ROLE OF TRACE ELEMENTS IN MITIGATING SALT STRESS OF SOYBEAN

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CERTIFICATE

This is to certify that the thesis entitled ***"ROLE OF TRACE ELEMENTS IN MITIGATING SALT STRESS OF SOYBEAN"*** *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* ***MIRA RAHMAN,*** *Registration No.* ***14-06223*** *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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Place: Dhaka, Bangladesh

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ABSTRACT

Like other abiotic stresses, salinity adversely affects the vital morphological, physiological and biochemical mechanisms of plants and ultimately leads to yield reduction worldwide. This experiment was carried out to study the morphological, physiological, biochemical, phenotypical and anatomical responses of soybean (*Glycine max* L. cv. BINA Soybean-5) upon exposure to different levels of salinity and to investigate the role of exogenous application of selenium (Se) and boron (B) in mitigating salt stress. Plants were treated with 0, 150, 300 and 450 mM NaCl at 20 and 35 DAS. Exogenous application of Se (0.50 µM Na_2SeO_4) and B (1 mM H_3BO_3) was done individually (Se, B) and combinedly (Se+B) at 20 DAS and continued at three days interval until pod filling stage under normal and saline condition. Plants exhibited a reduction in plant height, root fresh weight, root dry weight, shoot fresh weight, shoot dry weight, number of branches plant^{-1}, leaf area, relative water content and SPAD value under salinity in a dose-dependent manner, which were observed for assessing the growth and physiological responses. However, proline content and oxidative stress indicators such as MDA content and H_2O_2 content were increased with the increase of salinity. Consequently, it caused a reduction in number of flowers plant^{-1}, pod length, pods plant^{-1}, seeds pod^{-1}, seed yield plant^{-1}, stover yield and biological yield. In responses to 300 and 450 mM NaCl-induced salt stress, plant death occurred after completing the vegetative stage. Phenotypical and anatomical parameters showed a visible deleterious effect of different levels of salinity on growth and number of stomata, respectively. On the contrary, exogenous application of Se, B and Se+B reverted the negative effect of salinity. The combined application of Se+B showed a slight difference in result than Se or B alone. The findings indicated that exogenous application of Se, B and Se+B mitigated the adverse effects of salinity by upregulating physiological and biochemical processes and by enhancing growth parameters.

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LIST OF ABBREVIATIONS

APX	Ascorbate peroxidase
AsA	Ascorbic acid/Ascorbate
ATP	Adenosine triphosphate
BINA	Bangladesh Institute of Nuclear Agriculture
CAT	Catalase
Car	Carotenoid
Chl	Chlorophyll
cv.	Cultivar
DAS	Days after sowing
DHAR	Dehydroascorbate reductase
DW	Dry weight
EL	Electrolyte leakage
FAO	Food and Agriculture Organization
FW	Fresh weight
GPX	Glutathione peroxidase
GR	Glutathione reductase
GSH	Reduced glutathione
GSSG	Oxidized glutathione
H_2O_2	Hydrogen Peroxide
IAA	Indole acetic acid
LSD	Least Significant Difference
MDA	Malondialdehyde
mM	Milimolar
µM	Micromolar
MSI	Membrane stability index
$O_2^{\bullet-}$	Superoxide radical
$OH^{\bullet}$	Hydroxyl radical
PDC	Pyruvate decarboxylase
POD	Peroxidase
Pro	Proline
ROS	Reactive oxygen species
RWC	Relative water content
SOD	Super oxide dismutase

Chapter I

INTRODUCTION

As sessile organism, in the era of climate change, plants are becoming more prone to hostile environmental conditions day by day. Adverse environmental conditions which are detrimental to plants, termed as environmental or abiotic stresses (e.g., salinity, drought, heat, cold, flooding, toxic metals/metalloids etc.) cause significant reduction in quality and yield of crops (Hasanuzzaman *et al.,* 2016; Hasanuzzaman *et al.,* 2017). Abiotic stress plays a major role in the drastic reduction of crop growth, development and productivity (Vishwakarma *et al.,* 2017; Lohani *et al.,* 2020). Abiotic stress is considered as a potential threat to global food security for upcoming decades because it is responsible for nearly 50% crop yield reduction. By the year 2050, salinity and drought are expected to cause serious salinization of more than 50% of all available productive arable lands (Hasanuzzaman *et al*., 2019).

Among all abiotic stresses, salt stress is one of the most severe one, which greatly reduces crop productivity. About 20% of the cultivated land and 33% of the irrigated area in the world are salt-affected. Moreover, about 7% of the land content high rate of salt among the salt-affected area (Kibria *et al.*, 2017). The alarming issue is up to 50% of agricultural land loss may occur due to salinity by the next couple of decades. In the soil solution, sodium chloride (NaCl) and sodium sulfate (Na_2SO_4) are the most available soluble salts. An increase in salinity level, in most of the cases, indicates mainly an increase in Na^+ and Cl^- concentration. Both Na^+ and Cl^- ions produce critical conditions for plant survival, but between them, Cl^- is the more dangerous one (Hasanuzzaman *et al.,* 2013).

According to Shrivastava and Kumar (2015), salt stress is a potential threat to sustainable crop production. About 20 to 50% of crop productivity diminishes due to salinity in cultivated land area. In every minute, around 3 hectares of arable land are affected by salinity resulting in 10% increase in saline affected area every year. Because of climate change, the saline affected area has been increasing rapidly more than before (Reddy *et al.,* 2017).

Salinity induced growth and yield reduction occurs due to the wide spectrum alteration of physiological and biochemical activity of salt stressed plants. Salt stress affects plant physiology and biochemical activity by reducing water potential in the soil, causing ionic imbalance, ionic toxicity and higher production of reactive oxygen species (ROS), which ultimately leads to yield reduction (Hasanuzzaman *et al.,* 2019; Kamran *et al.,* 2020).

In combating salt stress, exogenous protectants (osmoprotectants, phytohormones, polyamines, antioxidants, and trace elements) showed promising results (Hasanuzzaman *et al.,* 2013). Trace elements increase plant stress tolerance by enhancing growth and physiological activities (Hasanuzzaman *et al.,* 2017). In adverse environments where soil application is not cost-effective, the foliar application is a judicious way to supply plant nutrients. In case of micronutrients, foliar application boasted the productivity of horticultural crops by 10% and other arable crops by 20%. Many investigations have been made and attempts have been taken to mitigate the hazardous effect of salt stress, including trace elements. After numerous investigations, selenium (Se) has been found to be an effective one in improving growth and inducing tolerance mechanisms against salt stress. Supplementation of Se mitigates salt stress by reducing Na^+ accumulation in plant parts, Na^+ compartmentalization, upregulating Na^+ and Cl^- ions transporter genes, chelation and boosting of the antioxidant defense system. Selenium protects plants from oxidative stress by triggering the detoxification of ROS, which is generated due to salt stress (Subramanyam *et al.,* 2019; Kamran *et al.,* 2020; Hasanuzzaman *et al.,* 2020a).

Boron has a vital role in lignin synthesizing, strengthening of the cell walls and indirectly protecting cell membranes in plants (Osakabe *et al.,* 2014). It is a crucial one for fruit production, from the very beginning of the process like flower initiation and bearing, growth and development of flower parts, development and elongation of the pollen tube, germination of pollen, seed production and ultimately the development of fruit. Not only fruit but also root development is aided by the B because of its role of facilitating sugar supply to the plant roots (Rattan, 2015). Konuskan *et al.* (2017) reported that B actively participates in crop growth and development as an essential micronutrient.

Shahverdi *et al.* (2018) observed that in stevia, foliar application of Se alone and combined application of B, Se and Fe significantly increased the growth and yield attributes. In another study, exogenous application of Se, B and Fe improved the overall root characteristics, growth parameters, water status, nutrient uptake, yield and yield attributes under salt stress in stevia. Especially, foliar application of Se and B effectively alleviated the deleterious effect of salt stress (Shahverdi *et al.,* 2020).

Soybean (*Glycine max* L.) is the most widely cultivated legume around the world because of its versatile uses and economic importance (Liu *et al.,* 2020). Soybean is one of the most multipurpose, nutritionally and economically important legumes due to its unique seed composition (Shea *et al*., 2020). Soybean seed contains about 18 to 22% oil and 38 to 56% vegetable protein with favorable amino acid (USDA, 2018). It is a prominent source of proteins and edible oil, it has valuable uses as food, feed and oilseedcrop (Liu *et al*., 2020). Globally, soybean is responsible for about 61% of total international oilseed production and occupied 6% of the world's cultivable area (SoyStat, 2019). According to USDA (2019), about 336.11 million tons of soybean produced around the world, from the cultivated area of 121.69 million hectares with an average yield of 2.76 ton ha^{-1}. The United States, Brazil and Argentina are the leading soybean producing countries in the world and responsible for 81% of the total production. In Bangladesh, 0.986 million tons of soybean produced in 59,445 hectares land area, while global production was 348.7 million tons in 124.9 million hectares area (FAOStat, 2018). BBS (2018) reported that the total soybean cultivated area was 59443.46 hectares and total production was 98699 tons in Bangladesh. In our country, the demand for soybean as poultry feed was 0.94-1.13 million tons in 2015. In Bangladesh, there are 80 oil refineries with a total production capacity of 2.9 million tons. But only 48% of production capacity is utilized, so there is a huge demand for soybean in these industries (USDA, 2017).

In previous investigations, the roles of Se and B in improving the salt tolerance of plants were studied in the laboratory and hydrophonic condition, mostly. But, the interaction role of Se and B, along with individual applications, were hardly studied in the field condition and yield traits were rarely observed. To reveal the role of Se and B in alleviating different levels of salinity morphological, physiological, biochemical, phenotypic, anatomical and yield traits were measured in the present study with the following objectives:

i. To investigate the morphological, physiological and biochemical responses of soybean to salt stress
ii. To investigate the protecting role of Se and B in soybean under salt stress
iii. To evaluate the productivity of soybean applied with Se and B under salt stress

Chapter II

REVIEW OF LITERATURE

2.1 Soybean

Soybean (*Glycine max* L.) is the most widely cultivated legume around the world because of its multipurpose uses and economic importance. As it is a prominent source of proteins and edible oils, it has valuable uses as food, feed and oilseedcrop (Liu *et al.,* 2020). In Bangladesh, Noakhali and Lakshmipur are the leading regions in the soybean production (Miah *et al.* 2015; Salam and Kamruzzaman, 2015). Miah *et al.* (2015) reported that due to the adaptation of improved soybean varieties living standard of soybean farmers has been improved. Improved soybean variety cultivation ha^{-1} has created an opportunity for additional employment of 6.1 men day^{-1}. In the high growing regions of soybean, the average yield is 1813 kg ha^{-1} and the net return is 25,599 Taka ha^{-1}. The BCR of soybean is 1.43 and the DRC value is 0.55. All of these facts clarify that in Bangladesh, domestic production is more profitable than importing soybean (Salam and Kamruzzaman, 2015).

2.2 Importance of soybean

Soybean has a very special seed composition and because of that, it has versatile uses. It is one of the most multipurpose, nutritionally and economically important legumes due to its unique seed composition (Shea *et al.,* 2020). Globally, in 2018 soybean alone was responsible for about 61% of total international oilseed production and occupied 6% of the world's cultivable area (SoyStat, 2019).

According to the United Soybean Board (2019), the two main products of soybean are meal and oil, which have various important industrial uses. Soybean meal is responsible for 70% of soybean's value. About 97% of soybean meal is used as feed for poultry and livestock in the USA. In case of uses, soybean oil has uses as fuel, solvent, cosmetics, foam, soap and candles. In food industry, high oleic acid containing soybean oil is popular as edible oil because it provides a trans-fat free solution.

Singer *et al.* (2019) reported soybean as a major source of protein and essential amino acid for humans and livestock because of its well-balanced amino acid profile. It supplies a significant amount of protein, amino acid, oil and carbohydrate. It contains about 25% linoleic and 3% linolenic acid. Moreover, it is also enriched with antioxidant and anticarcinogenic properties (Sharma *et al.*, 2014).

Legumes are popular around the world as restorative crops, green manuring crops and cover crops and can easily be included in cropping patterns as main crop or inter-crop. Besides, these legumes show moderate tolerance against different abiotic stresses (Hasanuzzaman *et al.*, 2016). For the hay and silage, soybean can be cultivated as a pasture crop (Heuze *et al.*, 2015).

Soybean is a potential source of bioenergy, corn grain ethanol and soybean biodiesel are used as biofuels (Adie and Krisnawati, 2015). Legumes have promising positive effects on soil health, such as in soil restoration, improving soil nitrogen pool by biological nitrogen fixation (BNF) and by increasing soil organic carbon stock (Dhakal *et al.*, 2016). Soybean improves soil nitrogen (N) pool by accumulating about 53-76 kg N ha^{-1} (Dabin *et al.*, 2016). Legumes improve the biological property of soil by associating with soil microbes and by fixing nitrogen to the soil without disturbing the soil natural biota (Prashar and Shah, 2016). Inter-cropping of legume increases biological diversity, the interaction between or among crop species, reduces fertilizer requirement and decreases the chance of crop failure (Meena *et al.*, 2016).

Legumes have a role in decreasing the negative effect of climate change by mitigating greenhouse gases (Hasanuzzaman *et al.*, 2019). According to Hasanuzzaman *et al.* (2019), by 2050 the world population is expected to reach 9 billion as a result, food production need to be increased by 70%. In the case of providing food security and maintaining sustainability in agriculture, legumes are considered as the jack of all trades. As a member of Fabaceae family, soybean also plays the above mentioned vital roles of the legumes.

2.3 Abiotic stress

Abiotic stress is not an individual one. It comprises all types of hostile environmental conditions that a plant may face in nature (Bechtold and Field, 2018). Among different types of environmental perturbation or abiotic stresses drought, flooding, salinity, toxic metal/metalloid stress, high temperature, low temperature, UV-radiation, pollutants etc. are the major abiotic stresses encountered by plants in nature (Hasanuzzaman *et al.,* 2016; Bechtold and Field, 2018). Abiotic stresses or environmental stresses are the potential threats for crop productivity for upcoming decades. Plants are sessile organisms are more prone to adverse environmental conditions day by day due to continuously changing climatic conditions. Abiotic stress adversely affects plant's morphological, physiological and biochemical activity, ultimately causes a reduction in productivity (Hasanuzzaman *et al.,* 2017; Vishwakarma *et al.,* 2017; Lohani *et al.,* 2020). Directly or indirectly, crop productivity is related to the economy, which means abiotic stresses are potential threats to the economy (Singh *et al.*, 2015).

According to Hasanuzzaman *et al.* (2017), the productivity of crops is decreasing due to environmental stresses because of climate change. On the other hand, the world population is growing rapidly as well as the demand is also increasing for food, fiber, oil and other products and by-product yielding crops with an issue of food security. Food security is one of the major concerns of the 2030 Sustainable Development Goals (SDGs). Farming communities are on the frontline facing tremendous challenges to harvest the potential crop yield and to maintain sustainability in the agriculture sector due to climate change (FAO, 2017). In counter to the growing demand of the rapidly increased population, plant productivity is decreasing because of the negative effect of abiotic stresses. Sometimes more than 50% of crop reduction occurs due to abiotic stresses. To ensure global food security, improving plant stress tolerance is a prerequisite. For improving plant stress tolerance understanding the response of plants towards abiotic stress is a vital point (Hasanuzzaman *et al.,* 2017).

The future challenge is to parallel the demand for the food, feed, fiber, oil and biofuel, for the increasing population, which is expected to reach about 9 billion before 2050, by mitigating abiotic stresses for maximizing productivity (Noreen *et al.,* 2018).

2.4 Salt stress

Salt stress is considered as one of the most detrimental one among the abiotic stresses. It reduces the productivity of about 6% of the land area. About 20% of the irrigated land area and 17% of the total arable area are already salt-affected. The alarming issue is up to 50% of agricultural land loss may occur due to salinity by the next couple of decades. In soil solution, sodium chloride (NaCl) and sodium sulfate (Na_2SO_4) are the most commonly found soluble salts. Moreover, calcium sulfate ($CaSO_4$), magnesium sulfate ($MgSO_4$), potassium nitrate (KNO_3), sodium bicarbonate ($NaHCO_3$), etc. are also found in the soil solution and most of these are partially soluble in the solution. An increase in salinity in most of the cases refers to mainly an increase in Na^+ and Cl^- ions. Both Na^+ and Cl^- ions produce toxic conditions for the plant, but between them, Cl^- is more dangerous (Hasanuzzaman *et al.,* 2013; Choudhury *et al.,* 2013).

According to Shrivastava and Kumar (2015), salt stress is a potential threat to sustainable crop production. About 20 to 50% of crop productivity diminishes due to salinity in cultivated land area. In every minute, around 3 hectares of arable land are affected by salinity resulting in 10% increase in saline affected area every year. Because of climate change saline affected area is increasing more rapidly than before (Reddy *et al.,* 2017). About 20% of the cultivated land and 33% of the irrigated area in the world are salt-affected already. About 7% of the land content high rate of salt among the salt-affected area (Kibria *et al.,* 2017). Salinity creates osmotic stress and ionic toxicity. Osmotic stress occurs due to the accumulation of a higher concentration of salt ions in the root zone outside of the root cell. Osmotic stress hinders the uptake of water and nutrient of the plants. In the later stage, a higher accumulation of salt inside the cell and tissues induces ionic toxicity. Both ionic and osmotic stresses are responsible for overproduction of ROS. Therefore, an excess concentration of ROS in plants induces deleterious oxidative stress, which causes oxidation of plant cells, along with the cell organelles and membranes. Moreover, oxidative stress hampers the plant growth, physiological activity, biochemical process, productivity and can also cause cell and plant death (Munns, 2005; Munns and Tester, 2008; Hasanuzzaman *et al.,* 2016; Choudhury *et al.,* 2017; Hasanuzzaman *et al.,* 2020b).

Seed germination, vegetative and reproductive growth and nutrient balance are adversely affected by salt stress (Hussain *et al.,* 2018). Salt concentration, duration, genotypes of the plants, phytochemical quenching capacity, age of the plant and environment are the key factors that dteremine the degree of deleterious effect of salt stress (Shahverdi *et al.,* 2018).

Kamran *et al.* (2020) reported salinity as one of the hazardous abiotic stresses causes significant reduction in the growth and yield of crops. Salinity induced growth and yield reduction occurs due to wide spectrum alteration physiological and biochemical activity of salt stressed plants. Salt stress affects plant physiology and biochemical activity by reducing water potential in the soil, causing ionic imbalance, ionic toxicity and overproduction of ROS, which ultimately leads to yield reduction.

2.4.1 Effect on plant growth

In numerous studies, salinity has been found to be a detrimental one in decreasing the growth traits of the plants (Kamran *et al.,* 2020). Accumulation of salt at the root zone and inside the plant cell causes osmotic stress and ionic toxicity, which negatively affects the morphological features of plants. Salinity causes a reduction in growth traits (seed germination rate, seedling growth, biomass accumulation, leaf growth, number of flowers, number of fruit, chlorophyll content and water content, etc.) of plants (Kataria and Verma, 2018).

In maize, under salinity, about 28 and 25% reduction observed in dry weight (DW) of shoot and root compared to control (Jiang *et al.,* 2017). In oat seedlings, 100 mM NaCl-induced salinity causes a noticeable reduction of the shoot and root length, fresh weight (FW) and DW of the root, FW and DW of the shoot and relative water content (RWC). About 26% reduction in total biomass occurred in saline conditions, compared to control (Sapre *et al.,* 2018).

Kataria *et al.* (2017) reported that 100 mM NaCl-induced salt stress reduced the shoot length and root length by 22 and 30%, respectively, in maize compared to control. Moreover, a similar decreasing trend was observed in the case of seedling DW when subjected to salt stress, compared to control.

Wu *et al.* (2018) reported that in cucumber, 13% reduction in leaf area was caused by 25 mM NaCl stress but increased plant height by 3.36%. Moreover, 41.67% leaf area and 17.68% plant height reduction was observed at 50 mM NaCl stress. At 75 mM NaCl salt stress 60.52% leaf area and 45.95% plant height reduction occurred. The highest dose was 100 mM NaCl, which caused 82.21% decrease in leaf area and 64.13% decrease in plant height.

With increasing salinity plant height, number of branches, leaf and biological yield and leaf mass ratio decreased in stevia. Reduction in dry matter production, plant growth and cell elongation occurred due to salt stress (Shahverdi *et al.,* 2018). Crop growth and development is greatly hampered because of salt stress (Hachicha *et al.,* 2018).

In BARI Gom 21 and BARI Gom 25, germination percentage significantly decreased by 14 and 18% in case of both varieties at 200 mM NaCl. The highest percentage of unhealthy seedlings about 78.97% observed in the case of BARI Gom 21. Shoot FW dropped at 17, 51, 65 and 93% for BARI Gom 21 and 10, 41, 77 and 86% for BARI Gom 25 when induced to 50, 100, 150 and 200 mM NaCl. Fresh weight of root reduced at the rate of 19, 49, 62 and 84% in BARI Gom 21 and 14, 38, 71 and 86% in BARI Gom 25 at 50, 100, 150 and 200 mM NaCl-induced salt stress (Hasanuzzaman *et al.,* 2018).

Height, leaf number, internodes' length, FW and DW of leaves and roots dropped in cucumber plants because of salt stress. Salt stress decreased the plant height, the number of leaves and length of internodes by 61, 17 and 34%, in comparison to control. About 30, 44, 44 and 46% reduction occurred in leaf FW, leaf DW, root FW and root DW, in comparison to control (Mohsin *et al.,* 2019).

According to Kordrostami and Rabiei (2019), salt stress is induced by the increase in the concentration of the inorganic salts in soil solution, which affects the growth and development of the plant and nutrient at different plant growth stages (germination to ripening) in different degrees. Plant growth stunting is considered one of the most prominent effects of salt stress. The decrease in the rate of leaf area expansion for hindering the increase in salt concentration in the stressed plants count as the immediate response of plants to salinity stress. The photosynthesis rate was significantly reduced in the salt stressed plant. Therefore, it imposes a negative effect on growth and development by reducing carbohydrate supply to the growing cells. Reduction in photosynthesis and stomatal conductance greatly hampers the growth parameters of the plant in the salt stress.

Plant height, the number of branches, the number of leaves, root FW, root DW, root volume, root area, root fitness, root diameter, root density, root length and RWC were reduced in stevia at 30, 60 and 90 mM NaCl stress, compared to control (Shahverdi *et al.,* 2020).

Rasool *et al.* (2020) reported that upon exposure to 50 to 200 mM NaCl stress in proso millet root length reduced by 30.23 to 50.43%, in comparison to control. Similarly, in foxtail millet root length decreased by 33.16 to 57.4% when subjected to 50 to 200 mM NaCl stress. In proso millet and foxtail millet, shoot length reduced by 17.47 to 43.12% and 11.66 to 34.50%, respectively, under NaCl stress. In seedlings of proso millet, biomass reduced by 24.75 to 58.94% at 50 to 200 mM salt stress, in comparison to control. Moreover, in foxtail millet seedlings, biomass decreased by 32.20 to 78.06% with the increment of salt stress (50 to 200 mM NaCl), in comparison to control. When subjected to 50 to 200 mM NaCl stress, FW of proso millet reduced by 23.31 to 60.08% and FW of foxtail millet reduced by 21.15 to 64.32%, compared to control.

2.4.2 Effect on plant physiology

Salt stress threatens plant growth and development, mainly in two ways. Firstly, it affects by imposing osmotic stress on the plant, which, caused by reducing soil water potential, leads to limited water uptake and ultimately creates physiological drought for plants. Secondly, it causes ion toxicity by excessive ions uptakes, particularly Na^+ and Cl^- which adversely affects the metabolic activity of plants (Choudhury *et al.,* 2013).

Salinity induced osmotic stress, ion-specificity, nutritional and hormonal imbalance and oxidative damage adversely affects the plant physiological activity. Along with shoot growth and physiological activity, root growth and physiological activity are also hampered due to the negative effect of salt stress. Salt stress ultimately leads to plant death-causing disorganization of cellular membranes, inhibiting photosynthesis, generating toxic metabolites and declining nutrient absorption (Hasanuzzaman *et al.,* 2013).

Nutrient uptake and water uptake are reduced through root due to salt accumulation, which hinders the plant growth and eventually results in plant death. Redox homeostasis alteration is a primary effect of salt stress. Overproduction ROS occurs due to alteration in electron flow from the central transport chain in organelles to oxygen-reduction pathways. Reactive oxygen species cause oxidation of requisite biomolecules (lipids, proteins, nucleic acids and carbohydrates). Deterioration in their properties and functions ultimately hampers the metabolic and physiological activity. Imbalance in the homeostasis at the cellular and subcellular levels occurs because of overproduction of ROS, results in cell death (Ivanova *et al.,* 2016).

Kataria *et al.* (2017) investigated that in maize 25, 50, 75 and 100 mM NaCl stress reduced the germination percentage in a dose-dependent manner. The maximum reduction of germination percentage (11%) occurred under the highest salt stress, in comparison to respective control. Moreover, salt stress also caused a reduction in water uptake.

In maize, salt stress decreased chlorophyll (Chl) and carotenoid (Car) content by 24 and 14%, respectively, in comparison to control. Under salt stress, photosynthesis rate, stomatal conductance and transpiration rate were significantly reduced. Remarkable damage of Chl ultrastructure was observed under salt stress. Compressed grana lamellae, disrupted stroma lamellae and distorted thylakoids were observed in deteriorated chloroplasts. In comparison to control, salt-treated plants showed higher electrolyte leakage and lipid peroxidation, which were about 1.9 fold and 2.7 fold, respectively (Jiang *et al.,* 2017).

Under 100 mM salt stress Chl content and protein content significantly decreased in oat seedlings compared to the positive control. Biochemical marker of salt stress like proline (Pro), malondialdehyde (MDA), hydrogen peroxide (H_2O_2), electrolyte leakage (EL), super oxide dismutase (SOD), peroxidase (POD) also increased remarkably in salt stress condition, in comparison to control. However, H_2O_2 content was about 50% more in salt-stressed seedling than control plants (Sapre *et al.*, 2018).

Salt stress-induced ionic and osmotic stress hampers leaf development, cell elongation, water uptake, photosynthesis, protein synthesis and enzymatic activity. Moreover, salinity causes leaf senescence and cell death (Kataria and Verma, 2018). Wu *et al*. (2018) reported that salt stress deteriorated photosynthetic apparatus after ultrastructural observation of mesophyll cell. Under salt stress, the contents of intermediates involved in Chl branch were decreased.

In the cucumber plant, salt stress remarkably destroyed the photosynthetic pigment. Chl (*a*+*b)* content and Car content decreased by 41 and 39%, in comparison to control. A remarkable increase in EL (222%) was observed in cucumber plants under salt stress, in comparison to control (Mohsin *et al.*, 2019).

Rasool *et al.* (2020) observed that upon exposure to 50 to 200 mM NaCl stress, RWC decreased by 2.36-36.31 and 18.5-57.70% in proso millet and foxtail millet, respectively, in comparison to control. When subjected to salt stress (50-200 mM NaCl) proso millet and foxtail millet exhibited a remarkable reduction of Chl a content by 32.10-91.82 and 32.51-94.80%, respectively, in comparison to the control. Noticeable reduction occurred in Chl b content by 53.93-97.03% in proso millet under 50-200 mM NaCl stress with respect to control. Similarly, in foxtail millet Chl b content decreased by 47.97-83.69% upon exposure to salt stress (50-200 mM NaCl), in comparison to control. So, the total Chl content reduced by 37.97-90.68 and 40.84-93.90% in foxtail millet and proso millet, respectively, compared to control when treated with 50-200 mM NaCl. Moreover, anthocyanin content and Car content were reduced by 26.47-88.97 and 13.62-50.99% in proso millet under salt stress compared to control. Similarly, in foxtail millet, anthocyanin content and Car content decreased by 30.28-96.80% and 20.90-71.02%, respectively, under salt stress in comparison to control. Lipid peroxidation increased under salt stress in both millet species with the increase of the NaCl concentration. Moreover, EL increased by 23.89 to 46.54% in proso millet and EL increased by 12.12 to 35.15% in foxtail millet upon exposure to 50-200 mM NaCl, in comparison to control.

2.4.3 Effect on yield

Manivannan *et al.* (2016) reported that salinity had multidimensional negative effect on crop growth and yield. Salinity induced vascular transportation and electrochemical gradients led to osmotic imbalance in plant and reduction of yield. Under salt stress, there are several factors which led to ultimate yield reduction like a decline in germination rate, lower number of healthy seedlings, stunting of the plant, decrease in photosynthesis rate and dry matter accumulation (Rahman *et al.*, 2016).

Upon exposure to salt stress (50, 100 and 150 mM NaCl), three boro rice varieties (BRRI dhan45, BRRI dhan47 and Nipponbare) exhibited a reduction in the number of effective tiller hill^{-1}, panicle plant^{-1}, filled grain panicle^{-1}, 1000-grain weight, straw yield and grain yield. The maximum reduction was observed under the highest level of salt stress (Naim *et al.*, 2017).

Hussain *et al.* (2019) reported that salinity and yield had a reverse relationship, an increase in salinity was responsible for a decrease in yield. In rice cultivar LYP9, salt stress reduced the yield about 14.3, 41.6 and 85.5% at low (1.08 to 1.16 dS m^{-1}), moderate (3.2 to 3.27 dS m^{-1}) and high (4.64 to 4.71 dS m^{-1}) salinity, in compare to control. About 4.7 and 52.3% yield reduction was observed in another rice cultivar named NPBA cultivar, at low and medium salt stress and no significant yield production was observed under high salinity.

The point of concern is that globally about 90% of food supply depends on only thirty crop species. All of these species undergoes significant yield reduction when subjected to moderate salinity, that’s why salinity is counted as a threat for food security in a world of rapidly growing population (Zörb *et al.*, 2019).

2.5 Salt induced oxidative stress and antioxidant defense system

Under 100 mM salt stress, Chl content and protein content significantly decreased in oat seedlings, compared to the positive control. Biochemical marker of salt stress like proline, MDA, H_2O_2, EL, SOD, POD also increased remarkably in salt stress condition, in comparison to control. Moreover, H_2O_2 content was 50% more in salt stressed seedlings compared to control (Sapre *et al.,* 2018).

While conducting an experiment Hasanuzzaman *et al.* (2018) found that, salt stress increased the H_2O_2 content and lipid peroxidation level (denoted by MDA content). Moreover, salt stress increased H_2O_2 by 63 to 98%, compared to the control condition when the salt (NaCl) concentration increased 100 mM to 200 mM. Moreover, a significant enhancement in MDA content was also observed by 60 to 129% when the NaCl concentration increased from 100 mM to 200 mM compared to control. However, catalase (CAT) was considered as one of the important H_2O_2 scavenging enzyme. The activity of CAT remarkably declined with the increase in salt concentration. Moreover, 41% of CAT activity decreased under high salinity and in low salinity, it decreased by 32% in compare to control. Similarly, dehydroascorbate reductase (DHAR) and glutathione reductase (GR) activities showed descending result with ascending salt concentration. High salt stress was responsible for 44% decrease in AsA and low salt stress was responsible for 17% decrease in AsA, compared to control. On the other hand ascorbate peroxidase (APX) increased with the increase in salt concentration. In comparisonto control, seedlings accumulated more oxidized reduced glutathione (GSH) under salt stress. The remarkable increase in oxidized glutathione (GSSG) content was noticed under salt stress, compared to control which was by 116% in high salt stress and by 54% in low salt stress.

Salt stress threats the antioxidant defense mechanism of plants and deteriorates the cellular organelles by producing excessive amount of ROS (superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^{\bullet}$) etc.) at the cellular level, ultimately by creating the oxidative stress (Hasanuzzaman *et al.,* 2018).

According to Ahmad *et al.* (2019), under salt stress, the production of different types of enzymatic and nonenzymatic antioxidants were observed at cellular level. In saline conditions, an excessive amount of ROS hindered the activity of plant cells. Scavenging and defense activity was observed against the excessive ROS and ROS-induced oxidative stress at the cellular level. Cellular properties and enzyme activity could be protected by maintaining the antioxidant level. A plant produce enzymatic antioxidants like SOD, POD, CAT, APX and GR were significantly active against the oxidative stress. Different types of nonenzymatic antioxidants such as proline, glycine betaine, sugars, polyols and phenols also played a vital role in protecting plants under salt-induced oxidative stress. Moreover, the amount of ROS at the cellular level determined the destructive or signaling roles of the ROS.

2.6 Effect of salt stress on soybean

2.6.1 Effect on growth

Kumari *et al.* (2015) investigated that upon exposure to 100 mM NaCl shoot length and root length reduced by 46.67 and 50%, respectively, compared to control. In salt-stressed plants, drastic reduction (75%) was observed in the number of leaves, compared to control. Plant FW decreased by 48.65% under salt stress, compared to control. Similarly, the number of lateral roots was reduced by 33.33% when subjected to salt stress, in comparison to control. Moreover, Chl content and leaf water content were decreased by 70 and 65.51%, respectively, compared to control.

According to Klein *et al.* (2015), salinity was responsible for a noticeable reduction in root FW and DW, shoot FW and DW, total FW and DW, plant height, root length, leaf number and area of leaf in seedlings of soybean. Upon exposure to 120 mM NaCl plant height, DW plant^{-1} and leaf area were reduced in two soybean cultivars (Jackson and Lee68), compared to control (Wei *et al.*, 2015).

Plant height, plant FW, plant DW, leaf area, root length, root DW, Chl a content, Chl b content, total Chl content and Chl a/b ratio were decreased at 25 and 50 mM NaCl-stressed soybean plants, in comparison to control (Baghel *et al.*, 2016).

Zhang *et al.* (2016) studied that shoot length, root length, shoot DW, leaf area, the total number of leaves plant^{-1}and root DW of soybean decreased under salt stress.

Furthermore, the number of lateral roots and the number of fibrous roots were reduced in salt stress, compared to control. Moreover, the color of root was deepened in salt-stressed plants, in comparison to control plants.

Shu *et al.* (2017) investigated that 150 mM NaCl-induced salt stress lingered the process of germination by regulating GA and ABA ratio in three soybean cultivars (Hedou-19, Nandou-12 and C-103). Moreover, the rate of germination percentage, length of radicle and germinated soybean seed FW were also reduced under salt stress, compared to control.

Akram *et al.* (2017) reported that 150 mM NaCl-induced salt stress significantly reduced the plant height and branch number plant^{-1} in different genotypes of soybean. In comparison to control, under salt stress 24.32, 19.93, 19.12, 15.50, 14.67, 14.03,12.09, 8.86, 8.61, 7.78 and 7.03% plant height reduction observed in BARI soybean-05, SBM-09, BINA Soybean-01, Sohag, BINA Soybean-03, BINA Soybean-02, BINA Soybean-04, BARI soybean-06, SBM-18, SBM-22 and SBM-15, respectively. Salinity declined the branch number plant^{-1} by 28.42, 21.88, 17, 16.79, 15.79, 12.33, 12.33, 11.70, 11.70, 11.55 and 10% in BINA Soybean-03, Shohag, SBM-22, BARI soybean-05, SBM-15, SBM-18, BINA Soybean-02, SBM-09, BINA Soybean-01, BARI soybean-06 and BINA Soybean-04 respectively, in comparison to control.

Kataria *et al.* (2017) investigated that 0, 25, 50, 75 and 100 mM NaCl decreased the shoot length and root length in a dose-dependent manner, compared to control. Moreover, DW of seedling was also reduced when subjected to salt stress, compared to corresponding control.

Hamayun *et al.* (2017) reported that shoot length, leaf area, Chl content and transpiration rate decreased under 70 and 140 mM NaCl-induced salt stress, compared to control. Similarly, FW and DW also showed a decreasing trend under salt stress, in comparison to control.

Upon exposure to 0, 50 and 75 mM NaCl shoot weight, root weight and protein content were reduced. Root length reduced by 20.28 and 35% when subjected to 50 and 75 mM NaCl, compared to control. In comparison to control, sharp reduction occurred in root surface area, by 56.74 and 78.65% under 50 and 75 mM NaCl stress, respectively. Root diameter decreased by 23.93and 31.08% under 50 mM and 75 mM NaCl stress. Plant exhibited a noticeable reduction in root volume by 58.95 and 78.95% upon exposure to 50 mM and 75 mM NaCl stress (Egamberdieva *et al.,* 2017).

Salinity adversely affects plant growth, root architecture traits and biomass yield. Root architecture traits are assessed as root length, root volume, root DW, root FW and the number of nodules formed plant^{-1}, negatively affected by salt stress. Moreover, at 40 and 80 mM NaCl length of the root decreased by 32.8 and 59.4%, respectively, compared to control. The volume of root also decreased by 29.2 and 54.2% at 40 and 80 mM NaCl-induced salt stress. Similarly, at 40 and 80 mM NaCl the FW of the root reduced by 33.7 and 57.4%. Moreover, DW of the root reduced by 45.5 and 72.7% at 40 and 80 mM NaCl, respectively. Salinity also reduced the shoot growth and plant biomass. Moreover, Shoot DW and shoot FW were negatively affected by salt stress. Moreover, at 40 and 80 mM NaCl length of shoot declined by 22.8 and 39.3%, compared to control (El-Eswai *et al.*, 2018).

When subjected to different levels of salt stress, growth parameters were negatively affected. Shoot length was reduced by 9.7, 23 and 35.39% at 100, 200 and 300 mM NaCl stress, compared to control. Root length followed a similar trend. Root length reduced by 2.6, 12.46 and 24.08% when exposed to 100, 200 and 300 mM NaCl, compared to control. Moreover, shoot FW decreased by 9, 16.11 and 60.18% at 100, 200 and 300 mM NaCl, in comparison to control. Soybean plants exhibited a reduction in root FW under 100, 200 and 300 mM NaCl by 10.6, 20.28 and 35.48%, in comparison to control plants (Khan *et al.*, 2019).

According to Kataria *et al.* (2019) investigated that leaf weight and leaf size were significantly reduced under salt stress. The leaf area reduction was 16, 28 and 38% and the reduction rate of leaf weight was 12, 15 and 31%, respectively, compared to control at 50, 75 and 100 mM NaCl stress. Moreover, 150 mM NaCl-induced salt stress remarkably reduced the root FW. Fresh weight plant^{-1} also decreased when subjected to salt stress (Cheng *et al.*, 2020).

Soliman *et al.* (2020) reported that when subjected to 100 mM NaCl, shoot length and shoot DW plant^{-1} decreased by 40 and 29%, respectively, in comparison to control. Intercellular CO_2 and stomatal conductance reduced by 26.55 and 22.13%, respectively, compared to control. Furthermore, net Chl content, Car content and photosynthesis decreased by 46.22, 40.23 and 42.12%, respectively, in comparison to control.

2.6.2 Effect on physiology

Upon exposure to 0, 50, 100, 150, 200, 250 and 300 mM NaCl, eight soybean genotypes exhibited a reduction in seed germination. However, lower seed germination was observed at 200, 250 and 300 mMsalt stress in a dose-dependent manner (Kan *et al.,* 2015). Under 120 mM NaCl photosynthesis rate and K^+ content decreased in two soybean cultivars (Jackson and Lee68), compared to the corresponding control. On the contrary, leaf EL, root EL and Na^+/K^+ ratio enhanced in two soybean cultivars under salinity, in comparison to control (Wei *et al.*, 2015).

Salinity reduced the stomatal conductance, which ultimately responsible for a decrease in the photosynthesis rate of 3 soybean genotypes (S111-9, S113-6 and Melrose). Salinity declined the photosynthesis rate of about 13.6 to 34.1% in 3 soybean genotypes, in comparison to control. Better photosynthesis rate was observed in S111-9 and S113-6 than Melrose (He *et al.,* 2016). When subjected to 0, 25 and 50 mM NaCl-induced salt stress net photosynthesis rate, stomatal conductance, intercellular CO_2 concentration and transpiration rate were reduced in a dose-dependent manner (Baghel *et al.,* 2016).

Ji *et al.* (2016) reported that MDA content, SOD activity, POD activity and Na^+/K^+ ratio of leaves increased due to imposition of 200 mM NaCl in a duration-dependent manner, compared to control. On the other hand, Chl content was decreased due to salinity and the maximum chlorosis was in the highest duration of salt stress. Moreover, MDA content, SOD activity, POD activity and Na^+/K^+ ratio were also enhanced in root under 200 mM NaCl stress, in comparison to respective control.

Shu *et al.* (2017) studied that compared to control MDA content enhanced under 150 mM salt stress. Similarly, the activity of SOD, POD and CAT also showed the increasing trend under salt stress, in comparison to control. Moreover, salt stress reduced the GA and ABA ratio, compared to control.

Kataria *et al.* (2017) investigated that when subjected to 25, 50, 75 and 100 mM NaCl stress, germination percentage decreased in a dose-dependent manner. The maximum reduction of germination percentage (20%) was occurred under 100 mM NaCl-induced salt stress, in comparison to control. Moreover, water uptake was also decreased under salt stress.

According to El-Eswai *et al.* (2018), nutrient uptake, Chl content, transpiration rate, photosynthesis rate, stomatal conductance, soluble proteins content, soluble sugar content, total phenolics and flavonoids contents were reduced due to salinity. Phosphorus and nitrogen uptake significantly reduced by salinity. Soluble proteins content decreased by 13.5 and 26% at 40 and 80 mM NaCl, respectively. Soluble sugar content reduced by 10.1 and 24.2% at 40 and at 80 mM NaCl salt stress. Moreover, Chl content also decreased by 18.5 and 28.3% at 40 and 80 mM NaCl-induced salinity, compared to control. Proline content significantly increased by 2.4 times and 3.2 times, glycine betaine content enhanced by 1.7 times and 3 times under saline stress (40 and 80 mM NaCl). Compared to control, salt stress (40 mM NaCl and 80 mM NaCl) increased H_2O_2 content by 3 times and 3.7 times, in comparison to control. Under salt stress MDA content is enhanced by 2.2 times and 2.7 times, in comparison to control. High salinity significantly reduced gas-exchange parameters (transpiration rate, stomatal conductance and photosynthesis). In comparison to control, phenolic content mitigated by 19.6 and 35.4%, flavonoid content alleviated by 33.8 and 53.5% under 40 and 80 mM NaCl-induced salt stress.

Upon exposure to 100, 200 and 300 mM NaCl-incduced salt stress, SPAD value was reduced by 3.58, 8.70 and 12.16%, compared to control (Khan *et al.*, 2019).

Soliman *et al.* (2020) reported that lipid peroxidation, H_2O_2 content, Pro content, soluble sugar content, glycine betaine content, tocopherol and reduced glutathione content increased at 100 mM NaCl stress. On the other hand, RWC content, ascorbic acid content and phenol content decreased when subjected to 100 mM NaCl-induced salt stress.

2.6.3 Effect on yield

Soybean plants exhibits yield reduction when the salinity exceeds 5 dS m^{-1} (Ashraf and Foolad, 2007). Salinity affects the seed yield and quality of the soybean. Due to the salinity, the yield and yield attributes of soybean in terms of branches $plant^{-1}$, number of pods $plant^{-1}$ and 1000 seed weight significantly decreased. Oil content, protein content, mineral content, soluble carbohydrate content and amino acid content decreased under salinity (El-Sabagh *et al.*, 2015). According to Hasanuzzaman *et al.* (2016), soybean varieties encountered a significant reduction in quality traits and yield when subjected to salt stress. The number of pods $plant^{-1}$, the number of seed $plant^{-1}$ and seed weight was decreased under 0-50 mM NaCl-induced salt stress (Baghel *et al.*, 2016).

He *et al.* (2016) reported that, salinity decreased the yield and yield contributing parameters of 3 soybean genotypes (S111-9, S113-6 and Melrose). Salinity reduced the number of pod $plant^{-1}$ and the number of seed $plant^{-1}$ in S111-9, S113-6 and Melrose. The lowest reduction occurred in the number of pod $plant^{-1}$ (46.1%) and the number of seed $plant^{-1}$ 49.9% in S111-9 genotypes. Moreover, by 36.1and 36.7% reduction occurred in the number of pods and the number of seed $plant^{-1}$in S113-6. The highest reduction observed in Melrose, the number of pod $plant^{-1}$ (39.7%) and the number of seed $plant^{-1}$ (51.4%).

Akram *et al.* (2017) conducted an experiment by inducing salinity (150 mM NaCl) at the reproductive stage and used 11 genotypes of soybean to study the plant response towards the salinity stress. BINA Soybean-1, BINA Soybean-2, BINA Soybean-3, BINA Soybean-4, BARI soybean-5, BARI soybean-6, Shohag, SBM-9, SBM-15, SBM-18 and SBM-22 were the 11 genotypes. Salinity is responsible for sharp decline in number of pod $plant^{-1}$, BINA Soybean-03 (51.69%), SBM-15 (49.05%), BARI soybean-06 (48.17%), BARI soybean-05 (46.80%), BINA Soybean-04 (44.80%), BINA Soybean-01 (43.24%), Sohag (42.21%), SBM-22 (40.03%), SBM-18 (38.27%), BINA Soybean-02 (33.72%) and SBM-09 (31.93%) respectively. Moreover, 150 mM NaCl induced salt stress reduced the number of seed pod^{-1} for 11 soybean genotypes, in BINA Soybean-04 (9.55%), SBM-22 (8.25%), Sohag (7.32%), BINA Soybean-02(6.31%), BARI soybean-05 (6.47%), BINA Soybean-03 (5.53%), SBM-15 (5.45%) and SBM-09 (5.08%), respectively, compared to control. In comparison to control, 150 mM NaCl stress caused a reduction in the number of seed plant^{-1}, by 53.85, 51.35, 50.28, 49.98, 49.46, 46.22, 45.16, 44.96, 40.44, 37.83 and 35.27% in BINA Soybean-03, SBM-15, BARI soybean-05, BARI soybean-06, BINA Soybean-04, Sohag, BINA Soybean-01, SBM-22, SBM-18 and SBM-09, respectively. According to an analysis of yield performance, BINA Soybean-1 and BINA soybean-2 showed the maximum tolerance against the salinity stress. On the other hand, BINA soybean-4 was the one with minimum tolerance.

In an experiment, Farhangi-Abriz and Ghassemi-Golezani (2018), observed a significant reduction in biomass and seed yield under salinity stress in soybean. About 39% plant biomass reduction and about 44% yield reduction occurred under salt stress.

Total biomass accumulation, rate of photosynthesis, crop yield and harvest index decreased as the salinity level increased at 50, 75 and 100 mM NaCl. The maximum reduction of photosynthesis 54% occurred at 100 mM salt stress compared to normal conditions. The reduction in photosynthesis rate caused low carbon accumulation and led to lower yield. As the salinity increased, the number of pod plant^{-1} remarkably decreased (Kataria *et al.*, 2019).

2.6.5 Oxidative stress in soybean under salt stress

While working with 11 soybean genotypes, Akram *et al.* (2017) reported that 150 mM NaCl-induced salt stress was responsible for remarkable enhance in H_2O_2 content and MDA content in the cellular level. BINA Soybean-1, BINA Soybean-2, BINA Soybean-3, BINA Soybean-4, BARI soybean-5, BARI soybean-6, Sohag, SBM-9, SBM-15, SBM-18 and SBM-22 were the 11 soybean genotypes. Compared to control

H_2O_2 content was the maximum in BINA Soybean-03 (150.49%). The maximum MDA content was also recorded in BINA Soybean-03, which was about 63.43% in comparison to control. High salinity enhanced the level of osmolytes, the activities of antioxidant enzymes (APX, CAT, SOD and POD) and reduced the amount of ROS in soybean (El-Eswai *et al.,* 2018).

According to Kataria *et al.* (2019), in soybean, activities of major ROS-scavenging antioxidant enzymes like SOD, APX, GR and POD significantly enhanced under salinity, compared to control. But the reduction in α-tocopherol content occurred with an increase in salt concentration from 0 to 100 mM NaCl. Soliman *et al.* (2020) studied that activity of SOD, CAT, APX and GR increased under 100 mM NaCl, compared to control.

2.7 Foliar spray of Se and B

In combating salt-induced oxidative stress, increasing plant growth and yield exogenous protectants (osmoprotectants, phytohormones, polyamines, antioxidants and trace elements) showed promising result (Hasanuzzaman *et al.,* 2013). Trace elements increase plant stress tolerance by enhancing growth and physiological activities (Hasanuzzaman *et al.,* 2017).

In developing countries, malnutrition is becoming more prominent issue day by day. The staple crop is the key factor to fight hunger in developing countries and as it is not sufficient to supply proper nutrients, so it fuels the malnutrition issue. Biofortification is gaining popularity to fight back malnutrition. Biofortification of nutrients and vitamins such as selenium (Se), zinc (Zn), iron (Fe), folic acid, vitamin A and lysine can mitigate the deficiency of these nutrients in staple crops and other crops (Dixit *et al.,* 2018).

According to Noreen *et al.* (2018), due to climate change, plants are more exposed to abiotic stresses, which are the hindering factors of plant growth and productivity. By foliar application and soil application plant nutrients are supplied. In adverse environments where soil application is not cost-effective foliar application is a judicial way to supply plant nutrients. In the case of micronutrients, foliar application boasted the productivity of horticultural crops by 10% and other arable crops by 20%.

2.7.1 Effect of Se on plant growth and salt stress mitigation

In many studies, supplementation of a trace amount of Se dramatically improved the plant growth and physiological activity by mitigating the adverse effect under different abiotic stresses, including salt stress (Chauhan *et al.,* 2017; Mroczek-Zdyrska *et al.,* 2017; Jiang *et al.,* 2017; Djanaguiraman *et al.,* 2018; Subramanyam *et al.,* 2019). The application of Se protects plant under salt stress by improving the protection mechanism (Hasanuzzaman *et al.,* 2020a). Moreover, numerous studies have reported the role of Se in the improvement of yield and yield contributing parameters (Nawaz *et al.,* 2016).

Under salt stress, foliar application of Se increased FW, DW, pod length, pod weight and other yield parameters. Exogenous application of 10 µM Se at 50 mM NaCl achieved maximum fresh and dry weights. Foliar application of Se at 50 mM NaCl significantly increased leaf area. All yield parameters showed the lowest result under 50 mM NaCl and on the other hand, foliar application 5 µM Se without salinity achieved the highest yield performance. Under salinity, induced by 50 mM NaCl, foliar application of 10 µM Se produced the highest number of seed pod^{-1} and significantly increased the number of pod plant^{-1} in cowpea. Foliar application of 10 µM Se under 50 mM NaCl gained the highest 100 seed weight (20.7 g). A similar positive effect was observed in case of yield plant^{-1} under 50 mM NaCl salt stress with the application of 10 µM Se foliar spray. Foliar application of Se under salt stress showed a positive effect on photosynthetic pigment content, Pro content, protein content, total soluble sugar content, the activity of SOD and activity of POD. Exogenous application of 10 µM Se at 50 mM NaCl achieved the highest Car content. The highest rate of SOD activity was observed under 50 mM salt stress with foliar application of 10 µM Se (Manaf *et al.,* 2016).

Jiang *et al.* (2017) investigated that supplementation of 1 µM Se improved the growth and development of maize and also noticeably enhanced the shoot and root DW by 17 and 18%, respectively, under salinity in comparison to control. A similar increasing trend was observed due to the application of Se under salinity in case of Chl content (13%), Car content (7%) and photosynthesis rate (19%), compared to control. Application of 1 µM Se also increased the stomatal conductance and transpiration rate under salinity. Moreover, 100 mM NaCl-induced structural damage of chloroplasts ameliorated due to supplementation of 1 µM Se. More integrated internal lamella, thicker grana lamellae and better shape of thylakoids were observed in Se treated plants. Supplementation of 1 and 5 µM Se reduced the EL by 30 and 18% at 100 mM NaCl. Foliar applied 1 µM Se improved the K^+ ions in shoot, on the other hand, decreased Na^+ ions in root of maize. Moreover, 1 µM Se enhanced the activity of SOD (13%) and POD (24%) in maize at 100 mM NaCl-induced stress.

Khalifa *et al.* (2016) studied that the growth traits, RWC, Chl content, Car content, soluble sugars content and yield increased under salinity due to application of 16 and 32 µM Se, in comparison to control.

Shalaby *et al.* (2017) reported that leaf number, leaf area plant, DW of leaf and Chl content was increased under saline condition due to the application of Se. The activity of CAT and APX increased by foliar and soil-applied Se under salinity, compared to control. On the other hand, salinity increased the EL and supplementation of Se decreased the EL in salt stressed plants.

Moussa and Hassen (2018) reported that in common bean at 100 mM NaCl application of 5 and 10 µM Se increased the dry matter by 71.1 and 100% respectively, compared to control. Supplementation of 5 and 10 µM Se increased the yield traits under saline condition. Application of 5 and 10 µM Se increased the number of pod (73.17 and 117.07%) at 100 mM NaCl stress, in comparison to control. Moreover, 5 and 10 µM Se increased the seed yield by 51.21 and 73.90% under salinity, in comparison to control. Foliar application of 5 and 10 µM Se increased the Chl content by 72.17 and 152.1% in saline condition, compared to control. Foliar applied Se increased the Car content by 281.53% and RUBPCase activity by 183.3% under salinity, compared to control. Similarly, an increasing trend was also observed in photosynthesis rate which was by 150.52 and 313%, compared to control due to exogenous application of 5 and 10 µM Se, respectively under salinity. Moreover, at 100 mM NaCl stress foliar applied 5 and 10 µM Se enhanced the RWC (33.33 and

60.46%) and membrane stability index (MSI) (37.38 and 64.04%) in common bean. However, in saline condition, supplementation of 5 µM Se reduced the MDA content, Pro content and EL by 10.47, 52.33 and 16.6%, respectively, compared to control. Similarly, supplementation of 10 µM Se diminished the MDA content, Pro content and EL by 14.74, 39.33 and 11.11%, respectively. Moreover, the application of Se increased the SOD, POD and CAT activity under salinity, in comparison to control.

Ashraf *et al.* (2018) investigated in maize, exogenous application of 20 mg L^{-1} Se improved the growth and Chl content, antioxidant activity (SOD, POD and CAT) and declined the MDA and H_2O_2 content under 12 dS m^{-1} salt stress.

According to the study of Elkelish *et al.* (2019a), the application of 5 µM Se modulated the deleterious effect of 100 mM NaCl-induced salt stress in wheat. Supplementation of 5 µM Se enhanced the growth, RWC, photosynthetic pigments content, photosynthesis rate, Pro content, sugar content, antioxidant enzymatic activity and also reduced the H_2O_2 content at 100 mM NaCl stress.

Astaneh *et al.* (2019) studied that Se-supplemented onion had better root biomass, bulb diameter, bulb height and photosynthetic pigment content. Moreover, the application of Se reduced the ion leakage under saline condition.

While working on rice, Subramanyam *et al.* (2019) found that Se had positive effects in mitigating salt stress but the optimal concentration is very vital in achieving the positive effects. Both seed priming and foliar spray of Na_2SeO_4 provided protection against salt-induced (150 mM NaCl) damages. When the plants were supplemented with Se (6 mg L^{-1} Na_2SeO_4), they increased the biomass. Exogenous Se also increased the Pro contents and decreased H_2O_2 content and MDA content, which helped in alleviating salinity-induced oxidative stress stress in rice plants. It was due to the enhanced activity of antioxidant enzymes (SOD, APX, CAT, and GSH-Px) in Se-supplemented plants.

Rasool *et al.* (2020) studied the role of 1 µM foliar-applied Se on proso millet and foxtail milletunder 50 to 200 mM NaCl-induced salt stress. According to their study, the exogenous application of 1 µM Se caused the highest increase in biomass (49.09

and 293.28%), RWC (14.49 and 20.42%), EL (15.62 and 49.18%), thiobarbituric acid reactive species (42.34 and 34.20%) and photosynthetic pigment content (228.86 and 507.22%) in both proso millet and foxtail millet respectively, in comparison to maximum salt stress (200 mM NaCl).

According to Kamran *et al.* (2020), as salt stress threats plant productivity, many investigations have been made and attempts have been taken to mitigate the hazardous effect of salt stress, including phytohormones, osmoprotectants, antioxidant, polyamines and trace elements. After numerous investigations, Se has been found to be an effective one in improving growth and inducing tolerance mechanisms against salt stress. Selenium mitigates salt stress by reducing Na^+ accumulation in plant parts, Na^+ compartmentalization, upregulating Na^+ and Cl^- ions transporter genes, chelation and boosting of the antioxidant defense system. Selenium protects plants from oxidative stress by triggering the dismutation of ROS generated due to salt stress.

2.7.2 Effect of B on plant growth and salt stress mitigation

Boron is an important element for lignin synthesizing, strengthening the cell wall and also protecting the cell membranes of plants, indirectly (Osakabe *et al.,* 2014). Boron is a vital element which plays crucial roles in fruit production, from the very beginning of the process like flower initiation and retention, growth and development of flower parts, development and expansion of pollen tube, pollen germination, seed production and ultimately the development of fruit. Not only fruit but also root development is aided by the B because of its role of facilitating sugar supply to the plant roots (Rattan, 2015).

Jabeen and Ahmad (2011) reported that growth and development decreased under salinity in a dose-dependent manner. On the the contrary, foliar-applied B increased the growth parameters remarkably under different levels of salinity and control, in comparison to control. Plant height decreased by 13.5 and 36.5% at 6.1 dS m^{-1} and 10.8 dS m^{-1} salinity, respectively, in comparison to control. Supplementation of B increased the plant height under normal and saline conditions. Similarly, stem diameter $plant^{-1}$ decreased by 15.8% at 6.1 dS m^{-1} and by 35.8% at 10.8 dS m^{-1}, in comparison to control. Moreover, number of leaves $plant^{-1}$ also showed a decreasing trend under salinity. Number of leaves plant^{-1} decreased by 10.2% at 6.1 dS m^{-1} and by 18.4% at 10.8 dS m^{-1}, compared to control. Supplementation of B increased the stem diameter and number of leaves plant^{-1} in saline and non saline condition. However, the highest reduction of average leaf area plant^{-1} occurred under the maximum salt stress, which was by 38.9%, in comparison to control. Application of B, along with Mn enhanced the average leaf area plant^{-1} by 22.5%, compared to control. Fresh weight plant^{-1} decreased by 20.9 and 41.1% under lower and severe salinity, respectively, compared to control. Similarly, under low and severe salinity dry weight plant^{-1} was also decreased by 22.6 and 43.7%, in comparison to the corresponding control. However, foliar application of B noticeably increased all the growth parameters under different levels of salinity, compared to control. Furthermore, yield and yield contributing parameters also showed a decreasing trend like growth parameters under salt stress. But, foliar application of B alleviated the negative effect of different levels of salinity and increased the yield and yield contributing parameters. The maximum salinity caused floral head diameter reduction by 37% compared to control. Foliar supplementation of B along with Mn increased the floral head diameter under salinity. The number of seed head^{-1} and weight of seed head^{-1} decreased under different levels of salinity. On the other hand, foliar supplementation of B remarkably increased them under salinity. Moreover, salinity showed a detrimental effect on pollen viability and pollen germination. On the contrary, foliar-applied B remarkably enhanced the pollen germination rate and pollen viability.

Jabeen *et al.* (2013) investigated that foliar application of B enhanced the yield and yield quality under salt stress in sunflower. Exogenous application of B increased the number of seed, the weight of seed, seed oil content, palmitic, stearic and linolenic fatty acid content in sunflower under salt stress.

Foliar application of B along with Mn and Zn increased the root length, shoot length, plant FW and DW in methi under NaCl stress (Ibrahim and Faryal, 2014). Zafar-ul-Hye *et al.* (2016) investigated that in wheat plant height, the number of tillers, grain spike, grain yield, straw yield and biological yield increased due to application of B in saline condition.

Shahverdi *et al.* (2017) studied that combined application of Se, Fe and B increased the growth parameters in stevia under saline condition. Especially, root growth traits like root length, root volume, root diameter and root DW increased at 3, 6, 9 dS m^{-1} NaCl stress, compared to control. Moreover, application of B along with Se and Fe increased the photosynthetic pigment content in saline condition, compared to respective control.

Konuskan *et al.* (2017) reported that B actively participated in crop growth and development as an essential micronutrient. In corn B significantly affected the protein ratio, starch ratio, oil yield and oil components. A higher concentration of B at 6 and 8 mg m^{-1} while foliar applying enhanced the oleic acid, palmitic acid and stearic acid accumulation in corn.

Shahverdi *et al*. (2018) observed that in stevia the foliar application of selenium alone and combined application of B, Se and Fe significantly increased the growth and yield. Gul *et al.* (2019) reported that in cowpea, supplementation of 5 ppm B increased the plant height, length of root, FW of plant, DW of plant, RWC, Chl a content, Chl b content, total Chl content, Chl a/b ratio, net carbohydrate and net protein at 2.5 and 5 dS m^{-1} salt stress. Moreover, K^+ content in stem, root and leaves also increased due to the application of B under saline condition. However, the application of B decreased the Na^+ content in stem, root and leaves.

Foliar application of B, Se and Fe increased the plant height, number of branches, number of leaves, root FW, root DW, root volume, root area, root fitness, root diameter, root density, root length, RWC, leaves yield and biological yield at under 30, 60 and 90 mM NaCl stress, in comparison to control (Shahverdi *et al.*, 2020).

In previous studies, salt stress decreased the growth and productivity of crops including soybean. However, application of Se and B increased the growth, development and productivity of plants under saline and nonsaline conditions.

Chapter III

MATERIALS AND METHODS

In this chapter a brief description experiment is presented.

3.1 Experimental Site

This experiment was conducted at the Department of Agronomy, Sher-e-Bangla Agricultural University farm, Dhaka-1207, under the Agro-ecological zone of Madhupur Tract, AEZ-28 from November, 2019 to January, 2020. The land area is located at 23°41′N latitude and 90°22′E longitude.

3.2 Weather condition of the experimental site

Sher-e-Bangla Agricultural University farm, is located in the sub-tropical climatic zone. High temperature, high humidity and heavy rainfall are the salient characteristics of this zone. Other climatic features are scattered rainfall with low temperature during rabi season (October-March) and monsoon rain with gusty winds in kharif season (April-September). The weather data of the experimental site was recorded by the meteorological center, Dhaka (Appendix I).

3.3 Materials

3.3.1 Plant material

BINA Soybean-5 was used as the plant material for conducting the experiment. The variety was released in 2017 by Bangladesh Institute of Nuclear Agriculture (BINA). Year-round cultivation of this variety is possible. Characteristics of BINA Soybean-5:
Primary number of branches: 2-3

Seed pod^{-1}: 2-3
Seed: creamy white in colour and medium in size
Protein content: 43.5%

Oil content: 18.2%

Carbohydrate content: 27%

3.4 Treatments

The plants were treated with the following 16 treatments:

1. Control
2. Se (0.50 µM Na_2SeO_4)
3. B (1 mM H_3BO_3)
4. Se+B
5. S_1 (150 mM NaCl)
6. S_1+Se
7. S_1+B
8. S_1+Se+B
9. S_2 (300 mM NaCl)
10. S_2+Se
11. S_2+B
12. S_2+Se+B
13. S_3 (450 mM NaCl)
14. S_3+Se
15. S_3+B
16. S_3+Se+B

Stress treatments (mild, moderate and severe) were applied at 20 DAS and at 35 DAS. Trace elements (Se, B and Se+B) were applied as a foliar spray at 20 DAS. Foliar application of trace elementswere continued at 3 days interval until the pod filling stage. Foliar application of B was done with freshly made 1 mM H_3BO_3 solution. In case of Se, 1 mM stock solution of Na_2SeO_4 was prepared and stored in the refrigerator. It was diluted to 0.50 µM for every foliar application of Se.

3.5 Design and layout of the experiment

The experiment was laid out in randomized complete block design (RCBD) with three replications. There were two sets of pot for conducting this experiment. One set was for measuring the growth, physiological and biochemical parameters (destructive data) and another one was for measuring yield and yield contributing parametrs.

3.6 Seed collection

Seeds of BINA Soybean-5 were collected from Oilseed division of Bangladesh Institute of Nuclear Agriculture, Mymensingh.

3.7 Soil preparation for pot

To prepare well pulverized and healthy soil for the experiment, soil was collected and then sun-dried and crushed. After that recommended basal dose of organic manures and fertilizers were incorporated with the prepared soil. Besides, the fertilizers and organic manures, Furadan®5 G was also mixed with the soil at the recommended dose to protect the seedlings from insect, mites and nematodes. Each pot was filled up with well pulverized soil containing organic manures, fertilizers and insecticide.

3.8 Fertilizer application

Fertilizer and manure dose for BINA Soybean-5 as follows:

Fertilizers	Dose (kg ha^{-1})
Cowdung	5000
Urea	25-30
Triple superphosphate	60-70
Murate of potash	35-40
Gypsum	35-45

All fertilizers and manures were incorporated during final soil preparation.

3.9 Seed sowing technique

Before seed sowing pot soil was irrigated with sufficient water to achieve the field capacity of soil for seed sowing. After that, twenty healthy seeds were sown at 5 cm depth in each pot.

3.10 Intercultural operations

3.10.1 Gap filling and thinning

Gap filling and thinning was done at 12 DAS to maintain the uniform plant density in each pot.

3.10.2 Weeding, mulching and irrigation

The pots were kept weed free by regular observation and hand weeding. Mulching and irrigation applications were done when needed.

3.10.3 Plant protection measure

Hairy caterpillar attacked the plants at 25 DAS. Sumithion$^{®}$57 EC was applied twice at 7 days interval (25 DAS and 33 DAS) to protect the plants. Furadan$^{®}$5G was mixed with the soil to protect the plants from insects, mites and nematodes.

3.11 General observation of the experimental pots

Plants were observed on a regular basis not only to find out the visual differences among the treatments but also to protect the plants from diseases, insects and pests.

3.12 Collection of data

The yield and yield contributing parameters were measured at harvest. Growth, physiological, biochemical, phenotypical and anatomical parameters were recorded after the completion of the treatment duration.

Data were collected on the following parameters:

3.12.1 Crop growth parameters:

- Plant height
- Root fresh weight plant^{-1}
- Root dry weight plant^{-1}
- Shoot fresh weight plant^{-1}
- Shoot dry weight plant^{-1}
- Number of branches plant^{-1}
- Leaf area

3.12.2 Physiological parameters:

- SPAD value of leaf
- Relative water content
- Proline content

3.12.3 Oxidative stress indicators

- Lipid peroxidation
- H_2O_2 content

3.12.4 Yield and yield contributing parameters

- Number of flowers plant^{-1}
- Number of pods plant^{-1}
- Pod length

- Number of seeds pod^{-1}
- Seed yield plant^{-1}
- Stover yield plant^{-1}
- Biological yield plant^{-1}

3.12.5 Phenotypical observation

3.12.6 Anatomical observation

3.12.7 Correlation among growth, physiological and biochemical parameters

3.12.8 Correlation among yield parameters

3.13 Measurement procedures of growth parameters

3.13.1 Plant height

The height of the plants was recorded after the duration of the treatment was completed, at 7 days interval from 28 DAS to 42 DAS. The height of the plant was counted starting from the ground level up to the apex of the leaves. Plant height of five plants from each pot were measured, then averaged and the average plant height was considered as the plant height.

3.13.2 Root fresh weight plant^{-1}

After the completion of the treatment duration, sample plants were randomly uprooted from each pot. After that the root of sample plants were washed, weighed and the weight was averaged to determine the shoot FW.

3.13.3 Root dry weight plant^{-1}

After measuring the FW of shoot, plant shoot samples were oven dried at 70 °C for 48h, followed by weighing. The average DW was considered as the shoot dry weight for each pot**.**

3.13.4 Shoot fresh weight plant^{-1}

After the completion of the treatment duration, sample plants were randomly uprooted from each pot. After that the shoot of sample plants were weighed and averaged to determine the shoot FW.

3.13.5 Shoot dry weight plant^{-1}

After measuring the FW of shoot, plant shoot samples were oven-dried at 70 °C for 48h, followed by weighing. The average DW was considered as the shoot dry weight for each pot.

3.13.6 Number of branches plant^{-1}

After the completion of the treatment duration, branches of five plants were counted and then averaged to determine the number of branches for plant^{-1}.

3.13.7 Leaf area

For leaf area measurement, first leaf images were taken by a digital camera and the area was calculated by using Image-J software.

3.14 Procedures of measuring physiological parameters

3.14.1 SPAD value

After completion of the treatment duration, five leaves were randomly selected from each pot. The top, middle and bottom of each leaflet was measured with at LEAF (FT Green LLC, USA) as at LEAF value. Then, the values were then averaged, total chl content was determined by the conversion of at LEAF value into SPAD units.

3.14.2 Relative water content

Leaf relative water content was measured according to Barrs and Weatherly (1962). The FW of the entire leaf disc was weighed, then floated on water in Petri dishes and kept in the dark place. Twelve hours later, after removing the extra surface water, the leaf disc was weighed again, which was considered as turgid weight (TW). Then, DW was weighed after drying at 80 °C for 48h. By using the following formula, the RWC of the leaf was calculated.

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

3.14. 3 Measurement of proline content

In leave tissues, free proline content was estimated ccording to the method of Bates *et al.* (1973). Fresh leaf tissue (0.25 g) was homogenized well in 5 ml of 3% sulfosalicylic acid with pre-cooled mortar and pestle on ice. After homogenization, the homogenate was centrifuged at 11,500×g for 15 min. 1 ml of the supernatant was than mixed with 1 ml of acid ninhydrin (1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid) and 1 ml of glacial acetic acid. The mixture was placed at 100°C in a water bath for 60 minutes, and then the reaction was terminated by placing the tube in ice bath for 15 min, after the reaction mixture get cooled, 2 ml of toluene was added with the reaction mixture and thoroughly vortexed for 20-30sec. The upper aqueous layer (toluene layer) was immediately transferred to new test tube and kept in room temperature for next 10 minutes. Finally, the optical density of the chromophore containing toluene was read spectrophotometrically at 520 nm using toluene as a blank. The amount of proline was calculated from the standard curve using laboratory grade proline.

3.15 Procedure of measuring oxidative stress indicators

3.15.1 Measurement of lipid peroxidation

According to Heath and Packer (1968), with slight modification by Hasanuzzaman *et al.,* (2012), Malondialdehyde (MDA) content was estimated to determine the level of lipid peroxidation in leaf tissues. Leaf samples (0.5 g) were homogenized in 3 mL 5% (w/v) trichloroacetic acid (TCA), and the homogenate was centrifuged at 11,500 × g for 15 min. After that, the supernatant (1 mL) was mixed with 4 mL of thiobarbituric acid (TBA) reagent (0.5% of TBA in 20% TCA). The reaction mixture was heated at 95 °C for 30 min in a water bath and then quickly cooled in an ice bath and centrifuged again at 11,500 × g for 10 min. The absorbance of the colored supernatant was measured at 532 nm and was corrected for non-specific absorbance at 600 nm. MDAcontent was estimated by using extinction coefficient 155 mM^{-1} cm^{-1} and expressed as nmol g^{-1} FW.

3.15.2 Determination of hydrogen peroxide content

Hydrogen peroxide (H_2O_2) was determined according to the method of Yu *et al.* (2003). Leaf tissue (0.5 g) was homogenized with 3 mL of 50 mM potassium–phosphate (K–P) buffer (pH 6.5) at 4 °C. The homogenate was centrifuged at 11,500 × g for 15 min. The supernatant (2 mL) was mixed with 666.4μLof 0.1% TiCl4 in 20% H_2SO_4 (v/v) and was kept at room temperature for 10 min. After that, the mixture was again centrifuged at 11,500 × g for 12 min. The supernatant was then measured spectrophotometrically at 410 nm to determine H_2O_2 content using the extinction coefficient 0.28 μM^{-1} cm^{-1} and was expressed as nmol g^{-1} FW.

3.16 Procedures of measuring yield and yield contributing parameters

3.16.1 Number of flowers plant^{-1}

After the completion of the treatment duration, flowers of five plants were counted and then averaged to determine the number of branches for plant^{-1}.

3.16.2 Number of pods plant^{-1}

After germination 5 plants were allowed to grow in each pot. The total number of pods from each pot were collected and counted, then averaged to measure the number of pod plant^{-1}**.**

3.16.3 Pod length

Randomly 10 pods were taken and then pod length was measured and then averaged.

3.16.4 Number of seeds pod^{-1}

From each pot ten pods were selected randomly. After that, seeds were counted from the individual pod and then averaged to determine the number of seed pod^{-1}.

3.16.5 Seed yield plant^{-1}

The total seeds were separated manually and then sun-dried to reduce the moisture content at the recommended level and then weighed. The seed weight was averaged to determine the seed yield plant^{-1}.

3.16.6 Stover yield plant^{-1}

Above ground FW of plants from each pot were weighed, then averaged for measuring the stover yield plant^{-1}.

3.16.7 Biological yield plant^{-1}

Biological yield was calculated by using the following formula

Biological yield = Grain yield+stover yield

3.17 Phenotypical observation

Phenotypic features of seedlings were observed and pictures were taken with digital camera.

3.18 Anatomical observation

Anatomy of leaf transverse section was observed under a digital microscope.

3.19 Statistical analysis

The collected data were subjected to statistical analysis using CoStat v.6.400 (CoStat 2008) and two-way analysis of variance (ANOVA). Fisher’s least significant difference (LSD) test at the 5% level of significance was applied. Correlation analysis was done considering 1 and 5% level of significance by using SPSS software.

Chapter IV

RESULTS AND DISCUSSION

4.1 Growth parameters

4.1.1 Plant height

Upon exposure to 150, 300 and 450 mM NaCl-induced salt stress, a sharp reduction of the plant height was observed in a dose-dependent manner at 28 DAS, 35 DAS and 42 DAS, in comparison to control. The lowest plant height was observed under the highest salt stress (450 mM NaCl) at 28 DAS, 35 DAS and 42 DAS (Figure 1A, 2A, 3A).

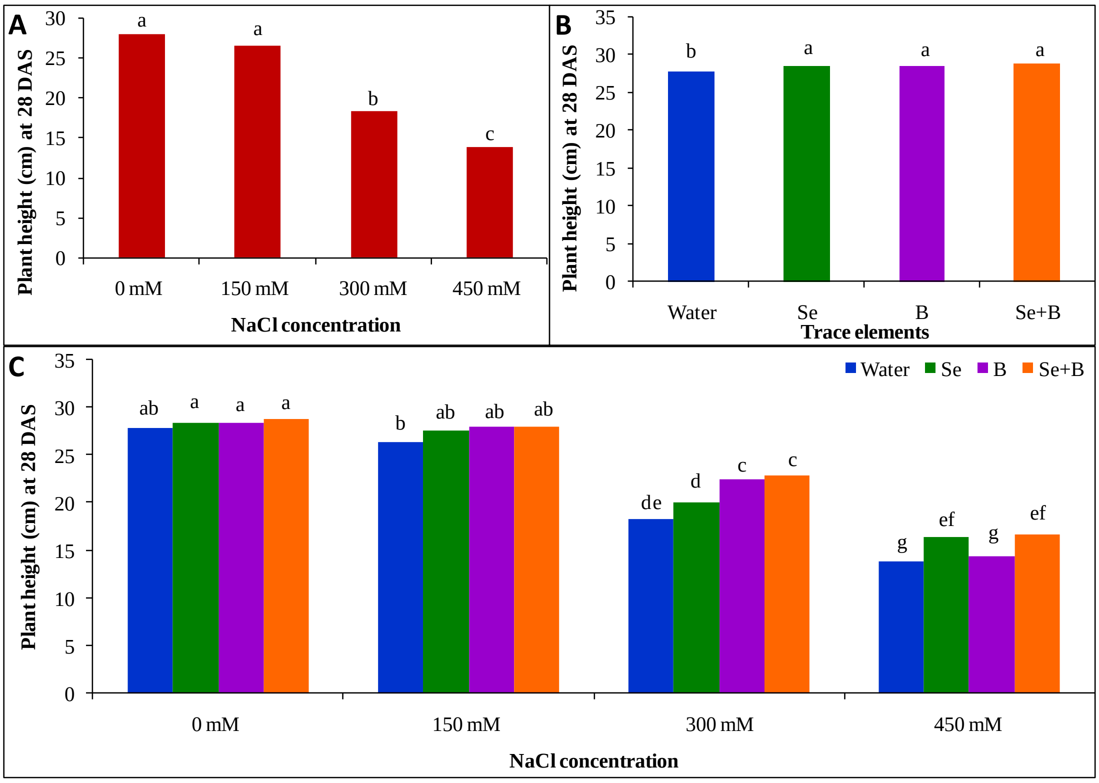

Figure 1. Effect of salinity (A), trace elements (B) and their interaction (C) on plant height of soybean at 28 DAS. Here, Se, B and Se+B indicate 0.50 µM Na_2SeO_4, 1 mM H_3BO_3 and 0.50 µM Na_2SeO_4+1 mM H_3BO_3. Values in a column with different letters are significantly different at $p \leq 0.05$ applying Fisher's LSD test

Moreover, at 28 DAS 5, 34 and 50% reduction occurred in the plant height, at 35 DAS 15, 36 and 47% reduction occurred in the plant height and at 42 DAS 17, 38 and 43% reduction occurred in the plant height under 150, 300 and 450 mM NaCl stress, respectively, compared to control (Figure 1A, 2A, 3A). On the contrary, exogenous application of Se, B and Se+B increased the plant height at 28 DAS, 35 DAS and 42 DAS, in comparison to the corresponding control (Figure 1B, 2B, 3B). Foliar application of Se increased the plant height by 2, 3 and 0.39% at 28, 35 and 42 DAS, in comparison to control (Figure 1B, 2B, 3B).

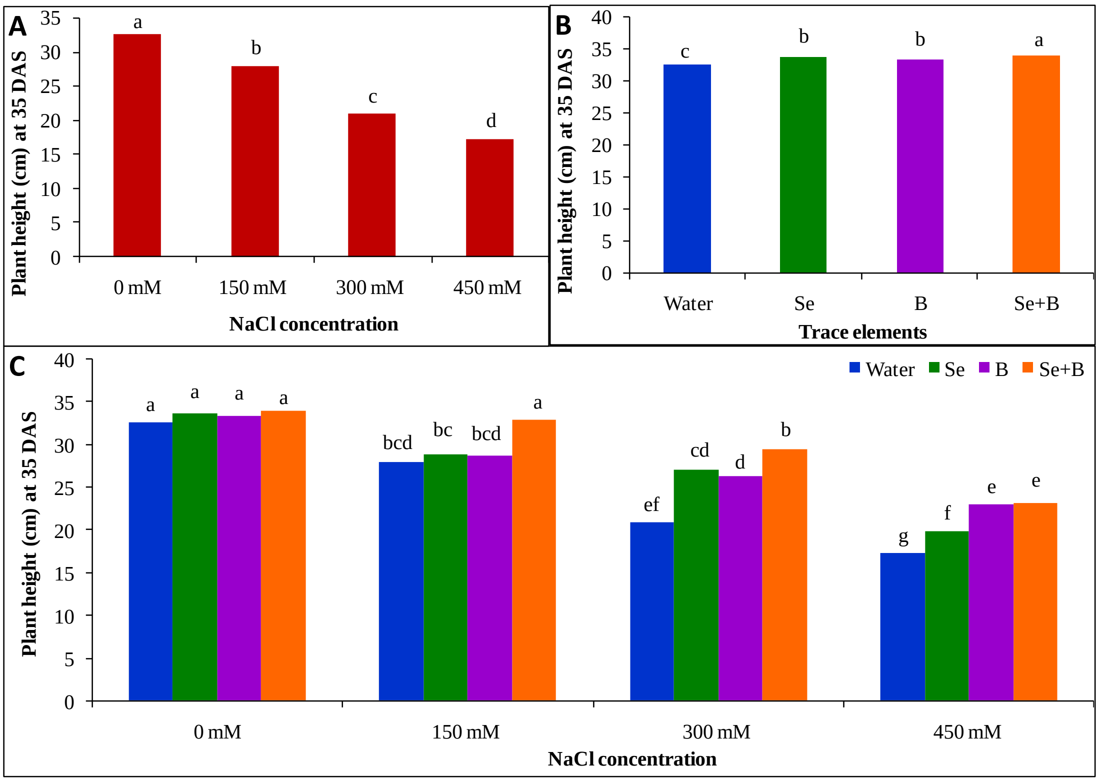

Figure 2. Effect of salinity (A), trace elements (B) and their interaction (C) on plant height of soybean at 35 DAS. Here, Se, B and Se+B indicate 0.50 µM Na$_2$SeO$_4$, 1 mM H$_3$BO$_3$ and 0.50 µM Na$_2$SeO$_4$+1 mM H$_3$BO$_3$. Values in a column with different letters are significantly different at $p \leq 0.05$ applying Fisher's LSD test

Exogenous application of B increased the plant height by 2, 3 and 0.19% at 28, 35 and 42 DAS, in comparison to control (Figure 1B, 2B, 3B). Moreover, combined application Se+B increased the plant height by 3, 4 and 1%, in comparison to corresponding control (Figure 1B, 2B, 3B). The application of trace elements (Se, B and Se+B) increased the plant height under salt stress and control (Figure 1C, 2C, 3C). Foliar supplementation of Se+B increased the plant height by 5, 24 and 20% at 28 DAS under 150, 300 and 450 mM NaCl-induced stress, compared to control (Figure 1C). When plants were subjected to mild, moderate and severe salinity, the application of Se+B increased the plant height by 18, 41 and 34% at 35 DAS (Figure 2C). Similarly, exogenous supplementation of Se+B increased the plant height by 12, 37 and 16% at 150, 300 and 450 mM NaCl-induced salt stress at 42 DAS, compared to control (Figure 3C).

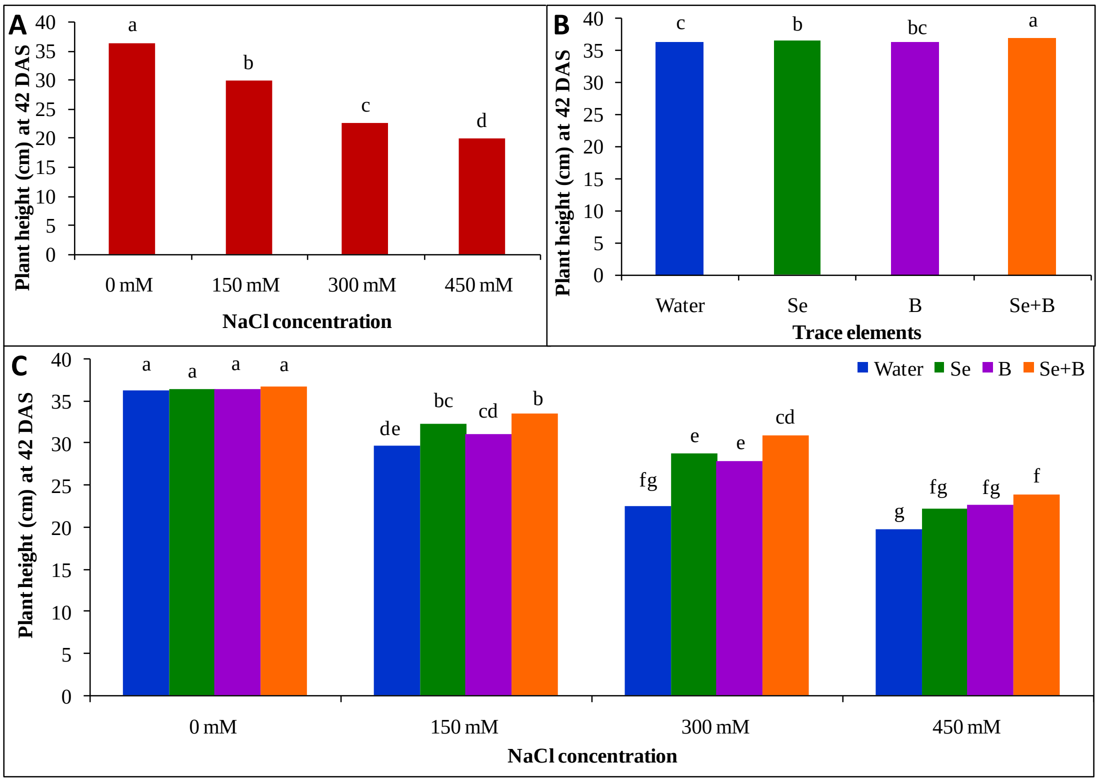

Figure 3. Effect of salinity (A), trace elements (B) and their interaction (C) on plant height of soybean at 42 DAS. Here, Se, B and Se+B indicate 0.50 µM Na_2SeO_4, 1 mM H_3BO_3 and 0.50 µM Na_2SeO_4+1 mM H_3BO_3. Values in a column with different letters are significantly different at $p \leq 0.05$ applying Fisher's LSD test

Salinity put detrimental effect on plant height. At an earlier stage, a reduction in the plant height occurs due to the accumulation of a higher salt concentration outside the root zone. In the later stage, a higher accumulation of salt inside the cellular level of the plant causes a reduction in plant height. In other words, plant height decreases due to salt-induced osmotic stress at an earlier stage and at later stage ionic stress is responsible for plant height reduction (Munns, 2005; Munns and Tester, 2008).The deleterious effect of salt stress was also observed in the present study, in a dose-dependent manner (Figure 1A, 2A, 3A). Salt-induced reduction in plant height was observed in soybean in several previous studies and in different soybean genotypes such as BINA Soybean-01, BINA Soybean-03, BINA Soybean-02, BINA Soybean-04, BARI soybean-05, SBM-09, BARI soybean-06, SBM-18, SBM-22 and SBM-15 and Sohag (Klein *et al.,* 2015; Akram *et al.,* 2017). As salt stress imposes both ionic and osmotic stress in the plant, it negatively affects the cell division and cell elongation process as well as normal cell functioning (Hasanuzzaman *et al.,* 2013; Shahverdi *et al.*, 2018). Salinity hinders the growth of meristematic tissues and inhibits the reducing carbohydrate supply to the growing cells. Reduction in photosynthesis and stomatal conductance greatly hampers the growth parameters of the plant in the salt stress (Kordrostami and Rabiei, 2019). These may have caused a reduction in the plant height in the present study. On the other hand, exogenous application of Se and B reverted the negative effect of salt stress on soybean in case of plant height (Figure 1C, 2C, 3C). Among the beneficial trace elements, Se is one of the most effective one in case of increasing growth traits and physiological activities in plants (Hasanuzzaman *et al.,* 2020a). Foliar application of Se, especially at the low concentration, was more effective in enhancing the plant height of wheat than higher concentration under salt stress (Shahzadi *et al.,* 2017; Elkelish *et al.,* 2019). Combined application of Se+B+Fe increased the plant height in stevia under salt stress. Moreover, the application of Se+B showed better performance in mitigating salt stress (Shahverdi *et al.,* 2018, 2020). Boron is a crucial factor for the processes like respiration, synthesis of proteins, transportation of sugars and carbohydrate metabolism. More importantly, growth hormone like IAA is also associated with B (Hansch and Mendel, 2009). In a previous study, Ullah *et al.* (2012) found that B plays a vital role in cell division of the actively growing region, especially the region near the shoot and root tips. For the growth and development of shoot and root B is required. Moreover, B transports the water, nutrients and carbohydrates needed for the meristematic (actively growing) region of the plants (Rattan, 2015). Application of Se, B and Se+B showed positive reflection in increasing plant height, in the present study at 28, 35 and 42 DAS (Figure 1C, 2C, 3C).

4.1.2 Root fresh weight plant^{-1}

Root FW decreased in comparison to control, upon exposure to 150, 300 and 450 mM NaCl-induced salt stress. Severe salinity showed the highest reduction in root FW. In comparison to control severe, moderate and mild salt stress resulted in 37, 24 and 13% reduction in root FW (Figure 4A). On the other hand, supplementation of Se, B and Se+B increased the root FW by 2, 3 and 4% when compared to control (Figure 4B). However, exogenous spraying of Se, B and Se+B mitigated the adverse effect of salt stress. Root FW increased by 13, 28 and 25% under mild, moderate and severe salinity in Se+B-supplemented plants, compared to control plants (Figure 4C).

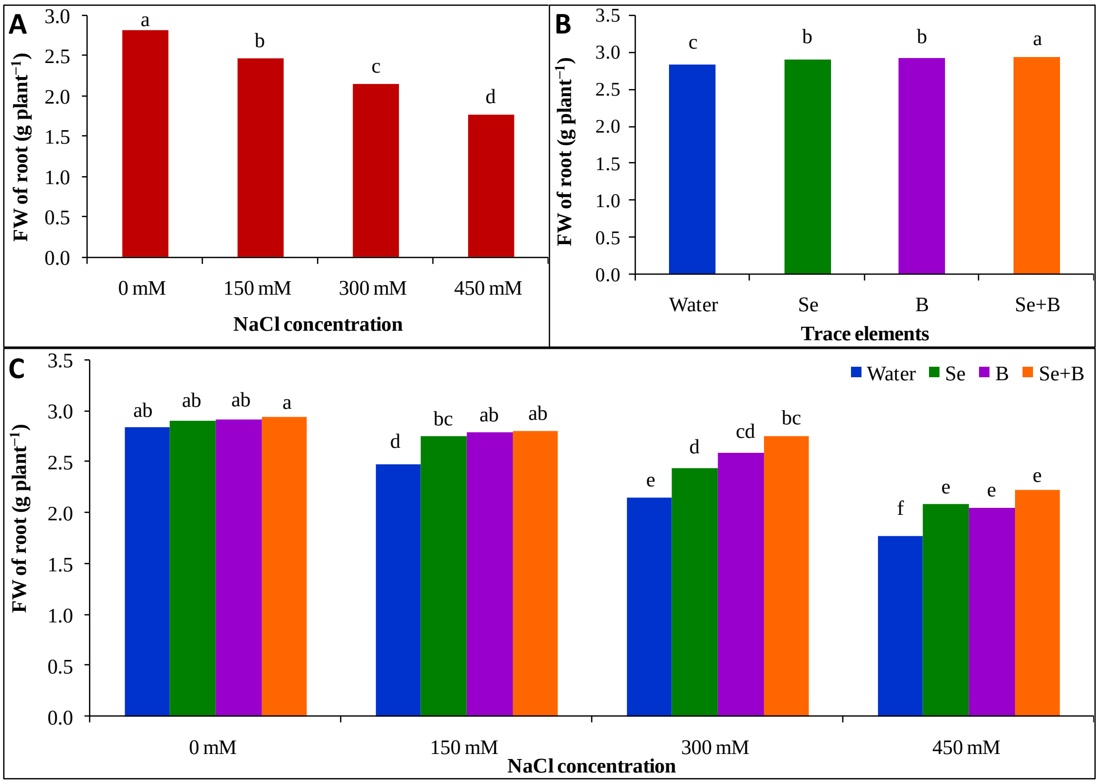

Figure 4. Effect of salinity (A), trace elements (B) and their interaction (C) on root fresh weight plant^{-1} of soybean. Here, Se, B and Se+B indicate 0.50 µM Na_2SeO_4, 1 mM H_3BO_3 and 0.50 µM Na_2SeO_4+1 mM H_3BO_3. Values in a column with different letters are significantly different at $p \leq 0.05$ applying Fisher's LSD test

Root FW depends on the root growth traits like root length, root volume, root diameter, root fitness, root area and root density (Shahverdi *et al.*, 2020). Root FW decreased in responses to different levels of salinity with the increase of the salt concentration (Figure 4A). In soybean plants, salt stress decreased the root FW (Klein *et al.*, 2015; El-Eswai *et al.*, 2018). Moreover, salinity reduced the root FW in cucumber (Mohsin *et al.*, 2019) and stevia (Shahverdi *et al.*, 2020). In a previous investigation, Sapre *et al.* (2018) reported that root volume and root length decreased due to salinity and ultimately decreased of root FW. Shahverdi *et al.* (2020) reported that different levels of salinity caused the reduction in root growth parameters such as length of root, volume of root, fitness of root, area of root, diameter of root, density of root and area of root which consequently reduced the root FW. In this experiment foliar application of Se, B and Se+B reverted the adverse effect of salinity on root FW. Plants treated with Se exhibited higher RWC, Chl levels, increased photosynthesis rates which increased the root FW. Moreover, Se plays a role in reduction of oxidative damage by enhancing the activity of enzymes (Hasanuzzaman *et al.*, 2020b). Boron plays a role in elongation and cell division of actively growing parts of the shoot and root tip. It helps in root growth by supplying sugars, water and nutrients needed for growth of the root. Moreover, it is also involved in growth and development of root volume in different legumes, including soybean (Rattan, 2015).

4.1.3 Root dry weight plant^{-1}

In response to the different level of salinity (150, 300 and 450 mM NaCl) root DW reduced sharply, in comparison to control plants. The maximum reduction occurred at the highest salinity level. Salt stressed plants exhibited 40, 56 and 71% reduction in root DW under mild, moderate and severe salinity, compared to control (Figure 5A). On the other hand, the exogenous spraying of Se, B and Se+B played the reverse role and increased the root DW. Supplementation of Se, B and Se+B increased the root dry weight by 6, 3 and 10%, compared to control (Figure 5B). Exogenous spraying of Se, B and Se+B increased the root DW under normal and saline condition. Combined application of Se+B increased 27, 19 and 24% root DW under 150, 300 and 450 mM NaCl-induced salt stress, in comparison to control (Figure 5C).

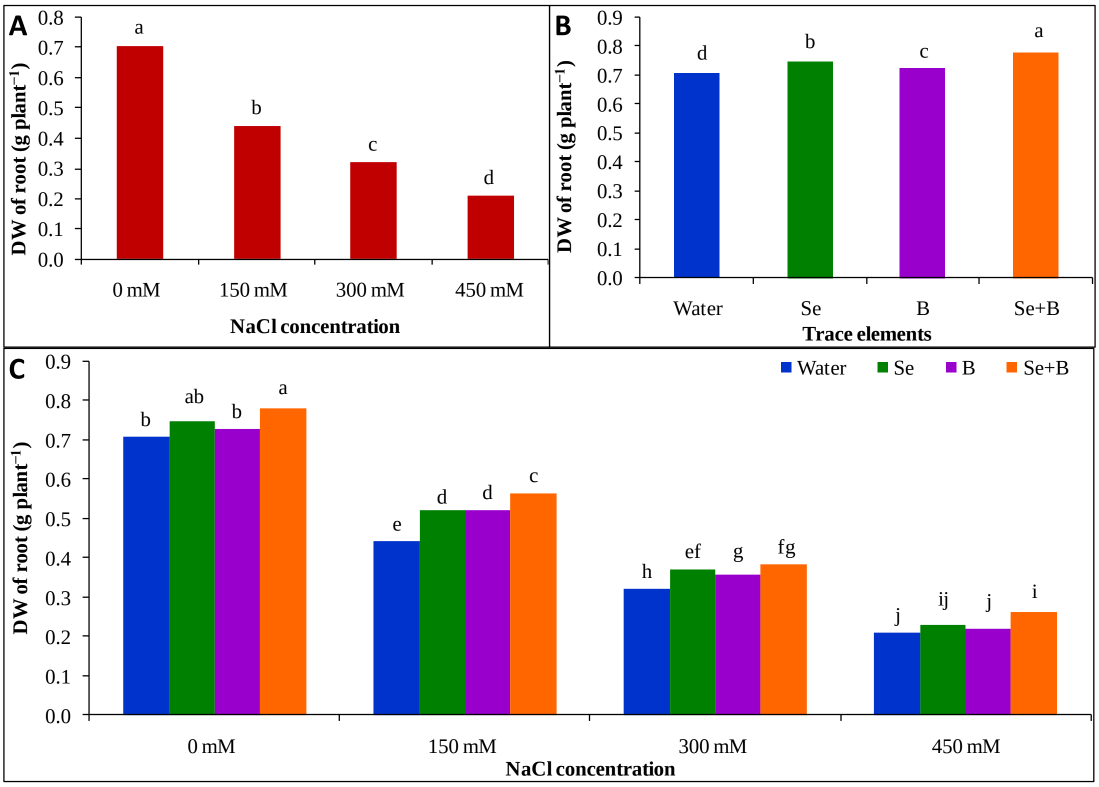

Figure 5. Effect of salinity (A), trace elements (B) and their interaction (C) on root dry weight plant^{-1} of soybean. Here, Se, B and Se+B indicate 0.50 µM Na_2SeO_4, 1 mM H_3BO_3 and 0.50 µM Na_2SeO_4+1 mM H_3BO_3. Values in a column with different letters are significantly different at $p \leq 0.05$ applying Fisher's LSD test

Root DW is associated with root growth traits and biomaas production (Shahverdi *et al.*, 2020). Upon exposure to different levels of salinity, plants exhibited reduction in root FW as well as reduction in root DW, as observed in this study (Figure 5A). Klein *et al.* (2015) and El-Eswai *et al.* (2018) reported that salt reduced the root DW along with root volume, root length and root FW in soybean. Similar results were observed in maize (Jiang *et al.*, 2017), oat (Sapre *et al.*, 2018), cucumber (Mohsin *et al.*, 2019), stevia (Shahverdi *et al.*, 2020) etc. According to Sapre *et al.* (2018), reduction in shoot length, root length, shoot FW and RWC occurred under salinity in oats which ultimately leads to lower DW. Reduction in biomass production, plant growth and cell expansion occurs due to salt stress. Moreover, reduction in the root traits like root FW, root volume, root area, root length, root diameter and root density occurred when subjected to different levels of salinity, leading to reduction in the root DW (Shahverdi *et al.*, 2018, 2020). On the contrary, foliar application of Se, B and Se+B increased the root DW in saline condition (Figure 5C). Application of Se increased the DW of root in maize at 100 mM NaCl (Jiang *et al.*, 2017). Combined application of Se+B increased the length, volume, FW, diameter, fitness, density and area of the root under different levels of salinity which ultimately increased the DW of root (Shahverdi *et al.*, 2020). Numerous previous investigations showed that application of Se improves the growth, development, yield and yield attributing parameters including the root growth by improving physiological and biochemical processes such as photosynthesis, C-assimilation, enzymatic activity etc. (Hasanuzzaman *et al.*, 2020a). Boron ensures growth and development of the root by providing sugars, nutrient and water needed for the root growth and development activity (Rattan, 2015). Therefore, Se, B and Se+B application also increased the root DW, in this study (Figure 5C).

4.1.4 Shoot fresh weight plant^{-1}

Shoot FW plant^{-1} was negatively affected by the different salt treatments. All the salt treatments caused a sharp decline in shoot FW. The maximum reduction of shoot FW occurred at 450 mM NaCl, which was 80% lower than control. Similarly, the imposition of 150 and 300 mM NaCl caused 39 and 49% reduction in shoot FW, respectively, in comparison to control (Figure 6A). On the other hand, exogenous application Se, B and Se+B reverted the negative effect of salt stress on shoot FW. Shoot FW was the highest in Se+B treated plants under normal conditions. Supplementations of Se, B and Se+B increased the shoot FW by 4, 4 and 7%, compared to control (Figure 6B). Compared to single supplement, the combined application of Se+B showed better results in case of increasing shoot FW under different salinity levels and control. Combined application of Se+B increased the shoot FW by 46, 68 and 231% under 150, 300 and 450 mM NaCl stress, in comparison to the control (Figure 6C).

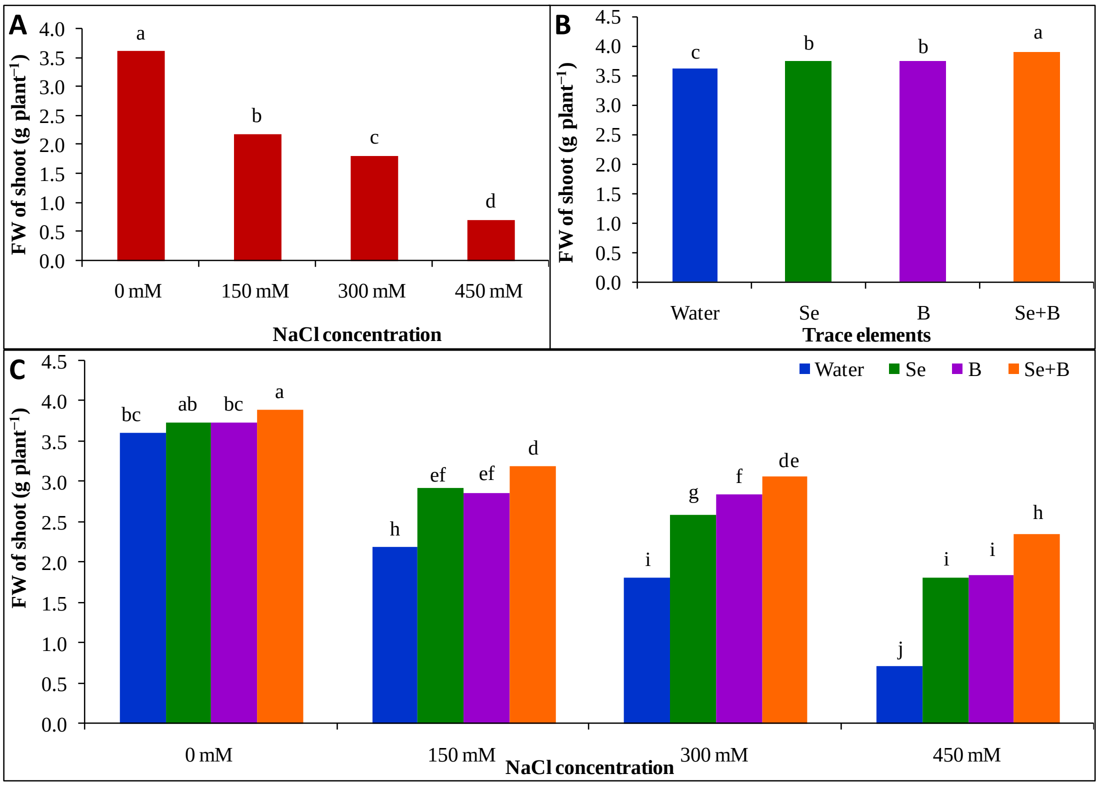

Figure 6. Effect of salinity (A), trace elements (B) and their interaction (C) on shoot fresh weight plant^{-1} of soybean. Here, Se, B and Se+B indicate 0.50 µM Na_2SeO_4, 1 mM H_3BO_3 and 0.50 µM Na_2SeO_4+1 mM H_3BO_3. Values in a column with different letters are significantly different at $p \leq 0.05$ applying Fisher's LSD test

Shoot FW depends on plant growth and development, which means shoot FW is related with cell expansion and cell division. Salinity reduces plant growth and development as well as the shoot FW by reducing the carbohydrate supply to the growing cells (Kordrostami and Rabiei, 2019). In this experiment, shoot FW decreased with the increase in salt concentration (Figure 6A). Moreover, in different studies, salinity reduced the shoot FW in soybean (Klein *et al.*, 2015; Akram *et al.*, 2017; El-Eswai *et al.*, 2018). Similar results were observed in some other crops like wheat (Elkelish *et al.*, 2019), lentil (Hossain *et al.*, 2019), maize (Ashraf *et al.*, 2018), oat (Sapre *et al.*, 2018), rice (Rahman *et al.*, 2017) etc. Salt stress hinders plant growth and development by creating osmotic stress and ionic toxicity (Choudhury *et al.*, 2013; Hasanuzzaman *et al.*, 2013). On the other hand, the application of Se, B and Se+B increased the shoot FW sharply, in comparison to the control (Figure 4B, 4C). Shoot FW increased due to the application of Se in maize under salt stress (Ashraf *et al.*, 2018). Supplementation of Se increased the nutrient uptake and translocation in plants. Application Se increased the Chl content, Car content, photosynthesis rate, transpiration rate and stomatal conductance. Supplementation of Se also alleviates the structural damage of chloroplast under salinity (Jiang *et al.*, 2017). Shoot FW increased after the application of B in sunflower under salt stress (Jabeen and Ahmad, 2011; Jabeen *et al.*, 2013). Boron is crucial for actively growing region like shoot tip, root tip, new leaf and developing bud. Not only the healthy conductive tissues but also nutrient, carbohydrate and water transportation to the actively growing regions are maintained by B (Rattan, 2015). All of these positive effects of Se and B leads to increment of the shoot FW in plants (Figure 6B, 6C).

4.1.5 Shoot dry weight plant^{-1}

Upon exposure to 150, 300 and 450 mM NaCl-induced salt stress, shoot DW decreased remarkably. Severe salt stress (450 mM NaCl) caused the maximum reduction in shoot DW. However, plants exihibited 53, 67 and 78% declined in shoot DW, compared to control under low, moderate and severe salinity (Figure 7A). In contrary, exogenous application of Se, B and Se+B increased the shoot DW sharply. Combined application of Se+B showed better performance in increasing the shoot DW rather than individual application of Se and B. Application of Se, B and Se+B increased the shoot DW by 4, 2 and 11%, compared to control (Figure 7B). Moreover, application of Se+B increased the shoot DW both in normal condition and saline condition, compared to control. Under different salinity levels Se+B treated plants showed 39, 71 and 71% increase in shoot DW, in comparison to corresponding control (Figure 7C).

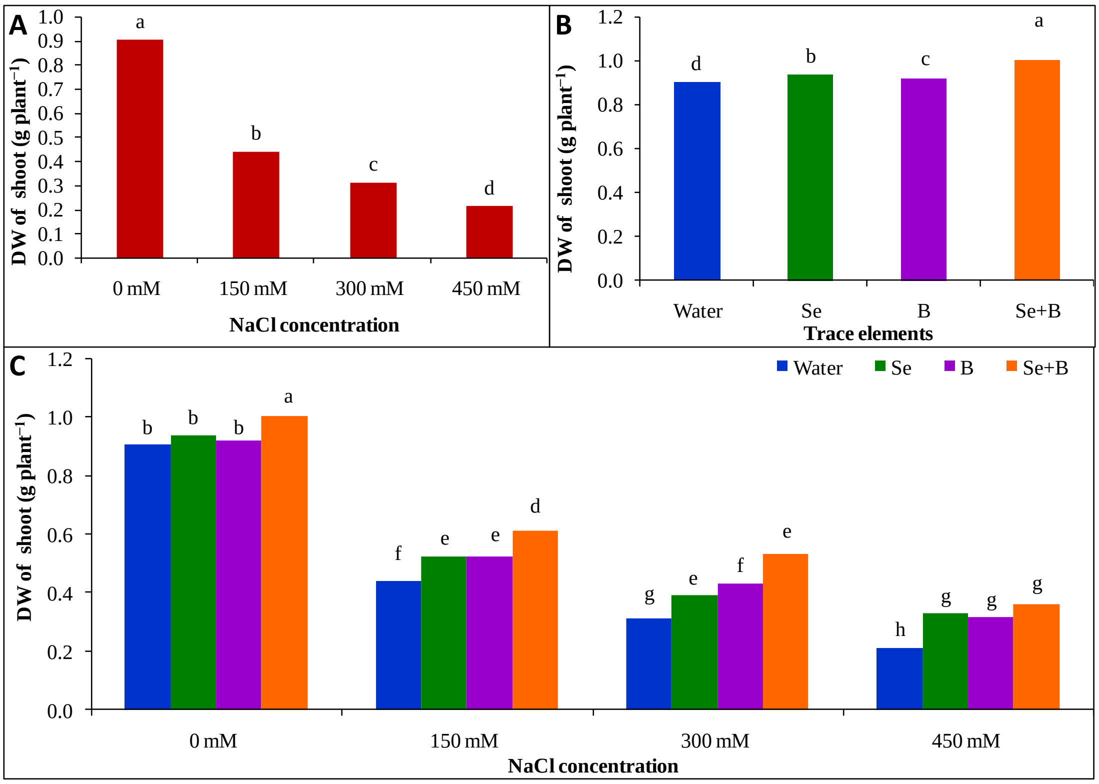

Figure 7. Effect of salinity (A), trace elements (B) and their interaction (C) on shoot dry weight plant^{-1} of soybean. Here, Se, B and Se+B indicate 0.50 µM Na_2SeO_4, 1 mM H_3BO_3 and 0.50 µM Na_2SeO_4+1 mM H_3BO_3. Values in a column with different letters are significantly different at $p \leq 0.05$ applying Fisher's LSD test

As shoot DW is associated with shoot FW, noticeable reduction in shoot FW has led to sharp reduction in shoot DW plant^{-1} under salt stress in a dose-dependent manner, in the present study (Figure 7A). Salinity decreased the shoot DW in soybean plants in several studies (Klein *et al.*, 2015; El-Eswai *et al.*, 2018). Salt stress showed similar results in case of shoot DW in previous studies of various crops like wheat (Elkelish *et al.*, 2019), lentil (Hossain *et al.*, 2019), maize (Ashraf *et al.,* 2018), oat (Sapre *et al.,* 2018), maize (Jiang *et al.,* 2017), rice (Rahman *et al.,* 2016), etc. Salt-induced osmotic stress and ionic toxicity hampers the plant growth and development. Reduction in photosynthesis and stomatal conductase greatly hinders the growth traits of the plant under saline condition (Kordrostami and Rabiei, 2019). These might cause the reduction in carbon assimilation and lower shoot DW in this study. On the contrary, application of Se, B and Se+B increased the shoot DW under non-saline and saline conditions (Figure 7C). In soybean plant, Se application increased the shoot DW (Ardebili *et al.*, 2014). Application of Se increased the shoot DW in maize under salinity (Ashraf *et al*., 2018). Moreover, application of Se enhances the photosynthetic pigments. Along with quenching the ROS, supplementation of Se maintains the enzymatic and non-enzymatic activity in plants and also protects the tissues from oxidative stress induced damage (Hasanuzzaman *et al.,* 2013; Chauhan et al., 2017). Boron is crucial for actively growing region. In other words, B is vital for plant growth and development. Moreover, B plays an important role in water, nutrient and sugar supply to the meristematic zone like shoot tips, root tips, new leaf etc. (Rattan, 2015). Positive effect of Se and B was reflected in shoot DW under salinity, in this study (Figure 7C).

4.1.6 Number of branches plant^{-1}

Remarkable decline was recorded in branch number plant^{-1} under low, moderate and severe salt stress compared to control. The lowest number of branches plant^{-1} was observed at the highest level of salinity treatment (450 mM NaCl) which was 70% lower than control. Similarly, 45% reduction was caused by moderate level of salinity and 9% reduction was caused by mild salinity level (Figure 8A). However, exogenous application of Se, B and Se+B increased the branch number plant^{-1} by 5, 5 and 28%, in comparison to control (Figure 8B). Compared to individual spray, combined application of Se+B spray effectively enhanced the number of branches plant^{-1} under saline and non saline condition. Moreover, under 150, 300 and 450 mM NaCl-induced stress 18, 46 and 90% enhancement occurred in the branch number plant^{-1} as a result of exogenous application of Se+B, in comparison to control (Figure 8C).

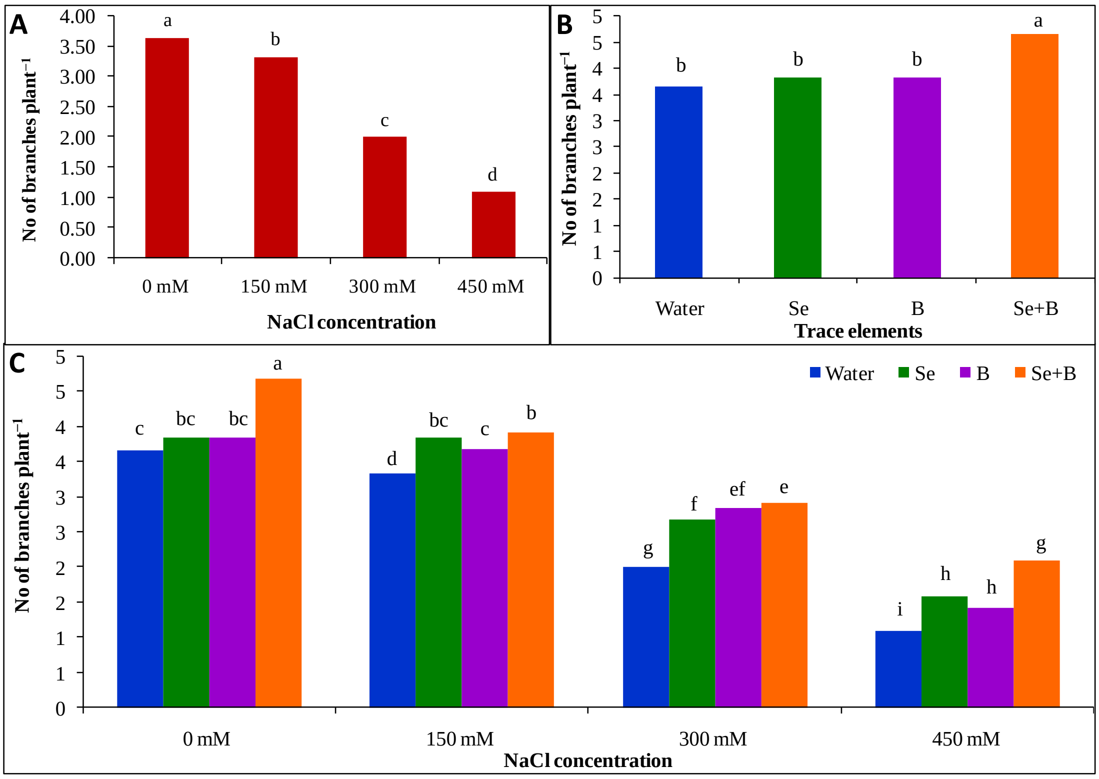

Figure 8. Effect of salinity (A), trace elements (B) and their interaction (C) on number of branches plant^{-1} of soybean. Here, Se, B and Se+B indicate 0.50 µM Na_2SeO_4, 1 mM H_3BO_3 and 0.50 µM Na_2SeO_4+1 mM H_3BO_3. Values in a column with different letters are significantly different at $p \leq 0.05$ applying Fisher's LSD test

Salinity reduced the branch number plant^{-1} in different soybean genotypes like BINA Soybean-03, Sohag, BARI soybean-05, BINA Soybean-02, BINA Soybean-01, BARI soybean-06 and BINA Soybean-04 etc (Akram *et al.*, 2017). Reduction in the branch number due to salinity was also observed in different studies of soybean (El-Sabagh *et al.*, 2015; Hamayun *et al.*, 2010). Similarly, decreasing trend in the branch number plant^{-1} also observed in this study when subjected to different level of salt stresses (Figure 8A). Along with aging the old branches, the new branch formation is greatly hampered under salinity (El-Sabagh *et al.*, 2015). Salt-induced ionic and osmotic stress has deleterious effect on cell elongation and cell division, in other words on plant growth and development. Salinity negatively affects the branching of the soybean plants (Hasanuzzaman *et al.*, 2016). Moreover, salinity limits the growth of meristematic tissues by reducing carbohydrate supply to the growing cells (Kordrostami and Rabiei, 2019). On the other hand, application of Se, B and Se+B increased the number of branch plant^{-1} (Fig. 8). Combined application of Se+B increased the branch number plant^{-1} in stevia (Shahverdi *et al.,* 2018, 2020). Application of Se in a low concentration (5 μM) alleviated the damaging effect of salinity (Elkelish *et al.,* 2019). In previous studies, application of Se increased the growth and growth attributing parameters under different levels of salinity in maize (Ashraf *et al.,* 2018; Jiang *et al.,* 2017) and in cowpea (Manaf, 2016). Boron is a crucial micronutrient for plant growth and development which is associated with actively growing shoot tip, root tip, new leaf, developing bud, protein synthesis, transportation of water, transportation of nutrient, carbohydrate metabolism and growth hormone (Hansch and Mendel, 2009; Rattan, 2015). In this study, application of Se, B and Se+B increased the branch number plant^{-1} by mitigating the adverse effect of salinity (Figure 8C).

4.1.7 Leaf area

Upon exposure to different level of salt stresses reduction in the leaf area occurred. Leaf area slightly decreased under mild and moderate salinity. Severe salinity caused the highest reduction in leaf area. Leaf area was decreased by 5, 9, and 24% under 150, 300 and 450 mM salt stress, compared to corresponding control (Figure 9A). On the contrary, application of trace elements (Se, B and Se+B) increased the leaf area. Leaf area was increased by 4, 7 and 9% due to supplementation of Se, B and Se+B, compared to the control plants (Figure 9B). Supplementation of Se, B and Se+B increased the leaf area under salt stress and normal condition. Combined application of Se+B showed better result than individual application of Se and B, in case of increasing leaf area. Supplementation of Se+B increased the leaf area by 5, 10 and 15% under 150, 300 and 450 mM salt stress, in comparison to control (Figure 9C).

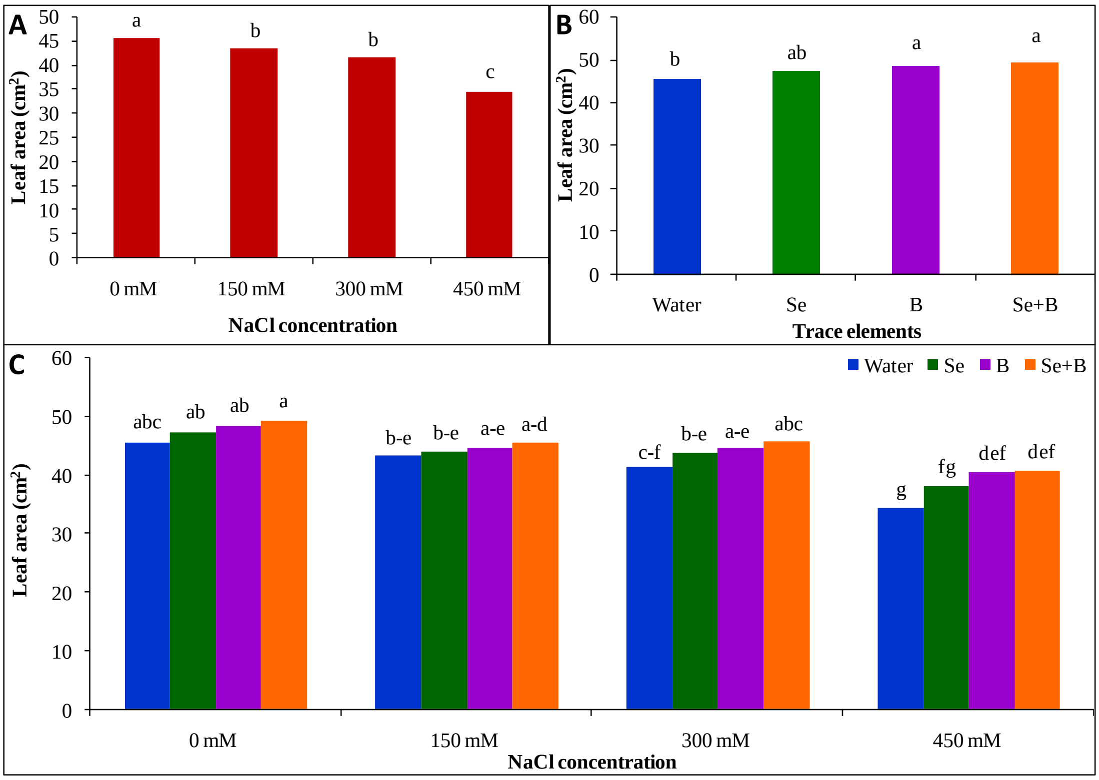

Figure 9. Effect of salinity (A), trace elements (B) and their interaction (C) on leaf area of soybean. Here, Se, B and Se+B indicate 0.50 µM Na_2SeO_4, 1 mM H_3BO_3 and 0.50 µM Na_2SeO_4+1 mM H_3BO_3. Values in column with different letters are significantly different at $p \leq 0.05$ applying Fisher's LSD test

Like stunting of the plant height, reduction in the leaf area is one of the prominent effect of salinity (Wu *et al.*, 2018). In this study, plant exhibited reduction in the leaf area in a dose-dependent manner (Figure 9A). Reduction of the leaf area when subjected to salt stress is count as the immediate response of plants towards salinity to reduce the salt concentration in plants (Kordrostami and Rabiei, 2019). Along with cell division, normal functions of cells are also hampered in saline condition due to ionic stress and osmotic stress, consequently hinders the normal growth and development of plant parts (Hasanuzzaman *et al.*, 2013). Salt stress also inhibits the growth of meristematic tissues, by limiting carbohydrate supply to the region of growing cells (Kordrostami and Rabiei, 2019). All of these cause reduction in the leaf area under salt stress. On the contrary, supplementation of Se, B and Se+B increased the leaf area under saline and non saline condition (Figure 9C). Supplementation of Se alleviated the adverse effect of salt stress in plants by improving the antioxidant defense mechanism and by diminishing the amount of ROS. Application of Se has positive effect on cell division, cell elongation and normal cell functioning (Hasanuzzaman *et al.,* 2013; Chauhan *et al.,* 2017; Hasanuzzaman *et al.,* 2020a). As a micronutrient B is a effective one for plant growth and development which is associated with actively growing shoot tip, root tip, new leaf, developing bud, protein synthesis, transportation of water, transportationof nutrient, carbohydrate metabolism and growth hormone (Hansch and Mendel, 2009, Rattan, 2015). In this study, application of Se and B reverted the adverse effects of salinity and increased the leaf area (Figure 9C).

4.2 Physiological parameters

4.2.1 SPAD value

When exposed to 150, 300 and 450 mM NaCl-induced salt stress, plants exihibited reduction in the SPAD value under all saline treatments at 28, 35 and 42 DAS, in comparison to control. Moreover, the reduction of SPAD value increased with the increment of the concentration of the salinity and eventually the minimum SPAD value recorded in the highest salinity level at 28, 35 and 42 DAS (Figure 10A, 11A, 12A). However, mild, moderate and severe salinity resulted in 19, 14 and 5% decline in SPAD value at 28 DAS (Figure 10A), 3, 11 and 21% decline in SPAD value at 35 DAS (Figure 11A) and 16, 24 and 33% decline in SPAD value at 42 DAS (Figure 12A) compared to control, respectively. On the contrary, supplementation of Se, B and Se+B increased the SPAD value by 3, 2 and 7% at 28 DAS, in comparison to control (Figure 10B).

Similarly, supplementation of Se, B and Se+B enhanced the SPAD value by 2, 2 and 3% at 35 DAS, respectively, in comparison to control (Figure 11B). Moreover, supplementation of Se, B and Se+B increased the SPAD value at 42 DAS by 7, 3 and 9%, compared to control (Figure 12B). However, exogenous application of Se, B and Se+B reverted the negative effect of the salt stress and among them combined application of Se+B showed the better result than Se or B alone at 28, 35 and 42 DAS (Figure 10C, 11C, 12C).

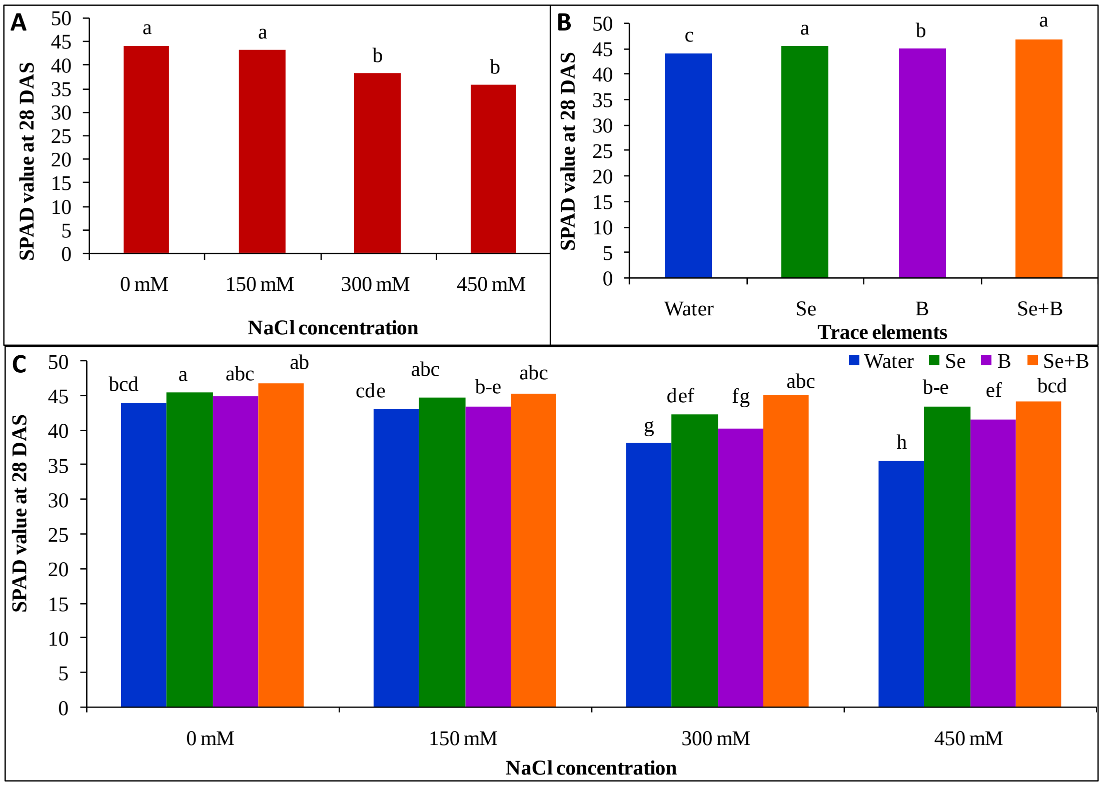

Figure 10. Effect of salinity (A), trace elements (B) and their interaction (C) on SPAD value of soybean at 28 DAS. Here, Se, B and Se+B indicate 0.50 µM Na_2SeO_4, 1 mM H_3BO_3 and 0.50 µM Na_2SeO_4+1 mM H_3BO_3. Values in a column with different letters are significantly different at $p \leq 0.05$ applying Fisher's LSD test

Under mild, moderate and severe salinity, at 28 DAS combined application of Se+B increased the SPAD value by 5, 18 and 24%, respectively, compared to the control (Figure 10C). Application of Se+B increased the SPAD value by 12, 8 and 18% under mild, moderate and severe salt stress at 35 DAS, in comparison to control (Figure 11C). Similarly, foliar spray of Se+B elevated the SPAD value 21, 35 and 41%, in comparison to the corresponding control plants at 45 DAS under mild, moderate and severe salinity level (Figure 12C).

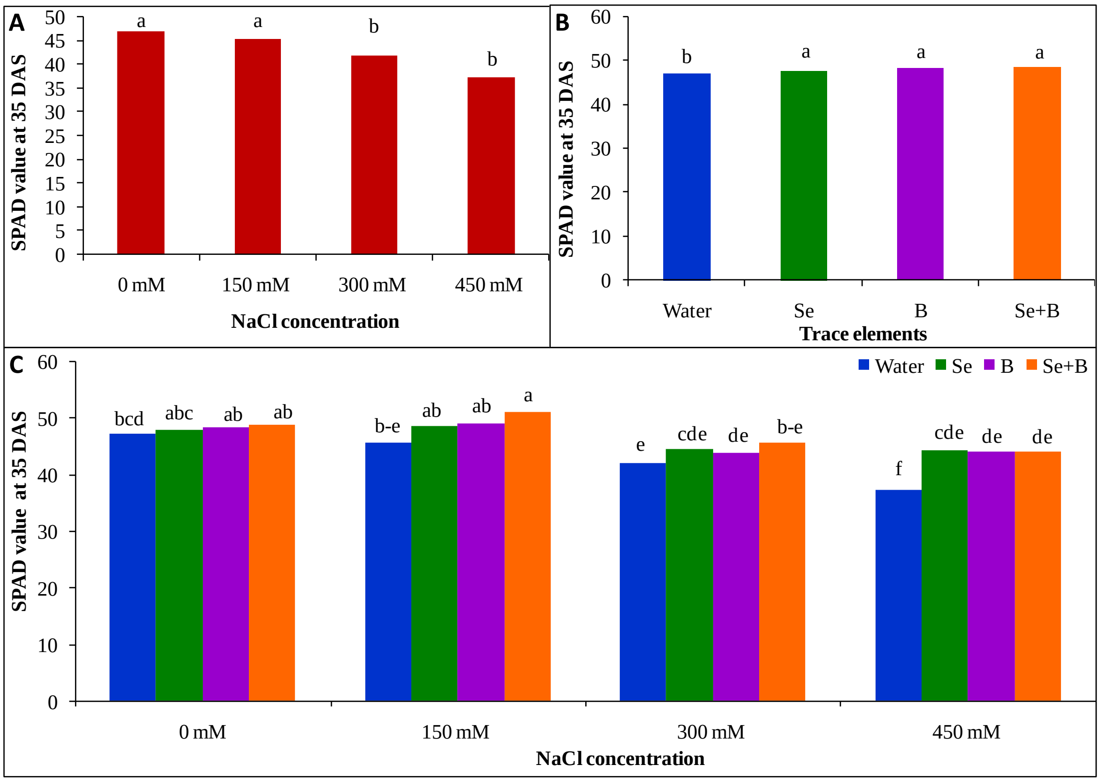

Figure 11. Effect of salinity (A), trace elements (B) and their interaction (C) on SPAD value of soybean at 35 DAS. Here, Se, B and Se+B indicate 0.50 µM Na_2SeO_4, 1 mM H_3BO_3 and 0.50 µM Na_2SeO_4+1 mM H_3BO_3. Values in a column with different letters are significantly different at $p \leq 0.05$ applying Fisher's LSD test

Salt stress is responsible for reduction in photosynthetic pigment content, chlorosis, necrosis and leaf senescence which ultimately causes reduction in photosynthesis rate (Khan *et al.*, 2017). In response to salt stress, soybean plant exihibited decrease in SPAD value in a dose-dependent manner at any age of the plant. Salinity caused slight decrease at earlier stage (at 28 DAS and at 35 DAS) but sharp decrease observed at later stage (at 42 DAS) in a time-dependent manner (Figure 10A, 11A, 12A).

Salinity reduced the stomatal conductase which ultimately responsible for decrease in photosynthesis rate of 3 soybean genotypes (S111-9, S113-6 and Melrose) which was 14 to 34% (He *et al.*, 2016). According to El-Eswai *et al.* (2018), in soybean nutrient uptake, Chl content, transpiration rate, photosynthesis rate and stomatal conductase were reduced by salt stress.

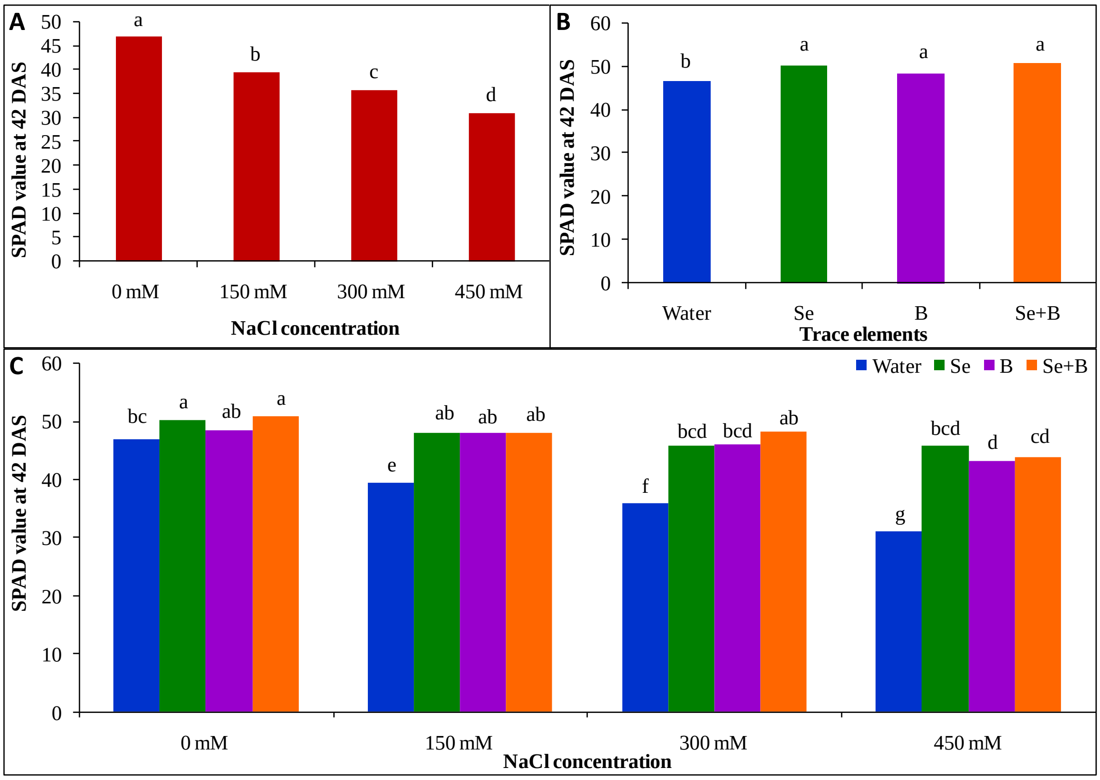

Figure 12. Effect of salinity (A), trace elements (B) and their interaction (C) on SPAD value of soybean at 42 DAS. Here, Se, B and Se+B indicate 0.50 µM Na_2SeO_4, 1 mM H_3BO_3 and 0.50 µM Na_2SeO_4+1 mM H_3BO_3. Values in a column with different letters are significantly different at $p \leq 0.05$ applying Fisher's LSD test

Under salt stress photosynthesis rate, stomatal conductase and transpiration rate sharply reduced. Salt stress decreased Chl and Car content by 24 and 14% respectively, in maize (Jiang *et al.*, 2017). Under salt stress Chl content and protein content significantly decreased in oat seedlings (Sapre *et al.*, 2018). Under 100 mM NaCl-induced salt stress Chl *(a+b)* content was reduced by 25%, in comparison to corresponding control in lentil (Hossain *et al.*, 2019). In cucumber plant, salinity remarkably damaged the photosynthetic pigments and reduced the photosynthesis rate. However, Chl *a*, Chl *b*, Chl *(a+b)* and Car content were sharply reduced at the rate of 41, 39, 40 and 36%, compared to control under salinity (Mohsin *et al.*, 2019). Salinity reduces the photosynthesis due to stomatal and non-stomatal factors like ultrastructural damage, reduction in photosynthetic pigments and Chl fluorescence (Khan *et al.*, 2017). Remarkable damage of chloroplast ultrastructure was observed under salt stress. Compressed grana lamellae, disrupted stroma lamellae and distorted thylakoids were observed in deteriorated chloroplasts under salinity (Jiang *et al*. 2017). Wu *et al.* (2018) reported that salt stress deteriorated photosynthetic apparatus after ultrastructural observation of mesophyll cell. On the other hand, supplementation of Se, B and Se+B increased the SPAD value at any stage of the plants, compared to corresponding control condition (Figure 10C, 11C, 12C). Moreover, under salinity supplementation of Se increased the Chl content and photosynthesis rate in maize (Jiang *et al.,* 2017; Ashraf *et al*., 2018). Supplementation of Se increases photosynthetic pigment synthesis, photosynthesis rate, gas exchange rate, accumulation of osmoprotectants and secondary osmolites. Se mitigates salt stress by reducing Na^+ accumulation in plant parts, Na^+ compartmentalization, upregulating Na^+ and Cl^- ions transporter genes, chelation and by improving of antioxidant defense system (Hasanuzzaman *et al.,* 2020a; Kamran *et al.*, 2020). Supplementation of 1 μM Se protected the chloroplast ultrastructure and enhanced the photosynthesis activity under salt stress in maize (Jiang *et al.*, 2017). Boron contributes to maintaining structural integrity of biomembranes including choloroplast (Hansch and Mendel, 2009). It reduces chlorosis by helping in assimilation of nitrates (Broschat, 2009). Therefore, supplementation of Se, B and Se+B also showed positive effect on SPAD value at 28, 35 and 42 DAS under saline and normal condition (Figure 10C, 11C, 12C).

4.2.2 Relative water content

Due to imposition of 150, 300 and 450 mM NaCl stress, RWC of leaf declined under all salinity level, compared to corresponding control. The highest decrease in RWC of leaf was observed at 450 mM NaCl-induced stress (26%), in comparison to control. Moreover, 150 and 350 mM NaCl-induced salt stress caused 19 and 25% reduction in RWC of leaf, respectively, compared to control (Figure 13A). On the contrary, application of trace elements (Se, B and Se+B) increased the RWC. The highest increase of RWC occurred due to combined application of Se+B (4%), compared to control. Individual application of Se and B increased the RWC by 3 and 2%, respectively, in comparison to control (Figure 13B). Moreover, foliar spray of Se, B and Se+B altered the diminishing effect of salinity under different salinity levels. Under mild, moderate and severe salinity Se+B spray elevated the RWC of leaf by 18, 21 and 20%, compared to control (Figure 13C).

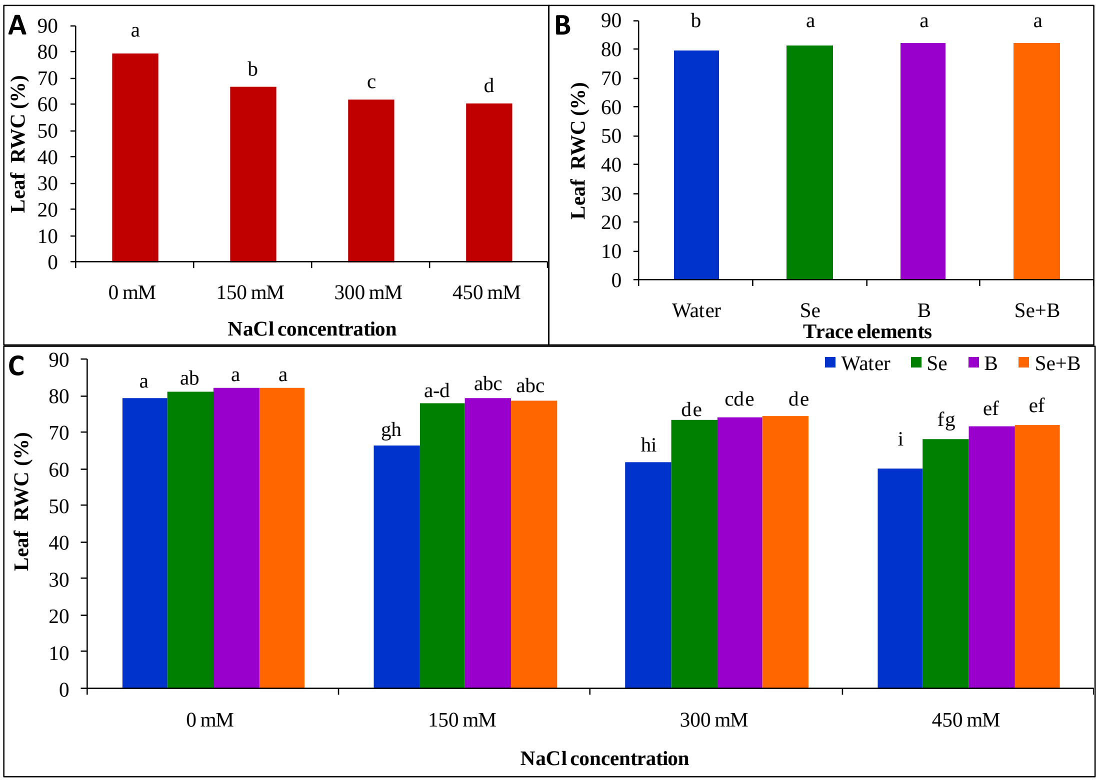

Figure 13. Effect of salinity (A), trace elements (B) and their interaction (C) on relative water content of soybean. Here, Se, B and Se+B indicate 0.50 µM Na_2SeO_4, 1 mM H_3BO_3 and 0.50 µM Na_2SeO_4+1 mM H_3BO_3. Values in a column with different letters are significantly different at $p \leq 0.05$ applying Fisher's LSD test

In response to salt stress, RWC decreased with the increase in the concentration of salt (Figure 13A). However, salinity decreased the RWC in soybean plants in numerous studies (Liu *et al.*, 2017). Similarly, decreasing trend in the RWC were also observed under salt stress in different studies such as in rice (Hasanuzzaman *et al.* 2014), in rice (Subramanyam *et al.*, 2019), in rapeseed (Mahmud *et al.*, 2020) etc. In saline condition, RWC decreased due to osmotic stress. Osmotic stress lowers the water retention capacity and turgor of the cell, consequently causes reduction in RWC under salinity (Hasanuzzaman *et al.*, 2014). Foliar application of Se, B and Se+B increased the RWC in salt-stressed and control plants (Fig. 13C). In cowpea, application of Se increased the RWC under salinity (Manaf, 2016). In lettuce, Se-supplementation increased the RWC under salinity (Khalifa *et al.*, 2016). Elkelish *et al.* (2019), reported that application of Se increased the RWC in wheat. Due to high concentration of salt ions in the rootzone conductivity of the root reduced and leads to lower water uptake and RWC. However, the Se (Na_2SeO_4) application reduced Na^+ accumulation and improved the root growth and enhanced the water translocation to the shoot and increased RWC (Subramanyam *et al.*, 2019). Boron plays a crucial role in improving RWC by transporting water to the different plant parts, specially in the growing regions (Rattan, 2015).

4.2.3 Proline content

Upon exposure to 150, 300 and 450 mM NaCl-induced salt stress, plants showed higher amount of Pro accumulation under all salinity treatments, in comparison to control both at 30 DAS and 45 DAS. However, in comparison to control the higher salinity level was responsible for the higher Pro accumulation in plants and the maximum Pro content was recorded under 450 mM NaCl-induced stress both at 30 DAS and 45 DAS (Figure 14A, 15A). Sharp increase of the Pro content was observed under mild, moderate and severe salinity level at 30 DAS which was 88, 131 and 180%, in comparison to the corresponding control (Figure 14A). Similarly, remarkable enhancement in Pro content occurred under mild, moderate and severe salinity which was 49, 99 and 142%, compared to control condition at 45 DAS (Figure 15A). On the contrary, supplementation of Se, B and Se+B decreased the Pro content sharply at 30 DAS. Among the trace elements treatments combined application of Se+B caused the highest reduction in Pro content (11%), compared to control (Figure 14B). Similarly, supplementation of Se, B and Se+B reduced the Pro content by 12, 15 and 17% at 45 DAS, in comparison to control (Figure 15B). Moreover, foliar application of Se, B and Se+B diminished the Pro content both in saline and non saline condition, compared to control at 30 DAS and 45 DAS. In comparison to individual foliar spray, Se+B spraying showed better result at 30 and 45 DAS, in case of diminishing the Pro content (Figure 14C and 15C). Application of Se+B spray decreased 20, 19 and 12% of proline content under mild, moderate and severe salinity level, compared to control at 30 DAS (Figure 14C). Similarly, Pro content was decreased by 10, 27 and 17% at 45 DAS under 150, 300 and 450 mM NaCl-induced stress, in comparison to control (Figure 15C).

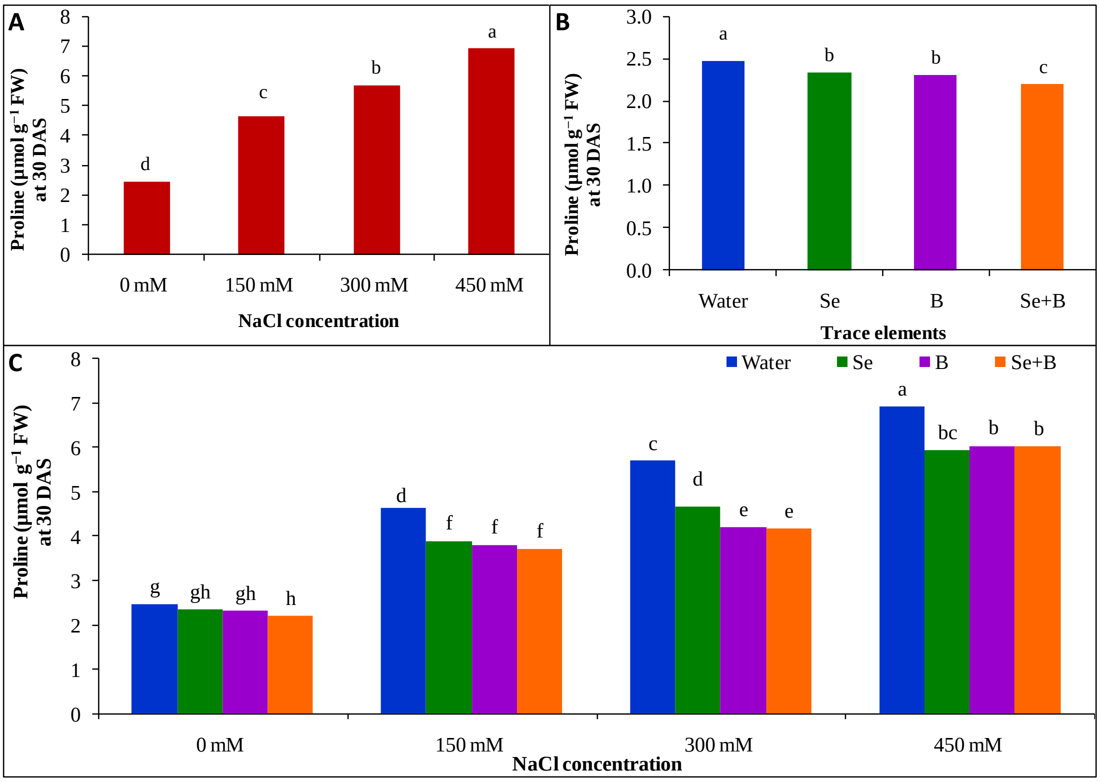

Figure 14. Effect of salinity (A), trace elements (B) and their interaction (C) on proline content of soybean at 30 DAS. Here, Se, B and Se+B indicate 0.50 µM Na_2SeO_4, 1 mM H_3BO_3 and 0.50 µM Na_2SeO_4+1 mM H_3BO_3. Values in a column with different letters are significantly different at $p \leq 0.05$ applying Fisher's LSD test

Proline content increased in response to salt stress at any age in a dose-dependent manner (Figure 14A, 15A). In previous studies of soybean, Pro content increased due to salt stress (Liu *et al.*, 2017). In addition, similar increasing trend in Pro content was observed under salinity in rice (Hasanuzzaman *et al.* 2014), in lentil (Hossain *et al.*, 2019), in rice (Subramanyam *et al.*, 2019), in rapeseed by 109 and 184% (Mahmud *et al.*, 2020), in wheat (Mohsin *et al.*, 2020) etc.

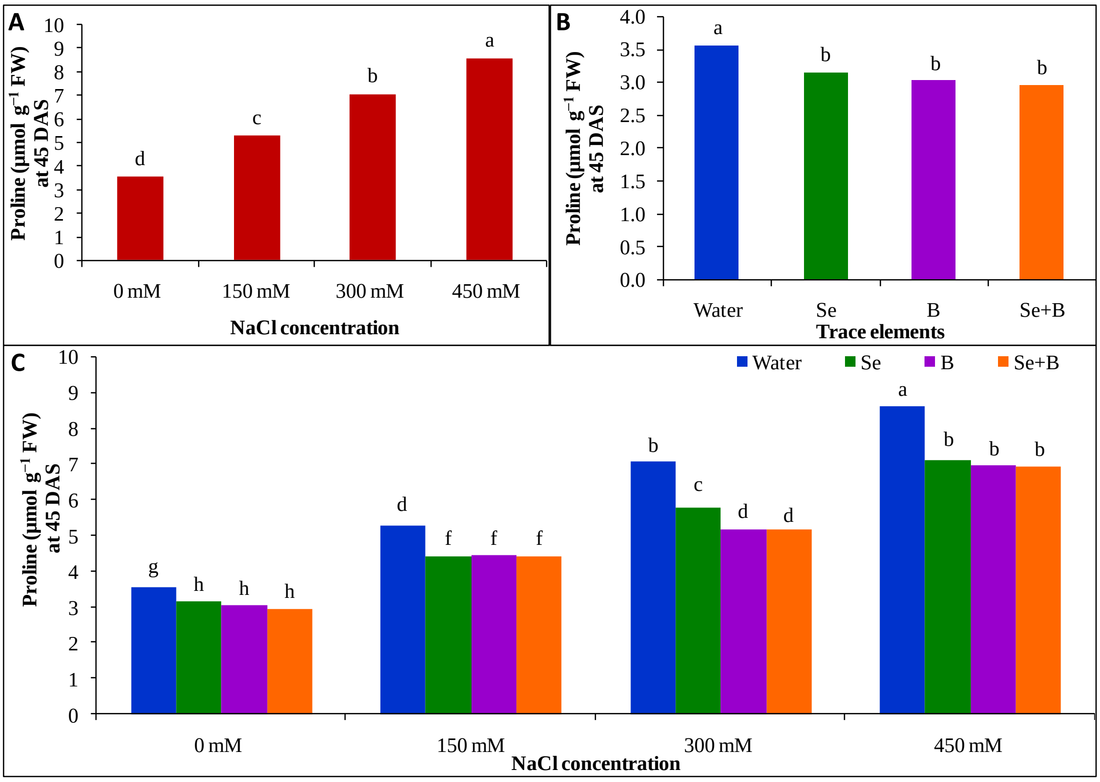

Figure 15. Effect of salinity (A), trace elements (B) and their interaction (C) on proline content of soybean at 45 DAS. Here, Se, B and Se+B indicate 0.50 μM Na_2SeO_4, 1 mM H_3BO_3 and 0.50 μM Na_2SeO_4+1 mM H_3BO_3. Values in a column with different letters are significantly different at $p \leq 0.05$ applying Fisher's LSD test

However, overproduction of ROS is prominent indicator of salt stress which induces oxidative stress. Excessive ROS production occurs under salt stress due to ionic toxicity and osmotic stress. Under stress condition Pro production occurs to maintain the equilibrium between ROS and defense mechanism (Hasanuzzaman *et al.*, 2020b). On the contrary, exogenous application of Se, B and Se+B diminished the Pro content at any age in saline condition (Figure 14C, 15C). According to Ardebili *et al.* (2014), Se application reduced the Pro content in soybean. In cowpea, application of Se decreased the Pro content (Manaf, 2016). Elkelish *et al.* (2019a) reported that application of Se decreased the Pro content in wheat. As an antioxidant Se plays a vital role in accumulation of osmoprotectants and secondary metabolites to enhance the tolerance of plants towards salt-induced oxidative stress. It enhances antioxidant defense mechanism and reduces accumulation of ROS (Hasanuzzaman *et al.*, 2020a).

Thus, Se ultimately reduces the Pro content in plants. Boron plays a role in maintaining enzymatic activity and structural intregrity (Hansch and Mendel, 2009).

4.3 Oxidative stress indicators

4.3.1 Lipid peroxidation (MDA content)

Salt-induced oxidative stress is responsible for deteriorating lipid membrane, termed as lipid peroxidation. A vital indicator of lipid peroxidation is MDA content. In order to estimate the lipid peroxidation level or oxidative stress, MDA content is measured (Hasanuzzaman *et al*., 2014).

The MDA content accelerated under salinity, upon exposure to 150, 300 and 450 mM NaCl-induced salt stress, with the increase of the salt concentration at 30 and 45 DAS (Figure 16A, 17A). Moreover, the maximum MDA content was recorded in the highest salinity level (450 mM NaCl) which was 172 and 132% at 30 and at 45 DAS (Figure 16A, 17A), respectively, in comparison to control. Similarly, under mild and moderate salinity MDA content was increased by 112 and 142% at 30 DAS (Figure 16A). Moreover, MDA content was increased by 34 and 100% at 45 DAS, compared to normal plants at 150 and 300 mM NaCl stress (Figure 17A). On the other hand, foliar application of Se, B and Se+B decreased the MDA content by 9, 5 and 13% at 30 DAS, in comprison to control (Figure 16B). Similarly, exogenous application of Se, B and Se+B diminished the MDA content by 0.12, 0.26 and 4% at 45 DAS, compared to control (Figure 17B). On the contrary, exogenous application of Se, B and Se+B reverted the negative effect of the salt stress. Combined application of Se+B showed better result in diminishing the level of MDA content at 30 and 45 DAS both in saline and non saline condition (Figure 16C, 17C). Moreover, MDA content was reduced due to Se+B application by 19, 23 and 9% under mild, moderate and severe salinity level compared to the corresponding control at 30 DAS (Figure 16C). However, Se+B application decreased the level of MDA content by 21, 29 and 21% under mild, moderate and severe salinity at 45 DAS, in comparison to control (Figure 17C).

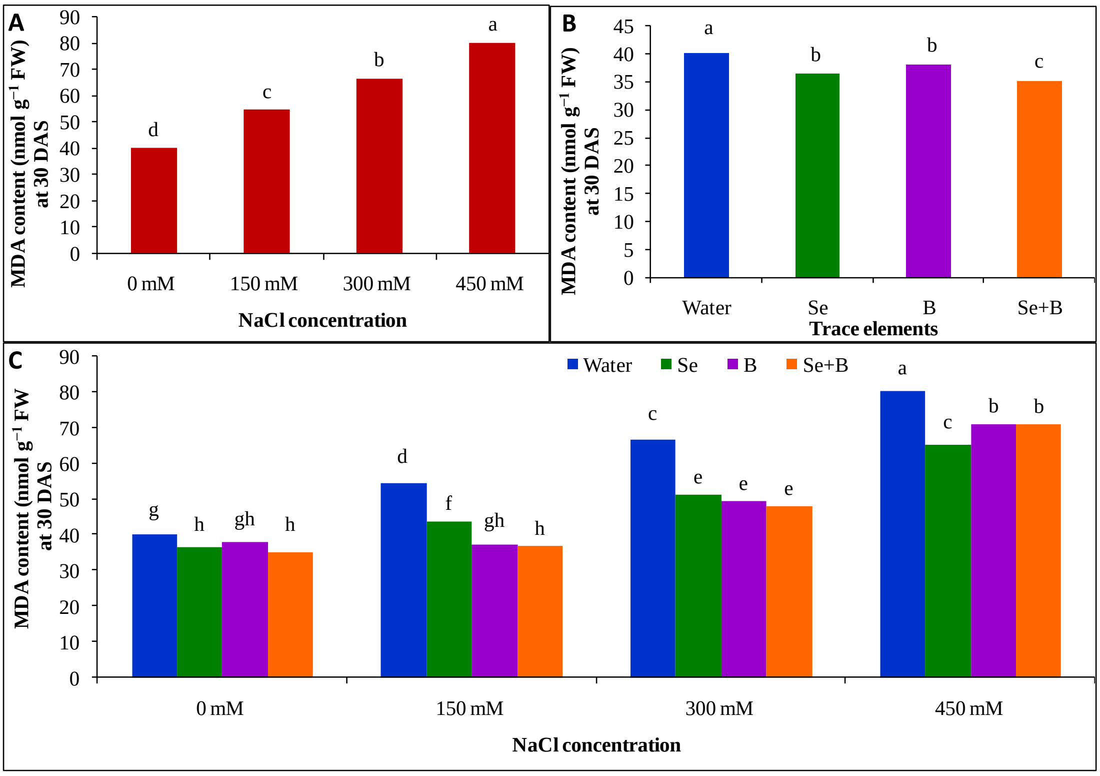

Figure 16. Effect of salinity (A), trace elements (B) and their interaction (C) on MDA content of soybean at 30 DAS. Here, Se, B and Se+B indicate 0.50 µM Na_2SeO_4, 1 mM H_3BO_3 and 0.50 µM Na_2SeO_4+1 mM H_3BO_3. Values in a column with different letters are significantly different at $p \leq 0.05$ applying Fisher's LSD test

Sharp increase in MDA content occurred in response to salinity, in a dose-dependent manner (Figure 16A, 17A). In previous studies, MDA content increased under salt stress in soyben (Akram *et al.*, 2017; El-Eswai *et al.*, 2018). Moreover, similar results was observed in rice (Hasanuzzaman *et al.*, 2014), in lentil (Hossain *et al.*, 2019), in cucumber (Mohsin *et al.*, 2019), in rice (Subramanyam *et al.*, 2019), in rapeseed (Mahmud *et al.*, 2020), in wheat (Mohsin *et al.*, 2020) etc.

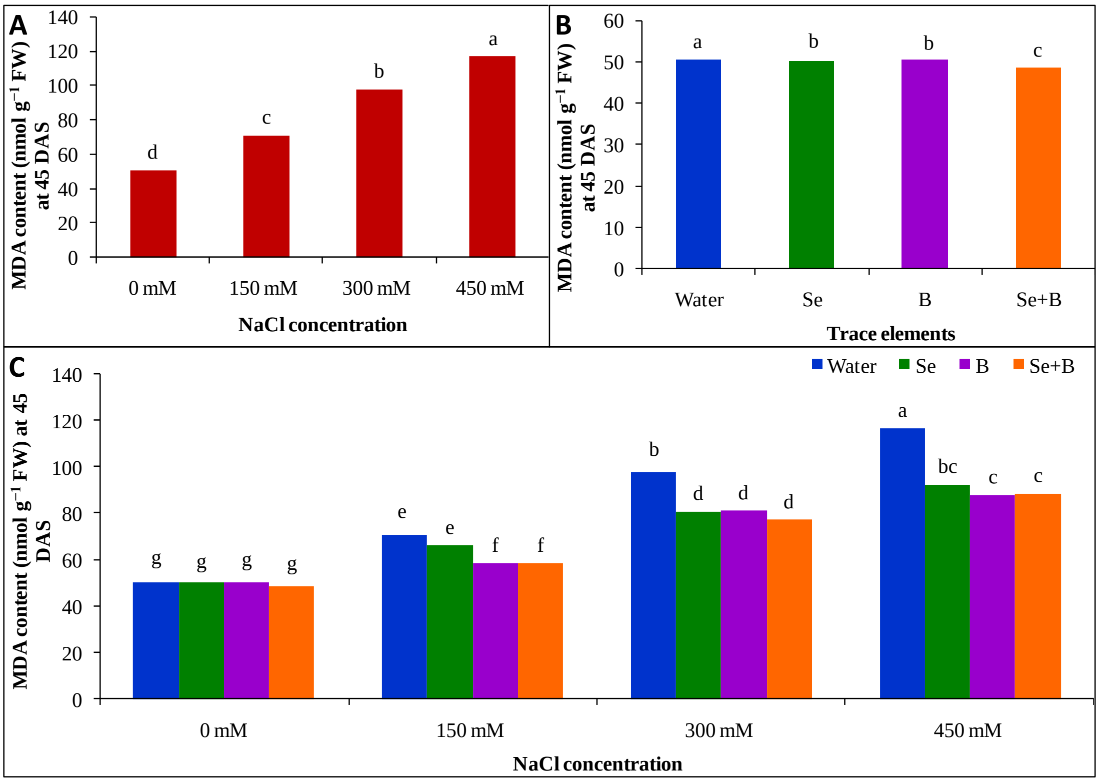

Figure 17. Effect of salinity (A), trace elements (B) and their interaction (C) on MDA content of soybean at 45 DAS. Here, Se, B and Se+B indicate 0.50 µM Na_2SeO_4, 1 mM H_3BO_3 and 0.50 µM Na_2SeO_4+1 mM H_3BO_3. Values in a column with different letters are significantly different at $p \leq 0.05$ applying Fisher's LSD test

However, salinity causes excess production of ROS which is responsible for oxidative stress. Therefore, lipid peroxidation (indicated by MDA content) and reduction in membrane intregity occurs in plants due to salt induced oxidative stress. Hence, MDA content accelerates when plants are subjected to salt stress-induced oxidative stress (Hasanuzzaman *et al.*, 2016; Hasanuzzaman *et al.*, 2020b). On the contrary, foliar application of Se, B and Se+B diminished the MDA content sharply in saline condition and slightly in non-saline condition at the early and later stages (Figure 16C, 17C). However, Se plays a vital role in regulating osmoprotectants and secondary metabolites. Moreover, Se reduces lipid peroxidation (MDA content) by improving the enzymatic and non-enzymatic activity and by reducing ROS induced oxidative stress (Hasanuzzaman *et al.*, 2020a). Boron plays a protective role against excessive lipid peroxidation by maintaining membrane intregrity (Hansch and Mendel, 2009).

4.3.2 H_2O_2 content

In response to 150, 300 and 450 mM NaCl treatments, H_2O_2 content of the plants remarkably increased in all salt-treated plants with the increase of the salt concentration, in comparison to control at 30 and 45 DAS. Moreover, H_2O_2 content was the maximum in the highest level of salinity both at 30 and 45 DAS (Figure 18A, 19A). However, H_2O_2 content was sharply increased by 40, 93 and 131% under mild, moderate and severe salinity at 30 DAS, in comparison to control (Figure 18A). Similarly, H_2O_2 content was increased by 112, 142 and 172% at 45 DAS under mild, moderate and severe salinity, in comparison to control (Figure 19A). However, foliar supplementation of Se, B and Se+B decreased the H_2O_2 content at 30 DAS by 6, 9 and 18%, in comparison to control (Figure 18B). Similarly, at 45 DAS exogenous application of Se, B and Se+B reduced the H_2O_2 content by 10, 8 and 16%, compared to control (Figure 19B). On the contrary, foliar application of Se, B and Se+B reduced the negative effect of salinity. Combined application of Se+B slightly diminished the level of H_2O_2 content under non saline condition but effectively decreased the H_2O_2 content under different salinity level, compared to the corresponding control (Figure 18C, 19C). However, application of Se+B diminished the H_2O_2 content by 17, 21 and 24% under mild, moderate and severe salinity at 30 DAS, compared to control (Figure 18C). Similarly, H_2O_2 content was decreased by 19, 22 and 21% at 45 DAS under mild, moderate and severe salinity, in comparison to control (Figure 19C).

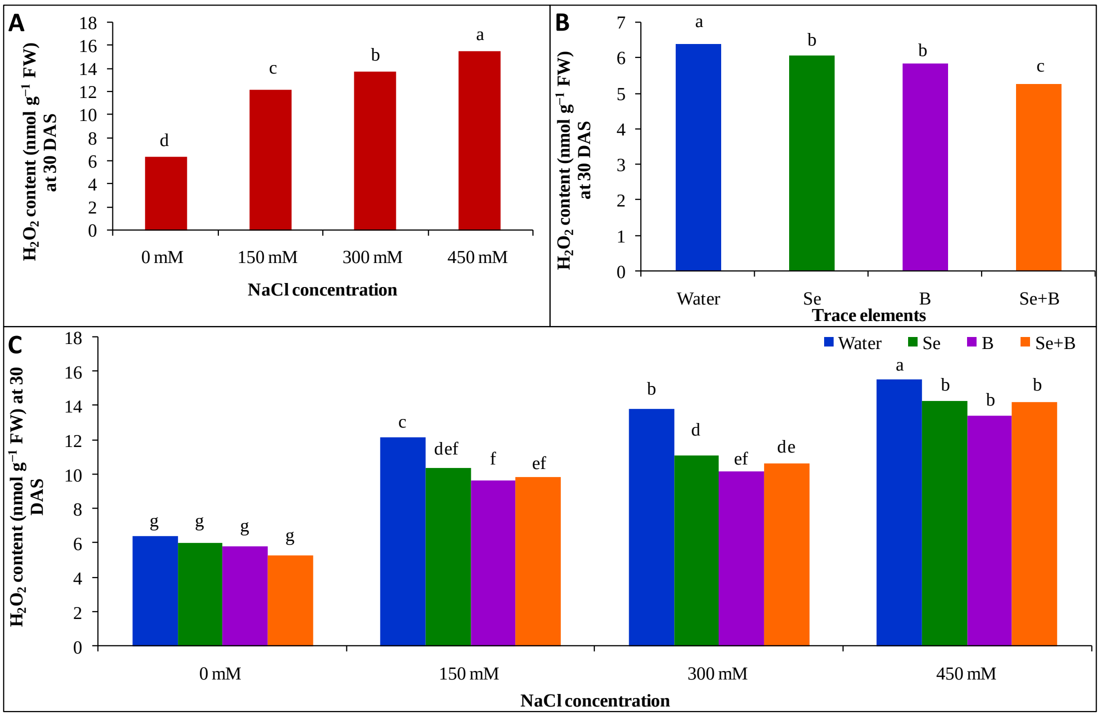

Figure 18. Effect of salinity (A), trace elements (B) and their interaction (C) on H_2O_2 content of soybean at 30 DAS. Here, Se, B and Se+B indicate 0.50 µM Na_2SeO_4, 1 mM H_3BO_3 and 0.50 µM Na_2SeO_4+1 mM H_3BO_3. Values in a column with different letters are significantly different at $p \leq 0.05$ applying Fisher's LSD test

Salt-induced osmotic stress and ionic stress is responsible for higher production of the ROS in plants (Hasanuzzaman *et al.*, 2020b). Remarkable increase in the H_2O_2 content was observed in a dose-dependent manner when exposed to salt stress (Figure 18A, 19A). When subjected to salinity H_2O_2 content increased in soybean (Akram *et al.*, 2017). Under salt stress H_2O_2 content was increasd in rice (Hasanuzzaman *et al.*, 2016), in cucumber (Mohsin *et al.*, 2019), in wheat (Mohsin *et al.*, 2020), in rice (Subramanyam *et al.*, 2019), in rapeseed (Mahmud *et al.*, 2020) etc. Salinity increases H_2O_2 content which resulted in oxidative damages in plants (Ashraf *et al.*, 2018). However, salt stress is responsible for the excessive production of ROS. Thus, salinity-induced excessive ROS like H_2O_2 content leads to oxidative damage of the plants (Hasanuzzaman *et al.*, 2014; Hasanuzzaman *et al.*, 2016; Hasanuzzaman *et al.*, 2020b). Exogenous application of Se, B and Se+B reduced the H_2O_2 content in soybean plants, in comparison to control (Figure 18C, 19C). Application of Se decreased the H_2O_2 content in maize (Ashraf *et al.*, 2018), in rice (Subramanyam *et al.*, 2019), in wheat (Elkelish *et al.*, 2019) etc.

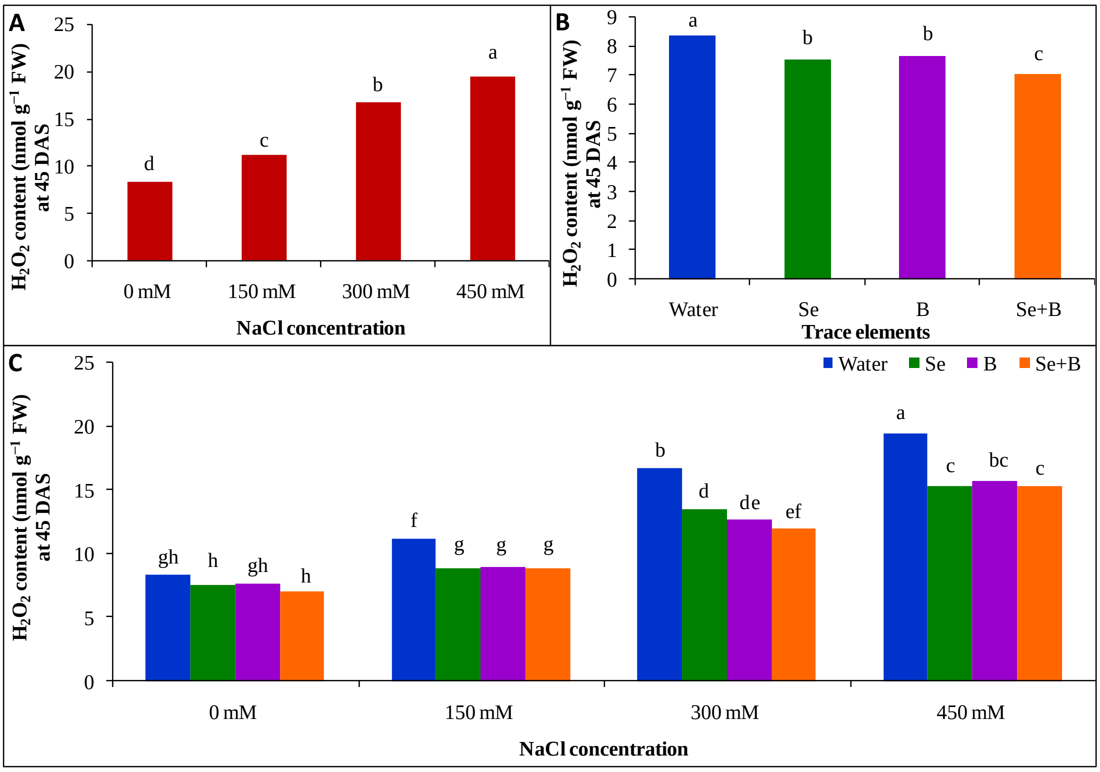

Figure 19. Effect of salinity (A), trace elements (B) and their interaction (C) on H_2O_2 content of soybean at 45 DAS. Here, Se, B and Se+B indicate 0.50 µM Na_2SeO_4, 1 mM H_3BO_3 and 0.50 µM Na_2SeO_4+1 mM H_3BO_3. Values in a column with different letters are significantly different at $p \leq 0.05$ applying Fisher's LSD test

According to Kamran *et al.* (2020), as salt stress threats plant productivity, many investigations have been made and attempts have been taken to mitigate the hazardous effect of salt stress by applying phytohormones, osmoprotectants, antioxidant, polyamines and trace elements. After numerous investigations Se has been found to be an effective one in improving growth and inducing tolerance mechanism against salt stress. Selenium mitigates salt stress by reducing Na^+ accumulation in plant parts, Na^+ compartmentalization, upregulating Na^+ and Cl^- ions transporter genes, chelation and boosting of antioxidant defense system. Selenium protects plants from oxidative stress by triggering the detoxification of ROS, generated due to salt stress (Hasanuzzaman *et al.*, 2020b). Boron plays a protective role against excessive ROS by maintaining membrane intregrity, metabolic activity and enzymatic activity (Hansch and Mendel, 2009; Rattan, 2015).

4.4 Yield Parameter

Under normal condition plant uses most of the energy and resources in growth and metabolic processes. However, resource allocation for growth and development decreased with the increasing levels of salinity. Major portion of energy and resources are used in reducing of salt stress instead of vegetative and reproductive growth (Munns and Gilliham, 2015; Zörb *et al.*, 2019). Soybean is a moderately salt tolerant plant which means it is able to produce 80% biomass under 110 mM NaCl-induced stress, compared to control. Further increase in salinity level will decrease the growth and yield of soybean (Munns and James, 2003). In this study, 300 and 450 mM NaCl-induced stress caused over production of ROS and disrupted the equilibrium of ROS and antioxidant defense system of the plant. Disruption in ion homeostesis is a prominent effect of salt stress. Moreover, ROS is responsible for oxidative damge of lipids, proteins, nucleic acid and carbohydrates. Deterioration in their properties and functions of the cell organelles ultimately hampers the physiological and biochemical activity of plants. Imbalance in the ion homeostasis at cellular and subcellular level occurs because of over production of ROS, results in cell death (Ivanova *et al.*, 2016, Hasanuzzaman *et al.*, 2020b). That’s why in response to 300 and 450 mM NaCl-induced salt stress plant death occurred, after completing the vegetative stage in this study (Plate 1, 2).

4.4.1 Number of flowers plant^{-1}

When the plants were subjected to 150, 300 and 450 mM NaCl-induced salt stress, the number of flowers plant^{-1} sharply decreased with the increment of the concentration of NaCl. Severe salinity level resulted in the minimum number of flowers plant^{-1} which was 71% lower than the control plants. Moderate salinity reduced 53% and mild salinity reduced 26% flower number plant^{-1}, compared to control plants, respectively (Figure 20A). Among the trace elements, combined application of Se+B showed better result than Se or B alone and the highest flower number plant^{-1} was observed in Se+B treated plants under normal condition. Supplementation of Se, B and Se+B increased the number of flowers plant^{-1} by 9, 24 and 37%, in comparison to the corresponding control (Figure 20B). However, supplementation of Se, B and Se+B increased the flower number plant^{-1} both in saline and non saline condition. Under severe, moderate and mild salinity flower number plant^{-1} was increased by 120, 38 and 33% due to Se+B supplementation, compared to control (Figure 20C).

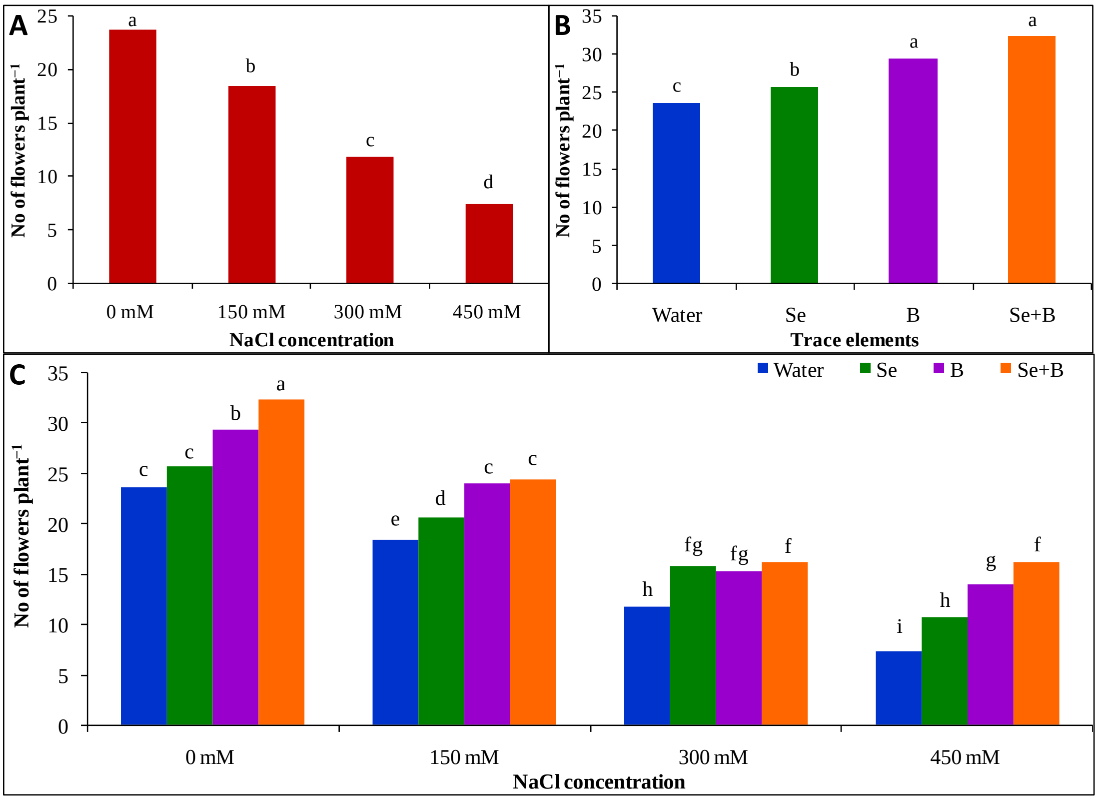

Figure 20. Effect of salinity (A), trace elements (B) and their interaction (C) on number of flowers plant^{-1} of soybean. Here, Se, B and Se+B indicate 0.50 µM Na_2SeO_4, 1 mM H_3BO_3 and 0.50 µM Na_2SeO_4+1 mM H_3BO_3. Values in a column with different letters are significantly different at $p \leq 0.05$ applying Fisher's LSD test

In legumes, salinity negatively affects the the number of flower plant^{-1}. Salt stress causes reduction in the number of flower and also responsible for flower abortion (Khan *et al.*, 2017). In response to mild, moderate and severe salinity the number of flower plant^{-1} remarkably reduced, in compare to control (Figure 20A). El-Sabagh *et al.* (2015) reported that salinity reduced the yield and yield attributes in soybean. Supplementation of Se, B and Se+B increased the flower number plant^{-1}, compared to the corresponding control (Figure 20). The number of flowers plant^{-1} is associated with yield and application of Se has positive effects on plant growth, development, yield and yield contributing parameters (Hasanuzzaman *et al*., 2020a). Boron is involved in flower production and bearing, growth and expansion of pollen tube, germination of pollen, seed and fruit bearing and plays a crucial role (Rattan, 2015).

4.4.2 Number of pods plant^{-1}

In response to exposure upon salt stress the number of pods plant^{-1} was noticeably decreased. However, the number of pods plant^{-1} was reduced by 38% under 150 mM NaCl-induced stress, compared to control plants (Figure 21A). On the contrary, exogenous application of Se, B and Se+B reverted the adverse effect of the salt stress. Application of Se slightly increased the pods plant^{-1}, application of B increased the pod plant^{-1} by 10% and combined application of Se+B increased the pod plant^{-1} by 11%, compared to control. The results showed that combined application of Se+B gave better result than individual application of Se and B (Figure 21B). However, compared to individual supplementation combined supplementation of Se+B showed better result under salinity and normal condition. Application of Se+B increased number of pods plant^{-1} by 3.18% under normal condition and by 36% under mild salinity, in comparison to control (Figure 21C).

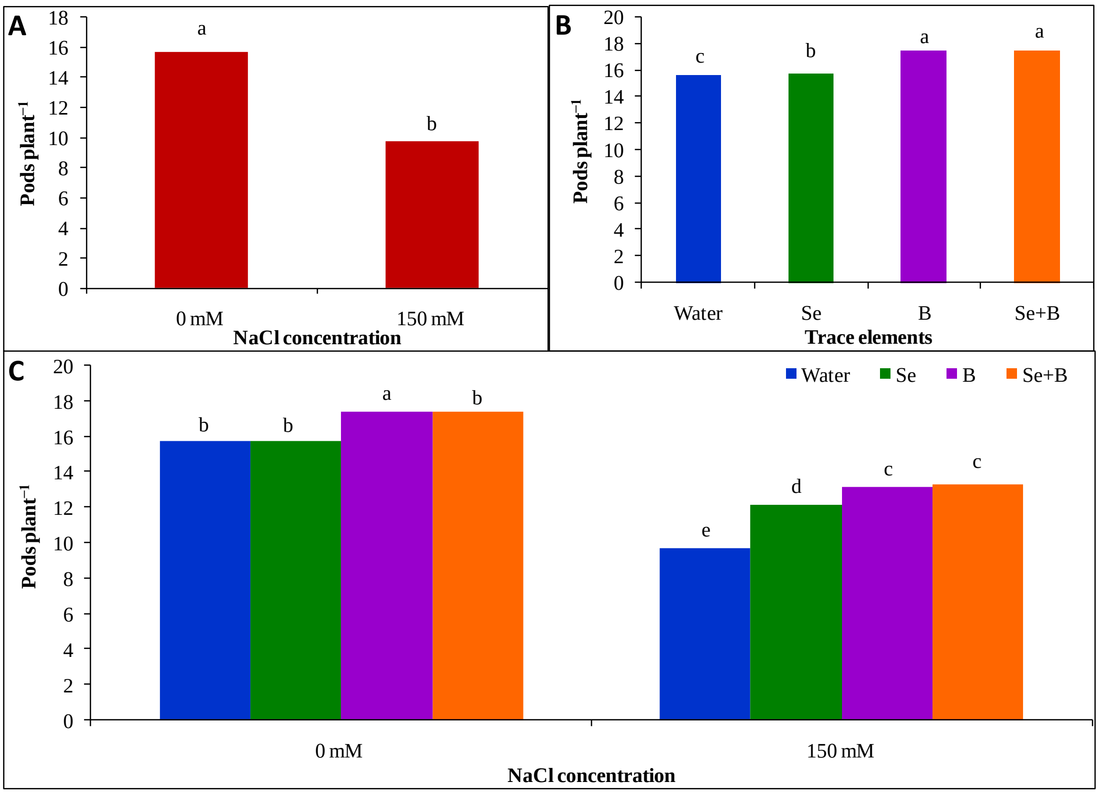

Figure 21. Effect of salinity (A), trace elements (B) and their interaction (C) on number of pods plant^{-1} of soybean. Here, Se, B and Se+B indicate 0.50 µM Na_2SeO_4, 1 mM H_3BO_3 and 0.50 µM Na_2SeO_4+1 mM H_3BO_3. Values in a column with different letters are significantly different at $p \leq 0.05$ applying Fisher's LSD test

Salinity decreases the number of flowers plant^{-1} and also causes abortion of flowers, which ultimately leads to the reduction in the number of pods plant^{-1} (Khan *et al.*, 2017). Mild salinity decreased the number of pods plant^{-1}, compared to control (Figure 21). Salinity decreased the number of pods plant^{-1} in several genotypes of soybean, ranging from 52 to 32% in BINA Soybean-03, BARI soybean-06, BARI soybean-05, BINA Soybean-04, BINA Soybean-01, Sohag, BINA Soybean-02 and SBM-09, respectively, compared to control (Ghassemi-Golezani *et al.*, 2009; Akram *et al.*, 2017). Main determinants of grain yield in grain legumes as well as in soybean are the number of pods plant^{-1}, seeds pod^{-1} and the weight of single grain. Reduction occurred in number of flowers and also in pollen production due to salinity, which ultimately reduces the number of pods plant^{-1}, seeds pod^{-1} and the seed weight (Dhingra and Varghese, 1993). As a result of salinity pod abortion occurrs which causes reduction in the number of pods in legume crops (Khan *et al.*, 2016). On the contrary, supplementation of Se, B and Se+B increased the number of pods plant^{-1}, compared to control (Figure 21B, 21C). Supplementation of Se (Na_2SeO_4) reduces the excessive concentration of Na^+ and Cl^- ions in root zone and improves the root growth, water availability and nutrient uptakes. Selenium assists in nutrient absorption and translocation within different parts of the plants which increases growth and productivity. Suitable Se supplementation reduces oxidative damage. Therefore, supplementation of Se increases the growth and productivity under salt stress by increasing physiological and biochemical activities and by mitigating negative effect of salt stress (Zhang *et al.*, 2006; Shahzadi *et al.*, 2017; Astaneh *et al.*, 2019; Subramanyam *et al.*, 2019; Hasanuzzaman *et al.*, 2020a). Boron plays a role in bearing of flowers, pollen formation, elongation and growth of pollen tube, germination, fixation of nitrogen and assimilation of nitrates (Broschat, 2009). Accordingly, application of Se and B increased the number of pods plant^{-1} in the present study (Figure 21C).

4.4.3 Pod length

Upon exposure to salt stress pod length was sharply reduced, compared to control. Mild salinity (150 mM NaCl) caused 9% reduction in the pod length, compared to control (Figure 22A). On the other hand, foliar application of Se, B and Se+B increased the pod length by 5, 30 and 32%, in comparison to control. Combined application of Se+B increased the pod length more than single application of Se and B (Figure 22B). Foliar application of Se, B and Se+B increased the pod length under saline and non saline condition. Combined application of Se+B increased the pod length more than individual application of Se and B under saline and normal condition. Pod length was increased due to Se+B application by 32% in normal condition and by 20% under mild salinity, in comparison to control (Figure 22C).

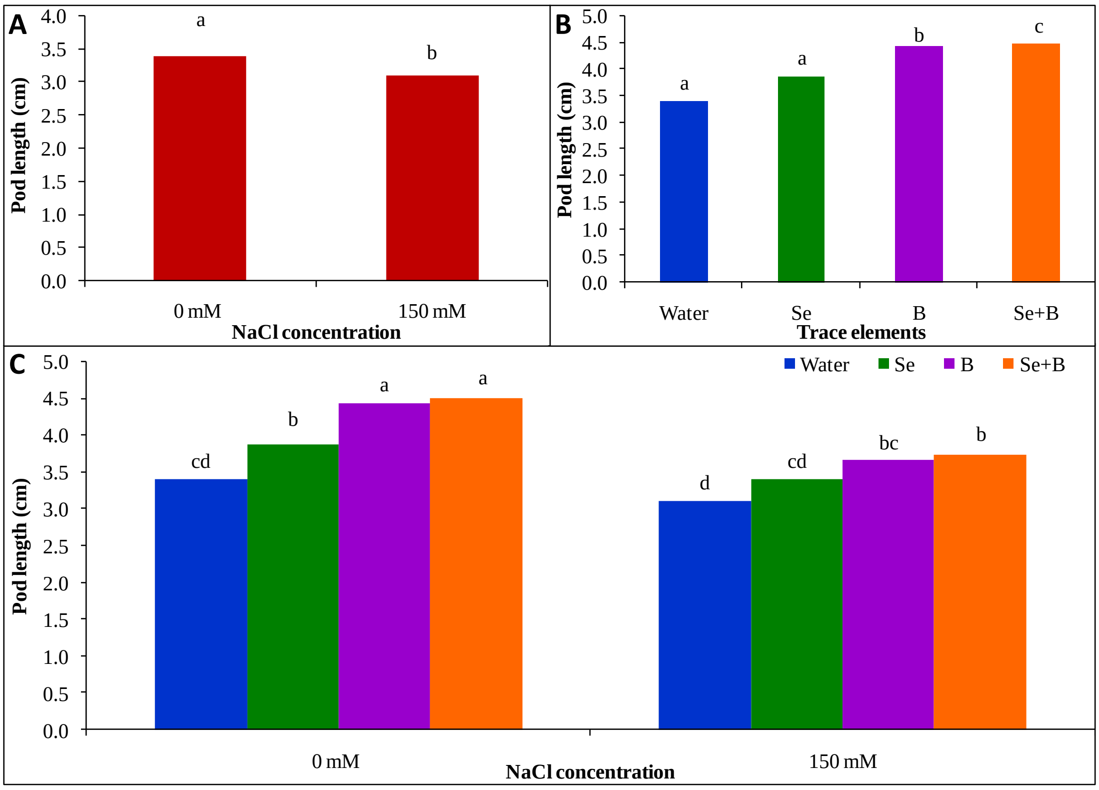

Figure 22. Effect of salinity (A), trace elements (B) and their interaction (C) on pod length of soybean. Here, Se, B and Se+B indicate 0.50 µM Na_2SeO_4, 1 mM H_3BO_3 and 0.50 µM Na_2SeO_4+1 mM H_3BO_3. Values in a column with different letters are significantly different at $p \leq 0.05$ applying Fisher's LSD test

Salinity decreases the length of pollen tube, fertilization and grain numbers ultimately resulted in lower seed number, pod length and seed yield (Dhingra and Varghese, 1993). Salinity affects the light harvesting, carbon fixation, nutrient uptake, nutrient assimilation, grain development and yield formation, which affects the pod length (Farooq *et al.*, 2017). Salinity reduces the uptake of several nutrients and leads to reduction in yield and yield attributing parameters (El Sayed, 2011). Salt induced excessive ROS production decreases the stomatal conductase which restricts transpiration and reduces CO_2 influx in Calvin Cycle (Abogadallah, 2010). These are the reasons of decreasing pod length under salinity. In contrary, application of Se, B and Se+B increased the pod length under saline and non-saline condition (Figure 22). Selenium increased the root growth, water uptake, nutrient uptake and nutrient translocation in different plant parts which increased the growth and productivity. Moreover, application of Se increased the pod length under salt stress by increasing tolerance against salt stress (Zhang *et al.*, 2006; Shahzadi *et al.*, 2017; Astaneh *et al.*, 2019; Subramanyam *et al.,* 2019; Hasanuzzaman *et al.*, 2020a). Boron is required for production of flowrers, retention of flowers, elongation of pollen tube, pollen germination, formation of seed and fruit (Rattan, 2015).

4.4.4 Number of seeds pod$^{–1}$

Imposition of salt stress caused marked decline in the number of seeds pod$^{–1}$, in comparison to control. Moreover, plants exihibited 35% decrease in the number of seeds pod$^{–1}$ under 150 mM salt stress, in comparison to control (Figure 23A). Application of Se, B and Se+B increased the number of seeds pod$^{–1}$ by 6, 14 and 16%, compared to control (Figure 23B). However, under saline and non saline condition the exogenous application of Se, B and Se+B increased the number of seeds pod$^{–1}$, in comparison to control. Moreover, combined application of Se+B showed better result than Se or B alone in both saline and normal condition. Combinedly, Se+B increased the number of seeds pod$^{–1}$ by 16% under normal condition and by 38% under saline condition, in comparison to the control (Figure 23C).

Upon exposure to salt stress number of seeds pod$^{–1}$ decreased, compared to control (Figure 23). When subjected to salt stress different soybean genotypes exhibited reduction in number of seeds pod$^{–1}$, compared to control. Salinity induced stress decreased the number of seed in BINA Soybean-04, SBM-22, Sohag, BINA Soybean-02, BARI soybean-05, BINA Soybean-03, SBM-15 and SBM-09 ranging from 9.55 to 5.08%, compared to control (Akram *et al.,* 2017).

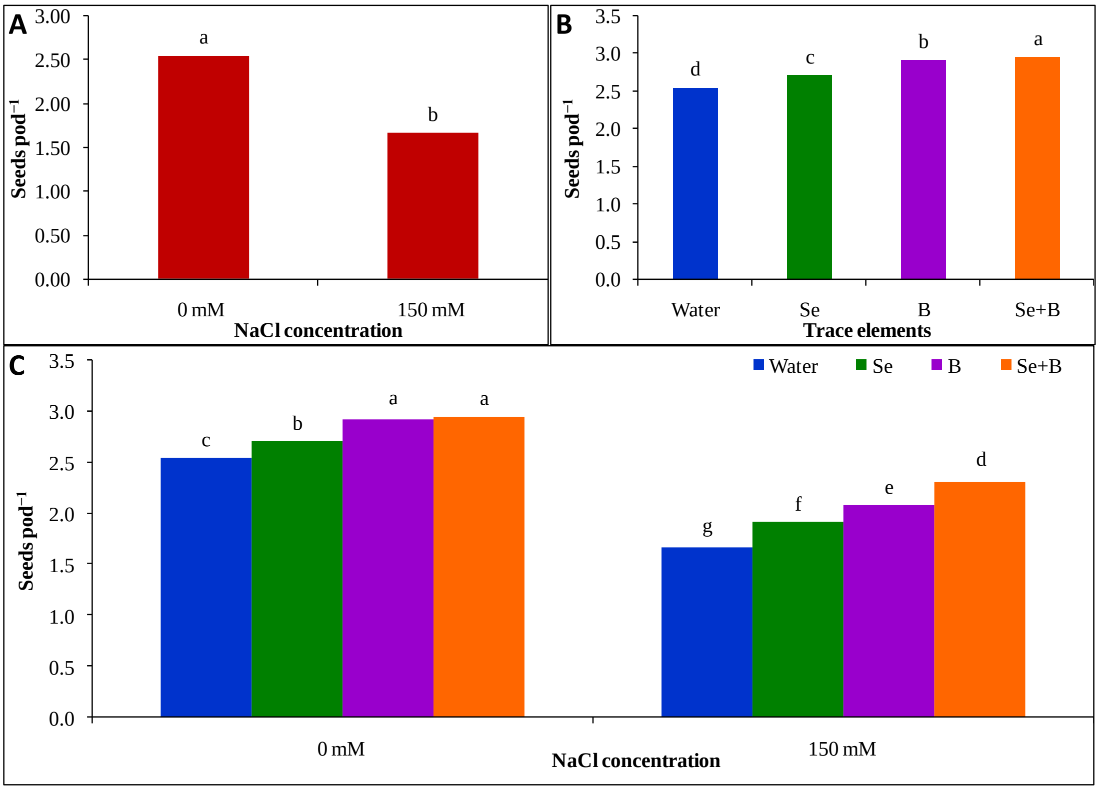

Figure 23. Effect of salinity (A), trace elements (B) and their interaction (C) on seeds pod^{-1} of soybean. Here, Se, B and Se+B indicate 0.50 µM Na_2SeO_4, 1 mM H_3BO_3 and 0.50 µM Na_2SeO_4+1 mM H_3BO_3. Values in a column with different letters are significantly different at $p \leq 0.05$ applying Fisher's LSD test

Salt-induced stress decreased the number of flowers and also the pollen production, which ultimately reduces the number of pods plant^{-1}, seeds pod^{-1} and the seed weight. Salinity decreased the length of pollen tube, fertilization and grain numbers ultimately resulted in lower seed number and seed yield (Dhingra and Varghese, 1993). In this study, application of Se, B and Se+B increased the number of seeds pod^{-1}, compared to control (Figure 23C). Supplementation of Se increases the root growth, water uptake, nutrient uptakes, light harvest, C-assimilation and several nutrients translocation within different plant parts which increases growth and production. Application of Se maintains ionic balance and reduces the oxidative damages (Zhang *et al.*, 2006; Shahzadi *et al.*, 2017; Astaneh *et al.*, 2019; Subramanyam *et al.*, 2019; Hasanuzzaman *et al.*, 2020a). Boron is crucial element for formation, growth and development of seed and fruit from the very beginning of the process (Rattan, 2015;

Broschat, 2009). Application of Se, B and Se+B increased the seed pod^{-1} in the present study (Figure 23C).

4.4.5 Seed yield plant^{-1}

When subjected to salt stress plant exhibited reduction in seed yield plant^{-1}, in comparison to control. However, mild salinity decreased the seed yield by 26%, compared to control (Figure 24A). On the other hand, foliar application of Se, B and Se+B reverted the negative effect of salinity and increased the seed yield plant^{-1} by 3, 4 and 6%, in comparison to control (Figure 24B). Seed yield was higher in Se+B-supplemented plants than single supplementation of B and Se, compared to control under salinity and normal condition. In comparison to control, application of Se+B increased the seed yield by 6 and 24% under mild salinity and control, respectively (Figure 24C).

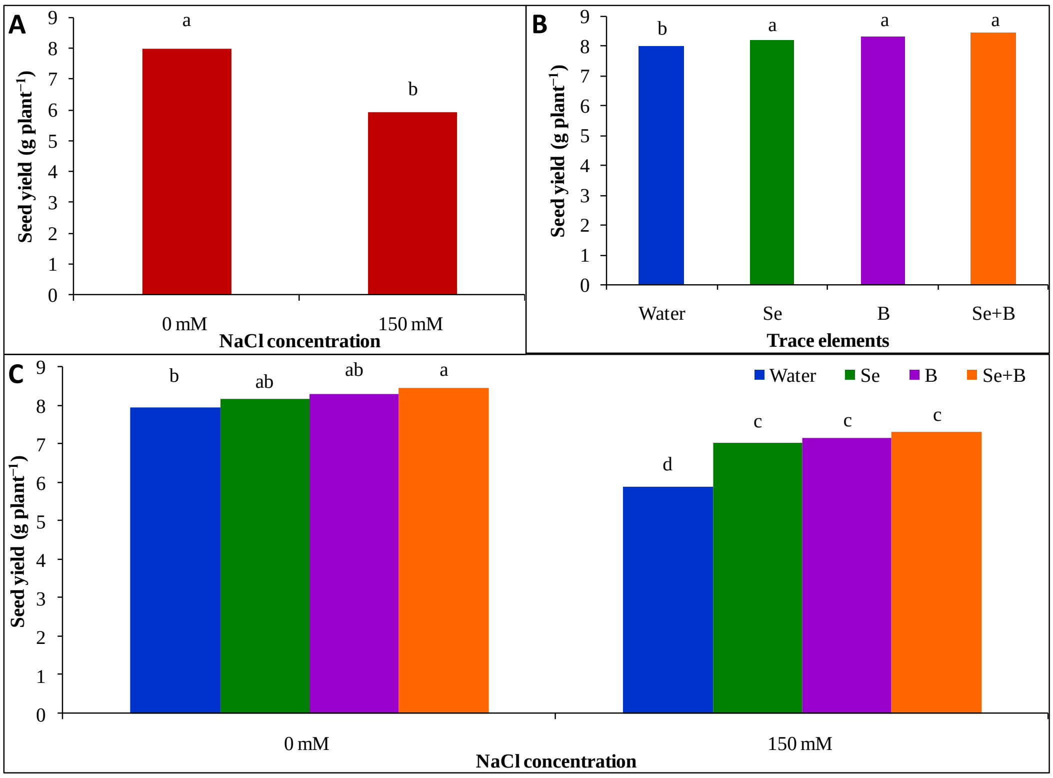

Figure 24. Effect of salinity (A), trace elements (B) and their interaction (C) on seed yield of soybean. Here, Se, B and Se+B indicate 0.50 µM Na_2SeO_4, 1 mM H_3BO_3 and 0.50 µM Na_2SeO_4+1 mM H_3BO_3. Values in a column with different letters are significantly different at $p \leq 0.05$ applying Fisher's LSD test

Salinity decreased the seed yield plant^{-1} compared to control plants, as obseved in this study (Figure 24). Salinity decreased the seed yield plant^{-1} in BINA Soybean-03, BINA Soybean-04, SBM-15, BARI soybean-06, BARI soybean-05, Shohag, SBM-09, SBM-22, SBM-18, BINA Soybean-01 and BINA Soybean-02 ranging from 51 to 38%, in comparison to control (Akram *et al.,* 2017). Yield reduction under salinity is related with reduced carbon assimilation, disturpted hormonal regulation, lower nutrient uptake, ion toxicity and osmotic stress, delayed flowering and less number of flower of pods in grain legumes, as well as in soybean (El-Sayed *et al.,* 2011; Khan *et al.,* 2016). Reduction in CO_2 intake accelerates the respiration and causes the lower C-assimilation and therefore, causes seed yield reduction (Abogadallah, 2010). In this study, salinity deacresed the flower number, branch number, SPAD value, root and shoot FW, root and shoot dry weight, ultimately reduced the seed yield. On the other hand, application of Se, B and Se+B increased these parameters in this study and thus, played a role in increasing the seed yield under saline and normal condition (Figure 24C).

4.4.6 Stover yield

When subjected to salt stress, plants showed reduction of the stover yield, in comparison to control. However, mild salinity decreased the stover yield by 3.31%, compared to control (Figure 25A). On the other hand, foliar application of Se, B and Se+B reverted the negative effect of salinity and increased the stover yield, in comparison to control. Application of Se and B increased the stover yield slightly but combined application of Se+B increased the stover yield by 10%, compared to control (Figure 25B). Stover yield was higher in Se+B-supplemented plants than single supplementation, compared to control under salt stres and normal condition. In comparison to control, application of Se+B increased the stover yield by 4 and 10% under mild salinity and non saline condition (Figure 25C).

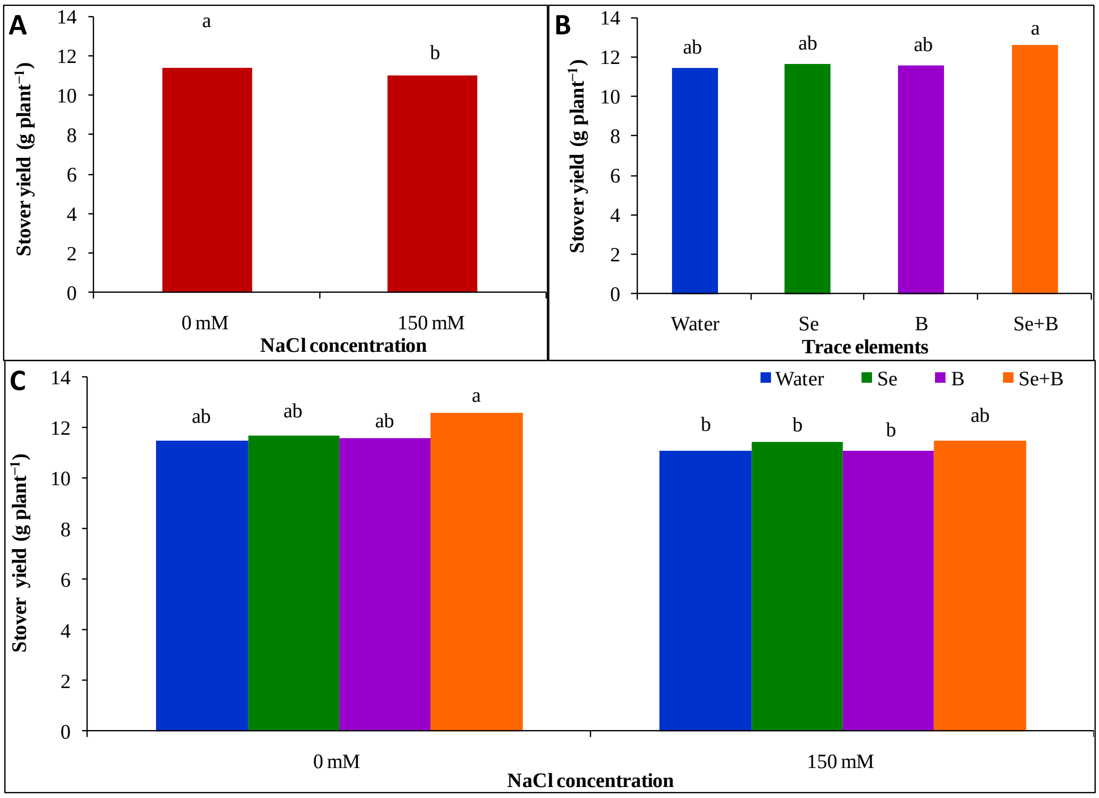

Figure 25. Effect of salinity (A), trace elements (B) and their interaction (C) on stover yield of soybean. Here, Se, B and Se+B indicate 0.50 µM Na_2SeO_4, 1 mM H_3BO_3 and 0.50 µM Na_2SeO_4+1 mM H_3BO_3. Values in a column with different letters are significantly different at $p \leq 0.05$ applying Fisher's LSD test

Stover is associated with the biomass accumulation. Salinity reduced the stover yield, compared to control condition (Figure 25A). Moreover, in previous studies, salinity decreased the shoot FW and shoot DW in soybean plants (Klein *et al.*, 2015; El-Eswai *et al.*, 2018). Salt stress showed similar results in case of shoot FW and DW in other studies of various crops like wheat (Elkelish *et al.*, 2019), lentil (Hossain *et al.*, 2019), maize (Ashraf *et al.*, 2018; Jiang *et al.*, 2017), oat (Sapre *et al.*, 2018), rice (Rahman *et al.*, 2016) etc. Salt-induced osmotic stress and ionic toxicity hampers growth and development of the plant. Reduction in photosynthesis and stomatal conductase greatly hampers the growth parameters of the plant under the salt stress (Kordrostami and Rabiei, 2019). These might cause the reduction in C-assimilation and lower biomass accumulation which ultmimately reduced the stover yield, in this study. On the contrary, supplementation of Se, B and Se+B increased the stover yield under salt stress. Exogenously applied of Se increased all growth parameters of this study and ultimately, increased the stover yield. As a micronutrient B is crucial for growth and development. It is responsible for the cell division, growth and development of different plant parts, specially the actively growing regions (Ullah et al., 2012; Rattan, 2015). All of these positive effects of Se, B and Se+B-supplementation assisted to increase the dry matter production and led to increment of stover yield in soybean plants (Figure 25B, 25C).

4.4.7 Biological yield

Upon exposure to salt stress biological yield decreased, in comparison to control. However, 150 mM NaCl-induced salt stress decreased the biological yield by 13%, compared to control (Figure 26A). On the contrary, exogenous application of Se, B and Se+B showed positive effect on biological yield, in comparison to control. Foliar application of Se, B and Se+B increased the biological yield by 5, 2 and 12%, in comparison to control (Figure 26B). However, in saline and normal condition exogenous application of Se, B and Se+B increased biological yield, compared to control. Moreover, combined application of Se+B increased the biological yield more than the individual application of Se and B. Under mild salinity and normal condition Se+B increased the biological yield by 10 and 12%, compared to control (Figure 26C).

In soybean, salinity is responsible for reduction in growth traits and biomass accumulation which causes the reduction in biological yield. Salinity reduced the biological yield of soybean, compared to control (Figure 26A). Salinity adversely affects the availability, competitive nutrient uptake, light harvesting and carbon assimilation and also the translocation process of the nutrients to the different parts of the plants.

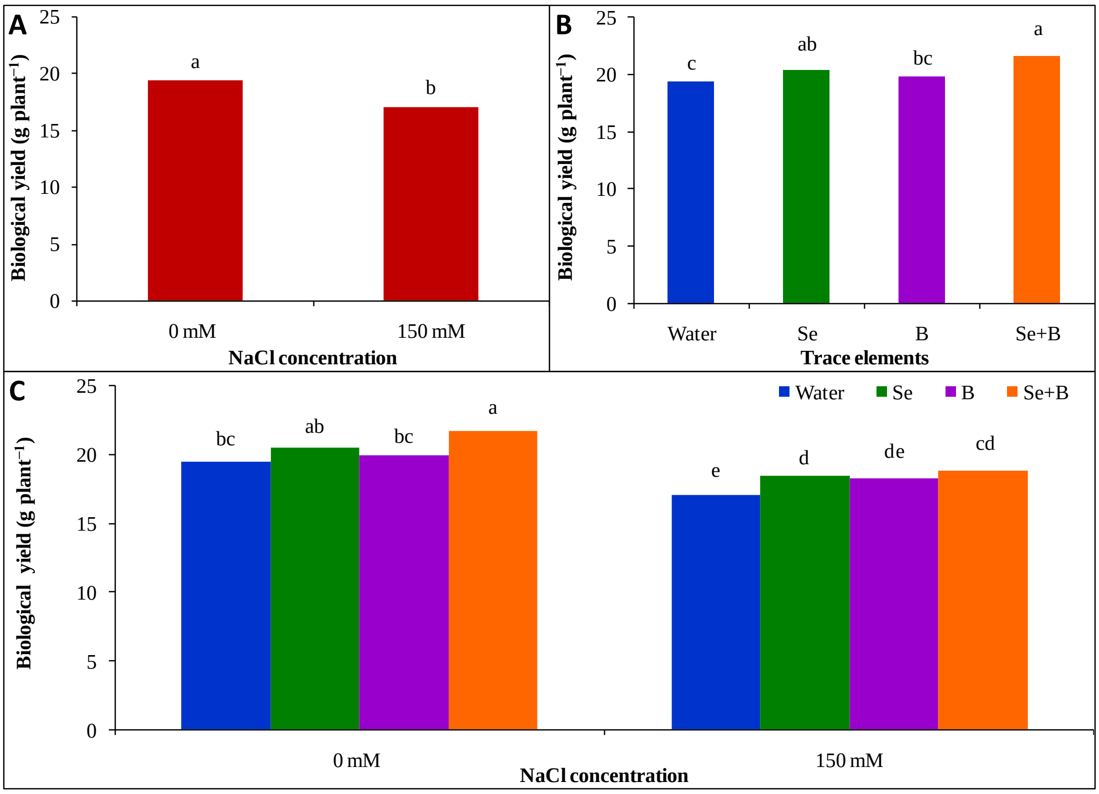

Figure 26. Effect of salinity (A), trace elements (B) and their interaction (C) on biological yield of soybean. Here, Se, B and Se+B indicate 0.50 µM Na_2SeO_4, 1 mM H_3BO_3 and 0.50 µM Na_2SeO_4+1 mM H_3BO_3. Values in a column with different letters are significantly different at $p \leq 0.05$ applying Fisher's LSD test

Salinity interferes with several nutrients uptake, including B. Salinity causes oxidative damage and reduction in biomass accumulation of soybean (El-Sayed *et al.,* 2011, Hasanuzzaman *et al*., 2016). In this study, salinity decreased the plant height, shoot FW, shoot DW, root FW, root DW, branch number, flower number, RWC and SPAD value. Moreover, salinity increased the Pro content, MDA content and also H_2O_2 content which indicates oxidative damages. However, all of these causes ultimately reduced the biological yield in this study. On the contrary, application of Se, B and Se+B increased all of these parameters and consequently increased the biological yield under normal condition and mild salt stress (Figure 26B, 26C).

4.5 Phenotypic observation

Phenotypic appearance of soybean seedlings were observed under different levels of salinity (150, 300 and 450 mM NaCl), compared to control. Sharp reduction of shoot length and root length occurred under salinity in a dose-dependent manner (Figure 27). Foliar application of Se, B and Se+B increased the shoot growth under 150 mM NaCl, compared to control (Figure 28). Similarly, under 300 and 450 mM NaCl stress exogenous Se, B and Se+B improved the soybean shoot growth, in comparison to control (Figure 29, 30).

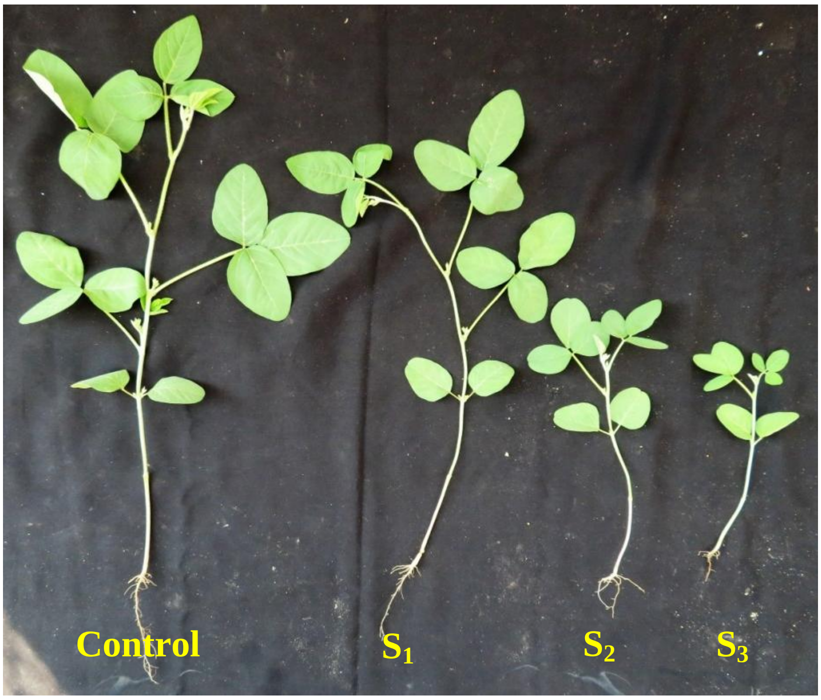

Figure 27. Phenotypic appearance of soybean seedlings treated with different levels of salinity compare to control. Here, S_1=150 mM NaCl, S_2=300 mM NaCl and S_3=450 mM NaCl

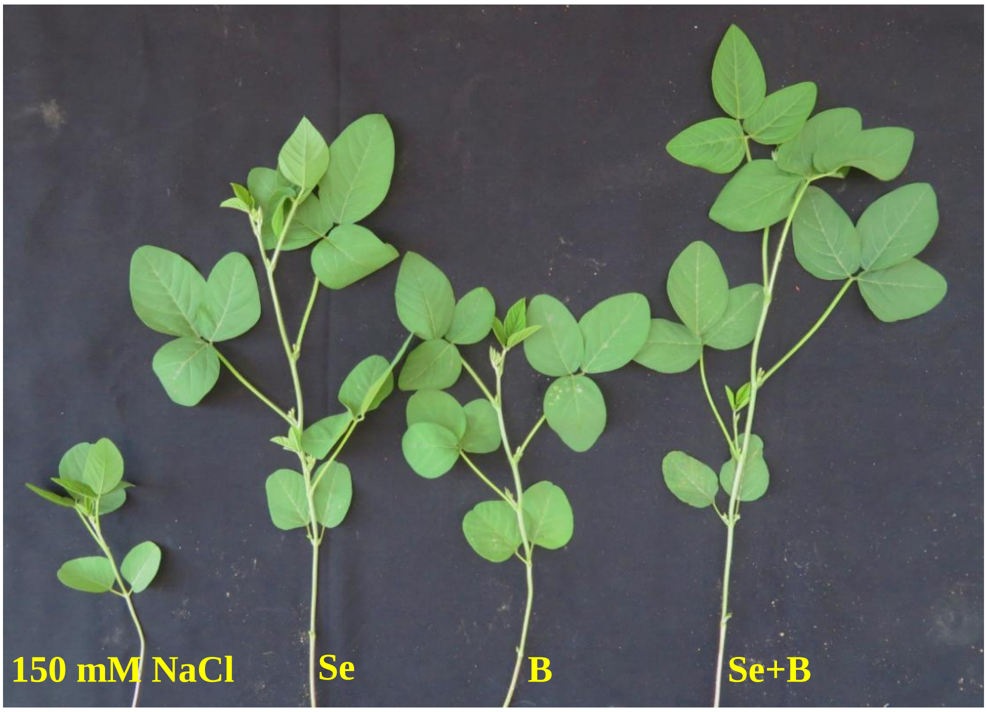

Figure 28. Phenotypic appearance of shoot of soybean seedlings at 28 DAS treated with 150 mM NaCl with or without Se, B and Se+B. Here, Se and B indicates 0.50 µM Na_2SeO_4 and 1 mM H_3BO_3

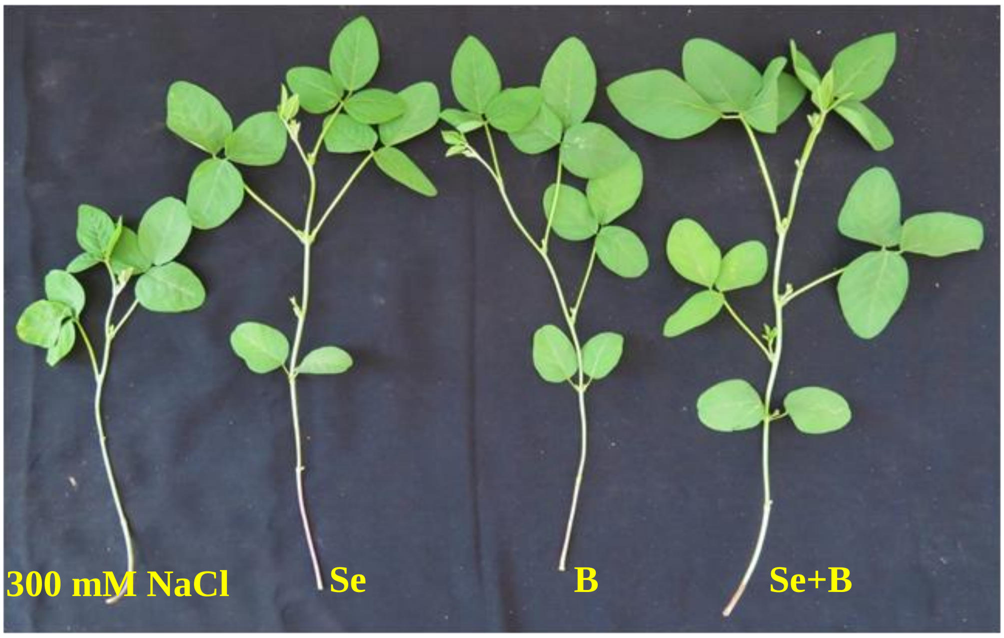

Figure 29.Phenotypic appearance of shoot of soybean seedlings at 28 DAS treated with 300 mM NaCl with or without Se, B and Se+B. Here, Se and B indicates 0.50 µM Na_2SeO_4 and 1 mM H_3BO_3

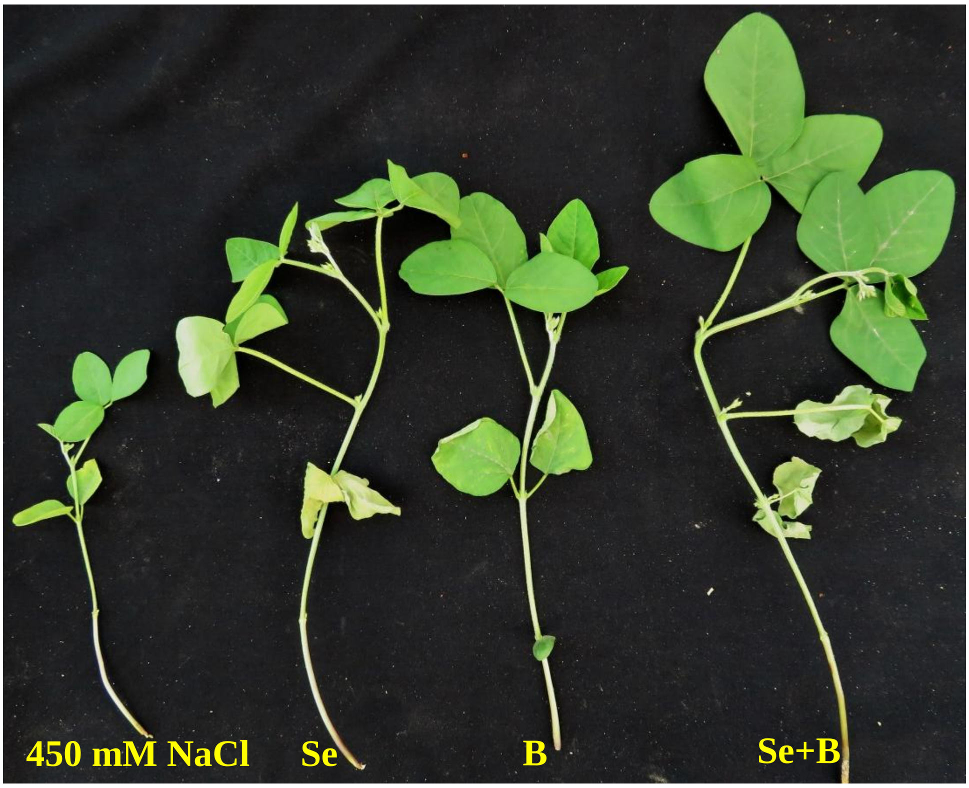

Figure 30. Phenotypic appearance of shoot of soybean seedlings at 28 DAS treated with 450 mM NaCl with or without Se, B and Se+B. Here, Se and B indicates 0.50 µM Na_2SeO_4 and 1 mM H_3BO_3

Stunting of the plant height is considered as one of the prominent effect of salinity. At earlier stage reduction in plant height occurs due to osmotic stress. In other words, the accumulation of higher concentration of salt outside the root zone causes the reduction in the plant height. In later stage, higher accumulation of salt inside the cell and tissues (ioninc toxicity) causes the reduction in plant height (Munns, 2005; Munns and Tester, 2008). Sharp decrease in plant height was observed in the present study, in a dose-dependent manner (Figure 27). Salt-induced reduction in plant height was observed in soybean in several studies and in different soybean genotypes such as BINA Soybean-01, BINA Soybean-03, BINA Soybean-02, BINA Soybean-04, BARI soybean-05, SBM-09, BARI soybean-06, SBM-18, SBM-22 and SBM-15 and Sohag (Klein *et al.*, 2015; Akram *et al.*, 2017). Salinity negatively affects the cell division and cell elongation process as well as normal cell functioning of the plants (Hasanuzzaman *et al.*, 2013; Shahverdi *et al.*, 2018). Salinity hinders the growth of meristematic tissues and inhibits carbohydrate supply to the growing cells. Reduction in photosynthesis and stomatal conductance greatly hampers the growth parameters of the plant in the salt stress (Kordrostami and Rabiei, 2019). These may have caused the reduction in plant height in the present study. On the other hand, exogenous Se and B application reverted the negative effect of salt stress on soybean in case of plant height (Figure 28, 29, 30). Among the beneficial trace elements Se is one of the most effective one in case of increasing growth traits and physiological activities in plants (Hasanuzzaman *et al.,* 2020a). Combined application of Se+B+Fe increased the plant height in stevia under salt stress. Moreover, application of Se+B showed better performance in mitigating salt stress (Shahverdi *et al.*, 2018, 2020). Boron is crucial one for the processes like respiration, synthesis of proteins, transportation of sugars and carbohydrate metabolisom. More importantly, growth hormone like IAA is also associated with B (Hansch and Mendel, 2009). Moreover, Ullah *et al.* (2012) found that B played vital role in cell division of the actively growing region. For the growth and development of shoot and root B is required. Moreover, B transports water, nutrients and carbohydrates needed for the meristematic region of the plants (Rattan, 2015). Application of Se, B and Se+B showed positive reflection in case of increasing plant height, in the present study at 150, 300 and 450 mM NaCl stress (Figure 28, 29, 30).

4.6 Anatomical observation

Upon exposure to 150, 300 and 450 mM NaCl stress number of stomata decreased remarkably in a dose-dependent manner, in comparison to control (Figure 31 A-D). However, the highest number of stomata observed in control (Figure 31A). On the other hand, the minimum number of stomata observed at 450 mM NaCl stress (Figure 31D). However, salinity level and number of stomata reversly propotional. Mild salinity caused lower reduction in number of stomata (Figure 31B). Moderate salinity caused moderate reduction in stomatal number (Figure 31C). However, severe salinity caused the highest reduction in the number of stomata and degradation of cell wall (Figure 31D). Mitsuya *et al.* (2000) reported that salinity was responsible for degradation of cytoplasm. In potato leaves, salinity reduced the number of stomata and chloroplast.

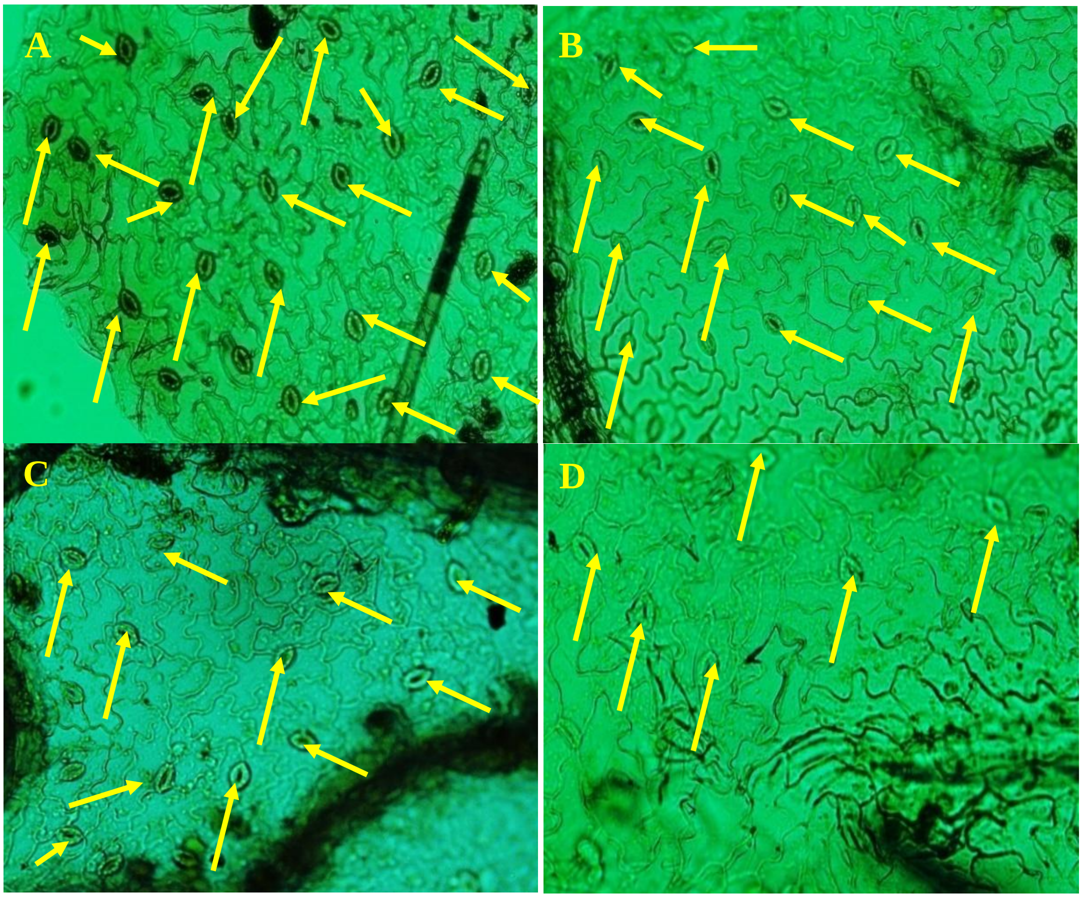

Figure 31. Anatomical appearance of transverse section of soybean leaves treated with A) 0 (control), B) 150, C) 300 and D) 450 mM NaCl at 42 DAS. Here, yellow arrow indicates the stomata

4.7 Correlation analysis

4.7.1 Correlation among growth, physiological and biochemical parameters

The correlation analysis revealed that plant height, shoot FW, shoot DW, root FW and root DW, number of branches plant^{-1}, number of flowers plant^{-1} and leaf area were positively interlinked with each other and negatively linked with salinity. On the contrary, Pro content, MDA content and H_2O_2 content were negatively interlinked with the growth parameters and physiological parameters but positively linked with salinity. However, Pro content, MDA content and H_2O_2 content were negatively linked with the RWC and SPAD value. Oxidative stress indicators were negatively interlinked with the growth and physiological parameter. On the contrary, oxidative stress indicators were positively interlinked with salinity (Figure 32).

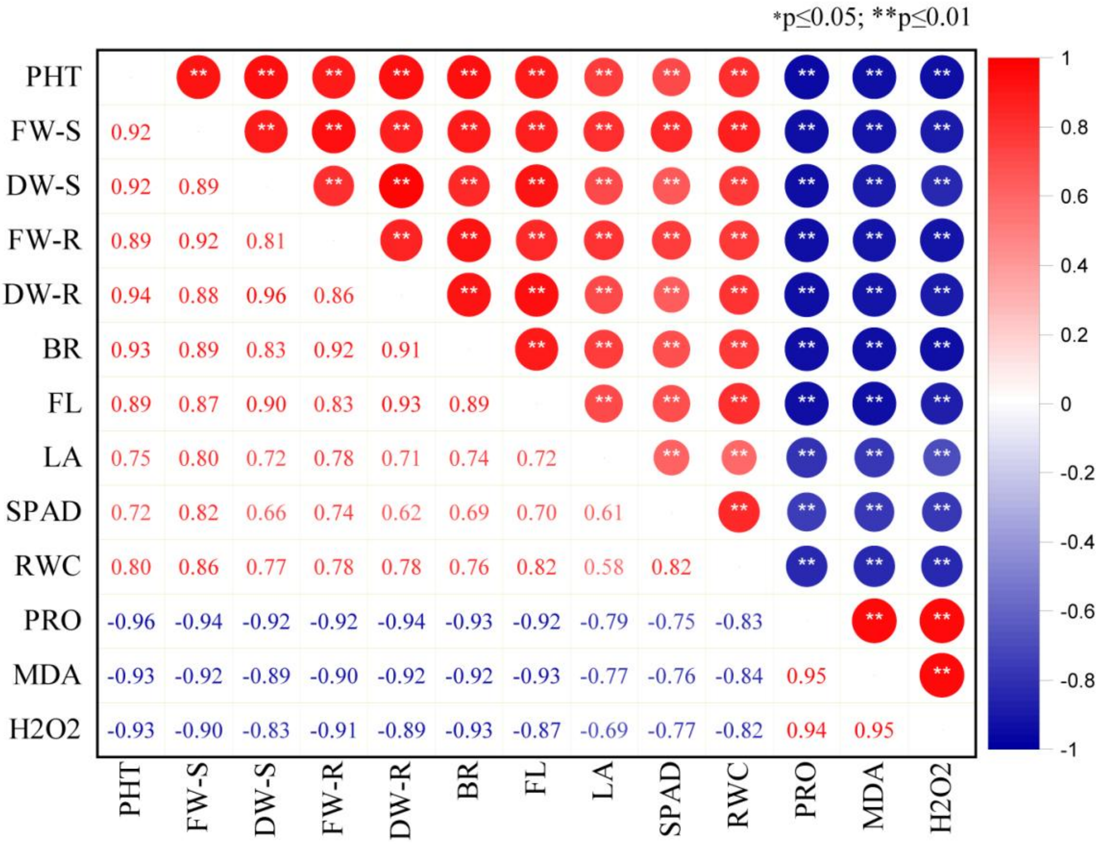

Figure 32. Correlation matrix (n=48) among growth, physiological and biochemical parameters. Upper tringle presents the significane level of correlations and the lower triangle represents the correlation coefficient. Here, PHT-plant height at 42 DAS, FW-S-shoot fresh weight, DW-S-shoot dry weight, FW-R-root fresh weight, DW-R-root dry weight, BR-number of branch plant^{-1}, FL-flower number plant^{-1}, LA-leaf area, SPAD-SPAD value at 42 DAS, RWC-relative water content, PRO-Pro content at 42 DAS, MDA-MDA content at 42 DAS, H_2O_2- H_2O_2 content at 42 DAS

4.7.2 Correlation among yield and yield contributing parameters

Yield and yield contributing parameters were positively interlinked. The increase in yield contributing parameters caused the increase in yield. The pod length was positively interlinked with the seed pod^{-1}. However, pod plant^{-1}, pod length, seed pod^{-1} were positively linked with the seed yield (Figure 33).

In the present study, after measuring different parameters it was observed that plant responded to salt stress in a dose-dependent and time-dependent manner. Under different levels of salt stress growth parameters (plant height, root FW, root DW, shoot FW, shoot DW, branches number plant^{-1} and leaf area) were reduced, compared to control. The maximum reduction of the growth parameters were observed under the highest level of salt stress. On the other hand, application of Se, B and Se+B increased the growth parameters under 150, 300 and 450 mM NaCl-induced salt stress. When subjected to mild, moderate and severe levels of salt stress, physiological activity of plants was disrupted which was estimated by measuring the physiological parameters viz. SPAD value, relative water content and proline content. Upon exposure to salt stress both SPAD value and relative water content were reduced. Under severe salt stress the highest reduction of SPAD value and relative water content was observed. Supplementation of Se, B and Se+B increased the SPAD value and RWC by reverting the negative effect of salt stress. However, Pro content and oxidative stress indicators (MDA content and H_2O_2 content) were increased in response to 150, 300 and 450 mM NaCl-induced salt stress in a dose-dependent manner. Foliar application of Se, B and Se+B diminished the Pro, MDA and H_2O_2 content by mitigating the salt stress. Similarly, yield parameters (flowers number plant^{-1}, number of pod plant^{-1}, pod length, number of seed pod^{-1}, seed yield plant^{-1}, stover yield plant^{-1} and biological yield plant^{-1}) were negatively affected when subjected to salt stress. In this study, it was observed that upon exposure to 300 and 450 mM NaCl-induced salt stress, plant death occurred after completing the vegetative stage, plants produced yield only under 150 mM NaCl-induced salt stress. Exogenous application of Se, B and Se+B increased the yield parameters under salt stress. Plants subjected to more damage with the increment in the concentration and duration of salt stress. Though, combined application of Se+B showed slightly better performance in result than Se or B alone. But, it was clearly observed that, exogenous application of Se, B and Se+B reverted the negative effect of the different levels of salt stress, in this study. Therefore, this study can be regarded as a milestone for further investigations in mitigating salt stress.

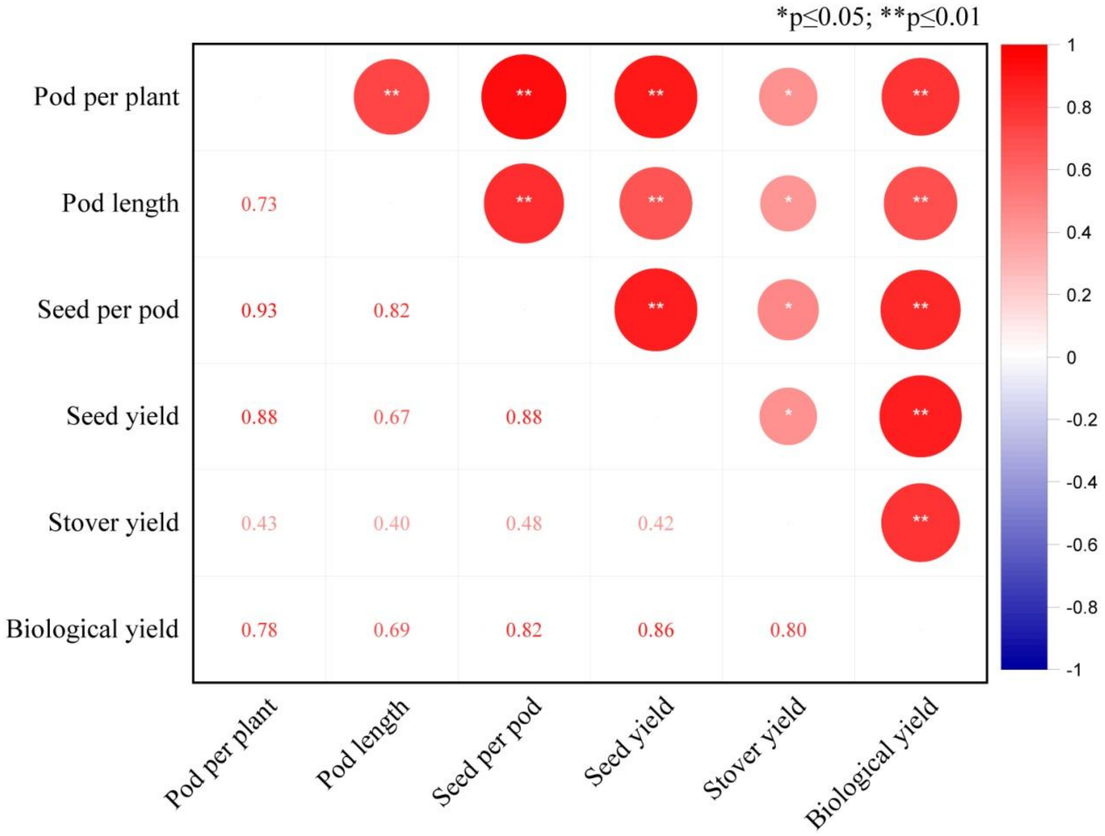

Figure 33. Correlation matrix (n=24) among yield parameters. Upper tringle presents the significane level of correlations and the lower triangle represents the correlation coefficient

Chapter V

SUMMARY AND CONCLUSION

This experiment was conducted to study the morphological, physiological, biochemical, phenotypical and anatomical responses of plants upon exposure to different levels of salinity and to investigate the mitigation of salinity by exogenous application of Se, B and Se+B.

The experiment was located at Sher-e-Bangla Agricultural University farm, Dhaka-1207, under the Agro-ecological zone of Madhupur Tract, AEZ-28 from November, 2019 to January, 2020.

The experiment was laid out in randomized complete block design (RCBD) with three replications. There were two sets of pot for conducting this experiment. One set was for measuring the growth, physiological, biochemical and anatomical parameters (Destructive data) and another one was for measuring yield attributes and yield. The experiment was consisted of 16 treatments. Stress treatments were applied at 20 DAS and at 35 DAS. Trace elements (Se, B and Se+B) were exogenously applied at 20 DAS. Foliar application were continued every 3 days interval until the pod filling stage.

Plant height, root FW, root DW, shoot FW, shoot DW, number of branches plant^{-1} and leaf area were observed for assessing the growth. For physiological responses SPAD value, RWC and Pro content were observed. Moreover, MDA content and H_2O_2 content were measured as oxidative stress indicators. Yield parameters viz. number of flowers plant^{-1}, number of pods plant^{-1}, pod length, number of seeds pod^{-1}, seed yield plant^{-1}, stover yield plant^{-1} and biological yield plant^{-1} were measured.

Sharp reduction of plant height was observed at 28, 35 and 42 DAS upon exposure to 150, 300 and 450 mM NaCl-induced salt stress, compared to control with the increase of the salt concentration. The lowest plant height was observed at 450 mM NaCl-induced salt stress at 28, 35 and 42 DAS. Application of Se, B and Se+B showed better result compared to control, at all the stages.

In comparison to control, severe, moderate and mild salt stress resulted in 37, 24 and 13% reduction in the root FW. On the contrary, exogenous spray of Se, B and Se+B mitigated the adverse effect of salt stress. However, root FW was increased by 13, 28 and 25% under mild, moderate and severe salinity in Se+B-supplemented plant, compared to the corresponding control.

The maximum reduction of root DW occurred in the highest salinity level. Salt-stressed plants exihibited 40, 56 and 71% reduction under mild, moderate and severe salinity in root DW, in comparison to control. Combined application of Se+B increased 27, 19 and 24% root DW under 150, 300 and 450 mM NaCl-induced salt stress, compared to control.

The maximum reduction of the shoot FW was occurred in 450 mM NaCl-stress which was 80% lower than control. Similarly, imposition of 150 and 300 mM NaCl caused 39 and 49% reduction in shoot FW, in comparison to control. On the other hand, exogenous application Se, B and Se+B reverted the negative effect of salinity on shoot FW. Compared to single supplement, combined application of Se and B showed better results under different salinity levels, in case of increasing shoot FW.

Severe salt stress (450 mM NaCl) caused the maximum reduction in shoot DW. However, shoot DW exihibited 53, 67 and 77% declined, compared to control under low, moderate and severe salinity. On the contrary, under different salinity levels Se+B treated plants showed 39, 71 and 72% increase in shoot DW, compared to the corresponding control.

The minimum branches number plant^{-1} was observed under the highest level of salinity treatment (450 mM NaCl) which was 70% lower, compared to control Compared to individual spray, combined application of Se+B spray effectively enhanced the number of branches plant^{-1} under saline and non saline condition. Moreover, under 150, 300 and 450 mM NaCl-induced stress 17, 46 and 90%

enhancement occurred in branch number plant^{-1}, due to exogenous application of Se+B, in comparison to corresponding control.

Plant exhibited the highest reduction in leaf area by 26% under 450 mM NaCl-induced salt stress, in comparison to control. Application of Se+B increased the leaf area under mild, moderate and severe salinity by 5, 10 and 15%, in comparison to control.

The reduction of SPAD value increased with the increment of the concentration of salinity. The minimum SPAD value recorded in the highest salinity level at 28, 35 and 42 DAS. On the other hand, exogenous application of Se, B and Se+B reverted the negative effect of the salt stress at 28, 35 and 42 DAS. Under mild, moderate and severe salinity, at 28 DAS combined application of Se+B increased the SPAD value by 5, 18 and 24%, respectively, compared to control. Application of Se+B increased SPAD value by 12, 8 and 18% under mild, moderate and severe salt stress at 35 DAS, in comparison to control. Similarly, foliar spray of Se+B elevated the SPAD value 21, 35 and 41%, respectively, compared to the corresponding control plants at 45 DAS under mild, moderate and severe salinity.

Due to imposition of 150, 300 and 450 mM NaCl stress, RWC of leaf sharply declined under all salt treatments, compared to control. The highest decrease in RWC of leaf was observed at 450 mM NaCl-induced stress which was 26% lower, compared to control. Moreover, 150 and 350 mM NaCl-induced salt stress caused 19 and 25% reduction in RWC of leaf. Under mild, moderate and severe salinity Se+B spray elevated the RWC of leaf by 18, 21 and 20%, in comparison to control .

Compared to control, the higher salinity level was responsible for the higher Pro accumulation in plants and maximum Pro content was recorded under 450 mM NaCl-induced stress which was by 180 and 142% at 30 and 45 DAS, respectively. On the other hand, foliar application of Se, B and Se+B diminished the Pro content both in saline and non saline condition, compared to control at 30 and 45 DAS. Though, single and combined application showed nearly similar result in mitigating Pro content.

The maximum MDA content was recorded in the highest salinity level (450 mM NaCl) which was 172 and 133% at 30 and 45 DAS, respectively, compared to control. However, combined application Se+B showed slightly better result than individual application of trace elemnents in diminishing MDA content both at 30 and 45 DAS, respectively, in comparison to control.

Under mild, moderate and severe salinity H_2O_2 content was sharply increased by 41, 93 and 131% at 30 DAS, in comparison to control. Similarly, H_2O_2 content was increased by 112, 142 and 172% at 45 DAS under mild, moderate and severe salinity, compared to control. Combined application of Se+B showed poor result at 30 DAS in diminishing H_2O_2 content. Moreover, at 45 DAS combined application of Se+B and single application showed nearly similar result in decreasing H_2O_2 content.

In this study, in response to 300 and 450 mM NaCl-induced salt stress plant death occurred, after completing the vegetative stage.

Severe salinity level resulted in the minimum number of flowers plant^{-1} which was 71% lower than the control plants. On the other hand, supplementation of Se, B and Se+B increased the flower number plant^{-1} both in saline and non saline condition. Under severe, moderate and mild salinity the flower number plant^{-1} was increased by 120, 38 and 33% due to Se+B supplementation, in comparison to control plants.

Number of pods plant^{-1} was reduced by 38% under 150 mM NaCl-induced stress, in comparison to control plants. However, compared to individual supplementation combined supplementation of Se+B showed better result under salinity than normal condition. Application of Se+B increased number of pods plant^{-1} by 36% under mild salinity, compared to the corresponding control.

Mild salinity (150 mM NaCl) caused 9% reduction in pod length, compared to control. However, combined application of Se+B increased the pod length more than individual application of Se and B. Pod length was increased due to Se+B application by 32% in normal condition and by 20% in salinity, compared to control.

Plants exhibited 35% reduction in number of seeds pod^{-1} at 150 mM salt stress, compared to control. However, combined application of Se+B showed better result than Se or B alone. Combinedly, Se+B increased the number of seeds pod^{-1} by 16% under normal condition and by 38% under saline condition, in comparison to control.

Upon exposure to mild salinity the seed yield decreased by 26%, compared to control. Seed yield was higher in Se+B-supplemented plants than single supplementation, in comparison to control under salinity and normal condition. In comparison to corresponding control, application of Se+B increased the seed yield by 6.23 and 24% under mild salinity and non saline condition.

Mild salinity decreased the stover yield by 3.31%, compared to control. In comparison to the corresponding control, application of Se+B increased the stover yield by 3.61 and 10% under mild salinity and non saline condition.

Upon exposure to 150 mM NaCl-induced salt stress the biological yield decreased by 13%, compared to control. However, combined application of Se+B increased the biological yield more than the individual application of Se and B. Under mild salinity and normal condition Se+B increased the biological yield by 10 and 12%, compared to control.

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PLATES

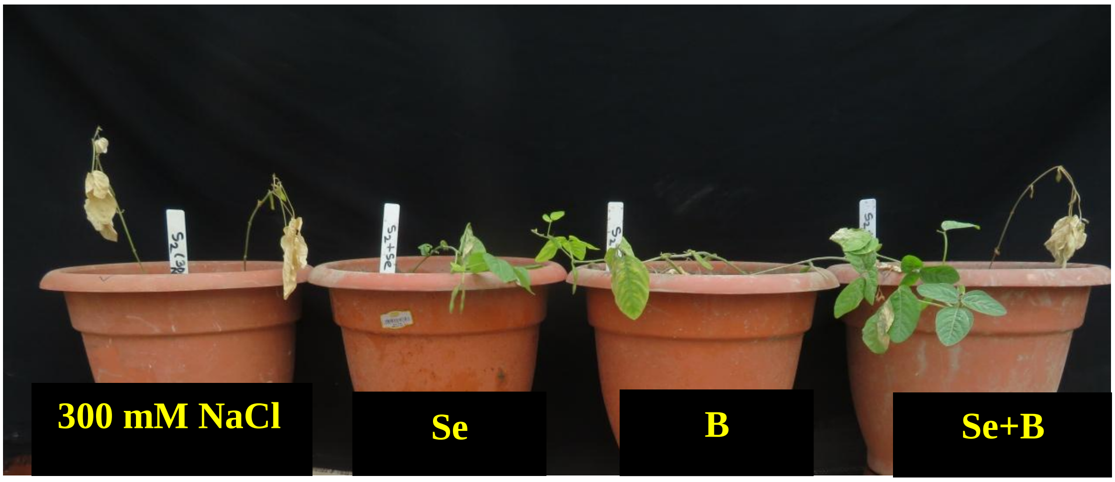


Plate 1. Plant died at 300 mM NaCl stress at 54 DAS

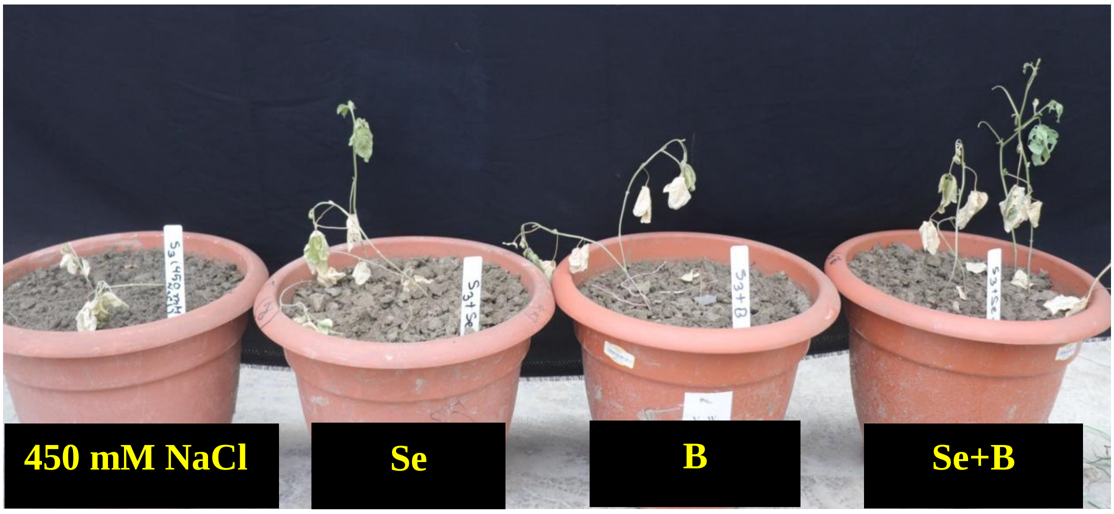
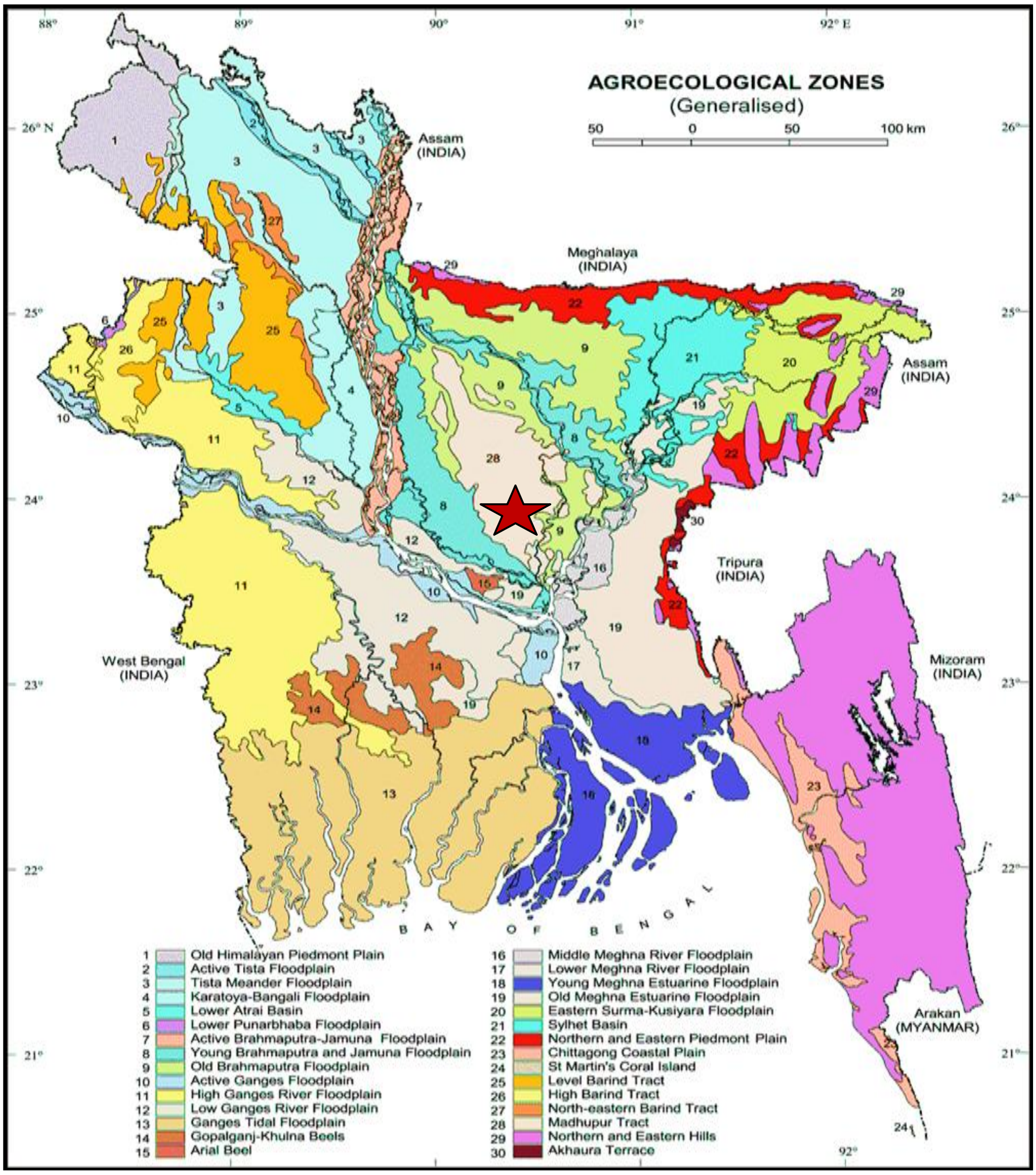


Plate 2. Plant died at 450 mM NaCl stress at 47 DAS

APPENDICES

Appendix I. Map showing the location of the experiment



★ Mark shows the experiment location in the map of Bangladesh

Appendix II: Mean square values and degree of freedom (DF) of plant height at 28, 35 and 42 DAS of soybean under different level of salinity

Source of variation	DF	Mean square value of		
		Plant height at 28 DAS	Plant height at 35 DAS	Plant height at 42 DAS
Salinity	3	452.304	330.723	428.059
Trace elements	3	9.151	39.452	32.561
Salinity×Trace elements	9	3.127	12.081	6.383
Error	30	1.347	2.024	1.576

Appendix III: Mean square values and degree of freedom (DF) of shoot FW, shoot DW, root FW and root DW of soybean under different level of salinity

Source of variation	DF	Mean square value of			
		Shoot FW	Shoot DW	Root FW	Root DW
Salinity	3	8.006	0.927	1.555	0.573
Trace elements	3	2.282	0.037	0.278	0.11
Salinity×Trace elements	9	0.239	0.006	0.035	0.001
Error	30	0.015	0.001	0.011	0.001

Appendix IV: Mean square values and degree of freedom (DF) of number of branch plant^{-1}, number of flower plant^{-1} and leaf area of soybean under different level of salinity

Source	DF	Mean square value of		
		Number of branch plant^{-1}	Number of flower plant^{-1}	Leaf area
Salinity	3	14.926	612.485	195.949
Trace elements	3	1.527	82.942	32.428
Salinity×Trace elements	9	0.106	8.728	390.744
Error	30	0.020	1.482	0.083

Appendix V: Mean square values and degree of freedom (DF) of SPAD value at 28 DAS, 35 DAS and 42 DAS of soybean under different level of salinity

Source	DF	Mean square value of		
		SPAD value at 28 DAS	SPAD value at 35 DAS	SPAD value at 42 DAS
Salinity	3	50.146	107.493	140.223
Trace elements	3	55.209	42.155	241.557
Salinity×Trace elements	9	7.892	5.273	16.680
Error	30	2.014	4.389	3.962

Appendix VI: Mean square values and degree of freedom (DF) of RWC and Pro content (at 30 DAS and 45 Das) of soybean under different level of salinity

Source	DF	Mean square value of		
		RWC	Pro content at 30 DAS	Pro content at 45 DAS
Salinity	3	423.518	31.979	0.010
Trace elements	3	217.194	1.880	38.578
Salinity×Trace elements	9	27.055	0.185	0.388
Error	30	7.905	0.030	0.043

Appendix VII: Mean square values and degree of freedom (DF) of MDA and H_2O_2 content at 30 DAS and 45 DAS of soybean under different level of salinity

Source	DF	Mean square value of			
		MDA at 30 DAS	MDA at 45 DAS	H_2O_2 content at 30 DAS	H_2O_2 content at 45 DAS
Salinity	3	2754.829	5143.756	149.973	174.493
Trace elements	3	424.357	603.834	9.586	2.243
Salinity×Trace elements	9	46.248	105.158	1.572	3.200
Error	30	4.042	12.708	0.345	0.407

Appendix VIII: Mean square values and degree of freedom (DF) of number of pod plant^{-1}, pod length, number of seed pod^{-1}, seed yield plant^{-1}, stover yield plant^{-1} and biological yield plant^{-1} of soybean under different level of salinity

Source	DF	Mean square value of					
		Number of pod plant^{-1}	Pod length	Number of seed pod^{-1}	Seed yield plant^{-1}	Stover yield plant^{-1}	Biological yield plant^{-1}
Salinity	3	19.344	0.736	0.673	2.656	0.682	6.392
Trace elements	3	7.856	0.178	0.128	0.879	0.132	1.023
Salinity×Trace elements	9	2.345	0.056	0.045	0.218	0.039	0.672
Error	30	0.272	0.024	0.008	0.98	0.027	0.134