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INFLUENCE OF FOLIAR APPLICATION OF ZINC SULFATE ON PEA (*Pisum sativum* L.) UNDER SALINE CONDITIONS

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Abstract

Plants have to face different harsh environmental conditions among which salinity is a major widespread abiotic stress for plants. Salinity imposes serious threats to the growth of plants, soil fertility, and agriculture production. Pea stands as a versatile crop, rich in protein and offering high nutritional value; however, its vigor is hampered by salt stress. Within plant mechanisms, zinc assumes a pivotal role. A pot-based experiment was conducted to explore the response of two pea cultivars (Tiger and EP-190). Employing a completely randomized design with 3 replicates, the study aimed to uncover the potential impacts of foliar-applied $ZnSO_4$ (at concentrations of 0, 500, and 1000 ppm) on pea plants under the influence of NaCl stress (at levels of 0 and 120 mM). The outcomes revealed that salt stress exerted a negative influence on all growth parameters, the fresh and dry weights of roots and shoots, as well as physiological factors, antioxidant enzymes, and plant yield. In contrast, the levels of MDA, H_2O_2, and membrane permeability surged in the presence of salt stress, although these effects were mitigated with the application of foliar $ZnSO_4$ spray. Notably, the application of $ZnSO_4$ at 500 ppm exhibited enhanced effectiveness in maintaining chlorophyll content, boosting antioxidant enzymes, and enhancing overall growth and yield attributes in the presence of both 0 and 120 mM NaCl. Overall, the cultivar Tiger (in contrast to EP-190) had comparatively higher yield under salt stress and $ZnSO_4$ treatment in terms of the number and weight of seeds. Hence, foliar $ZnSO_4$ may improve the productivity of pea under the control and stressful environment.

Keywords: Foliar application Zinc sulfate, *Pisum sativum* L., Antioxidants Enzymes, Saline and non-saline conditions

Introduction

The impacts of salinity persistently influence both plant growth and yield (Chrysagargyris *et al.*, 2018). Throughout the globe, about one-third of cultivated area is salt affected. In this way, salt stress acts as a limiting factor for food production. Declining of fresh water, high evapotranspiration, and rise of the water table may increase the salt load in water irrigation, and agricultural malpractices are considered the most factors that intensify this problem (Koyro *et al.*, 2013). Salinity is recognized as a prominent abiotic factor that

frequently imposes restrictions on growth and yield of plants across diverse regions. (Mantri *et al*., 2012). Such circumstances can induce changes in the rates of biosynthesis and photosynthesis, along with modifications in enzyme activity (Qin *et al*., 2019). Salinity stress hinders the functioning and overall yield and growth of *Pisum sativum*. Excess of Na^+ and Cl^- concentrations lowered water potential in chloroplast which immediately altered photosynthetic rates in pea plants (Gupta *et al.,* 2021). Salinity stress causes both short and long-term modifications in plants. This stress can ultimately destroy crucial biomolecules by escalating ROS production (Ishrat *et al.,* 2022).

Pea is a very popular crop grown in many Middle Eastern countries (Parihar *et al.,* 2020). It is an annual plant that possesses edible seeds and is also called a garden pea. It considered as main leguminous crop and an important agricultural component in under-developed countries (Desoky *et al.,* 2017). The field pea is a desirable leguminous crop grown during cooler seasons. Its green pods are consumed by humans, and its dried seeds are valued as a pulse. This popularity stems from its notable protein content (25–35%) containing essential amino acids. Moreover, the seeds offer a rich supply of essential minerals including iron and calcium; vitamins such as tocopherols niacin, thiamine and folic acid (Yang *et al.,* 2022). Pea holds a versatile role, providing nourishment to both human beings and livestock (Bashir *et al*., 2019). As a leguminous plant, pea forage plays a crucial role in livestock nutrition and herbage production. Among legume grains, pea ranks third globally, following common beans and soybean. Notably, as a leguminous crop, pea has the capability to fix 50-150 kg of atmospheric nitrogen per hectare (Voor *et al*., 2020).

Pea cultivation holds a significant position in Pakistan's agricultural economy. The cultivated area spans around 10,000 hectares, yielding approximately 72,000 kilograms. While the average pea production in the country lags behind that of other nations, the significance of pea as a crucial dietary staple sets it apart from other locally grown pulses (Bashir *et al*., 2019). Moreover, peas like other leguminous crops also play a big role in crop rotation practices, leading to increased production in other crops and improved soil fertility. Following the cultivation of leguminous crops, there is a noticeable increase in organic matter content. As a result, the demand for leguminous crops is progressively on the rise (Maftuna *et al*., 2022).

Legumes crops yield and growth are reduced under salinity stress due to disturbances in hormonal regulation and osmotic balance (Nadeem *et al.,* 2019). Owing to their limited tolerance mechanisms, leguminous plants are particularly vulnerable to the effects of salt stress and struggle to thrive in saline environments. (Gupta & Huang, 2014). *Pisum sativum* is sensitive to various stresses including oxidative stress, drought and salinity. (Yousef *et al.,* 2020).

Multiple studies have described that many of the pea genotypes are adversely affected by salt stress above 100 mM concentration (Noreen & Ashraf, 2009; Grozeva *et.al.* 2023). Salt stress leads to a reduction in dry matter content, leaf size and an increase in the root/shoot ratio. These combined effects contribute to a decline in overall yield (Munns & Tester, 2008; Manchanda & Garg, 2008).Soil infertility occurs in the presence of

adequate salt and pea crops are not resistant to it, this inhibits overall plant growth and affects nodule formation, hence productivity declines (Yousef *et al.,* 2020). To optimize the toxicity caused by salt stress, enhancing the soil with zinc has been identified as a potential method to mitigate the toxicity due to sodium and chloride ions (Ali *et al.,* 2022; Ramzan *et al.,* 2023).

Zinc is acknowledged as a crucial nutrient vital for achieving optimal plant growth, given its significant involvement in fundamental cellular processes and its role in safeguarding critical elements such as chlorophyll from oxidation (Cherif *et al*., 2010). When plants are subjected to salt stress, foliar application of micronutrients and macronutrients has shown greater efficacy compared to other soil-based nutrient application methods (El-Fouly *et al*., 2011). Multiple studies have reported the positive effects of applying Zn, highlighting its growth-promoting effects (Ibrahim & Ramadan, 2015). It has been suggested that supplementing plants with Zn is a valuable strategy for safeguarding plants against the negative impacts of salinity.

Within this framework, the present study was undertaken to determine the responses of various pea cultivars to salinity conditions. Additionally, the investigation aimed to explore the potential advantages associated with foliar application of $ZnSO_4$ in promoting the growth of pea plants under conditions characterized by salt-induced stress.

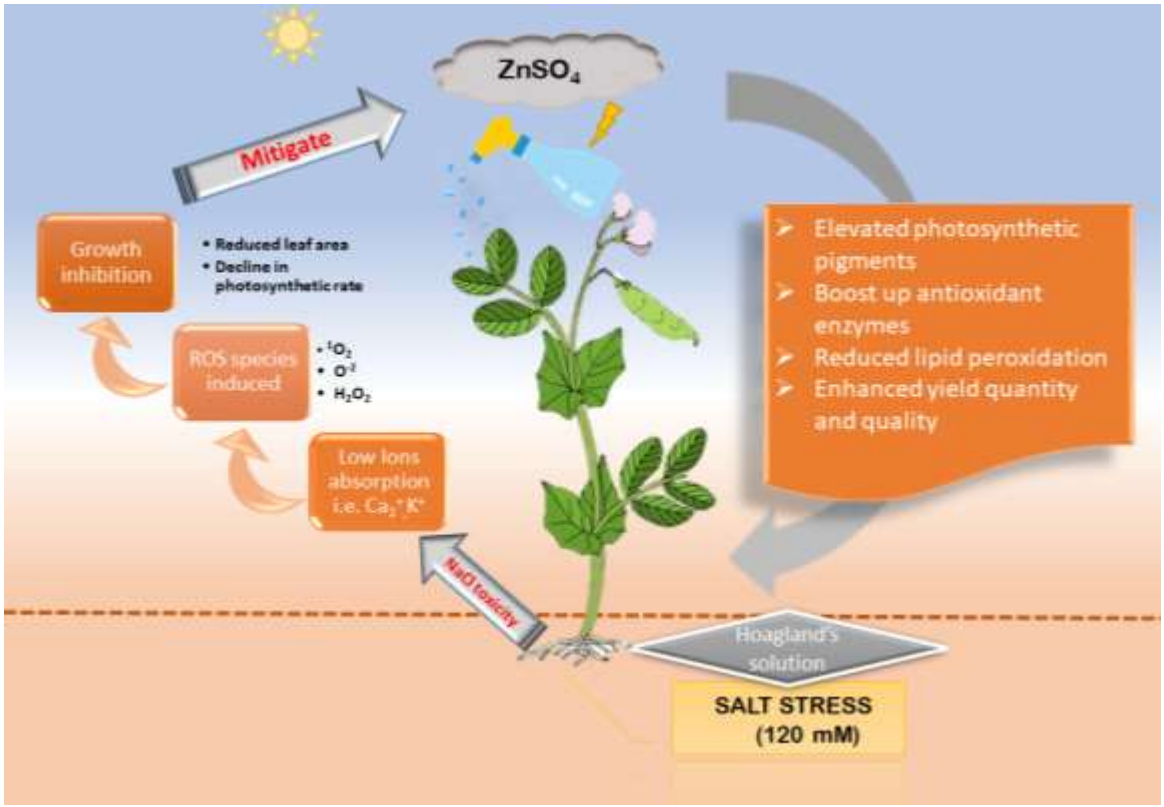

Fig 1: Effect of applying zinc sulfate via foliar application on pea plants (*Pisum sativum* L.) under NaCl stress

Materials and Methods

The pea cultivars were cultivated in the research area of the University of Education Lahore, with all assessments conducted within the Botany Department's laboratories. Seeds of two distinct pea cultivars, namely Tiger and EP-190, were taken from the Ayub Agriculture Research Institute in Faisalabad (AARI). The experimental setup was organized following a completely randomized design, comprising 3 replicates and a total of 36 pots, all filled with a sand medium. Three different concentrations of $ZnSO_4$ (0, 500 and 1000 ppm) and 2 levels of NaCl (0 and 120 mM) were given. Both cultivars have 5 seedlings in each pot after thinning and Hoagland's solution was used at regular intervals throughout plant development. The final level of salt stress 120 mM was maintained in two applications to avoid shock to the plants.

Growth and yield parameters analysis

For the growth analysis, following the sampling process, the roots were detached from the shoots of each plant. A digital weight balance was used to get the fresh weight of both roots and shoots. The dry weights of the shoots and roots were obtained subsequent to subjecting the samples to 48 hours of oven-drying at 70°C. Measurements of shoot and root lengths were taken using a meter rod, noted in centimeter units.

No. of pods and seeds per plant were calculated from each pot manually. The weight of fresh seeds in grams were measured with electronic balance.

Chlorophyll determination

Plant chlorophyll content was determined using the method described by Arnon (1949). Initially, 0.5 grams of fresh leaves were weighed and subsequently crushed in 10 ml of 80% acetone. The samples were then left to stand for 24 hours, after which the absorbance was measured at 480 nm, 645 nm, and 663 nm utilizing a spectrophotometer (Model T60 UV).

The formula employed for chlorophyll measurement is:

Chl. a=[12.7(OD663)-2.69(OD645)]×Vol of acetone/1000×weight

Chl. b=[22.9(OD645)-4.68(OD663)]×Vol of acetone/1000×wieght

Membrane permeability (%)

Membrane permeability was evaluated by (Yang *et al.,* 1996) protocol. Equally matured leaves were taken from each pot and these leaves were cut into small sections. These small pieces of leaves then kept in 20 ml of distilled water and vortexed for 5s to record the initial reading i.e. EC_0. Samples were refrigerated at 4°C for about one day to note the EC_1 reading. In addition these samples were autoclaved at 120°C for 24 hours to estimate the final reading EC_2.

The relative permeability calculated as follows:

$$RMP\ (\%)=(EC_1-EC_0/EC_2-EC_0)\times 100$$

Malondialdehyde determination (MDA)

Method described by Cakmak & Horst (1991) was employed to assess MDA contents. For this purpose, 0.5 g leaf sample was rinsed with 1% TCA (trichloroacetic acid) solution (3 mL). The mixture was centrifuge at 20,000 g for 15 min. Filtrate (0.5 mL), after centrifugation, was added to 3 mL of 0.5% TBA (Thiobarbituric acid). The

mixture was allowed to stand for a duration of 30 minutes. Subsequently, it was subjected to heating using a hot water bath for 15 minutes at a temperature of 95°C, followed by immediate cooling of the entire mixture in an ice bath. The treated samples were then analyzed for absorbance at 523 nm and 600 nm using a spectrophotometer (Model T60 UV).

$$MDA(nmol.cm^{-1})=1000[(Abs523-Abs600nm)/155]$$

H_2O_2 Determination

The method described by Velikova *et al*. (2000) was employed to assess H_2O_2 levels in plants. A plant sample (0.5 g) was ground in 5 ml of 0.1% TCA while maintaining an ice bath. After centrifugation of the crushed material, the resulting supernatant was used for analysis. To the obtained supernatant, 1 ml of potassium iodide was added, along with a 0.5 mL of potassium phosphate buffer (pH 7.0). The mixture was then subjected to vortexing, and the absorbance was recorded at 390 nm utilizing a UV-Visible spectrophotometer (Model T60 UV).

Determination of Anthocyanin

The measured plant sample (0.5 g) was grounded with 5 mL of buffer in an ice bath. This solution was centrifuged and supernatant was taken to record readings at 600 nm with the help of a spectrophotometer (Model T60 UV).

Antioxidant enzyme and protein (extract preparation)

For the preparation of the extract, a leaf sample weighing (0.5 g) was placed in a 50 mM phosphate buffer under cold conditions, ensuring that the pH remained at 7.8. Subsequently, the sample was subjected to centrifugation at 15,000 rpm for a duration of 20 minutes at a temperature of 4°C. The resulting extract, intended for the subsequent analysis of various antioxidant enzymes.

Total Soluble Proteins

The soluble proteins were measured by the Bradford method (1976). Plant extract (0.1 ml) was taken as earlier prepared and 2 ml of Bradford reagent was mixed in a test tubes. Finally left these mixture at room temperature for 5 min to note the absorbance by using spectrophotometer (Model T60 UV) at 595 nm.

Catalase and Peroxidase

Catalase and Peroxidase activities were determined following the method described by Chance and Maehly (1955). For the Catalase assay, the reaction mixture included 0.1 ml of plant sample extract, 5.9 mM H_2O_2, and 50 mM buffer with a pH of 7.0. The reaction was initiated by adding the enzyme extract, and the solution's absorbance was recorded at 240 nm at 20-second intervals for 1 minute using a spectrophotometer.

As for the Peroxidase assay, the reaction mixture comprised 40 mM H_2O_2, 20 mM guaiacol, and 50 mM buffer in a test tube. The enzyme extract (0.1 ml) was added to start the reaction. Subsequently, the solution's absorbance was monitored at 470 nm every 20 seconds using a spectrophotometer (Model T60 UV). The activity of Peroxidase was defined based on an absorbance change of 0.01 units per minute.

Ascorbate peroxidase (APX)

The procedure outlined by Nakano and Asada (1981) was adhered to for the assessment of APX activity. In this regard, the reaction

mixture consisted of EDTA (100 µM), H_2O_2 (100 µM), ascorbate (250 µM), 25 mM sodium phosphate buffer, and 0.1 ml of plant extract. The spectrophotometer (Model T60 UV) was utilized to measure readings at 290 nm at intervals of 20 seconds for two minutes.

Ions Concentration

To determine the ion concentration, digestion methods was utilized (Wolf, 1982). Firstly, the plant sample were dried in oven for 24 hours 70°C. Subsequently, the dried samples underwent digestion using concentrated sulfuric acid and were left for a period of one day. To facilitate this process, a hot plate was employed to heat the digestion flasks, with heating being intermittently sustained by introducing hydrogen peroxide (H_2O_2) into the digestion flask until the mixture achieved a colorless state. The concentrations of sodium and potassium cations were then determined utilizing a Flame photometer (Sherwood Model 360) to obtain readings.

Statistical Analysis

CoStat was used for data analysis (version 6.303, cohort software). All collected data were analyzed for ANOVA at 5% alpha (level of significance).

Results and Discussion

The present work described the results of morpho-physiological, and biochemical parameters of 2 cultivars i.e. Tiger and EP-190 under NaCl stress and alleviating potentiality of $ZnSO_4$ under salinity (Table 1).

Salinity has detrimental effects on plant growth and development. It subjects plants to osmotic stress, induces toxicity from specific ions like sodium (Na^+), disrupts the function of key cytosolic enzymes due to oxidative stress, and perturbs the equilibrium of intracellular potassium within plant cells. (Sairam & Srivastava, 2002; Safdar *et al*., 2019).

Zinc holds a significant importance in plant metabolism. Micronutrients like zinc contribute to the production of auxins and play a significant part in processes like chlorophyll synthesis, carbohydrate metabolism, and protein formation (Gondal *et al.*, 2021). Zinc's involvement extends to plant nitrogen metabolism as well. It facilitates the amalgamation of various amino acids, which are essential building blocks for enzymes and proteins (Galili *et al*., 2016).

Cultivar Tiger exhibited an improvement in shoot fresh and dry weight at the 500 ppm $ZnSO_4$ concentration, while EP-190 demonstrated maximal shoot fresh and dry weight at the 1000 ppm $ZnSO_4$ level, in comparison to the control, as depicted in Figure 2. Notably, salinity led to decrease fresh and dry shoot and root weights in both cultivars (Tiger and EP-190) when contrasted with plants experiencing non-saline conditions. This trend aligns with previous observations in Turnip (Noreen *et al*., 2010).

Interestingly, the combined application of salt and $ZnSO_4$ resulted in augmented fresh and dry shoot and root weights at concentrations of 500 ppm and 1000 ppm in both cultivars, as compared to when only subjected to salt stress. The influence of Zn treatment was evident in the enhancement of overall growth parameters, including shoot length, fresh and dry root and shoot weights, which corresponds with findings reported by Borah and Saikia (2021).

The fresh and dry root weights of both Tiger and EP-190 cultivars displayed an increase in mean values at the 500 ppm $ZnSO_4$ level. Moreover, under salt stress, there was a notable decline in shoot length and root length in the Tiger cultivar compared to EP-190. In terms of plant height, the application of $ZnSO_4$ positively impacted growth, a trend previously documented by Srivastav *et al*. (2019) in chickpeas.

Chlorophyll a and b content showed a maximum value at 500 ppm of $ZnSO_4$ when given alone and under salt stress to the pea plants in both cultivars, as proved earlier in Glycine soja (Jiang *et al*., 2014). Salinity had a considerable diminishing effect on the levels of both chlorophyll a and b in both the Tiger and EP-190 cultivars, as compared to the control group. This trend has been previously documented in turnip by various studies (Ashraf *et al*., 2014; Eryilmaz 2014; Noreen *et al*., 2010). Furthermore, the concentration of chlorophyll a and b was relatively higher in the Tiger cultivar compared to EP-190. Notably, plants treated with 1000 ppm of $ZnSO_4$ exhibited notably reduced levels of chlorophyll a and b. Anthocyanin content was reduced under salt stress with reference to control in both cultivars. Moreover, when $ZnSO_4$ (i.e.500 ppm and 1000 ppm) was applied under salt stress, anthocyanin concentration improved compared to salt-stress plants. While under control conditions in both cultivars, zinc dropped down anthocyanin concentration, which has been previously observed in proso millet (Mushtaq *et al*., 2023).

Salinity has the potential to significantly disrupt regular metabolic processes by inducing oxidative damage to lipids, proteins, and nucleic acids (Gill & Tuteja, 2010). MDA, relative membrane permeability, and H_2O_2 content of both cultivars (Tiger and EP-90) shoot up in salt-treated plants as compared to plants under control conditions, with the same results noted in turnip (Noreen *et al*., 2010) and pea (Noreen & Ashraf, 2009; Yasar *et al*., 2016). Plants treated with three levels of concentrations of $ZnSO_4$ (0. 500 ppm and 1000 ppm) dropped the content i.e. H_2O_2, MDA, and relative membrane permeability as compared to salt-treated plants (Ruiz-Torres *et al*., 2021). Tiger showed less membrane permeability than EP-190 at $ZnSO_4$ i.e.1000 ppm. MDA increased in both cultivars (Tiger and EP-190) when expose to salt stress.

Zn is an important element of various biomolecules like lipids and proteins, it participates in carbon metabolism and acts as co-factor of auxins (Hassan *et al*., 2020). The protein content demonstrated an increase at the 500 ppm $ZnSO_4$ level in comparison to the 1000 ppm concentration. This consistent pattern was observed in both Tiger and EP-190 cultivars, irrespective of whether the plants were subjected to salt stress or maintained under normal control conditions.Plants treated with $ZnSO_4$ showed a significantly increased effect on total soluble protein at both concentrations (500 ppm and 1000 ppm) in both cultivars under salt and control conditions. Similar studies have been conducted on chickpeas (Srivastav *et al*., 2019) and fava beans (Reda *et al*., 2014) under control and salt stress respectively.

All studied antioxidant enzyme activities greatly enhanced during stress conditions, alike findings by (Noreen & Ashraf 2009; Eryilmaz 2014). Upon treatment of plants with $ZnSO_4$ at concentrations of 500 ppm and 1000 ppm in

contrast to the control group, noticeable improvements were observed in terms of enzymatic activity, specifically in the case of ascorbate peroxidase (APX), POD, and CAT. Interestingly, the co-application of salt and $ZnSO_4$ resulted in even higher levels of APX activity compared to the treatment with $ZnSO_4$ alone.Notably, the 500 ppm $ZnSO_4$ concentration exhibited greater activity in enzymes such as ascorbate peroxidase (APX), catalase (CAT), and peroxidase (POD) compared to the 1000 ppm concentration under conditions involving salt-induced stress as well as normal control conditions. The same has been observed in common beans that a low amount of $ZnSO_4$ had a better antioxidative activity (Salehi *et al*., 2021). However, in EP-190 activity of catalase slightly declined at 1000 ppm under salt-stressed and control plants from their respective control.

The sodium ions raised while potassium ions concentration declined in (root and shoot) when cultivars Tiger and EP-190 were directly exposed to salt stress as compared to control, which was earlier observed in turnip and Lavandula by (Noreen *et al*., 2010; Chrysargyris *et al*., 2018). Salt stress caused a reduction in K^+/Na^+ previously noted by (Ghonaim *et al*., 2021). Salinity badly affects the ions absorption in plants in EP-190 as compared to Tiger. The amount of Na^+ decreased in plants solely treated with $ZnSO_4$. Plants exclusively treated with $ZnSO_4$ had boosted the potassium ions under salt and control conditions as reported by (Chrysargyris *et al*., 2018; Adekiya *et al*., 2018). Salt stress caused a reduction in the yields as indicated by 100-seed weight, seeds per pod, and number of pods in each plant as compared to control in both cultivars. Increased number of pods, seeds, and 100-seed weight were recorded when the pea plant exposed to the $ZnSO_4$ (500 ppm and 1000 ppm) in both cultivars as compared to the control condition. The same results were recorded earlier in chickpea by Srivastav *et al*., 2019. In both cultivars, $ZnSO_4$ treatment under salt stress had almost the same results at two levels. Almost the same trend is shown by cultivars at two levels under control and stress conditions. When treated with $ZnSO_4$.fertilizer, different ions absorption boosted and hence it increased the yield of the plant as previously found in sweet potatoes (Adekiya *et al*., 2018). The zinc application increased the yield characters i.e. number of seeds per pod, and weight of the seeds in pea plants as previously observed (Borah & Saikia 2021). Moreover, under control conditions, zinc sulfate elevated pea yield as same results noted by (Mikusova *et al*., 2019; Pandey *et al*., 2013). The application of $ZnSO_4$ on leaves has been found to lessen the damaging effects of high salinity. This treatment promotes growth, boosts the antioxidant defense system, and increases the overall soluble protein content. As a result, this approach leads to improved flower and pod production, as well as higher total pea yield. Similar positive effects were previously seen in eggplant (Salim *et al*., 2019).

Furthermore, $ZnSO_4$ exhibits a beneficial effect when used for seed priming. It enhances the availability and uptake of zinc, subsequently boosting the yield of both maize and pea crops. This has been determined through research (Sharma *et al*., 2021).

Conclusion

This study's findings suggest that externally applying $ZnSO_4$ to plant leaves can

effectively counteract the detrimental impacts of salinity on pea plants. Salinity was observed to have negative effects on various growth parameters such as root and shoot fresh weights, root and shoot dry weights, root and shoot lengths, as well as chlorophyll and anthocyanin levels, along with yield parameters including pod numbers per plant, seed count per pod, and seed weight. Concurrently, physiological indicators like malondialdehyde (MDA), membrane permeability, and H_2O_2 content increased under salt-induced stress.

The application of $ZnSO_4$ through foliar means was found to enhance growth, chlorophyll levels, and yield while mitigating the adverse consequences of salinity in both pea cultivars. This effect was achieved by reducing levels of MDA and reactive oxygen species. Notably, the application of 500 ppm of $ZnSO_4$ demonstrated greater efficacy in alleviating the harsh impact of salt stress and fostering improved growth rates, leading to higher pea plant yields

Future recommendations

Past a certain threshold, increasing the concentration of micronutrients can lead to a decrease in plant yield. In future, various other concentrations of zinc sulfate may be applied at different growth stages of plants. This will serve to assess the plant's response to micronutrients and aim to achieve more valuable and higher yields.

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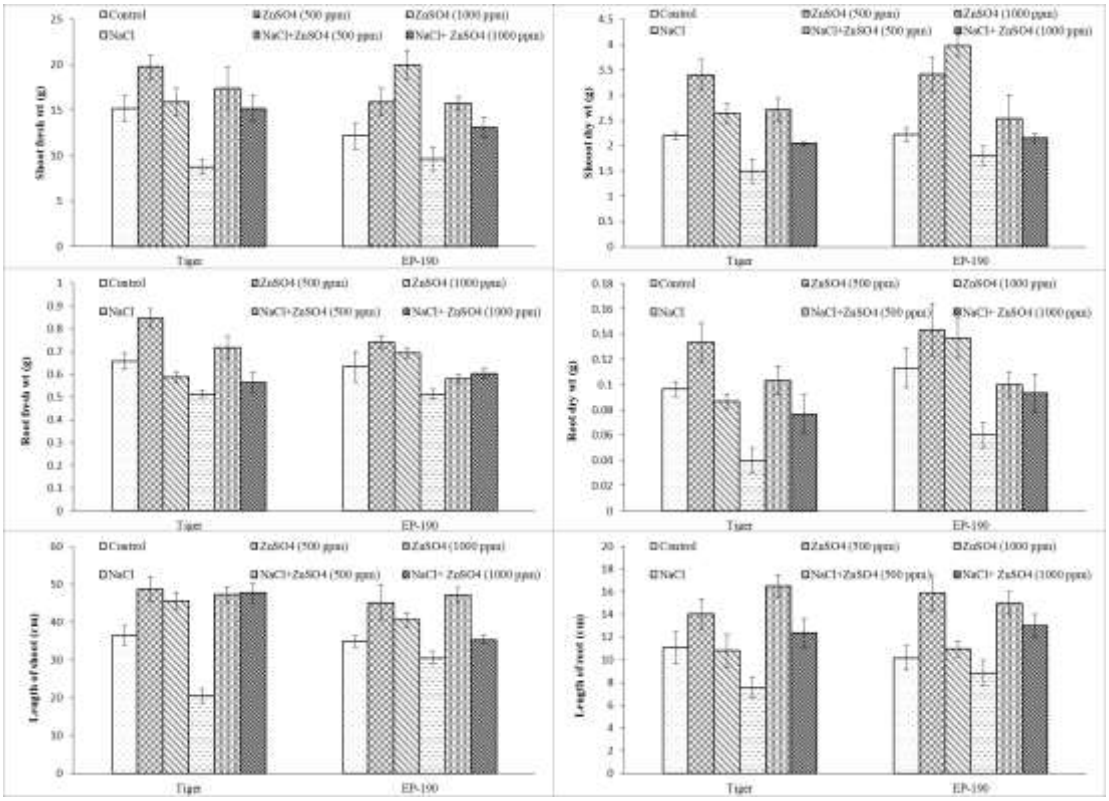

Fig. 2 Shoot fresh weight & dry weight; root fresh weight & dry weight, and shoot & root lengths of 2 cultivars of 29 days old pea plants grown under NaCl stress and Zinc sulfate (0, 500 and 1000 ppm).

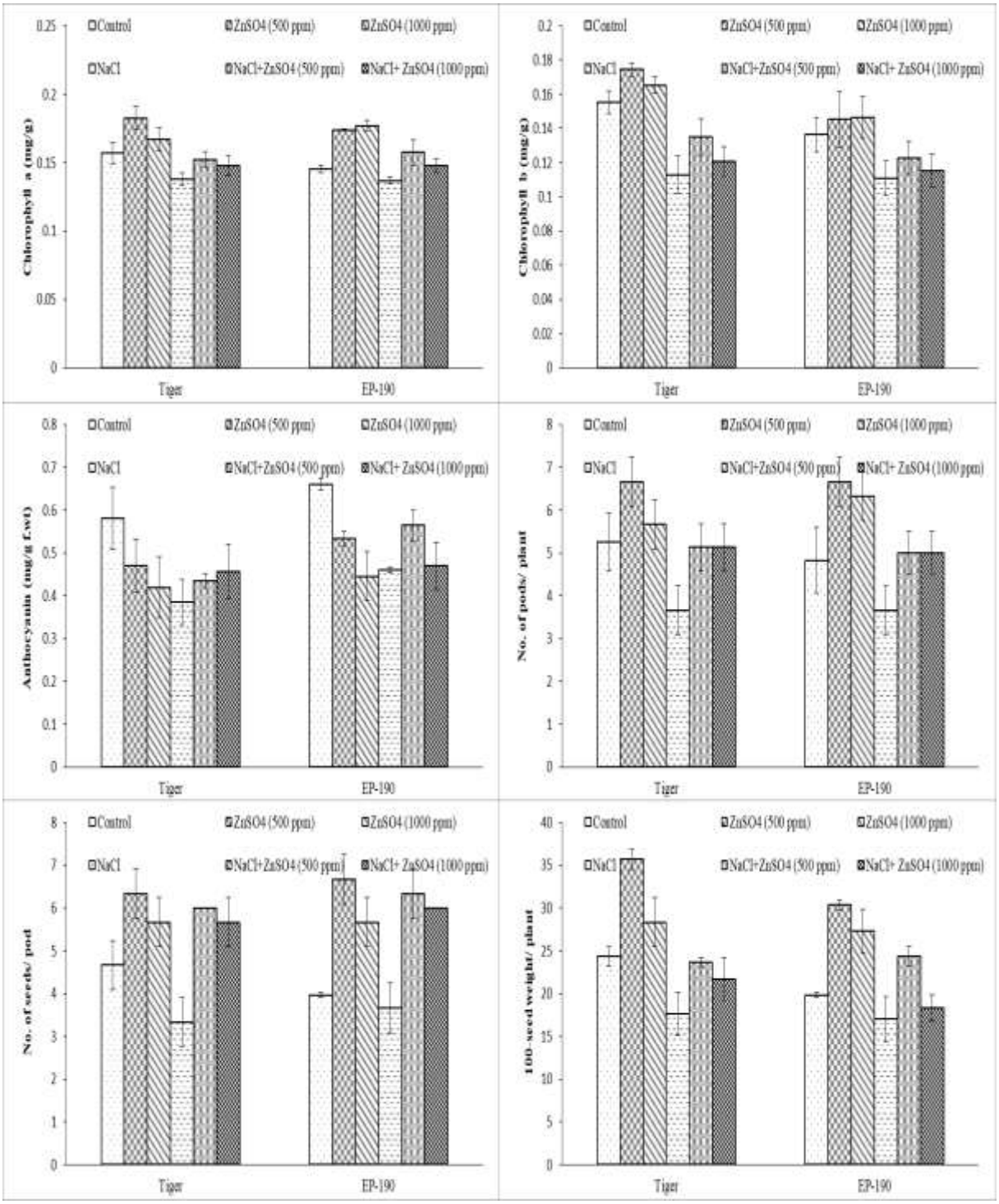

Fig. 3 Chlorophyll a, chlorophyll b, anthocyanin, no. of pods plant^{-1}, no. of seeds pod^{-1} and weight of 100-seed 2 cultivars of 29 days old pea plants grown under NaCl stress and Zinc sulfate (0, 500 and 1000 ppm).

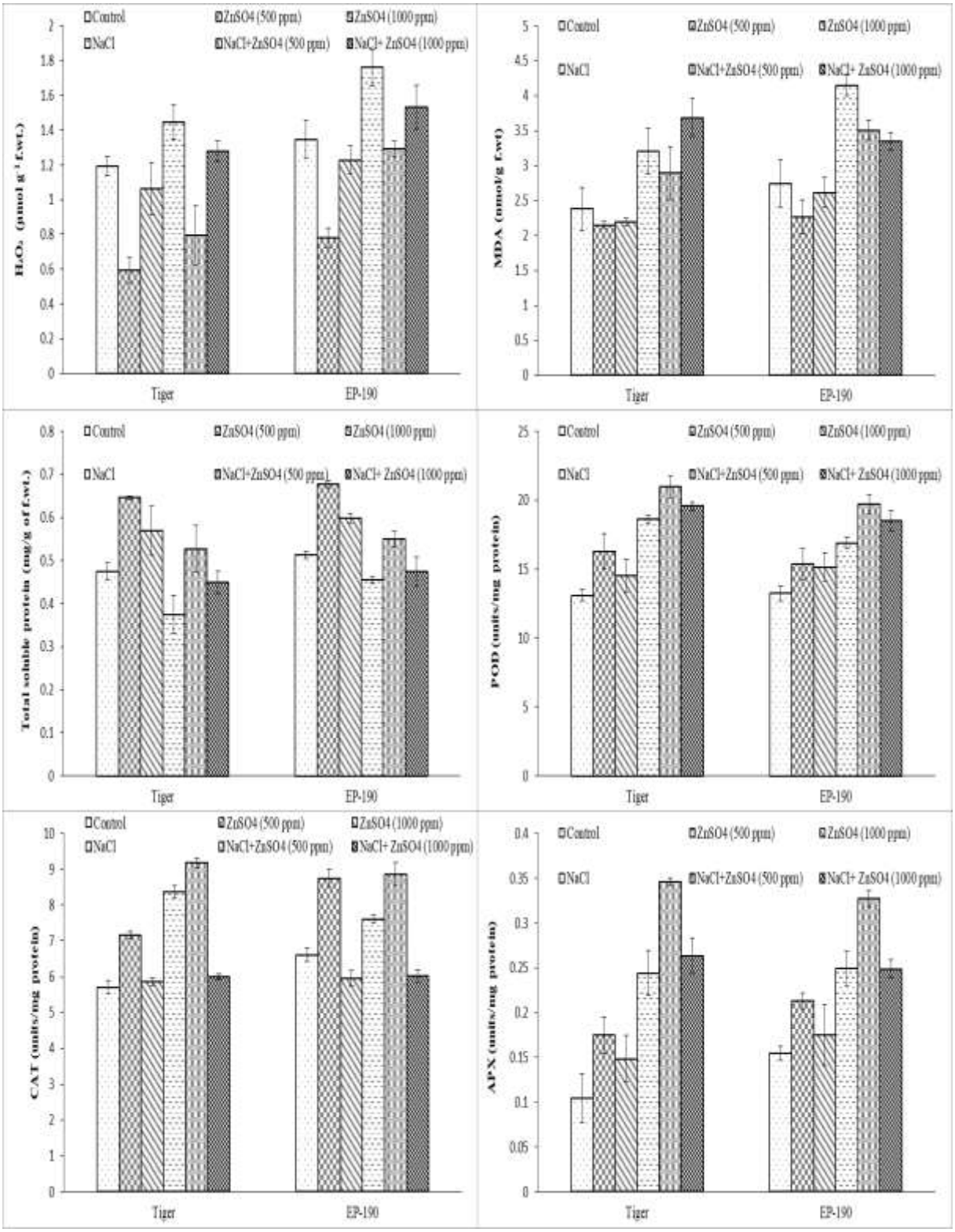

Fig. 4 H_2O_2, MDA, Total soluble proteins, peroxidase, catalase and ascorbate peroxidase activities of 2 cultivars of 29 days old pea plants grown under NaCl and Zinc sulfate (0, 500 and 1000 ppm).

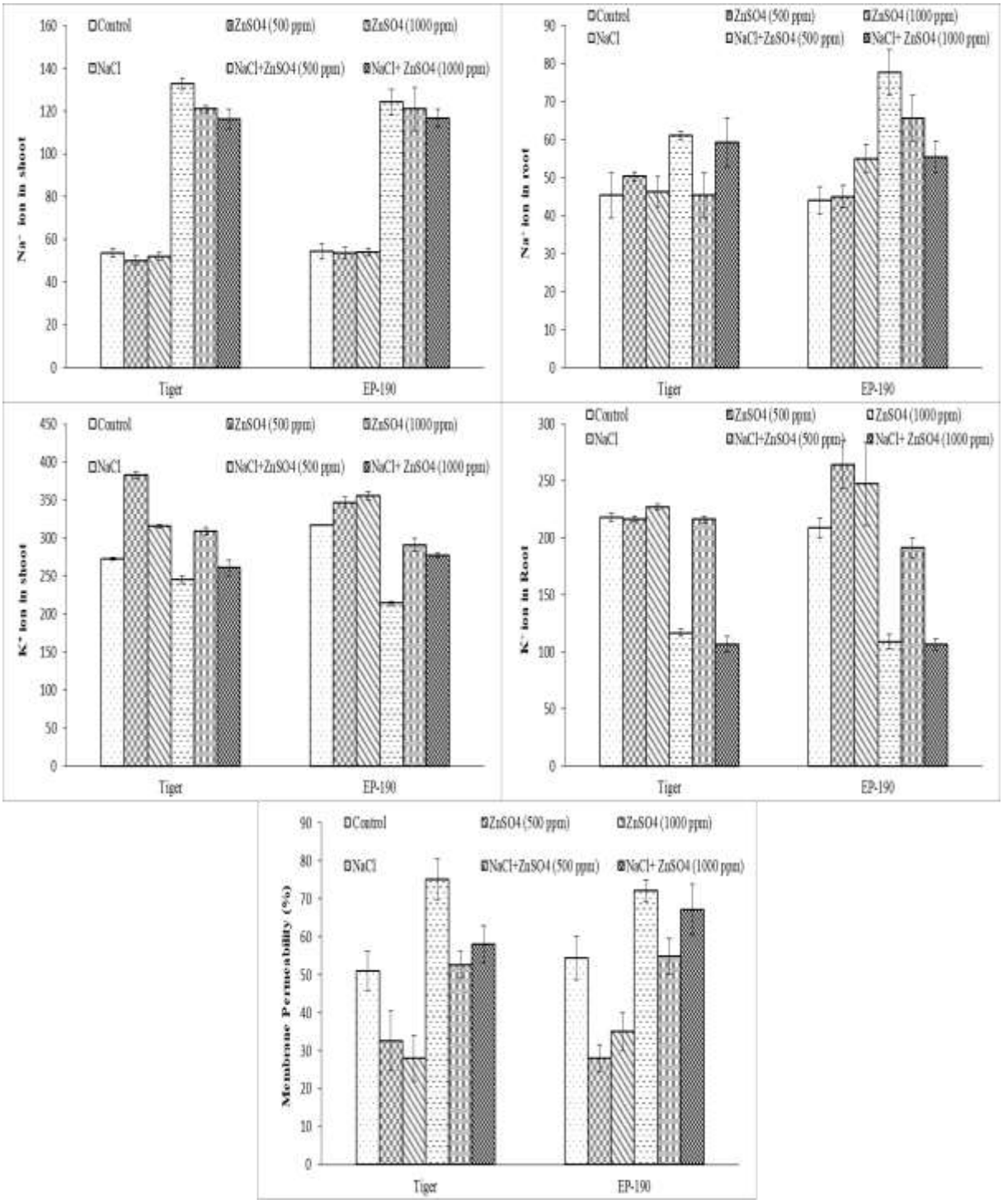

Fig. 5 Sodium ions in shoot and root, Potassium ions in shoot and root and membrane permeability of 2 cultivars of 29 days old pea plants grown under NaCl and Zinc sulfate (0, 500 and 1000 ppm).

TABLE 1: Mean square values from ANOVA of the data on the different growth, physiological, biochemical and yield parameters of the two cultivars of pea (*Pisum sativum* L.) grown for 29 days under salt stress and Zinc sulfate (500 and 1000 ppm)

Source of variation	**df**	**Shoot fresh wt.**	**p-value**	**Shoot dry wt.**	**p-value**	**Root fresh Wt.**	**p-value**	**Root dry wt.**	**p-value**	**Anthocyanin**	**p-value**	**No. of pods/plant**	**p-value**	**No. of seeds/plant**	**p-value**	**100-seed weight**	**p-value**
Cultivar	1	7.7748ns	0.060	0.651**	0.003	0.0036ns	0.103	0.003***	0.004	0.037393	0.001	0.0003ns	0.978	0.1002ns	0.532	50.17***	0.001
NaCl	1	90.59***	0.000	6.51***	0.000	0.111***	0.000	0.013***	0.000	0.028737	0.002	15.34***	0.000	0.9669ns	0.610	465.8***	0.000
ZnSO4	2	111.7***	0.000	3.75***	0.000	0.066***	0.000	0.005***	0.000	0.017359	0.004	7.533***	0.000	19.22***	0.000	232.0***	0.000
Cultivar * NaCl	1	0.002ns	0.975	0.3115*	0.031	0.0013ns	0.311	0.0004ns	0.117	0.000600	0.625	0.0625ns	0.675	0.4669ns	0.184	14.062ns	0.055
Cultivar * $ZnSO_4$	2	10.4578*	0.013	0.516**	0.001	0.028***	0.000	0.00067*	0.355	0.004883	0.159	0.1836ns	0.595	0.2086ns	0.446	0.1319ns	0.963
NaCl * $ZnSO_4$	2	8.83364*	0.023	0.334**	0.009	0.00680*	0.011	0.00061*	0.047	0.04596	0.000	0.3469ns	0.382	0.7252ns	0.074	14.465*	0.028
Cultivar*NaCl*$ZnSO_4$	2	22.11***	0.004	0.456**	0.003	0.0015ns	0.311	0.0002ns	0.250	0.00143	0.564	0.2858ns	0.449	0.2086ns	0.446	14.0208*	0.030
Error	24	1.9991<-		0.059<-		0.0012<-		0.0001<-		0.0024<-		0.3461<-		0.2502<-		3.4513<-	
Source of variation	**df**	**Shoot length**	**p-value**	**Root length**	**p-value**	**Chl a**	**p-value**	**Chl b**	**p-value**	**RMP**	**p-value**	**Total soluble proteins**	**p-value**	**POD**	**p-value**	**APX**	**p-value**
Cultivar	1	39.69*	0.016	0.613ns	0.512	0.0001ns	0.665	0.002***	0.002	9.5069ns	0.177	0.013***	0.001	4.40726*	0.015	0.00191*	0.036
NaCl	1	129.2***	0.001	0.034ns	0.877	0.003***	0.000	0.011***	0	423.6***	0.000	0.105***	0.000	177.3***	0.000	0.125***	0.000
ZnSO4	2	862.7***	0.000	107***	0.000	0.002***	0.000	0.0007**	0.003	224.4***	0.000	0.063***	0.000	20.97***	0.000	0.019***	0.000
Cultivar * NaCl	1	14.44ns	0.130	0.122ns	0.769	0.0001ns	0.215	0.00054*	0.027	0.0625ns	0.911	0.0002ns	0.063	3.8262*	0.023	0.0050**	0.001
Cultivar * $ZnSO_4$	2	124.9***	0.000	0.055ns	0.960	0.0001ns	0.914	0.0001ns	0.415	10.131ns	0.149	0.0009ns	0.354	0.5469ns	0.444	0.0004ns	0.383
NaCl * $ZnSO_4$	2	93.51***	0.000	14.3***	0.001	0.0001ns	0.882	0.0000ns	0.685	10.548ns	0.139	0.0019ns	0.149	0.1225ns	0.829	0.00178*	0.207
Cultivar*NaCl*$ZnSO_4$	2	66.69***	0.000	5.9908*	0.025	0.00012*	0.484	0.0000ns	0.966	9.5208ns	0.166	0.0006ns	0.545	0.5534ns	0.439	0.00005n	0.877
Error	24	5.8802<-		1.383<-		0.0000<-		0.0009<-		4.9236<-		0.0009<-		0.0009<-		0.0004<-	
Source of variation	**df**	**CAT**	**p-value**	**H_2O_2**	**p-value**	**MDA**	**p-value**	**Na^+ (shoot)**	**p-value**	**Na^+ (root)**	**p-value**	**K^+ (shoot)**	**p-value**	**K^+ (root)**	**p-value**		
Cultivar	1	0.587***	0.004	0.62***	0.000	1.113***	0.000	1ns	0.818	306.2***	0.001	69.444ns	0.142	169ns	0.336		
NaCl	1	9.088***	0.000	0.89***	0.000	10.41***	0.000	4298***	0.000	1534***	0.000	3881***	0.000	71645**	0.000		
ZnSO4	2	19.26***	0.000	1.04***	0.000	0.531**	0.001	140.58**	0.003	88.3611*	0.028	14800**	0.000	12151**	0.000		
Cultivar * NaCl	1	3.310***	0.000	0.0779*	0.011	0.0277ns	0.497	53.777ns	0.103	240.25**	0.003	1681***	0.000	2116 **	0.002		
Cultivar * $ZnSO_4$	2	0.332***	0.000	0.015ns	0.255	0.27821*	0.018	30.583ns	0.215	27.583ns	0.291	2302***	0.000	362.33ns	0.148		
NaCl * $ZnSO_4$	2	2.251***	0.000	0.008ns	0.480	0.0150ns	0.775	93.5277*	0.015	304.3***	0.000	2.3333ns	0.926	6874***	0.000		
Cultivar*NaCl*$ZnSO_4$	2	0.748***	0.000	0.009ns	0.419	0.4206**	0.004	11.861ns	0.538	308.5***	0.000	1616.***	0.000	1083**	0.006		
Error	24	0.0342<-		0.010<-		0.0585<-		18.666<-		21.222<-		30.111<-		175.78<-			