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Effect of foliar application of proline on morpho-biochemical and yield attributes of mustard (*Brassica juncea* L.) under salt stress

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Abstract

Salinity is one of the most serious issue that hampered agricultural production in the arid and semi-arid climates in the world. In this study, the effect of the foliar application of proline (20 mM) on morpho-biochemical and yield attributes were investigated in mustard (*Brassica juncea* L.) cv. “BARI Sarisha-14” at 150 mM NaCl stress. At 30 days after sowing (DAS), plants were sprayed with 20 mM proline in the presence or absence of NaCl stress. The results showed that NaCl stress decreased the growth, biomass accumulation, SPAD value and yield of the mustard plants. However, Na^+/K^+ ratio significantly increased under NaCl stress. Exogenous application of proline reversed the negative effect of NaCl stress in mustard by increasing the growth, SPAD value and yield parameters of the plants whereas addition of proline decreased the Na^+/K^+ ratio in NaCl-stressed mustard plant. These results suggest that proline alleviate growth and yield inhibition via regulation of photosynthesis efficiency and ion homeostasis in mustard under NaCl stress.

Keywords: Salinity, proline, growth, yield, Na^+/K^+ ratio

Introduction

Salinity is one of the abiotic stresses that negatively effects on growth, development, and yield of plants (Zorb *et al.* 2019; Van *et al.* 2020) ^[31, 25]. Approximately 830 million hectares area in the globe is affected with salinity and sodicity stresses (Petal *et al.* 2018) ^[19]. Thus, it is crucial to find out studies on related alleviation strategies. Salinity stress triggers osmotic and ion toxicity as increases the uptake of Na^+ ions, and this inhibits K^+ uptake, and thereby disruption of ion homeostasis in plant, leading to decrease plant growth (Huang *et al.* 2014; Junedi *et al.* 2023) ^[11, 13]. Additionally, Salinity stress also changes the ultrastructural cell components, damage the photosynthesis machinery, elevated the reactive oxygen species (ROS), and regulation of the enzymatic activities, which decline the growth and yield attributes of crops (Hasanuzzaman *et al.* 2014) ^[9]. Proline is an essential amino acid that accumulates in plant tissues to increase salinity tolerance (Hayat *et al.* 2012) ^[10]. It has been reported that application of proline effectively lessens the salt-induced impairments in plants (Huang *et al.* 2009; Sobahan 2024) ^[12, 24]. Additionally, this solutes contribute to osmotic adjustment, improved leaf relative water content, protects photosystem II (PSII) from photo-damage, improved various photosynthetic attributes, increased the level of stress protective proteins and scavenging ROS (Ashraf & Foolad 2007; Khedr *et al.* 2003) ^[4, 15]. Mustard [*Brassica juncea* (L.), family Brassicaceae] is a fast-growing oil seed crop, which accounts for 12% of the total annual global production of oil. (FAOSTAT, 2014) ^[6]. However, its growth and yield are drastically affected by salinity (Priya *et al.* 2021) ^[20]. Thus, this study was carried out to know the effect of proline on growth, biomass, SPAD value, Na^+/K^+ ratio and yield of mustard under NaCl stress.

Materials and Methods

Plant Material and Treatment

A pot trial was performed at Bangladesh Open University, Gazipur, Bangladesh. The healthy seeds of mustard [*Brassica juncea* (L.)] cv. BARI Sarisha-14 were obtained from Bangladesh

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Agriculture Research Institute (BARI), Gazipur. Each pot contained 12 kg of air-dried soil and fertilized with the BARI suggested dose of fertilizers (Urea, TSP, MoP, Gypsum and ZnSO_4 @138, 51, 63, 60 and 4 kg ha⁻¹, respectively). The study was conducted in a Completely Randomized Design (CRD) arrangement having three replications. The following treatments were applied: i) Control (-NaCl), ii) 20 mM proline, iii) 150 mM NaCl and iv) 150 mM NaCl treatments with proline (150 mM NaCl + 20 mM proline). Intercultural operation was done at 7 DAS, kept five plants pot⁻¹. Plants were irrigated with salt solution (150 mM NaCl, pure salt) at 20 DAS, and maintained field capacity by irrigating with either water (control) or salt solution. For proline application, a solution of 20 mM proline (Wako, Japan) was prepared by dissolving in water and sprayed at 9 am each 2 days interval.

Measurement of Growth Parameters

The plants were removed from pot soil at 40 DAS for measuring the growth attributes. The height of the plant was measured on a centimeter scale. The plants were separated from root and weighted to record plant and root fresh weight. Then, plant and root were placed in an oven (70 °C for 72 h) to record dry weight.

Measurement of Leaf Area

The leaf area was recorded by a digital leaf area meter (LICOR 3100) in leaves at 40 DAS.

Determination of Soil Plant Analysis Development (SPAD) Value

The concentration of leaf chlorophyll value (SPAD value) was measured by a SPAD meter (Konica, Minolta SPAD-502 Plus, Inc., Japan). The mean SPAD value was measured from three readings in same leaf.

Determination of Total Na⁺ and K⁺ Contents

The amounts of Na⁺ and K⁺ were measured according to Yoshida *et al.* (1976) [29]. The shoots of the treated mustard plants were oven dried, grind and then sieve with mesh. One hundred milligram of the sample was kept into conical flasks, and 10 ml of 1N HCl was added, while shaking was avoided for keeping the sample submerged. The samples were kept at room temperature. Then, samples were filtrated and diluted to 100 ml. The Na⁺ and K⁺ content were estimated by a flame photometry (Flame Photometer 410, UK).

Determination of Seed Weight and Yield Contributing Attributes

At maturity stage, siliqua were collected and counted the number of siliqua per plant. After that siliqua length was measured on a meter scale. Seeds were removed from siliqua and counted the number of seeds by seed counter machine. The weight of seeds per plant was measured by weight scale.

Statistical Analysis

One-way ANOVA (analysis of variance) with the statistix10 software was used to analyze the data. Means were compared using the least significant difference (LSD) test at $P < 0.05$ level of significance.

Results and Discussion

Growth and Biomass Accumulation

Figure 1 shows that NaCl stress hampered the growth parameters of mustard plants. However, the detrimental effects of NaCl stress was attenuated in mustard plants with the application of proline. Comparism with the control plants, salt stress significantly decreased plant height by 16.81%. However, the application of proline showed significant increased plant height by 12.55% under salt stress (Figure 1A). In the presence of salt stress, plant fresh weight (FW) and dry weight (DW) were significantly declined by 44.57% and 46.56%, respectively, compared to the controls. However, application of proline attenuated the reduction of plant FW and DW by 54.98% and 62.85%, respectively, compared to the stressed plants (Fig.1B, C). On the other hand, salt stress significantly reduced root length by 27.53%. Conversely, plant treated with proline did not alter root length of mustard plants (Fig. 1D). Root FW and DW were significantly declined by 39.42% and 44.34%, respectively in salt-stressed mustard, compared to the controls (Fig.1 E, F). However, application of proline attenuated the reduction of root FW and DW by 62.72% and 31.92%, respectively, while root FW did not show significant effect, compared to the stressed plants alone (Fig.1 E, F). In the previous studies observed that salt stress had a detrimental impact on growth and biomass accumulation of mustard plants (Verma *et al.* 2023; Zaman *et al.* 2015) [26, 30]. However, the use of proline increased the growth of mustard plants under salt stress, suggesting that it may inhibit the salt-induced osmotic stress (Sobahan 2024) [24] and ionic stress (Fig. 3). Foliar application of proline improved plant growth under salt stress has been observed in several crops, such as mustard (Wani *et al.* 2016) [28], maize (de Freitas *et al.* 2018) [5], carrot (Qirat *et al.* 2018) [21], alfalfa (Guo *et al.* 2022) [7], tomato (Reddy and Basha 2023) [22] and jute (Sobahan 2024) [24].

Leaf Area and SPAD Value

As illustrated in fig. 2A, NaCl stress has noticeably reduced leaf area. There was significantly decrease in leaf area by 55.65% under salt stress compared to the control. However, application of proline increased leaf area in salt-stressed mustard plants by 69.51% compared to corresponding stressed plants (Fig. 2A). Salt stress significantly dropped SPAD value by 23.49% in salt-stressed seedlings of mustard compared to the control plants. However, the application of proline to salt-stressed plants demonstrated enhance SPAD value by 18.31% under salt stress condition (Fig. 2B). Our findings illustrated that proline effectively counteracts the salt-induced decrease in leaf area and SPAD value, in line with previous studies involving different plant species, such as mustard (Wani *et al.* 2016) [28], jute (Sobahan 2024) [24] and proso millet (Alam and Hasanuzzaman 2023) [3].

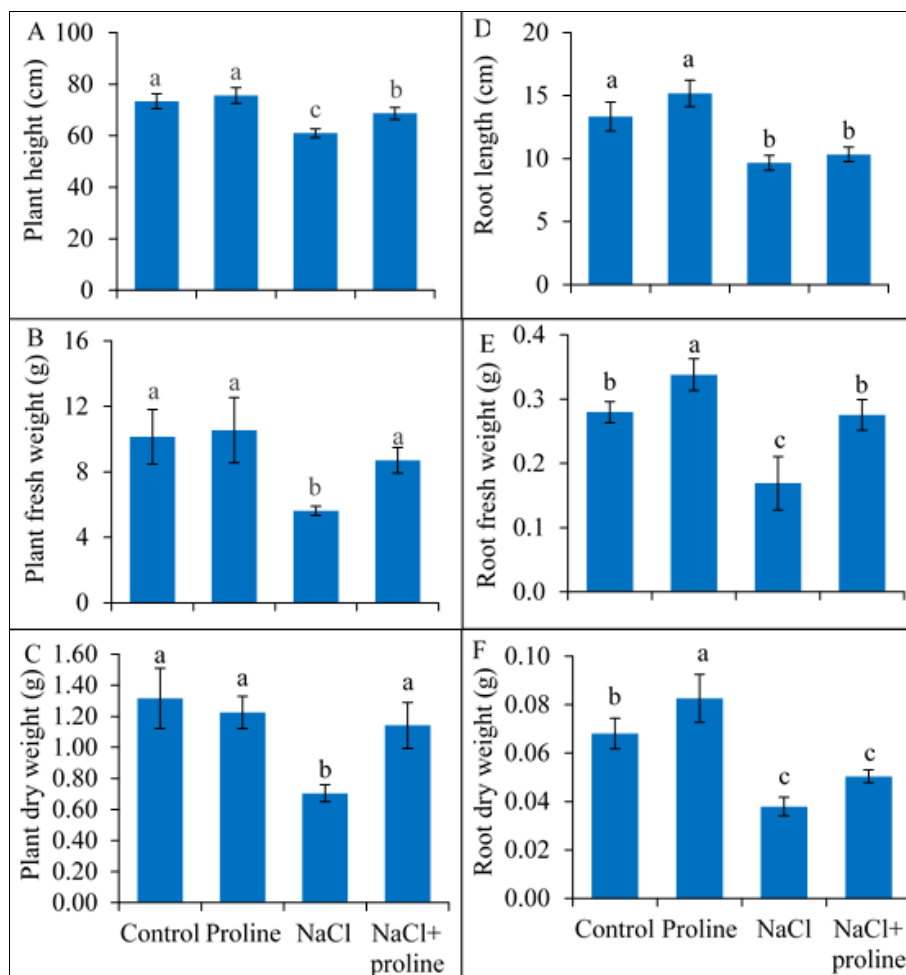


Fig 1: Effect of proline (20 mM) on plant height (A), plant fresh weight (B), plant dry weight (C), root length (D), root fresh weight (E) and root dry weight (F) of mustard under NaCl stress (150 mM). Data are mean $\pm$ SD (standard deviation). Error bars represent LSD value at a 5% level of significance. Dissimilar letters denote significant differences between treatments at $P < 0.05$.

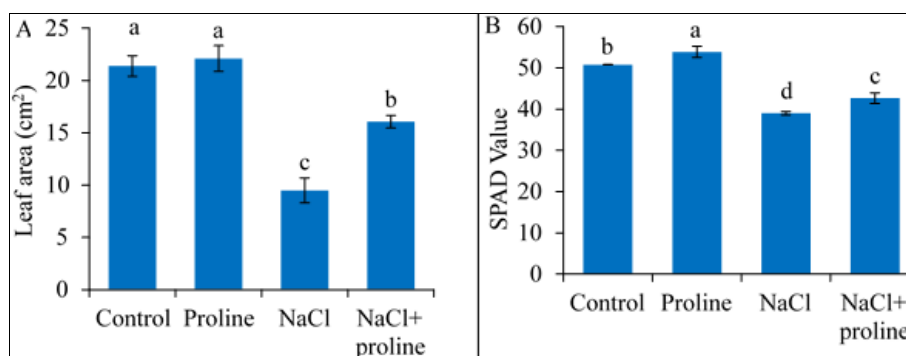


Fig 2: Effect of proline (20 mM) on leaf area (A) and SPAD value (B) of mustard under NaCl stress (150 mM). Data are mean $\pm$ SD (standard deviation). Error bars represent LSD value at a 5% level of significance. Dissimilar letters denote significant differences between treatments at $P < 0.05$.

Na⁺, K⁺ and Na⁺/K⁺ Ratio

Salt stress induces an ionic imbalance by elevating Na⁺ ion and depleting K⁺ ion (Luo *et al.* 2023) ^[17]. In this study, shoot Na⁺ content of mustard increased significantly by 96.00% under salt stress as compared to control. However, application of proline decline Na⁺ content by 11.29% in salt-stressed mustard plants compared to NaCl stress plant alone (Fig. 3A). To compare with control, K⁺ content decreased by 23.76% in mustard plant after NaCl treatment. However, foliar application of proline lessened the reduction of K⁺ content by 23.54% in salt-stressed mustard plant compared to salt stress alone (Fig. 3B). The Na⁺/K⁺ ratio showed a significant increase by 161.70% in shoot of mustard during salt stress while the application of proline significantly

decreased Na⁺/K⁺ ratio by 26.31% in salt stressed mustard compared to salt stress alone (Fig. 3C). According to this research, NaCl stress caused high Na⁺ ion uptake and increased Na⁺/K⁺ ratio value, which reduces plant growth (Fig. 1) via lower water movement in plant. Huang *et al.* (2014) ^[11] reported that K⁺ acts a crucial function in keeping ion homeostasis and regulating plant growth under salt stress condition. In the present study, proline functions ion homeostasis through maintaining the Na⁺/K⁺ ratio by the increased K⁺ content, and reduced Na⁺ concentration in salt-stressed mustard plant. These findings align with studies in jute (Sobahan 2024) ^[24], rice (Koc *et al.* 2024) ^[16], *Phaseolus vulgaris* (Abdelhamid *et al.* 2013) ^[2] and sunflower (Khan *et al.* 2014) ^[14] treated with Proline.

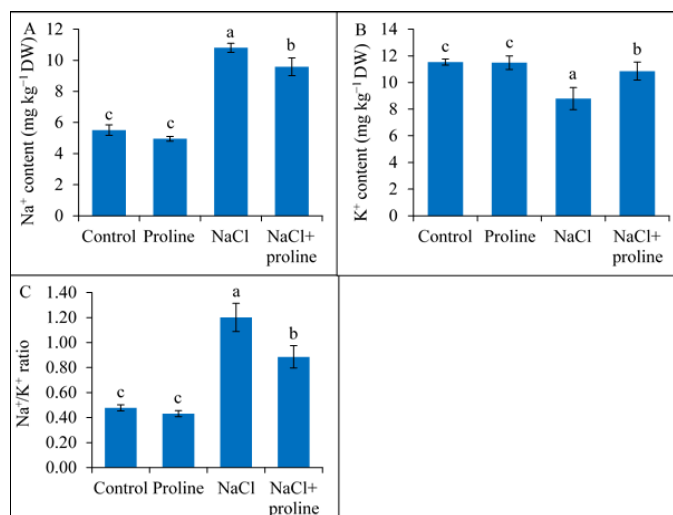


Fig 3: Effect of proline (20 mM) on Na⁺ (A), K⁺ (B) and Na⁺/K⁺ ratio (C) in shoot of mustard under NaCl stress (150 mM). Data are mean $\pm$ SD (standard deviation). Error bars represent LSD value at a 5% level of significance. Dissimilar letters denote significant differences between treatments at $P < 0.05$.

Yield attributes and Seed Weight

The data revealed that NaCl stress decreased silique length,

Table 1: Effect of proline (20 mM) on silique length, silique number plant⁻¹, seed plant⁻¹ and seed weight plant⁻¹ of mustard under NaCl stress (150 mM)

Treatments	Silique length (cm)	Silique number plant ⁻¹	Seeds plant ⁻¹	Seed weight (g plant ⁻¹)
Control	3.8 $\pm$ 0.173a	13.55 $\pm$ 1.57a	310.88 $\pm$ 29.56b	0.634 $\pm$ 0.031a
Proline	3.9 $\pm$ 0.10a	14.77 $\pm$ 2.52a	372.77 $\pm$ 45.42a	0.639 $\pm$ 0.069a
NaCl	2.56 $\pm$ 0.05c	8.32 $\pm$ 0.57b	235.77 $\pm$ 16.17c	0.401 $\pm$ 0.028c
NaCl+proline	3.03 $\pm$ 0.11b	12.66 $\pm$ 1.20a	320.77 $\pm$ 13.03ab	0.539 $\pm$ 0.017b
CV (%)	2.70	13.68	9.17	7.66

Note- Dissimilar letters denote significant differences between treatments at $P < 0.05$.

Conclusion

In conclusion, salt stress decreased the growth, biomass accumulation, SPAD value and seed weight of mustard, whereas increased Na⁺/K⁺ ratio. Addition of proline to salt-stressed plants improve growth and seed weight of mustard by keeping ionic balance and the increment of SPAD value.

References

- Abdallah MMS, El-Bassiouny HMS. Impact of exogenous proline or tyrosine on growth, some biochemical aspects and yield components of quinoa plant grown in sandy soil. *Int J Phar Tec*. 2016;9(7):12-23.
- Abdelhamid MT, Rady M, Osman AS, Abdalla MA. Exogenous application of proline alleviates salt-induced oxidative stress in *Phaseolus vulgaris* L. plants. *J Hort Sci Biotech*. 2013;88:439-446.
- Alam MM, Hasanuzzaman M. Enhancing salt stress tolerance and yield parameters of proso millet through exogenous proline and glycine betaine supplementation. *Bangladesh Agron J*. 2023;26:75-83.
- Ashraf M, Foolad MR. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ Exp Bot*. 2007;59:206-216.
- de Freitas PAF, de Souza Miranda R, Marques EC, Prisco JT, Gomes-Filho E. Salt tolerance induced by exogenous proline in maize is related to low oxidative damage and favorable ionic homeostasis. *J Plant Growth Regul*. 2018;37:911-924.
- FAOSTAT. Oil crop primary data. FAO Statistics Division, Rome, Italy. 2014. <http://www.fao.org/faostat/en/#data/QC> [verified 8 June 2017].
- Guo S, Ma X, Cai W, Wang Y, Gao X, Fu B, *et al*. Exogenous Proline Improves Salt Tolerance of Alfalfa through Modulation of Antioxidant Capacity, Ion Homeostasis, and Proline Metabolism. *Plants*. 2022;11(21):2994.
- Hasan A, Hafiz HR, Siddiqui N, Khatun M, Islam R, Mamun AA. Evaluation of wheat genotypes for salt tolerance based on some physiological traits. *J Crop Sci Biotechnol*. 2015;18:333-340.
- Hasanuzzaman M, Alam M, Rahman A, Hasanuzzaman M, Nahar K, Fujita M. Exogenous proline and glycine betaine mediated upregulation of antioxidant defense and glyoxalase systems provides better protection against salt-induced oxidative stress in two rice (*Oryza sativa* L.) varieties. *BioMed Res Int*. 2014;2014:757219.
- Hayat S, Hayat Q, Alyemeni MN, Wani AS, Pichtel J, Ahmad A. Role of proline under changing environments: a review. *Plant Signal Behav*. 2012;7(11):1456-66.
- Huang C, Wei G, Jie Y, Wang L, Zhou H, Ran C, *et al*. Effects of concentrations of sodium chloride on photosynthesis, antioxidative enzymes, growth and fiber yield of hybrid ramie. *Plant Physiol Biochem*. 2014;76:86-93.
- Huang Y, Bie Z, Liu Z, Zhen A, Wang W. Protective role of proline against salt stress is partially related to the

- improvement of water status and peroxidase enzyme activity in cucumber. *Soil Sci Plant Nutri.* 2009;55(5):698-704.
13. Junedi MA, Mukhopadhyay R, Manjari KS. Alleviating salinity stress in crop plants using new engineered nanoparticles (ENPs). *Plant Stress.* 2023;9:100184.
 14. Khan A, Iqbal I, Ahmed I, Nawaz H, Nawaz M. Role of proline to induce salinity tolerance in sunflower (*Helianthus annuus* L.). *Sci Technol Develop J.* 2014;33:88-93.
 15. Khedr AHA, Abbas MA, Wahid AAA, Quick WP, Abogadallah GM. Proline induces the expression of salt-stress responsive proteins and may improve the adaptation of *Pancreaticum maritimum* L. to salt-stress. *J Exp Bot.* 2003;54:2553–2562.
 16. 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 Physiol Biochem.* 2024;214:108914.
 17. Luo D, Song F, Lu M, Shi Y, Ma Q. Salt-stress-induced ion transport contributes to K⁺/Na⁺ homeostasis in roots of ping'ou hybrid hazelnut. *Forests.* 2023;14(8):1651.
 18. Orsini F, Pennisi G, Mancarella S, Al Nayef M, Sanoubar R, Nicola S, *et al.* Hydroponic lettuce yields are improved under salt stress by utilizing white plastic film and exogenous applications of proline. *Sci Hort.* 2018;233:283-293.
 19. Patel R, Yadav RC, Avtar R, Jangra S, Boken GD, Singh B, Yadav NR. Genetic diversity analysis for salinity tolerance in Indian mustard [*Brassica juncea* (L.)] using SSR markers. *Int J Curr Microbial App Sci.* 2018;7:1776-1785.
 20. Priya K, Sharma P, Choudhary OP, Sardana V. Regulation of salinity tolerance in *Brassica juncea* (L.) introgression lines: osmoprotectants, antioxidative molecules and ionic content. *GSC Adv Res Rev.* 2021;6(3):116-131.
 21. Qirat M, Shahbaz M, Perveen S. Beneficial role of foliar-applied proline on carrot (*Daucus carota* L.) under saline conditions. *Pak J Bot.* 2018;50:1735-1744.
 22. Reddy MS, Osman Basha P. Ameliorating effects of proline application in *Solanum lycopersicum* L. under salt stress. *Asian J Res Biol.* 2023;6:56-64.
 23. Sakr M, El-Sarkassy N, Fuller M. Osmoregulators proline and glycine betaine counteract salinity stress in canola. *Agronomy for Sustainable Development.* 2012;32(3):747-754.
 24. Sobahan MA. Effect of foliar application of proline on photosynthetic attributes, osmolyte and ion homeostasis in jute (*Corchorus olitorius* L.) under salt stress. *Asian J Res Crop Sci.* 2024;9(4):310-21.
 25. Van Zelm E, Zhang Y, Testerink C. Salt tolerance mechanisms of plants. *Annu Rev Plant Biol.* 2020;71:403-433.
 26. Verma T, Bhardwaj S, Raza A, Djalovic I, Prasad PV, Kapoor D. Mitigation of salt stress in Indian mustard (*Brassica juncea* L.) by the application of triacontanol and hydrogen sulfide. *Plant Signal Behav.* 2023;18(1):2189371.
 27. Wang L, Zuo Q, Zheng J, You J, Yang G, Leng S. Salt stress decreases seed yield and postpones growth process of canola (*Brassica napus* L.) by changing nitrogen and carbon characters. *Sci Rep.* 2022;12:17884.
 28. Wani AS, Ahmad A, Hayat S, Tahir I. Is foliar spray of proline sufficient for mitigation of salt stress in *Brassica juncea* cultivars? *Environ Sci Pollut Res.* 2016;23:13413-13423.
 29. Yoshida SDA, Forno JH, Cock KA, Gomez. Laboratory manual for physiological studies of rice. 3rd ed. Los Banos, Philippines: International Rice Research Institute; 1976:83.
 30. Zaman MM, Mannan M, Samanta SC. Salt induced changes in dry matter accumulation and yield of mustard (*Brassica juncea*). *Bangladesh Agron J.* 2015;18(1):65-70.
 31. Zorb C, Geilfus CM, Dietz KJ. Salinity and crop yield. *Plant Biol (Stuttg).* 2019;21 Suppl 1:31-38.