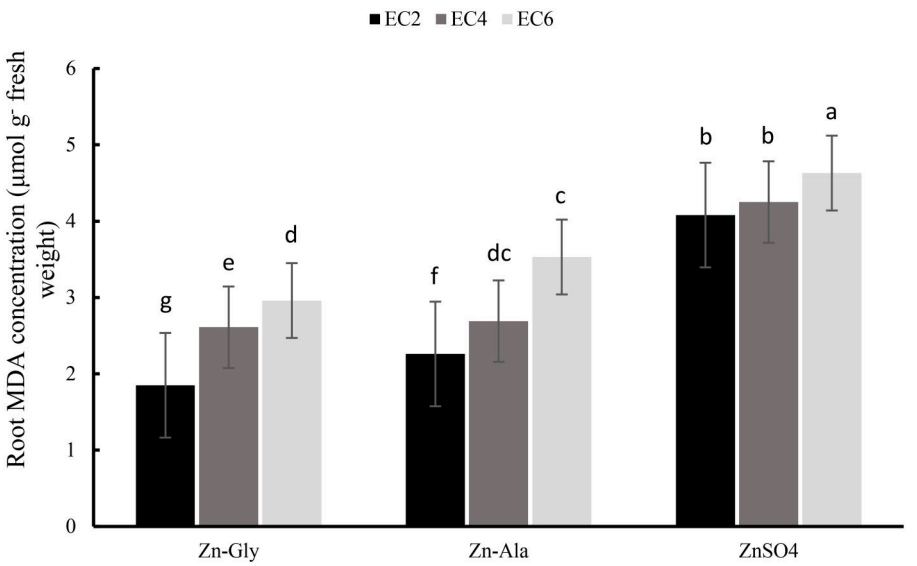

Figure 10. The effect of different Zn-amino acids complexes (i.e. Zn-Gly, Zn-Ala) on root MDA concentration of the wheat (*Triticum aestivum*, cvs. Kavir) under three salinity levels (ECs: 2, 4 and 6 dS m^{-}) in comparison with $ZnSO_4$.
Note Zn-Gly: Zn-Glycine; Zn-Ala: Zn-Alanine; MDA: malondialdehyde. Values are as the means of three replicates and different letters denote statistical differences (Duncan's Multiple Range Test at $P \leq 0.05$).

conditions (Cakmak and Marschner 1993). However, the use of Zn-AAc under salinity conditions resulted in a significant increase in the shoot (8 to 10 mg kg^{-}) and grain (22 to 35 mg kg^{-}) Zn concentration compared to $ZnSO_4$ application. Amino acids improve Nitrogen (N) nutrition status in the plant (Mirbolook et al. 2021) that which leads to an increase in the number and activity of Fe and Zn-transporter proteins on the root cell membranes. Increasing the metal transporters can increase Zn uptake by plant roots (Murata et al. 2008). On the other hand, Zn-AAc nutrition could reduce the detrimental effect of salinity. Physiological mechanisms that explain the role of zinc in reducing salinity stress in plants are still not well understood, but researchers have shown that zinc plays a vital role in the detoxification of O_2 free radicals (Cakmak 2000) and also that this element leads to maintaining the integrity of cell membranes (Welch 1986).

In addition to the importance of Zn nutrition on the reduction of the negative effect of salinity, the positive effects of amino acids on increasing plant resistance to salinity were reported (Çavuşoğlu et al. 2007). One of the probable reasons for the effect of Zn-AAc on the reduction of salinity stress on wheat is maintaining hormone balance within plant tissues (Çavuşoğlu et al. 2007, Hoque et al. 2007, Iqbal et al. 2018). Researchers have reported that the reduction of plant growth in saline conditions is due to hormonal imbalance in plant tissues (Fricke et al. 2004, Akhiyarova et al. 2005). The results of this study indicated the positive effect of Zn-AAc on the grain yield and shoot of wheat in saline conditions. Amino acids have motivational effects on plant growth. The positive effect of complexes of glycine and alanine with Zn on plant growth and yield of bread wheat has been indicated by Mirbolook et al. (2020a). The stimulating effect of Zn-AAc on wheat growth parameters may be due to the role of amino acids in cell division and plant development (Abdul-Qados El Bassiouny and Mostafa 2008). In this study, the stimulating effect of Zn-Gly on grain yield and 1000-grain weight was higher than Zn-Ala and $ZnSO_4$. This might be due to various uptake rates of the studied Zn-AAc by plant roots. Zn-Gly has a simple structure and small size of less than one μm and it can easily pass-through root pores and enter into phloem either individually or along with amino acids in the form of a complex (Mirbolook et al. 2021).

One of the possible reasons for reduced plant growth under salt stress is excessive production of reactive oxygen species (ROS) and loss of cell membrane integrity (Khan et al. 2020; Marschner 2011). In this study, the root MDA concentration was used as an indicator of lipid peroxidation and the severity of oxidative damage on cell membranes (Naidu et al. 1992). The MDA is the final product of lipid peroxidation and could be used as an indicator for evaluating the injurious effects of free radicals including superoxide (O^{-}_{2}), hydroxyl radical (OH°), and hydrogen peroxide (H_2O_2) produced under salinity stress on plant tissues (Azevedo et al. 2009). In this research, salinity increased the root MDA concentration in plants treated with $ZnSO_4$. Based on the results, with increasing EC up to 6 dS m^{-}, the highest shoot Zn concentration was observed in plants supplied with Zn-Gly and Zn-Ala, and MDA concentration in the root of these plants was reduced by 36% and 23% compared to $ZnSO_4$. Tavallali et al. (2010) also reported that Zn nutrition could play a protective role against oxidative stresses such as salinity. This process should be done by inhibiting the oxidation of membrane lipids and facilitating the function of its proteins. In the conditions of salt stress, more sodium absorption and potassium leakage leads to disturbance in the integrity of the cell membrane (Marschner 2011). But in the application of Zn-AAc, the result indicated that shoot K concentration was increased by about 37% in plants supplied with Zn-Gly at ECs, 4 and 6 dS m^{-} in comparison with Zn-Ala and $ZnSO_4$. This could be due to the role of Zn in cell membrane integrity and glycine in the growth stimulation of wheat. The result of Mirbolook et al. (2020a) about the effect of Zn-amino complexes on wheat plants showed that there is a positive correlation between plant growth, glycine application, and Zn uptake. The accumulation of mineral ions such as K in plant cells under saline conditions can improve osmotic regulation, protein synthesis, and root membrane integrity (Marschner 2011). Cuin and Sh (2007) reported the role of some amino acids on root K leakage in barley and show that application of amino acids reduced root K leakage by improving cell membrane integrity.

In addition to enhancing shoot K concentration, exposure to salinity increased the shoot Na concentration of wheat; although the magnitude of this increase was less (about 1.8 to 3.72 mg kg^{-}) in the plants supplied by Zn-Gly and Zn-Ala in comparison to $ZnSO_4$. The reduction of shoot Na concentration due to the Zn supply has also been reported by Yahya (1998, Hassanpouraghdam et al. 2020). According to Alpaslan et al. (1999), Zn can control the entry and exit of Na and K ions from plasmalemma. The role of amino acids in reducing the damage caused by salinity is related to their role in increasing the synthesis of proteins and improving the integrity of the cell membrane. In addition, amino acids might affect the intracellular distribution of Na in cell plants (Ghasemi et al. 2014). The results of Zhang et al. (2009) showed that amino acid application did not affect Cd absorption while reducing Cd-induced damages by altering the intracellular distribution of Cd in the plant cells. In this study, increasing salinity level reduced grain Na concentration in plants supplied *via* Zn-Gly and Zn-Ala comparison with $ZnSO_4$. It may be due to changes in the distribution of Na in plant tissues by amino acid application (Khan et al. 2020). Contrary to Na, the concentration of K in grain is reduced by increasing salinity. Perhaps the reduction of K translocation from shoot to grain led to this reduction because K activity in the phloem is low. Also, Na and K can compete for accumulation in grain (Ramoliya et al. 2004). In saline conditions, N metabolism and protein synthesis in the leaves can lead to K and Na imbalance in plant tissues and lead to more Na accumulation than K in the grain (Ramoliya et al. 2004). Despite the Na increasing and K decreasing in the grain, K/Na ratios in plants supplied with Zn-Gly and Zn-Ala were 17.5% and 5% higher than those supplied with $ZnSO_4$ at ECs 4 and 6 dS m^{-}. This indicates the importance of Zn-AAc in nutrient balance under salinity stress. The high K/Na ratio is often known as a good parameter under salinity stress (Francois et al. 1994; Zhu 2003).

Shoot Ca concentration also increased by increasing soil salinity which could be due to the presence of Ca salts used as salinization ions in the preparation of a salt solution. Similar results

have been observed in high salinity (>8.4 dS m^{-}) in *Salvadora persica* (Rouphael et al. 2012) and watermelon species (Khoshgoftar et al. 2006). Studies show that increasing shoot calcium concentration can play an effective role in reducing the effects of salinity. In fact, plants increase calcium absorption to improve high sodium conditions (Alpaslan et al. 1999). However, Ca mobility in the xylem is low which reduces Ca translocation from shoot to grain. It may be the reason for the decrease of Ca concentration in wheat grain by increasing salinity level.

In environmental stresses, also activity of plant antioxidant enzymes changes (Gao et al. 2008) and these have a vital role in the plant defense system against oxidative stresses induced by salinity. The positive effects of amino acids on increasing plant resistance to salinity might be due to an increase in the activity of antioxidant enzymes. In this experiment, the application of Zn-Gly and Zn-Ala increased the activity of root CAT and SOD enzymes and thus reduced MDA concentration compared with $ZnSO_4$. On the other hand, Zn-AAc nutrition could protect cell membranes against oxidative stress by increasing the activity of antioxidant enzymes and eliminating ROS in plant cells.

Higher activity of root CAT and SOD enzymes in wheat supplied with Zn-Gly and Zn-Ala may also be due to the improvement of nutrient status in the plant. In all salinity levels, Zn-AAc had higher shoot Zn concentrations than $ZnSO_4$. These results indicate that the activity of these enzymes depends on the Zn. Glycine and alanine can bind to nucleic acids and membrane phospholipids and improve the activity of enzymes such as CAT (Abdul-Qado). According to the results, the effect of Zn-AAc on improving salinity-induced damages could be due to the role of both Zn in activating Zn-containing antioxidant enzymes such as CAT and SOD, and amino acids in increasing the antioxidant capacity of wheat plants against saline conditions.

Conclusion

The results of this study showed the importance of the Zn nutritional status in increasing the resistance of wheat to salinity after the use of Zn-AAc (as Zn source). Salinity reduced shoot dry matter and grain yield of wheat and induced oxidative damage on root cell membranes. Increased MDA production under salinity stress was evidence of damage caused by oxidative stress in wheat plants. Adding Zn in the form of a complex with amino acids reduced the destruction of root cell membranes by increasing shoot and grain Zn concentrations in saline conditions. This was accompanied by higher concentrations of K and Ca in the shoot and K to Na ratio in the shoot and grains of wheat. The application of Zn-Gly and Zn-Ala increased the activity of CAT and SOD enzymes, which could explain the positive influence of Zn-AAc in the reduction of salt-induced oxidative damage on wheat. Based on the results, it seems that the Zn-AAc can supply Zn and increase the salt stress tolerance of wheat in the soil.

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Disclosure statement

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