Original Research Article

Foliar Application of Proline Enhances Salt Stress Tolerance of Jute (*Corchorus olitorius* L.) by Regulating Photosynthetic Attributes, Osmolytes and Ion Homeostasis

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ABSTRACT

Soil salinity is one of the abiotic stresses that hamper plant growth and development. Foliar application of proline mitigates the detrimental effect of salt stress in plants. The effect of proline on salt-induced inhibitory effects in the jute (*Corchorus olitorius* L.) plant was investigated. The experiment consisted of four treatments: control, proline, salt stress, and salt stress + proline. Salt stress significantly reduced growth, biomass accumulation, leaf area, stress tolerance index (%), photosynthetic pigments, SPAD value and K^+/Na^+ ratio in jute plants. Consequently, salt stress increased lipid peroxidation and proline content. Exogenous application of proline lessened the reduction of growth, leaf area, stress tolerance index (%), photosynthetic pigments, SPAD value and K^+/Na^+ ratio in jute plants. Proline also increased oxidative stress tolerance by increasing proline accumulation and reducing lipid peroxidation in jute plants. The present study suggests that proline comprehensively alleviates the detrimental effects of salt stress on jute plants.

Keywords: Salt stress; growth; jute; proline.

1. INTRODUCTION

Salt stress is an ~~vital~~ abiotic stress ~~that~~ with detrimental effect on plant growth and plant yield ~~of plants~~ [1]. Induced by ~~due to~~ the climate changes and the degradation of the environment caused by human interference, this stress has been becoming an utmost warning to food security. One-third of the world's food supply comes from irrigated lands, but around 20% of the lands are affected by salinity stress which reduces more than 50% of the yield of annual crops [2]. Salt stress induces both ionic and osmotic stresses in plants, leading to alteration of metabolic processes, accumulation of reactive oxygen species (ROS) and lipid membrane peroxidation [3]. Salt stress reduces the physiological functions of the plants like photosynthesis and osmotic adjustment [4]. Thus, salinity decreases agricultural productivity through reductions in plants' photosynthetic and water use efficiency, and fresh and dry biomass [5]. Plants deploy different mechanisms to adapt to salinity stress, including morpho-physiological, biochemical, and molecular mechanisms [6].

Proline is an osmolyte accumulated under abiotic stresses including salinity [7]. Proline can improve salt tolerance by several factors including crop growth stage and Na^+/K^+ ratio (sentence need revision) [8]. Proline is one of the highly beneficial ~~compatible~~ molecules, and plays an important role in plant salt tolerance by osmoregulation. Moreover, proline attenuated salt stress via quenching ROS, up-regulating stress-protective proteins and reducing oxidation of lipid membranes in plants [9,10].

Jute (*Corchorus olitorius* L.), is a widely cultivated natural bio-fiber crop around the world. It is used as a good source of biopolymer which is an alternative to environment pollutant polythene [11,12]. Jute can grow readily in saline soils, while high salinity constrains its growth, and development [3]. Based on the high potential roles of proline, the present study aimed to investigate the effects of exogenous application of proline on plant growth, photosynthetic pigments, proline accumulation, MDA content as well as ion homeostasis in jute plants under saline conditions.

2. MATERIAL AND METHODS

2.1. Plant materials, growth conditions, and stress treatments

Jute seeds (*C. olitorius* L. cv. BJRI-08) were collected from Rangpur Regional Station, Bangladesh Jute Research Institute (BJRI). Plants were irrigated with salt solution (150 mM NaCl) 20 days after sowing, and maintained field capacity by irrigating with either water (control) or salt solution. For proline application, a solution of 25 mM proline (Wako, Japan) was prepared by dissolving in water and sprayed at 9 am every day. At 30 DAS (full name), different parameters were measured. The study was conducted in a CRD(full name) arrangement having three replications. The following treatments were applied: control, proline @ 25 mM, NaCl @ 150 mM and NaCl @ 150 mM + proline @ 25 mM.

2.2. Measurement of shoot and root length

Shoot and root length were measured by measuring the scale from the plant camp to the apex of the stem and root, respectively.

2.3. Estimation of fresh weight and dry weight of shoot and root

Shoots and roots were separated and weighted to estimated fresh weight (FW). Then, the dry weight of roots and shoots (oven-dried at 70ºC for 72 h) were determined.

2.4. Estimation of root and shoot ratio

The fresh root and shoot length ratio (root length: shoot length) of the seedlings was estimated.

2.5. Measurement of leaf area

The leaf area was measured with a digital leaf area meter (LICOR 3100).

2.6. Stress tolerance index

Stress tolerance indices (STI) were calculated using following the formulae [13]:

SFWSTI = Shoot fresh weight of stress plant / Shoot fresh weight of control plant × 100

RFWSTI = Root fresh weight of stress plant / Root fresh weight of control plant × 100

SDWSTI = Shoot dry weight of stress plant / Shoot dry weight of control plant × 100

RDWSTI = Root dry weight of stress plant / Root dry weight of control plant × 100

SLSTI = Shoot length of stress plant / Shoot length of control plant × 100

RLSTI = Root length of stress plant / Root length of control plant × 100

2.7. Determination of the photosynthetic pigments

Leaf (100 mg, fresh weight) was completely extracted with 10 ml of 80% acetone. The absorbance of the extract was monitored at 645, 663, and 470 nm, respectively, for chlorophyll a, chlorophyll b, and carotenoids content. The following equations were used for calculation [14]: Chlorophyll a = 11.75 A_{663} - 2.350 A_{645}, Chlorophyll b = 18.61 A_{645} - 3.960 A_{663}, Total chlorophyll a+ b = chlorophyll a + chlorophyll b and Total carotenoid = (1000A_{470} - 2.270 Chl a - 81.4 Chl b)/227.

2.8. Determination of SPAD (full name) value

SPAD value was recorded using a SPAD meter (Konica, Minolta SPAD-502 Plus, Inc., Japan). Expanded leaf was used for the measurement of the SPAD value.

2.9. Determination of proline content

Proline content was assayed according to [15]. Leaf sample (0.25 g) was homogenized in 1 ml aqueous sulfosalicylic acid (3 % w/v) by the mortar with liquid nitrogen. The homogenate was centrifuged at 12000 rpm for 15 min. The extracted mixture was reacted with 2 ml of glacial acetic acid and 2 ml ninhydrin reagent and heated in the water bath at 95°C for 1 h. The reaction mixture was stopped in an ice bath. The sample was mixed with 4 mL toluene and vortex with a vortex ~~meter~~. After that, the chromophore absorbance was recorded at 520 nm. A calibration curve was prepared by using L-proline (ref).

2.10. Determination of MDA Content

Lipid peroxidation was determined by measuring malondialdehyde (MDA) formation using the thiobarbituric acid method [16]. Leaf sample (0.5 g, fresh weight) was homogenized with 2 ml of trichloroacetic acid (TCA, 5%) and centrifuged at 12,000 rpm for 15 min. Then, 1 ml of the supernatant was mixed with 2 ml of 20% TCA containing thiobarbituric acid (TBA, 0.5%). The mixture was then placed in a boiling water bath for 25 min. After cooling, the absorbance of supernatant was read at 532 nm and 600 nm. The concentration of MDA was calculated using an extension co-efficient of 155 mM^{-1} cm^{-1}.

2.11. Measurement of Na^+ and K^+ content

Leaf Na^+ and K^+ contents were estimated with a Na^+ and K^+ meter (Horiba, Kyoto, Japan). Fresh leaf sample was oven-dried and then the sample was heated (95°C for 30 min) with distilled water and cold at room temperature. The sample was put to the sensor of the meter, and value was recorded as ppm.

2.12. Statistical analysis

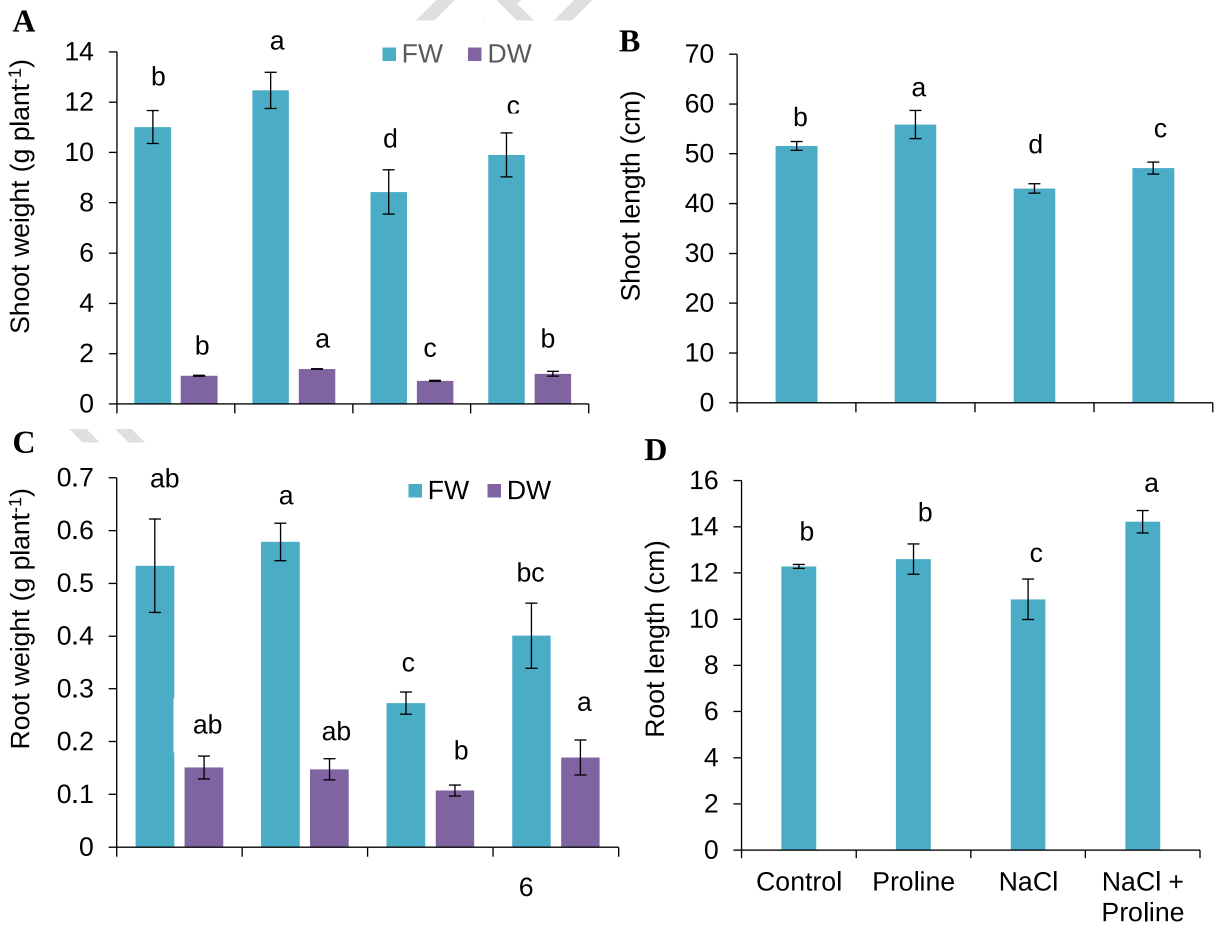
One-way analysis of variance (ANOVA) with the Cropstat10 software was used to analyze data. Least-significant-difference (LSD) test at $P \leq 0.05$ level of significance was carried out to evaluate statistical significance.

3. RESULTS AND DISCUSSION

3.1. Effect of proline on growth attribute and biomass accumulation under salt stress condition

The effect of proline on the growth attributes of jute plant under salt stress conditions is presented in Fig. 1. The results showed that salt stress significantly ($p \leq 0.05$) decreased shoot FW, shoot DW, shoot length, root FW, root DW and root length compared to the control (Figs. 1A-D). These results might be due to altering cytosolic K^+/Na^+ ratio, and sequestrating Na^+ and Cl^- into vacuoles, as well as reducing the internal osmotic potential, which impairs the cellular metabolism and ultimately reduces the growth of the seedlings [17,18]. Furthermore, proline significantly ($p<0.05$) increased shoot length, shoot FW, shoot DW, root length, root FW and root DW in salt-stressed plant (Figs. 1A-D). These results are consistent with previous studies, where the application of proline increased plant growth under salt stress [19,20]. Similarly, in 2016, Alam et al. [8] suggested that proline enhances the salt tolerance of maize by increasing the K+/Na+ ratio and improving nutrient uptake, especially P uptake. ~~that proline supplies plants with energy for growth as a result helping the plant to survive under stressful conditions~~. In addition, [21] (Author name should be added) observed that exogenous application of proline significantly increases in growth parameters ~~traits~~ (plant height, shoot, root fresh, and dry weight) on quinoa plants.

Leaf area is one of the factors that play an important role in photochemical reaction and carbon fixation, which affects plant growth and biomass. The leaf area of jute was significantly (p≤0.05) affected by salt stress as it dropped by 26.37%, compared to control (Fig. 1E). However, exogenous proline treatment significantly (p≤0.05) alleviated the inhibitory effect of salt stress on the leaf area of jute as it increased by 24.85%. This is consistent with the results of [22] (Author name should be added) who observed that the application of proline has significantly increased the leaf area in canola under salt stress conditions. The phenotypic appearance of salt-stressed jute plants is shown in Fig. 2. The growth of salt-treated jute plants was hindered due to salt stress but was improved by proline application compared to the respective salt-stressed (Fig. 2).



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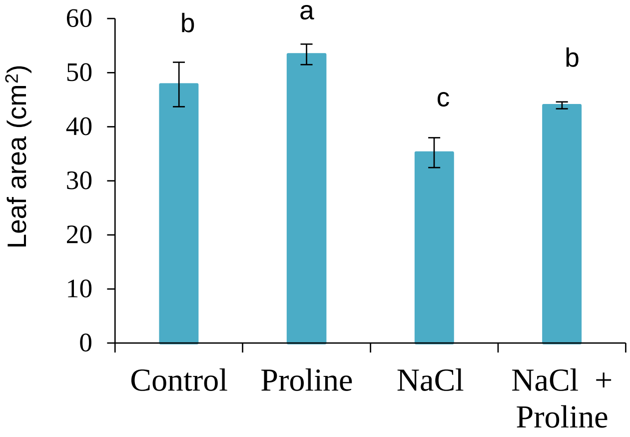

Fig. 1. Effect of foliar application of proline on the shoot fresh weight (FW) and shoot dry weight (DW) ~~of shoot~~ (A), shoot length (B), root FW-fresh weight () and root DW ~~dry weight~~ () ~~of root~~ (C) and root length (D) of jute under salt stress conditions. Data are mean ± standard deviation. Values with different letters ~~in the same columns~~ showed differ statistically among themselves (p≤0.05)

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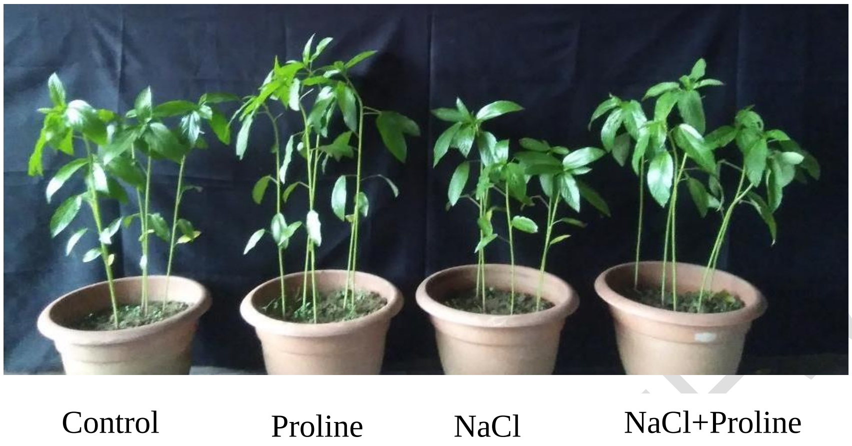

Fig. 2. Phenotypic appearance of jute plants as boosted by proline supplementation under salt stress condition

3.2. Effects of proline on root/shoot ratio and stress tolerance index under salt stress conditions

In this study, salt stress increased the root/shoot length ratio by 8.40% in jute (Fig. 3A). However, the application of proline to the salt-stressed plant significantly (($p \leq 0.05$) increased the root/shoot ratio by 16.66% compared to ~~that of the~~ salt-stressed control plants ~~alone~~. These results are similar to **(**Author name should be added) [23] who indicated that exogenous proline application increases the root/shoot ratio of wheat under stress conditions. Fig. 3B shows that jute plants grown with foliar spray of proline have the highest value of stress tolerance index (STI %) and it was lowest in control salt-stressed plants. STI (%) was calculated from the ratio of stressed plants and control plants. Proline application lessened the reduction of the SLSTI, SFWSTI, SDWSTI, RLSTI, RFWSTI and RDWSTI value by 9.63%, 17.10%, 30.48%, 30.68%, 52.94% and 59.72%, respectively under salt stress. In the alfalfa plant, ~~observed~~ results showed that supplementation of proline significantly increased STI under salt stress conditions [24].

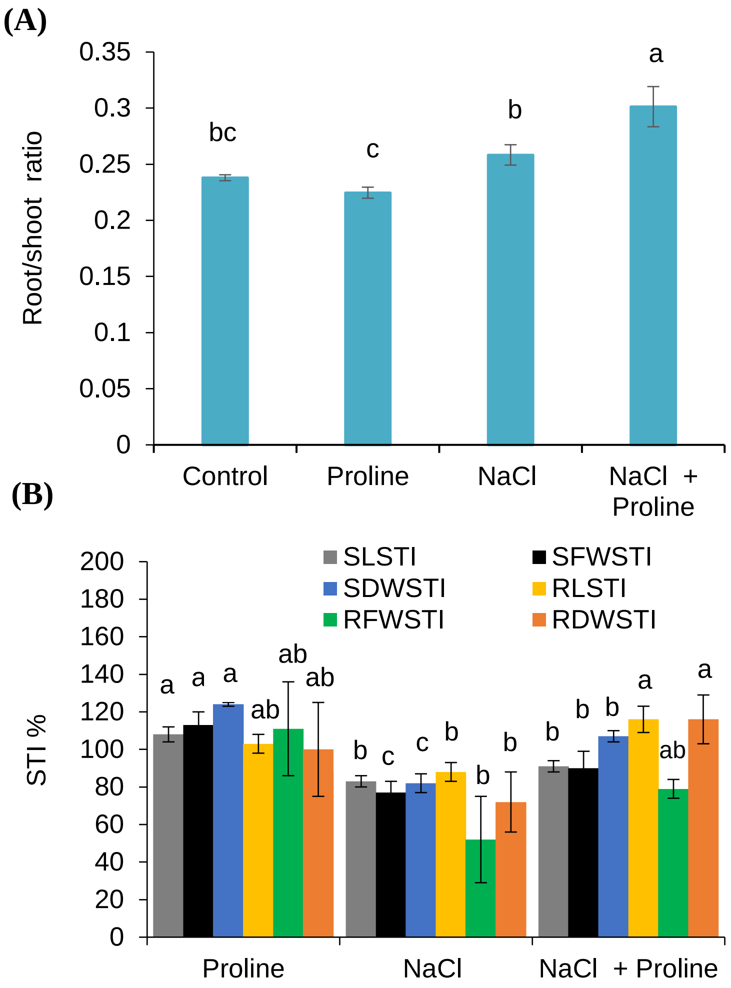

Fig. 3. Root/shoot length ratio (A) and salt tolerance index (STI) % (B) of jute as improved by proline under salt stress. SL, shoot length; SFW, shoot fresh weight; SDW, shoot dry weight; RL, root length; RFW, root fresh weight; RDW, root dry weight. Vertical bars represent LSD value at a 5% level of significance. Different letters indicate statistically significant differences between treatments means at p≤0.05

3.3. Effect of proline on photosynthetic pigments and SPAD value under salt stress conditions

The photosynthetic pigment chlorophyll plays an important role in capturing light energy and is essential for photosynthesis. Thus, the measurement of chlorophyll content can be used ~~to determine the level of~~ as plant stress marker [25]. In this study, salt stress caused a significant ($p \leq 0.05$) decrease in the chl a, chl b, chl a+b and carotenoid content ~~as~~ compared ~~with their~~ to control. However, the foliar application of proline significantly lessened the reduction in the content of photosynthetic pigments in salt-stressed jute plants compared to that of the salt-treated plants alone (Figs. 4A-D). In addition, salt stress decreased significantly ~~($p \leq 0.05$)~~ the SPAD value in jute by 6.38%, compared to ~~that of~~ control plants. Proline application ~~of significantly ($p \leq 0.05$)~~ increased the SPAD value by 6.65% in salt-stressed jute plants, compared to ~~that of~~ control salt-stressed plants ~~alone~~ (Fig. 4E). Similar results were observed by (Author name should be added) [26] on rice, Author name should be added [27] on celery's and Author name should be added [28] on quinoa plants. Moreover, Author name should be added [10] found that exogenous application of proline improved PSII activity by higher carotenoid accumulation in Aloe vera plants. Salt-induced chlorophyll depletion may be the result of the inhibition of chlorophyll biosynthesis followed by an increase in ethylene production [29]. In addition, Author name should be added [30] indicated that chlorophyll degradation under salt stress can be attributed to the chlorophyllase enzymes.

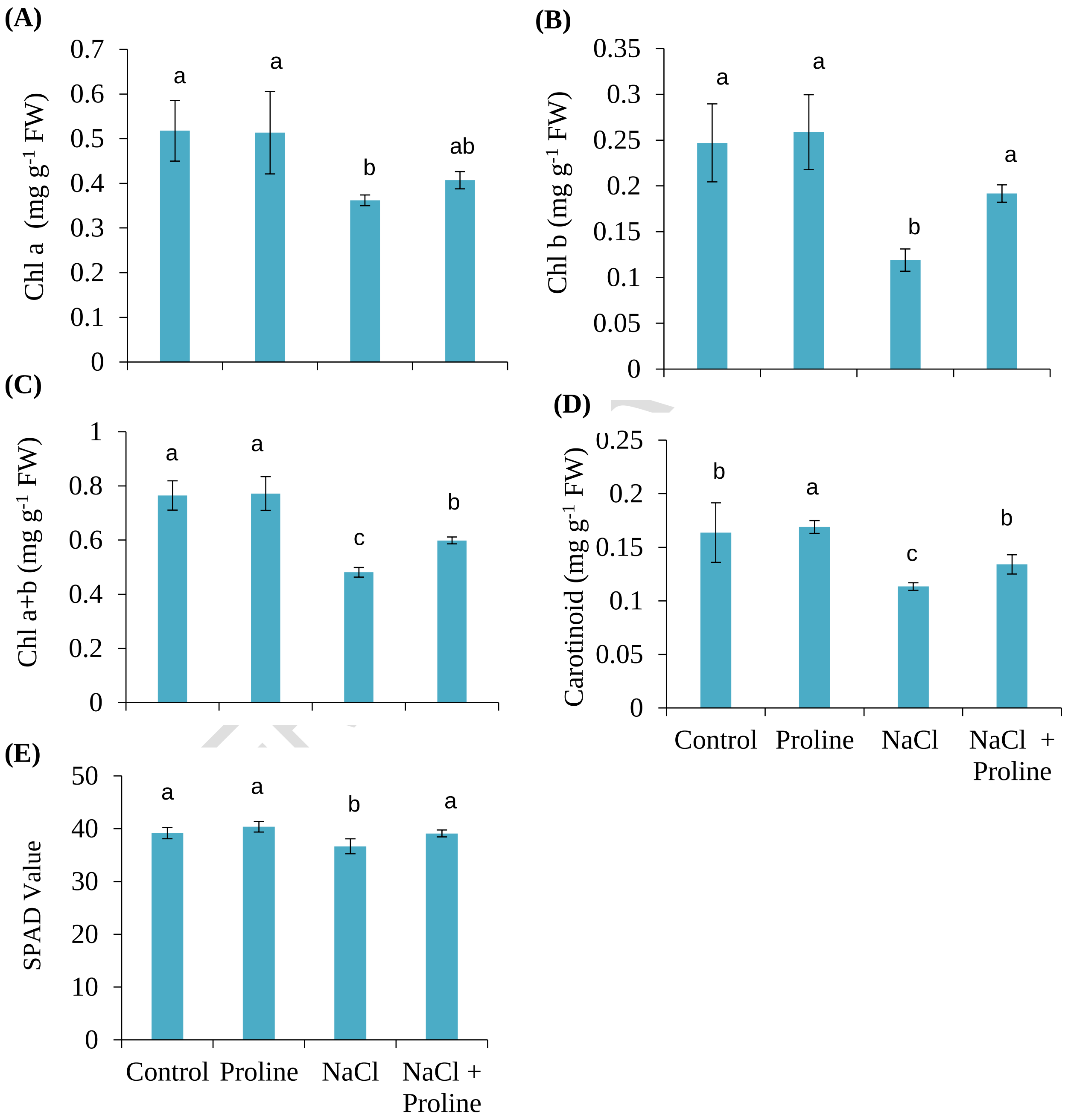

Fig. 4. Contents of photosynthetic pigments, Chl a (A), Chl b (B), Chl a+b (C), carotenoids (D), and SPAD value (E) in leaves of jute plants treated with proline under salt stress conditions.

Data are mean ± standard deviation. Values with different letters in the same columns showed differ statistically among themselves ($p \leq 0.05$)

3.4. Effect of foliar application of proline on osmoregulation and lipid peroxidation under salt stress condition

Proline accumulation is thought as an important physiological parameter for plant adaptation to salt conditions [31]. In this study, salinity significantly ~~($p \leq 0.05$)~~ enhanced the proline content by 127.22% in jute compared to control. However, exogenous proline significantly ~~($p \leq 0.05$)~~ enhanced concentrations of proline by 29.69% in leaves of salt-stressed jute plants compared to control salt stressed plants ~~alone~~ (Fig. 5A). Increased concentration of proline under salinity conditions was also observed in jute [3] and sunflower plants [32]. Mitigation of salt-induced toxicity caused by increasing accumulation of proline under salt stress has been reported in sorghum [33], maize [34], sainfoin [35] and *Aloe vera* [10]. High levels of proline under stress conditions reduced the osmotic potential of tissues, and thus involved in the osmotic adjustment [36]. It has been shown that proline protects enzymes and increases membrane stability under salinity stress [37]. These results suggested that foliar application of proline improved the salt tolerance by increasing proline accumulation.

MDA is one of the important end products of membrane lipid peroxidation, reflecting the degree of damage to the membrane in plants under biotic and abiotic stresses [38]. In this study, salt stress significantly ~~($p \leq 0.05$)~~ increased the concentrations of MDA in the jute leaf compared ~~with the~~ to control (Fig. 5B). Similar results were found in jute [3] and sugar beet [31]. However, proline treatment significantly ($p \leq 0.05$) reduced MDA concentrations compared with the salt treatment alone (Fig. 5B). It was reported that exogenous proline alleviates oxidative damage by enhancing the stability of lipids in membranes [39,40,27]. These results indicated that proline could maintain membrane stability under salinity stress in jute plants.

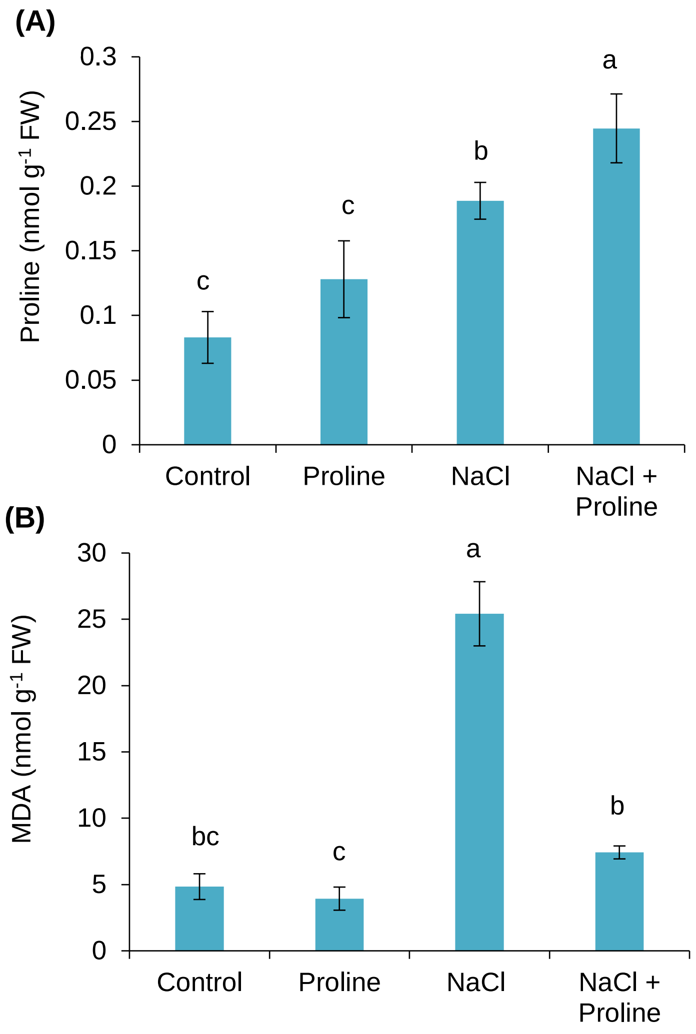

Fig. 5. Proline content (A), and MDA content (B) of jute as affected by proline under salt stress. Vertical bars represent LSD value at a 5% level of significance. Different letters indicate statistically significant differences between treatments means at p≤0.05

3.5. Effect of proline foliar application on ion homeostasis under salt stress condition

Under salt stress conditions, sustaining ion homeostasis is one of the adaptive strategies that tolerant plants use to cope with salt stress. These strategies may help the plant to prevent potentially toxic effects of the build-up of ions like Na^+ and Cl^- that cause various types of damage to lipids, proteins and nucleic acids [41,42,43]. In the present study, salt stress significantly ($p \leq 0.05$) increased Na^+ concentration in the leaf compared to the control. Exogenous application of proline decreased Na^+ accumulation in the leaf of jute (Fig. 6A). Salt stress significantly ($p \leq 0.05$) decreased K^+ content compared to the control. However, proline treatment significantly increased K^+ content in salt-stressed jute plant (Fig. 6B). It was also found that salt stress significantly decreased the K^+/Na^+ ratio in plants compared with the control ($p \leq 0.05$). In addition, the K^+/Na^+ ratio was significantly higher in NaCl + proline-treated jute plants than in salt-treated plants alone ($p \leq 0.05$) (Fig. 6C). Thus, exogenous application of proline might lead to a reduction in Na^+ uptake and salt-induced toxicity. These results suggested that exogenously applied proline played a crucial role in regulating the K^+/Na^+ ratio under salinity conditions, which was reflected in higher water content and reduced membrane damage under salt conditions (Fig. 5B). Application of proline in a foliar spray decreased Na^+ content and increased K^+/Na^+ ratio in *Phaseolus vulgaris* [44]. More recently, Author name should be added [26] reported that external application of proline decreased Na^+ content, but increased the K^+ content and the K^+/Na^+ ratio in salt-stressed *Zea mays*. Also, Author name should be added [45] demonstrated that exogenous proline alleviated the negative effect of NaCl stress, and enhanced K^+ content, and reduced Na^+ concentration in *Helianthus annuus*. Compared to salt-stressed rice plants, exogenous proline was shown to reduce Na^+ accumulation and increase the K^+/Na^+ ratio under salt stress [19]. These results illustrated that exogenous proline application improved salt tolerance by maintaining a low Na^+ content, a high K^+ content and higher K^+/Na^+ ratio in jute plants.

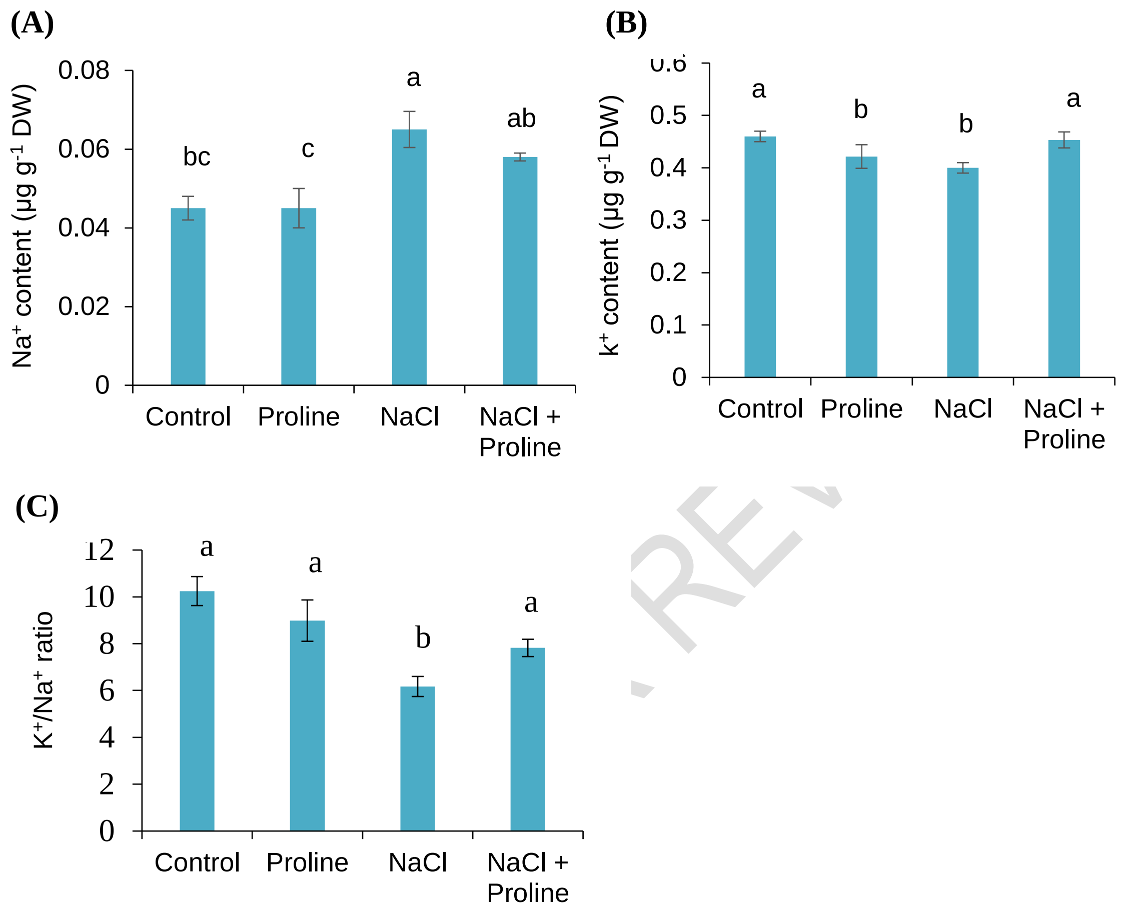

Fig. 6. Effect of foliar application of proline on Na^+ content (A), K^+ content (B) and K^+/Na^+ ratio (C) of jute under salt stress condition. Vertical bars represent LSD value at a 5% level of significance. Different letters indicate statistically significant differences between treatments means at p≤0.05

4. CONCLUSION

In conclusion, salt stress ~~inhibited~~ decreased the growth, photosynthetic efficiency and K^+/Na^+ ratio of jute plants, ~~with~~ which consequently increased proline accumulation and lipid peroxidation. Proline treatment alleviated salinity effects by restoring ionic equilibrium, improving photosynthetic pigments, proline accumulation ~~of~~ and reducing lipid peroxidation in jute plants.

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