

# Alleviative Effects of Zinc on Biomass Yield and Antioxidative Enzymes Activity in Leaves of Soybean (*Glycine max* L.) Under Salt Stress

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## To cite this article:

Sadia Afrin, Nahid Akhtar, Tahmina Khanam, Feroza Hossain. Alleviative Effects of Zinc on Biomass Yield and Antioxidative Enzymes Activity in Leaves of Soybean (*Glycine max* L.) Under Salt Stress. *American Journal of Agriculture and Forestry*.

Vol. 9, No. 3, 2021, pp. 147-155. doi: 10.11648/j.ajaf.20210903.18

Received: April 30, 2021; Accepted: May 25, 2021; Published: June 7, 2021

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**Abstract:** The present study was conducted to determine the interaction effects of zinc availability and salt stress in Bangladeshi soybean cultivar (cv. Shohag) whether zinc can alleviate the hazardous effects of salt stress or not. In this study, the plants are grown in zinc treated soil and also exposed to increasing (0, 50, 100, 150, 200, and 250 mM NaCl) levels of salinity. The results showed that the dry weight of root, stem, leaves, petioles and total dry weight were significantly reduced by salinity. The activities of antioxidant enzymes, lipid peroxidation, proline content were significantly affected by salt stress. Zinc supplementation helped the plants to cope with the salinity stress by improving the total dry weight. The antioxidant enzyme activities including catalase (CAT) and ascorbate peroxidase (APX) and proline content increased in response to salinity. The extent of lipid peroxidation noticed in salt stressed plants. However, zinc application enhanced catalase and ascorbate peroxidase activity as well as proline content in growing plants at different salt concentrations. The interaction between zinc and salinity significantly reduced lipid peroxidation. Application of zinc to salt-stressed plants ameliorates the salinity induced hazardous effects by enhancing the activities of antioxidant enzymes such as CAT and APX and Proline content.

**Keywords:** Soybean, Salinity, Zinc, Biomass, Antioxidant Enzyme, Lipid Peroxidation

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## 1. Introduction

Among other natural disasters, salinity is one of the most pernicious abiotic stresses which reduces crop production worldwide. Salinity causes ion toxicities, osmotic stress, ionic imbalance, oxidative stress, genotoxicity which limits normal growth and development of plants by diminishing cell division and expansion, reducing photosynthetic efficiency, modifying metabolic processes [1–7]. High level of salinity induces reactive oxygen species (ROS) generation in plant cell [8, 9]. ROS formation takes place in chloroplasts, mitochondria, peroxisomes, plasma membrane of plant cells. [10–12]. These ROS can act as signaling molecules, mediating many key physiological processes. But overproduction of ROS causing damages to proteins, lipids, and eventually leading to cell death [5, 13, 14]. Therefore, plants possess various defense mechanisms including

enzymatic and non-enzymatic defense systems to combat the harmful effects of ROS. Protection against oxidative stress is mediated by an enzymatic antioxidant mechanism that includes catalase (CAT), superoxide dismutase (SOD), peroxidase (POX) and enzymes of the ascorbate-glutathione cycle as ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) [15–17] and non-enzymatic antioxidants such as phenolics, flavonoids, tocopherol, ASC and GSH [18–20]. In plants, superoxide dismutase scavenges superoxide anions and converts them to hydrogen peroxide [21, 22]. Catalase is the second line of defense which converts lethal hydrogen peroxide to water and molecular oxygen [23]. Another versatile antioxidant enzyme is ascorbate peroxidase which makes use of ascorbate (AsA) as electron donor and scavenges H<sub>2</sub>O<sub>2</sub>. Ascorbate peroxidase and catalase both are H<sub>2</sub>O<sub>2</sub> scavengers, where increase APX activity can overcome

salinity-induced reduction in CAT activity [13, 24]. The combined activities of the multiple forms of these enzymes in different subcellular compartments adjust ROS production rates and elimination rates, while maintaining  $\text{H}_2\text{O}_2$  at the levels required for cell signaling [23]. It was reported that an overaccumulation of  $\text{H}_2\text{O}_2$  enhance malondialdehyde (MDA) content assuring salinity induced oxidative stress condition in plant [25–27]. Evidence suggested that when plants are exposed to abiotic stress, the oxidative cell damage in plants is controlled by the ability of the antioxidative systems to function [28].

Proline accumulation is one of the most common adaptive mechanism in plant under salinity stress. It was stated that elevated salt tolerance is associated with the high level of endogenous proline accumulation in plants [29–32]. This metabolite has a plenty of defensive properties, including stabilizing of cellular structure and decrease in harm of photosynthetic device, redox status balance, cytosolic pH upkeep, stabilization of protein structure [33]. The reason of high proline content is the enhanced activities of ornithine amino transferase (OAT) and pyrroline -5-carboxylate reductase (P-5-CR), the enzymes involved in proline biosynthesis [34] as well as the inhibition of proline oxidase, proline dehydrogenase (PDH), proline catabolizing enzymes [35].

Now-a-days researchers have adopted different strategies to improve crop production and quality. For instance, micronutrient supplementation at required levels maintains plant growth and enhances crop yield by limiting the detrimental effects of stressors [36–38]. However, Zn is considered an essential plant nutrient, which plays an integral part in the formation of chlorophyll, protein, lipid, carbohydrate, enzymes cofactor (DNA and RNA polymerase) and the action of hormones. [39]. Zinc is the main constituents of zinc finger and this protein acts as a structural motif crucial for DNA binding [40].

Soybean is mostly cultivated as oil yielding crop throughout the world that often suffers from salinity problem. It is salt sensitive crop. Global crop production is hampered due to the increase levels of salinity in large arable land. Zinc plays a vital role in protecting critical cellular components such as chlorophyll content by preventing their oxidation [41, 42] and IAA metabolism [43]. Several workers concluded that noxious effects of  $\text{Na}^+$  and  $\text{Cl}^-$  ion can be alleviated by supplementing the soil with zinc [8, 14, 44–48]. Thus, this study was conducted with the aims of evaluating the effects of zinc on growth and the changes in antioxidant enzymes in soybean in response to salinity.

## 2. Materials and Methods

### 2.1. Plant Materials and Treatments

The experiments were conducted in 2019 at Plant Physiology and Biochemistry Laboratory of Botany Department, Jahangirnagar University, Savar, Dhaka, Bangladesh. For this experiment certified seeds of soybean (cv. Shohag) were collected from Bangladesh Agricultural Research Institute

(BARI). The soil samples were dried and crushed and sufficient water was added to saturate the soil. 10 mg/kg zinc ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ) was mixed thoroughly with soil, and pots were filled with zinc treated soil. The seeds were surface sterilized with 0.1% sodium hypochlorite for 3 minutes followed by washing 3 times with distilled water. The experiment was carried out in randomized block design with three replications. There were five treatments at different concentrations for the experiment viz 50, 100, 150, 200 and 250 mM NaCl, distilled water considered as control and the cultivar were also exposed to salinity stress along with zinc sulfate. Seeds of uniform size were directly sown on 4<sup>th</sup> February 2019. Distilled water was applied in all pots up to the emergence of seedling. After seedling establishment distilled water in control pots and 12.5 mM NaCl solution were applied in salt treatment. When the first leaf appeared, actual amount of NaCl solution were applied. The salt solutions were applied till maturity. The plants were kept under natural sunshine till harvesting. After 120 days plants were harvested, and leaves, petioles, stems and roots were separated to determine their dry weight.

### 2.2. Enzyme Assays

For the measurement of the activity of CAT, the leaf samples (100 mg FW) were homogenized to a fine powder with a mortar and pestle. Subsequently, the leaf samples were added to 1ml of extraction buffer containing 50 mM potassium phosphate (pH 7.8) and 1 mM Na-EDTA, and 20 mg of PVPP. The crude homogenate was centrifuged at 15,000 rpm for 10 minutes at 4°C. The supernatant of the sample was used in the assay of the activity of CAT. For the measurement of the activity of CAT the supernatant was applied to PD-10 desalting column filled with sephadex G-25 to desalt the supernatant. The activity of CAT was determined by the method of Aebi [49]. The supernatant (20  $\mu\text{l}$ ) was added to 970  $\mu\text{l}$  of 50 mM potassium phosphate buffer (pH 7.8) containing 1 mM Na-EDTA. After the measurement of blank solution at 240 nm for 1 minute with the spectrophotometer (ROTINA 420R), 10  $\mu\text{l}$  of 880 mM  $\text{H}_2\text{O}_2$  was added. Then the absorbance of the sample solution was measured for 2 minutes at 240 nm with the spectrophotometer (ROTINA 420R). The activity of CAT was calculated using the extinction coefficient,  $0.04 \text{ mM}^{-1} \text{ cm}^{-1}$ . In the present study, CAT activity was expressed as  $\mu\text{kat}$  of  $\text{H}_2\text{O}_2 \text{ m}^{-2}$ .

For the measurement of the activity of APX, leaf samples (50 mg FW) were homogenized to a fine powder with a mortar and pestle. Subsequently, leaf samples were added to 0.5 ml of extraction buffer containing 50 mM potassium phosphate (pH 7.0), 1 mM Na-EDTA and 5 mM L-ascorbic acid, and 10 mg of PVPP. The crude homogenate was centrifuged at 15000 rpm for 10 minutes at 4°C. The supernatant of the sample solution was used in the assay of the activity of APX. The activity of APX was determined by the method of Nakano and Asada [50]. The supernatant (20  $\mu\text{l}$ ) was added to 910  $\mu\text{l}$  of 50 mM potassium phosphate buffer (pH 7.0) and 50  $\mu\text{l}$  of 5 mM L-ascorbic acid solution. After the measurement of blank solution at 290 nm for 1 minute with the spectrophotometer (ROTINA 420R), 20  $\mu\text{l}$  of 50 mM  $\text{H}_2\text{O}_2$  was added. Then the

absorbance of the sample solution was measured for 2 minutes at 290 nm with the spectrophotometer (ROTINA 420R). The activity of APX was calculated using the extinction coefficient,  $2.8 \text{ mM}^{-1} \text{ cm}^{-1}$ . In the present study, APX activity was expressed as  $\text{nkat of ascorbate m}^{-2}$ .

### 2.3. Lipid Peroxidation

Lipid peroxidation was determined by estimating the malondialdehyde (MDA) content according to Health and Packer [51]. Leaf samples (50 mg FW) was homogenized in 0.1ml of 0.05% BHT and 0.9 ml of 20% TCA and 10 mg of PVPP. The crude homogenate was centrifuged at 15,000 g for 10 minutes at 4°C. The supernatant of the sample (400  $\mu\text{l}$ ) was added to 1.6 ml of 0.5% TBA and was incubated at 95°C for 30 minutes. The reaction was stopped by placing the test tubes in an ice bath. The samples were then centrifuged at 15,000 g for 5 minutes at 4°C. The absorbance of the sample solution was measured at 600 nm and 532 nm with the spectrophotometer (ROTINA 420R). The amount of MDA was calculated using the extraction coefficient of  $155 \text{ mM}^{-1} \text{ cm}^{-1}$ .

### 2.4. Proline Content

Leaf samples were collected from the three soybean plants of each treatment in each of the three replications for the experiment. Extraction procedure and colorimetric

determination with acidic ninhydrin reagent (2.5 gm ninhydrin/100 ml of solution containing glacial acetic acid, distilled water and orthophosphoric acid 85% at a ratio of 6: 3: 1) were carried out. Samples of 0.25 gm leaf fresh weight were grinded in a mortar after the addition of a small amount (5ml) of quartz sand and lome of 3% (w/v) aqueous sulfosalicylic acid solution. The homogenate was filtered through 2 layers of glass fiber filter and the clear filtrate was then used in the assay. Glacial acetic acid and ninhydrin reagent (1ml each) were added to 1ml of the filtrate. The closed test tubes with the reaction mixture were kept in a boiling water bath for 1hour and the reaction was terminated in a water bath of room temperature (21°C) for 5 min. Reading were taken immediately at a wave length of 546 nm. The proline concentration was determined from a standard curve and calculated on a fresh weight basis.

### 2.5. Statistical Analysis

The experiment was arranged out in randomized block design with three replications. Statistical analysis of the collected data were performed using Analysis of Variance (ANOVA). All statistical analysis were performed with the SPSS statistical package (SPSS program 16.00). Data were presented as means for each treatment and means were compared using the Duncan test at the 5% probability level.

**Table 1.** Effects of zinc on dry matter production of soybean cv Shohag under salinity.

Treatments	Root dry weight (gm/plant)	Stem dry weight (gm/plant)	Leaf dry weight (gm/plant)	Petiole dry weight (gm/plant)	Total dry weight (gm/plant)	Root/shoot ratio
0 mM NaCl	$2.33 \pm 0.22$ a	$4.92 \pm 0.62$ a	$1.24 \pm 0.34$ a	$0.023 \pm 0.012$ a	$8.51 \pm 0.46$ a	$0.38 \pm 0.03$ a
50 mM NaCl	$1.38 \pm 0.05$ b (59.23)	$2.51 \pm 0.77$ b (51.02)	$1.21 \pm 0.20$ ab (97.58)	$0.016 \pm 0.001$ ab (69.56)	$5.13 \pm 0.6$ b (60.28)	$0.37 \pm 0.04$ a (97.36)
50 mM NaCl + Zn	$1.71 \pm 0.08$ b (73.39)	$3.12 \pm 0.59$ b (63.41)	$1.49 \pm 0.22$ ab (120.16)	$0.024 \pm 0.004$ ab (104.35)	$6.34 \pm 0.77$ b (74.50)	$0.37 \pm 0.04$ a (97.37)
100 mM NaCl	$0.50 \pm 0.08$ c (21.46)	$1.84 \pm 0.36$ bc (37.39)	$0.98 \pm 0.13$ abc (79.03)	$0.014 \pm 0.018$ ab (63.67)	$3.34 \pm 0.39$ c (39.24)	$0.18 \pm 0.02$ b (47.37)
100 mM NaCl + Zn	$0.56 \pm 0.05$ c (24.03)	$2.84 \pm 0.76$ b (57.72)	$1.18 \pm 0.51$ abc (95.16)	$0.016 \pm 0.008$ abc (69.56)	$4.60 \pm 0.63$ c (54.05)	$0.14 \pm 0.01$ b (36.84)
150 mM NaCl	$0.29 \pm 0.04$ d (12.45)	$1.59 \pm 0.19$ c (32.31)	$0.87 \pm 0.14$ bcd (70.16)	$0.008 \pm 0.003$ ab (35.89)	$2.75 \pm 0.27$ cd (32.31)	$0.12 \pm 0.03$ b (31.58)
150 mM NaCl + Zn	$0.39 \pm 0.03$ d (16.74)	$2.19 \pm 0.57$ bc (44.51)	$0.94 \pm 0.24$ bcd (75.80)	$0.013 \pm 0.008$ bc (56.52)	$3.53 \pm 0.78$ d (41.48)	$0.13 \pm 0.04$ b (34.21)
200 mM NaCl	$0.23 \pm 0.02$ d (9.87)	$1.47 \pm 0.36$ c (29.88)	$0.69 \pm 0.17$ cd (55.64)	$0.007 \pm 0.004$ b (30.77)	$2.39 \pm 0.39$ de (28.08)	$0.11 \pm 0.02$ b (28.15)
200 mM NaCl + Zn	$0.25 \pm 0.09$ e (10.73)	$1.49 \pm 0.34$ c (30.28)	$0.79 \pm 0.22$ cd (63.71)	$0.013 \pm 0.004$ bc (56.52)	$2.52 \pm 0.40$ de (29.61)	$0.11 \pm 0.07$ b (28.94)
250 mM NaCl	$0.20 \pm 0.06$ d (8.58)	$1.19 \pm 0.21$ c (24.19)	$0.42 \pm 0.20$ d (33.87)	$0.005 \pm 0.001$ b (19.66)	$1.82 \pm 0.36$ e (21.39)	$0.13 \pm 0.07$ b (34.21)
250 mM NaCl + Zn	$0.22 \pm 0.05$ e (9.44)	$1.25 \pm 0.27$ c (25.40)	$0.47 \pm 0.13$ d (37.90)	$0.007 \pm 0.003$ c (30.43)	$1.93 \pm 0.24$ e (22.68)	$0.13 \pm 0.04$ b (34.21)

\* Average value of 3 plants in each treatment. \* Means in a column followed by the same letter do not differ significantly at 5% level and  $\pm$  means standard deviation \* Values within parenthesis indicate percentage relative to the control.

## 3. Results

### 3.1. Biomass

Dry matter production in roots decreased by 40.77%, 78.54%, 87.55%, 90.13% and 91.42% under salinity treatments of 50, 100, 150, 200 and 250 mM NaCl,

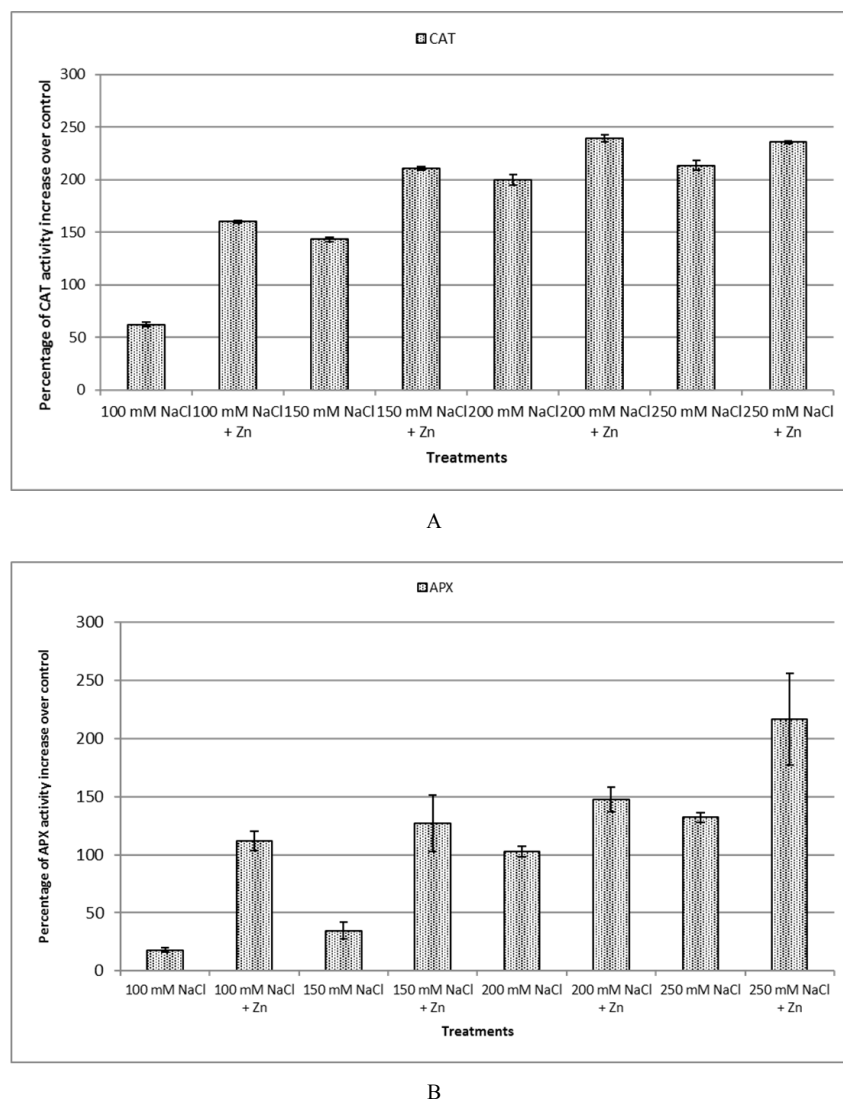
respectively (Table 1) (significant at  $<0.05$ ). Zinc application on NaCl stressed soybean plants increased the dry matter production in roots by 23.91%, 12.00%, 34.48%, 8.00% and 10.00% under salinity levels at 50, 100, 150, 200 and 250 mM respectively (Table 1). Dry weight of stems decreased by 48.98%, 62.61%, 67.69%, 70.12% and 75.81% under salinity treatments of 50, 100, 150, 200 and 250 mM NaCl,

respectively (Table 1) (significant at  $<0.05$ ). Whereas, zinc application on NaCl stressed soybean plants increased the dry weight of stems by 24.30%, 54.35%, 37.74%, 1.36% and 5.04% under salinity levels at 50, 100, 150, 200 and 250 mM respectively (Table 1). The dry weight of leaves decreased by 2.42%, 20.97%, 29.84%, 44.36% and 66.13% under salinity treatments of 50, 100, 150, 200 and 250 mM NaCl, respectively (Table 1) (significant at  $<0.05$ ). Zinc application on NaCl stressed soybean plants increased the dry weight of leaves by 28.00%, 20.41%, 8.04%, 14.49% and 11.90% under salinity levels at 50, 100, 150, 200 and 250 mM, respectively (Table 1). The dry weight of petioles decreased by 30.34%, 36.33%, 64.11%, 69.23% and 80.34% under salinity treatments of 50, 100, 150, 200 and 250 mM NaCl, respectively (Table 1) (significant at  $<0.05$ ). Zinc application on NaCl stressed soybean plants increased the dry weight of petioles by 50.00%, 14.29%, 62.50%, 85.71% and 40.00% under salinity levels at 50, 100, 150, 200 and 250 mM respectively (Table 1). Root/shoot ratio decreased by 2.64%, 52.63%, 68.42%, 71.85% and 65.79% under salinity treatments of 50, 100, 150, 200 and 250 mM NaCl,

respectively (Table 1) (significant at  $<0.05$ ). Results showed that root/shoot ratio was not significantly affected by zinc supplementation (Table 1).

### 3.2. Antioxidant Enzyme Activities

NaCl treatments increased CAT activity over the control with a maximal increase of 162.04%, 243.06%, 299.80% and 313.47% under salinity levels at 100, 150, 200 and 250 mM, respectively (Figure 1A) (significant at  $<0.05$ ). Zn application further increased CAT activity in salt stressed plants by 60.49%, 27.75%, 13.12% and 7.09% under salinity levels at 100, 150, 200 and 250 mM, respectively (Figure 1A). NaCl treatments increased APX activity over the control with a maximal increase of 117.99%, 134.67%, 202.64% and 232.19% under salinity levels at 100, 150, 200 and 250 mM respectively (Figure 1B) (significant at  $<0.05$ ). Zn application further increased APX activity in salt stressed plants by 79.36%, 68.61%, 14.59% and 36.45% under salinity levels at 100, 150, 200 and 250 mM respectively (Figure 1B).



**Figure 1.** Changes in catalase (CAT) (A) and ascorbate peroxidase (APX) (B) activities in leaves of soybean cultivar Shohag as affected by salinity and zinc.

### 3.3. Lipid Peroxidation

The oxidative damage was observed as malondialdehyde (MDA) content, which is a product of lipid peroxidation. Results showed that lipid peroxidation was significantly influenced by salt stress. MDA content was higher under saline conditions, compared with control. Salinity levels at 100, 150, 200, and 250 mM NaCl, caused 151.96%,

208.14%, 224.76% and 280.94% increase in leaf MDA content in non-zinc application plants, respectively (Figure 2) (significant at  $<0.05$ ). However, lipid peroxidation significantly reduced by 18.83%, 10.44%, 9.08% and 12.62% at 100, 150, 200, and 250 mM NaCl, compared with NaCl treatments without zinc application, respectively (Figure 2).

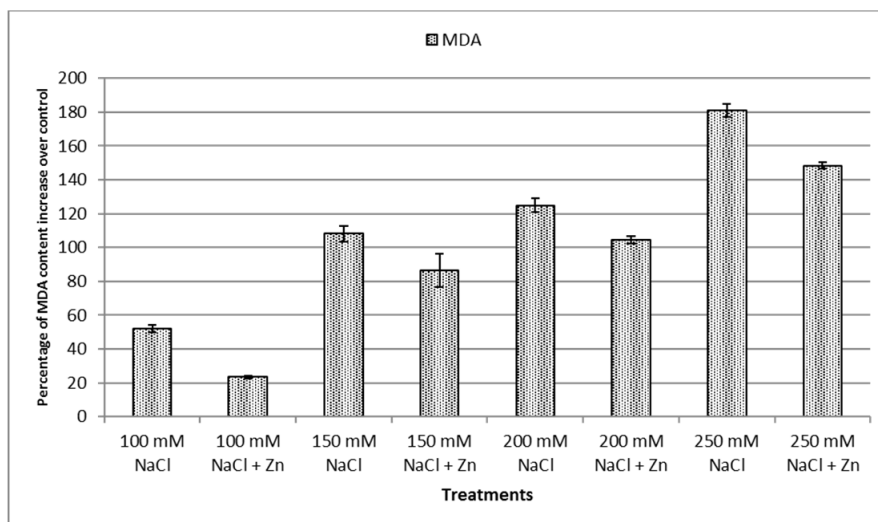


Figure 2. Changes in MDA content in leaves of soybean cultivar Shohag as affected by salinity and zinc.

### 3.4. Proline Content

The proline content in leaf tissue was significantly increased with increasing salinity. The free proline content was significantly enhanced in the stressed plants over control plants in both conditions (with and without zinc application). NaCl treatments increased proline content over the control

with a maximal increase of 315.02%, 404.05%, 461.81% and 568.68% under salinity levels at 100, 150, 200 and 250 mM respectively (Figure 3) (significant at  $<0.05$ ). However, with zinc application proline content significantly increased at 100, 150, 200 and 250 mM NaCl by 7.87%, 10.02%, 7.26% and 6.47% in leaves (Figure 3).

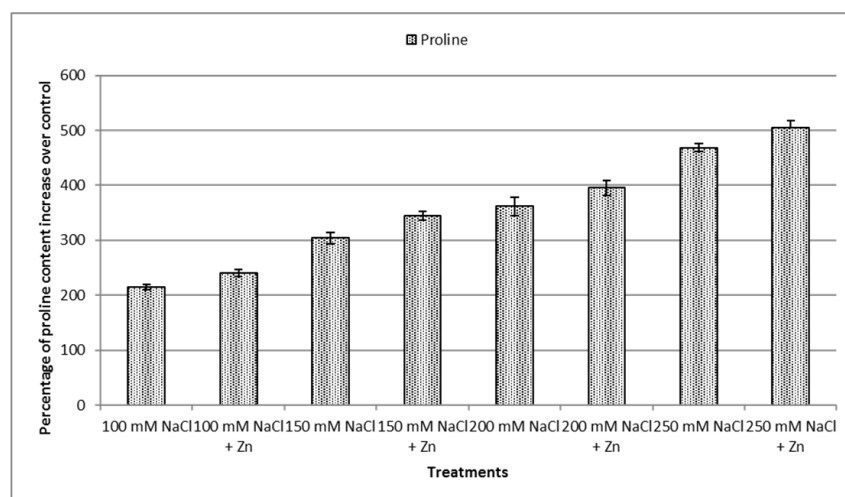


Figure 3. Changes in proline content in leaves of soybean cultivar Shohag as affected by salinity and zinc.

## 4. Discussion

In the present experiment, salinity caused a decrease in total dry weight of plants by reducing root DW, stem DW, petiole DW and leaf DW (Table 1). Khan *et al.* [52] observed

a decrease in the growth and biomass yield of soybean due to salinization. Higher salinity levels counteract the normal growth of plants by negatively affecting various physiological and biochemical processes such as photosynthesis, antioxidant enzyme activity, nitrogen

metabolism, ionic homeostasis [53, 54] osmotic stress and low absorption of essential elements [55], proline metabolism [56]. On contrary, the total dry weight of salt-affected soybean plants increased with zinc application. Weisany *et al.* [45] and Jiang *et al.* [48] stated that Zn could increase dry weight of soybean plants under both normal and salinity stress conditions. Zinc has positive effects on the uptake and partitioning of important mineral elements [46, 57]. It plays a protective role by maintaining structural integrity of plasma membrane [46], increasing the natural auxin synthesis, thereby activating the cell division [58], accumulating phospholipid [59], improving protein synthesis [60] and inhibiting excessive  $\text{Na}^+$  and  $\text{Cl}^-$  uptake [57, 61, 62].

Plants activate various antioxidant enzymes to mitigate the harmful effects of oxidative stress [23]. The enhancement of ROS production in saline plants induces an increase in antioxidant enzymes activity [4, 8, 40, 46]. In this study, salinity stress up-regulated the activities of CAT and APX in soybean plants. (Figure 1A and B). These results are analogous with earlier reports on, wheat and barley [63], mung bean [25], soybean [45], mustard [8]. Catalase is a tetramer containing heme group and helps in detoxification of ROS by converting 26 million of  $\text{H}_2\text{O}_2$  into water within a minute [64]. APX is the only enzyme that can scavenge  $\text{H}_2\text{O}_2$  in chloroplast while CAT is absent. Moreover, different isoforms of APX such as cytosolic APX (cAPX), mitochondrial APX (mtAPX), peroxisomal APX (pAPX) are  $\text{H}_2\text{O}_2$  scavengers involved in AsA-GSH cycle [65]. Abbaspour [66] reported that the increase in APX activity could be due to the high concentration of intracellular  $\text{H}_2\text{O}_2$  induced by salt stress. But zinc supplementation further increased CAT and APX activities under salinity stress (Figure 1A and B). Similar result was found by Weisany *et al.* [45] who suggested that Zn plays an important role in regulating the transmembrane redox homeostasis, thereby neutralizing the hazardous effects of ROS produced by oxidative stress by increasing activity of antioxidant enzymes. In contrast, reduction in catalase activity was observed in many plants species treated with zinc under salinity [8, 59]. Zn is indirectly required for a high activity of enzymes involved in  $\text{H}_2\text{O}_2$  detoxification such as CAT [2]. The use of zinc is believed to increase the activity of the enzyme APX. This may be due to zinc's ability to participate in the biosynthesis of antioxidant enzymes [67].

MDA concentrations is an indicator of oxidative stress which determine the extent of lipid peroxidation.  $\text{H}_2\text{O}_2$  induced membrane damage leads to acceleration of the Haber-Weiss reaction by generating hydroxyl free radicals, leading to increased lipid peroxidation [68]. Our study reported a significant increase in MDA content in leaves of soybean plants under the action of salt (Figure 2). The extent of lipid peroxidation occurs due to salt stress has been reported by several workers [27, 63, 69, 70]. However, consequences of an increase in  $\text{H}_2\text{O}_2$  are the overproduction of membrane fatty acids, the denaturation of proteins as well as impeding DNA integrity [71, 72]. Zinc plays an indispensable role in controlling the formation and detoxification of free oxygen

radicals following oxidation of lipid membranes [73]. Kawano *et al.* [74] concluded that  $\text{Zn}^{2+}$  have an inhibitory effects on membrane bound, NADPH oxidase. Results showed a significant decline in concentrations of MDA in leaves of soybean plants exposed to salt and zinc (Figure 2). These results are corresponding with previous research on soybean [45, 68], mustard [8], eggplant [59].

In our study, proline content was significantly enhanced in leaves of salt stressed soybean plants (Figure 3). Proline is considered as an osmolytes which contributes to osmotic adjustment, stabilizing subcellular structure, detoxifying free radicals as well as buffering cellular redox potential under salinity stress [68]. The findings are in accordance with other investigations [4, 33]. Proline's high accumulation aids in the maintenance of tissue water potential under stress conditions, accordingly offering an important resistance strategy [8, 36, 75, 76]. In saline plants, the activity of key enzymes involved in proline synthesis is increased, leading to an increase in proline [4]. In this study, the application of Zn increased the proline content of soybean plants under influence of salinity (Figure 3). Similar results were observed by other authors [8, 59, 77]. Zinc application induces a low availability of solutes, improves the accumulation of proline and allows plant cell to maintain a higher water content. Interestingly, many plants have lower proline content [45, 68]. According to Weisany *et al.* [45] the application of zinc in salted plants results in low proline content may be due to dilution effect.

## 5. Conclusions

Based on the study, it is concluded that salt stress adversely affects the biomass, antioxidant enzymes activities (CAT and APX), lipid peroxidation (MDA) and proline content in soybean plants. Zinc application to NaCl-stressed plants reduced lipid peroxidation and enhanced proline content and antioxidant enzymes activities. Thereby, zinc promotes plant growth under stress condition. Hence, it can be concluded that zinc could partially alleviate the adverse effects of salinity and provide better tolerance capacity to salt stress through osmotic adjustment as well as modulating the antioxidant enzymes activity and proline content.

## Abbreviations

APX- Ascorbate peroxidase; AsC- Ascorbic acid; BHT- Butylated hydroxytoluene; CAT- Catalase; DHAR- Dehydroascorbate reductase; GSH- Reduced glutathione;  $\text{H}_2\text{O}_2$ - Hydrogen peroxide; IAA- Indole-3-acetic acid; MDA- Malondialdehyde; MDHAR- Monodehydroascorbate reductase; POX- Peroxidase; PVPP- Polyvinyl pyrrolidone; ROS- Reactive oxygen species; SOD- Superoxide dismutase; TCA- Trichloroacetic acid.

## Acknowledgements

This study was supported by a grant from University Grants Commission, Bangladesh.

## References

- [1] N. Akhtar, F. Hossain, A. Karim, Influence of calcium on water relation of two cultivars of wheat under salt stress, *Int. J. Environ.* 2 (2013) 1–8. <https://doi.org/10.3126/ije.v2i1.9202>.
- [2] A. S. Wani, A. Ahmad, S. Hayat, Q. Fariduddin, Salt-induced modulation in growth, photosynthesis and antioxidant system in two varieties of *Brassica juncea*, *Saudi J. Biol. Sci.* 20 (2013) 183–193. <https://doi.org/10.1016/j.sjbs.2013.01.006>.
- [3] K. Yan, H. Shao, C. Shao, P. Chen, S. Zhao, M. Brestic, X. Chen, Physiological adaptive mechanisms of plants grown in saline soil and implications for sustainable saline agriculture in coastal zone, *Acta Physiol. Plant.* 35 (2013) 2867–2878. <https://doi.org/10.1007/s11738-013-1325-7>.
- [4] P. Ahmad, A. A. A. Latef, A. Hashem, E. F. Abd Allah, S. Guzel, L. S. P. Tran, Nitric oxide mitigates salt stress by regulating levels of osmolytes and antioxidant enzymes in chickpea, *Front. Plant Sci.* 7 (2016). <https://doi.org/10.3389/fpls.2016.00347>.
- [5] N. A. Anjum, A. Sofo, A. Scopa, A. Roychoudhury, S. S. Gill, M. Iqbal, A. S. Lukatkin, E. Pereira, A. C. Duarte, I. Ahmad, Lipids and proteins—major targets of oxidative modifications in abiotic stressed plants, *Environ. Sci. Pollut. Res.* 22 (2015) 4099–4121. <https://doi.org/10.1007/s11356-014-3917-1>.
- [6] M. Hussein, A. Embiale, A. Husen, I. M. Aref, M. Iqbal, Salinity-induced modulation of plant growth and photosynthetic parameters in faba bean (*Vicia faba*) cultivars, *Pakistan J. Bot.* 49 (2017) 867–877.
- [7] S. Rehman, G. Abbas, M. Shahid, M. Saqib, A. B. Umer Farooq, M. Hussain, B. Murtaza, M. Amjad, M. A. Naeem, A. Farooq, Effect of salinity on cadmium tolerance, ionic homeostasis and oxidative stress responses in conocarpus exposed to cadmium stress: Implications for phytoremediation, *Ecotoxicol. Environ. Saf.* 171 (2019) 146–153. <https://doi.org/10.1016/j.ecoenv.2018.12.077>.
- [8] P. Ahmad, M. A. Ahanger, M. N. Alyemeni, L. Wijaya, D. Egamberdieva, R. Bhardwaj, M. Ashraf, Zinc application mitigates the adverse effects of NaCl stress on mustard [*Brassica juncea* (L.) czern & coss] through modulating compatible organic solutes, antioxidant enzymes, and flavonoid content, *J. Plant Interact.* 12 (2017) 429–437. <https://doi.org/10.1080/17429145.2017.1385867>.
- [9] K. Nahar, M. Rahman, M. Hasanuzzaman, M. M. Alam, A. Rahman, T. Suzuki, M. Fujita, Physiological and biochemical mechanisms of spermine-induced cadmium stress tolerance in mung bean (*Vigna radiata* L.) seedlings, *Environ. Sci. Pollut. Res.* 23 (2016) 21206–21218. <https://doi.org/10.1007/s11356-016-7295-8>.
- [10] V. A. Dmitrieva, E. V. Tyutereva, O. V. Voitsekhovskaja, Singlet oxygen in plants: Generation, detection, and signaling roles, *Int. J. Mol. Sci.* 21 (2020) 3237. <https://doi.org/10.3390/ijms21093237>.
- [11] S. K. Kohli, K. Khanna, R. Bhardwaj, E. F. Abd Allah, P. Ahmad, F. J. Corpas, Assessment of subcellular ROS and NO metabolism in higher plants: Multifunctional signaling molecules, *Antioxidants.* 8 (2019) 641. <https://doi.org/10.3390/antiox8120641>.
- [12] G. Noctor, C. H. Foyer, Intracellular redox compartmentation and ROS-related communication in regulation and signaling, *Plant Physiol.* 171 (2016) 1581–1592. <https://doi.org/10.1104/pp.16.00346>.
- [13] S. S. Gill, N. Tuteja, Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants, *Plant Physiol. Biochem.* 48 (2010) 909–930. <https://doi.org/10.1016/j.plaphy.2010.08.016>.
- [14] A. H. Saeidnejad, Alleviative effects of zinc on physiological properties and antioxidants activity of maize plants under salinity stress, *Int. J. Agric. Crop Sci.* 5 (2013) 529–537.
- [15] C. H. Foyer, G. Noctor, Ascorbate and glutathione: The heart of the redox hub, *Plant Physiol.* 155 (2011) 2–18. <https://doi.org/10.1104/pp.110.167569>.
- [16] S. Chawla, S. Jain, V. Jain, Salinity induced oxidative stress and antioxidant system in salt-tolerant and salt-sensitive cultivars of rice (*Oryza sativa* L.), *J. Plant Biochem. Biotechnol.* 22 (2013) 27–34. <https://doi.org/10.1007/s13562-012-0107-4>.
- [17] H. AbdElgawad, G. Zinta, M. M. Hegab, R. Pandey, H. Asard, W. Abuelsoud, High salinity induces different oxidative stress and antioxidant responses in maize seedlings organs, *Front. Plant Sci.* 7 (2016) 1–11. <https://doi.org/10.3389/fpls.2016.00276>.
- [18] B. Gupta, B. Huang, Mechanism of salinity tolerance in plants: Physiological, biochemical, and molecular characterization, *Int. J. Genomics.* 2014 (2014). <https://doi.org/10.1155/2014/701596>.
- [19] Z. F. Rakhmankulova, E. V. Shuyskaya, A. V. Shcherbakov, V. V. Fedyaev, G. Y. Biktimerova, R. R. Khafisova, I. Y. Usmanov, Content of proline and flavonoids in the shoots of halophytes inhabiting the South Urals, *Russ. J. Plant Physiol.* 62 (2015) 71–79. <https://doi.org/10.1134/S1021443715010112>.
- [20] S. Talbi, M. C. Romero-Puertas, A. Hernández, L. Terrón, A. Ferchichi, L. M. Sandalio, Drought tolerance in a Saharian plant *Oudneya africana*: Role of antioxidant defences, *Environ. Exp. Bot.* 111 (2015) 114–126. <https://doi.org/10.1016/j.envexpbot.2014.11.004>.
- [21] R. Biczak, Quaternary ammonium salts with tetrafluoroborate anion: Phytotoxicity and oxidative stress in terrestrial plants, *J. Hazard. Mater.* 304 (2016) 173–185. <https://doi.org/10.1016/j.jhazmat.2015.10.055>.
- [22] L. A. del Río, F. J. Corpas, E. López-Huertas, J. M. Palma, Plant superoxide dismutases: Function under abiotic stress conditions, in: *Antioxidants Antioxid. Enzym. High. Plants*, Springer International Publishing, 2018: pp. 1–26. [https://doi.org/10.1007/978-3-319-75088-0\\_1](https://doi.org/10.1007/978-3-319-75088-0_1).
- [23] M. Hasanuzzaman, M. H. M. B. Bhuyan, F. Zulfikar, A. Raza, S. M. Mohsin, J. Al Mahmud, M. Fujita, V. Fotopoulos, Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator, *Antioxidants.* 9 (2020) 1–52. <https://doi.org/10.3390/antiox9080681>.
- [24] P. Ahmad, C. A. Jaleel, M. A. Salem, G. Nabi, S. Sharma, Roles of enzymatic and nonenzymatic antioxidants in plants during abiotic stress, *Crit. Rev. Biotechnol.* 30 (2010) 161–175. <https://doi.org/10.3109/07388550903524243>.

- [25] P. Ahmad, M. A. Ahanger, P. Alam, M. N. Alyemeni, L. Wijaya, S. Ali, M. Ashraf, Silicon (Si) supplementation alleviates NaCl toxicity in mung bean [*Vigna radiata* (L.) Wilczek] through the modifications of physio-biochemical attributes and key antioxidant enzymes, *J. Plant Growth Regul.* 38 (2019) 70–82. <https://doi.org/10.1007/s00344-018-9810-2>.
- [26] Y. W. Cheng, X. W. Kong, N. Wang, T. T. Wang, J. Chen, Z. Q. Shi, Thymol confers tolerance to salt stress by activating anti-oxidative defense and modulating Na<sup>+</sup> homeostasis in rice root, *Ecotoxicol. Environ. Saf.* 188 (2020) 109894. <https://doi.org/10.1016/j.ecoenv.2019.109894>.
- [27] M. A. Ahanger, R. A. Mir, M. N. Alyemeni, P. Ahmad, Combined effects of brassinosteroid and kinetin mitigates salinity stress in tomato through the modulation of antioxidant and osmolyte metabolism, *Plant Physiol. Biochem.* 147 (2020) 31–42. <https://doi.org/10.1016/j.plaphy.2019.12.007>.
- [28] T. Demiral, I. Türkan, Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance, *Environ. Exp. Bot.* 53 (2005) 247–257. <https://doi.org/10.1016/j.envexpbot.2004.03.017>.
- [29] M. Ashraf, M. R. Foolad, Roles of glycine betaine and proline in improving plant abiotic stress resistance, *Environ. Exp. Bot.* 59 (2007) 206–216. <https://doi.org/10.1016/j.envexpbot.2005.12.006>.
- [30] M. Boscaiu, C. Lull, J. Llinares, O. Vicente, H. Boira, Proline as a biochemical marker in relation to the ecology of two halophytic *Juncus* species, *J. Plant Ecol.* 6 (2013) 177–186. <https://doi.org/10.1093/jpe/rts017>.
- [31] S. Sripinyowanich, P. Klomsakul, B. Boonburapong, T. Bangyeekhun, T. Asami, H. Gu, T. Buaboocha, S. Chadchawan, Exogenous ABA induces salt tolerance in indica rice (*Oryza sativa* L.): The role of OsP5CS1 and OsP5CR gene expression during salt stress, *Environ. Exp. Bot.* 86 (2013) 94–105. <https://doi.org/10.1016/j.envexpbot.2010.01.009>.
- [32] M. M. F. Mansour, E. F. Ali, Evaluation of proline functions in saline conditions, *Phytochemistry*. 140 (2017) 52–68. <https://doi.org/10.1016/j.phytochem.2017.04.016>.
- [33] D. Hmidi, C. Abdelly, H. ur R. Athar, M. Ashraf, D. Messedi, Effect of salinity on osmotic adjustment, proline accumulation and possible role of ornithine-δ-aminotransferase in proline biosynthesis in *Cakile maritima*, *Physiol. Mol. Biol. Plants*. 24 (2018) 1017–1033. <https://doi.org/10.1007/s12298-018-0601-9>.
- [34] D. H. Kohl, J. J. Lin, G. Shearer, K. R. Schubert, Activities of the pentose phosphate pathway and enzymes of proline metabolism in legume root nodules, *Plant Physiol.* 94 (1990) 1258–1264. <https://doi.org/10.1104/pp.94.3.1258>.
- [35] R. P. Kandpal, C. S. Vaidyanathan, M. U. Kumar, K. S. K. Sastry, N. A. Rao, Alterations in the activities of the enzymes of proline metabolism in Ragi (*Eleusine coracana*) leaves during water stress, *J. Biosci.* 3 (1981) 361–370. <https://doi.org/10.1007/BF02702623>.
- [36] M. A. Ahanger, R. M. Agarwal, N. S. Tomar, M. Shrivastava, Potassium induces positive changes in nitrogen metabolism and antioxidant system of oat (*Avena sativa* L. cultivar Kent), *J. Plant Interact.* 10 (2015) 211–223. <https://doi.org/10.1080/17429145.2015.1056260>.
- [37] S. N. Siddiqui, S. Umar, M. Iqbal, Zinc-induced modulation of some biochemical parameters in a high- and a low-zinc-accumulating genotype of *Cicer arietinum* L. grown under Zn-deficient condition, *Protoplasma*. 252 (2015) 1335–1345. <https://doi.org/10.1007/s00709-015-0767-8>.
- [38] M. A. Ahanger, R. M. Agarwal, Salinity stress induced alterations in antioxidant metabolism and nitrogen assimilation in wheat (*Triticum aestivum* L.) as influenced by potassium supplementation, *Plant Physiol. Biochem.* 115 (2017) 449–460. <https://doi.org/10.1016/j.plaphy.2017.04.017>.
- [39] M. M. Hussein, N. H. Abou-Baker, The contribution of nano-zinc to alleviate salinity stress on cotton plants, *R. Soc. Open Sci.* 5 (2018). <https://doi.org/10.1098/rsos.171809>.
- [40] V. Tavallali, M. Rahemi, M. Maftoun, B. Panahi, S. Karimi, A. Ramezani, M. Vaezpour, Zinc influence and salt stress on photosynthesis, water relations, and carbonic anhydrase activity in Pistachio, *Sci. Hortic. (Amsterdam)*. 123 (2009) 272–279. <https://doi.org/10.1016/j.scienta.2009.09.006>.
- [41] J. Cherif, N. Derbel, M. Nakkach, H. von Bergmann, F. Jemal, Z. Ben Lakhdar, Analysis of in vivo chlorophyll fluorescence spectra to monitor physiological state of tomato plants growing under zinc stress, *J. Photochem. Photobiol. B Biol.* 101 (2010) 332–339. <https://doi.org/10.1016/j.jphotobiol.2010.08.005>.
- [42] M. P. Zago, P. I. Oteiza, The antioxidant properties of zinc: Interactions with iron and antioxidants, *Free Radic. Biol. Med.* 31 (2001) 266–274. [https://doi.org/10.1016/S0891-5849\(01\)00583-4](https://doi.org/10.1016/S0891-5849(01)00583-4).
- [43] H. Marschner, Mineral Nutrition of Higher Plants, 2nd ed., Academic Press, London, 1995.
- [44] S. Torabian, M. Zahedi, A. Khoshgoftarmanesh, Effect of foliar spray of zinc oxide on some antioxidant enzymes activity of sunflower under salt stress, *J. Agric. Sci. Technol.* 18 (2016) 1013–1025.
- [45] W. Weisany, Y. Sohrabi, G. Heidari, A. Siosemardeh, K. Ghassemi-Golezani, Changes in antioxidant enzymes activity and plant performance by salinity stress and zinc application in soybean (*Glycine max* L.), *Plant Omics*. 5 (2012) 60–67.
- [46] W. Weisany, Y. Sohrabi, G. Heidari, A. Siosemardeh, H. Badakhshan, Effects of zinc application on growth, absorption and distribution of mineral nutrients under salinity stress in soybean (*Glycine max* L.), *J. Plant Nutr.* 37 (2014) 2255–2269. <https://doi.org/10.1080/01904167.2014.920386>.
- [47] S. Zafar, M. Y. Ashraf, S. Anwar, Q. Ali, A. Noman, Yield enhancement in wheat by soil and foliar fertilization of K and Zn under saline environment, *Soil Environ.* 35 (2016) 46–55.
- [48] W. Jiang, X. H. Sun, H. L. Xu, N. Mantri, H. F. Lu, Optimal concentration of zinc sulfate in foliar spray to alleviate salinity stress in *Glycine soja*, *J. Agric. Sci. Technol.* 16 (2014) 445–460.
- [49] H. Aebi, 3] Catalase *in Vitro*, *Methods Enzymol.* 105 (1984) 121–126. [https://doi.org/10.1016/S0076-6879\(84\)05016-3](https://doi.org/10.1016/S0076-6879(84)05016-3).
- [50] Y. Nakano, K. Asada, Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts, *Plant Cell Physiol.* 22 (1981) 867–880. <https://doi.org/10.1093/oxfordjournals.pcp.a076232>.



- [51] R. L. Heath, L. Packer, Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation, *Arch. Biochem. Biophys.* 125 (1968) 189–198. [https://doi.org/https://doi.org/10.1016/0003-9861\(68\)90654-1](https://doi.org/https://doi.org/10.1016/0003-9861(68)90654-1).
- [52] M. S. A. Khan, M. A. Karim, M. M. Haque, M. M. Islam, A. J. M. S. Karim, M. A. K. Mian, Influence of salt and water stress on growth and yield of soybean genotypes, *Pertanika J. Trop. Agric. Sci.* 39 (2016) 167–180.
- [53] N. Misra, A. K. Gupta, U. N. Dwivedi, Changes in free amino acids and stress protein synthesis in two genotypes of green gram under salt stress, *J. Plant Sci.* 5 (2010) 75–85. <https://doi.org/10.3923/jps.2006.56.66>.
- [54] M. Ashraf, Some important physiological selection criteria for salt tolerance in plants, *Flora* 199 (2004) 361–376. <https://doi.org/10.1078/0367-2530-00165>.
- [55] H. Abeer, E. F. Abd-Allah, G. El-Didamony, S. Alwhibi Mona, D. Egamberdieva, P. Ahmad, Alleviation of adverse impact of salinity on faba bean (*Vicia faba* L.) by arbuscular mycorrhizal fungi, *Pakistan J. Bot.* 46 (2014) 1987–2003.
- [56] N. Misra, A. K. Gupta, Effect of salt stress on proline metabolism in two high yielding genotypes of green gram, *Plant Sci.* 169 (2005) 331–339. <https://doi.org/10.1016/j.plantsci.2005.02.013>.
- [57] W. Weisany, Y. Sohrabi, G. Heidari, A. Siosemardeh, K. Ghassemi-Golezani, Physiological responses of soybean (*Glycine max* L.) to zinc application under salinity stress, *Aust. J. Crop Sci.* 5 (2011) 1441–1447.
- [58] E. A. Ali, A. M. Mahmoud, Effect of foliar spray by different salicylic acid and zinc concentrations on seed yield and yield components of mungbean in sandy soil, *Asian J. Crop Sci.* 5 (2013) 33–40. <https://doi.org/10.3923/ajcs.2013.33.40>.
- [59] A. Galal, Exogenous application of zinc mitigates the deleterious effects in eggplant grown under salinity stress, *J. Plant Nutr.* 42 (2019) 915–927. <https://doi.org/10.1080/01904167.2019.1584221>.
- [60] E. Ebrahimian, A. Bybordi, Exogenous silicium and zinc increase antioxidant enzyme activity and alleviate salt stress in leaves of sunflower, *J. Food, Agric. Environ.* 9 (2011) 422–427.
- [61] P. Y. Yousuf, A. Ahmad, A. H. Ganie, M. Iqbal, Salt stress-induced modulations in the shoot proteome of *Brassica juncea* genotypes, *Environ. Sci. Pollut. Res.* 23 (2016) 2391–2401. <https://doi.org/10.1007/s11356-015-5441-3>.
- [62] P. Y. Yousuf, A. Ahmad, I. M. Aref, M. Ozturk, Hemant, A. H. Ganie, M. Iqbal, Salt-stress-responsive chloroplast proteins in *Brassica juncea* genotypes with contrasting salt tolerance and their quantitative PCR analysis, *Protoplasma* 253 (2016) 1565–1575. <https://doi.org/10.1007/s00709-015-0917-z>.
- [63] M. Zeeshan, M. Lu, S. Sehar, P. Holford, F. Wu, Comparison of biochemical, anatomical, morphological, and physiological responses to salinity stress in wheat and barley genotypes deferring in salinity tolerance, *Agronomy* 10 (2020) 1–15. <https://doi.org/10.3390/agronomy10010127>.
- [64] N. Mehla, V. Sindhi, D. Josula, P. Bisht, S. H. Wani, An introduction to antioxidants and their roles in plant stress tolerance, in: *React. Oxyg. Species Antioxid. Syst. Plants Role Regul. under Abiotic Stress*, Springer Singapore, 2017: pp. 1–23. [https://doi.org/10.1007/978-981-10-5254-5\\_1](https://doi.org/10.1007/978-981-10-5254-5_1).
- [65] S. Pandey, D. Fartyal, A. Agarwal, T. Shukla, D. James, T. Kaul, Y. K. Negi, S. Arora, M. K. Reddy, Abiotic stress tolerance in plants: Myriad roles of ascorbate peroxidase, *Front. Plant Sci.* 8 (2017) 581. <https://doi.org/10.3389/fpls.2017.00581>.
- [66] H. Abbaspour, Effect of salt stress on lipid peroxidation, antioxidative enzymes, and proline accumulation in Pistachio plants, *J. Med. Plants Res.* 6 (2012) 526–529. <https://doi.org/10.5897/jmpr11.1449>.
- [67] A. Amiri, B. Baninasab, C. Ghobadi, A. H. Khoshgoftarmanesh, Zinc soil application enhances photosynthetic capacity and antioxidant enzyme activities in almond seedlings affected by salinity stress, *Photosynthetica* 54 (2016) 267–274. <https://doi.org/10.1007/s11099-016-0078-0>.
- [68] M. R. Sofy, Effects of gibberellic acid, paclobutrazol and zinc on growth, physiological attributes and the antioxidant defence system of soybean (*Glycine max*) under salinity stress, *Int. J. Plant Res.* 6 (2016) 64–87.
- [69] K. A. Abdelaal, L. M. EL-Maghraby, H. Elansary, Y. M. Hafez, E. I. Ibrahim, M. El-Banna, M. El-Esawi, A. Elkelish, Treatment of sweet pepper with stress tolerance-inducing compounds alleviates salinity stress oxidative damage by mediating the physio-biochemical activities and antioxidant systems, *Agronomy* 10 (2020). <https://doi.org/10.3390/agronomy10010026>.
- [70] I. Lalarukh, M. Shahbaz, Response of antioxidants and lipid peroxidation to exogenous application of alpha-tocopherol in sunflower (*Helianthus annuus* L.) under salt stress, *Pakistan J. Bot.* 52 (2020) 75–83. [https://doi.org/10.30848/PJB2020-1\(41\)](https://doi.org/10.30848/PJB2020-1(41)).
- [71] N. Tuteja, P. Ahmad, B. B. Panda, R. Tuteja, Genotoxic stress in plants: Shedding light on DNA damage, repair and DNA repair helicases, *Mutat. Res. - Rev. Mutat. Res.* 681 (2009) 134–149. <https://doi.org/10.1016/j.mrrev.2008.06.004>.
- [72] G. Habibi, Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) generation, scavenging and signaling in plants, in: *Oxidative Damage to Plants Antioxid. Networks Signal.*, Elsevier Inc., 2014: pp. 557–584. <https://doi.org/10.1016/B978-0-12-799963-0.00019-8>.
- [73] B. J. Alloway, Zinc in soils and crop nutrition. IZA, Brussels, Belgium and IFA, Paris, France, 2008.
- [74] T. Kawano, N. Kawano, S. Muto, F. Lapeyrie, Retardation and inhibition of the cation-induced superoxide generation in BY-2 tobacco cell suspension culture by Zn<sup>2+</sup> and Mn<sup>2+</sup>, *Physiol. Plant.* 114 (2002) 395–404. <https://doi.org/10.1034/j.1399-3054.2002.1140309.x>.
- [75] P. Ahmad, M. Sarwat, N. A. Bhat, M. R. Wani, A. G. Kazi, L. S. P. Tran, Alleviation of cadmium toxicity in *Brassica juncea* L. (Czern. & Coss.) by calcium application involves various physiological and biochemical strategies, *PLoS One* 10 (2015) e0114571. <https://doi.org/10.1371/journal.pone.0114571>.
- [76] P. Ahmad, A. Hashem, E. F. Abd-Allah, A. A. Alqarawi, R. John, D. Egamberdieva, S. Gucel, Role of *Trichoderma harzianum* in mitigating NaCl stress in Indian mustard (*Brassica juncea* L.) through antioxidative defense system, *Front. Plant Sci.* 6 (2015). <https://doi.org/10.3389/fpls.2015.00868>.
- [77] M. M. Aldinary, The protective effect of presoaking seed and foliar treatments on growth and metabolism of *Vigna sinensis* L. plants under salt stress condition, Al-Azhar University, 2015.