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ZINC PRIMING TRIGGERS OSMOREGULATION TO ENHANCING GROWTH OF SOYBEAN (*Glycine max* L.) UNDER SALINITY

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Abstract

Soil salinity is becoming an alarming issue in crop production and increasing trend with a disastrous effect in near future. An experiment was conducted following completely randomized design to investigate the responses of zinc (Zn) priming (0.5 and 1.0 mM $ZnSO4\cdot 7H_2O$) upon exposure to different salt concentrations (50, 100, and 150 mM NaCl) in mitigating salt-induced damages in soybean (*Glycine max* L.). Results evidenced that shoot and root length, stem diameter, number of branches, number of leaves, and shoot and root biomass was reduced in a dose-dependent manner compared to control seedling. In a contrary, Zn priming resulted in the improvement of the parameters, particularly at a lower dose of salt. Moreover, leaf reduced relative water content and proline content were revived in primed seed in comparison with non-primed stressed plants. These triggers soybean plants' tolerance to salt stress. It was concluded that priming seeds with lower concentration of Zn (0.5 mM) could alleviate the salt stress through improving plant growth characteristics, relative water content while decreasing proline content.

Introduction

Recently, it has been reported that worldwide approximately 20% of land under cultivation, as well as more than 30% of irrigated land, are being affected by salinity (FAO, 2019). Continuous malpractices in crop cultivation including poor irrigation and drainage system, improper application of mineral fertilizers, intrusion of wastewater or saltwater to cultivable land, and unyielding soil degradation can accentuate salt stress conditions in agricultural land. Under salt stress plants are exposed to both osmotic and ionic stress which starts disturbing from germination, changing plant morphological, physiological, and biochemical attributes and ultimately reducing crop yield and quality within a very short period of time (Hasanuzzaman *et al*., 2021).

Soybean is one of the most popular legumes due to its versatile usage and can withstand a wide array of environmental conditions but different types of adversities like salt stress, extreme temperatures, drought, metal/metalloid toxicity, imbalanced nutrition, ultraviolet radiation, ozone etc. often reduce the yield and quality of crop production (Hasanuzzaman *et al.*, 2022). Under salt stress, the morphological attributes like plant height, root and shoot fresh weight (FW) and dry weight (DW), leaf number, and branch number are seen to decrease notably in a dose-dependent manner (Sadak *et al*., 2020). In addition, the salient physiological and biochemical characteristics of soybean like chlorophyll contents, carotenoid contents, relative water content (RWC), antioxidant enzymes activities are hampered by an increased rate of proline (Pro) content due to imposition of salinity (Soliman *et al*., 2020; Rahman *et al*., 2021) which resulted loss in both yield and quality of soybean. However, different protective tactics such as using salt tolerant genotypes, seed priming, changing faulty agronomic practices, using stress elicitors etc. are now being applied to minimize the losses. In mitigation of salt stress of soybean, seed priming using micronutrient showed the satisfactory result with an improved plant growth characteristic and withstand better under stress, compared to control or no priming through enhancing antioxidant activities (Mangena *et al.*, 2020).

Zinc (Zn) is an important micronutrient that helps in maintaining the stability of cell membrane structure and functions as well. Moreover, Zn is proved to be advantageous over other treatments in the alleviation of potassium ion content in leaves, photosynthetic pigments, RWC, stomatal conductance, seed yield while lowering sodium ion (Na^+) content, malondialdehyde and electrolyte leakage percentage and subsequently increasing strong antioxidant defense activities to better withstand in water stress condition caused by salinity (Yusefi-Tanha *et al*., 2020; Osman *et al*., 2021). Though Zn is very beneficial in soybean cultivation and recorded to be a protective tool in crop salt stress resistance as a priming agent, very little work has been done to explore the performance of Zn priming in soybean under salt stress condition. The present study aimed to investigate the role of Zn priming in conferring soybean salt tolerance.

Materials and Methods

Seeds of soybean (cv. BARI Soybean-6) were collected from the Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh. The entire experiment was conducted with three replications following a completely randomized design (CRD). After collecting, mature, healthy, and uniform-sized seeds were sorted manually and selected for the priming treatment. Before priming, seeds were washed with distilled water (dH_2O) three times to remove adhering dusts. After then primed with two different concentrations of zinc sulfate ($ZnSO_4 \cdot 7H_2O$), viz. 0.5 and 1.0 mM Zn by immersing for 3 h in the dark at room temperature. Seeds were then washed again for three times with dH_2O and air-dried to obtain a safe moisture content (Al-Zahrani *et al.*, 2022). Both primed and non-primed seeds were sown in 14 L plastic pots (35 cm diameter) in well-prepared soil, mixed with organic matter, urea, triple superphosphate, muriate of potash, gypsum and boric acid as recommended by BARI (2019). At 21 days after sowing (DAS), salinity treatment was imposed on the sets of plants by irrigating with 50, 100, and 150 mM NaCl solution and a uniform field capacity was maintained up to 42 DAS by measuring moisture percentage of the soil with a moisture meter. Relevant morphological and physiological parameters were observed at 21 days after the initiation of salinity treatment.

Measurement of Growth Parameters

Growth parameters were obtained from the five randomly selected plants from each treatment at 42 DAS after 21 d later from the initiation of salt treatment. For measuring the shoot and root length, plants were uprooted and washed with running tap water to remove the adhering soil. Then shoot length was measured from the base to the tip of the uppermost leaf, whereas root length was also measured from the base to the longest branch of the root by using a measuring scale and expressed in centimeters (cm). By using a slide caliper stem diameter was measured from three different points of five randomly selected plants and expressed in millimeters (mm).

Primary branches were counted manually from five randomly selected plants and the values were averaged for determining the number of branches plant^{-1}. Similarly, fully expanded leaves were counted and averaged for the determination of the number of leaves plant^{-1}.

Determination of fresh weight and dry weight of shoot and roots

Five randomly selected plants were uprooted, washed with dH_2O and then roots were separated from shoots. After that, by using a digital balance fresh weight (FW) of roots and shoots was measured. Then sun-dried to reduce the initial moisture and followed by oven-drying for 72 h at 80 °C and weighed again for measuring dry weight (DW) and expressed as g plant^{-1}. The summation of FW and DW of root and shoot was considered as total FW and DW and expressed as g plant^{-1}. Parameters were taken at 21 d later from the imposition of salt stress at 42 DAS.

Estimation of Relative Water Content (RWC) of leaf

The procedure of Barrs and Weatherly (1962) was followed to estimate the RWC of the leaf at 42 DAS after 21 d of salt stress imposition. Immediately after plucking three randomly selected leaves lamina were weighed for the FW and then dipped in dH_2O for 24 h by covering with filter paper. The leaf lamina was weighed again after 24 h for the turgid weight (TW) by removing excess adhering water with blotter paper. These laminas were then oven-dried at 80 °C for 48 h and weighed for DW. After then, RWC of the leaf was calculated by using the following formula:

$$\text{RWC (\%)} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$$

Determination of Proline Content

Proline content was determined at 42 DAS (21 d of salt exposure) by following the method of Bates *et al*. (1973). A leaf sample of 0.5 g was homogenized with 3% sulfosalicylic acid and the homogenate was centrifuged for 12 min at 11,500 ×g. Then the 1 mL of aliquot was mixed with 1 mL glacial acetic acid and 1 mL acid ninhydrin solution in a falcon tube and heated at 100 °C in a water bath for 60 min. After cooling at room temperature, 5 mL toluene was added to the mixture and vortexed and kept for a while to separate a colored chromophore. Then the optical density of the chromophore was observed at 520 nm by using a spectrophotometer. Finally, the Pro content was estimated by plotting the absorbance values against a standard curve and was expressed as μmol g^{-1} FW.

Statistical analysis

Statistical analysis was done by using CoStat v.6.400 computer-based software (CoHort Software, Monterey, CA, USA) (CoStat, 2008). The mean values were compared by applying Tukey's HSD test and the value was considered as statistically significant at $p \leq 0.05$.

Results

Root length, Shoot length, and Stem diameter of Plants

Noticeable reduction of root length was observed by 29, 28, and 29% in 50, 100, and 150 mM NaCl stressed plants, respectively compared to the control plants (Figure 1A). Priming with 0.5 and 1.0 mM Zn improved root length by 41 and 32%, respectively under 50 mM salt-stressed plants in comparison to the corresponding salt-stressed plants only. But under 100 and 150 mM NaCl treated plants priming with Zn did not improve root length in comparison to the salt-stressed plants alone.

Upon exposure to 50, 100, and 150 mM NaCl, shoot length was declined by 22, 30, and 41%, respectively in comparison to the control plants (Figure 1B). When compared to the corresponding unprimed salt-stressed plants, 0.5 mM Zn priming improved shoot length by 31 and 20% in 50 and 150 mM NaCl stressed plants, respectively, whereas 1.0 mM Zn improved by 29 and 19%, respectively. Under 100 mM NaCl treated plants priming did not enhance shoot length compared to the corresponding salt-affected plants alone.

Similarly, stem diameter was also decreased by 17, 29, and 34% in salt-affected plants compared to the untreated control plants (Figure 1C). The highest increase in stem diameter was found by 13, 19, and 18% in plants under 50, 100, and 150 mM NaCl treated plants with 0.5 mM Zn priming, respectively in comparison to the corresponding salt-stressed plants without priming.

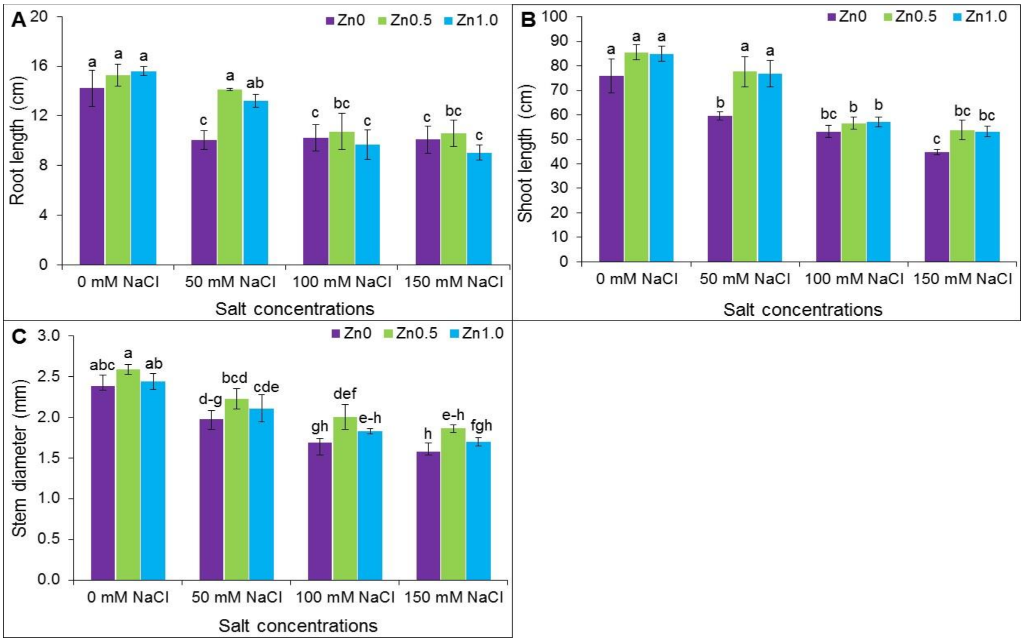

Fig. 1. Shoot length (A), root length (B), and stem diameter (C) of soybean as affected by different concentrations of NaCl and seed priming with zinc sulfate ($ZnSO_4 \cdot 7H_2O$). Denote Zn0.5 and Zn1.0 seed priming with 0.5 and 1.0 mM $ZnSO_4 \cdot 7H_2O$, respectively. Mean value (± SD) was calculated by $p \leq 0.05$ applying Tukey's HSD test.

Number of branches and leaves plant^{-1}

Salt stress significantly affects branch and leaf number of soybean plants. Branch number reduced by 57% in 50 mM, 70% in 100 mM and 82% in 150 mM, whereas leaf number was declined by 47% in 50 mM, 49% in 100 mM and 58% in 150 mM NaCl stressed plants in comparison to the control plants. Priming with 0.5 and 1.0 mM Zn increased branch number by 75 and 45%, and leaf number by 42 and 33% in 50 mM salt-affected plants compared to its corresponding unprimed salt-stressed plants only (Figure 2A, B).

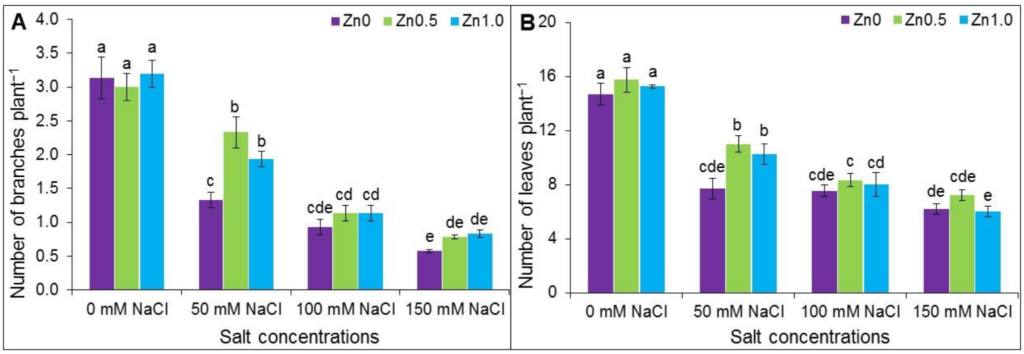

Fig. 2. Number of branches plant^{-1}(A) and the number of leaves plant^{-1}(B) of soybean as affected by different concentrations of NaCl and seed priming with zinc sulfate ($ZnSO_4 \cdot 7H_2O$). Denote Zn0.5 and Zn1.0 seed priming with 0.5 and 1.0 mM $ZnSO_4 \cdot 7H_2O$, respectively. Mean value (± SD) was calculated by $p \leq 0.05$ applying Tukey's HSD test.

Fresh and dry weight of roots and shoots

The highest reduction of root FW and DW was observed by 76 and 73% in 150 mM salt-affected plants, respectively compared to the control plants. Priming with 0.5 and 1.0 mM Zn in 50 mM NaCl conditions, improved root FW by 159 and 136%, and root DW by 147 and 140%, respectively in comparison to the corresponding salt-treated plants without priming. Under 100 and 150 mM salt stressed plants priming did not enhance root FW and DW compared to the salt-affected plants alone (Figure 3A, B).

Upon exposure to 50, 100, and 150 mM NaCl, shoot FW was declined by 60, 68, and 78%, and shoot DW was declined by 59, 68, and 78%, respectively compared to the control plants. Priming with Zn (0.5 mM) enhanced shoot FW by 51, 31, and 44%, and shoot DW by 65, 41, and 44% in 50, 100, and 150 mM NaCl treated plants in comparison to its corresponding salt-affected plants without priming. On a contrary, improvement of shoot FW (by 42%) and shoot DW (by 52%) were found in 50 mM salt stressed plants when priming was done with 1.0 mM Zn compared to the salt-stressed plants only (Figure 3C, D).

The highest reduction of total FW and DW was also found in salt-affected soybean plants. But priming with Zn enhanced biomass accumulation in salt-stressed plants compared to its salt-treated plant alone (Figure 3E, F).

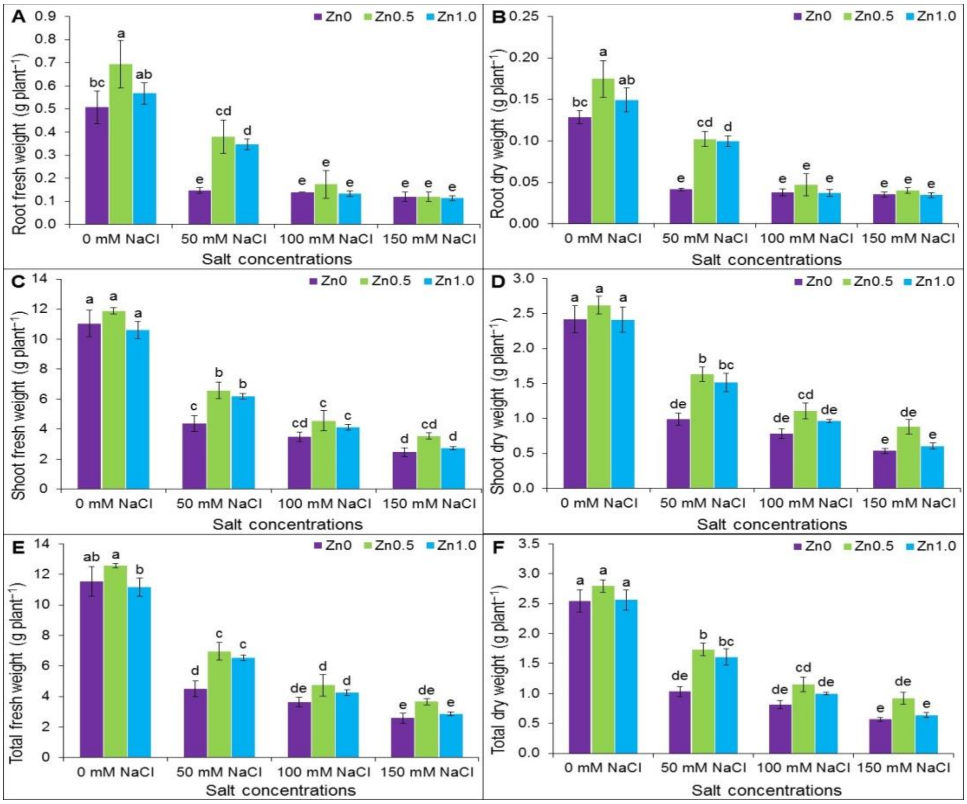

Fig. 3. Root fresh weight **(A),** root dry weight **(B)**, shoot fresh weight **(C)**, shoot dry weight **(D)**, total fresh weight **(E)**, and total dry weight **(F)** of soybean as affected by different concentrations of NaCl and seed priming with zinc sulfate ($ZnSO_4{\cdot}7H_2O$). Denote, Zn0.5 and Zn1.0 as seed priming with 0.5 and 1.0 mM $ZnSO_4{\cdot}7H_2O$, respectively. Mean value (± SD) was calculated at $p \leq 0.05$ applying Tukey's HSD test.

Relative Water Content and Proline Accumulation in Plants

Leaf RWC was decreased by 11, 13, and 18% in 50 mM, 100 mM, and 150 mM NaCl treated plants compared to the controls. Zinc priming at 0.5 and 1.0 mM increased RWC by 9, 8, and 7%, and by 5, 6, and 6% in 50, 100, and 150 mM salt-stressed plants, respectively compared to the corresponding unprimed salt-affected plants only (Figure 4A).

Proline accumulation was upgraded by 103% in 50 mM, 193% in 100 mM, and 333% in mM NaCl treated plants in comparison to its untreated controls plants. When compared to the corresponding salt-stressed but unprimed plants, Pro content reduced by 27, 38, and 14% with 0.5 mM Zn, and by 30, 25, and 7% with 1.0 mM Zn in plants treated with 50, 100, and 150 mM NaCl, respectively (Figure 4B).

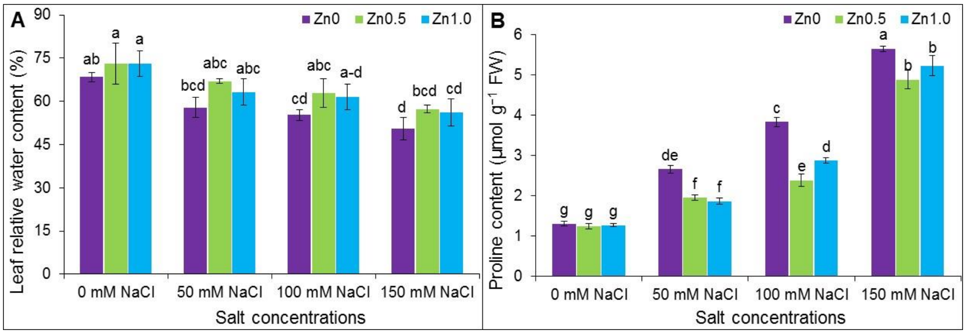

Fig. 4. Leaf relative water content (A) and proline content (B) of soybean as affected by different concentrations of NaCl and seed priming with zinc sulfate ($ZnSO_4{\cdot}7H_2O$). Denote, Zn0.5 and Zn1.0 as seed priming with 0.5 and 1.0 mM $ZnSO_4{\cdot}7H_2O$, respectively. Mean value (± SD) was calculated at $p \leq 0.05$ applying Tukey's HSD test.

Phenotypic Appearance

Phenotypic pictures of salt-affected soybean plants with/without Zn priming are shown in Figure 5. Growth was retarded when salt stress was imposed on soybean plants at different concentrations, priming with Zn improved plants growth compared to the corresponding unprimed salt-stressed plants (Figure 5).

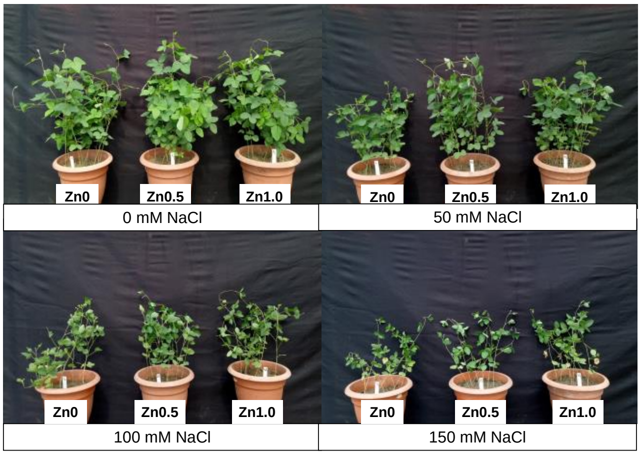

Fig. 5. Phenotypic appearance of soybean plants as affected by different concentrations of NaCl and seed priming with zinc sulfate ($ZnSO_4{\cdot}7H_2O$). Denote, Zn0.5 and Zn1.0 as seed priming with 0.5 and 1.0 mM $ZnSO_4{\cdot}7H_2O$, respectively.

Discussion

Seed priming protects plant from cell damage, elevates water status, dry matter production and stress tolerance with the desired yield under stress condition (Abdel Hamid *et al*., 2019).

A higher concentration of NaCl hinders uptake and translocation of both nutrients and water and leads to an altered cell formation and growth retardation which is a very common phenomenon in salt stressed plants (Hasanuzzaman *et al*., 2022). Upon exposure to salt stress, the growth attributes of soybean i.e. shoot and root length, stem diameter, branches and leaves number were reduced and the lowest parameters were recorded at higher salt concentrations (Figure 1, 2). These findings are in line with other studies on soybean (Sadak *et al*., 2020; Rahman *et al*., 2021) whereas it has been proved that the growth retardation of soybean increases with increasing salt concentrations. However, alleviation of these growth parameters was noticeable in seedlings pre-treated with Zn priming. Although both doses of priming helped in attenuating the loss, lower concentration of priming (0.5 mM $ZnSO_4{\cdot}7H_2O$) performed better in most of the parameters, the dose-dependent changes of priming are in accordance with another study (Soliman *et al*., 2020), whereas it was concluded that higher dose priming may show toxicity with an undesirable outcome.

The fresh and dry mass of above-ground and below-ground parts of soybean including the total plant FW and DW were decreased in a dose-dependent manner (Figure 3) and the higher doses (100 and 150 mM NaCl) exposure showed the lowest weight compared to control soybean. But the root structure was affected greatly more than other plant parts even under low (50 mM NaCl) salt concentrations. As the destructive Na^+ ion of salt stress is getting into the plant through the root system, it causes a huge reduction of root growth with a disturbed root structure (Silva *et al*., 2021). As Zn priming advantages plants in imbibition of nutrients during seed soaking, it may help in better performance compared to without priming. The positive effect of Zn priming under salt stress in growth parameters are also reported in other crops like maize (Imran *et al*., 2018) and wheat (Spańo *et al*., 2020).

When plants are imposed to higher salt concentrations, an excess amount of Na^+ and Cl^- ions are accumulated in root cells and restrain plants from water absorption through root zone and causes osmotic stress included with lower water potentiality (Munns and Tester, 2008) and ultimately results in reduced RWC.

Fig. 6. Possible changes of soybean by zinc priming under salt stress which indicates the salt stress tolerance ability of this priming.

Soybean without any salt stress showed a higher percentage of RWC and that was opposite to salt stressed plant and the lowest value was in the highest salt concentrations. Conversely, Zn primed soybean subjected to salt stress, exhibited comparatively higher leaf RWC than an unprimed seedling. When plants are treated with Zn, higher accumulation of organic osmolytes were recorded under salinity that ultimately resulted in higher turgor and water potentiality (Iqbal *et al*., 2018) and it might be the reason behind the uplifted RWC in primed seed. Similar results of salt stress mitigation in leaf RWC, through priming, has been recorded by Mangena (2020) and reported a positive association between soybean plant and priming with a better establishment against salt stress. Proline in plant cells act as an osmolyte that enhances protection against cellular membrane degradation and activates enzyme coherence under stress conditions (Suresh *et al*., 2017). So, enhancement of Pro content indicated a higher accumulation of organic solute in response to salt stress and the maximum was in higher NaCl accumulation whereas control soybean exhibited the lowest Pro content. Zinc priming in both concentrations diminished the rate of Pro content in this experiment which is not a common phenomenon but is supported by another study by Sheteiwy *et al.* (2020). This may be caused due to osmotic adjustment ability of this soybean variety under salt stress conditions. With the application of Zn, a possible reduction in ROS generated damage to plants lead to a better adaptability of the plant to salinity (Sofy *et al*., 2020).

In a nutshell, Zn priming can uphold the prime morphological and physiological attributes of soybean in salt stress conditions to some extent (Figure 6) and this phenomenon indicates the salt stress tolerance ability of this priming. Furthermore, lower concentration (0.5 mM) priming can give a satisfactory outcome even under higher salt concentrations compared to a higher level (1.0 mM) Zn priming.

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