

Original Research Article

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

Comment [WU1]: It would be better to change the title of the article. effect of Foliar Application of Proline on Photosynthetic Attributes, Osmolytes and Ion Homeostasis in Jute (*Corchorus olitorius* L.)

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 a vital abiotic stress that detrimental effect on plant growth and yield of plants [1]. This stress has been becoming an utmost warning to food security due to the changes in climate and degradation of the environment caused by human interference. 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 [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 proline on plant growth, photosynthetic pigments, proline accumulation, MDA content as well as ion homeostasis in jute plants under saline conditions.

Comment [WU2]: You need to have a definition of saline soil. There are various definitions for soil salinity. generally, soils with an electrical conductivity (solution) of more than 4 dS/m are called saline soils (Lamond and Whitney, 1992).

Comment [WU3]: Give a brief explanation for each of these mechanisms.

Comment [WU4]: Explain the botany of jute.

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 at 20 days after sowing with salt solution (150 mM NaCl) and maintained field capacity by irrigating with 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, different parameters were measured. The study was conducted in a CRD arrangement having three replications. The following treatments were applied: control, proline @ 25 mM, NaCl @ 150 mM and NaCl @ 150 mM + proline @ 25 mM.

Comment [WU5]: Why was the salinity treatment applied 20 days after cultivation? Why did you use a 150 mM salt solution? Give a reference?
What salt did you use?

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 70°C 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]:

$$\text{SFWSTI} = \text{Shoot fresh weight of stress plant} / \text{Shoot fresh weight of control plant} \times 100$$

$$\text{RFWSTI} = \text{Root fresh weight of stress plant} / \text{Root fresh weight of control plant} \times 100$$

$$\text{SDWSTI} = \text{Shoot dry weight of stress plant} / \text{Shoot dry weight of control plant} \times 100$$

$RDWSTI = \text{Root dry weight of stress plant} / \text{Root dry weight of control plant} \times 100$

$SLSTI = \text{Shoot length of stress plant} / \text{Shoot length of control plant} \times 100$

$RLSTI = \text{Root length of stress plant} / \text{Root length of control plant} \times 100$

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 = $(1000A_{470} - 2.270 \text{ Chl a} - 81.4 \text{ Chl b})/227$.

2.8. Determination of SPAD 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.

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 $155 \text{ mM}^{-1} \text{ cm}^{-1}$.

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 30min) 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 sequestering 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, [8] suggested that proline supplies plants with energy for growth as a result helping the plant to survive under stressful conditions. In addition, [21]

observed that exogenous application of proline significantly increases in growth 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 ($p \leq 0.05$) affected by salt stress as it dropped by 26.37%, compared to control (Fig. 1E). However, exogenous proline treatment significantly ($p \leq 0.05$) 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] 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).

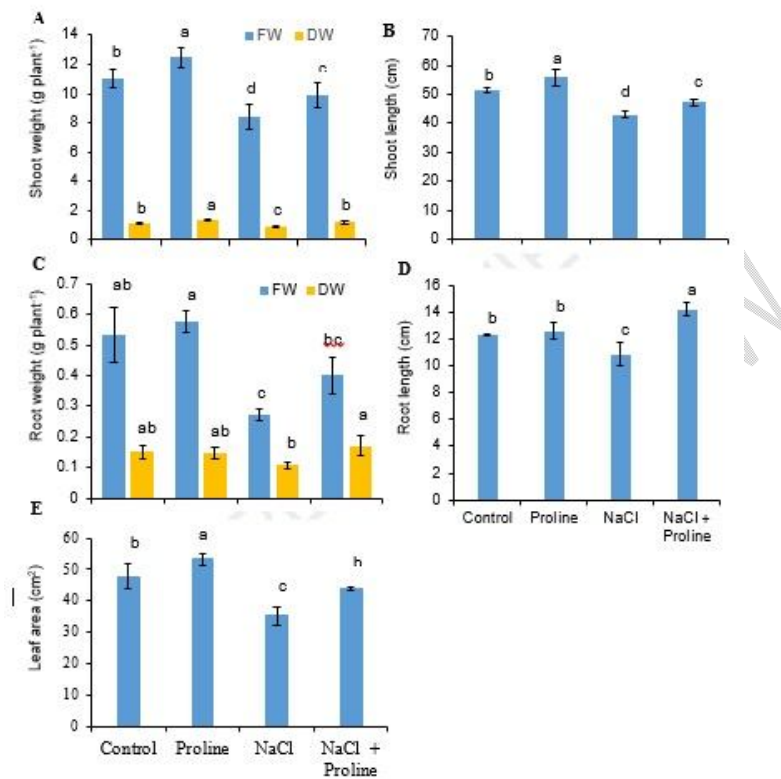


Fig. 1. Effect of foliar application of proline on the fresh weight (FW) and dry weight (DW) of shoot (A), shoot length (B), fresh weight (FW) and dry weight (DW) of root (C) and root length (D) of jute under salt stress conditions. Data are mean \pm standard deviation. Values with different letters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

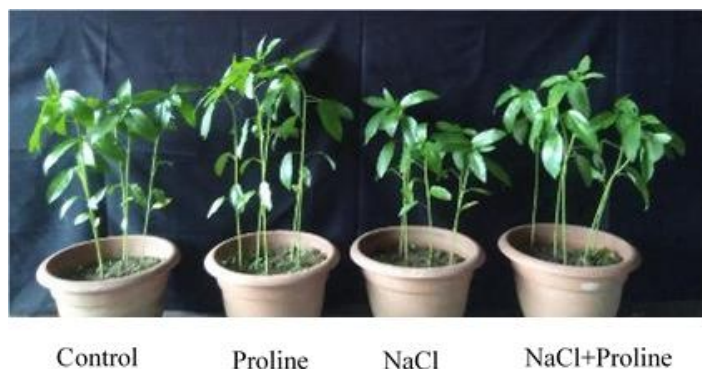


Fig. 2. Phenotypic appearance of jute plants as boosted by proline supplementation under salt stress condition

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 plant alone. These results are similar to [23] who indicated that exogenous proline 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 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, [24] observed that supplementation of proline significantly increased STI under salt stress conditions.

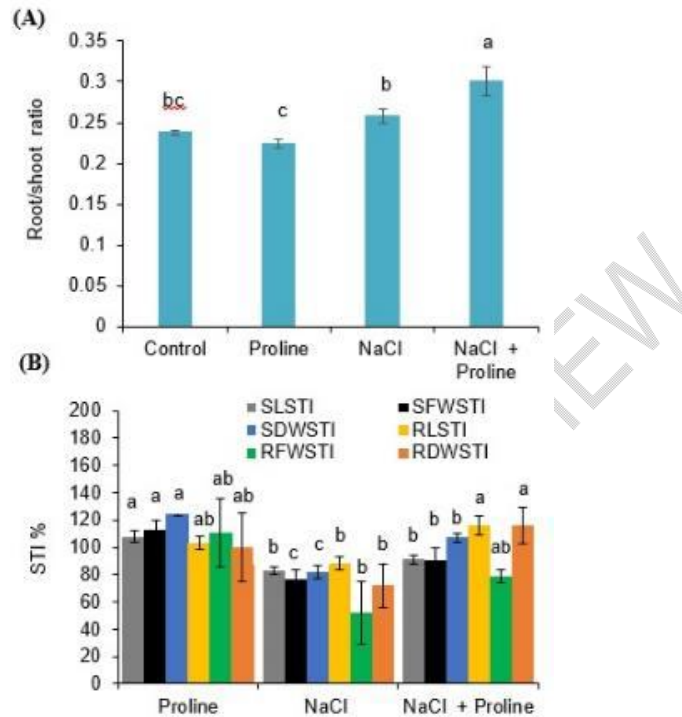


Fig. 3. Root/shoot length ratio (A) and salt tolerance index (STI) % (B) of jute as improved by proline under salt stress. SL, shoot length; SFW, shoot fresh weight; SDW, shoot dry weight; RL, root length; RFW, root fresh weight; RDW, root dry weight. Vertical bars represent LSD value at a 5% level of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

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 plant stress [25]. In this study, salt stress caused a significant ($p \leq 0.05$)

decrease in the chl a, chl b, chla+b and carotenoid content as compared with their 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 significantly ($p \leq 0.05$) decreased the SPAD value in jute by 6.38%, compared to that of control plants. Application of proline significantly ($p \leq 0.05$) increased the SPAD value by 6.65% in salt-stressed jute plants, compared to that of salt alone (Fig. 4E). Similar results were observed by [26] on rice, [27] on celery's and [28] on quinoa plants. Moreover, [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, [30] indicated that chlorophyll degradation under salt stress can be attributed to the chlorophyllase enzymes.

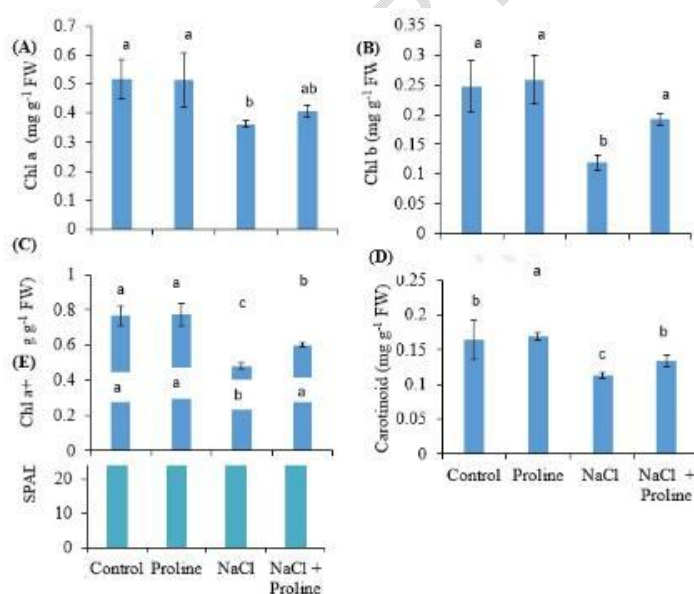


Fig. 4. Contents of photosynthetic pigments, Chl a (A), Chl b (B), Chl a+b (C), carotenoid (D), and SPAD value (E) in leaves of jute plants treated with proline under salt stress conditions. Data are

mean \pm 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 salt stress 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 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.

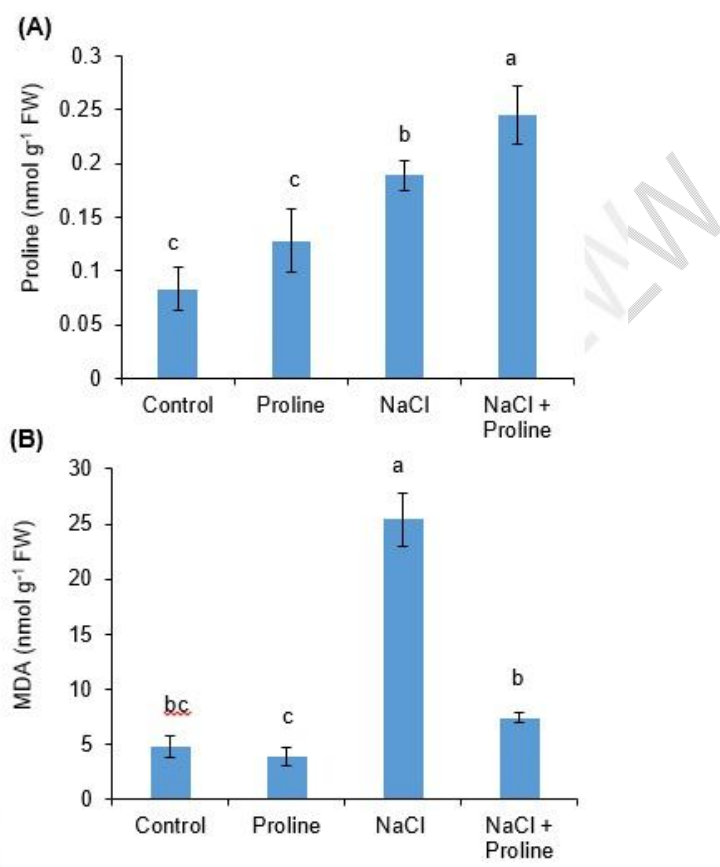


Fig. 5. Proline content (A), and MDA content (B) of jute as affected by proline under salt stress. Vertical bars represent LSD value at a 5% level of significance. Different letters indicate statistically significant differences between treatments means at $p \leq 0.05$

3.5. Effect of foliar application of proline 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, [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, [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 improved salt tolerance by maintaining a low Na^+ content, a high K^+ content and higher K^+/Na^+ ratio in jute plants.

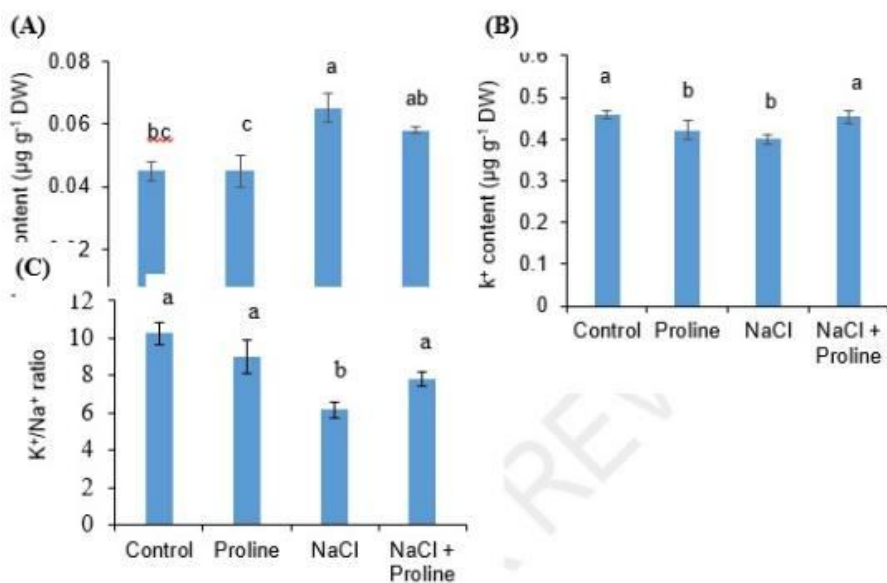


Fig. 6. Effect of foliar application of proline on Na⁺ content (A), K⁺ content (B) and K⁺/Na⁺ ratio (C) of jute under salt stress condition. Vertical bars represent LSD value at a 5% level of significance. Different letters indicate statistically significant differences between treatments means at p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

4. CONCLUSION

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

REFERENCES

1. Canter LW. Environmental Impact of Agricultural Production Activities. Broken Sound Parkway NW: CRC Press, Boca Raton.2018.
2. Singh. Biochemical, physiological, and molecular approaches for improving salt tolerance in crop plants-a review. In: Engineering practices for management of soil salinity. CRC Press, USA. 2018; 159-208.
3. Hasanuzzaman M, Raihan MRH, Khojah E, Samra BN, Fujita M, Nahar K. 2021. Biochar and chitosan regulate antioxidant defense and methylglyoxal detoxification systems and enhance salt tolerance in jute (*Corchorus olitorius* L.). *Antioxidants*. 2021; 10: 2017.
4. Garg N, Bhandari P. Interactive effects of silicon and arbuscular mycorrhiza in modulating ascorbate-glutathione cycle and antioxidant scavenging capacity in differentially salt-tolerant *Cicer Arietinum* L. Genotypes subjected to long-term salinity. *Protoplasma*. 2016; 253: 1325-1345.
5. Shahid SA, Zaman M, Heng L. Soil salinity: Historical perspectives and a world overview of the problem. In guideline for salinity assessment, mitigation and adaptation using nuclear and related techniques. Springer: Cham, Switzerland. 2018; 43-53.

6. Kumar S, Singh AK, Mohapatra T. Epigenetics: history, present status and future perspective. *Indian Journal of Genetics and Plant Breeding*. 2017; 77: 445-463.
7. Slama S, Bouchereau A, Flowers T, Abdelly C, Savoure A. Diversity, distribution and roles of osmoprotective compounds accumulated in halophytes under abiotic stress. *Annals of Botany*. 2015;115: 433-447.
8. Alam R, Das DK, Islam MR, Murata Y, Hoque MA. Exogenous proline enhances nutrient uptake and confers tolerance to salt stress in maize (*Zea mays* L.). *Progressive Agriculture*. 2016; 27(4): 409-417.
9. Hossain MA, Kumar V, Burritt D, Fujita M, Makelaa P. 2019. "Proline metabolism and its functions in development and stress tolerance", Springer Nature; Cham, Switzerland: Osmoprotectant-mediated abiotic stress tolerance in plants. 2019; 41-72.
10. NakhaieA., Habibi G. &Vaziri A. Exogenous proline enhances salt tolerance in acclimated Aloe vera by modulating photosystem II efficiency and antioxidant defense. *South African Journal of Botany*.2022;147: 1171-1180.
11. Akram NA, Shafiq MA, Iqbal M, Ahmad P. Advances in salt tolerance of some major fiber crops through classical and advanced biotechnological tools: a review. *Journal of Plant Growth Regulation*.2020; 40: 891-905.
12. Mukul MM. Perspective chapter: Nutraceutical diversity of eco-friendly jute and allied Fiber (JAF) Crops in Bangladesh. *Population Genetics*. 2022; 33.
13. Wilkins D. A technique for the measurement of lead tolerance in plants. *Nature*, 1957; 180: 37-38.
14. Lichtenthaler HK. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods of Enzymology*.1987; 48:350-382.
15. Bates LS, Waldren RP, Teari D. Rapid determination of free proline for water stress studies. *Plant Soil*.1973; 39: 205-207.
16. Madhava Rao KV, Sresty TVS. Antioxidative parameters in the seedlings of pigeon pea (*Cajanuscajan* L. Millapaugh) to Zn and Ni stresses. *Plant Science*.2000;157:113-128.

17. Guo S, Ma X, Cai W, Wang Y, Gao X, Fu B, Li S. Exogenous proline improves salt tolerance of alfalfa through modulation of antioxidant capacity, ion homeostasis, and proline metabolism. *Plants*. 2022; 11: 2994.
18. Balasubramaniam T, Shen G, Esmaeili N, Zhang H. Plants' response mechanisms to salinity stress. *Plants (Basel)*.2023; 2(12): 2253.
19. Sobahan MA, Akter N, Ohno M, Okuma E, Hirai Y, Mori IC, Nakamura Y, Murata Y. Effects of exogenous proline and glycinebetaine on the salt tolerance of rice cultivars. *Bioscience Biotechnology and Biochemistry*. 2012; 76: 1568-1570.
20. Wani AS, Ahmad A, Hayat S, Tahir I. Is foliar spray of proline sufficient for mitigation of salt stress in *Brassica juncea* cultivars? *Environmental Science and Pollution Research*. 2016; 23: 13413-13423.
21. Abdallah M, El-Bassiouny H. Impact of exogenous proline or tyrosine on growth, some biochemical aspects and yield components of quinoa plant grown in sandy soil. *International Journal of Pharm Tech Research*.2016; 9: 12-23.
22. Sakr M, Naser El-Sarkassy N, Fuller M. Osmoregulators proline and glycine betaine counteract salinity stress in canola. *Agronomy for Sustainable Development*. 2012; 32 (3): 747-754.
23. Genc OT. Effect of different concentration of exogenous proline applications on cadmium accumulation and mineral nutrition (K, Mg, Na, and Ca) of common wheat (*Triticum aestivum*). *Biotech Studies*.2021; 30(2): 86-91.
24. El Moukhtari A, Cabassa-Hourton C, Crilat E, Carol P, Lamsaadi N, Hidri R, FarissiM, Savoure A. Salt stress is alleviated by either proline or silicon but not by their combination in alfalfa (*Medicago sativa* L.) inoculated with a salt-tolerant *Ensifer meliloti* Strain. *Journal Plant Growth Regulation*. 2023; 42: 4048-4062.
25. Kalaji HM, Jajoo A, Oukarroum A, Brestic M, Zivcak M, Samborska IA, Cetner MD, Lukasik I., GoltsevV, Ladle RJ. Chlorophyll a fluorescence as a tool to monitor

- physiological status of plants under abiotic stress conditions. *Acta Physiologiae Plantarum*.2016; 38: 1-11.
26. Koc YE, Aycan MM, Mitsui TT. Exogenous proline suppresses endogenous proline and proline-production genes but improves the salinity tolerance capacity of salt-sensitive rice by stimulating antioxidant mechanisms and photosynthesis. *Plant Physiology Biochemistry*.2024; 214: 108914.
 27. Gao Y, Zhang J, Wang C, Han K, Hu L, Niu T, Yang Y, Chang Y, Xie J. Exogenous Proline Enhances Systemic Defense against Salt Stress in Celery by Regulating Photosystem, Phenolic Compounds, and Antioxidant System. *Plants (Basel)*. 2023; 12(4): 928.
 28. Abdallah MM-S, El Sebai, TN, Ramadan AAEl-M, El-Bassiouny HMS. Physiological and biochemical role of proline, trehalose, and compost on enhancing salinity tolerance of quinoa plant. *Bulletin of the National Research Centre*. 2020; 44: 96.
 29. Khan MA, Abdullah Z. Reproductive physiology of two wheat cultivars differing in salinity tolerance under dense saline-sodic soil. *Journal Food Agriculture Environment*. 2003; 1: 185-189.
 30. Rady MM, Varma CB, Howladar SM. Common bean (*Phaseolus vulgaris*,L.) seedlings overcome NaCl stress as a result of presoaking in *Moringa oleifera* leaf extract. *Scientia Horticulturae*. 2013; 162: 63-70.
 31. Wu GQ, Liang N, Feng RJ, Zhang JJ. Evaluation of salinity tolerance in seedlings of sugar beet (*Beta vulgaris* L.) cultivars using proline, soluble sugars and cation accumulation criteria. *Acta Physiologia Plantarum*. 2013; 35: 2665-2674.
 32. Wu GQ, Jiao Q, Shui QZ. Effect of salinity on seed germination, seedling growth, and inorganic and organic solutes accumulation in sunflower (*Helianthus annuus*L.). *Plant Soil Environment*. 2005; 61: 220-226.
 33. de Freitas PAF, de Carvalho HH, Costa JH, Miranda RdeS, da Cruz Saraiva KD, de Oliveira F D B, Coelho DG, Prisco JT, Gomes-Filho E. Salt acclimation in sorghum

- plants by exogenous proline: physiological and biochemical changes and regulation of proline metabolism. *Plant Cell Reports*. 2019;38: 403-416.
34. de Freitas PAF, DE Souza MR, Marques EC, Prisco JT, Omes-Filho E. Salt tolerance induced by exogenous proline in maize is related to low oxidative damage and favorable. *Journal Plant Growth Regulation*.2018; 37: 911-924.
 35. Wu G-Q, Feng R-J, Li S-J, Du Y-Y. Exogenous application of proline allivates salt-induced toxicity in sainfoin seedlings. *The Journal of Animal & Plant Sciences*. 2017; 27(1): 246-251.
 36. Farkhondeh R, Nabizadeh E, Jalilnezhad N. Effect of salinity stress on proline content, membrane stability and water relations in two sugar beet cultivars. *International Journal Agricultural. Science*.2012; 2(5): 385-392.
 37. WutipraditkulN, Wongwean P, Buaboocha T. Alleviation of salt-induced oxidative stress in rice seedlings by proline and /or glycine betaine. *Biologia Plantarum*. 2015; 59: 1-7.
 38. Eraslan F, Inal A, Pilbeam DJ, Gunes A. Interactive effects of salicylic acid and silicon on oxidative damage and antioxidant activity in spinach (*Spinacia oleracea* L. CV. Matador) grown under boron toxicity and salinity. *Plant Growth Regulation*.2008;55: 207-219.
 39. Hasanuzzaman M, Alam MM, Rahman A, Hasanuzzaman M, Nahar K, Fujita M. Exogenous proline and glycinebetaine mediated upregulation of antioxidant defense and glyoxalase systems provides better protection against salt-induced oxidative stress in two rice (*Oryza sativa* L.) varieties. *BioMed Research International* 2014; 2014: 17.
 40. Sobahan MA, Akter N, Murata Y, Munemasa S. Exogenous proline and glycinebetaine mitigate the detrimental effect of salt stress on rice plants. *Silpakorn University Science and Technology Journal*. 2016; 10: 38-43.
 41. Zhu Y, Gong H. Beneficial effects of silicon on salt and drought tolerance in plants. *Agronomy for Sustainable Developop*. 2014;34:455–472.

42. Bargaz A, Zaman-Allah M, Farissi M, Lazali M, Drevon J-J, Maougal RT, Carlsson G. Physiological and molecular aspects of tolerance to environmental constraints in grain and forage legumes. *International Journal of Molecular Sciences*. 2015;16:18976-19008.
43. Rizwan M, Ali S, Ibrahim M, Farid M, Adrees M, Bharwana SA. Mechanisms of silicon-mediated alleviation of drought and salt stress in plants: a review. *Environmental Science and Pollution Research*. 2015; 22: 15416-15431
44. Abdelhamid MT, Rady M, Osman AS, Abdalla MA. Exogenous application of proline alleviates salt-induced oxidative stress in *Phaseolus vulgaris* L. plants. *Journal of Horticultural Science and Biotechnology*. 2013; 88: 439-446..
45. Khan, A., Iqbal, I., Ahmed, I., Nawaz, H., & Nawaz, M. (2014). Role of proline to induce salinity tolerance in sunflower (*Helianthus annuus* L.). *Science Technology and Development Journal*, 33, 88-93.