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Exogenously applied ZnO nanoparticles induced salt tolerance in potentially high yielding modern wheat (*Triticum aestivum* L.) cultivars

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ABSTRACT

Salinity stress is one of the potential threats that adversely affect the productivity of many cereal crops worldwide. Spraying plants with nano-Zn particles may lessen effectively such negative impacts on plants; yet its mode of action is still not well explored. This study was performed to evaluate the effects of spraying nano-Zn particles with varying concentrations (0, 20, 50 and 80 mg L^{-1}) on two wheat cultivars irrigated with saline water (EC = 6.3 dS m^{-1}) versus a non-saline one. The key results revealed that root and shoot weights decreased significantly under salinity stress conditions, while improved considerably with nano-Zn-particles foliar application up to 50 mg nano-Zn L^{-1}; thereafter significant reductions occurred. Also, shoot and root lengths as well as plant leaf area index improved considerably owing to this foliar application. Clearly, roots and shoots weights of wheat plants sprayed with nano-Zn particles under salinity stress conditions exhibited higher values than the corresponding ones that was grown under non-saline conditions without nano-Zn-particles applications. Unexpectedly, this foliar spray led to significant reductions in plant pigments and also in enzymatic and

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non-enzymatic antioxidants in plants. Yet, this foliar spray enhanced formation of total soluble sugars and proline, and raised significantly Ca contents in wheat roots and shoots, and to some extent K contents. In conclusion, the foliar application of nano-Zn particles increased plant growth under salty stress conditions via two parallel processes, i.e., stimulating formation of osmolytes and stimulating nutrient uptake which may, in turn, increase plant metabolism.

1. Introduction

Salinity stress adversely affects the productivity of cereal crops/glycophytes aggravating agribusiness worldwide and along with global climate change and population explosion is a risk for food security (Lalarukh et al., 2022). It has deteriorated 20% of total irrigated land area decreasing one-third production of food (Mansoora et al., 2021). Around the world 20%–50% decline in agronomic yield is mainly due to salinity and water deficit consequent upon reallocation of photosynthetic energy from yield to plants defenses against stress (Amjad et al., 2021a). Out of the total 230 Mha irrigated land, 45 Mha is deteriorated due to salt deposition. In Pakistan out of 6 Mha of salt affected arable land 2.67 Mha is situated in Punjab and further accelerating a 0.02 to 0.04 Mha each year (Yaseen et al., 2021). Due to deficiency of good quality water irrigation of arable land with underground brackish water is a compulsion (Bukhari et al., 2021).

Osmotic stress as well as ion toxicity and nutritional disparity are main consequences of salt stress (Amjad et al., 2021b). Salt stress inhibits plant growth as well as productivity by altering the metabolic pathways of plants (Rafeeq et al., 2020). Salt stress triggers production of reactive oxygen species (ROS), disturbance in antioxidant defense mechanisms, photosynthetic process and hormones imbalance in plants (Yaseen et al., 2021). Other important processes concerned with growth, i.e., nutrients availability, water relation, photosynthetic pigments, seed germination, photosynthetic mechanism and productivity are mainly affected by salt stress (Hanin et al., 2016). Therefore, plant survival under saline condition requires highly activated protective mechanisms to diminish the effects of salinity induced production of toxic metabolites (Mansoora et al., 2021).

Zinc (Zn) is an essential micronutrient as it stimulates various plant enzymes which catalyze sugar metabolism, auxin production, protein synthesis and pollen production and its deficiency reduces crop productivity (Amjad et al., 2021b). Zinc regulates and maintains gene expression that is essential for making plants stress tolerant. Zinc performs other functions like tryptophan synthesis, maintaining membranous structures, cell division and photosynthesis. Protein synthesis is regulated by zinc where it serves as co-factor (Marschner et al., 2011). So, adequate Zn supply enhances forage, vegetable and cereal productivity (Amjad et al., 2021b). Zinc deficiency in the people of developing countries is a major health hazard due to high consumption of cereal based diet and is amongst the ten fundamental health related problems worldwide (Domingues et al., 2016). Plant breeders have developed modern wheat cultivars with high grain yield but low in Zn content due to poor bioavailability of Zn in 50% of the wheat fields, universally (Cakmak and Kutman, 2018).

Nanotechnology is domineering agricultural technique these days due to its potential benefit in food quality enhancement, reduction in agricultural input by the use of nano scale pesticides, fertilizers and increased yield of agricultural products (Prasad et al., 2017). Nano materials due to their smaller size (billionth of a meter) have large surface area, are more reactive in most biological processes and are ecofriendly (Prasad et al., 2014). Currently, metal oxide nanoparticles such as FeO, CeO_2, Al_2O_3, TiO_2 and ZnO have been applied in agricultural industry and their role in disease control, toxicity and enhanced cereal production in abiotic stresses is under investigation (Zheng et al., 2016).

Wheat though the leading staple food with 736 million metric tons per annum production suffers substantial decline in yield due to salinity worldwide (Lalarukh et al., 2022). Plants respond to salinity stress by closing stomata, inhibiting shoot and foliage growth, reducing number of tillers, altering reproductive development and lowering the carbohydrate production thus drastically affecting crop yield (Dawood et al., 2019). Deposition of soluble salts in rooting medium of plant results in poor growth, kernels germination, nutrient uptake, impaired photosynthesis, membrane damage and toxins accumulation as a result of osmotic stress, nutritional/hormonal disparity, ion toxicity and oxidative damage (Amjad et al., 2021a,b). Mostly wheat cultivated soils are deficient in Zn and in alkaline soil. Zinc availability decreases to plant 30 times with a unit rise in soil pH and above 8 pH, it binds to the soil (Sadeghzadeh et al., 2013). Agronomic bio fortification strategy has been practiced to provide crops with adequate Zn through soil and foliar fertilizers. Zinc fertilizers mostly in the form of zinc sulfate are used in large quantity however, use of ZnO nanoparticles in subtle amount as exogenous application on cereal crops is cost effective and might be the possible solution of Zn malnutrition. Two main sources of Zn content in seeds are (1) absorbed from the soil by roots and (2) translocate from vegetative parts during grain filling stage (Cakmak and Kutman, 2018). Therefore, Zinc oxide NPs can be applied through these two sources to sustain level of available Zn to the plant. Therefore, to curtail all bearings on the food security and environment, nanotechnology especially use of ZnO nanoparticles for the yield enhancement in modern wheat cultivars under saline condition could be the only possible solution. It is thought that nano-Zn can lessen Na-absorption under saline conditions (Lalarukh et al., 2022). The main objectives of the present study are: (i) Evaluation of modern wheat cultivar with high salinity tolerance. (ii)

Investigate the potential role of ZnO nanoparticles foliar applications for ameliorating salinity stress of the tested wheat varieties, and identify the main responsible mechanism. (iii) An examination of morpho-physiological and biochemical responses of potentially high yielding varieties to salt stress and (iv) Recommendation of salt tolerant, high yielding bread wheat cultivar to the stakeholders, to maximize yield in soils having high pH value.

2. Materials and methods

The pot-based experiment was conducted to evaluate the effects of nano feeding of ZnO particles on wheat plant, grown under salinity stress. The seeds of two wheat cultivars named as Inqilab 91 and Pasban 90 were procured from Ayub Agriculture Research Institute (AARI) Faisalabad, Pakistan. Inqilab 91 has lower level of salinity stress tolerance while Pasban 90 has much greater salt stress tolerance potential than Inqilab 91 cultivar (Zafar et al., 2022).

Three-fourth of loam and one-fourth of organic compost were mixed up thoroughly. Clay pots were taken, and each pot was filled up with 10 kg soil (mixture of loam and compost). Half pots were seeded with wheat variety Inqilab 91 and remaining half with Pasban 90. Each pot was sown up with 10 seeds in uniform depth and space. Half pots were supplied with good quality water and other half with salty water (EC=6.3 dS m^{-1}) per pot 1 liter every week. All pots were sprayed with varying concentration (0, 20, 50 and 80 mg L^{-1}) of ZnO nanoparticles at booting stage. Experiment was conducted in complete randomize design (CRD) with four replications. Plants were sampled two weeks after foliar application of ZnO nanoparticles and yield at maturity to study various morpho-physiological and biochemical parameters.

2.1. Morphological characteristics

After sample collection, fresh and dry weight of root and shoot, length of roots and shoots, length and width of flag leaf along with leaf number per plant and number of tillers were recorded.

2.2. Photosynthetic pigments

2.2.1. Chl. a, b and carotenoids

Pigments of chlorophyll *a*, *b* and carotenoids were evaluated by using Arnon (1949) technique. Weighed 0.1 g leaf pieces (chopped) were kept in 5 ml acetone (80%) at 4 °C overnight. With the help of spectrophotometer absorbance of the mixture was recorded at 480 nm, 645 nm and 663 nm.

2.3. Enzymatic antioxidants

0.1 g weighed fresh leaf homogenized in 2 ml phosphate buffer with 7.8 pH was centrifuged at 12,000 rpm for 20 min. The supernatant was conserved at chilling temperature of 20 °C and enzymatic antioxidants were determined.

2.3.1. Catalase (CAT) and Peroxidase (POD) determination

Catalase and peroxidase analysis were done using protein-based technique of Chance and Maehly (1955). For CAT evaluation, 1 mL hydrogen peroxide was added with 0.1 mL enzyme extract along with 1.9 mL phosphate buffer with pH 7.8. Absorbance change was recorded at 240 nm with time interval of 30 s for 2 min.

For POD determination, 100 μL of 40 mM H_2O_2, mixed up with 100 μL of 20 mM guaiacol, 750 μL phosphate buffer and 50 μL enzyme extract in same cuvette. Absorbance change was recorded at 470 nm for every 30 s for 2 min via spectrophotometer.

2.4. Total soluble proteins

Using pestle and mortar, 0.1 g weighed leaf was homogenized in 5 mL phosphate buffer having pH 7.8 and subjected to centrifugation at 12,000 rpm for 20 min following Bradford method (1976). Bradford reagent was prepared by adding 0.2 g Coomassie Brilliant Blue G250 along with 10 mL ethanol, 17 mL 85% H_3PO_4 and 3 mL distilled water in beaker; diluted up to 200 mL and subjected to filtration until the appearance of brown color. 0.1 mL above sample with 4 mL Bradford reagent (dye) in test tube was vortex with recorded absorbance change after 30 min at 595 nm via spectrophotometer.

2.5. Non enzymatic antioxidants

2.5.1. Ascorbic acid

Using methodology of Bates et al. (1973), 0.1 g fresh weighed leaf was crushed in 2 mL 5% Trichloroacetic acid (TCA) buffer via pestle–mortar and centrifuged at 12,000 rpm for 15 min at 4 °C. 0.5 ml 2% dinitrophenyl hydrazine was added in 1 mL supernatant with 1 drop of thiourea prepared in 17% ethanol and boiled for 15 min using water bath. After cooling, 1.5 mL of 80% H_2SO_4 was added in same test tube at chilled temperature of 0 °C. Absorbance change was noted at 530 nm via spectrophotometer.

2.6. Malondialdehyde (MDA)

Lewis et al. (1999) technique was followed to determine malondialdehyde. Centrifugation of 0.1 g fresh weighed leaf, ground in 1 mL (0.1%) TCA was done at 10,000 rpm for 10 min. 1 mL extract with 4 mL 0.5% thio barbibutyric acid (TBA), heated in water bath for 30 min at 95 °C followed by cooling via ice bath was subjected to spectrophotometer to measure absorbance at 532 nm and 600 nm.

2.7. Organic osmolytes

2.7.1. Free proline

Bates et al. (1973) technique was used to determine free proline. 0.1 g fresh leaf ground in 2 mL 3% sulfosalicylic acid, and then was filtered. 1 mL filtrate with addition of 1 mL acid ninhydrin and 1 mL glacial acetic acid was heated in water bath at 100 °C for 1 h. After being cooled at chilling temperature in ice bath, 2 ml proline was added and vortexed for 1 min, forming two layers. Above layer was taken and absorbance recorded with spectrophotometer at 520 nm.

2.8. Total soluble sugars

1 g weighed fresh leaf was ground in 10 mL distilled water and boiled for 1 h. After filtration, 1 mL filtrate was diluted up to 5 mL. 600 μL of above prepared filtrate with addition of 3 mL freshly prepared anthrone reagent alongside walls of test tube was poured, briefly vortexed followed by heating at 90–95 °C for 20 min. Upon cooling, absorbance change was recorded at 620 nm with distilled water used as blank (Yoshida and Coronel, 1976).

2.9. Ion analysis (Na^+, K^+, Ca^{2+})

Wolf (1982) technique was adopted for measuring Na^+, K^+ and Ca^{2+} in roots and shoots. In 0.1 g dried sample of roots and shoots was added 2 mL conc. H_2SO_4 in digestion flask and kept overnight. Mixture was heated at 370 °C for 30 min using hot plate and cooled at room temperature. 1 mL H_2O_2 was added and heated again at 370 °C until it became colorless. Diluted up to volume of 50 mL with distilled water and filtered. Analysis was done using flame photometer to determine Na^+, K^+ and Ca^{2+} ions.

2.10. Statistical analysis

Statistical analysis of data was done by a post hoc test, which was performed to measure specific differences between treatments by using Duncan's Multiple Range test (DMRT) by two-way ANOVA test. The treatment means were analysis of the variance and separation of means at 5% significance level ($p \leq 0.05$).

3. Results and discussion

3.1. Effect of nano-Zn foliar application on the fresh weights of wheat roots and shoots subjected to salinity stress

The effect of applied treatment was significant on shoot fresh weight, root fresh weight, shoot dry weight and root dry weight on both varieties of wheat with 50 mg L^{-1} nano-Zn particles concentration as a most effective treatment. Fresh and dry weights of wheat roots and shoots decreased significantly under salinity stress conditions (Fig. 1). This is because salinity stress affected negatively many physiological (Saddiq et al., 2021) and biochemical activities in plants (Zafar et al., 2022); hence, this stress suppressed its growth (Poustini and Siosemardeh, 2004). The negative effects of soil salinity were also noticed on roots and aboveground parts of *Lupinus termis* (Abdel Latef et al., 2017), *Solanum tuberosum* (Mahmoud et al., 2020) and *Rosmarinus officinalis* (Hassanpouraghdam et al., 2020) and *Vicia faba* (Ragab et al., 2022). Spraying plants with nano-Zn-particles led to significant improvements in the investigated growth parameters following the pattern of 50>80>20>0 mg nano-Zn L^{-1}; yet the growth performance attained under salinity stress conditions were still lower than those achieved under non-stress conditions. Mostly, this foliar application played considerable roles in the alleviation of salt stressed wheat plants (Abou-Zeid et al., 2021) via regulating their tolerance mechanisms (Zafar et al., 2022). A point to note is that the effect of soil salinity seemed to be less pronounced on fresh and dry weights of both roots and shoots of the two salt tolerant wheat cultivars versus spraying plants with nano-Zn. In this context, roots sprayed with nano-Zn particles (50 mg L^{-1}) under salinity stress conditions were 4.25 and 2.79 folds higher than that grown under the non-stressful salinity conditions of Pasban and Inqalab wheat verities, respectively. Similarly, shoots sprayed with nano-Zn particles (50 mg L^{-1}) under stress condition were 4.40 and 2.23 folds higher than the corresponding ones that did not receive nano-Zn-particles of Pasban and Inqalab wheat verities, respectively and grown under non-stressful salinity conditions. This is a good point towards improving the growth of wheat plants under salinity stress conditions. The following sections would highlight the mechanisms that were responsible of increasing salinity stress tolerance in plants owing to this foliar application.

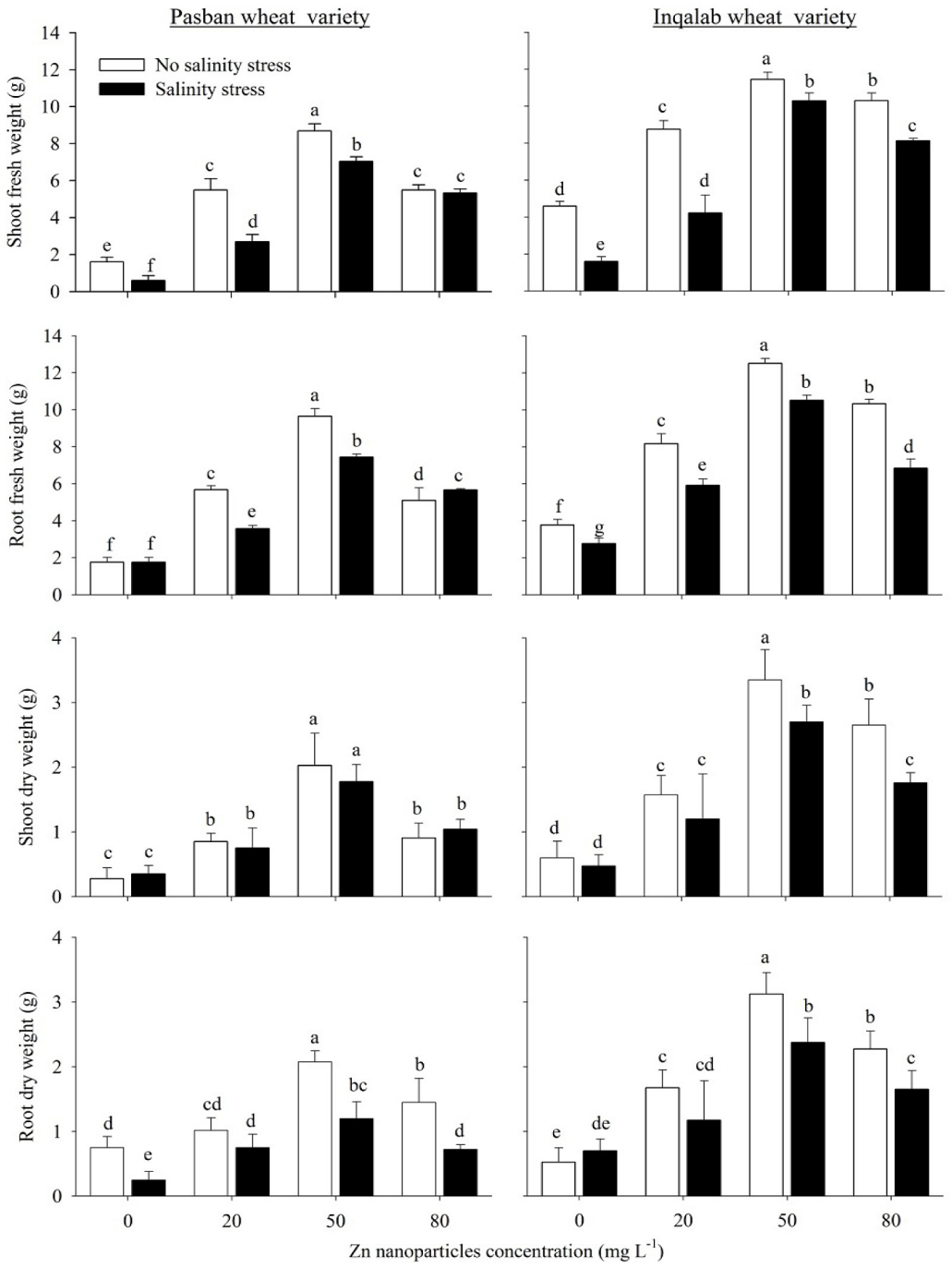

Fig. 1. Effect of nano-Zn foliar application on fresh and dry weights of wheat roots and shoots subjected to salinity stress. Similar letters indicate no significant variations among treatments.

3.2. Effect of nano-Zn foliar application on wheat leaf area index, root and shoot lengths subjected to salinity stress

Results showed a significant difference in root and shoot lengths and leaf area index of wheat as effect of applied amendments while salinity stress diminished significantly shoot and root lengths of the two wheat cultivars of study as well as their leaf area index (Fig. 2). It was observed that Zn foliar applications at all concentrations were significantly different from control groups of both varieties. This is because this abiotic stress affects negatively plant cell division and expansion (Lalarukh et al., 2022). Our findings agree with those of El-Bassiouny et al. (2022) who recorded significant

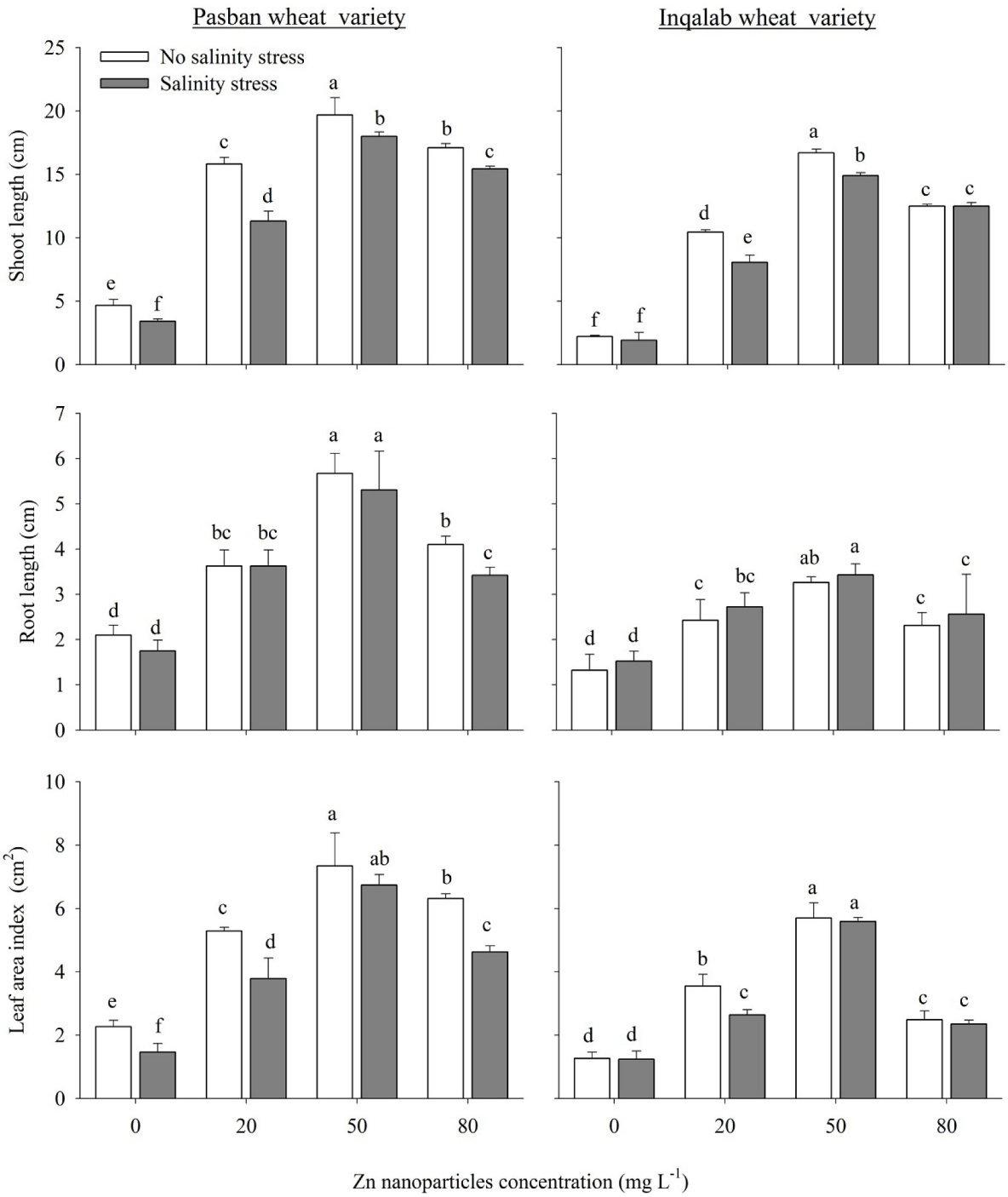

Fig. 2. Effect of nano-Zn foliar application on the leaf area index, root and shoot lengths of wheat. Similar letters indicate no significant variations among treatments.

reductions in plant morphological parameters under stressful conditions such as salinity (Mahmoud et al., 2020) and drought (El-Bassiouny et al., 2022). Likewise, Mahmoud et al. (2020) noticed significant increases in plant height owing to Zn foliar application. On the other hand, significant improvements were observed in these growth parameters when sprayed with nano-Zn particles at a rate up to 50 mg Zn L^{-1}; afterwards, significant reductions occurred. The outcomes of salinity stress on the above-mentioned growth parameters were almost mislaid comparable with the effect of the foliar

application of nano-Zn-particles, i.e., spraying plants with Zn-nano-particles improved these parameters with at least 2–4 folds higher than the control when strayed with a rate of 50 mg Zn L^{-1}.

3.3. Effect of nano-Zn foliar application on pigment contents in wheat plants subjected to salinity stress

Results from Fig. 3 showed that Zn foliar spray significantly increased chlorophyll A, B and total chlorophyll contents only at higher dose rate but still lower in concentration than controlled plants. Salinity stress conditions raised significantly chlorophyll A, b and total contents in the two cultivars of study while recorded no significant effect on the carotenoids. These results contradict the findings of Babaei et al. (2017) who recorded significant reductions in chlorophyll pigments in plants. Probably, these cultivars alleviate salt stress via enhancing plant growth by increasing the contents of plant pigments. Spraying plants with nano-Zn particles as a foliar spray decreased significantly these pigments in plants, especially under salinity stress conditions. This finding was also noticed by Abdel Latef et al. (2017) in lupin (*Lupinus termis*) plants. It is worth mentioning that this result probably did not contradict the findings which indicate that this foliar spray improved significantly plant growth parameters, especially with increasing its rate of application up to 50 mg L^{-1} as the rate of plant growth owing to this spray could be much higher than the rate of formation of these pigments in plant. On the other hand, the usage of 80 mg Zn L^{-1} as a foliar spray raised significantly the contents of chlorophyll A, B and the total contents versus the lower application doses; yet these parameters were still below that of the control. This might occur because of the reductions that took place in plant growth when using this level of application versus the lower application Zn-levels.

3.4. Effect of nano-Zn foliar application on the enzymatic (catalase) and non-enzymatic (ascorbic acid AsA) antioxidants, organic osmolytes (proline and TSS) and Malondialdehyde (MDA) in wheat leaves subjected to salinity stress

Foliar applied nano-Zn at all concentrations caused significant effect on enzymatic, non-enzymatic antioxidants, osmolytes and oxidative stress indicators in both cultivars (Table 1). Results showed that salinity stress decreased significantly the soluble protein contents in leaves of Pasban cultivars while this content increased in Inqalab cultivar. In all cases, protein content decreased significantly in plants that were sprayed with nano-Zn especially with increasing the level of application. Likewise, enzymatic (catalase) and non-enzymatic (ascorbic acid AsA) antioxidants decreased in plants that were subjected to salinity stress and additionally decreased more with the foliar application of nano-Zn, especially with increasing its level of application. These results contradict those of Babaei et al. (2017) and Abdel Latef et al. (2017) who recorded significant increases in the enzymatic antioxidant (catalase) in plants sprayed with nano-Zn. Although these antioxidants are needed to increase plant tolerance to salinity (Noohpisheh et al., 2021); yet the decrease in the activities of antioxidant enzymes may indicate that this nano-product lessened successfully the implications of salinity stress in plants.

On the other hand, organic osmolytes (proline and TSS) increased significantly in leaves of the studied wheat cultivars under salinity stress conditions and furtherly increased with the foliar application of nano-Zn. This modulation (proline and soluble sugar) could improve the osmotic protection of plant cells (Babaei et al., 2017; El-Bassiouny et al., 2022). Although, the levels of MDA was elevated significantly in wheat leaves subjected to salinity stress; yet it decreased considerably with the foliar application of nano-Zn particles up to 50 mg L^{-1}. This result was also confirmed by Hassanpouraghdam et al. (2020) and Zafar et al. (2022) who indicated the success of this foliar spray in reducing the concentrations of MDA contents in plants. It seems that the level 80 mg nano-Zn L^{-1} was not toxic for plants as the levels of MDA stood below the levels of the control; yet its effectiveness in ameliorating salinity stress was not confirmed in this study.

3.5. Effect of nano-Zn foliar application on Ca, K and Na contents in both roots and shoots of wheat subjected to salinity stress

Results from Table 2 indicate that nano-Zn foliar application had a profound effect on the mineral ion concentrations of wheat. The concentrations of Ca, K and Na in plant parts were taken into account, in this study, because salinity stress may considerably lessen the concentrations of K and Ca in plant shoots and roots while increase Na content in these plant parts (Aazami et al., 2021; Noohpisheh et al., 2021). In this context, K is an important nutrient for crop production and stress tolerance (Zörb et al., 2014) via increasing the water potential of root vacuoles and maintaining xylem-sap flow under stress conditions (Hawkesford et al., 2012). In case of Ca, it takes part in osmoregulation as a second messenger in cells and also through increasing the membrane-stability (Hawkesford et al., 2012). Our results indicate that salinity stress did not affect significantly nutrient contents in either roots or shoots of wheat cultivars for the plants that did not receive nano-Zn particles as a foliar spray. This probably indicates that these cultivars are salt-tolerant ones. Application of nano-Zn particles (up to 50 mg L^{-1}) raised significantly Ca contents in wheat roots and shoots, and to some extent K. On the other hand, it recorded no significant impacts on Na contents in either plant roots or shoots. This probably indicates that Na uptake increased by plants with this foliar spray due to the increases that took place in plant growth. The concentrations of K and Ca in plants grown under salinity stress conditions when sprayed with 50 mg Zn L^{-1} was significantly higher than the corresponding ones that were not sprayed with nano-Zn while grown under non stress conditions. This finding indicates that this application might increase plant growth via stimulating their uptake of nutrients; hence increase plant metabolism.

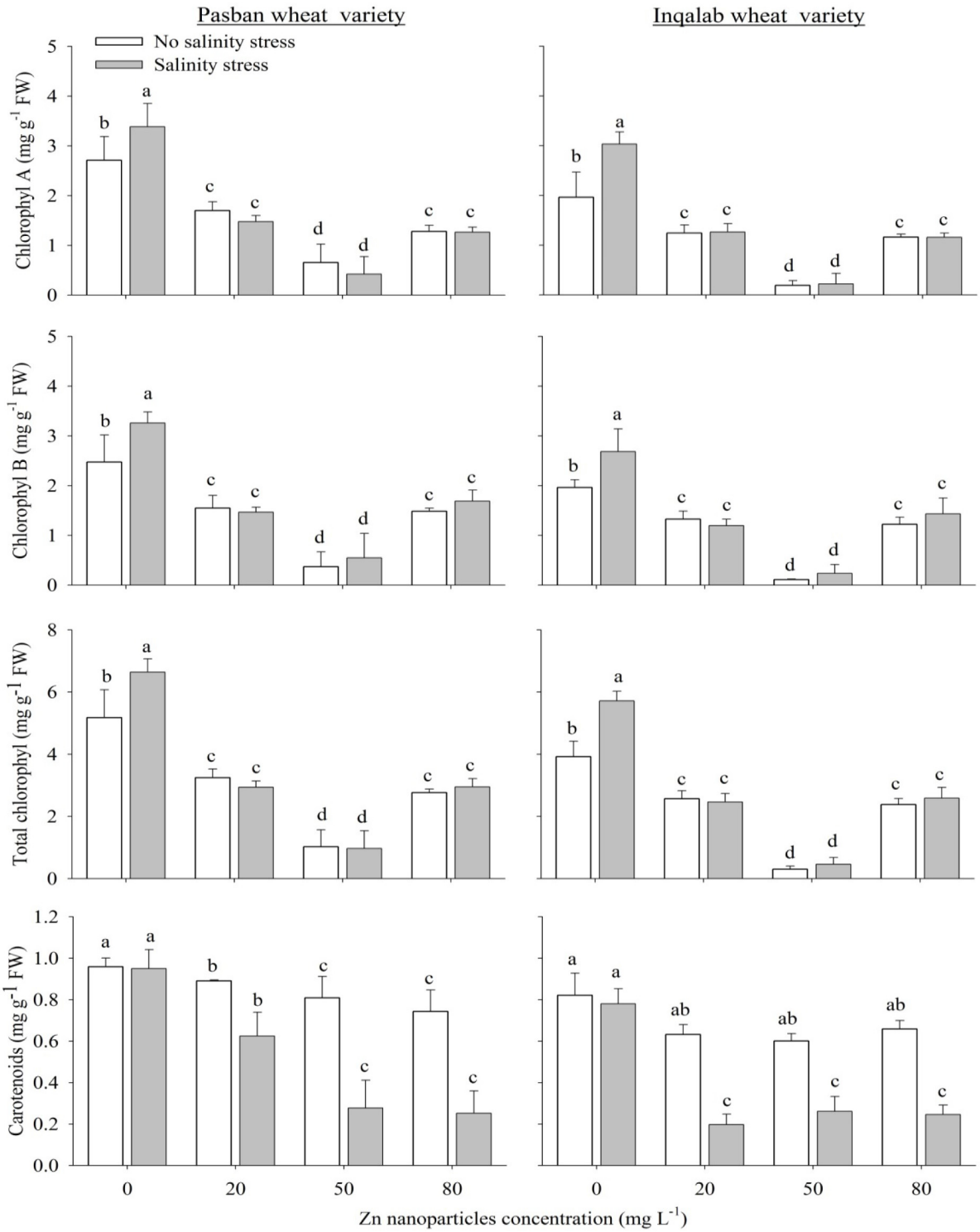

Fig. 3. Effect of nano-Zn foliar application on pigment contents in wheat plants subjected to salinity stress. Similar letters indicate no significant variations among treatments.

Table 1
Effect of nano-Zn foliar application on the enzymatic (catalase) and non-enzymatic (ascorbic acid AsA) antioxidants, organic osmolytes (proline and TSS) and Malondialdehyde (MDA) in wheat leaves.

Zn nano particles level (mg L^{-1})	Status	Total soluble proteins, mg g^{-1} DW	Catalase, mg $protein^{-1}$	AsA, $\mu g\ g^{-1}$ DW	MDA, $\mu mol\ g^{-1}$ DW	Proline, $\mu g\ g^{-1}$ DW	TSS, $\mu mol\ g^{-1}$ DW
		Pasban wheat variety					
0		8.54 ± 0.18a	1.47 ± 0.1a	0.62 ± 0.04ab	23.0 ± 1.243e	23.44 ± 3.4b	0.12 ± 0.01b
20	No salinity	7.83 ± 0.21b	1.33 ± 0.09a	0.41 ± 0.17b	28.43 ± 2.42de	29.05 ± 8.54ab	0.15 ± 0.03ab
50	stress	6.36 ± 0.09d	1.03 ± 0.06b	0.38 ± 0.1b	26.38 ± 3.64e	42.63 ± 5.52a	0.17 ± 0.02ab
80		5.38 ± 0.06e	0.85 ± 0.05b	0.41 ± 0.17b	27.86 ± 2.0e	35.19 ± 12.13ab	0.15 ± 0.04ab
0		7.19 ± 0.15c	1.30 ± 0.05a	0.73 ± 0.2a	39.7 ± 11.4bc	21.09 ± 7.9b	0.14 ± 0.03ab
20	Salinity	5.59 ± 0.17e	0.85 ± 0.19b	0.50 ± 0.11ab	37.40 ± 4.6b-d	29.29 ± 13.7ab	0.17 ± 0.04ab
50	stress	4.80 ± 0.18f	0.36 ± 0.32c	0.59 ± 0.13ab	29.11 ± 9.13de	41.27 ± 13.6a	0.18 ± 0.03a
80		3.79 ± 0.18 g	0.26 ± 0.06c	0.39 ± 0.2b	29.44 ± 3.37de	33.65 ± 13.8ab	0.16 ± 0.04ab
		Inqalab wheat variety					
0		7.77 ± 0.2b	1.39 ± 0.15a	0.72 ± 0.2a	30.04 ± 3.02de	33.58 ± 10.9ab	0.13 ± 0.01b
20	No salinity	6.66 ± 0.23c	1.24 ± 0.09ab	0.40 ± 0.16bc	29.71 ± 8.0de	39.86 ± 4.9ab	0.15v0.03ab
50	stress	5.65 ± 0.73e	0.81 ± 0.16c	0.19 ± 0.06d	27.67 ± 7.7e	44.09 ± 6.5a	0.17 ± 0.04ab
80		4.87 ± 0.08f	0.36 ± 0.18de	0.38 ± 0.13b-d	27.35 ± 6.64e	35.33 ± 14.6ab	0.15 ± 0.02ab
0		8.72 ± 0.42a	1.10 ± 0.2b	0.88 ± 0.17a	48.17 ± 5.75a	29.31 ± 9.7b	0.15 ± 0.02ab
20	Salinity	7.68 ± 0.32b	0.85 ± 0.16c	0.22 ± 0.09cd	40.71 ± 5.79ab	38.68 ± 4.8ab	0.17 ± 0.04ab
50	stress	6.49 ± 0.14c	0.55 ± 0.09d	0.35 ± 0.03b-d	26.21 ± 8.77e	41.00 ± 8.6ab	0.19 ± 0.01a
80		5.66 ± 0.62e	0.30 ± 0.14e	0.46 ± 0.06b	31.60 ± 6.51c-e	34.22 ± 3.7ab	0.17 ± 0.05ab

ASA: ascorbic acid, MDA: Malondialdehyde; TSS: total soluble sugar, FW: fresh weight. Similar letters indicate no significant variations among treatments.

Table 2
Effect of nano-Zn foliar application on Ca, K and Na contents in both roots and shoots of wheat subjected to salinity stress.

Zn nano particles level (mg L^{-1})	Status	Ca (mg g^{-1} DW)		K (mg g^{-1} DW)		Na (mg g^{-1} DW)	
		Shoot	Root	Shoot	Root	Shoot	Root
		Pasban wheat variety					
0		1.00 ± 0.58cd	1.25 ± 0.29ab	30.50 ± 6.56ab	16.38 ± 2.9d	20.38 ± 2.32a	15.63 ± 3.4ab
20	No salinity	1.50 ± 0.41bc	1.00 ± 0.41ab	27.75 ± 2.99ab	22.13 ± 1.9c	18.38 ± 2.66a	14.50 ± 1.87b
50	stress	2.25 ± 0.29a	1.75 ± 0.65a	48.00 ± 2.58a	42.25 ± 2.75a	17.88 ± 2.06a	14.13 ± 3.07b
80		1.00 ± 0.41cd	0.88 ± 0.48b	23.00 ± 7.96c	32.00 ± 2.6b	18.00 ± 2.04a	14.25 ± 2.75b
0		0.63 ± 0.25d	1.38 ± 0.48ab	29.00 ± 3.37ab	11.50 ± 1.3e	23.25 ± 5.3a	19.38 ± 4.05a
20	Salinity	1.00 ± 0.41cd	1.00 ± 0.41ab	34.00 ± 7.62b	17.75 ± 3d	20.88 ± 3.6a	17.63 ± 0.85ab
50	stress	1.75 ± 0.65ab	1.13 ± 0.63ab	47.25 ± 4.35a	34.25 ± 2.2b	19.50 ± 3.4a	16.75 ± 2.1ab
80		1.13 ± 0.48b-d	1.00 ± 0.71ab	32.25 ± 2.75b	25.25 ± 2.5c	19.50 ± 3.6a	17.00 ± 2.16ab
		Inqalab wheat variety					
0		0.75 ± 0.29cd	1.50 ± 0.41ab	26.25 ± 3.4ab	13.50 ± 2.65f	20.88 ± 5.63a	17.50 ± 2.6a-c
20	No salinity	1.38 ± 0.48b-d	1.00 ± 0.41b	26.00 ± 2.16ab	19.25 ± 1.71e	19.88 ± 4.03a	16.25 ± 2.36a-c
50	stress	2.13 ± 0.48a	1.88 ± 0.25a	42.00 ± 3.56a	38.25 ± 2.06a	17.50 ± 3.03a	14.50 ± 3.54c
80		1.00 ± 0.41b-d	1.13 ± 0.48b	20.25 ± 7.09c	28.50 ± 1.7c	17.50 ± 4.1a	15.63 ± 3.01bc
0		0.63 ± 0.25d	0.88 ± 0.25b	27.00 ± 3.74ab	9.00 ± 0.8 g	22.88 ± 2.14a	19.88 ± 1.55a
20	Salinity	1.25 ± 0.29b-d	1.38 ± 0.25ab	30.50 ± 7.42b	13.50 ± 3.1f	21.50 ± 3.37a	19.13 ± 0.85ab
50	stress	1.50 ± 0.71a-c	1.00 ± 0.41b	41.50 ± 5.97a	30.00 ± 0.8b	19.75 ± 1.04a	17.13 ± 2.63a-c
80		1.75v0.65ab	2.00 ± 0.71a	29.00 ± 2.83b	22.25 ± 1.5d	20.88 ± 2.69a	17.63 ± 2.14a-c

DW: dry weight. Similar letters indicate no significant variations among treatments.

4. Conclusions

Spraying plants with nano-Zn particles lessen effectively the negative impacts of salinity on wheat plants. Application of nano-Zn particles enhanced the growth of either Inqilab 91 or Pasban 90 wheat verities in terms of, root and shoot (fresh and dry) weights, shoot and root lengths as well as plant leaf area index. Furthermore, nano-Zn foliar spray led to significant reductions in plant pigments (carotenoids, chlorophyll A, B and their total contents) and also in enzymatic (catalase and peroxidase) and non-enzymatic (ascorbic acid) antioxidants in plants. Yet, this foliar spray enhanced formation of total soluble sugars and proline in both cultivars of study. Moreover, this spray raised significantly Ca contents in wheat roots and shoots, and to some extent K contents. It could be concluded that, the foliar application of nano-Zn particles increased plant growth under salty stress conditions via two parallel processes, i.e., stimulating formation of osmolytes and stimulating nutrient uptake which may, in turn, increase plant metabolism.

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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.

Data availability

Data will be made available on request.

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