MITIGATION OF SALT STRESS IN SOYBEAN PLANT AT VEGETATIVE STAGE BY EXOGENOUS APPLICATION OF ZINC

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*This is to certify that the thesis entitled **"MITIGATION OF SALT STRESS IN SOYBEAN PLANT AT VEGETATIVE STAGE BY EXOGENOUS APPLICATION OF ZINC"** submitted to the Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (MS) in AGRONOMY**, embodies the results of a piece of bonafide research work carried out by **Maliha Rahman Falguni,** Registration No. **14-06056** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that such help or source of information, as has been availed during the course of this investigation has been duly acknowledged and style of this thesis has been approved and recommended for submission.

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ABSTRACT

In this 20th century, due to global warming, several abiotic stresses (salinity, flooding, drought and metal/ metalloid toxicity) have evolved which drastically effect our environment and life. Among all abiotic stresses, salinity is now-a-days very drastic issue for our agriculture sector and poses a great threat on our food security which is an alarming issue in our world. Micronutrients application have proven to increase tolerance in plants under salt stress. Considering this issue, a pot experiment was carried out on soybean plant (BARI soybean 5) to investigate the salinity induced growth and physiological effect under salinity (5 and 10 dS m^{-1} NaCl) and the role of exogenous application (priming and foliar application) of zinc in reducing salinity in soybean plant. In this experiment, $ZnSO_4.7H_2O$ is used as a protectant against salinity. The experiment was conducted in a completely randomized design (CRD) with three replications consisting of nine treatments T_1: Control, T_2: Priming with 1 mM $ZnSO_4$. $7H_2O$, T_3: Foliar application of 0.5% $ZnSO_4.7H_2O$, T_4: 5 dS m^{-1} salinity, T_5: 5 dS m^{-1} salinity + priming with 1 mM $ZnSO_4.7H_2O$, T_6: 5 dS m^{-1} salinity + foliar application of 0.5% $ZnSO_4$. $7H_2O$, T_7: 10 dS m^{-1} salinity, T_8: 10 dS m^{-1} salinity + priming with 1 mM $ZnSO_4$. $7H_2O$ and T_9: 10 dS m^{-1} salinity + foliar application of 0.5 % $ZnSO_4$. $7H_2O$. After germinating seed, treatments were applied on plants in vegetative stage (20 DAS and 30 DAS). All the growth and physiological parameters of soybean plants were negatively affected by both saline conditions at vegetative stage. Zn priming and foliar spray showed the better performance in increasing plant height, root length, fresh and dry weight of root and shoot, chlorophyll content and leaf relative water content in vegetative stage under saline condition, compared to control. Exogenous application of Zn reduced lipid peroxidation (MDA), hydrogen peroxide and proline in saline affected plants, compared to plants under salt stress. This study indicates that Zn is an effective protectant in improving tolerance and increasing the growth and physiology of soybean plants under salt stress.

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LISTS OF ABBREVIATIONS

AsA	Ascorbic acid/ ascorbate
APX	Ascorbate peroxidase
BARI	Bangladesh Agricultural Research Institute
CAT	Catalase
Chl	Chlorophyll
cv.	Cultivar
DAS	Days after sowing
DW	Dry weight
EBL	24-epibrassinolide
et al.	*et alibi* (and others)
FAO	Food and Agriculture Organization
FW	Fresh weight
GR	Glutathione reductase
H_2O_2	Hydrogen peroxide
LSD	Least significant difference
MDA	Melondialdehyde
MEJA	Methyl jasmonate
$O_2^{\bullet -}$	Superoxide radical
POD	Peroxidase
Pro	Proline
ROS	Reactive oxygen species
RWC	Relative water content
SOD	Superoxide dismutase
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid

Chapter I

INTRODUCTION

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Among several abiotic stresses, salinity is a serious problem triggering drastic yield (Hasanuzzaman *et al*., 2016). Salinity affected areas are distributed all over the world, and it is estimated that 6% of world´ s total land area and one third of irrigated land are being harmed by varying degree of salt accumulation (Rahman *et al*., 2017). Increased level of salinity causes poor germination, retarded growth and reproducibility and negatively influences physiological process like photosynthesis, transpiration, respiration membrane properties and metabolic activities.

While many countries of the world are suffering from salt stress, Bangladesh is no exception. Salinity is one of significant concern for coastal areas in Bangladesh. It is estimated that 20% of total area of Bangladesh and 53% of seaside areas are hampered by saline condition. About 2.85 million hectares of arable land including 1.2 million hectares of seaside areas were hampered due to salinity. As a result of low land use, low food production, low yield and no food stability in the coastal areas are occurred by food insecurity (Akher *et al*., 2018).

All over the world, soybean is widely grown legume crop due to its cheap protein source, edible oil content, nitrogen fixing ability and forage properties (Sadak *et al*., 2020). In Bangladesh, total cultivated area of soybean was 59490 ha and total production was 98699 tons in our country (BBS 2017-2018).

Among various crops, legumes are more sensitive to salinity. Soybean, as a legume crop is very much affected by salinity (Shu *et al*., 2017; EL Sabagh *et al*., 2019). Nodulation and nitrogen fixing efficiency are affected by salinity and as a result, it reduces nodule production (EL Sabagh *et al*., 2019). As salinity affects more cultivable fields, soybean cultivation is under pressure. Salinity impeded growth of soybean seedlings by limiting synthesis and nutrient pool translocation and reduced soybean production by low germination and post-germinative growth.

Al though salinity is a complex global problem and cannot be mitigated simply. Plants develop some tolerance mechanisms like salt excretion, exclusion, vaculor transportion, changes in photosynthetic pathways to maintain their normal growth and physiology. Breeding salt tolerant varieties, agronomic management, supplementation with nutrients (seed priming, feeding with irrigation water, pre-treatment, foliar spray and injecting) and application of organic amendments etc. are remediation strategies against saline condition (Akher *et al*., 2018).

Among various strategies, supplementation with nutrients is very effective and practical solutions. From many previous studies, we found that micronutrients are proven to increase tolerance against salinity. Zn is required for Plant growth, metabolism and root cell membrane integrity (Said-Al Ahl *et al*., 2010). In crops, Zn deficiency is very frequent. Soybean production are affected by Zn deficiency and it is needed for chl formation, nodulation, growth hormone stimulation, enzymatic activity and methods of regeneration (Thenua *et al*., 2014; Sharifi *et al*., 2016). Zinc is able to scavenge ROS including radicles in the superoxide radicles ($O_2^{\bullet -}$) in the hydrogen peroxide (H_2O_2) under saline condition and alleviates effects of salinity on phyto-hormonal levels (Tavallali, 2016).

Zinc is applied to the plants in many ways. But due to low solubility in soil, exogenous application of Zn is preferred in many ways. Among various ways, priming is the most suitable, low-cost and effective method that enhances rapid and uniform emergence under saline condition and it also provides high vigorous plant and improved the performance in many field crops (Dai *et al*., 2017).

Zinc could be applied in various ways which include soil application of Zn, treatment of seedlings with Zn before transplanting and foliar spray of Zn. Among them, Foliar application is the most suitable and efficient method because small amount of Zn can be applied when required (Verma and Neue, 1984; Alpaslan *et al*., 1999; Yildirim *et al*., 2008; Tzortzakis, 2010). Low solubility of Zn is one of the causes of its deficiency in crops so that foliar application of Zn is most suitable method and several studies found that Exogenous application of Zn can increase crop yield (Sharifi *et al*., 2016). Foliar application of Zn showed better results in mustard, sunflower, maize and millets under salinity (Mahmoud, 2010; Ahmad *et al*., 2017; Wasaya *et al*., 2017; Hussein *et al*., 2018).

The present study is under taken to observe the role of exogenous application (priming and foliar application) of zinc sulfate on soybean seedlings growth under different levels of salinity. We have shown how morphological and physiological growth were regulated by zinc under saline condition.

This research has following objectives:

i. To investigate the salinity induced growth and physiological effects in soybean plant at vegetative stage
ii. To understand the role of zinc on soybean plant at vegetative stage in mitigating salinity
iii. To compare the role of priming and foliar application on soybean plant against salinity

Chapter II

REVIEW OF LITERATURE

2.1 Soybean

Soybean (*Glycine max*) is most widely used oil seed crops all over the world for its good source of protein and other nutritive values. Soybean has high source of vegetable proteins (40-50%) and oil content (20-30%). Soybean is a popular crop among developing countries not just as an oilseed crop but a source of essential fatty acids and secondary metabolites (Sabagh *et al*., 2019). In Bangladesh, total cultivated area of soybean was 59490 ha and total production was 98699 tons in our country (BBS 2017-2018).

2.2 Importance of soybean

Soybean is an annual oilseed crop of fabaceae family. It is popular oilseed crop among the world. Soybeans have a high protein and oil content. Soybean and other soy products like soybean meal is very popular around the world and is an important protein source for animal feed (Terzic *et al.*, 2019).

Soybean can fix atmospheric nitrogen through symbiotic nitrogen fixation. There are four well-known species of *Bradyrhizobium* include: *Bradyrhizobium japonicum*;.*Bradyrhizobium elkanii*; *Bradyrhizobium lianingense* and *Bradyrhizobium* sp.are nitrogen fixers and important for plant growth and seed production (Pagano and Miransari, 2015).

Due to heavy of use of chemical N fertilizer, the productivity of land is declining and also soil and water resources are destroyed slowly. The production of soybean can reduce the use of inorganic fertilizers and maintain the sustainable agriculture. In previous studies, we found that after maize cultivation, soybean cultivation under notill reduced soil erosion, fuel usage, insecticidal activity and carbon emissions (Pagano and Mriansari, 2015).

Soybean as a legume crop can act as a green manuring and cover crop to reduce soil erosion (Hasanuzzaman *et al*., 2016). Soybean is an excellent bioenergy source **(**Krisnawati *et al*., 2015).

2.3 Abiotic stress

Now-a-days global warming is an alarming issue in the world. Global climate change poses a great threat to sustainable agriculture. Influence and intensity of abiotic stresses have risen due to climate change. Abiotic stresses involve salt stress, drought, flooding, heat stress and metal stress. Due to impact of abiotic stress, plant suffers from morphological and physiological disorders like disrupted stomatal conduction, distruption of photosynthetic pigments and reduction of yield. Oxidative stress is induced by increased number of ROS or free radicals. Due to overproduction of ROS, it causes ion leakage from the cell membrane and reduced photosynthesis rate (Hasanuzzaman *et al*., 2017).

Salinity is excessive accumulation of salt in soil which severely hampers plant growth and physiology. Salt stress has two phases: i) osmotic phase and ii) Ionic phase. The intial respond under salt stress is water absorption constraints, retarded growth, physiology and yield.

Drought is a serious problem now-a-days which affects sustainable agriculture and poses a major threat for global food security. Drought in plants results from unavailability of soil water to meet the demand of a crop at a specific period. For surviving in extreme dry conditions in various climatic regions, plants have developed to tolerate drought with an array of morphological, physiological and biochemical adaptations like drought resistance, drought escape and drought tolerance (Basu *et al*., 2016 ; Salgado *et al*., 2020).

Waterlogging is known as a significant problem in Asia particularly in areas where waterlogging occurs frequently because of excessive rainfall, inappropriate irrigation and poor drainage facilities. Flooding hampers the availability of O_2 and CO_2 in soil and also retards aerobic respiration and photosynthesis ultimately limits growth and development (Joshi, 1966).

Now-a-days, increasing temperature is one of major consequences in recent areas. Temperatures, which are higher than normal temperature, are termed as heat stress (Mathur *et al*., 2014; Dahal *et al*., 2019).

Being a sessile organism, plants have to face consequences of climate change including heavy metal stress. Due to rapid industrialization, various types of pollutants emit into the soil and atmosphere and toxify the environment. This also reduce the productivity of soil and causes toxicity in plants. Metal stress is major threat for global food security worldwide.

2.4.1 Effect of salt stress on crop morphology

To observe the positive effects of salicylic acid in mitigating salt stress, *Torreya grandis* were grown under saline condition (0%, 0.2% and 0.4% NaCl) in a study and they observed that 30 days of salt treatment reduced the dry weight of shoot by 12.1% and 29% and dry weight of root by 9.9% and 25 5% under 0.2% and 0.4% NaCl, compared to plants with SA application (Li *et al*., 2014).

In a study, salt stress (50, 100 and 200 mM NaCl) were imposed on two common bean genotypes, high-yielding Tema and low-yielding Djadida after three weeks of their growth and they observed that with increasing salinity treatment, shoot and root dry weight decreased. In Tema, Shoot and root dry weight were decreased by 30% and 59% and in Djadida decreased by 27% and 61%, under 200 mM NaCl in comparison to plants without stress (Taibi *et al*., 2016).

An experiment of Billah *et al*. (2017) showed that 29 commercial maize hybrids were screened against salinity (12 dS m^{-1}). After 18 days salinity treatment, data were collected. They observed that 12 dS m^{-1} NaCl decreased shoot length by 30.29% in Super gold, 41.80% in 900M gold and 37.84% in PS-999 and also reduced dry matter above 50% in all maize hybrids, compared to plants without stress.

In wheat plant, 200 mM NaCl reduced fresh weight of root and shoot significantly in BARI Gom 21 and BARI Gom 25 varieties by 84%, 80% and 93%, 86% and wheat seedlings treated with 100 mM NaCl reduced dry weight by 60% and 54.7% at 8 days and 16 days (Fardus *et al*., 2018).

2.4.2 Effect of salt stress on crop physiology

Role of proline on two Malaysian cultivars MR 220 and MR 232 were studied where they found that chl content were increased by 7%, 26%, 84% and 93% in 100 mM, 200 mM, 300 mM and 400 mM NaCl concentrations, respectively (Deivanai *et al*., 2011).

To know the of positive effects of 24-epibrassinolide (EBL) in mitigating salt stress (10 mM NaCl) and maximum reduction was observed in photosynthesis (24%), stomatal conductance (37%), Chl *a* (58%) and Chl *b* (46%) under NaCl treatment (10 mM) but application of EBL increased photosynthesis and total chl contents under salt stress(Shahid *et al*., 2012).

Li *et al*. (2014) observed the positive impact of salicylic acid on mitigating salinity in *Torreya grandis* and after 30 days of salt treatments (0%, 0.2% and 0.4%), they found that without SA treatment, Chl *(a+b)* was reduced by 99% and 24.1% under salinity (0.2% and 0.4% NaCl).

An experiment was carried out on common bean (*Phaseolus vulgaris*) genotypes i) high yielding genotype Tema and ii) low yielding genotype Djadida under salt stress. They observed that, Chl *a*, Chl *b* and total carotenoid content was reduced by 52%, 33% and 18% in high yielding bean genotype Tema and by 57%,43% and 19% in low yielding genotype Djadida under saline condition (200 mM NaCl) (Taibi *et al*., 2016).

Salinity negatively affected the photosynthesis rate in soybean plant in experiment of Kataria *et al*. (2019) and they observed that the maximum reduction of photosynthesis (54%) was observed in soybean plant under 100 mM salt stress compared to normal condition.

2.4.3 Effect of salt stress on crop yield

Salt stress effects the growth and physiology of plant and ultimately it hampers the yield of crops.

Khanam *et al*. (2018) reported that panicle number, total grain number and number of plant was reduced by 55.07%, 27.51% and 67.1% in BR 55 rice cultivar and 64.17%, 32.33% and 76.68% in BR 43 rice cultivar significantly under saline condition. In pea plant, both NaCl treatments (1 and 10 mM) reduced seed yield by 15% and 32% control to unstressed plants (Shahid *et al*., 2011).

2.4.4 Oxidative stress and antioxidant defense system in salt induced crop

In high saline condition, (10 mM NaCl), nitrate reductase (NRA), nitrite reductase, superoxide dismutase, catalase and peroxidase activities enhanced significantly. EBL (10 µM) treatment positively mitigated negative effects of salt stress and increased NRA (14%), NiRA (26%), SOD (58%),CAT (87%) and POD (10%) activities significantly in common bean (Shahid *et al*., 2011).

In a study, two common bean (*phaseolus vulgaris*) genotypes, 1) high yielding genotype Tema and 2) low yielding genotype Djadida were grown under salinity to investigate the variations among antioxidant defense system. They observed that, NaCl treatment led to gradual increase in MDA content. High Saline condition increased MDA level by 44% and 56% in high yielding Tema and low yielding Djadida (Taibi *et al*., 2016)

2.5.1 Effect of salt stress on morphology of soybean

Yoon *et al*. (2009) investigated the positive role of Methyl Jasmonate (MEJA) in mitigating salt stress in soybean plant in hydroponics solution. They observed that salinity (60 mM NaCl) resulted a decline in crop growth, photosynthesis and transpiration rate, compared to control and the shoot length was decreased by 34.75% under saline condition (60 mM NaCl).

Under medium salinity (80 mM NaCl), the length of soybean hypocotyls and root were decreased by 60% and 28% and fresh weight of hypocotyls and roots were reduced by 42% and 56% in comparison to control (Sobhanian *et al*., 2010).

Under saline condition (0, 50, 100 and 200 mM NaCl), Amirjani *et al.* (2011) explored the negative effects of salt stress on growth mineral composition and Pro content of soybean plants. Plant height and fresh weight were recorded at four days after treatment. Plant height was decreased by 30, 47 and 76% and fresh weight was decreased by 32, 54 and 76% under 50, 100 and 200 mM NaCl. A soybean plant (var. William) was grown to investigate the role of zinc (Zn) (0.355 mg kg^{-1}) to lessen salt induced damage and they observed that the shoot length was declined by 24%, 32% and 47% under salinity (33, 66 and 99 mM NaCl) but Zn application showed a increase in the shoot length by 23% and 41% under 66 and 99 mM NaCl.

With increasing salinity level (0 to 15 dS m^{-1}), NaCl gradually negatively affected the averages of final germination percentage, germination index, seedlings vigor index in soybean cultivars. Highest mean germination time (46.45%), germination index (34.25%) and seedling vigor index (83.07%) were observed under 15 dS m^{-1} NaCl (Kandil *et al*., 2015).

A study was carried to understand the positive function of Ca on soybean plants under salt stress. They observed that (100 mM NaCl) salt stress decreased biomass accumulation. In soybean plant, plant height, fresh biomass and dry biomass were decreased by 37.67%, 25.79% and 38.81% under saline (100 mM NaCl) condition (Elkelsih *et al*., 2019).

Soybean plants were used in an experiment to determine the positive role of potassium fertilizers in mitigating salt stress. After two weeks of salt treatment, they observed that potassium fertilizer showed the positive effect on plant height and root, shoot fresh weight under mild salinity (6 dS m^{-1}). But under severe salinity (12 dS m^{-1}), potassium fertilizer did not show the significant effects and reduced plant height, root and shoot fresh weight of soybean plant (Adhikari *et al*., 2020).

2.5.2 Effect of salt stress on physiology of soybean

To observe the physiological attributes of soybean genotypes with different salt tolerance levels under saline condition. In soybean cultivar Shohag, Na^+ accumulation in the root and shoot were higher under saline condition (50 and 100 mM NaCl), compared to non-saline condition (Mannan *et al*., 2013).

In a study, potassium fertilizers were used to mitigate salt stress. After two weeks of salt treatment, they observed that under medium saline condition (6 dS m^{-1}), Chl and carotenoid contents were decreased compared to unstressed soybean plant (Adhikari *et al*., 2019).

Elkelish *et al*. (2020) investigated the role of Ca (2 mM $CaSO_4$. $2H_2O$) on alleviating salt stress on soybean plant where they observed that 100 mM NaCl reduced chl, carotenoids, photosynthesis rate and stomatal conductance by 45.18%, 30.72%, 41.88% and 35.98% , compared to control.

In a study, Sadak *et al*. (2020) observed the role of cysteine in mitigating salt stress in soybean plant and they found that with increasing salinity, Chl *a*, Chl *b* and carotenoids of soybean plant were decreased under saline condition (3000 to 6000 mg L^{-1}) but the application of cysteine effectively alleviated the negative consequences of salt induced damage.

2.5.3 Effect of salt stress on yield of soybean

Sadak *et al*. (2020) reported that saline water reduced pod weight and seed number $plant^{-1}$ of soybean. In soybean plant, protein and oil content was decreased under salinity (60 mM NaCl) (Parveen *et al*., 2016).

In a study, among soybean cultivars, the relative number of seeds per pod was decreased by 92% and 79% and the relative 100-seed weight was alleviated by 50% and 40%, respectively under 50 and 100 mM NaCl (Mannan *et al*., 2013).

2.5.4 Oxidative stress and antioxidant defense system of soybean

Soybean plant faces osmotic stress due to over-production of ROS which causes growth inhibition and yield reduction in crop. During osmotic stress, soybean plants exhibit some antioxidant defense systems which include complex activities of non-enzymes (ascorbate, glutathione, α-tocopherol), enzymes (SOD, APX, GR and CAT) and osmolyte production to encounter salt stress.

Sadak *et al*. (2020) reported that H_2O_2 level was increased by 43.7% and 71.3% under low saline condition (3000 mg L^{-1}) and high saline condition (6000 mg L^{-1}). But cysteine treatment (40 mg L^{-1}) reduced this percentage by 20.4%, 40.4% and 37.8% under saline condition. They observed that MDA content was increased by 42% and 78% under low salinity (3000 mg L^{-1}) and high salinity (6000 mg L^{-1}) but cysteine application reduced this percentage by 20.8, 33.0 and 28.8% under salinity level (0, 3000 and 6000 mg L^{-1}).

2.6 Role of priming under salt stress

Among different strategies developed to mitigate salt-induced adverse effects, priming is the most popular physiological technique to mitigate salt stress. Priming is the process of seed hydration and drying to increase the pre-germinative metabolism for quick germination, seedling development and better yield (Oliveira *et al*., 2019; Waqas *et al*., 2019). Under salinity, the normal physiological process of seed hampered is due to low water potential and restricts the translocation of nutrients from the soil water.

Afzal *et al*. (2006) observed the positive effect of seed priming with abscisic acid, salicylic acid or AsA on *Triticum aestivum* cv. Auqab under saline condition (4 and 15 dS m^{-1}) showed better salt tolerance. Maximum germination, shoot fresh weight and root fresh weight were obtained in seeds primed with 50 ppm ascorbic acid and lowest mean germination time was observed in 50 or 100 ppm ascorbic acid treatments.

2.7 Effect of foliar application under salt stress

Due to salt stress, plant suffers from several disorders. They are unable to take sufficient nutrients from the soil and this cause nutrient deficiency. To cope with this problem, essential micronutrients can be supplied to the plants for better growth and performance. Foliar application is the most effective process that helps in grain enrichment and yield development.

To observe the role of KOH in mitigating salt stress in sunflower plants, foliar application of KOH (0, 0.5, 1.0, 1.5 and 2.0%) was imposed on sunflower plants under saline condition. They observed that salinity reduced root and shoot fresh weight but potassium nitrate application improved these growth parameters and maximum increase was found in 1% of KOH in control and stressed conditions. Maximum increase in yield attributes was found in 0.5% KOH level (Akram *et al*., 2007).

Qayyum *et al*. (2007) observed that effect of foliar application of 24-epibrassinolide (24-epiBL; 0, 0.0125 and 0.025 mg L^{-1}) in mitigating salt stress (150 mmol L^{-1} NaCl) in wheat plant (S-24). They found that Chl *a* and Chl *b* were increased at 0.025 mg L^{-1} level of 24-epiBL application under salt stress.

Soliman *et al*. (2015) investigated that foliar application of ZnO and Fe_3O_4 on moringa plant (*Moringa peregrina*) showed positive result against saline condition (0, 3000, 6000 and 9000 ppm). Salt stress drastically affected all growth parameters, chl and carotenoids contents. They observed that Moringa plant sprayed with Hoagland solution containing ZnO and Fe_3O_4 (60 mg L^{-1}) showed an increase in all growth parameters and reduced Na^+ concentrations under saline conditions, compared to control.

In an experiment, thirteen days old *Oryza sativa* L. cv. BRRI dhan 47 seedlings were subjected to 200 mM NaCl in absence or presence of 2 mM $CaCl_2$ and 2 mM ethylene glycol tetra acetic acid for three days. They observed that supplementation of Ca improved plant growth, relative water content and also increased antioxidant activities in salt stressed rice plant compared to control (Rahman *et al*., 2017).

2.8 Role of zinc on crop growth

Kanai *et al*. (2009) showed that poor growth and small brown spots on leaves were observed in rice and maize under Zn deficiency and interveinal leaf chlorosis and leaf motling symptoms were observed in Zn deficient citrus trees.

In barley (*Hordeum vulgare* L.), Zn primed seed increased germination and seedling development and priming with 1% $ZnSO_4$ solution (for 16 hrs) substantially improved crop growth, grain yield and grain Zn content in maize (Ajouri *et al*., 2004 ; Harris *et al*., 2007).

Zinc is required for chl biosynthesis and shortage of this element causes reduction in plant photosynthesis and plant performance and quality of crop.

Zinc deficiency appears mostly on young leaves of plants. Inter-node distance and leaf size will be short in monocot and dicot plants. Shoot is more likely to be affected by Zn deficiency than root (Mousavi *et al*., 2013).

2.9 Role of zinc under salt stress

Zinc is an essential micronutrient for plant growth and metabolism. Zn minimizes the harmful consequences of salt stress by increasing enzymatic activities (Torabian *et al*., 2016).

An experiment was carried out to know the role of Zn ($ZnSO_4$. $7H_2O$) (0.25 and 5 mg kg^{-1} soil) on wheat genotype (cv. ceyhan) under salt stress (0, 5 and 10 dS m^{-1}) They observed that sufficient supply of Zn ($ZnSO_4$. $7H_2O$) increased dry matter of shoot by 37%, compared to low Zn supply. Sufficient Zn supply enhanced shoot dry matter production by 20% and 53% at 5 and 10 dS m^{-1} salt treatments (Eker *et al*., 2013).

A study of Gulmezoglu *et al*. (2016) showed that green bean (*Phaseolus vulgaris*) genotypes (Seker Fasulye and local genotype) were primed with $ZnSO_4$. $7H_2O$ under saline condition (50, 100 and 150 mM NaCl) and they reported that Zn priming effectively reduced the consequences of salt stress and reduced sodium and calcium concentrations in green bean genotypes under salt stress (100 mM NaCl), compared to control plants

Hussein *et al*. (2018) reported that foliar application of Nano-Zinc (100 and 200 ppm) on cotton plant increased growth parameters (root Fw and Top/ root ratio) under salt stress. In sunflower variety Olsion, foliar application of Zinc oxide (2 g L^{-1}) enhanced pro content and SOD activities under salt stress (100 mM NaCl), compared to plants without zinc appliaction (Torabian *et al*., 2016).

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Chapter III

MATERIALS AND METHODS

This section delivers a detailed information about the experimental time, site, climatic condition, seed or planting materials, treatments, experimental design and layout, cultivation procedure, fertilizer application, intercultural operations, data collection and statistical analysis of the experiment.

3.1 Location

The experiment was performed at shed house of the Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka during October 2019 to March 2019 (Rabi Season) to investigate the positive role of exogenous applications (seed priming and foliar application) with Zinc sulfate (1 mM $ZnSO_4.7H_2O$ for priming) and (0.5% $ZnSO_4.7H_2O$ for foliar application) on Soybean seedlings growth under two saline conditions (5 and 10 dS m^{-1} NaCl).

3.2 Characteristics of soil

The soil was non-calcareous dark grey soil and belonged to Madhupur tract (AEZ 28)

3.3 Materials

3.3.1 Plant material

Soybean variety (BARI soybean 5) was used in this experiment. This variety was released in 2002. Plant height of 40-60 cm, capsule/plant 40-60, seeds/capsule 2-3, 100 seed weight 9-14 g. In rabi season sowing at mid December to mid January, sowing in kharif season mid July to August. Crop duration is 90-100 days. Yield 1.6-2.0 ha^{-1}.

3.3.2 Chemicals

Zinc sulfate hyptahydrate was used as a source of zinc for priming (1 mM $ZnSO_4$. $7H_2O$) and foliar application (0.5% $ZnSO_4.7H_2O$).

3.3.3 Soil preparation

Plastic pot (11 inch depth and 12 inch depth) was used in this experiment. Twelve Kilogram soil with recommended fertilizers and manures were put in each pot. The collected soil was sun dried and crushed. The soil, organic manure and fertilizers were incorporated well before placing soil in the pot. Each pot was filled with 12 kilogram soil

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3.3.4 Seed collection

Healthy and uniform sized soybean seed was gathered from Bangladesh Agricultural Research institute (BARI).

3.4 Experiment Design

The experiment was performed with three replications in completely randomized design. In this experiment, two sets of pots are used for measuring vegetative growth.

3.5 Fertilizer application

Fertilizers like organic manure, urea, triple superphosphate, muriate of potash, gypsum and Furadan 5G ® were used in the experimental pots at required rate. At final pot preparation before seeding all fertilizers had been applied with the soil.

3.6 Priming method

Before priming, we did trial on priming with zinc sulfate heptahydrate (1 mM $ZnSO_4.7H_2O$) for 1, 2 and 3 h. 3h priming showed the best result. So soybean seeds were soaked in 1mM $ZnSO_4$. $7H_2O$ solution in the dark at 25℃ for 3h. Then seeds were cleaned three times with fresh water and were air dried to safe moisture content. Seed were prepared for sowing. Control seeds were not primed.

3.7 Seed sowing technique

Fifteen seeds are sown in each pot. After germinating seeds, seven plants are allowed to grow in that pot.

3.8 Treatments

The experiment had nine treatments and three replications. Treatments consisted of

1. control
2. priming with 1 mM $ZnSO_{4.}\ 7H_2O$
3. Foliar application of 0.5% $ZnSO_{4.}\ 7H_2O$
4. 5 dS m^{-1} salinity
5. 5 dS m^{-1} salinity + Priming with 1 mM $ZnSO_4.7H_2O$
6. 5 dS m^{-1} salinity + Foliar application of 0.5% $ZnSO_4.\ 7H_2O$
7. 10 dS m^{-1} salinity
8. 10 dS m^{-1} salinity + Priming with 1 mM $ZnSO_4.\ 7H_2O$ and
9. 10 dS m^{-1} salinity + Foliar application of 0.5% $ZnSO_4.7H_2O$.

After germinating seeds, treatment was given at 20 DAS and 30 DAS (Vegetative stage)

3.9 Foliar application

5g $ZnSO_4.7H_2O$ granules was dissolved in 1000 ml distilled water to make 0.5% solution. Tween 20 as a cementing agent was added in the dissolved solution. Foliar application was employed from 25 DAS to 65 DAS and was given 2 days interval.

3.10. Intercultural operations

3.10.1 Gap filling and Thinning

After sowing seeds, continuous observation was kept. It was observed that no single seed failed to germinate, Gap filling was done when needed. Thinning was done to maintain seven seedlings

3.10.2 Weeding and irrigation

Sometimes there were some weeds observed in pots which were uprooted manually. Irrigation was given to maintain field capacity moisture level.

3.10.3 Plant protection measure

In sometimes aphid had appeared. Recommended insecticide was applied to control the aphid.

3.11 Collection of data

Growth and physiological data were taken each time after each treatment (30 DAS and 45 DAS). Plant height and SPAD value were taken with 5 days of interval until fruiting stage.

Data was collected on following parameters:

3.11.1 Crop growth parameters

- Plant height
- Root length
- Fresh weight of root
- Dry weight of root
- Fresh weight of shoot
- Dry weight of root

3.11.2 Physiological and biochemical parameters

- Relative water content (RWC)

- SPAD value
- Electrolyte leakage (leaf and root)
- MDA content
- H_2O_2 content
- Proline content

3.12. Procedure of sampling for growth study during crop growth period

3.12.1 Plant height

Soybean plant height was assessed following methods. Using the weighing scale, plant height was determined from the ground level to the highest tip of leaves. With each container, the average height of five plants were known as the height of the plant.

3.12.2 Fresh weight plant^{-1}

Five sample plants were harvested from each pot following completion of treatments (30 and 45 DAS). Then they washed the plants in water. Then the plants were divided into shoot and root. After that both shoot and root were then weighted in a balance and combined to provide fresh weight plant^{-1} and data taken after the treatment period was finished.

3.12.3 Dry weight plant^{-1}

Five sample of shoot and root of soybean plants were oven-dried after weighing the fresh weight, holding a temperature of 72° C for 48 hours. Then the plants were weighted and averaged in an electrical equilibrium to provide a dry weight plant^{-1}.

3.12.4 Root length plant^{-1}

Root length was counted from the ending point of shoot to the tip of root as the length of root of plant. The average length of five plants was considered as the root length of the plant for each pot. Root length plant^{-1} was recorded at 30 DAS and 45 DAS.

3.12.5 Leaf Relative water content

According to Barrs and Weatherly (1962), the relative water content (RWC) was recorded in this experiment as fresh weights and floating on distillated water in the petri plates and held in darkness. Whole leaf lamina was recorded after 8 hr, and then leaf disks were further weighed and treated as the turgid weight (TW) by elimination of excess surface water. After drying, 48 h, DW was finally tested at 80 ° C at 30 DAS and 45 DAS were taken. Leaf RWC was calculated using the following formula:

RWC (%) = $\frac{Fw - Dw}{Tw - Dw} \times 100$

3.12.6 SPAD Value

Any pot had five leaves picked randomly. At the LEAF (FT Green LLC, USA) as a weight, the top and bottom of each leaflet is calculated. The average chl content was determined and then the overall ch content was recorded by converting a LEAF in SPAD units. The value of SPAD was taken from 25 DAS to 65 DAS for 5 days.

3.12.7 Electrolyte leakage

Electrolyte leakage (EL) was recorded at 30 DAS. To measure EL, 0.5 g leaf samples were put in falcon tube with 15 ml distilled water. The falcon tubes then incubated in a water bath at 40 °C for about 1 hr. After cooling, electrical conductivity (EC_1) was recorded with a conductivity meter. Samples were again incubated in an autoclave machine for about 1 hr and electrical conductivity (EC_2) were measured after cooling the samples. EL was calculated using the following formula (Zhang *et al*., 2006):

EL (%) = $\frac{EC1}{EC2} \times 100$

3.13 Procedure of measuring oxidative stress indicators

3.13.1 Measurement of lipid peroxidation

The level of lipid peroxidation has been measured (Heath and Packe, 1968) Malondialdehyde (MDA) content which is slightly altered by Hasanuzzaman *et al*. (2017). The 3 mL 5% (w / v) trichloroacetic acid (TCA) was homogenized with leaf sample (0.5 g) and the homogeneous one was centrifugated with 11.500 × g of for 15 min. 4 mL thiobarbituric acid (TBA) reagent is mixed with supernatant (1 mL) (0.5% TBA in 20% TCA) . For 30 minutes, the reaction mechanism was heated to 95 °C in a bath of water, and then rapidly refreshed in the ice bath. The colored supernatant absorption was estimated at 532 nm and calibrated at 600 nm for non-specific absorbance. Calculated by the usage of 155 mM^{-1} cm-001 and expressed as a nmol g^{-1} FW, MDA content was calculated.

3.13.2 Determination of hydrogen peroxide (H_2O_2) content

The process Yu *et al*. (2003) has been used to calculate the hydrogen peroxide (H_2O_2). A 3 mL potassium-phosphate (K – P) of 50 mL (pH 6.5) at 4 °C homogenated leaf tissue (0.5 g) was present. At 11500 × g for 15 min, the homogenate was centrifuged. The supernatant (2 mL) was dissolved in 20 percent H_2SO_4 (v / v) with 666, 4 µL 0, 1 percent $TiCl_4$ and processed at room temperature for ten minutes. The mixture was then again centrifuged for 12 minutes. Then Spectrophotometrically calculated with the extinction coefficient 0.28 μM^{-1} cm^{-1} was then measured as supernatant at 410 nm and measured as nmol g^{-1} FW.

3.13.3 Measurement of proline content

Bates *et al*. (1973) stated that proline content (Pro) was taken. In 5 mL of sulfosalicylic acid, fresh leaf tissue (0.5 grams) was homogenized in ice-cold condition and homogenous was centrifuged at 11, 500 × g of sulfosalicylic acid for 15 minutes. The supernatant (1 mL) was combined with 1 mL of ninhydrin acid (1 mL) and 1 mL of glacial acetic acid (100° C) for 1 hour. Then the mixture was moved into a test tube and cooled on ice. Through the cooled mixture of Toluene (2 mL) was added and properly dissolved using a vortex machine. In a few minutes, the spectrophotometric reading of chromophore containing toluene was 520 nm. In relation to a standard curve of known pro-concentration, the pro quality of the sample was calculated

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3.14. Statistical analysis

Data accumulated from different parameters were subjected to analysis using CoStat v.6.400 (CoStat 2008) and one way analysis of variance (ANOVA). For finding out mean differences among the replications, Fisher's least significant difference (LSD) test at the 5% level of significance was applied.

Chapter IV

RESULTS AND DISCUSSION

4.1 Crop growth parameters

4.1.1 Plant height

A reduction was observed at both doses of salinity, mild (5 dS m^{-1}) and severe (10 dS m^{-1}) salinity. Compared to control, plant height were reduced by 24.9%, 38.9%, 34.6% and 35.32% under mild salinity and by 33.2%, 41.3%, 42.5% and 42.5% under severe salinity at 25, 30, 35 and 40 DAS, respectively. But zinc priming effectively enhanced the plant height compared to plants with salt stress alone. Plant height was increased by 25.2%, 43%, 43.1% and 43.2% under mild salinity and by 31.9%, 48%, 24.1% and 24.4% under severe salinity due to Zn priming at 25, 30, 35 and 40 DAS, compared to Zn un-treated plants. Foliar application of zinc also mitigates the negative effects of salinity (mild and severe) and enhanced plant height by 18.9%, 31.7%,23.8% and 24.5% and 27.8%, 22.9%, 25.9% and 26.5% at 25, 30, 35 and 40 DAS, compared to plants without Zn supplementation under salinity.

In this study, mild (5 dS m^{-1}) and severe (10 dS m^{-1}) salinity reduced plant height of soybean plant. This reduction in soybean plant height occurred due to water deficiency caused by a reduction in synthesis and translocation of nutrient pool and reduction in seedlings vigor (Parveen *et al*., 2016). But zinc priming effectively reduced that negative effects of salt stress and increased plant height in soybean. Zinc application reduces salinity induced damage and has a positive effect on the absorption and division of major mineral elements (Weisany *et al*., 2012). Plant height was increased in soybean, chickpea and lupine plants under salinity through Zn priming (Seyedi *et al*., 2012; Latef *et al*., 2016; Dai *et al*., 2017).

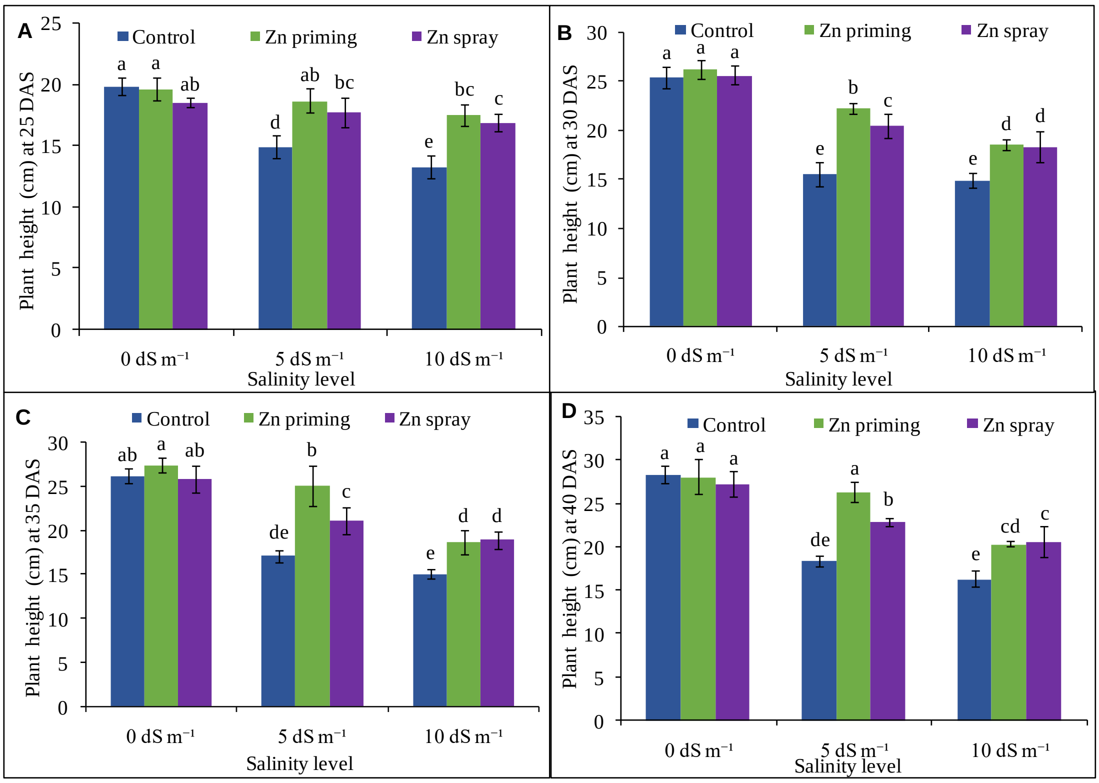

Figure 1. Effect of Zn priming and Zn foliar spray on plant height of *G. max* at (A) 25 DAS, (B) 30 DAS, (C) 35 DAS and (D) 40 DAS under mild (5 dS m^{-1}) and severe (10 dS m^{-1}) salinity. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

Zinc foliar spray also showed effective results in soybean under salt stress. Similar results were found after foliar spray of Zn in soybean, mustard, cotton and wheat (El-Fouly *et al*., 2011; Weisany *et al*., 2012; Ahmad *et al*., 2017; Hussein *et al*., 2018)

4.1.2 Root length

Both levels of salinity reduced soybean root length at vegetative stage (30 and 45 DAS). Compared to control, 22.8% and 36.21% reduction were observed under mild salinity and 46.1 % and 44.9% reduction were observed under severe salinity at 30 and 45 DAS, respectively. Zinc primed plants showed better results at vegetative stages and increased root length by 21.18% and 25.7% under mild salinity and by 57.24% and 31.61% under severe salinity compared to plants without Zn supplementation under salinity at 30 and 45 DAS, respectively.

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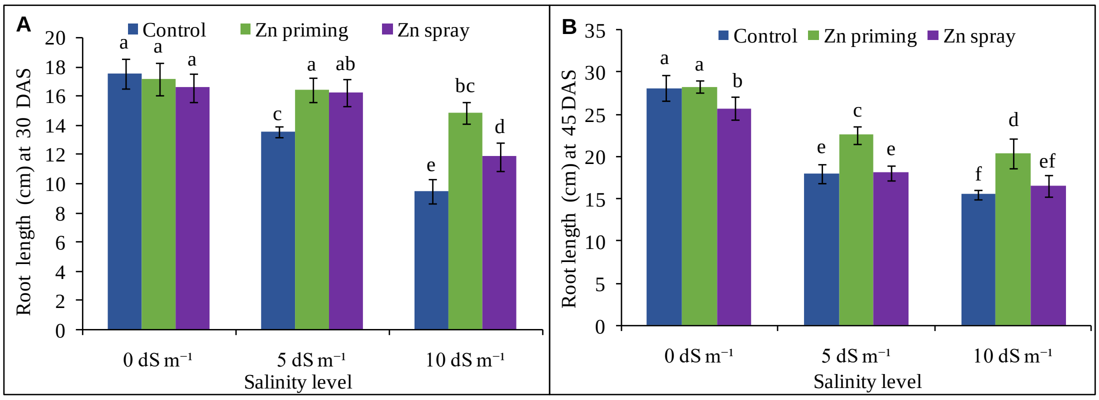

Figure 2. Effect of Zn priming and Zn foliar spray on root length of *G. max* at (A) 30 DAS and (B) 45 DAS under mild (5 dS m^{-1}) and severe salinity (10 dS m^{-1})**.** Mean (±SD) was calculated from three replicates for each treatment. Values in column with different letters are significantly different at $p \leq 0.05$ applying LSD test

Foliar application of Zn also reduced the negative effects of salt stress and increased root length by 19.95% and 25.4% at 30 DAS and by 0.5% and 6.9% at 45 DAS compared to plants without Zn supplementation under salinity.

Root length was decreased at both salinity levels (mild and severe) in soybean plants. Suppression of plant growth and reduction in cell elongation is the primary effect of salinity on plants (Munns and James, 2003). Zinc priming showed a positive effect on root length in both saline conditions in this experiment. Zinc priming also mitigated the negative effects and increased root length of soybean and lupine in previous studies (Dai *et al*., 2017; Latef *et al*., 2017). Foliar application of Zn had marked increase in root length under mild salinity and not under severe salinity

4.1.3 Fresh weight of root

Fresh weight of soybean roots were significantly reduced at all salt doses (5 dS m^{-1} and 10 dS m^{-1}). Fresh weight of root were decreased by 55.4% and 60.6% under mild salinity and 48.7% and 65.85% under severe salinity at 30 and 45 DAS, compared to control. . Due to Zn priming, fresh weight of root were increased by 55.3% and 56.35% under mild salinity and 27.49% and 15.46% under severe salinity at 30 and 45 DAS, compared to plant without Zn supplementation under salinity.

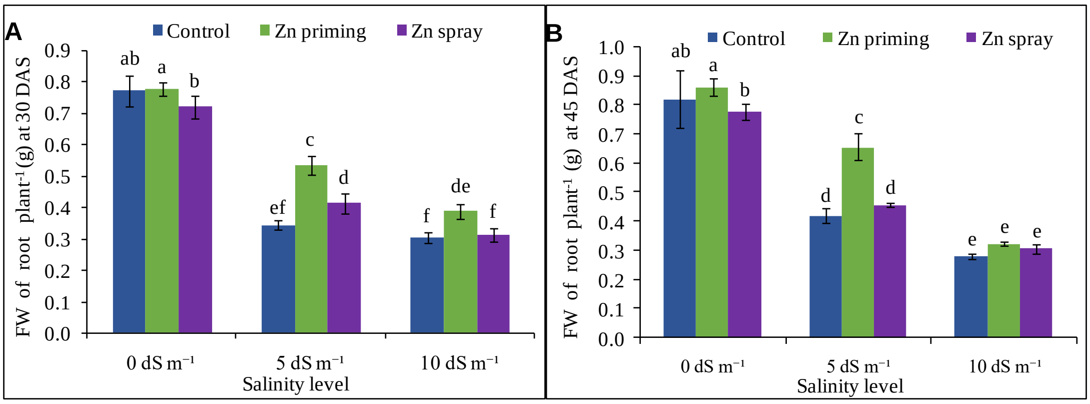

Figure 3. Effect of Zn priming and Zn foliar spray on fresh weight of root of *G. max* at (A) 30 DAS and (B) 45 DAS under mild salinity (5 dS m^{-1}) and severe salinity (10 dS m^{-1}). Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

This reduction in meristem is occurred due to the reduced cellular water content under salinity. But Zn application regulated the growth of soybean plant by improving biomass production and growth under salinity. Zinc priming showed effective results in ameliorating negative effects and increased fresh and dry weight of soybean under mild salinity but did not show any significant results under severe salinity. Similar results were observed in green bean genotypes and lupine plants under salinity (Gulmezoglu *et al.*, 2016; Latef *et al.*, 2017). Zinc primed seed positively affects these growth parameters under salinity. But foliar application of Zn did not show any remarkable results in mitigating the harmful effects of salt stress in both 30 and 45 DAS.

4.1.4 Dry weight of root

Dry weight of soybean root was decreased by 54.7% and 66.5% under mild salinity and 69% and 76% under severe salinity at 30 and 45 DAS. Zinc priming increased dry weight of root by 51.1% and 89.2% under mild salinity and by 35.67% and 15.1% under severe salinity at 30 and 45 DAS, compared to plants without Zn supplementation under salinity. But foliar application of Zn did not showed any significant result in mitigating salinity.

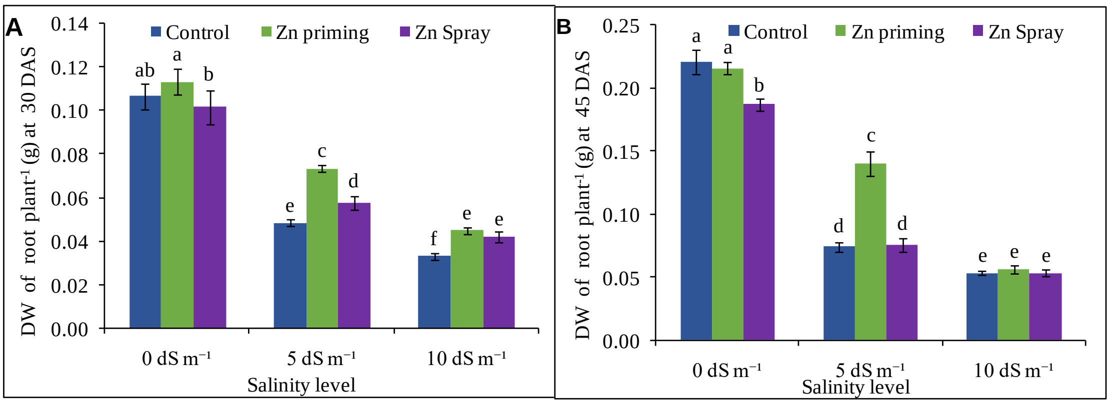

Figure 4. Effect of Zn priming and Zn foliar spray on dry weight of root of *G. max* at (A) 30 DAS and (B) 45 DAS under mild salinity (5 dS m^{-1}) and severe salinity (10 dS m^{-1}). Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.1.4 Fresh weight of shoot

Similar to root, all doses of salt stress affected the fresh and dry weight of shoot negatively. Compared to control, 57.9% and 71.60 % reduction and 74.22% and 80.71% reduction of fresh weight of shoot were observed under mild and severe salinity at 30 and 45 DAS. But primed soybean plant showed better results than salt stressed plants

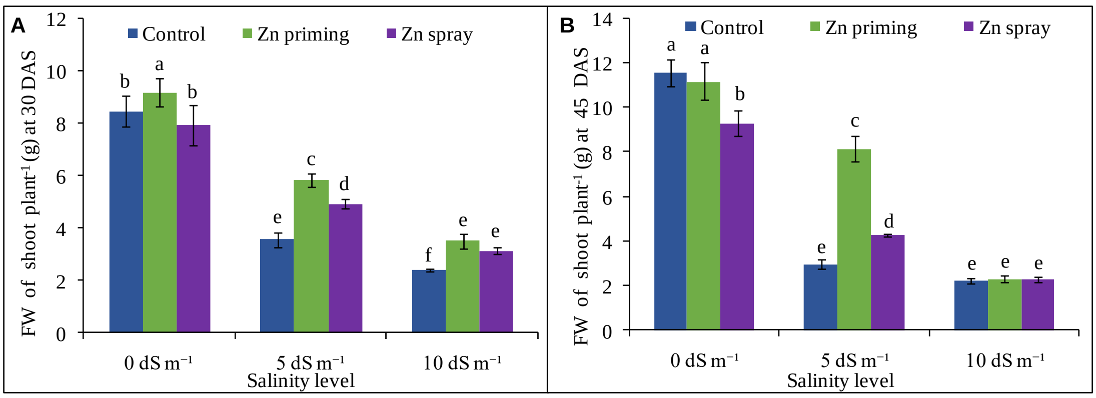

Figure 5. Effect of Zn priming and Zn foliar spray on fresh weight of shoot of *G. max* at (A) 30 DAS and (B) 45 DAS under mild (5 dS m^{-1}) and severe salinity (10 dS m^{-1}).). Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

Zinc priming increased the fresh weight by 63.30% and 45.7% under mild and severe salinity at 30 DAS. Fresh and dry weight of shoot of soybean plant was reduced at all doses of salinity in this study. Previous studies found that the typical symptoms of saline injury to plants decreased water content which affects cellular elongation process and ultimately causes retarded growth in soybean plant (El Sabagh *et al*., 2015). Seed priming with Zn plays a significant part in mitigating negative effect of mild salinity in this study but not severe salinity. The similar results found that ZnO priming alleviated the harmful consequences of salinity and increased fresh and dry weight of shoot in lupine plants (Latef *et al*., 2017).

4.1.5 Dry weight of shoot

Shoot dry weight was decreased by 64.43% and 79.41% under salinity and 75.24% and 78.64% under severe salinity at 30 and 45 DAS. Zinc Primed soybean plant increased the dry weight of shoot by 47.98% and 41.1% and 89.3% and 17.8% under salt stress (5 dS m^{-1} and 10 dS m^{-1}) at 30 DAS and 45 DAS, compared to plant without Zn supplementation under salinity. But compared to priming, foliar application of Zn did not contribute any significant results in mitigating salt stress

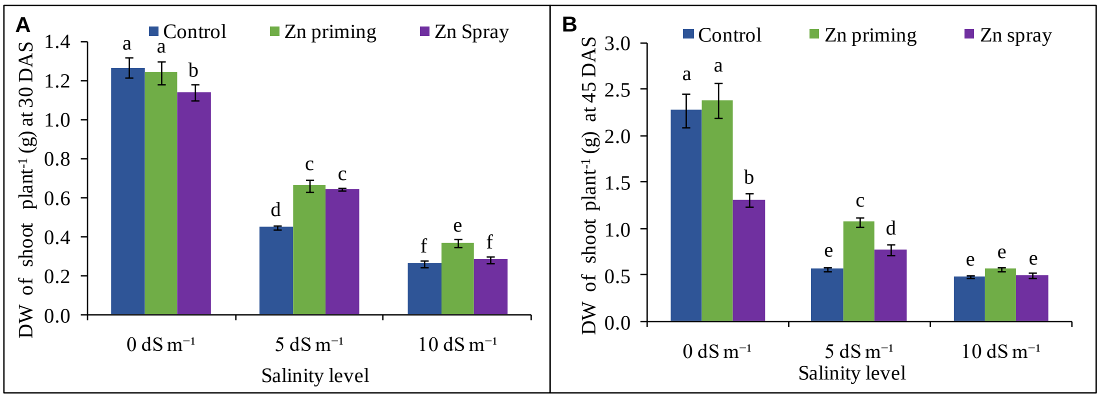

Figure 6. Effect of Zn priming and Zn foliar spray on dry weight of shoot of *G. max* at (A) 30 DAS and (B) 45 DAS under mild salinity (5 dS m^{-1}) and severe salinity (10 dS m^{-1}). Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Also positive results were found in mild salinity conditions but not in severe saline conditions with foliar spray of Zn in this study.

4.1.5 SPAD Value

Upon exposure to salinity treatments, SPAD value was decreased markedly in soybean plants. SPAD value were decreased by 8.8%, 9.0%, 16.3% and 16.9% under mild salinity (5 dS m^{-1}) and by 10.2%, 9.1%, 17.2% and 15.7% under severe salinity (10 dS m^{-1}) at 25, 30, 35 and 40 DAS, compared to control plants. Zinc supplementation effectively reduced the negative effect of salt stress. El Sabagh *et al*. (2015) reported that total chlorophyll content was decreased in soybean due to destruction of chloroplast structure and initiation of chlorophyll degrading enzymes under salinity.

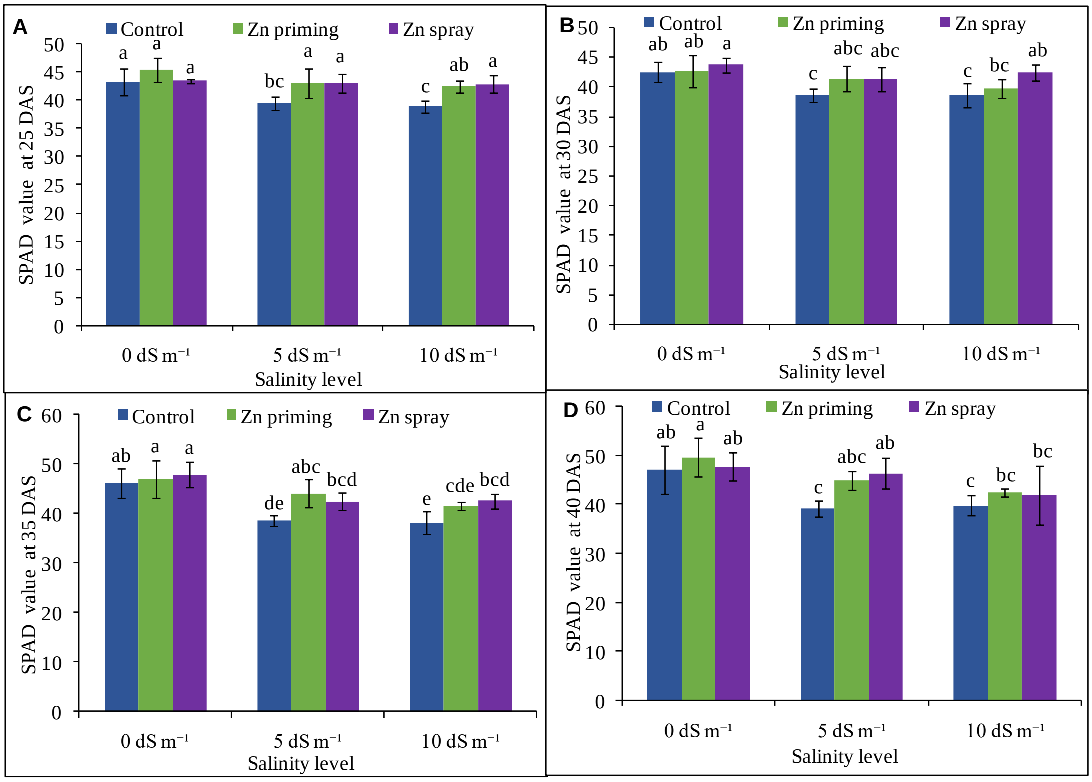

Figure 7. Effect of Zn priming and Zn foliar spray on SPAD value of *G. max* at (A) 25 DAS, (B) 30 DAS, (C) 35 DAS and (D) 40 DAS under mild (5 dS m^{-1}) and severe salinity (10 dS m^{-1}). Mean (±SD) was calculated from three replicates for each treatment. Values in column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Zinc primed soybean plant increased SPAD value by 8.8%, 7.0%, 14.4% and 14.4% under mild salinity and by 9.0%, 3.1%, 9.0% and 6.7% under severe salinity at 25, 30, 35 and 40 DAS, compared to plants without Zn priming. Also foliar application of Zn increased SPAD value by 8.8%, 7.0%, 10.2% and 18.5% under mild salinity and by 10.2%, 10%, 11% and 5.5% under severe salinity at 25, 30, 35 and 40 DAS, compared to un-treated plants under salinity.

Reduction of SPAD value was observed in soybean plants under both mild and severe salinity. SPAD value is referred to be an effective photosynthetic ability marker in plants. In this study Zn priming mitigates the negative effects of salt stress and increased SPAD value in soybean plants under both saline conditions. Similar results were found with Zn priming in soybean and lupine plants under saline conditions (Dai *et al*., 2017; Latef *et al*., 2017). Zn foliar application also effectively mitigated the harmful effects of salinity in this study. Foliar application of Zn showed effective results in soybean, mustard and maize under salinity (Ahmad *et al*., 2017; Wasaya *et al*., 2017).

4.1.6 Relative water content

Compared to control, leaf relative water content (RWC) of soybean plant decreased at all salt treatments (mild and severe salinity) significantly. 27.4% and 21.4% reduction were observed under mild salinity and 41.1% and 36.56% reduction were observed under severe salinity at 30 and 45 DAS, compared to un-treated plants. The reduction in relative water content of soybean plant indicated low water availability due to salinity treatment (El Sabagh *et al*., 2015). But zinc primed soybean plant effectively maintained RWC in salt stressed plants. Zinc primed plants increased RWC by 39% and 10 % under mild salinity and by 37.2% and 26.6% under mild salinity at 30 DAS and 45 DAS, compared to plants without Zn priming. Foliar application of Zn showed better result at 30 DAS under salt stress and increased RWC by 30.4% and 54.7% under mild and severe salinity, compared to plants without Zn supplementation.

In this study, a reduction in RWC of soybean plant under salinity was observed. A reduction in relative water content denotes a loss of turgor that resulted in limited water availability for cell extension process.

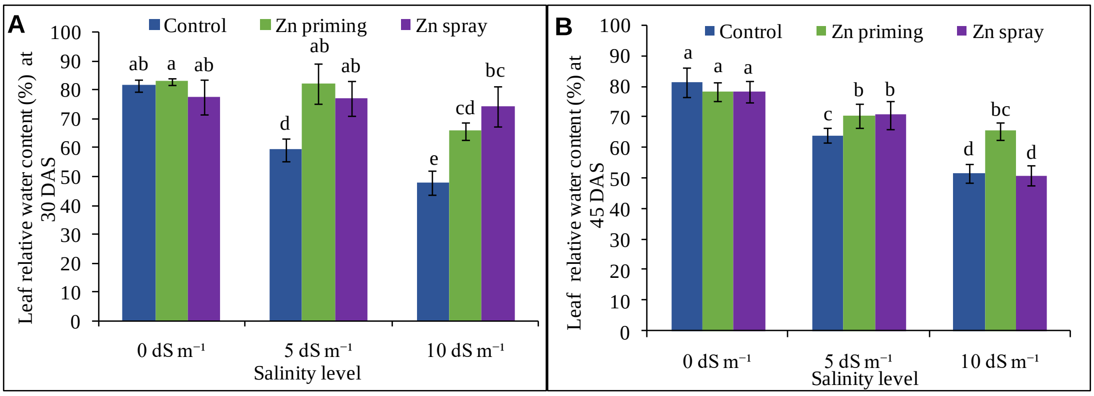

Figure 8. Effect of Zn priming and Zn foliar spray on leaf relative water content of *G. max* at (A) 30 DAS and (B) 45 DAS under mild (5 dS m^{-1}) and severe salinity (10 dS m^{-1})**.** Mean (±SD) was calculated from three replicates for each treatment. Values in column with different letters are significantly different at $p \leq 0.05$ applying LSD test

Zinc priming increased the relative water content in green bean genotypes, lupine and millets (Gulmezoglu *et al*., 2016; Latef *et al*., 2017; Rameshraddy *et al*., 2017).In this study Zn foliar application also increased RWC in soybean plant. In a study, we found that foliar application of Zn reduced salinity-induced cause and increased leaf relative water content in mustard (Ahmad *et al*., 2017).

4.1.7 Electrolyte leakage of leaf

In case of salinity experiment, electrolyte leakage (EL) is considered one of the important criteria for identification of primary site of ion-specific salt injury in plasma membrane (Hnilickova *et al*., 2019). In this experiment, EL increased with increasing NaCl concentration. Under mild and severe salinity, EL increased by 61.8% and 100%, compared to control at 30 DAS. Due to Zn priming, 13.7% and 15.12% reduction was observed under mild (5 dS m^{-1}) and severe (10 dS m^{-1}) salinity at 30 DAS, compared to plants without Zn priming. Also foliar application of Zn reduced EL by 15.1% and 12% under mild and severe salinity at 30 DAS, compared to un-treated plants.

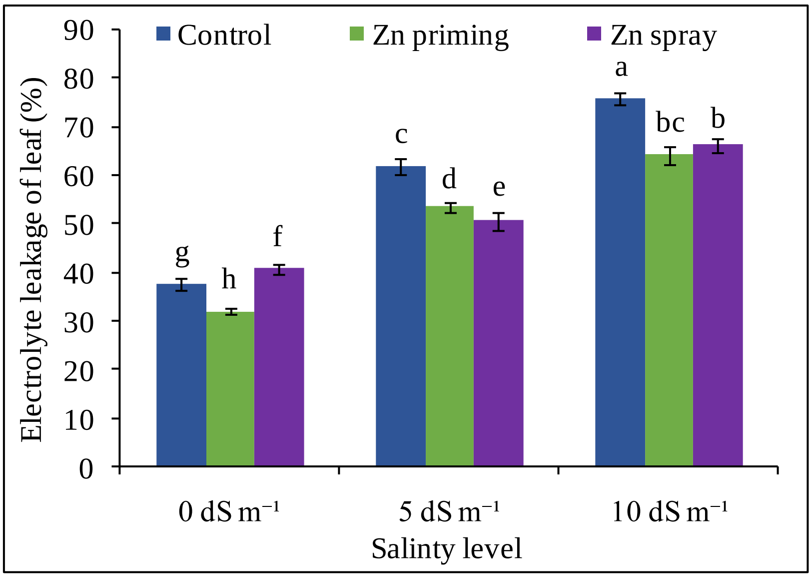

Figure 9. Effect of Zn priming and Zn foliar spray on electrolyte leakage of leaf of *G. max* at 30 DAS under mild salinity (5 dS m^{-1}) and severe salinity (10 dS m^{-1}). Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

In this experiment, electrolyte leakage was higher in both saline conditions (mild and severe). El Sabagh *et al*. (2015) reported that Higher EL was occurred due to salinity indicated specific injury on membrane. Both exogenous application of zinc (priming and foliar application) play an effective role in mitigating salinity induced damage and decreased electrolyte leakage. Previous findings showed that Zn was effective in decreasing Na^+ concentration in plants and mitigating salt injury in green bean genotypes and mustard (Gulmezoglu *et al*., 2016; Ahmad *et al*., 2017).

4.1.8 Electrolyte leakage of root

Similar to shoot, electrolyte leakage (EL) of root had increased under all salt treatments (mild and severe). At 30 DAS, mild and severe salinity increased EL by 64.3% and 100%, compared to control plants. Zinc primed seeds alleviated the harmful effects of saline condition and reduced EL by 13.7% and 15.12% under mild and severe salinity, compared to plants without Zn priming. Under mild and severe salinity, foliar application of Zn also reduced EL by 18.1% and 12.5%, compared to plant without Zn application.

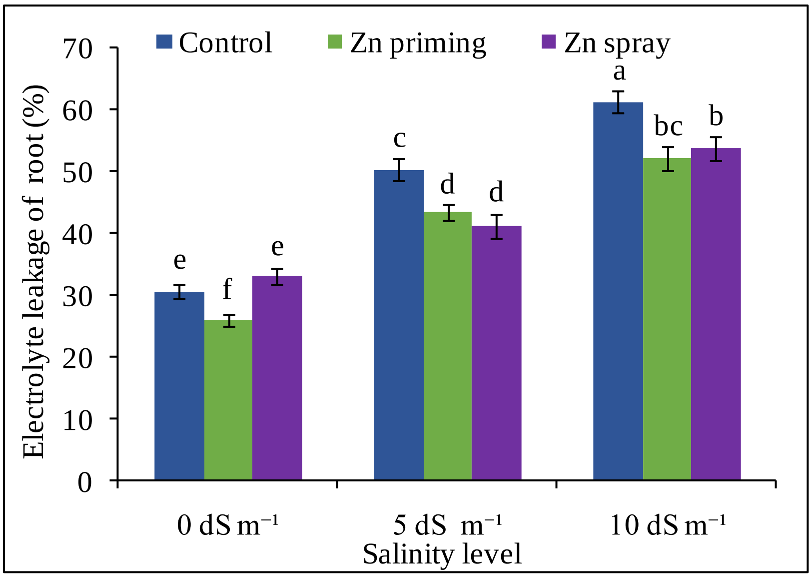

Figure 10. Effect of Zn priming and Zn foliar spray on electrolyte leakage of root of *G. max* under mild salinity (5 dS m^{-1}) and under severe salinity (10 dS m^{-1}**).** Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

Under all salt treatments, EL of root increased due to mild and severe salinity and Zn has an effective role in mitigating saline induced stress in this experiment. Both Zn priming and Zn foliar application mitigated the negative effects of salinity.

4.2 Biochemical parameters

4.2.1 Lipid peroxidation

In presence of NaCl, MDA content (indication of lipid peroxidation) was increased in untreated soybean plants. Mild salinity and severe salinity caused an increase in MDA content by 31.3% and 53.96% at 30 DAS, compared to control plants.

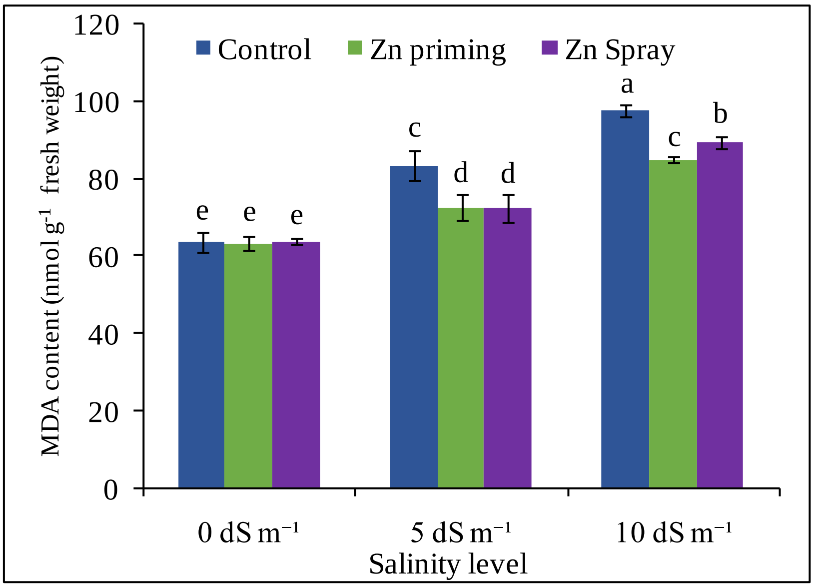

Figure 11. Effect of Zn priming and Zn foliar spray on MDA content of *G. max* under mild salinity (5 dS m^{-1}) and under severe salinity (10 dS m^{-1}). Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

But due to zinc priming, MDA content was decreased by 12.8% and 13.0% under mild and severe salinity, compared to plant without Zn priming at 30 DAS. Also foliar application also reduced MDA content by 13% and 8.0% under salinity (mild and severe) at 30 DAS, compared to plants without Zn supplementation.

4.2.2 H_2O_2 content

The content of H_2O_2 (Hydrogen peroxide) was also increased by 29.5% and 46.5% under mild and severe salinity, compared to control plants at 30 DAS. Zinc primed plants reduced H_2O_2 content by 15% and 18% under salinity (mild and severe) at 30 DAS, compared to plants without Zn priming. Foliar application of Zn also reduced H_2O_2 content by 12.1% and 5.4% under salinity (mild and severe) at 30 DAS, compared to plants without Zn supplementation.

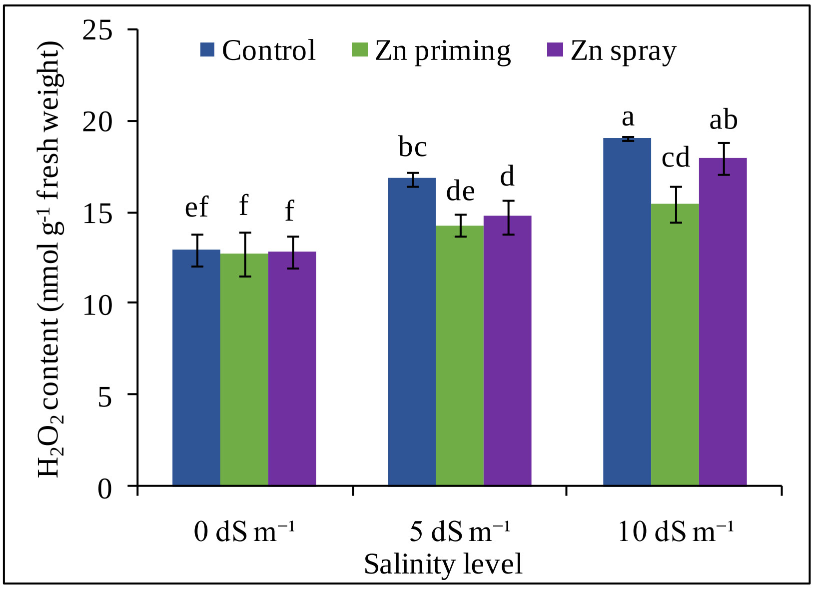

Figure 12. Effect of Zn priming and Zn foliar spray on H_2O_2 content of *G. max* under mild salinity (5 dS m^{-1}) and under severe salinity (10 dS m^{-1}). Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

The content of H_2O_2 was higher in both saline conditions (mild and severe) compared to control plants. But exogenous application of Zn decreased the H_2O_2 production in this study. Zinc has a positive role in mitigating NaCl induced effects. Similar results were found in soybean plant and mustard plants through Zn priming and Zn foliar spray (Ahmad *et al*., 2017; Dai *et al*., 2017).

4.2.3 Proline content

Upon exposure to salinity, proline content (Pro) was increased in soybean plant in this study. Mild and severe salinity caused an increase in Pro content by 96.3% and 230.2% at 30 DAS, compared to control plants. But Zn primed soybean plants reduced Pro content by 17.3% and 18.7% under mild and severe salinity at 30 DAS compared to plants without Zn priming. Foliar application also reduced Pro content by 6.3% and 8.9% under mild and severe salinity at 30 DAS, compared to plants without Zn supplementation.

In our study, exogenous application (priming and foliar spray) of Zn improved the Pro accumulation. Dai *et al.* (2017) observed that Zn priming induced Pro accumulation under salinity in soybean plants. Foliar application of Zn also showed similar results in mustard and pistachio seedlings under saline conditions (Tavallali, 2016 and Ahmad *et al.*, 2017).

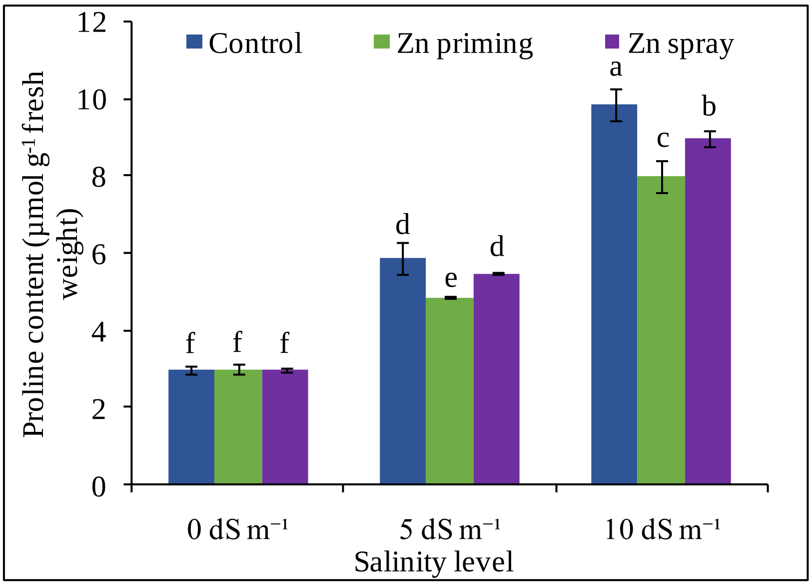

Figure 13. Effect of Zn priming and Zn foliar spray on proline content of *G. max* under mild salinity (5 dS m^{-1}) and under severe salinity (10 dS m^{-1}). Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

Chapter V

SUMMARY AND CONCLUSION

A pot experiment was carried out at agronomy shed house of the department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka during October 2019 to March 2019 (Rabi Season) to investigate the role of exogenous applications (seed priming and foliar application) with Zinc sulfate (1 mM $ZnSO_4$. $7H_2O$ for priming) and (0.5% $ZnSO_4$. $7H_2O$ for foliar application) on soybean seedlings growth under two level of salinity (5 dS m^{-1} and 10 dS m^{-1}). Healthy and uniform BARI soybean 5 seeds were used in this experiment. The experiment was laid out in a completely randomized design with three replications. In this experiment, two sets of pots were used for measuring vegetative growth.

The experiment consists of nine treatments: 1) control, 2) priming with $ZnSO_4.7H_2O$, 3) foliar application of $ZnSO_4.7H_2O$, 4) salinity 5 dS m^{-1}, 5) salinity 5 dS m^{-1} +priming with $ZnSO_4.7H_2O$, 6) salinity 5 dS m^{-1} + foliar application of $ZnSO_4.7H_2O$, 7) salinity 10 dS m^{-1}, 8) salinity 10 dS m^{-1} + priming with $ZnSO_4.7H_2O$ and 9) salinity 10 dS m^{-1} +foliar application of $ZnSO_4.7H_2O$, 6) salinity 5 dS m^{-1} + foliar application of $ZnSO_4.7H_2O$, 7) salinity 10 dS m^{-1}, 8) salinity 10 dS m^{-1} + priming with $ZnSO_4.7H_2O$ and 9) salinity 10 dS m^{-1} + foliar application of $ZnSO_4.7H_2O$.

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Soybean seeds were primed with $ZnSO_4.7H_2O$ for 2 hrs before sowing. After germinating seeds, treatments were given at Vegetative stage (20 and 30 DAS). Foliar application of $ZnSO_4$. $7H_2O$ were employed from 25 DAS to 65 DAS. Growth, physiological and biochemical parameters were taken each time after the completion of treatment (30 DAS and 45 DAS). Plant height, SPAD value, leaf area plant^{-1}, fresh and dry weight of plant^{-1}, shoot length^{-1}, root length^{-1} and electrolyte leakage (root and shoot) were taken for vegetative growth. Biochemical parameters (MDA, H_2O_2 and proline) were measured at vegetative stages.

The highest damaging effects were observed mostly in 45 DAS. Plant height was recorded to reduce mostly at vegetative stage (45 DAS). But Zn primed seed showed highest plant height at 45 DAS under mild salinity but not severe salinity. Foliar application of Zn did not show any significant increase in plant height. SPAD value was observed to decrease at 45 DAS under mild and severe saline condition but both priming and foliar application of zinc showed better result at vegetative stage. Leaf relative water content was reduced at vegetative stage but both priming and foliar application of zinc showed better result at vegetative stage under mild salinity but not severe salinity. Root length was reported to decrease at vegetative stage (30 and 45 DAS). But Zn primed plant showed significant result at vegetative stage under mild salinity. A reduction was noticed in fresh weight and dry weight of soybean plant at vegetative stage but Zn primed soybean plant showed a better result. But foliar application did not show any significant result in this case.

Electrolyte leakage of root and shoot were increased significantly at vegetative stage. Zinc primed soybean plant caused a significant reduction in electrolyte leakage but foliar application did not show any significant result in this parameters. MDA content indicates oxidative damage caused by salinity and in this study, MDA and H_2O_2 content was higher in salinity induced soybean seedlings than control plants under mild and severe saline conditions. Priming and foliar application of Zn caused a reduction in MDA and H_2O_2 content in soybean plant. Pro content was significantly higher under mild and severe salinity at reproductive stage. But foliar application of Zn showed a significant reduction in Pro content compared to Zn priming in soybean seedlings under both saline conditions

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From above findings, it was observed that soybean plant reacts to salinity depends on the degree of severity and. The longer the soybean plant under saline condition, the more the damage effects occur. We know that Zn is an essential micronutrient for plant and has positive role in plant physiological processes and Zn deficiency is considered as one of critical micronutrient deficiency under saline condition. Zn has the ability to scavenge ROS including superoxide radicals ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) under saline condition and also mitigated the harmful effects of salinity on phytohormones levels. In this experiment, we observed that Zn primed soybean seedlings showed effective results in most cases under saline condition and in few cases foliar application of Zn showed better results. Priming is an effective technique in now-a-days that imparts stress tolerance against biotic and abiotic stresses due to quick cellular defense system and effective energy metabolism. Foliar application is the most suitable and efficient method because small amount of nutrients can be applied when required.

So both priming and foliar application of Zn is very convenient technique for our farmers. Gaining food security for the growing population amidst the declining farmland is one of biggest challenges for Bangladesh. Zn acted as a beneficial element for eliminating salinity stress. Exogenous application of micronutrients could be an adaptive strategy to mitigate salinity and improve soybean production in our coastal areas. Further studies are required to know the effects of different doses of salinity on yield and yield contributing parameters of soybean plant. Investigation should be carried out to know the role of different doses of zinc in improving tolerance under different stresses.

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PLATES

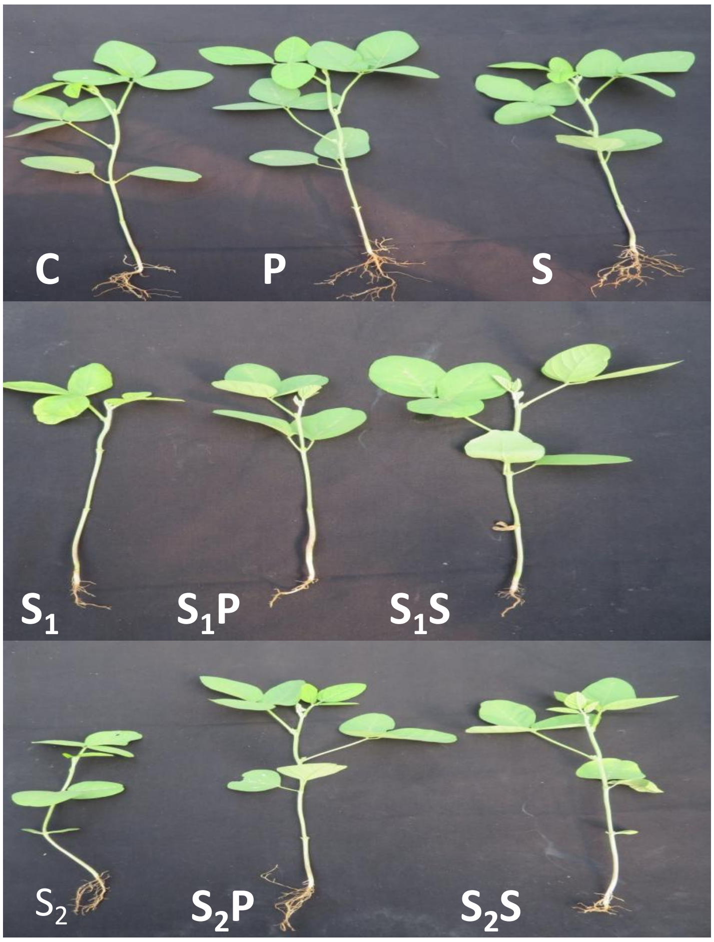

Plate 1. Phenotypic variation of soybean plants under mild (5 dS m^{-1}) and severe (10 dS m^{-1}) salinity at 30 DAS, C= Control, P= Priming (1 mM $ZnSO_4.7H_2O$), S= Foliar spray (0.5% $ZnSO_4.7H_2O$), S_1= 5 dS m^{-1}, S_1P= 5 dS m^{-1} + 1 mM $ZnSO_4.7H_2O$, S_1S= 5 dS m^{-1} + 0.5% $ZnSO_4.7H_2O$, S_2= 10 dS m^{-1}, S_2P= 10 dS m^{-1} + 1 mM $ZnSO_4.7H_2O$ and S_2S= 10 dS m^{-1} + 0.5% $ZnSO_4.7H_2O$.

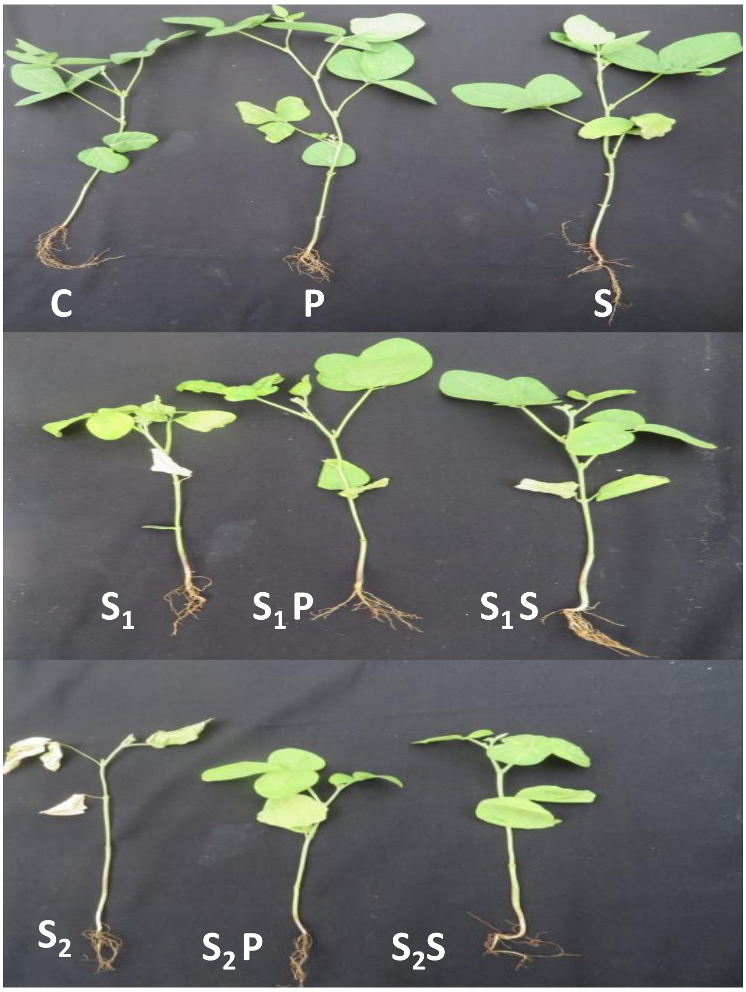

Plate 2. Phenotypic variation of soybean plants under mild (5 dS m^{-1}) and severe (10 dS m^{-1}) salinity at 45 DAS. Here, C= Control, P= Priming (1 mM $ZnSO_4.7H_2O$), S= Foliar spray (0.5% $ZnSO_4.7H_2O$), S_1= 5 dS m^{-1}, S_1P= 5 dS m^{-1} + 1 mM $ZnSO_4.7H_2O$, S_1S= 5 dS m^{-1} + 0.5% $ZnSO_4.7H_2O$, S_2= 10 dS m^{-1}, S_2P= 10 dS m^{-1} + 1 mM $ZnSO_4.7H_2O$ and S_2S= 10 dS m^{-1} + 0.5% $ZnSO_4.7H_2O$.

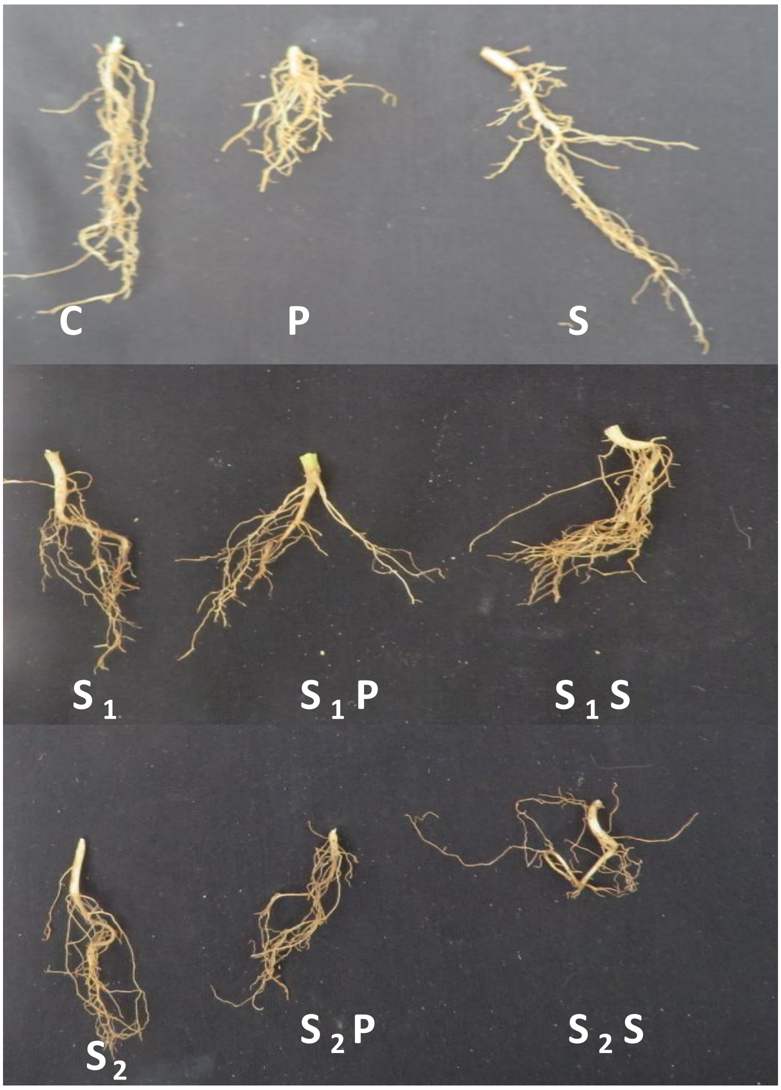

Plate 3. Phenotypic variation of roots of soybean plants under mild (5 dS m^{-1}) and severe (10 dS m^{-1}) salinity at 30 DAS. Here, C= Control, P= Priming (1 mM $ZnSO_4.7H_2O$), S= Foliar spray (0.5% $ZnSO_4.7H_2O$), S_1= 5 dS m^{-1}, S_1P= 5 dS m^{-1} + 1 mM $ZnSO_4.7H_2O$, S_1S= 5 dS m^{-1} + 0.5% $ZnSO_4.7H_2O$, S_2= 10 dS m^{-1}, S_2P= 10 dS m^{-1} + 1 mM $ZnSO_4.7H_2O$ and S_2S= 10 dS m^{-1} + 0.5% $ZnSO_4.7H_2O$.

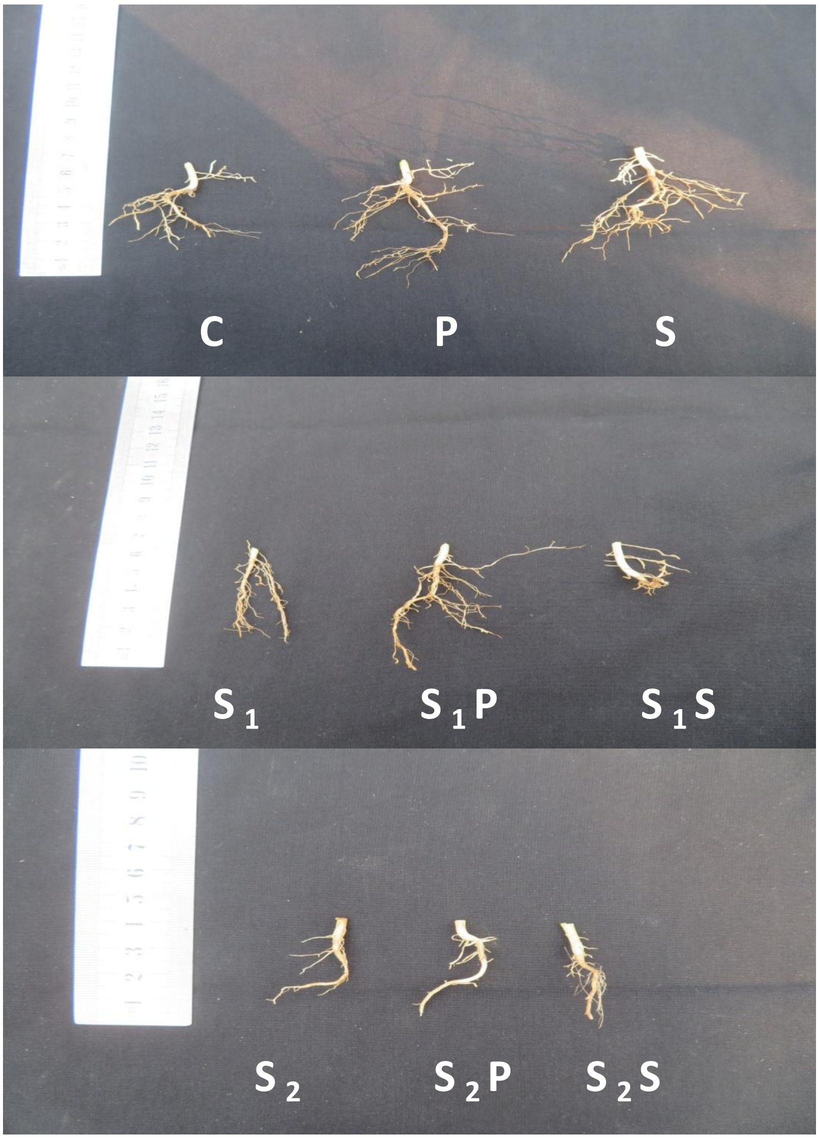

Plate 4. Phenotypic variation of root of soybean plant under mild (5 dS m^{-1}) and severe (10 dS m^{-1}) salinity at 45 DAS. Here, C= Control, P= Priming (1 mM $ZnSO_4.7H_2O$), S= Foliar spray (0.5% $ZnSO_4.7H_2O$), S_1= 5 dS m^{-1}, S_1P= 5 dS m^{-1} + 1 mM $ZnSO_4.7H_2O$, S_1S= 5 dS m^{-1} + 0.5% $ZnSO_4.7H_2O$, S_2= 10 dS m^{-1}, S_2P= 10 dS m^{-1} + 1 mM $ZnSO_4.7H_2O$ and S_2S= 10 dS m^{-1} + 0.5% $ZnSO_4.7H_2O$.

APPENDICES

Appendix I: Means square value and degree of freedom (DF) of plant height (25, 30, 35, 40 and 45 DAS) of soybean plant under salt stress.

Source of variation	DF	Means square value of				
		Plant height				
		25 DAS	30 DAS	35 DAS	40 DAS	45 DAS
Treatments	8	14.122	56.44	61.403	61.13	73.95
Errors	18	0.781	1.093	1.608	1.485	1.310

Appendix-II: Means square value and degree of freedom of SPAD value (25, 30, 35, 40 and 45 DAS) of soybean plant under salt stress

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Source of variation	DF	Means square value of SPAD Value				
		25 DAS	30 DAS	35 DAS	40 DAS	45 DAS
Treatments	8	12.050	10.222	35.733	41.255	44.580
Errors	18	2.959	3.305	5.553	11.51	2.968

Appendix-III: Means square value and degree of root fresh weight (30 and 45 DAS) and root dry weight (30 and 45 DAS) of soybean plant under salt stress.

Source of variation	DF	Means square value of			
		Root fresh weight		Root dry weight	
		30 DAS	45 DAS	30 DAS	45 DAS
Treatments	8	0.119	0.166	0.003	0.016
Error	18	0.001	0.166	0.001	0.001

Appendix IV: Means square value and degree of shoot fresh weight (30 and 45 DAS) and shoot dry weight (30 and 45 DAS) of soybean plant under salt stress.

Source of variation	DF	Means square value of			
		Shoot fresh weight		Shoot dry weight	
		30 DAS	45 DAS	30 DAS	45 DAS
Treatments	8	19.410	0.003	0.507	1.689
Error	18	0.173	0.001	0.001	0.009

Appendix-V: Mean square value and degree of freedom of root length (30 and 45 DAS) and leaf relative content (RWC) (30 and 45 DAS) of soybean plant under salt stress.

Source of variation	DF	Means square value of			
		Root length		Leaf relative content	
		30 DAS	45 DAS	30 DAS	45 DAS
Treatments	8	22.458	73.693	434.45	368.95
Error	18	0.777	1.432	24.38	12.85

Appendix-VI: Mean square value and degree of freedom of electrolyte leakage (%) of root and shoot (30 DAS) of soybean plant under salt stress.

Source of variation	DF	Means square value of	
		Electrolyte leakage of root	
		30 DAS	30 DAS
Treatments	8	424.81	644.83
Errors	18	2.44	1.868

Appendix-VII: Means square value and degree of freedom of MDA, H_2O_2 and proline content (30 DAS) of soybean plant under salt stress.

Source of variation	DF	Means square value of		
		MDA Content (30 DAS)	**H_2O_2 content (30 DAS)**	**Proline content (30 DAS)**
Treatment	8	479.41	15.88	20.969
Error	18	5.97	0.678	0.096