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Ameliorating the effect of salinity on barley using chemically synthesized zinc oxide nanoparticles

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Abstract

Barley (*Hordeum vulgar L.) was observed in the current study for mitigating the adverse effects of salinity using zinc oxide nanoparticles (ZnO-NPs) by ameliorating pigments, antioxidants, total protein and sugar contents, and* ionic and growth parameters. A pot experiment in the greenhouse was conducted at the Government College Women's University of Faisalabad with a randomized complete block design (RCBD) replicated three times. The treatments comprised of different levels i.e., i) control (no ZnO-NPs), ii) T1 (200 mg L^{-1} ZnO-NPs), iii) T2 (400 mg L^{-1} ZnO-NPs). Salt stress was induced by supplying NaCl (100 mM) to the roots at the rate of 100 mM L^{-1}- and 150-mM L^{-1} NaCl on the 10th day after seed sowing. Results showed that ZnO-NPs inhibited growth-reducing factors due to salinity in barley by improving net photosynthesis, chlorophyll contents, stomatal conductance, total soluble protein and sugars, root and shoot length, and dry and fresh weight. There was less chloroplast observed per cell in barley leaves when exposed to higher levels of salinity and untreated replicates. Statistical analysis revealed that ZnO-NPs decreased the production of H_2O_2, MDA, AsA, Proline, phenolic, and sodium ion accumulation in roots and shoots by 50-60%. Higher salinity in untreated plants was observed to show the physiological activities less supportive of the growth and yield of barley. The salt accumulation in plant tissues developed due to salinity was observed to damage the structural organization in the photosynthetic apparatus and ultimately reduce the photosynthetic activities.

Keywords: salt stress, *Hordeum vulagre* L., Antioxidant enzymes, ZnO-NPs

Full length article **Corresponding Author*, e-mail: A.sami@tu.edu.sa

1. Introduction

Salinity induced stress has generally been reported to involve certain mechanisms including ionic imbalance and hyper-osmotic stress. If the stress is caused by excess amount of NaCl, sodium enters in the cell cytosol by potassium channels and begins to accumulate reaching the toxic level. A few cultivars have hardly been breeded till date for better sodium exclusion or improved osmotic adjustment leading to salt tolerance [1]. This is the reason for salinity-induced perturbance in potassium homeostasis is considered as secondary importance usually [2-3] which should be signified. [4] Observed that by overcoming such constraints can open new avenues for plant agronomic practices and breeding purpose to introduce salt tolerance. Salinity negatively affects photosynthetic process as observed [5]. This effect can be mitigated by limiting the CO_2 diffusion towards chloroplast by inhibiting the effects of salinity on stomatal conductance [6] and ultimately this process affects the decline in photosystem-II related with stress consequently resulting the inhibition of photo damage of PSII [7]. [8] on the other side reported the maximum photochemical efficiency, was not much affected by salinity. However, the increase in electron flux to oxygen resulted in accumulation of reactive oxygen species (ROS) but the absorbed light energy utilized for CO_2 fixation was restricted by NaCl treatment [9]. To combat the problems related to abiotic stress including water and salinity stress, the use of nanoparticles (NPs) has gained the interest in agriculture by the beginning of 21st century [10-11] and over 231 products from nanotechnology are now available for various agricultural practices. Nanoparticles have gained the interest to minimize the plant growth dependence on fertilizers for sustainable crop production with maximum food security to fulfill the needs of nutrition for rapidly growing population in the world [5], [12]. Nanoparticles based on Zinc are the most used NPs for agriculture and other industries [8], [13] and their production is more 10 to 100 times greater than other nanoparticles [6-7]. They are being highly used in medicines, solar cells, food and cosmetic industry [14-15] that inevitably lead their transfer to the environmental sciences [16]. Increase in the application of nanoparticles and their accumulation in biological systems, may be increased by the help of plants that can play a very crucial role in their transport in food chain [17-18]. There are both positive and negative effects of NPs on plant growth and development

used in agriculture with respect to soil conditions [19-22]. The application of ZnO NPs increased the protein and chlorophyll contents in wheat [23-25]. However, seed treated with ZnO NPs at high concentration reduced the plant growth by 80% and the chlorophyll contents were also decreased by 50% in *Arabidopsis thaliana* [26-27]. The toxic effects of NPs on growth of *A. thaliana* also revealed lower concentrations of ZnO NPs that improved the plant growth and development [28-30]. Nanoparticles if once reached into plant cells, they begin to accumulate in vascular bundle and stem stele and are then transferred to aerial parts of the plant [31-32] via cellular pores [33] through apoplast or either symplast pathway [19] and begin to interact with the sub-cellular and cellular organelles. During their penetration and transfer from one part of plant to another, it passes through metabolic pathways [34]. It has been obvious from recent studies that ZnO NPs accumulate in different sub-cellular organelles like vacuoles, chloroplast and cell membranes and modulates the cellular organization [35-37]. However the transforming effect f ZnO NPs on plants especially cereals grown I saline soils is not well known, and so to fill the gap of studies, current research work was designated to evaluate the effect of ZnO NPs on barley grown in saline soils in semiarid agro-climatic conditions. ZnO NPs can possibly be synthesized using certain physiochemical methods such as sol-gel process, laser vaporization, precipitation, ball mining and micro emulsion [38-39]. Zinc is one of an essential micronutrient in plants that plays significant role in metabolism. Zinc activates many enzymes involved in protein synthesis, cell membrane integrity, controls auxin synthesis pathway, lipid and carbohydrate metabolism by activating RNA polymerase, dismutase, carbonic dehydrogenase and dehydrogenase [40-41] observed the effect of ZnO NPs by seed priming on wheat and found that ZnO NP linearly improves the growth rate, total pigments and biomass of cereals. A substantially higher level of Zinc concentration in grains, roots and shoots was observed and verified to be used as a source of Zn to mitigate Zn deficiency in plants. As the osmotic pressure of soil is increased, field crops face exceptional difficulty in growing in saline soils (abiotic stress) because roots lose the role of efficient water absorption. In addition, concentrations of sodium and chloride ions due to due to elevated salinity stress, induces toxicity to plants in order to remove these ions [42-43]. In short, Salinity is the most destructive stress with tremendous impacts on plant growth especially cereals like barley by decreasing germination rate, growth and development, nutrient and water uptake [44-45], photosynthetic pigments, enzymatic activity, yield and yield components [46]. Barley is a world's most useful and strategic cereal crop cultivated as major staple food crop around the globe [47], [3] but barley yield is severely affected by abiotic stresses especially water deficiency and excessive salts that will be observed to be mitigated using nanoparticles. Hence, it was hypothesized that harmful effects of salinity can by reduced by using certain metallic oxide NPs like ZnO (chemically synthesized).

2. Materials and Methods

2.1. Experimental setup and layout

A pot experiment was led in a greenhouse with a controlled environment at the experimental site of Taif University For this trial; RCBD (randomized complete block design) with three replications was used.

2.2. Soil characteristics

The clay loam soil was obtained from the experimental site of the university, let to air dry, and then sieved using a 2 mm sieve. The soil was covered with a thin layered plastic sheet and sterilized under sunlight. The sun's heat increases the temperature of the soil, killing the majority of pathogens, pests and weeds [48] The soil chemical properties, including its EC=7.7 dSm^{-1}, pH=7.3, organic matter contents (OMC) =1.35%, available nitrogen (N) =0.033 ppm, phosphorous (P) =5.89 ppm, and available potassium (K) =31.9 ppm concentrations, were investigated prior to the experiment.

2.3. Seed sterilization

Surface sterilization of mature barley (Genotype B-14011) seeds for 5 min was done with 5% sodium hypochlorite solution and then continuously washed 4-5 times with double-distilled water, dried with blotting paper and then allowed to dry completely at room temperature.

2.4. Treatments

The treatments (3 sets of pots; untreated, 100 mM L^{-1} NaCl and 150 mM L^{-1} NaCl) were: i) control (no ZnO-NPs], ii) 200 mg L^{-1} ZnO-NPs, iii) 400 mg L^{-1} ZnO-NPs were set up under RCBD with each treatment replicated thrice.

2.5. Seed sowing

Ten seeds were sown initially in each pot that contained 15 kg of clay loamy soil. Each pot had a diameter of 25cm with a height of 30cm. After the seeds were fully germinated, six plants per pot were thinned.

2.6. Synthesis of ZnO-NPs

ZnO-NPs were made as a colloidal suspension using a sol-gel procedure. For this, 80 mL of 99.9% 2-propanol was dissolved in 8.78 g of 0.5 mol/L zinc acetate dihydrate at 50 ◦C. Using 2-propanol, the dilution of the solution was kept up to 840 mL. Following that, 160 mL of a 0.5 mol/L sodium hydroxide (NaOH) solution containing 99.5% sodium hydroxide was added within one minute at 0 °C for stirring. The mixture was boiled at 65 °C in a water bath for two hours. After seven days, the solvent was separated for ageing at room temperature by centrifugation of 7000 rpm for 15 minutes. The precipitate was dried for eight hours at 120 °C in an oven. The powder was then calcined at 400 °C for two hours in a muffle furnace [49-50].

2.7. Application of treatments and establishment of stress

The uniformly sized seeds were primed for 8 hours in an airtight bottle with continuous gentle shaking in distilled water as the control, 200 mg L^{-1} and 400 mg L^{-1} Zinc oxide nanoparticles (ZnO-NPs). The primed seeds were spread out on filter paper for eight hours, enabling the seeds to dry out until they returned to their pre-priming moisture level. In order to induce salt stress, NaCl (100 mM) was additionally supplied to the roots at the rate of 100 mM L^{-1} and 150 mM L^{-1} NaCl on the 10th day after seed sowing when seed were completely germinated. The soil moisture levels were maintained at 75% of the field capacity.

2.8. Harvesting and evaluation of growth characteristics

Plants were harvested after 40 days of plantation. Fresh weights and lengths of the roots and shoots were immediately measured following the harvest. Fresh plant samples were stored in a biomedical refrigerator at -30°C for additional examination. Three samples from each treatment were oven-dried at 65°C for three days in order to measure their dry weights and to perform an acid digestion for ionic content analysis.

2.9. Pigment composition

80% v/v acetone was used to extract the pigments from the leaves, and leaf samples of 30 mg were ground in ice-cold acetone and centrifuged for 5 minutes at 5000 g at 4 °C. Spectrophotometer was used to measure the extract's absorption (Model SM1200; Randolph, NJ, USA). Total chlorophyll, chlorophyll a, b, and carotenoids were quantified in accordance with Lichtenthaler's protocol [51].

2.10. Stomatal conductance and net photosynthesis

Fully developed leaves were placed in the camber of a portable infrared gas analyzer from all three plants per treatment to evaluate stomatal conductance (A.D.C., Hoddeson, UK). The measurements were taken six days after the establishment of salt stress [53]. The Reed et al. (1976) method was used to calculate net photosynthesis [52].

2.11. Total soluble proteins (TSP)

According to the Bradford (1976) method, 0.1 g of leaf was weighed and homogenized in 5 mL of phosphate buffer with a pH of 7.8. Bradford reagent was formed by combining 0.2 g of Brilliant Blue Coomassie G250 with 10 mL of ethanol, 17 mL of 85% H3PO4, and 3 mL of distilled water in a beaker. The liquid was filtered and diluted to 200 mL, which revealed a brown colour. Following a 30-minute vortex period in test tubes containing 0.1 mL of sample and Bradford reagent of 4 mL, the absorbance was measured at 595 nm with a spectrophotometer.

2.12. Total soluble sugars (TSS)

1 g of fresh leaf was dissolved in distilled water (10 mL), boiled for an hour, and then filtered to determine the total soluble sugar content. Filtrate (1 mL) was diluted with distilled water up to 5 mL. The filtrate was added to test tubes and mixed with 3 mL of the freshly prepared anthrone reagent. This mixture was immediately vortexed and then heated at 90 °C for 20 minutes. After that, it was cooled down at room temperature. The absorbance was determined at 620 nm using pure water as a blank following Yoshida et al. (1971) method [54].

2.13. Assay of free proline content

The proline contents were calculated using the Bates et al. (1973) 1 g of fresh leaves were extracted in 3% aqueous sulfo-salicylic acid. The extracted material was filtered. After that, toluene was heated to 100°C for 1 hour while being mixed with a glacial acetic acid and ninhydrin solution. The toluene phase was retained after the aspiration of the aqueous phase. The contents were measured colorimetrically at 520 nm [55].

2.14. Determination of total phenolic contents

1 g of frozen leaves was ground in 50 mL of methanol. The homogenate was agitated for an hour at room temperature. After that, the extract was filtered. The tannic acid was sued as standard value for estimation of concentration of total phenols. 1 ml of 10% Folin-Ciocalteu phenol, deionized water (10 ml) and extract (1 ml) were mixed. After waiting for five minutes, 2 ml of a 20% solution of sodium carbonate was added. The solution was kept in the dark and the absorbance at 750 nm was calculated [57].

2.15. Hydrogen peroxide

For the quantification of hydrogen peroxide (H_2O_2), 0.3 g of leaf sample and 3 ml trichloroacetic acid (TCA) were mixed. Samples were then centrifuged at 10,000 rpm for 10 min at 4 °C. The supernatant solution was then mixed with 0.75 mL of a buffer containing 10 mM potassium phosphate and 1.5 mL of 1 M potassium iodide. The H_2O_2 content was measured by comparing the samples' adsorption at 390 nm with its reference curve [56].

2.16. Electrolyte leakage

For the purpose of determining the EL, tubes containing distilled water were filled with 2-3 completely expanded leaves from representative plants, one from each replicate and left at room temperature and in the dark. Using a conductometer, the solutions' electrical conductivity (EC 1) was assessed after 24 hours of incubation. The samples' ultimate electrical conductivity was measured after they had been boiled for 30 minutes to remove all of the electrolytes (EC 2). The following formula was used to estimate the EL [58] : EL (%) = (EC 1/EC 2).100

2.17. Malondialdehyde content (MDA)

MDA was measured to determine the degree of lipid peroxidation in fresh leaves tissue according to Heath and Packer (1968). Using a pre-chilled pestle and mortar, 2.5 ml of w/v trichloroacetic acid (0.1%) was used to homogenize 0.5 g of leaf sample. For 15 minutes, the homogenate was spun at 10,000 rpm. To a 0.5 ml aliquot of the supernatant, 2 ml of freshly made 98% thiobarbituric acid in 20 % w/v of

TCA in a TBA reagent was added. The combination was boiled in water bath for 15 minutes at 95 °C., and then it instantly cooled. The absorbance at 532 nm was measured [59].

2.18. Determination of ascorbic acid (AsA)

Ascorbic acid was measured in accordance with Mukherjee and Choudhuri's (1983) protocol. A sample of 2g fresh leaves was homogenized with six percent Trichloroacetic Acid (TCA). After filtering and then centrifuging the extract at 1000 g for 20 min., the filtrate was diluted with TCA to make 10 ml. 4 mL of the extract, 2 mL of the 2% acidic dinitrophenyl hydrazine, and a few drops of the thiourea were mixed (10% in 70% ethanol). After cooling to room temperature and adding 5 ml of 80% v/v H_2SO_4, the mixture was heated for 15 minutes in a water bath. At 530 nm, the resultant color intensity was measured [60].

2.19. Ion analysis (Na^+)

The method defined by Wolf (1982) was used to determine the Na^+ concentrations in roots and shoots of barley plants. 2 mL of concentrated H_2O_2 was added to a digestion flask having 0.1 g of dried roots and shoots and left overnight. It was then boiled for 30 minutes on a hot plate at 370°C with the addition of H_2O_2 until clear liquid was obtained and was then let to cool down at room temperature. After filtering, distilled water was added to this solution to dilute up to 50 mL. Flame photometer was used to measure the Na^+ ions [61].

2.20. Statistical analysis

A completely randomized block design and the Duncan Multiple Range Test were utilized to statistically analyze the data using a post hoc test, which was used to determine specific changes between treatments (DMRT). A mean separation and a variance analysis with a 5% level of significance were the treatment means ($p \leq 0.05$). Additionally, a Pearson correlation was performed on a number of barley qualities produced both under salt stress and untreated group. Where required, a logarithmic data transformation was employed to create a distribution prior to analysis close to normal, were necessary. General percentage calculation was done using given formula:

$$\%age\ change = \frac{Difference\ between\ Pi\ an\ Po}{Po} \times 100$$

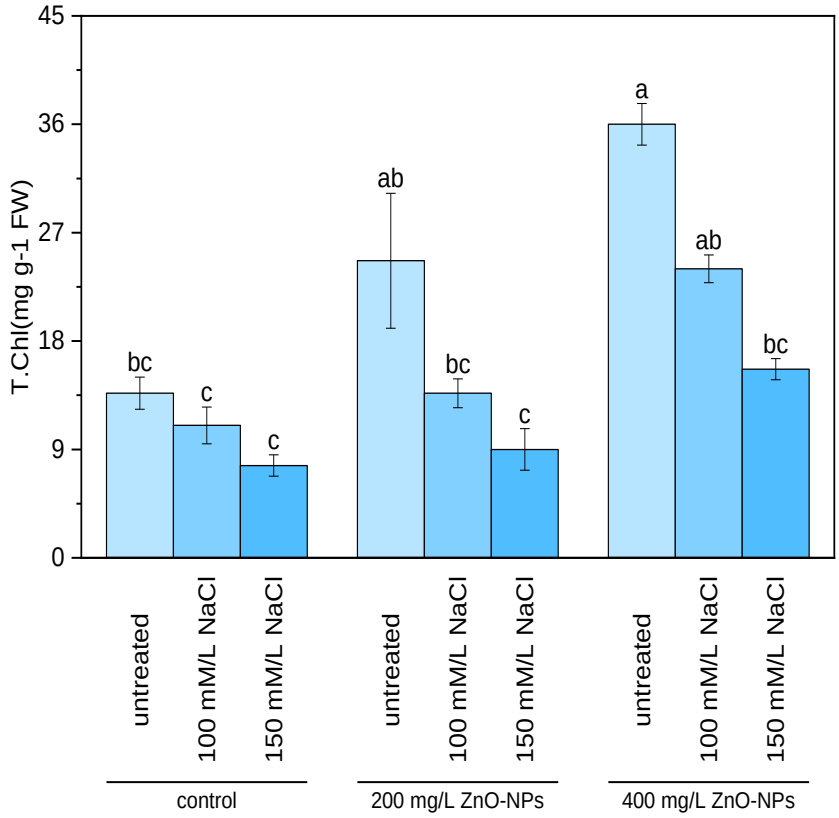
3. Results and discussion

Statistical analysis on different traits in observation under ZnO NPs on barley when grown in saline soil showed that there was an obvious difference among applied treatments. It was observed that ZnO NPs improved the net photosynthetic activity in barley significantly. Photosynthetic rate was increased by increasing levels of ZnO NPs and maximum photosynthesis was observed with 400 mg L^{-1} of ZnO NPs whereas barley variety without saline stress had maximum photosynthesis decreased with increasing level of salinity. Similar results were observed for total soluble proteins (TSP) and sugars (TSS). However, it was observed that hydrogen peroxide (H_2O_2), Malondialdehyde (MDA), proline contents, ascorbic acid (AsA), phenolics, root and shoot sodium levels were significantly decreased with increasing NPs level and minimum of them were observed with ZnO NPs at the rate of 400 mg L^{-1}. Results showed that net photosynthetic rate, TSP and TSS were increased by 24%, 43% and 74% respectively and decreased by salinity whereas H_2O_2, MDA, proline, AsA, phenolics, and sodium levels in plant were decreased by 54-62% varying with variable levels of ZnO NP concentration and it was observed that increasing salinity levels had adverse effects on plant growth. Hence, analysis of variance showed that chemically synthesized ZnO NPs had significant effect on physiological attributes of barley grown under saline soils in semi-arid climatic conditions as given in Table 1. Fig. 1 shows graphical representation on effects of varying levels of ZnO NPs on barley cultivated in differently concentrated saline soils. It was observed that total chlorophyll contents, stomatal conductance, shoot length and shoot fresh weigh were highest when treated with 400 mg L^{-1} of ZnO NPs but on the other side, at same level of nanoparticles the total chlorophyll contents were decreased with increasing the soil salinity and was highest with plants without salinity and lowest total chlorophyll along with stomatal conductance, shoot length and shoot fresh weight was observed at 150 mM L^{-1} of NaCl. Similar trends were followed by electrolyte leakage, chlorophyll a, b and carotenoids as illustrated in Fig. 2. Graphical representation on effects of varying levels of ZnO NPs on barley cultivated in differently concentrated saline soils is given in the Fig. 3. It was observed that shoot and root length, root and shoot fresh weight and root and shoot dry weight were highest when treated with 400 mg L^{-1} of ZnO NPs but on the other hand at same level of nanoparticles shoot dry wright were decreased with increasing the soil salinity and was highest with plants without salinity and lowest total chlorophyll was observed at 150 mM L^{-1} of NaCl. Pearson correlation was done to illustrate the change in different parameters of barley due to increase or decrease in other characters and it was observed that shoot fresh weight, shoot dry weight, root dry weight, root fresh weight, stomatal conductance, electrolyte leakages , chlorophyll contents, carotenoids and net photosynthesis along with TSS and TSP were positively correlated with each other whereas H_2O_2, MDA, AsA, Proline contents and phenolics were negatively associated among themselves but also with all other traits of plant and negatively affected the plant growth and development and also reduced the yield. Comparative analysis on different levels of ZnO NPs was studied in current experiment showed and effect of salinity along with different concentrations of salts on barley was observed. The mechanism of salt tolerance in barley after application of ZnO NPs was mitigated as there was significant increase in plant metabolism especially photosynthetic rate, pigment composition, total soluble protein and sugar contents were improved due to ZnO NPs application when compared with control.

Table 1. Effect of different levels of ZnO NPs on Net photosynthesis

	Main Effect of Treatments									
Treatment	Net photosynthesis (µmol $m^{-2}s^{-1}$)	TSP (mg g^{-1} FW)	TSS (µmol g^{-1} FW)	H_2O_2 (µmol g^{-1} FW)	MDA (µmol g^{-1} FW)	Proline (µg g^{-1} FW)	AsA (µg g^{-1} FW)	Phenolics (mg g^{-1} DW)	RNa (mg g^{-1} DW)	SNa (mg g^{-1} DW)
control	4.67±1.58b	1.41±0.24b	1.05±0.24c	3.94±0.98a	3.53±1.1a	3±0.67a	2.98±0.69a	3.88±1.39a	3.51±1.56a	4.12±1.71a
200 mg L^{-1} ZnO-NPs	3.56±2.46b	2.02±1.53b	2.17±1.43b	2.68±0.94b	2.51±0.97b	1.58±0.66b	1.82±0.77b	2.13±0.9b	2.2±1.15b	2.23±1.03b
400 mg L^{-1} ZnO-NPs	8.33±4.18a	3.77±0.93a	3.78±0.72a	1.74±0.95c	1.51±0.9c	1.08±0.5b	1.31±0.43b	1.26±0.75b	1.3±0.53b	1.31±0.65b
Salinity	**Main Effect of salinity**									
untreated	8.11±4.11a	3.49±1.53a	3.26±1.5a	2.11±0.77b	1.78±0.68b	1.77±1.24ab	1.74±0.81b	1.71±1.13b	1.58±0.79b	1.91±1.29b
100 mM L^{-1} NaCl	5.22±2.59ab	2.12±1.02b	2.21±1.3ab	2.24±1.18b	2±1.03b	1.36±0.63b	1.52±0.61b	1.82±0.85b	1.68±0.62b	1.76±0.64b
150 mM L^{-1} NaCl	3.22±1.72b	1.6±1.05b	1.55±1.11b	4.01±0.99a	3.78±1.01a	2.53±0.79a	2.84±0.86a	3.73±1.55a	3.76±1.56a	4±1.85a
ANOVA										
Total	5.52±3.51	2.4±1.43	2.34±1.45	2.79±1.3	2.52±1.27	1.89±1.02	2.04±0.94	2.42±1.5	2.34±1.45	2.56±1.67
CV	63.65	59.48	62.21	46.64	50.45	54.01	46.38	61.93	62.03	65.17

TSP (total soluble proteins), TSS (total soluble sugars), H_2O_2 (hydrogen peroxide), MDA (malondialdehyde), Proline, AsA (ascorbic acid), Phenolics, RNa (sodium in roots), and SNa (sodium in shoots) of barley under salt stress along with main effect of variety showing the tolerance against salinity at $p \leq 0.05$ (level of significance), Control= untreated, ZnO NPs = zincoxide nanoparticles.


T.Chl(mg g-1 FW)
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control
200 mg/L ZnO-NPs
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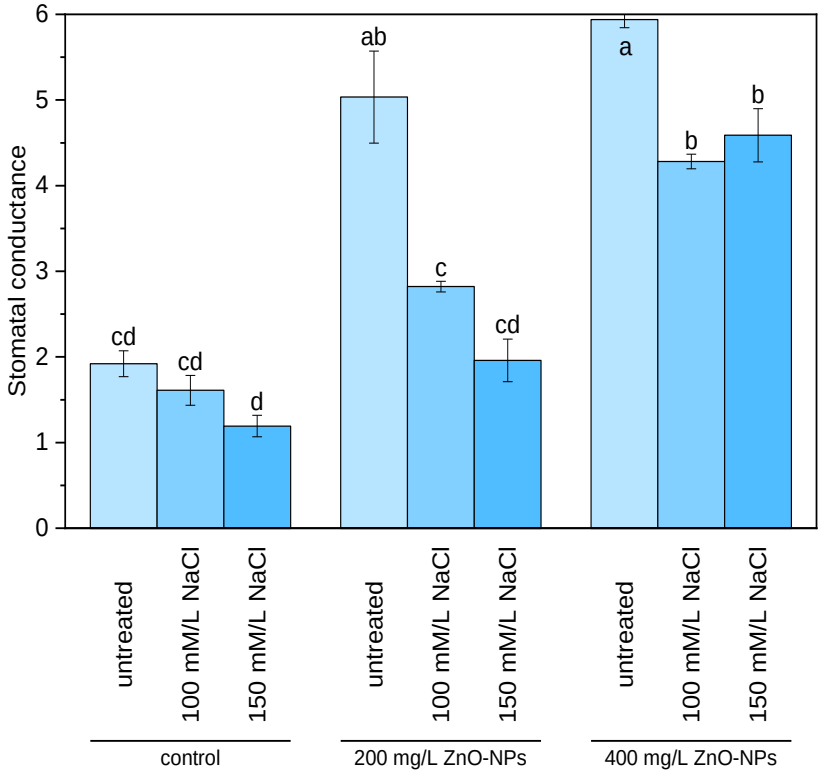
Stomatal conductance
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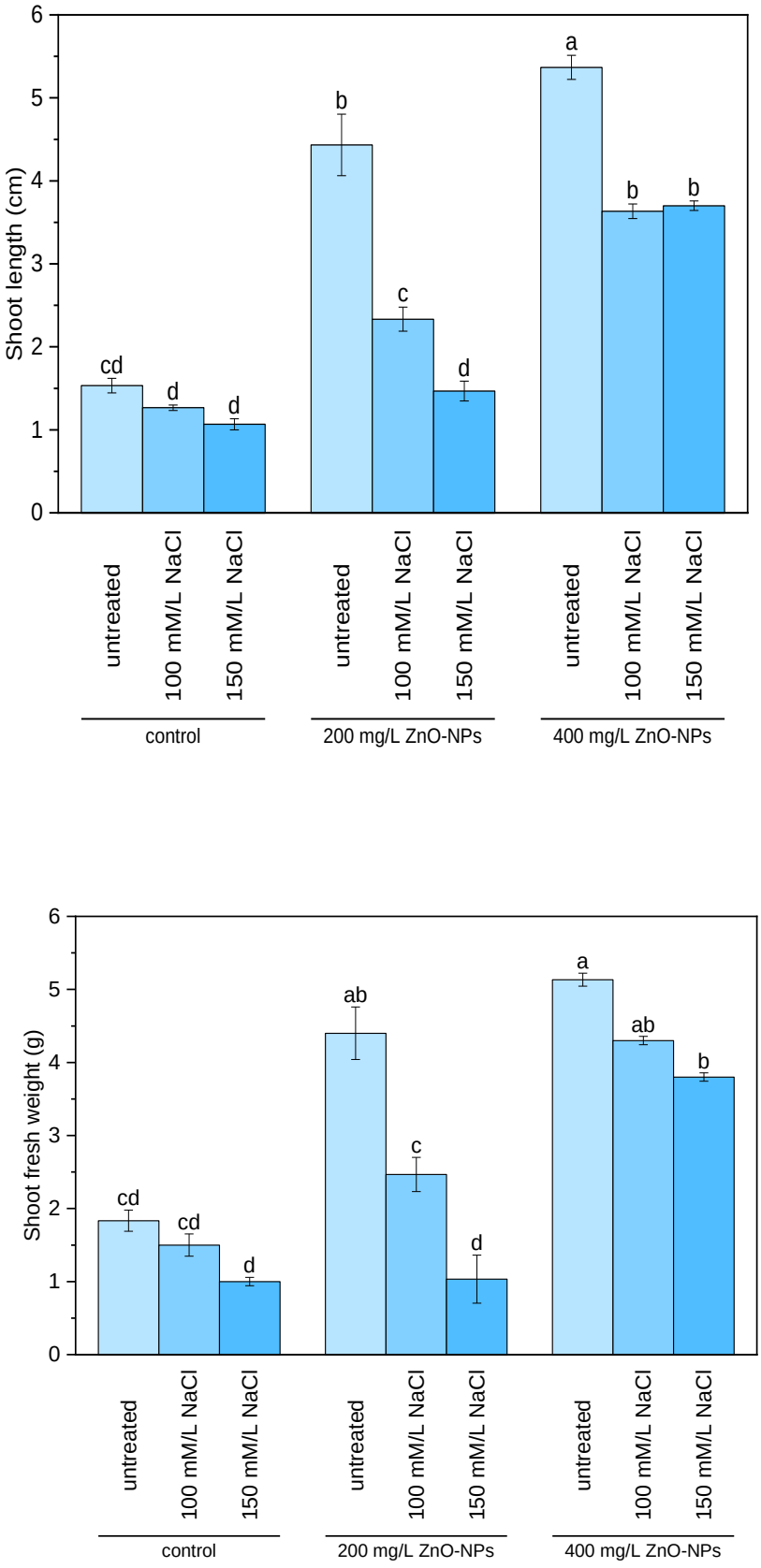
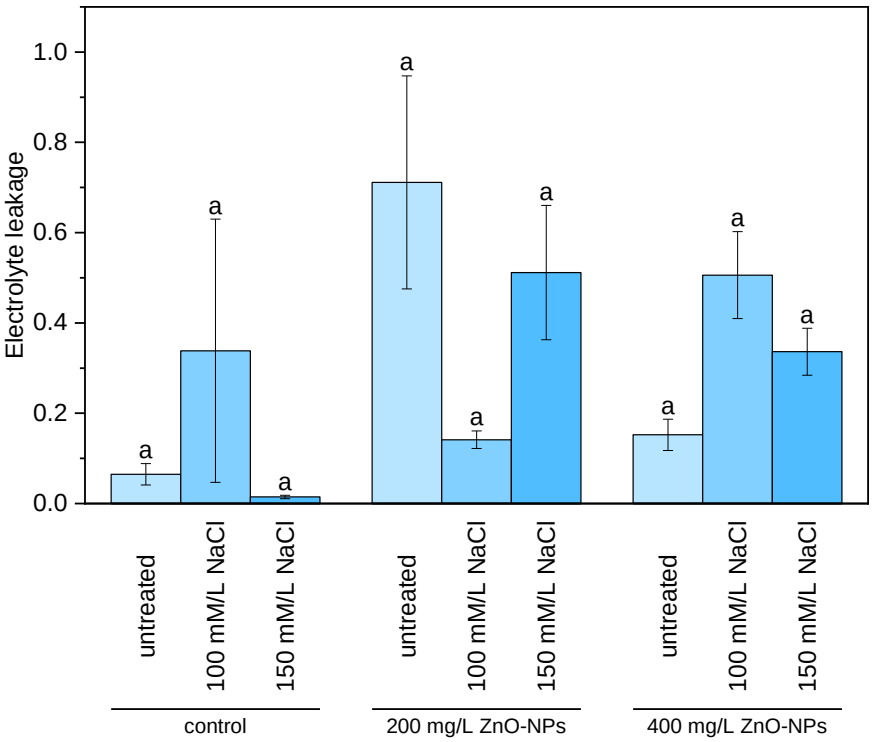


Figure 1. Effect of different levels of ZnO NPs on total chlorophyll contents, stomatal conductance, shoot length and shoot fresh weight of barley under salt stress along with main effect of variety showing the tolerance against salinity at $p \leq 0.05$ (level of significance), Control= untreated, ZnO NPs = zincoxide nanoparticles


Electrolyte leakage
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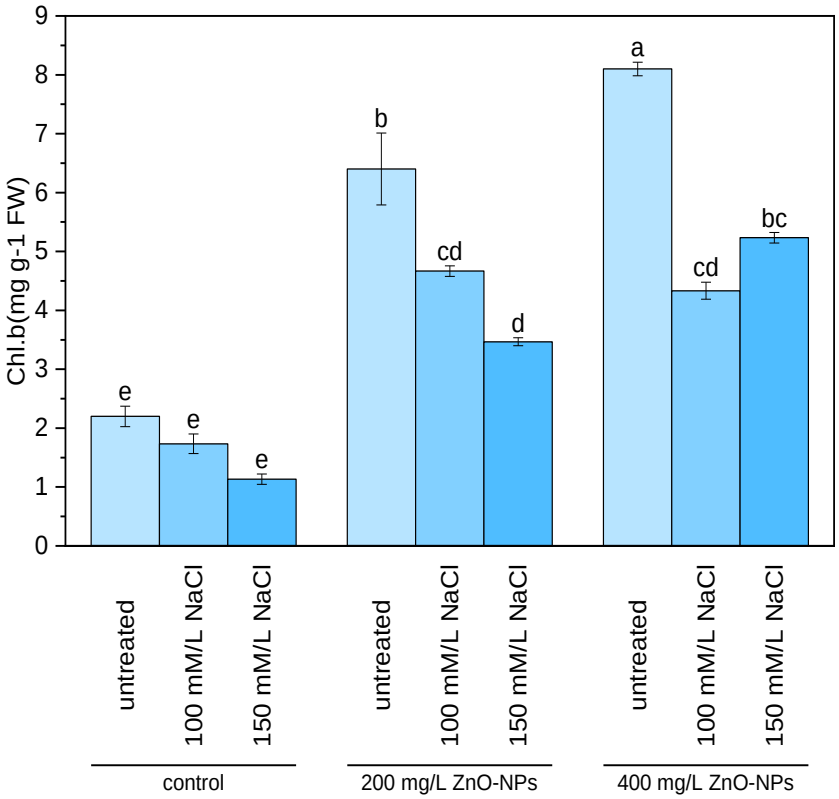
control
200 mg/L ZnO-NPs
400 mg/L ZnO-NPs


Chl.b(mg g-1 FW)
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control
200 mg/L ZnO-NPs
400 mg/L ZnO-NPs

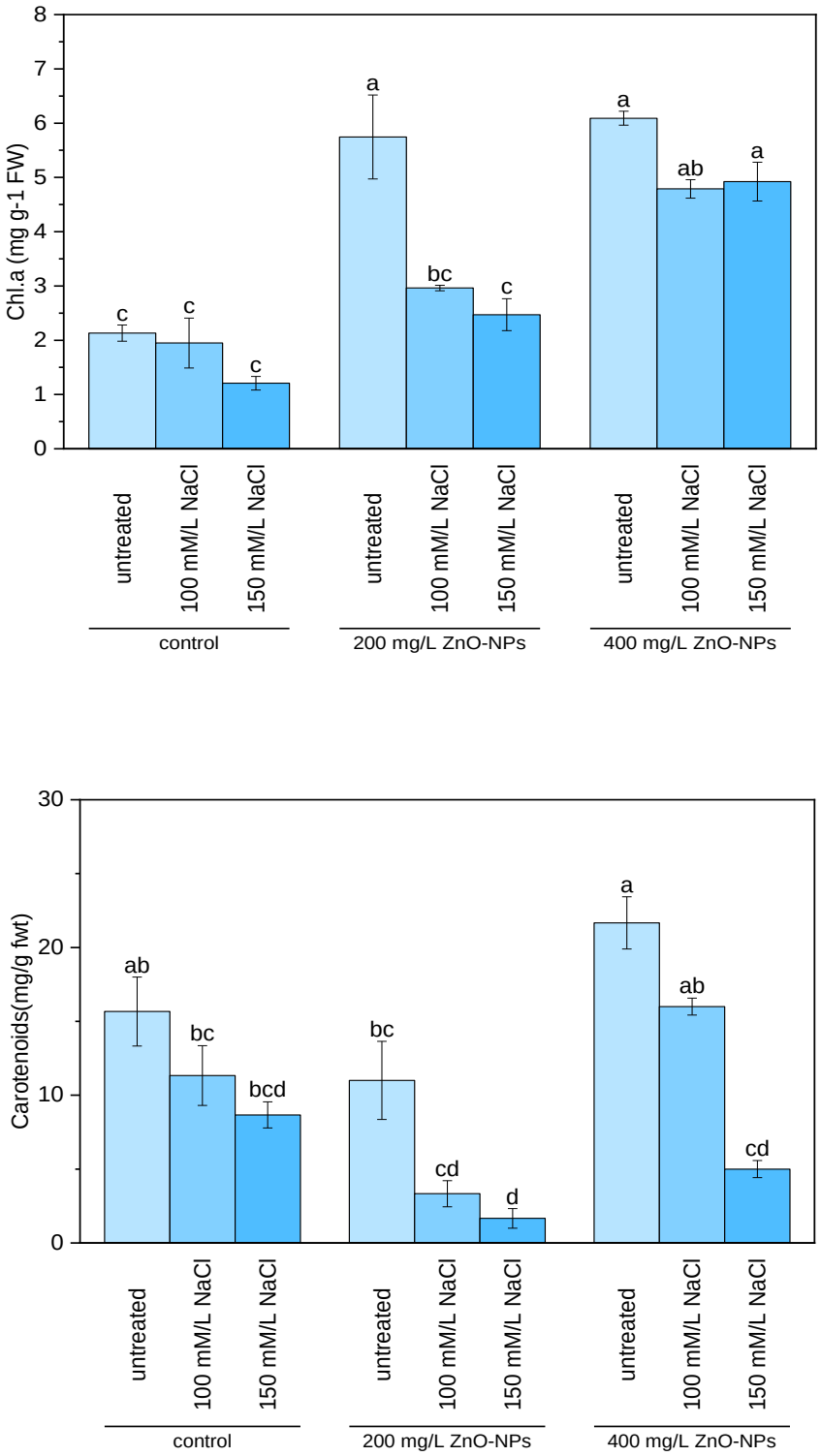
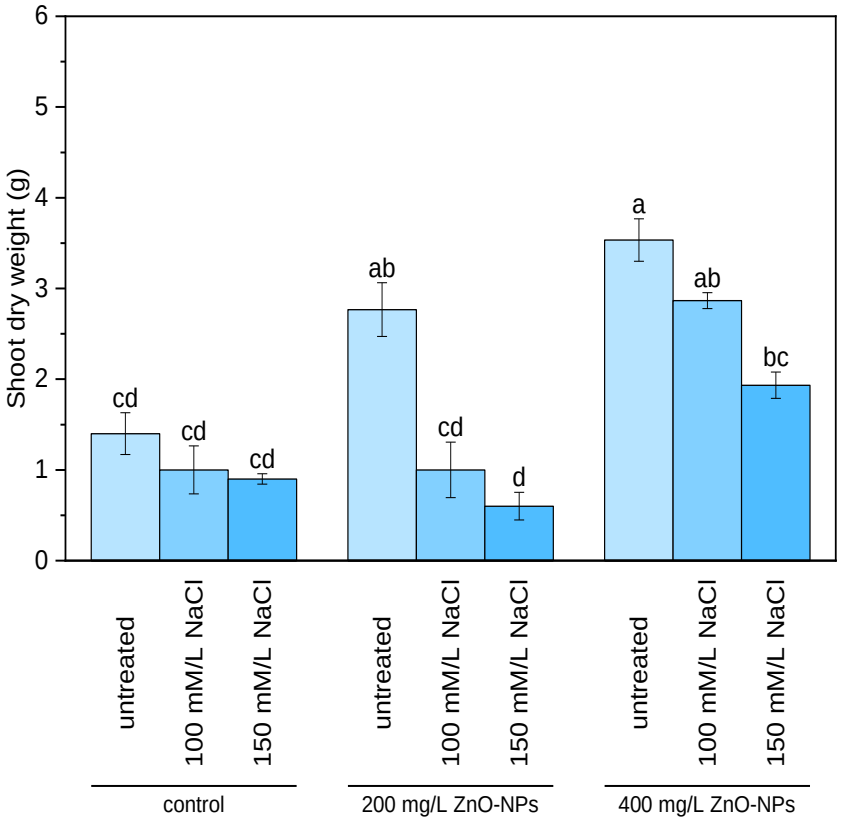


Figure 2. Effect of different levels of ZnO NPs on electrolyte leakage, chlorophyll a and b, and carotenoids in barley under salt stress along with main effect of variety showing the tolerance against salinity at $p \leq 0.05$ (level of significance), Control= untreated, ZnO NPs = zincoxide nanoparticles


Shoot dry weight (g)
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100 mM/L NaCl
150 mM/L NaCl
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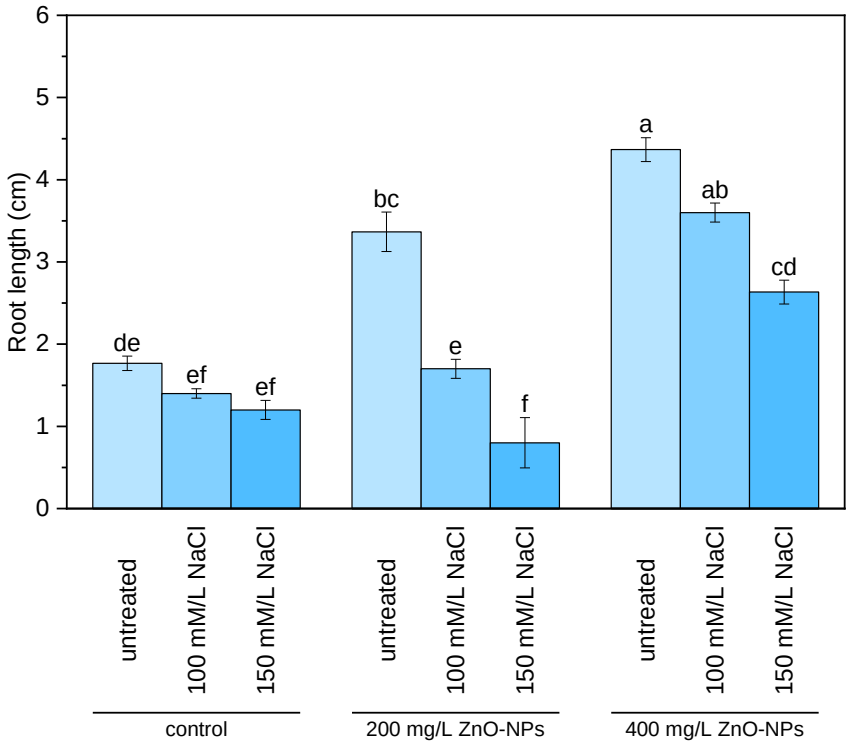
control
200 mg/L ZnO-NPs
400 mg/L ZnO-NPs


Root length (cm)
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150 mM/L NaCl
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control
200 mg/L ZnO-NPs
400 mg/L ZnO-NPs

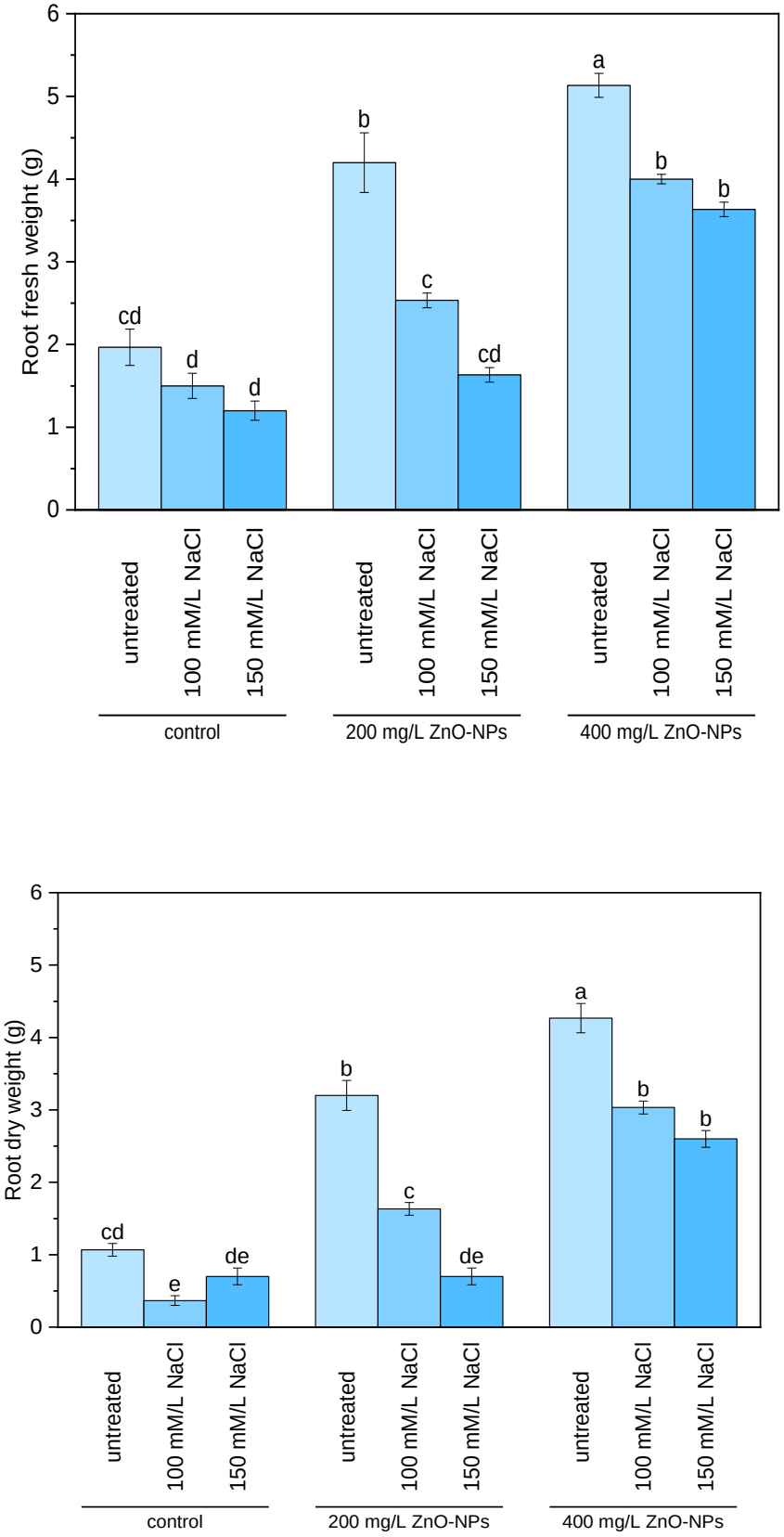

Figure 3. Effect of different levels of ZnO NPs on Shoot dry weight, root length, root fresh and dry weight of barley under salt stress along with main effect of variety showing the tolerance against salinity at $p \leq 0.05$ (level of significance), Control= untreated, ZnO NPs = zincoxide nanoparticles

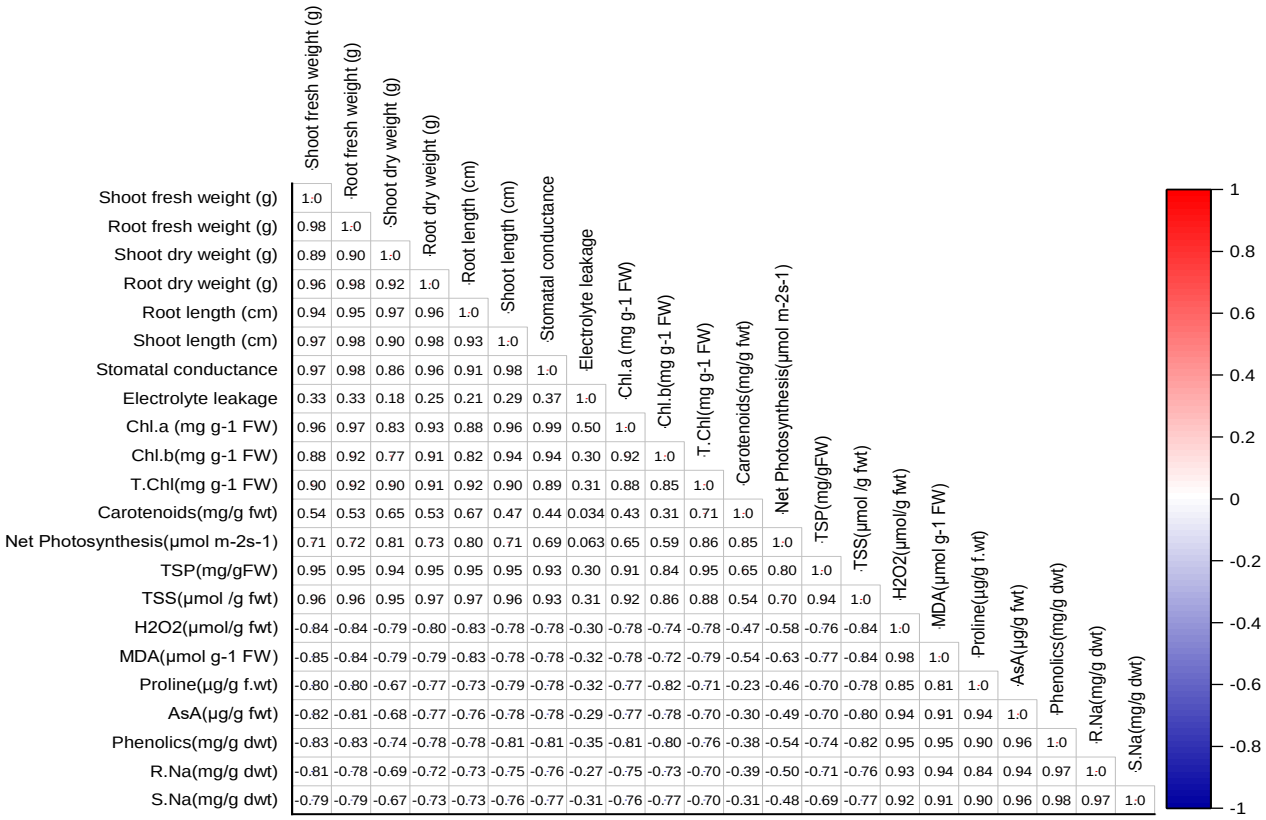

Figure 4. Figure showing correlation between different physiological, photosynthetic, biochemical and ionic constituents of barley growing under different levels of ZnO NPs under salt stress. Red color shows positively significant values while blue color shows negatively significant values. [Control= untreated, ZnO NPs = zincoxide nanoparticles]

The improvement can be associated with the fact that ZnO NPs help improvement in energy change in adaptation process through osmotic adjustment and ion exclusion and ultimately aided to plant growth in faba bean [62]. The toxicity of salt accumulation in barley was observed to reduce the photosynthetic pigment composition and chlorophyll a, b, total chlorophyll contents and carotenoids were reduced along with increased salt stress, but ZnO NPs mainly related with greater pigment composition as compared to control [63-64]. This increase was attributed to facilitation in CO_2 transport to carboxylation site of chloroplasts and [65] related it with repairing of photosystem II and chloroplast growth due to applied zinc oxide nanoparticles. Effect of ZnO NPs on total soluble proteins (TSP) and sugars (TSS) was observed to be positive and the results were similar to the findings Wan et al. (2020) who observed increased TSS and TSP in *sophora alopecuroides* grown under salinity treated with ZnO NPs [66]. This increment was referred to improved carbon and nitrogen metabolism in plants after promoted glycolysis and TCA cycle. Application of these nanoparticles however, reduced the rate of antioxidants produced due to salt stress including MDA, H_2O_2, AsA along with sodium ion accumulation in roots and shoots. These results were attributed to the prediction that (Zafar et al., 2022) [67] ZnO NPs reduced the salt generated suppression in biochemical traits and processes in spinach where nanoparticles activated the plant antioxidant system that minimized the oxidative stress damage it was observed that the shoot and root length along with their dry and fresh weights were decreased with increased levels of NaCl however this effect was mitigated by using zinc oxide nanoparticles [68] and plant biomass was improved with it. On the other hand, stomatal conductance and electrolyte leakage in barley during research was observed to be reduced under negative effects of increased salt accumulation [69-70] whereas this reduction was controlled by nanoparticles and higher levels. ZnO NPs were reported to have antagonistic effect against abiotic stress in plant [71-72] improving the plant physiological and

morphological characters [73-74]. In short, the hypothesis to mitigate the salt stress in barley using zinc oxide nanoparticles was strongly supported by results. However, there is need for future research to work on application method and stages of plant growth for ZnO NPs and there is need to work on other nanoparticles for improving agricultural commodities facing hazardous abiotic and biotic stress in different climates.

4. Conclusions

Current study evaluated the saline stress tolerance using different levels of applied nanoparticles (ZnO NPs) on morphology, physiology and chemical attributes of barley. The results were supportive to hypothesis that ZnO NPs improve plant growth and photosynthetic activities of barley and reduce the toxicity of NaCl. Results specifically, showed that the 400 mg/L of ZnO NPs improved net photosynthesis, stomatal conductance, electrolyte leakage, plant root and shoot systems, photosynthetic pigments, plant's growth and metabolic rates compared to other treatments. In terms of antioxidants production, least of MDA, H_2O_2, AsA, and sodium ion accumulation in root and shot were produced in ZnO NP treated plants as compared to control under salt stress. In conclusion, ZnO NPs treatment on barley can be recommended as a feasible agronomic practice to improve plant growth and metabolism grown under salinity.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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