Research Article

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Effect of salinity on the morphological, physiological and biochemical properties of lettuce (*Lactuca sativa* L.) in Bangladesh

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Abstract: This study aimed to explore the changes in morphological, physiological and biochemical characteristics of lettuce (*Lactuca sativa* L.) in response to salt stress when grown using hydroponic techniques. The seedlings were subjected to five different concentrations (0 mM, 50 mM, 100 mM, 150 mM, and 200 mM) of NaCl for three weeks. During the salt stress, morphological properties (shoot length, root length, total plant weight, leaf number) were measured in every week. After 21 days of salt stress, physiological properties (water content and relative water content) and biochemical properties (proline, protein, phenol, reducing and non-reducing sugar content) were measured. Morphological and physiological properties were found decreased gradually with increasing salt concentrations. Biochemical properties such as proline and protein content increased remarkably, and total phenol content decreased gradually with increasing salt concentrations. Reducing sugar accumulation was higher in all treatments except 50 mM in comparison to control. Non-reducing sugar accumulation was decreased in 100 mM and 200 mM treatment, similar in 150 mM treatment, and increased in 50 mM treatment when compared to control. These findings render lettuce a salt-sensitive plant at higher salt concentration. However, changes in characteristics were realistic up to 50 mM salt concentration.

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1 Introduction

Salinity is one of the most common abiotic factors that limit the productivity of crop plants. The area of land affected by salinity is continuously increasing day by day (Shrivastava and Kumar 2015). Because of soil salinity, about 4×10^4 hectare (ha) of land throughout the world every year loss the ability for agricultural production. Specialized agencies of the United Nations prepared a report that indicates about 50% of the irrigated area of the world is either salinized or has potential danger in the future (Ünlükara et al. 2008). Bangladesh has a coastal region, where increasing salinity is an alarming issue. About 20% of the country has a coastal area from which 53% of the land is affected by the salinity of varying degrees. In addition to that, more than 30% of the cultivable land in Bangladesh is in the coastal area, and about 1.056 million ha land out of 2.86 million ha are affected by differing degrees of salinity (Haque 2006). Due to the salinity problem, agriculture is greatly affected, and most of the land cannot be utilized for farming. Salinity significantly reduces crop productivity and limits the cultivation of potential crops. That's why soil salinity is considered as a significant limitation to agricultural development in the coastal regions of Bangladesh (Islam et al. 1999).

Salinity adversely affects plants leading to impair physiological and biochemical processes owing to the nutritional imbalance, osmotic stress, specific ion effects, water deficit and oxidative stress (Ashraf and Wu 1994; Marschner 1995). High levels of salts create a noxious effect on the whole plant which has resulted in a reduction of growth and productivity. Consequently, most plants adopt strategies to impede salt from the cells and many plants build up a tolerance against the presence of salt within

the cells (Parida and Das 2005). Soil salinity is implicated by ions including Na^+, Cl^-, SO_4^{2-}, HCO^{3-}, Ca^{2+}, Mg^{2+}, NO^{3-} and K^+ (Bernstein 1975). The salt NaCl is a critical component of salinity and responsible for water deficit induced by osmotic stress. Excess Na^+ and Cl^- ions affect critical biochemical processes (Munns and Tester 2008).

Hydroponics or soilless farming referred to the method of growing plants where essential nutrient components are provided in water (Kim et al. 2013). This system involves the placement of plant roots in either a static or continuously aerated nutrient solution (Nguyen et al. 2016; Shohael et al. 2017). Efficient nutrient regulation and efficient water use are the main advantages of hydroponics (Resh 2016). Several studies have been conducted under salinity stress using a hydroponics system in the medicinal and crop plants (Rahneshan et al. 2018; Alam et al. 2017), including lettuce (Garrido et al. 2014).

Lettuce (*Lactuca sativa* L.) is a favorite leafy vegetable consumed mostly as a salad and famous for direct consumption or food component. It is a nutritious vegetable rich in vitamins C, carotenoids, antioxidants, caffeic acid, and flavanols (Viacava et al. 2014). The nutritional components in lettuce are found to be involved in some health advantages including a reduction in the risk of cardiovascular disease and certain cancers (Hung et al. 2004). Lettuce is perceived to be a relatively salt-sensitive vegetable (Xu and Mou 2015). Seed germination, fresh and dry weight of shoot, and root weight of lettuce have been affected both by ionic and osmotic effects due to salinity (Barassi et al. 2006).

Salinity problems may give rise to salinity tolerance in plants. Salinity type, plants species concerned, plant growth stages may define tolerance or sensitivity (Botía et al. 1998). Plants adopt several strategies to endure salinity through the mechanism of osmotic adjustment which includes the accumulation of compatible solutes such as proline, soluble sugars and sugar alcohols (Hasegawa et al. 2000). Salinity has an impact on morphological, physiological and biochemical properties of the plant. Plant metabolism may alter due to the oxidative stress caused by the high amount of reactive oxygen species within the plant cell (Minh et al. 2016). Water content (WC) and relative water content (RWC) are the most common indicator of plant water status in terms of the physiological scenarios of cellular water deficit (Tanentzap et al. 2015). Water content (WC) is defined as the quantity of water present while relative water content (RWC) is concerned with the comparison of initial and turgid water contents on a percentage basis. WC and RWC provide insights into the water deficit and may act as a sign of the degree of stress implemented (Barrs and Weatherley 1962).

Salt can be added to the nutrient medium to investigate its effects on plants. It also facilitates a more efficient regulation of stress conditions and environmental parameters. The objectives of this study were to evaluate the effect of salt stress on morphological parameters such as root length, shoot length, plant weight, leaf number, fresh weight (FW) and dry weight (DW) as well as physiological properties such as water content (WC) in root and shoot, the relative water content (RWC) in leaf. This study also aimed to analyze changes in various biochemical properties such as proline content, protein content, total phenol, reducing and non-reducing sugars in response to salt stress.

2 Materials and methods

2.1 Experimental design, plant material, growing condition, and salinity treatment

The experiment was conducted at the Plant Biotechnology and Genetic Engineering Laboratory, Department of Biotechnology and Genetic Engineering, Jahangirnagar University, Dhaka-1342, Bangladesh (23°53'14" N 90°15'56" E). This experiment consisted of five treatments of NaCl (0 mM, 50 mM, 100 mM, 150 mM, 200 mM). The treatment with no NaCl (0 mM) served as the control. The design of the experiment was completely randomized. There were three replications of twenty seeds per experiment. The experimental design is depicted in Figure 1.

Fresh seeds (Chia Tai Co. LTD, Bangkok, Thailand) of lettuce (*Lactuca sativa* L.) were collected from the local market of Savar, Dhaka, Bangladesh. The seeds were germinated in moist tissue paper in disposable plastic Petri plates under low light condition. Following germination, the seedlings were transferred to the hydroponics system. The system for growing lettuce plants under hydroponics condition was established following the method of Shohael et al. (2017). Small sized plastic bowls equipped with Styrofoam were used as a container. The Styrofoam has a hole in the middle which was filled with foam block to support the plant seedling. This experiment was conducted using Hoagland's solution (Hoagland and Arnon 1950) as a nutrient medium. Each container was filled with 500 mL of nutrient medium. Then, the germinated seedlings were placed in the middle of the Styrofoam which was placed above the container. The composition of the medium is given in Table 1.

After 7 days of growth in hydroponics medium, the lettuce plants achieve reasonable growth and then salinity treatment was applied. New hydroponics medium

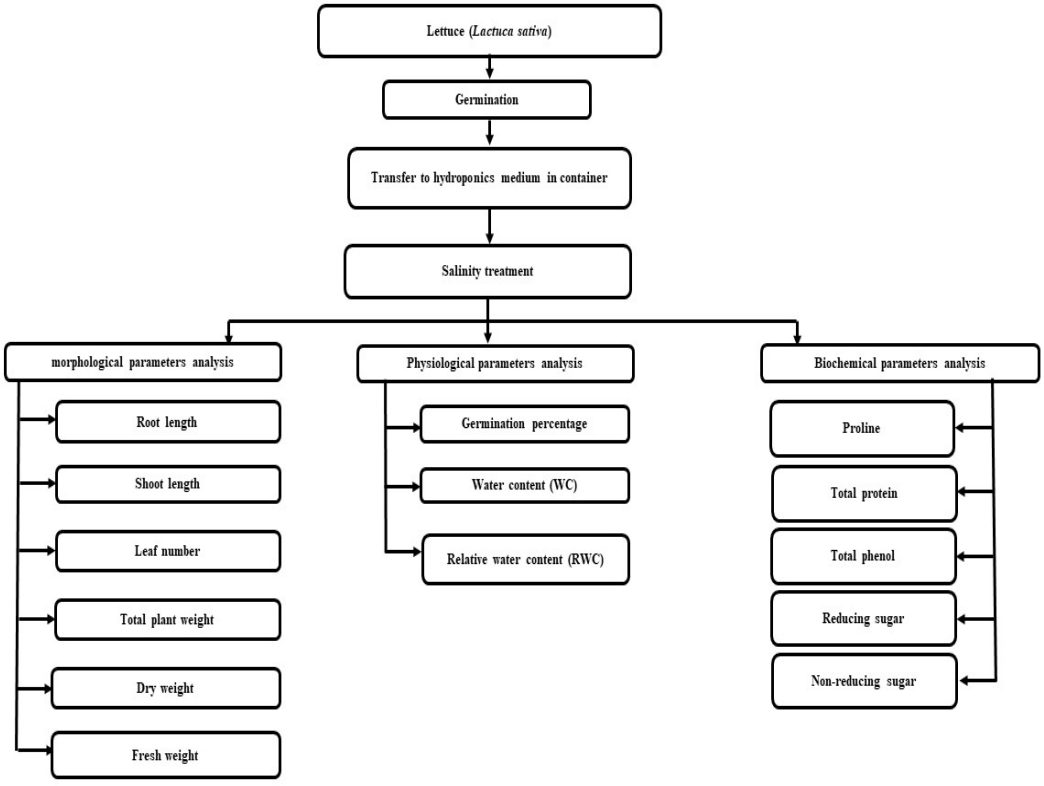

Figure 1: Experimental design.

Table 1: Composition of the hydroponics medium.

Stock solution	Chemicals name	Quantity (mg/L)
Macronutrients	KNO_3	505
	$Ca(NO_3)_2.4H_2O$	180
	NH_4NO_3	80
	$MgSO_4.7H_2O$	493
Micronutrients	H_3BO_3	2.86
	$MnSO_4.4H_2O$	1.81
	$ZnSO_4.7H_2O$	0.22
	$CuSO_4$	0.08
	$NaMoO_4.H_2O$	0.12
	KH_2PO_4	68
Fe-EDTA		22.5

with five different concentrations of NaCl (0 mM, 50 mM, 100 mM, 150 mM, 200 mM) was supplemented to the containers. Salt treatment was applied for three weeks. After three weeks of salt stress, the plants were harvested. For biochemical analysis, leaf samples were collected and stored. Biochemical properties (proline content, protein content, total phenol content, reducing and non-reducing sugar content) were measured using those samples. Another physiological property (germination percentage) was measured in a completely different experiment.

2.2 Analysis of morphological properties

During the salt treatment, morphological properties such as shoot length, root length, total plant weight, and leaf number were measured in every week. To estimate shoot length, root length, total plant weight, and leaf number, twenty plants from each experiment were considered. Shoot length and root length was measured as previously described (Abedin et al. 2002). Shoot length measured

using a scale from the root-shoot junction to the tip of the longest leaf. Root length measured from the root-shoot junction to the tip of the longest root. Total plant weight was measured in an electric balance (And Gulf EK600I precision electronic balance, U.A.E). Leaf number of every plant was counted accurately.

After three weeks of salt treatment, the rest of the morphological properties such as fresh weight (FW) and dry weight (DW) were measured. To estimate the fresh weight (FW) and dry weight (DW), one plant from each treatment was selected randomly. Root and shoot samples were separated by cutting the plants at the root-shoot junction, and the fresh weight was measured immediately root and shoot portions. For dry weight, samples were placed in a hot air oven at 65°C for 48 h. The measurements were done in an electric balance (And Gulf EK600I precision electronic balance, U.A.E).

2.3 Analysis of physiological properties

2.3.1 Germination percentage in response to salt stress

Germination percentage of lettuce seeds were measured in a separate experiment, in which 100 seeds were used for each treatment with three replications. Disposable Petri plates were equipped with layers of tissue paper onto which seeds were arranged. 5 mL of saline water of differing concentration was poured on each Petri plate daily. Germination percentage was calculated using the following formula:

Germination percentage (%) = (Germinated seeds/ Total seeds) × 100

2.3.2 Water content (WC) and relative water content (RWC)

Water content was determined according to the method as described previously (Garrido et al. 2014). To estimate the water content (WC), fresh weight (FW) and dry weight (DW) of roots and shoots were utilized. The water content (%) of different samples were calculated from the following formula:

WC (%) = (Fresh Weight-Dry Weight / Fresh Weight) × 100

The relative water content (RWC) of leaves was measured according to the protocol as previously described (Schonfeld et al. 1988). Leaves were collected by cutting at the base using a scalpel, and fresh weights were measured immediately. The leaves were then soaked in distilled water for 24 hours at room temperature. The turgid weight of leaves was measured after the leaves were blotted dry using tissue paper. The leaves were then placed in a hot air oven at 80°C for 48 h, and dry weights were then measured. The relative water content was calculated as follows:

RWC (%) = (Fresh Weight-Dry Weight)/ (Turgid Weight-Dry Weight) × 100

2.4 Analysis of biochemical properties

2.4.1 Determination of proline, total phenol, and total protein

Proline content was estimated by following the method as described previously (Bates et al. 1973) with minor modifications. A portion of tissues (500 mg) was homogenized in 5 mL of 3% 5-sulphosalicylic acid (Sigma Aldrich, USA) using a mortar and pestle. The extract (2 mL) was treated with 2 mL of ninhydrin reagent (Loba Chemie, India) and glacial acetic acid (Scharlau, Spain), and incubated in a boiling water bath at 100 °C for 30 min. After cooling the reaction mixture, it was eluted by adding 4 mL of toluene (Sigma Aldrich, USA) in the fume hood. Then, the mixture was vortexed, and the absorbance of chromophore-containing toluene was recorded at 520 nm using a UV-visible spectrophotometer (T60 UV-Visible Spectrophotometer, PG Instruments Ltd., United Kingdom). The proline concentration was determined using a calibration curve where L-proline (Carl Roth, Germany) was used as a standard and expressed as µmole proline/g leaves fresh weight.

The total phenol content was determined by the Folin-Ciocalteau method as described previously (Malik and Singh 1980). The extracts were taken in 10 mL glass tubes and total volume made to 3 mL with distilled water. Then the extracts were mixed with 0.5 mL Folin-Ciocalteau reagent (SRL, India) (1:1 with water) and 2 mL of 20% Na_2CO_3 (Alfa Aesar, UK). A blue colored complex, molybdenum blue developed in each tube, as the phenols undergo a redox reaction with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium. The tube containing the blue solutions were incubated for 1 min at 23°C, then cooled, and the solution absorbance was measured at 650 nm using a UV-visible spectrophotometer (T60 UV-Visible Spectrophotometer, PG Instruments Ltd., United Kingdom). The concentration of phenol was determined using gallic acid (Sigma Aldrich, USA) as standard. The concentration of phenol was expressed as milligram gallic acid equivalent (mg GAE)/g leaves fresh

weight.

Total proteins were estimated according to the method described by Bradford (Bradford 1976). The leaf sample (25 mg) was homogenized with 1 mL of TRIS-HCl (0.1 M) buffer and then 20 µL supernatant was mixed with 80 µL of distilled water. Then, 900 µL Bradford reagent (Biobasic, Canada) was added and incubated for 2 min. The absorbance was measured at 595 nm in a UV-visible spectrophotometer (T60 UV-Visible Spectrophotometer, PG Instruments Ltd., United Kingdom). The concentration of protein was determined using bovine serum albumin (Sigma Aldrich, USA) as standard. The concentration of protein was expressed as milligram bovine serum albumin equivalent (mg BSA)/g leaves fresh weight.

2.4.2 Determination of reducing sugar and non-reducing sugar

Reducing sugar was estimated according to the method described previously (Miller 1959). Fresh leaves (100 mg) were extracted with 80% ethanol (Merck, Germany). The supernatants were separated and evaporated in a water bath at 80°C. After evaporation, the leaf extract dissolved in 10 mL of distilled water and then 3,5-dinitrosalicylic acid reagent (Sigma Aldrich, USA) was added. The absorbance was measured at 530 nm wavelength using a UV-visible spectrophotometer (T60 UV-Visible Spectrophotometer, PG Instruments Ltd., United Kingdom). The quantity of reducing sugar was calculated using glucose (Duchefa biochemie, Netherlands) as standard and expressed as mg/g leaves fresh weight.

Non-reducing sugars were estimated according to the method described previously (Hedge et al. 1962). Fresh leaves (100 mg) were extracted with 5 mL of 2.5 N HCl (Sigma Aldrich, USA) and placed in a water bath for 3 h. Neutralization was performed by adding Na_2CO_3 (Alfa Aesar, UK), and centrifuged at 5000 × *g* for 15 min. After centrifugation, the supernatant was separated into 1 mL aliquot per tube. 4 mL of Anthrone reagent (Loba Chemie, India) was added to the tube, and the tubes were placed in hot water bath for 8 min. The absorbance was measured at 630 nm wavelength using a UV-visible spectrophotometer (T60 UV-Visible Spectrophotometer, PG Instruments Ltd., United Kingdom). The quantity of non-reducing sugar was calculated using glucose (Duchefa biochemie, Netherlands) as standard and expressed as mg/g leaves fresh weight.

2.5 Statistical analysis

The present study was performed in a completely randomized design (CRD). Significant differences among mean values were compared by Tukey's honestly significant difference (HSD) test at a level of significance of $P \leq 0.05$. All data were displayed as the mean ± standard error of the mean at least three independent biological replications. The statistical analysis was performed using Statistical Package for Social Science software (SPSS, version 16.0, IBM Corporation, NY).

3 Results

3.1 Effect of salinity on morphological properties

Shoot length, root length, total plant weight, and leaf number were significantly changed when plants were exposed to the salt stress conditions (Table 2). For shoots, in the first week, there was a reduction in length by 12%, 25%, 28%, and 33% respectively under 50 mM, 100 mM, 150 mM, 200 mM salt treatment compared to the control plants (Table 2). In the second week, there was 16%, 31%, 34%, 54% reduction observed in shoot length respectively under 50 mM, 100 mM, 150 mM, and 200 mM salt treatments compared to control (Table 2). Then in the third week, shoot length of treated seedlings were found decreased to 5%, 37%, 40%, 61% respectively under 50 mM, 100 mM, 150 mM, 200 mM salt treatment as compared to control (Table 2). The highest shoot length (17.5±0.20 cm) observed in control treatment while the lowest shoot length (6.8±0.21 cm) was observed in seedlings treated with 200 mM salt concentrations at the third week.

For root growth, similar results were found. In first week, root length significantly decreased to 2%, 3%, 23%, 27% respectively in response to 50 mM, 100 mM, 150 mM, 200 mM salt treatment compared to the control (Table 2). In the second week, under 50 mM, 100 mM, 150 mM, and 200 mM salt treatment, the root length found as 11%, 18%, 28%, and 42% lower respectively compared to the control (Table 2). Finally, in the third week, root length found as 7%, 21%, 31%, and 42% lower respectively under 50 mM, 100 mM, 150 mM, 200 mM salt treatments as compared to the control (Table 2).

Total plant weight increased up to 50 mM of salt treatment while the plant weight decreased remarkably in the seedlings treated with 100 mM, 150 mM, and 200 mM salt treatment. Salinity also affected the average leaf

number of plants. Leaf number of the plant increased notably up to 50 mM after three weeks of salt treatment. Leaf number did not increase with 100 mM treatment after three weeks compared to the first week. Average leaf numbers remained unchanged with 150 mM treatment in the second week, but decreased significantly in the third week. However, the leaf number (6±0.32) decreased dramatically when the plants were treated with 200 mM salt concentrations after three weeks. The effect of salinity on the plant morphology has been shown in Figure 2 and Figure 3.

Effect of salinity on the fresh and dry weight of root and shoot after three weeks of salt treatment was summarized in Table 3. Both fresh and dry weight of roots and shoots were significantly affected by salt treatment. Reduction in fresh and dry weight was observed with increasing salt concentrations. Highest shoot fresh weight (44.95±0.95 g) was found in untreated plants and lowest shoot fresh weight (5.72±0.27 g) was found in 200 mM salt concentration. Root fresh weight also showed a similar pattern of reduction where the highest root fresh weight was 18.06±0.43 g for control treatment and lowest root fresh weight was found as 0.99±0.04 g for 200 mM salt treatment.

Dry weights were also affected in the presence of salt treatment. Shoot dry weight was noticed highest (3.85±0.07 g) in control plant, and lowest (1.04±0.03 g) in 200 mM salt treatment. Also, root dry weight showed a similar reduction pattern; 1±0.10 g in control treatment and 0.12±0.01 g in 200 mM salt concentration.

Table 2: Effect of salt treatment (NaCl) on the Shoot length, root length, weight and leaf number of lettuce plants investigated three consecutive weeks after mitigation of salt stress.

Treatment	Time	Shoot length (cm)	Root length (cm)	Total plant Weight (g)	Leaf number
Control (0 mM)	First week	15±0.41a	15±1.08a	51.46±2.26a	12±0.63a
	Second week	16.75±0.63a	16.25±0.25a	59.13±2.56a	17±0.71a
	Third week	17.5±0.20a	16.5±0.28a	62.75±0.85a	17±0.41a
50 mM	First week	13.25±0.63ab	14.75±0.25a	41.15±2.25b	11±0.48a
	Second week	14±0.41b	14.50±0.29ab	55.12±2.21a	14±0.70b
	Third week	16.7±0.18a	15.4±0.23a	53.65±0.39b	14±0.19b
100 mM	First week	11.25±0.48bc	14.50±0.28a	35.59±0.90bc	10±0.29ab
	Second week	11.50±0.29c	13.25±0.48bc	30.24±1.74b	11±0.48bc
	Third week	11±0.35b	13±0.58b	13.55±0.21c	10±0.26c
150 mM	First week	10.75±0.75c	11.50±0.29b	28.46±1.45cd	8±0.87bc
	Second week	11±0.71c	11.75±0.63c	24.88±0.97b	8±0.65cd
	Third week	10.5±0.17b	11.4±0.20c	7.1±0.13d	7±0.28d
200 mM	First week	9+0.00c	11±0.58b	24.60±1.17d	6±0.85ce
	Second week	7.88±0.13d	9.5±0.29d	15.62±1.16c	6±0.75d
	Third week	6.8±0.21c	9.5±0.26d	6.95±0.22d	6±0.32d

Means with same letter within each column are not significantly different according to Tukey's HSD tests ($P < 0.05$).

Table 3: Effect of salt treatment on the fresh and dry weight of root and shoot after three weeks of salt treatment.

Treatment	Shoot Fresh Weight (g)	Root Fresh weight (g)	Shoot Dry weight (g)	Root Dry weight (g)
Control (0 mM)	44.95±0.95a	18.06±0.43a	3.85±0.07a	1±0.10a
50 mM	37.51±1.16b	16.70±0.30b	3.46±0.08b	0.51±0.02b
100 mM	8.74±0.64c	4.38±0.04c	1.58±0.06c	0.29±0.03c
150 mM	6.15±0.34c	1.06±0.02d	1.09±0.04d	0.14±0.01c
200 mM	5.72±0.27c	0.99±0.04d	1.04±0.03d	0.12±0.01c

Means with same letter within each column are not significantly different according to Tukey's HSD tests ($P < 0.05$).

3.2 Effect of salinity on physiological properties

Germination percentage was found to be decreased with increasing salt concentration. The highest germination percentage (99.0±0.58 %) was found in control (0 mM) treatment where the lowest germination percentage (76.13±3.89 %) was found in the highest concentration (200 mM) of NaCl. The effect of salinity on germination percentage was depicted in Figure 4.

Salt treatment significantly reduced the water content of root and shoot of lettuce seedlings. The impact of salt treatment on the water content of shoot and root was summarized in Table 4. Water content reduced gradually

Figure 2: Reduction in growth with increasing salt concentration (from left to right).

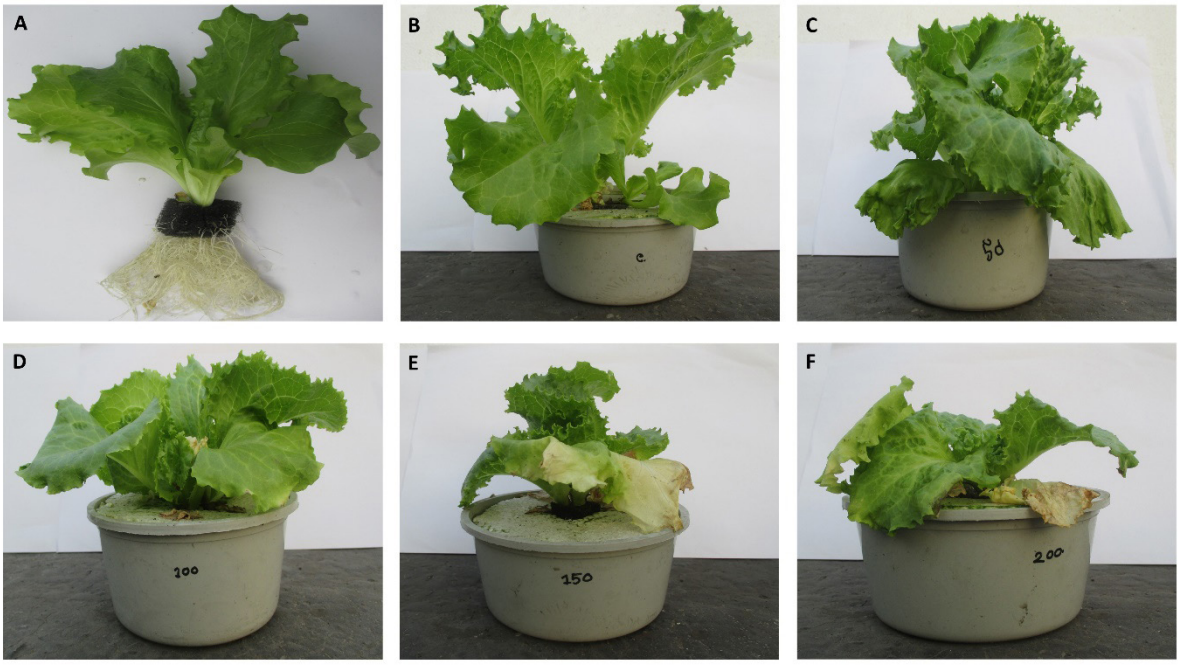

Figure 3: (A) Seedling before salt treatment (B) Control (0 mM) (C) 50 Mm (D) 100 mM (E) 150 mM (F) 200 mM.

with increasing concentration of NaCl. For the shoot, the highest water content (89.18±1.54 %) was found in the control plant, and the lowest (81.57±0.70 %) was found in the highest concentration (200 mM) of NaCl. The water content of shoot was found decreased by 1%, 7%, 8%, 9% respectively in 50 mM, 100 mM, 150 mM, 200 mM salt treatment as compared to the control. For root, the highest water content was 94.50±0.68 % in control treatment and the lowest was 82.73±0.98 % in 200 mM salt treatment. The water content of root was found decreased by 2%, 3%, 10%, and 12% respectively under 50 mM, 100 mM, 150 mM, and 200 mM salt treatment as compared to the control. Relative water content (RWC) was estimated from fresh leaf samples and found to be significantly influenced by salt treatment. Increasing salt concentrations resulted in a gradual reduction in relative water content. The highest relative water content (86.30±1.71 %) was found with control plants, and the lowest relative water content (57.16±1.84 %) was found with 200 mM salt treatment. The relative water content showed 2%, 19%, 27%, 34% reduction respectively under 50 mM, 100 mM, 150 mM, and 200 mM salt treatment as compared to the control.

3.3 Effect of salinity on biochemical properties

This experiment found a clear correlation between proline accumulation with the increasing salt concentrations (Figure. 5 A). Proline content was found higher in treated seedlings, and proline accumulation increased steadily with increasing salt concentrations (Figure 5 A). The highest proline content (54.21±0.58 μM) was observed in 200 mM salt treatment whereas the lowest proline content (12.90±0.69 μM) was observed from the untreated seedlings. However, the increased proline content was gradual as observed in all other salt treatments as compared to control.

Significant reduction in total phenol content was observed towards all salt treatments as compared to control treatment (Figure 5 B). Total phenol content was highest (34.23±0.21 mg) in control plants, where the gradual decrease was observed in other treatments (Figure 5 B). This led to the lowest total phenol content (18.58±1.78 mg) in the seedlings under 200 mM salt concentration. The reduction was 8%, 11%, 31%, and 46% lower respectively under 50 mM, 100 mM, 150 mM, and 200 mM salt treatments compared to the control treatment.

Increasing salt concentration has a significant effect on the total protein content of lettuce plant. The responses of the protein content to salt stress were exhibited in a dose-dependent manner. The lowest protein content (24.13±1.45 mg) was observed in untreated plants. The highest protein content (75.19±1.49 mg) was observed in plants treated with 200 mM NaCl as compared to control (Figure 5 C).

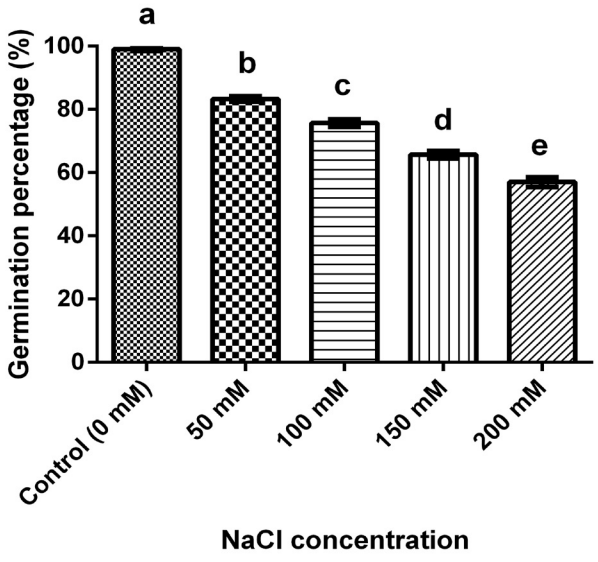

Figure 4: Effect of salt treatment on the germination percentage of lettuce seeds. Bars with different letters are significantly different according to Tukey's HSD tests ($P < 0.05$).

Table 4: Effect of salt treatment on the water content of shoot and root and relative water content of the leaf.

Treatment	Water content (Shoot) %	Water content (Root) %	Relative water content of leaf (RWC) %
Control (0 mM)	89.18±1.54a	94.50±0.68a	86.30±1.71a
50 mM	89.08±1.0a	92.92±2.19a	84.73±1.45a
100 mM	83.22±1.26b	92.80±0.59a	70.13±1.42b
150 mM	82.72±0.94b	85.30±1.07b	62.90±1.48bc
200 mM	81.57±0.70b	82.73±0.98b	57.16±1.84c

Means with the same letter within each column are not significantly different according to Tukey's HSD tests ($P < 0.05$)

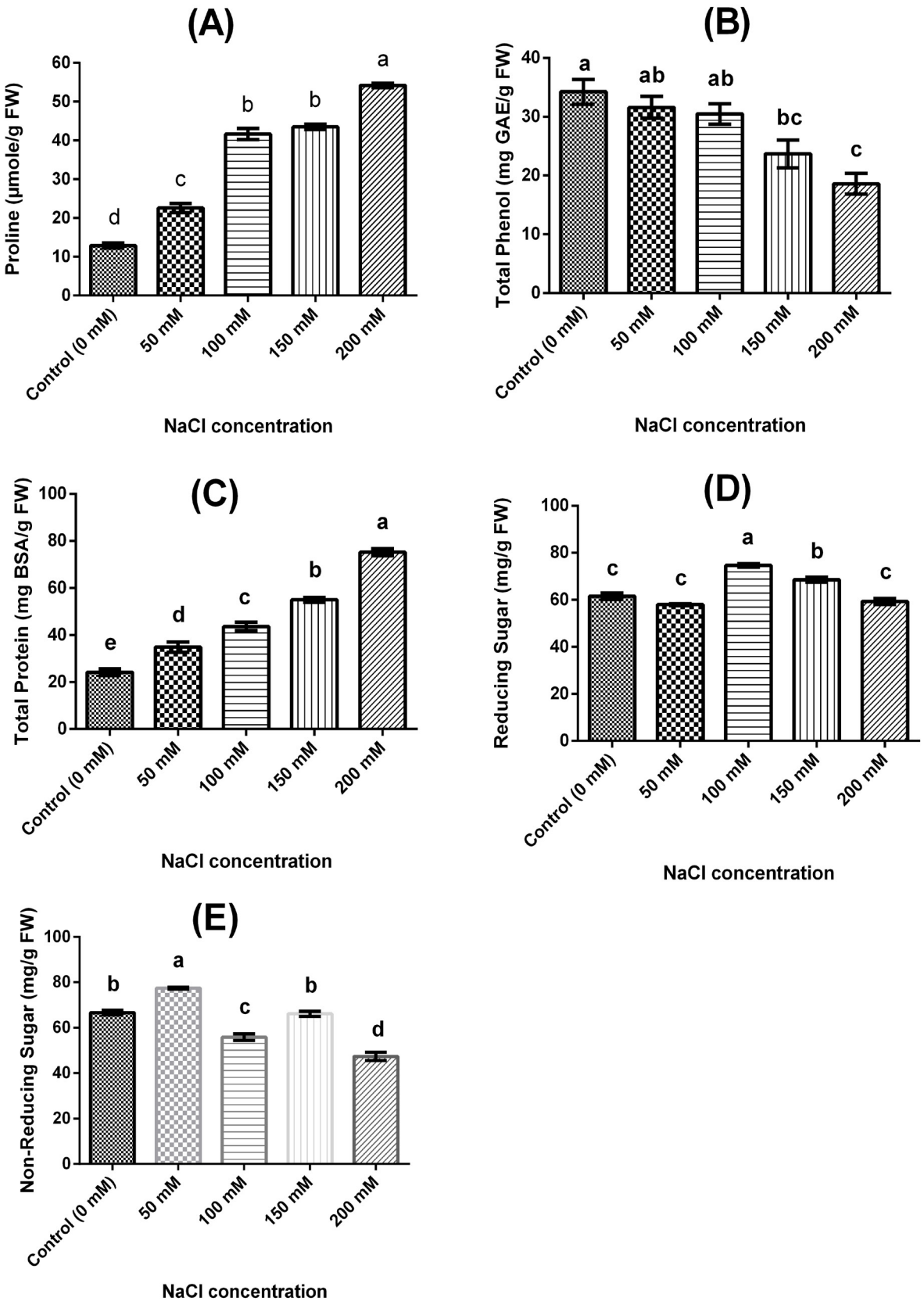

Figure 5: Effect of salt stress on the biochemical properties of lettuce. (A) Proline (B) Total phenol (C) Total protein (D) Reducing sugar (E) Non-reducing sugar. Bars with different letters are significantly different according to Tukey's HSD tests ($P < 0.05$).

The effect of increasing salt treatment resulted in an increase in reducing sugar in a stressed plant except for the plants under 50 mM salt treatment. Other salt treatments showed a significant increase in reducing sugar content than that of control treatment. The highest (74.62±0.78 mg) accumulation of reducing sugar was found in 100 mM salt treatment followed by 150 mM and 200 mM. The lowest (57.97±0.42 mg) reducing sugar accumulation was observed in 50 mM salt treatment, which was lower than the control treatment. Increasing salt concentration caused a reduction of non-reducing sugar content in stressed plants except for the plants under 50 mM salt treatment (Figure 5 D). A significant decrease in non-reducing sugar accumulation occurred in other salt treatments over the control treatment. The non-reducing sugar content was found to be highest (77.46±0.39 mg) in 50 mM treatment. The non-reducing sugar content was lowest (47.40±1.82 mg) in 200 mM salt treatment. The results obtained from the present study revealed that the plants in 150 mM salt treatment exhibit similar non-reducing sugar content as control treatment (Figure 5 E).

4 Discussion

Salinity limits crop productivity through its deleterious effects, which are the low osmotic potential of the soil solution, nutritional imbalance, specific ion effects. All these factors hamper plant growth and development (Ashraf and Harris 2004). Growth reduction is perceived to be the general observation in plants caused by salinity stress. Lower water potential in cells may cause stomatal closure and CO_2 assimilation leading to growth reduction (Zeng et al. 2002; Pattanagul and Thitisaksakul 2008). The present study found a significant growth reduction of lettuce plants after exposure to salinity stress. Similar growth reduction was investigated earlier in lettuce plants (Ünlükara et al. 2008; Al-Maskri et al. 2010; Ekinci et al. 2012; Turhan et al. 2014). The growth reduction was observed both for the root and shoot lengths. Total plant weight and average leaf number of the plant were also significantly affected by increasing salt concentrations. The present study found a gradual reduction in plant weight and average leaf number with increasing salt concentrations which was consistent with the study as previously described (Ünlükara et al. 2008; Al-Maskri et al. 2010). Total plant weight reduction with increasing salt concentrations was consistent with the results described in an earlier report (Garrido et al. 2014). However, Andriolo et al. (2005); and Garrido et al. (2014) reported that leaf number of lettuce was not affected by increasing salt concentrations. Fresh weight and dry weight of shoot and root were also significantly reduced with the increasing salt concentrations. Result found by Al-Maskri et al. (2010) and Ekinci et al. (2012) were consistent with the result in the present study. A similar result was also observed for rice and sorghum (Gill et al. 2001; Özdemir et al. 2004; Minh et al. 2016).

Salinity stress negatively affects the absorption of water due to the osmotic effect. Accumulation of salts in the root zone decreases the osmotic potential which leads to the reduction in water potential. This, in turn, reduces the amount of water available to the plant root system. Plants may balance its water potential by losing water, inducing a decrease in osmotic potential (Acosta-Motos et al. 2017). Therefore, plants may adapt to salt stress by reducing its water content. The present study found a reduction in water content in both shoot and root. A similar result was also found in several other studies (Al-Maskri et al. 2010; Garrido et al. 2014). The relative water content of lettuce leaf was also negatively affected by increasing salt concentrations. Up to date, no study addresses the changes in relative water content in lettuce plant caused by salinity stress. However, reduction in relative water content due to salt treatment have been investigated in rice and sorghum (Gill et al. 2001; Pattanagul and Thitisaksakul 2008).

Seed germination percentage is reduced, and germination is delayed by the imposition of salt stress. The accumulation of osmotic components such as Na and Cl ions are thought to interfere with the germination of seeds (Živković et al. 2007). In this investigation, the germination percentage was reduced significantly with the increasing exposure of salt. The onset of germination is also delayed gradually for each treatment. This result is evidenced by several other experimental studies (Anuradha and Rao 2001; Barassi et al. 2006; Bojović et al. 2010).

Saline stressed plants accumulate proline in larger amounts than control plants. Salt and water deficit both contribute to the accumulation of proline in the plants. Proline stabilizes cell membrane and acts to prevent NaCl from disrupting the cell membrane (Ashraf and Harris 2004). Proline may also act as a signaling molecule to activate multiple processes essential for the adaptation process (Maggio et al. 2002). The present investigation found a positive correlation between proline accumulation in lettuce plant with increasing salt concentrations. Proline concentration was also found increased on rice, tomato, soybean, pea and mangrove plants due to the salt stress (Waheed et al. 2006; Lee et al. 2010; Rajakumar 2013; Das et al. 2016; Gharsallah et al. 2016; Palliyath and Puthur 2018).

Phenolic compounds are secondary metabolites

responsible for plant responses to unfavorable or stress conditions. Salinity-induced oxidative stress may be overcome by the accumulation of phenolic compounds. However, studies show both increases and decrease in the phenolic compound as a response to salinity stress (Waśkiewicz et al. 2013). The present study found a gradual decrease in the phenolic compound with increasing salt concentrations. A similar result was found in Romaine lettuce in a previous study (Chisari et al. 2010; Kim et al. 2013). However, Mahmoudi et al. (2010) found an increase in phenolic content after 100 mM salt concentration in Romaine lettuce. Various stress proteins accumulate in plants in response to salt stress may provide storage of nitrogen for utilization after stress condition and help in osmotic adjustment (Ashraf and Harris 2004). The present study showed increased protein concentration with increasing salt concentrations. Increasing protein concentration was also found in rice and safflower (Özdemir et al. 2004; Javed et al. 2014).

Salinity stress induces the plant to accumulate sugar contents which protect biomolecules and membranes. Increased accumulation of reducing sugar was evidenced by many studies in rice (Dubey and Singh 1999; Pattanagul and Thitisaksakul 2008; Rajakumar 2013). However, the present study found an increase in reducing sugar content than control except for the treatment of 50 mM of NaCl. Non-reducing sugar accumulation was found to be increased only in 100 mM salt treatment and slight reduction than control found in others treatment. Reduction of non-reducing sugar content was evidenced by the result found in rice in a previous study (Rajakumar 2013).

5 Conclusion

Lettuce is one of the most popular vegetables in Bangladesh, cultivation and consumption are growing tremendously day by day. However, a large proportion of land is salinized, and presence of salt in irrigation of water may impose detrimental effect of the successful lettuce cultivation in Bangladesh. Our investigation demonstrates the effect of NaCl on the morphological, physiological and biochemical characteristics of lettuce. The results obtained from the present study revealed that salinity negatively affects lettuce plants, where the response varied depending upon different salt concentrations. A significant increase occurred in proline, protein and reducing sugar accumulation, and a decrease in phenol and non-reducing sugar was observed, which suggest that plants were trying to adapt to the stress condition. The plants at 50 mM salt concentration have encountered less detrimental effect; other treatments reveal a significant decrease in yield which was unavoidable. Therefore, further investigation into the properties that demonstrate the changes and robust mitigation process should be carried out. However, salt tolerant lettuce variety can be developed through extensive screening procedures or by applying genetic engineering methods if investigated further.

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