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EFFECTS OF $ZnSO_4$ ON GROWTH, SOME KEY PHYSIOLOGICAL PROCESSES AND IONIC HOMEOSTASIS OF DIFFERENT BREAD WHEAT (*TRITICUM AESTIVUM* L.) GENOTYPES UNDER SALINITY STRESS

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Abstract

Salinity stress is one of the most threating abiotic stresses limiting agricultural crop production worldwide by affecting crop growth and physiological processes and ion balance. Exogenous application of various chemicals can enhance the crop production by improving growth and physiological parameters and reducing specific ion toxicity. The purpose of the present study was to investigate the ameliorative role of Zn in wheat during salinity stress by bringing more land under cultivation to improve wheat production. A hydroponic experiment was performed to assess the rectifying effect of increased doses of zinc from recommended dose in different salinity levels i.e., moderate to strong salinity. Two doses of zinc (2 mM, 4 mM) and two levels of NaCl (70 mM, 140 mM) along with control were used to screen the twelve genotypes of wheat. Salinity stress decreased the growth parameters i.e., shoot and root length, fresh and dry biomass maximum up to 45% when the recommended dose of zinc was applied. Increasing the dose of zinc improved the growth attributes of seedling up to 21% under moderate to strongly saline conditions. In the same way, high doses of zinc increased the RWC, MSI and K^+ concentration up to 24%, 17% and 45% respectively decreasing the Na^+ conc. in plant aerial parts up to 27%. K^+ ion conc. was increased by increasing the zinc application rate showing synergistic effect of Zn with K^+, was improved by increasing the zinc dose showing increasing the tolerance of seedling against salinity stress. Two varieties i.e., Faisalabad-08 and Sehar-06 performed the best in terms of salinity tolerance and zinc use efficiency under saline conditions. It was concluded from the results that higher doses of Zn fertilization along with salt tolerant varieties can significantly increase the crop biomass and grain yield by improving growth and physiological processes and reducing Na^+ toxicity under saline conditions.

Key words: Salinity stress, Zinc, Wheat, Principal component analysis, Ranking of genotypes.

Introduction

Salinity stress adversely affects the crop growth and production globally by disturbing the whole metabolic system and physiological functions of plants (Msanne *et al*., 2011) and drastically decrease the crop yield (AbdElgawad *et al*., 2016; Negrao *et al*., 2017). Nearly 20% of irrigated land is affected by salinization causing the loss of productivity of cultivated soils and food insecurity (Shrivastava & Kumar, 2015). In Pakistan, 22.1 Mha area is under cultivation. Out of which, 12.67 Mha of area occurs in Punjab. Of this area about 6.30 Mha is salt affected and this figure is increasing (Anon., 2017). Salinity stress in crops causes loss of potential yield and thus minimizes the profit level and reduction of average crop production.

Different physio-biochemical defects including nutritional imbalance, impaired gas exchange, osmotic stress, Na^+ toxicity, electrolyte leakage or a mixture of all these factors reduces yield and productivity of plant (Siddiqui *et al.,* 2019; Sofy *et al.*, 2020). Salinity stress accelerates the production of reactive oxygen species (ROS) causing increase of oxidative stress, loss of cell water which causes osmotic stress and nutrients imbalance due to specific ion toxicity. Reduction in turgor pressure caused due to osmotic damage which inhibits photosynthetic parameters and growth of plant (Ashraf & Ashraf, 2016). Osmotic stress results due to loss of water absorption by the roots causing reduction in stomatal conductance, cell elongation and the excess of salts like Na^+ and Cl^- develops ionic stress (Tian *et al*., 2020). Reduction in photosynthesis is coupled with decrease in yield (Goussi *et al*., 2018). Uptake of vital nutrients like Zn is affected by Toxic ions present in saline soils (Ashraf & Ashraf, 2016). Furthermore, salt causes major damage to pigments, proteins, nucleic acids (DNA and RNA) and lipids due to oxidative burst caused by excessive ROS generation (Klein *et al*., 2018). Plants naturally possess antioxidant enzymes (POD, APX, SOD, CAT) and non-enzymatic antioxidant compounds (carotenoids, ascorbic acid, tocopherols, flavonoids and phenolics) (Ashraf *et al*., 2018).

Wheat (*Triticum aestivum* L.) is the staple crop all over the world. Wheat is of a paramount importance in Pakistan, about 9 million hectares of total land is used for wheat cultivation which is almost 80% of the total under cultivation area, 14% of value added in agriculture by wheat and 3% of the total GDP of the country. In recent years, about 22 to 26 million tons of wheat production per year is reported. About 37% of total food energy intake in Pakistan is contributed by wheat (Anon., 2017).

It is estimated, half of the cereal crops are grown on soils deficient in zinc (Zn) worldwide (Cakmak, 2008). In addition to its role in the biosynthesis of chlorophyll and carotenoids, Zn plays an important role in a wide variety of metabolic reactions (Aravind & Prasad, 2005a, 2005b). In response, Zn stimulates enzymes that enhance metabolism and growth. This produces Zn-dependent metalloproteins, which, in turn, plays significant role in transcription and replication of DNA by regulating gene expression (Barker & Pilbeam, 2015). Zn lowers the function of membrane-bound NADPH oxidase and limits the harmful effect of salt stress which in response reduces the development of ROS (Waraich *et al*., 2011). It strengthens membranes, DNA binding proteins and is helpful in the production of IAA and protein metabolism. Protection from oxidative damage, protein synthesis, gene regulation and DNA transcription are some other essential roles of (Zn) (Alloway, 2008). This micronutrient is now being used as soil amendment in wheat for development under environmental constraints and to enhance plant growth (Zafar *et al*., 2018).

It is difficult to distinguish between toxicity and deficiency of Zn physiologically (Saboor *et al*., 2021). Generally, it ranges from 10 mg kg^{-1} to 100 mg kg^{-1} in shoots, and its toxicity effects are also observed above 300 mg kg^{-1}. In salt affected soils this micronutrient deficiency damage is not only limited to affect the chloroplast structure but also 50-70% reduction in photosynthesis and causes toxicity from ROS as well (Alloway, 2008). However, plant species vary widely in threshold limits for Zn bioaccumulation and toxicity (Glińska *et al*., 2016; Grassi *et al*., 2020). Zn deficiency results in loss of membrane stability which may affect the Na uptake in salt affected plants. So, improvement in salt stress tolerance is directly linked with the improvement of Zn nutritional status (Akatas *et al*., 2006; Jan *et al*., 2019). This deficiency in food passes to the humans and affecting their immune system, enhancing susceptibility to infections (Rosado, 2003; Hotz & Brown, 2004).

Cereals are generally low in Zinc concentration and on saline soils, its deficiency is further increased. Supplementation of zinc fertilizers under saline condition can boost the crop tolerance against abiotic stress as well as increase the zinc concentration upto optimum levels. Keeping in view the above factors, this experiment was planned to check the response of wheat genotypes under higher salt concentration with different doses of zinc supplementation in hydroponics.

Material and Methods

Experimental details: A hydroponic study was carried out in the wire house of institute of Soil and Environmental Sciences (ISES), University of Agriculture Faisalabad to compare the different genotypes of bread wheat for tolerance against salinity stress with and without Zinc supplementation and selection of salt tolerant and zinc efficient varieties for further experimentation. Twelve different wheat genotypes i.e., AARI-11 (V1), Miraj-08 (V2), Pasban-90 (V3), Punjab-11 (V4), Sehar-06 (V5), Faisalabad-08 (V6), Shafaq-06 (V7), Borlaug-15 (V8), Zincol (V9), NARC-09 (V10), MH-97 (V11), AAS-11 (V12) were screened to check the salinity tolerance under different saline stress conditions. Impact of Zn on various physiological, developmental, and ionic parameters were monitored. Genotypes were arranged from Ayub Agricultural Research Center (AARI), Faisalabad and National Agricultural Research Center (NARC), Islamabad.

Wheat grains were sterilized with 1% Sodium hypochlorite solution prior to sowing. Nursery was shifted and transplanted to 100 Liter water tubs when it reached two leaf stages. Half strength Hoagland solution was used to comply with the nutritional requirements of seedling. Two level of Zinc (2 mM and 4 mM) by using the $ZnSO_4$ under two level of salinity stress (70 mM, 140 mM) were applied instantly after transplanting. Doses of Zinc i.e., 2 and 4 mM and Salt i.e., 70 mM and 140 mM were selected based on review and previous studies. Treatments and varieties used in the experiment were arranged according to the completely randomized design i.e., CRD factorial. The pH of water was maintained 5.5-6.5 on daily basis and adjusted with 1M HCl and 1M NaOH solution when required.

Measurement of growth, physiological and ionic parameter: After 45 days of transplantation crop was harvested. At the time of harvesting, growth and physiological parameters were recorded. Root and shoot length of seedlings were recorded in centimeter (cm) using meter scale. Plants were oven-dried at 65ºC for 48 hours followed by air drying. After removal of moisture contents, dry biomass of plants was calculated in grams using digital balance. Fully expanded green leaves from each replication was collected for measuring of relative water contents (RWC) and membrane stability index (MSI). For RWC, leaves were washed with distilled water and 0.5 g of green leaf was taken. Then these samples were placed in test tubes and 10 mL deionized water was added. The leaves were left for 4 hours for imbibition. After taking the imbibed weight, samples were oven dried for 72 hours according to the method described by (Weatherley, 1951). The RWC of seedling was calculated using the following equation.

$$RWC = (FW-DW)/ (TW-DW) * 100$$

MSI was determined from younger expanded leaves. After washing with distilled water, 0.2 g of fresh weight of these leaves was taken in the test tubes and 10 ml distilled water was added. Test tubes were put in water bath for 30 minutes at 40ºC. After 30 minutes first reading of EC was taken and labeled as C_1. Second EC labelled as C_2 was measured after placing the test tubes again in water bath at 100ºC for 15 minutes. MSI was measured by the method described by (Sairam *et al*., 2002).

$$MSI = [1-(C1/C2)] * 100$$

Samples were dried in a forced air driven oven at 65ºC for 72 hours then grinded to fine powder.0.5g Over dried powdered plant samples were digested using di-acid mixture i.e., HNO_3 and $HClO_4$ in 2:1 for the determination of ionic parameters. After digestion procedure, plant digest was diluted to 50 mM with deionized water. Na^+ and K^+ ion concentration in plant digest was determined by flame photometer (Jenway, PFP-7) while zinc concentration was determined using atomic absorption spectrophotometer (AAS).

Experimental design and statistical analysis: Experimental pots were laid out in completely randomized design (CRD) arrangement in triplicate. Data was analyzed by using analysis of variance (ANOVA) in two-way factorial (Steel *et al*., 1997) by statistical software Statistix 8.1 (USA). HSD (honest significant difference) test was used for the pairwise comparison of treatments and genotypes mean values at level of significance $p<0.05$. Wheat genotypes were ranked using Principal Component

Analysis (PCA) on the basis of total dry matter production in different environments i.e., 70 mM NaCl and 140 mM NaCl in statistical package xl-stat.

Results

Zinc application effects on growth attributes of different genotypes of wheat under salinity: Growth parameters including root and shoot length and oven dried biomass were recorded highest in 0 mM NaCl when recommended dose and 2 mM Zn was applied while it was decreased with 4 mM Zn application without salinity. With increasing level of salinity growth parameters were reduced significantly but application of Zn countered the salt stress and improved the growth parameters at both salinity levels with both Zn application rate (Table 1 a, b and c). However, in moderate salinity 2 mM Zn was proved best while in strong salinity 4 mM Zn level was proved best for reducing effect of salinity stress. Salinity stress i.e., 70 mM NaCl and 140 mM NaCl decreased up to 16% and 28% of shoot length (Table 1a), 11% and 34% of root length (Table 1b) and 15% and 45% of dry biomass (Table 1c) respectively in wheat seedling when the recommended dose of zinc was applied. Growth parameters were increased upto 7% to 21% by increasing the dose of zinc to 2 mM and 5%-11% when zinc dose was increased upto 4 mM in moderate to strong salinity. Performance of varieties, Faisalabad-08 and Sehar-06 was overall best in all growth parameters showing highest values of shoot length (46.3, 45.1) and dry biomass (3.57, 3.50) respectively while in root length Shafaq-06 showed the highest value (35.1) followed by Sehar-06 and Faisalabad-08.

Role of Zinc application on physiological parameters under salinity stress: Salinity stress and different doses of Zn affected the physiology of wheat seedling. By increasing the NaCl concentration RWC and MSI of wheat was decreased by 21% and 24% in 70 mM NaCl and 36% and 44% respectively in 140 mM NaCl Stress where RD of zinc was applied (Figs. 1,2). By increasing zinc concentration relative water contents and membrane stability index under moderate and strong salinity were improved. 2 mM zinc dose was better than that of 4 mM zinc dose in saline conditions. However, performance as Zinc concentration 2 mM was the best and increasing zinc dose to 4 mM in 0 mM NaCl negatively affected the RWC and MSI. By increasing the RD of zinc to 2 mM RWC was increased overall by 18% in 70 mM NaCl and 24% in 140 mM NaCl treatment. Same was the case with MSI 2 mM zinc dose increased the values to 14% and 17% compared to recommended dose of zinc. RWC (47.20) was the highest in Faisalabad-08 followed by Sehar-06 compared to all the other varieties showing their tolerance in physiological processes against salinity stress.

Ameliorating effect of zinc on Na^+ toxicity and K^+ deficiency: Ionic homeostasis was disturbed by increasing the salinity stress. Concentration of sodium ions (Na^+) was increased with the application of NaCl while by increasing the zinc dose Na^+ ions concentration in plant body was decreased (Fig. 3). Highest mean value of Na^+ (42) was observed in 140 mM NaCl with lowest zinc dose and the lowest Na^+ contents (8.2) were found in 0 mM NaCl with 2 mM zinc dose. Na^+ contents in plants were decreased upto 20% in moderate salinity and 14% in strong salinity when 2 mM zinc dose was applied. On the other hand, Na^+ concentration in seedling was decreased maximum upto 27% in 2mM Zn and 24% in 4 mM Zn application rate in moderate to strong salinity respectively showing the antagonistic effect of Na^+ ions with zinc application rate. NARC-09 (26.9) and Zincol (26.7) showed the maximum mean Na^+ concentration compared to Faisalabad-08 (21.4) and Sehar-06 (21.6) in all treatments. Potassium ion (K^+) contents in wheat seedling were observed highest (19.9) in 0 mM NaCl with 2 mM Zinc application rate (Fig. 4). K^+ ions concentration was decreased by maximum upto 29% and 45% in 70 mM NaCl and 140 mM NaCl concentration respectively while zinc dose was normal (i.e., RD Zn). By increasing the Zn to 2 mM the concentration of K^+ was increased max upto 46% in 70 mM NaCl salt stress and 29% in 140 mM NaCl stress. With the increase of zinc dose upto 4 mM K^+ ions were increased upto 4% in 70 mM and 30% in 140 mM Salt stress. Wheat genotype Faisalabad-08 and Sehar-06 were proved to be overall best in K^+ ion concentration with the max value of 16.4 mg g^{-1} in both. Maximum Zinc concentrations (268 mg kg ^{1}) were found under control conditions with 4 mM Zn dose application whereas Zn conc. was decreased in all genotypes of wheat when seedlings were subjected to salinity stress (Fig. 5). Application of 70 mM NaCl stress reduced the plant zinc concentration upto 26% whereas 140 mM NaCl stress level reduced Zn conc. maximum upto 50% in wheat genotypes. However, maximum Zn conc. was found in 4 mM Zn level under both salinity levels compared to 2 mM Zn level and recommended dose of Zn. Maximum zinc concentration was found in V9 i.e., Zincol followed by V6 i.e., Faisalabad-08.

Pearson correlation and ranking of wheat genotypes for salt tolerance: Different genotypes were screened in this experiment for their tolerance against NaCl induced salinity stress and zinc use efficiency under saline conditions. Principle component analysis factor scoring (Table 2) and biplot (Fig. 7a, b) were used to screen out best varieties. Faisalabd-08 and Sehar-06 showed the best factor scores in terms of both K^+/Na^+ ratio and total dry matter production. Biplot explained the GGE variation upto 87% in K^+/Na^+ ratio and about 78% in total dry matter production data. Faisalabad-08 (V6) and Sehar-06 (V5) performed overall best in all the environment i.e. in all salinity and zinc levels exhibiting the strong positive relationship under the varying environment. Pearson correlation analysis portrayed the negative relationship of Na^+ concentration with other measured parameters i.e., root and shoot growth analysis, physiological parameters, K^+ and Zn^{2+} concentration (Fig. 6). Zn concentration showed positive relationship with root and shoot growth parameters, tissue health parameters as well as K^+ concentration in plant tissue depicting the increment in those parameters by increasing the Zn dose under saline and non-saline conditions.

Table 1(a). Effect of zinc application on shoot length (cm) of wheat under salinity stress.

Varieties	Control			EC 70 mM			EC 140 mM			Mean
	Zn (RD)	Zn 2mM	Zn 4mM	Zn (RD)	Zn 2mM	Zn 4mM	Zn (RD)	Zn 2mM	Zn 4mM	
AARI-11	45.9 ± 1.4	46.7 ± 0.9	30. 7± 1.2	42.4 ± 1.6	46.7 ± 1.0	45.0 ± 1.8	36.3 ± 1.2	42.7 ± 1.2	37.3 ± 1.5	41.5 c
Miraj-08	48.1 ± 1.6	49.6 ± 2.1	29.7 ± 1.8	40.8 ± 1.4	48.1 ± 1.6	45.5 ± 2.5	35.9 ± 1.2	43.2 ± 2.5	39.8 ± 1.2	42.3 bc
Pasban-90	49.7 ± 0.8	45.3 ± 2.6	31.7 ± 1.3	39.7 ± 1.3	47.9 ± 2.1	43.2 ± 3.8	35.6 ± 1.7	42.3 ± 1.3	40.1 ± 1.1	41.7 bc
Punjab-11	49.8 ± 0.9	50.7 ± 0.9	28.8 ± 2.0	40.7 ± 1.5	47.4 ± 2.0	45.4 ± 0.9	32.7 ± 2.4	41.1 ± 1.1	39.9 ± 1.4	41.8 bc
Sehar-06	55.0 ± 1.8	57.7 ± 2.4	32.5 ± 1.8	43.2 ± 0.9	49.2 ± 0.6	46.8 ± 1.5	38.8 ± 2.1	43.0 ± 1.5	39.5 ± 0.3	45.1 a
Faisalabad-08	53.9 ± 1.9	57.8 ± 3.1	31.1 ± 1.7	47.7 ± 1.8	51.0 ± 1.4	50.0 ± 0.8	40.6 ± 2.1	43.7 ± 1.8	40.7 ± 1.3	46.3 a
Shafaq-06	51.1 ± 1.0	52.4 ± 2.4	30.2 ± 1.7	39.0 ± 1.6	46.3 ± 0.8	45.3 ± 0.7	36.0 ± 0.1	40.7 ± 0.9	37.9 ± 1.0	42.1 bc
Borlaug-15	48.7 ± 1.1	53.0 ± 2.1	32.0 ± 1.5	40.6 ± 0.9	44.6 ± 0.4	46.8 ± 2.0	32.4 ± 1.3	41.8 ± 1.4	38.4 ± 1.2	42.0 bc
Zincol	50.8 ± 1.3	56.0 ± 1.2	33.5 ± 1.6	39.7 ± 1.2	45.9 ± 1.2	51.8 ± 1.5	34.7 ± 2.3	44.7 ± 2.0	40.0 ± 1.0	44.1 ab
NARC-09	47.4 ± 1.3	43.7 ± 2.6	27.4 ± 1.0	39.5 ± 1.0	44.3 ± 2.1	43.7 ± 3.3	36.6 ± 1.5	40.4 ± 0.9	37.7 ± 1.0	40.1 c
MH-97	46.6 ± 1.2	47.5 ± 1.3	31.7 ± 1.1	41.3 ± 1.3	46.0 ± 1.1	42.7 ± 0.3	35.9 ± 2.1	40.2 ± 1.6	38.5 ± 1.3	41.1 c
AAS-11	48.1 ± 0.4	50.6 ± 1.8	32.3 ± 2.4	42.4 ± 1.6	45.7 ± 0.9	45.0 ± 2.5	34.9 ± 2.3	41.2 ± 0.4	36.9 ± 1.6	41.9 bc
Mean	49.6 A	50.9 A	31.0 F	41.4 C	46.9 B	45.9 B	35.9 E	42.1 C	38.9 D	

All the values in the main table are the means of three replicates ± Standard Error. Capital Letters in show difference in treatments while small letters show difference in Varieties.
HSD1 (Treatments) = 2.04, HSD2 (Varieties) = 2.48

Table 1(b). Effect of zinc application on root length (cm) of wheat under salinity stress.

Varieties	Control			EC 70 mM			EC 140 mM			Mean
	Zn (RD)	Zn 2mM	Zn 4mM	Zn (RD)	Zn 2mM	Zn 4mM	Zn (RD)	Zn 2mM	Zn 4mM	
AARI-11	36.33 ± 1.1	40.50 ± 3.1	34.27 ± 2.2	34.27 ± 2.9	37.80 ± 1.1	31.67 ± 4.0	26.33 ± 3.3	28.67 ± 3.5	26.53 ± 0.7	32.9 ab
Miraj-08	37.10 ± 1.5	44.33 ± 1.8	36.33 ± 2.2	30.10 ± 1.7	36.03 ± 2.1	29.50 ± 1.3	22.33 ± 3.7	30.27 ± 1.3	28.60 ± 0.9	32.7 ab
Pasban-90	37.23 ± 1.4	39.00 ± 0.6	36.50 ± 1.3	34.90 ± 4.6	36.90 ± 0.7	32.33 ± 3.6	22.20 ± 0.4	29.00 ± 2.0	26.87 ± 5.9	32.8 ab
Punjab-11	40.23 ± 2.5	40.20 ± 0.8	31.60 ± 0.9	36.87 ± 3.9	40.27 ± 4.0	28.00 ± 3.3	29.30 ± 7.4	32.67 ± 2.9	28.87 ± 4.7	34.2 ab
Sehar-06	38.90 ± 1.8	39.00 ± 2.1	33.07 ± 0.6	36.00 ± 1.7	41.80 ± 7.6	31.83 ± 2.2	23.13 ± 3.1	32.67 ± 3.5	26.17 ± 2.1	33.6 ab
Faisalabad-08	36.20 ± 1.6	38.67 ± 2.0	30.80 ± 0.4	32.17 ± 3.9	33.40 ± 2.5	29.67 ± 2.5	21.50 ± 0.5	29.33 ± 0.9	23.53 ± 1.8	30.6 b
Shafaq-06	43.67 ± 1.8	40.33 ± 1.2	34.80 ± 0.8	41.20 ± 1.4	36.00 ± 1.2	32.33 ± 2.1	26.00 ± 0.6	33.20 ± 1.2	28.40 ± 6.1	35.1 a
Borlaug-15	37.97 ± 4.1	39.67 ± 2.3	33.27 ± 2.3	36.57 ± 5.9	34.40 ± 2.5	32.00 ± 2.2	28.87 ± 6.4	30.73 ± 2.7	23.53 ± 6.8	33.0 ab
Zincol	36.07 ± 2.5	39.00 ± 1.2	31.93 ± 1.1	30.87 ± 1.1	35.10 ± 0.2	29.83 ± 1.5	23.30 ± 4.5	29.33 ± 0.9	30.77 ± 1.3	31.8 ab
NARC-09	41.30 ± 2.3	32.67 ± 1.2	31.73 ± 1.2	29.13 ± 1.9	32.13 ± 1.7	27.33 ± 1.2	23.20 ± 2.6	30.73 ± 0.9	23.33 ± 3.6	30.2 b
MH-97	36.67 ± 1.6	34.00 ± 2.3	32.02 ± 1.8	32.00 ± 1.8	36.30 ± 3.2	26.00 ± 2.2	26.67 ± 1.9	27.27 ± 1.2	25.23 ± 2.8	30.7 b
AAS-11	36.93 ± 1.9	38.00 ± 2.6	30.73 ± 0.3	33.90 ± 1.5	40.07 ± 1.6	30.33 ± 3.3	28.00 ± 3.6	31.00 ± 0.6	35.27 ± 2.1	33.8 ab
Mean	38.2 A	38.8 A	33.1 CD	34.0 BC	36.7 AB	30.1 DE	25.1 F	30.4 DE	27.3 EF	

All the values in the main table are the means of three replicates ± Standard Error. Capital Letters in show difference in treatments while small letters show difference in Varieties.
HSD1 (Treatments) =3.52, HSD2 (Varieties) = 4.28

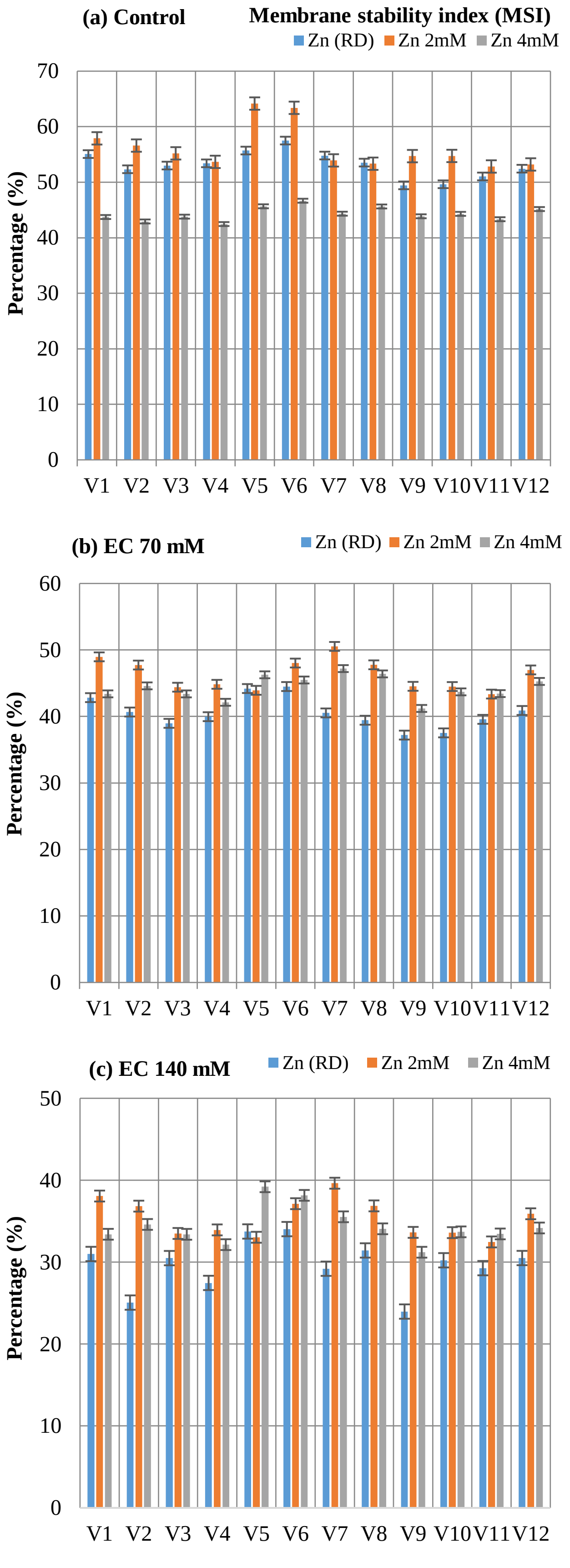

Fig. 1. Effect of zinc application on membrane stability index of wheat under salinity stress.
(a) Control, **(b)** EC 70mM, **(c)** EC 140mM. **V1**=AARI-11, **V2**=Miraj-08, **V3**=Pasban-90, **V4**=Punjab-11, **V5**= Sehar-06, **V6**=Faisalabad-08, **V7**=Shafaq-06, **V8**=Borlaug-15, **V9**=Zincol, **V10**=NARC-09, **V11**=MH-97, **V12**=AAS-11

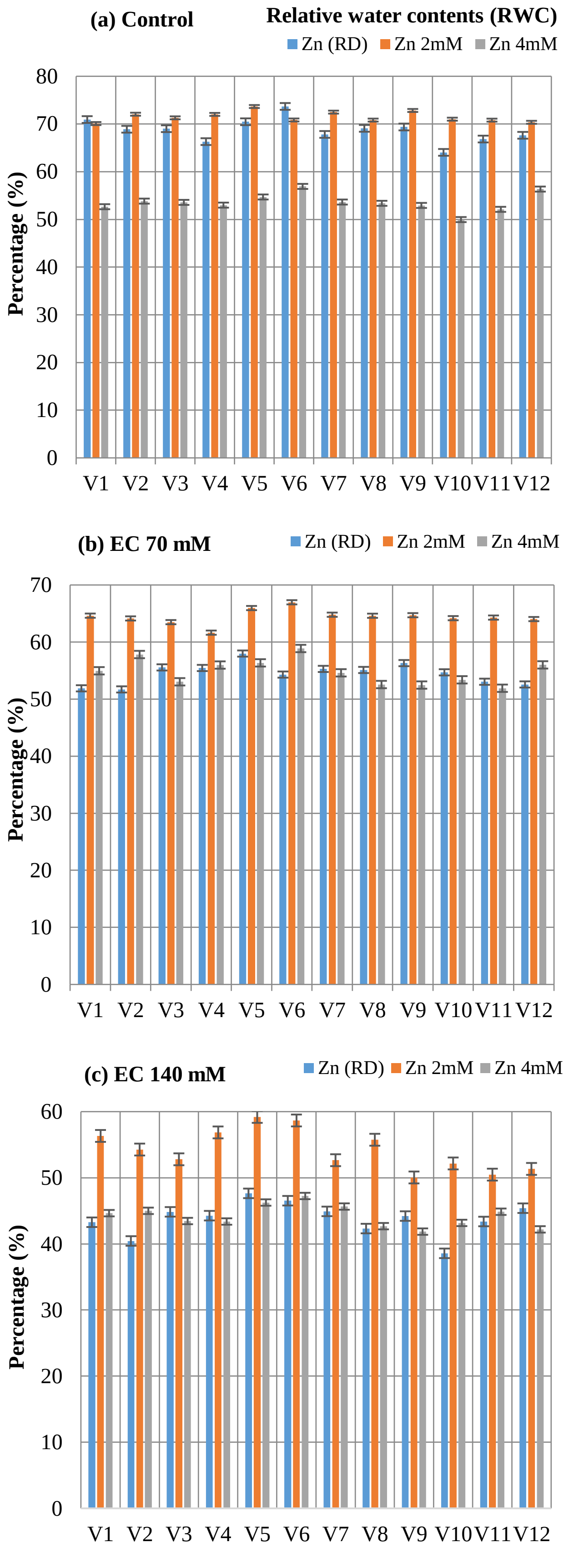

Fig. 2. Effect of zinc application on relative water contents (RWC) of wheat under salinity stress.
(a) Control, **(b)** EC 70mM, **(c)** EC 140mM. **V1**=AARI-11, **V2**=Miraj-08, **V3**=Pasban-90, **V4**=Punjab-11, **V5**= Sehar-06, **V6**=Faisalabad-08, **V7**=Shafaq-06, **V8**=Borlaug-15, **V9**=Zincol, **V10**=NARC-09, **V11**=MH-97, **V12**=AAS-11.

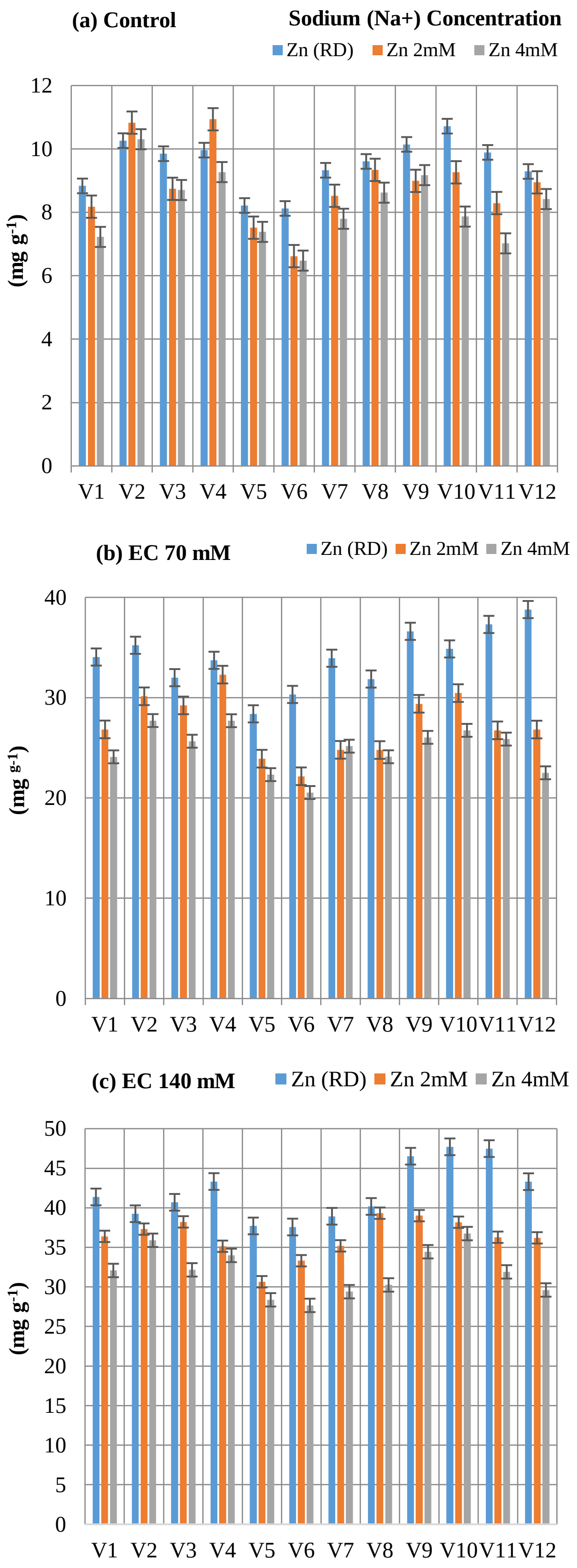

Fig. 3. Effect of zinc application on sodium concentration in wheat under salinity stress.
(a) Control, **(b)** EC 70mM, **(c)** EC 140mM. **V1**=AARI-11, **V2**=Miraj-08, **V3**=Pasban-90, **V4**=Punjab-11, **V5**= Sehar-06, **V6**=Faisalabad-08, **V7**=Shafaq-06, **V8**=Borlaug-15, **V9**=Zincol, **V10**=NARC-09, **V11**=MH-97, **V12**=AAS-11

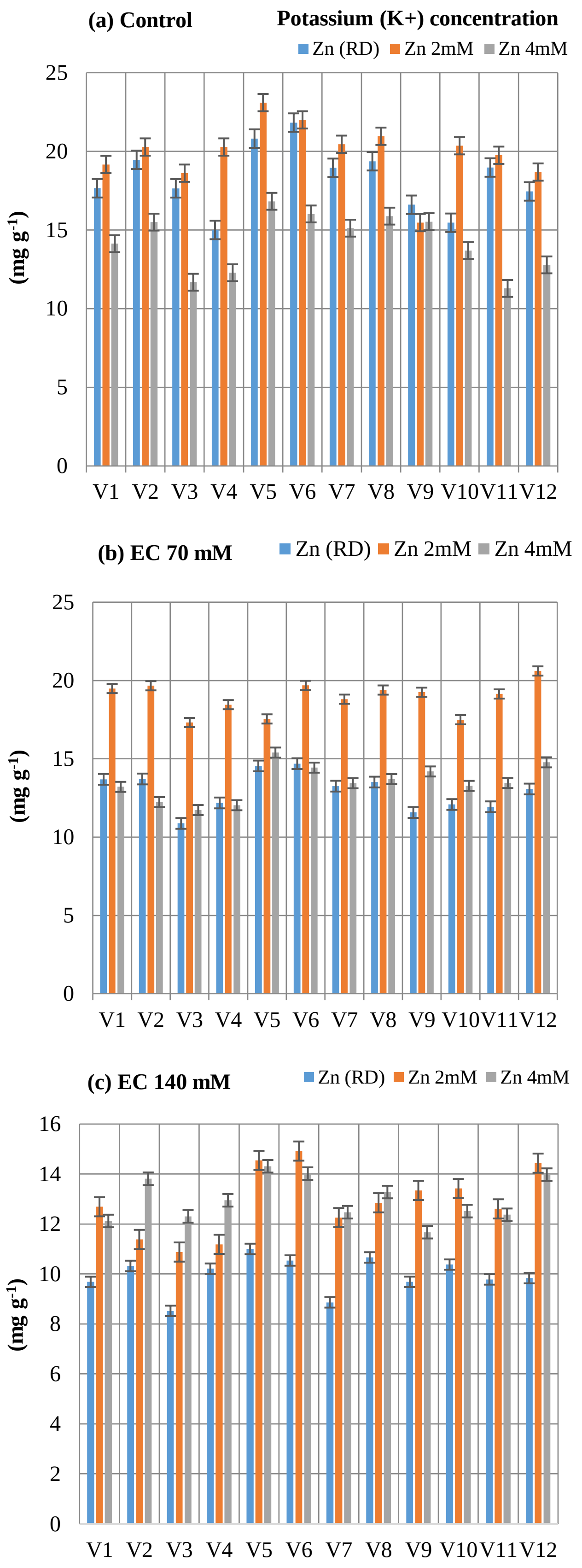

Fig. 4. Effect of zinc application on potassium concentration in wheat under salinity stress.
(a) Control, **(b)** EC 70mM, **(c)** EC 140mM. **V1**=AARI-11, **V2**=Miraj-08, **V3**=Pasban-90, **V4**=Punjab-11, **V5**= Sehar-06, **V6**=Faisalabad-08, **V7**=Shafaq-06, **V8**=Borlaug-15, **V9**=Zincol, **V10**=NARC-09, **V11**=MH-97, **V12**=AAS-11.

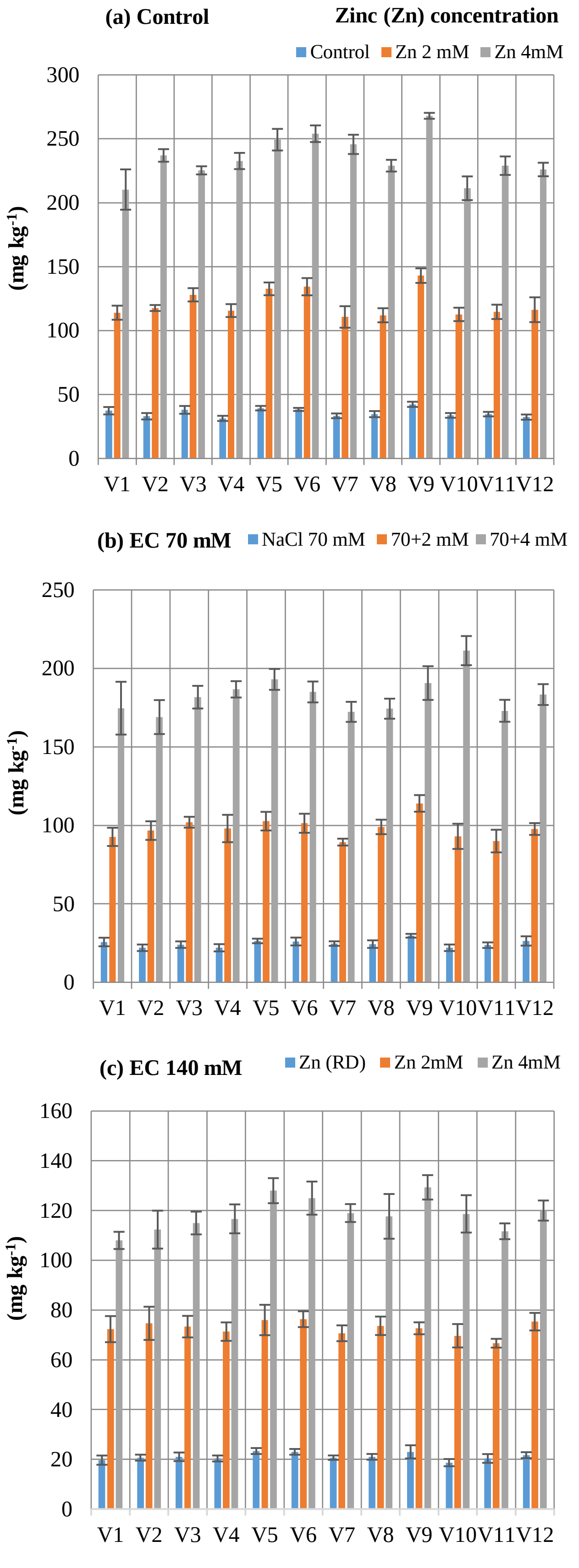

Fig. 5. Effect of zinc application on plant zinc concentration in wheat under salinity stress.
(a) Control, (b) EC 70mM, (c) EC 140mM. **V1**=AARI-11, **V2**=Miraj-08, **V3**=Pasban-90, **V4**=Punjab-11, **V5**= Sehar-06, **V6**=Faisalabad-08, **V7**=Shafaq-06, **V8**=Borlaug-15, **V9**=Zincol, **V10**=NARC-09, **V11**=MH-97, **V12**=AAS-11.

Table 1(c). Effect of zinc application on dry biomass (g) of wheat under salinity stress.

Varieties	Control			EC 70 mM			EC 140 mM			Mean
	Zn (RD)	Zn 2mM	Zn 4mM	Zn (RD)	Zn 2mM	Zn 4mM	Zn (RD)	Zn 2mM	Zn 4mM	
AARI-11	3.74 ± 0.3	4.09 ± 0.3	3.34 ± 0.2	3.32 ± 0.2	3.69 ± 0.2	3.02 ± 0.2	2.21 ± 0.1	2.46 ± 0.1	1.94 ± 0.1	3.09 c
Miraj-08	3.92 ± 0.4	4.27 ± 0.2	3.60 ± 0.1	3.63 ± 0.1	3.81 ± 0.3	3.30 ± 0.3	2.23 ± 0.1	2.43 ± 0.1	2.16 ± 0.2	3.26 bc
Pasban-90	3.95 ± 0.3	4.16 ± 0.3	3.34 ± 0.3	3.30 ± 0.3	3.83 ± 0.1	3.35 ± 0.2	2.14 ± 0.2	2.36 ± 0.2	2.02 ± 0.2	3.16 c
Punjab-11	3.75 ± 0.4	4.07 ± 0.5	3.49 ± 0.2	3.47 ± 0.2	3.77 ± 0.2	3.26 ± 0.1	2.26 ± 0.1	2.46 ± 0.1	2.13 ± 0.1	3.18 c
Sehar-06	4.75 ± 0.3	4.94 ± 0.3	3.61 ± 0.2	3.68 ± 0.2	4.01 ± 0.1	3.24 ± 0.1	2.48 ± 0.1	2.62 ± 0.1	2.22 ± 0.1	3.50 ab
Faisalabad-08	4.72 ± 0.4	5.07 ± 0.4	3.82 ± 0.2	3.75 ± 0.2	3.94 ± 0.1	3.53 ± 0.1	2.47 ± 0.1	2.68 ± 0.1	2.16 ± 0.1	3.57 a
Shafaq-06	3.94 ± 0.4	4.26 ± 0.2	3.52 ± 0.1	3.47 ± 0.1	3.90 ± 0.1	3.36 ± 0.1	2.15 ± 0.1	2.40 ± 0.1	1.95 ± 0.2	3.22 c
Borlaug-15	3.99 ± 0.5	4.49 ± 0.1	3.43 ± 0.1	3.41 ± 0.1	3.57 ± 0.2	3.01 ± 0.2	2.20 ± 0.2	1.44 ± 0.2	1.98 ± 0.1	3.06 c
Zincol	4.12 ± 0.7	4.40 ± 0.2	3.42 ± 0.2	3.43 ± 0.1	3.70 ± 0.2	3.35 ± 0.2	2.24 ± 0.1	2.43 ± 0.1	2.08 ± 0.2	3.24 c
NARC-09	3.90 ± 0.5	3.75 ± 0.3	3.43 ± 0.1	3.40 ± 0.1	3.65 ± 0.1	3.27 ± 0.1	2.28 ± 0.1	2.46 ± 0.2	2.00 ± 0.2	3.13 c
MH-97	4.07 ± 0.3	4.35 ± 0.1	3.43 ± 0.1	3.38 ± 0.1	3.60 ± 0.1	3.21 ± 0.1	2.14 ± 0.2	2.36 ± 0.1	1.95 ± 0.2	3.17 c
AAS-11	4.04 ± 0.2	4.04 ± 0.1	3.14 ± 0.2	3.47 ± 0.1	3.53 ± 0.1	3.08 ± 0.1	2.16 ± 0.2	2.38 ± 0.1	1.81 ± 0.2	3.07 c
Mean	4.08 B	4.32 A	3.46 DE	3.48 D	3.75 C	3.25 E	2.25 FG	2.37 F	2.03 G	

All the values in the main table are the means of three replicates ± Standard Error. Capital Letters in show difference in treatments while small letters show difference in Varieties.
HSD1 (Treatments) = 0.22, HSD2 (Varieties) = 0.26

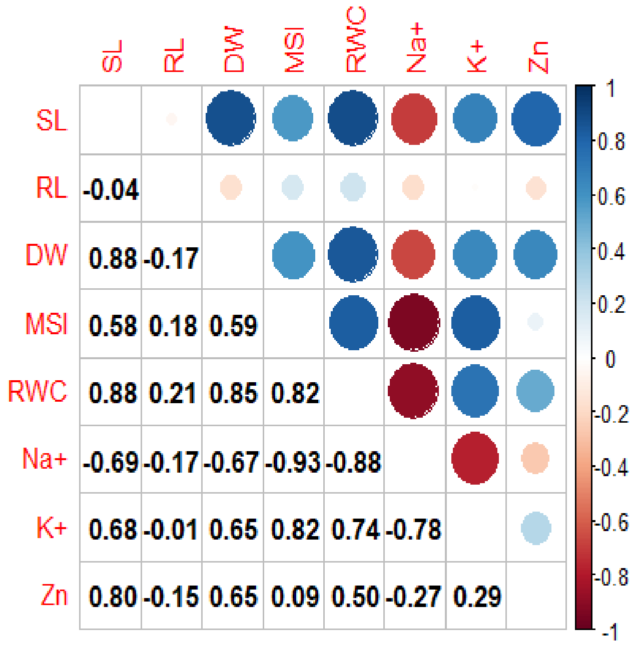

Fig. 6. Pearson Correlation Analysis of measured parameters with Na^+ toxicity. Whereas,
SL=Shoot length, RL=Root length, DW=Dry weight, MSI=Membrane stability index, RWC=Relative water contents, Na^+= Sodium conc., K^+=Potassium conc., Zn=Zinc.

Discussion

Plants respond to salinity stress by adopting various mechanisms. Different species and different genotypes have different tolerance level and respond to abiotic stresses and salinity. In our experiment, we checked the response of different genotypes of wheat against different salinity levels and effect of zinc doses on these genotypes under these levels and control. By increasing salinity levels growth parameters of all the wheat genotypes were reduced. Photosynthetic activity of plants is disturbed under higher level of salinity either by stomatal factors or non-stomatal factors, which disturbs the gaseous exchange, transpiration rate, reduction in CO_2 assimilation and ultimately retarded growth of plants (Arif *et al*., 2020; Pan *et al*., 2021). Soil salinity usually occurs simultaneously with nutrients deficiencies especially zinc deficiency in crop and cultivated soils. Zinc supply to the crop can enhance tolerance of wheat towards abiotic stresses especially salt stress by modulating antioxidant defense system, suppressing oxidative damage to plant root, diminishing electrolyte leakage from plant roots, lessening root cell permeability and stabilizing cell membrane of root (Daneshbakhsh *et al*., 2013). Reduction in plant growth and biomass production can be associated with ion toxicity due to higher level of Na^+ and Cl^- in plants in salinity stress. Zinc fertilization to plants can ameliorate the negative effects caused by restricting Na^+ and Cl^- ion uptake in higher levels of salts (Nadeem *et al*., 2020; Tolay, 2021). Our results proved that application of higher dose of zinc lessened the negative effect of salinity on growth parameters and root shoot length and fresh dry biomass was increased at both levels of salinity i.e., moderate, and strong salinity when zinc dose was increased from recommended to 2 mM and 4 mM. Application of zinc at higher rates i.e., 4 mM showed overall less values than 2 mM. This may be attributed with that higher rates of zinc toxicated the plants and negatively affected the plant growth and physiology (Salem, 2021). Our findings are in line with the experiments conducted by Daneshbakhsh *et al*., 2013; Hussein & Abou-Baker, 2018 and Tolay, 2021 in which zinc application impaired the salt damage and increased the plant growth under salinity stress. Zinc fertilization under salinity stress reduced the uptake of toxic ions and zinc itself is a co-enzyme of more than 60 enzymes. Due to this zinc fertilization, it happened to increase the plant tolerance under higher salt levels and growth attributes and biomass were improved in both salinity levels where the higher doses of zinc were applied compared to recommended dose of zinc fertilizer.

Salinity stress affects the water relations of plants causing reduction in turgor and water potential as well as less water use efficiency. This is due to limited water uptake of plants caused by salinity induced osmotic stress (Nadeem *et al*., 2022a, b, c). Plants treated with trace elements like zinc had better water and turgor potentials under saline conditions (Ashraf & Ashraf, 2012; Iqbal *et al*., 2018). In current experiment, higher salt levels decreased RWC and MSI of seedlings by increasing electrolyte leakage and further decreased with higher levels of salinity showing the negative correlation of RWC and MSI with salinity levels. However, by increasing the rate of zinc fertilizations, RWC and MSI of plants were improved significantly. Similar findings were also reported in rice crop (Nadeem *et al*., 2020) in which relative water contents and membrane stability index were improved under salinization with the application of exogenous zinc. This improvement may be attributed with the less ion leakage and cell membrane stability which helps plant to absorb more mineral nutrition under higher salt levels (Broadley *et al*., 2011) ultimately improving the plant morphological attributes. Zinc application under saline conditions limits the Na^+ and Cl^- uptake (Nadeem *et al*., 2020) improving salt tolerance of crop. It can act as ROS scavenger protecting the cell from oxidative damage in abiotic stresses (Jan *et al*., 2019). It helps plant to absorb more water from root (Hejazi Mehrizi *et al*., 2011; Salem, 2021). Moreover, less uptake of Na^+ and Cl^- due to zinc fertilization causes reduction in salinity induced hyperosmotic and hyper ionic stress stabilizing the membrane integrity (Yang & Guo, 2018). Potassium (K^+) acts as osmoregulator and osmo- protectant in plants in normal and stress environment. In our study, increase in zinc application dose (i.e., 2 mM Zn and 4 mM Zn) also improved the K^+ uptake in seedling under moderate as well as strong saline conditions. So, by increasing the concentration of K^+ in plants RWC and MSI were improved.

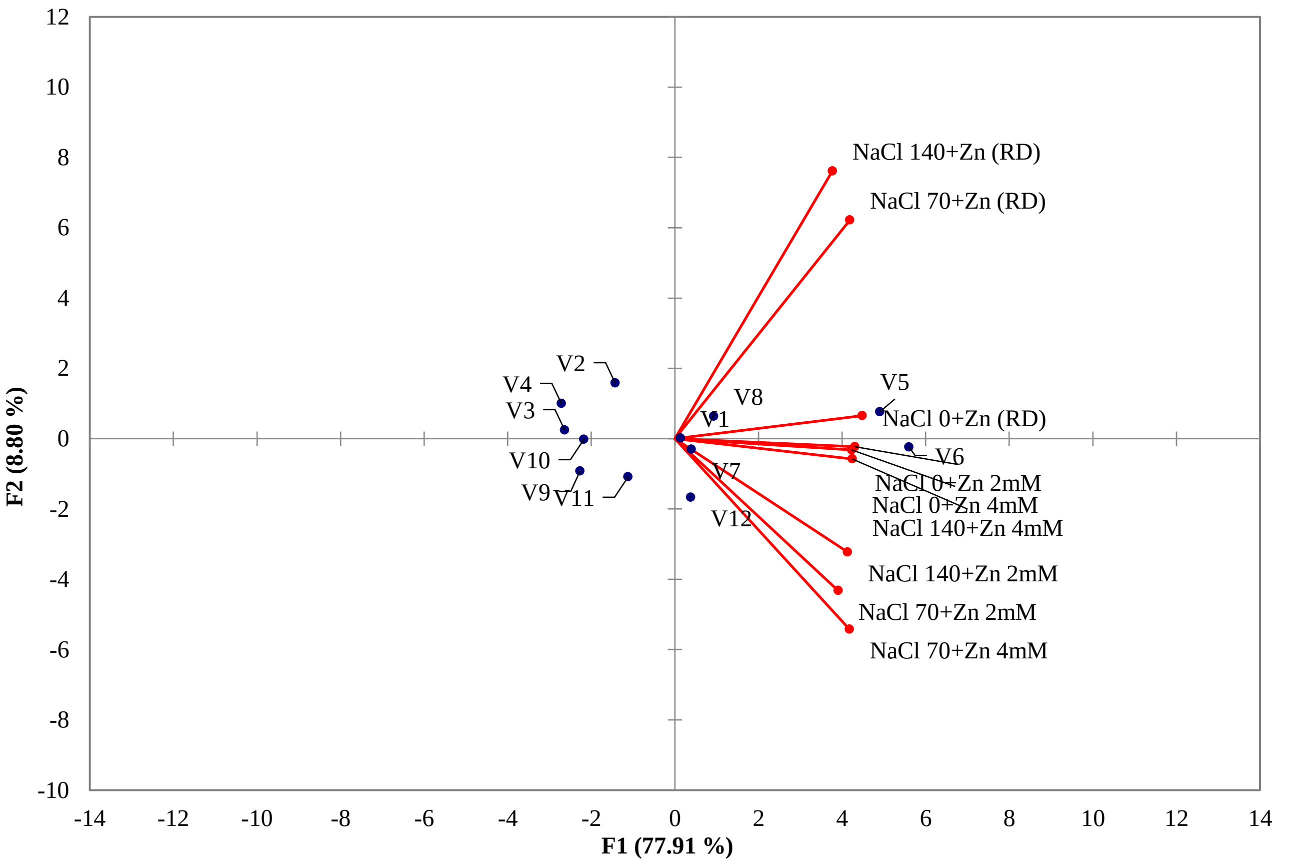

Fig. 7a. Biplot (axes F1 and F2: 86.71 %) for K+/Na+ Ratio.

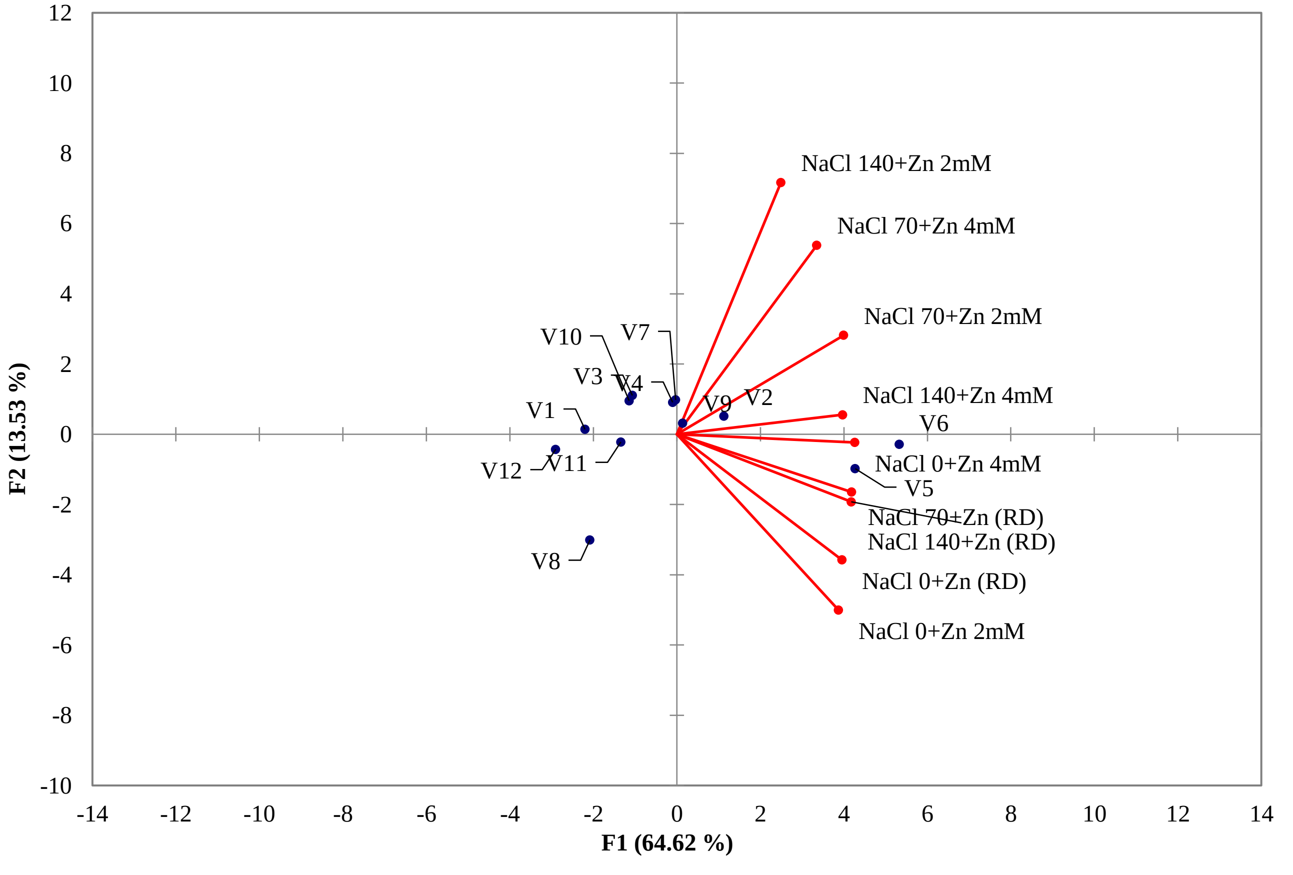

Fig. 7b. Biplot (axes F1 and F2: 78.15 %) for total dry weight.

Table 2. Factor scoring on the basis of K^+/Na^+ ratio and total dry weight.

Factor score K^+/Na^+ ratio			Factor score total dry weight		
Observation	**F1**	**F2**	**Observation**	**F1**	**F2**
V6	5.6011	-0.2363	V6	5.3285	-0.2868
V5	4.9063	0.7661	V5	4.2724	-0.9790
V8	0.9308	0.6338	V2	1.1272	0.5122
V7	0.3973	-0.3071	V9	0.1386	0.3176
V12	0.3816	-1.6655	V7	-0.0297	0.9783
V1	0.1302	0.0110	V4	-0.1014	0.9105
V11	-1.1194	-1.0879	V3	-1.0663	1.1053
V2	-1.4297	1.5832	V10	-1.1439	0.9545
V10	-2.1768	-0.0184	V11	-1.3415	-0.2199
V9	-2.2686	-0.9215	V8	-2.0811	-3.0062
V3	-2.6384	0.2474	V1	-2.2013	0.1412
V4	-2.7144	0.9952	V12	-2.9015	-0.4277

V1=AARI-11, **V2**=Miraj-08, **V3**=Pasban-90, **V4**=Punjab-11, **V5**= Sehar-06, **V6**=Faisalabad-08, **V7**=Shafaq-06, **V8**=Borlaug-15, **V9**=Zincol, **V10**=NARC-09, **V11**=MH-97, **V12**=AAS-11

Ionic imbalance is widely reported in plants under salinity stress. Na^+ ions and Cl^- ions concentrations are found to be toxic at higher at toxic levels in plants where NaCl or salinity is fed in growth medium (Ashraf *et al.*, 2018). Our results exhibited that increasing the dose of zinc fertilization could reduce the uptake of sodium ions in seedlings in all genotypes. Na^+ and K^+ ions showed inverse relationship. K^+ concentration was found decreasing while increasing the salinity levels. With the application of zinc in higher doses concentration of K^+ in plants was increased and Na^+ was decreased at higher salt levels showing that increasing dose of zinc fertilizer can reduce the Na^+ uptake and rectify the toxic effect of it (Fig. 4). Our finding were in line with similar results found in *Oryza sativa* L. (Nadeem *et al.*, 2020) and *Zea mays* L. (Iqbal *et al.*, 2018) in which zinc fertilization significantly rectified the Na^+ ion concentration and increased the K^+ and Zn^{2+} concentration in plants under salinity stress. Salinized soils having higher concentration of toxic ions like Na^+ and Cl^- usually deficit in macro and micronutrients like N, P, K, Ca, Zn and Fe because toxic ions i.e., Na^+ and Cl^- interfere with these elements and compete for uptake (Kaya *et al.*, 2018). Our results are in agreement with those of Eker *et al.*, 2013; Weisany *et al.*, 2014 and Saeidnejad *et al.*, 2016 in which zinc fertilization decreased the Na^+ ion concentration and increased macronutrients and micronutrients concentration including K^+ and Zn^{2+} in wheat and soybean plants.

Conclusion

Salinization is the most alarming abiotic stress for agricultural crop production and food security. Plants undergo many physiological stresses, ionic and nutritional imbalance in saline conditions. Increasing the dose of zinc from the recommended dose under moderate to strong saline conditions improved the growth and physiological attributes of all the selected genotypes and rectified the ion imbalance in seedling. It is concluded from the results that increasing the zinc dose upto a certain limit can ameliorate the toxic effect of salinity stress and ultimately growth and crop production is improved with higher zinc concentration in plants and soil. It is recommended that higher zinc dose than normal under saline condition may be applied to tackle the negative effect of salinity and improving plant growth and physiological processes.

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