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Exogenous proline enhances nutrient uptake and confers tolerance to salt stress in maize (*Zea mays* L.)

R Alam[1], DK Das[1], MR Islam[1], Y Murata[2], MA Hoque[1]

[1]Department of Soil Science, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh
[2]Graduate School of Natural Science and Technology, Okayama University, Okayama 700-8530, Japan

Abstract

Proline accumulation is the main adaptive mechanism to salinity in plants. The pot experiments were carried out to mitigate the adverse effects of NaCl stress on BARI Hybrid Maize-5 and Hybrid Maize Pacific-987 by exogenous application of proline. Plants were exposed to different concentrations of NaCl at vegetative stage. Proline solutions were sprayed over maize leaves at both vegetative and tasseling stages. NaCl stress caused significant reductions in plant growth of maize. NaCl stress at 50 mM drastically reduced the growth of maize plant. Salt stress also reduced reproductive growth, grain yield, chlorophyll contents, K^+/Na^+ ratio and nutrient (NPS) uptake in both maize varieties. Exogenously applied proline improved growth and grain yield of BARI Hybrid Maize-5 at 25 mM NaCl stress condition. Additionally, BARI Hybrid Maize-5 conferred tolerance to 50 mM NaCl stress with 25 mM proline. Proline application significantly increased K^+/Na^+ ratio and nutrient uptake by maize under salt stress. The present study suggests that proline improves salt tolerance of maize by increasing the K^+/Na^+ ratio and nutrient uptake, particularly P uptake.

Key words: K^+/Na^+ ratio, maize, nutrient uptake, proline, salt stress

 ***Corresponding Author:** anamul71@yahoo.com

Introduction

Soil salinity is a major concern to agriculture all over the world because it affects almost all plant functions. More than 6% of the world's land and one third of the world's irrigated land are significantly affected by soil salinity (Flowers and Yeo, 1995; FAO, 2008). A study shows that soil salinity has affected more than 100 million hectares of agricultural land worldwide (Athar and Ashraf, 2009). Salinity effects are more prominent in arid and semiarid regions where limited rainfall, and high evapotranspiration and temperature associated with poor water and soil management (Azevedo Neto et al., 2006; Jaleel et al., 2008).

Agriculture is the most important sector of Bangladesh's economy. Physiological stress in plants due to salinity is the major factor reducing crop yields in coastal areas of Bangladesh. The coastal areas of Bangladesh cover about 30% of the cultivable lands of the country. About 1.06 million hectares of land in Bangladesh are affected by salinity (SRDI, 2010). Increased soil salinity due to climate change would significantly reduce food grain production. According to the IPCC (2007), crop production may fall by 10-30% by 2050 in Bangladesh due to climate change.

Salt accumulation in plants causes osmotic stress and ionic toxicity, and induces nutritional deficiencies

(Hasegawa et al., 2000; Munns, 2002; Zhu, 2003). Salt stress disturbs cytoplasmic K^+/Na^+ homeostasis, causing an increase in Na^+ to K^+ ratio in the cytosol (Zhu, 2003). Further, salt stress can trigger oxidative stress due to the accumulation of reactive oxygen species. Plants have evolved a variety of adaptive mechanisms to respond to salt stress. One of the main adaptive mechanisms to salt stress in plants is the accumulation of compatible solutes. Proline is the most common compatible solute that occurs in a wide variety of plants. Increased levels of proline accumulated in plants correlate with improved salt tolerance (Hasegawa et al., 2000; Okuma et al., 2004; Ashraf and Foolad, 2007). Inorganic nutrients such as N, P and K play essential roles in plant metabolism. In addition to its role as an osmoprotectant, proline counteracts the adverse effects of various stresses on plants by affecting uptake and accumulation of inorganic nutrients (Ali et al., 2008) and reducing cellular damages and increasing antioxidant defense systems (Hoque et al., 2008; Banu et al., 2009).

Soil salinization is a major process of land degradation that decreases soil fertility and crop productivity. Appropriate management strategies with suitable cultivars having higher yield potential could contribute to the improvement of crop production in saline areas. In addition to the management practices, exogenous application of proline is a novel approach to minimize the adverse effects of salt stress on crop plants. So far, no report is available in Bangladesh about the role of proline in the mitigation of salt stress in plants. Maize is the third important cereal crop in Bangladesh after rice and wheat. In Bangladesh, maize can be grown in different agro-climatic zones round the year. Maize can contribute significantly towards solving food problems and thereby play a gainful role in economy of the country. Therefore, the present study aimed to evaluate the role of exogenous proline in nutrient uptake and improving yield of maize under salt stress conditions.

Material and Methods

Soil Collection and Pot Preparation**:** Two pot experiments were carried out at Net-house of the Department of Soil Science, Bangladesh Agricultural University (BAU), Mymensingh. Soils were collected from the Soil Science Field Laboratory, BAU. Equal size plastic pots were prepared with 8 kg soils. The soil was silt loam having pH 6.15, electrical conductivity (EC) 0.17 dS/m, exchangeable Na 0.35 meq/100 g soil, total N 0.11% and organic matter 1.90%.

Plant materials and treatments**:** Two hybrid maize varieties viz. BARI Hybrid Maize-5 and Hybrid Maize Pacific-987 were used as plant materials. Nine treatment combinations *viz.* control (no NaCl or proline), 25 mM NaCl, 25 mM NaCl+25 mM proline, 25 mM NaCl+50 mM proline, 25 mM NaCl+100 mM proline, 50 mM NaCl, 50 mM NaCl+25 mM proline, 50 mM NaCl+50 mM proline, 50 mM NaCl+100 mM proline were used for the two maize varieties. Three maize seeds were sown into each pot. One plant was kept in each pot and others were uprooted after emergence. The pure salt (NaCl) was used for developing salinity. Maize plants were exposed to different concentrations of NaCl at vegetative stage (6 weeks after sowing). On the same day, different doses of proline containing 0.1% Tween-20 were sprayed on the leaves at a volume of 25 mL per plant as per treatment. Similarly, proline was applied at tasseling stage as per treatment. The experiment was laid out in a completely randomized design with four replications.

Management practices, crop harvesting and data recording**:** Normal water was used as irrigation. Fertilization and other management practices were performed as and when required. The crops were harvested at full maturity. Root growth, plant dry matter, yield attributes and grain yield were recorded. Chlorophyll contents were measured from green leaves. N, P, K, S and Na contents were measured from grain and straw samples.

Determination of chlorophyll content**:** Chlorophyll contents were measured according to Porra et al. (1989). Fifteen days after first proline application, healthy green leaf was detached from the plants. An aliquot amount of leaf was suspended in 10 mL of 80% acetone, mixed well and kept at room temperature in the dark for 7 days. The supernatant was collected after centrifugation at 5000 rpm and the absorbance was recorded at 645 and 663 nm in a spectrophotometer.

Chemical analysis of plant and soil samples**:** Soil pH, EC, exchangeable Na, total N and organic carbon content were measured using standard methods. Grain and straw samples were analyzed for N, P, K, S and Na contents using the methods described by Khanam et al. (2001).

Statistical analysis**:** Data were analyzed statistically using analysis of variance with the help of software package MSTAT-C. The significant differences between mean values were compared by Duncan's Multiple Range Test. Differences at P<0.05 were considered significant.

Results and discussion

Root and vegetative growth of maize**:** Salt stress caused a significant reduction in root growth of both BARI Hybrid Maize-5 and Hybrid Maize Pacific-987 (Figure 1A). NaCl stress at 50 mM caused a drastic reduction in root growth of both maize varieties. Foliar application of proline resulted in a significant increase in root growth of both varieties at 25 mM NaCl stress but not at 50 mM NaCl stress conditions (Figure 1A).

Salt stress also caused a significant reduction in plant dry matter of BARI Hybrid Maize-5 (Figure 1B). No significant changes were observed in dry matter of Hybrid Maize Pacific-987 in response to 25 mM NaCl stress even application of proline. NaCl stress at 50 mM caused a drastic decrease in plant dry matter of both varieties. Proline application resulted in an increase in plant dry matter of BARI Hybrid Maize-5 under 25 mM NaCl stress, and this increase was significant when plants were treated with 50 mM proline. At 50 mM NaCl stress, 25 mM proline application significantly improved plant growth of

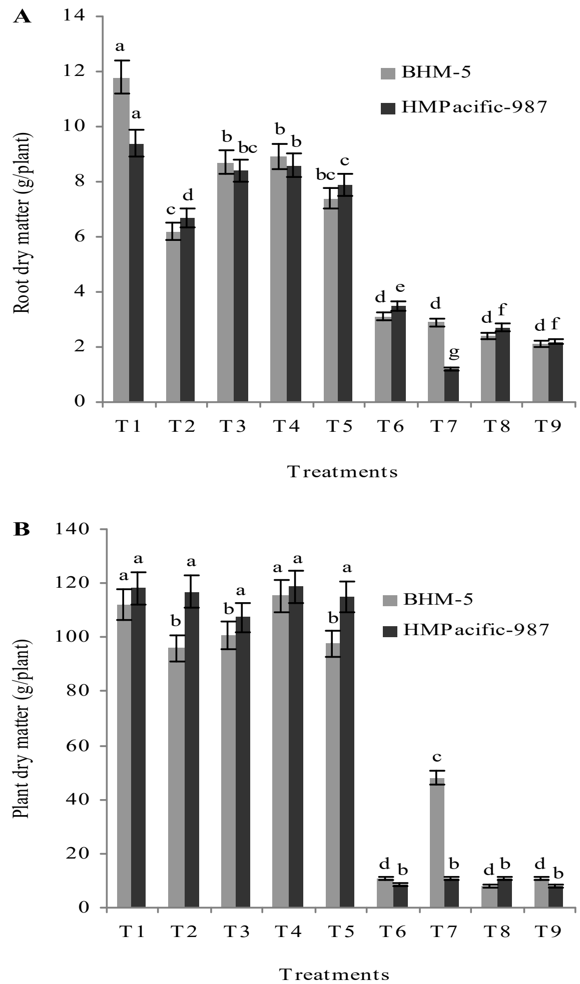

Figure 1. Effect of proline on the root (A) and plant (B) growth of BARI Hybrid Maize-5 (BHM-5) and Hybrid Maize Pacific-987 (HMPacific-987) under salt stress. Values represent the mean±SE of four replications. For the same maize variety, bars with the same letters are not significantly different at P<0.05. Treatments are T_1: control (no NaCl or proline), T_2: 25 mM NaCl, T_3: 25 mM NaCl+25 mM proline, T_4: 25 mM NaCl+50 mM proline, T_5: 25 mM NaCl+100 mM proline, T_6: 50 mM NaCl, T_7: 50 mM NaCl+25 mM proline, T_8: 50 mM NaCl+50 mM proline, T_9: 50 mM NaCl+100 mM proline.

BARI Hybrid Maize-5 although other doses of proline did not influence plant growth of both varieties (Figure 1B).

***Reproductive growth and grain yield of maize*:** Significant reductions in reproductive growth and grain yield of both maize varieties were observed in response to NaCl stress (Table 1). Neither BARI Hybrid Maize-5 nor Hybrid Maize Pacific-987 could produce cob as well as grains at 50 mM NaCl stress. Exogenous proline resulted in an increase in reproductive growth and grain yield of both varieties in response to 25 mM NaCl stress. The increasing effect of proline was significant on BARI Hybrid Maize-5. At 50 mM NaCl stress, BARI Hybrid Maize-5 produced cob and grains when plants were treated with 25 mM proline whereas Hybrid Maize Pacific-987 did not produce cob even application of proline (Table 1). Overall, low dose of proline application performed better in increasing growth and yield of maize under salt stress conditions (Table 1).

***Chlorophyll contents*:** Significant decreases in chlorophyll contents of both varieties were observed in response to salt stress (Table 2). NaCl stress at 50 mM caused a drastic decrease in chlorophyll-b content of BARI Hybrid Maize-5. No chlorophyll contents were detected from 50 mM NaCl-treated Hybrid Maize Pacific-987 plants because all plants died within 15 days even proline application. In some cases, proline application increased chlorophyll contents in both varieties under salt stress but these increases were not significant in most cases (Table 2).

***Nutrient uptake*:** We investigated whether exogenous proline influenced nutrient uptake by maize under salt stress (Table 3). Salt stress caused reductions in nutrient (NPS) uptake by straw and grain of both maize varieties. NaCl stress at 50 mM caused a drastic decrease in nutrient uptake by straw of both varieties. In most cases, exogenous proline resulted in an increase in N, P and S uptake by both varieties under salt stress. Under salt stress, P uptake by grain was approximately 4-fold higher in the presence of proline than in its absence (Table 3).

***K^+/Na^+ ratio*:** Significant decreases in K^+/Na^+ ratio in grain and straw of both varieties were observed in response to salt stress (Figure 2A, B).

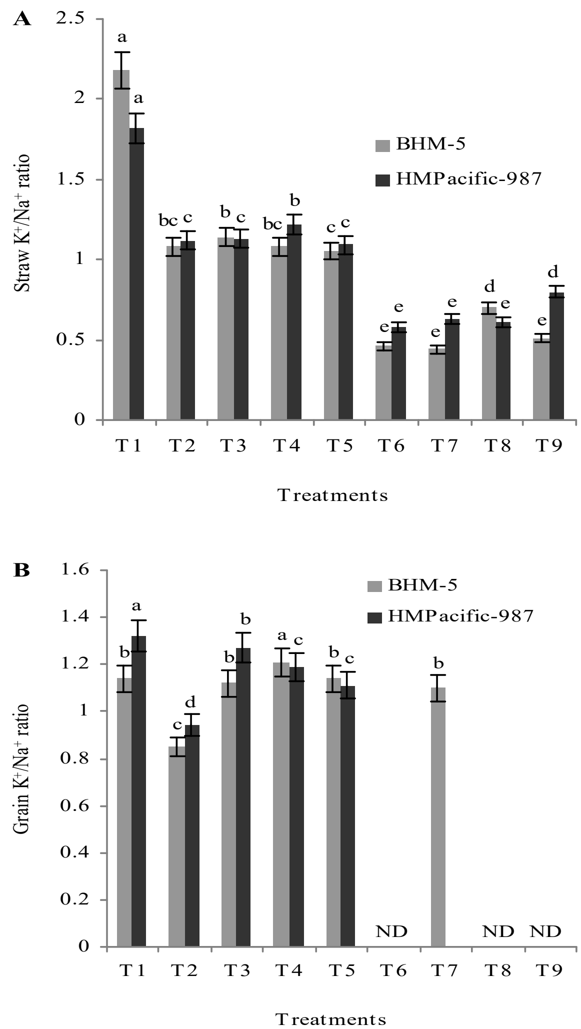

Figure 2. Straw K^+/Na^+ (A) and grain K^+/Na^+ (B) ratio in BARI Hybrid Maize-5 (BHM-5) and Hybrid Maize Pacific-987 (HMPacific-987) influenced by proline under salt stress. "ND" indicates data not determined. ND samples were not considered for statistical analysis. Values represent the mean±SE of four replications. For the same maize variety, bars with the same letters are not significantly different at $P<0.05$. Treatment details are shown in the legend of Figure 1.

On the contrary, proline application showed a significant increase in grain K^+/Na^+ ratio in both varieties response to salt stress (Figure 2B). In some cases, exogenous proline also increased straw K^+/Na^+ ratio in both varieties under salt stress (Figure 2A).

Table 1. Effect of proline on reproductive growth and grain yield of maize under salt stress

Treatments	**BARI Hybrid Maize-5**					**Hybrid Maize Pacific -987**				
	Cob length (cm)	Cob diameter (cm)	Grains/ cob (no.)	100-grain weight (g)	Grain weight (g/plant)	Cob length (cm)	Cob diameter (cm)	Grains/ cob (no.)	100-grain weight (g)	Grain weight (g/plant)
T_1	18.0a	13.3ab	327a	26.9b	85.7a	20.3a	12.9a	283a	27.3b	59.0a
T_2	14.7b	12.4b	263cd	21.7d	54.1c	20.2a	12.4a	237b	27.4b	52.1c
T_3	14.7b	13.2ab	285bc	23.4cd	63.2b	19.2ab	12.9a	282a	26.2c	55.8b
T_4	16.7ab	12.8b	271bcd	26.1bc	61.8b	19.5a	12.3a	275ab	27.9b	54.8bc
T_5	15.7ab	12.3b	287b	25.4bc	61.0b	18.0b	9.5b	42c	35.4a	20.8d
T_6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
T_7	11.3c	14.0a	252d	30.1a	50.3c	ND	ND	ND	ND	ND
T_8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
T_9	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
SE (±)	0.46	0.22	4.58	0.52	1.96	0.26	0.30	15.9	0.63	2.44
CV (%)	3.02	1.68	1.63	2.03	3.13	1.35	2.51	7.13	2.17	5.03

“ND” (not detected) indicates no plants survived during the data recording. ND samples were not considered for statistical analysis. Values represent the mean of four replications. Same letter in a column represents insignificant difference at $P<0.05$. Treatment details are shown in the legend of Figure 1.

Table 2. Chlorophyll contents in maize influenced by proline under salt stress

Treatments	**BARI Hybrid Maize 5**			**Hybrid Maize Pacific 987**		
	Chlorophyll a (µg/ml)	Chlorophyll b (µg/ml)	Total Chlorophyll (µg/ml)	Chlorophyll a (µg/ml)	Chlorophyll b (µg/ml)	Total Chlorophyll (µg/ml)
T_1	7.17a	4.54a	11.7a	6.49a	2.99b	9.48ab
T_2	6.66ab	3.51c	10.2bc	5.66b	2.01c	7.67b
T_3	6.48bc	3.49c	9.97bc	6.56a	3.16b	9.72ab
T_4	6.69ab	3.41c	10.1bc	6.69a	3.51a	10.2a
T_5	6.92ab	4.07b	11.0ab	6.48a	3.03b	9.51ab
T_6	6.03bc	2.36d	8.39d	ND	ND	ND
T_7	5.77c	3.52c	9.30cd	ND	ND	ND
T_8	ND	ND	ND	ND	ND	ND
T_9	ND	ND	ND	ND	ND	ND
SE (±)	0.13	0.11	0.21	0.13	0.10	0.21
CV (%)	1.95	3.22	2.11	1.96	3.43	2.28

“ND” (not detected) indicates no plants survived during the estimation of chlorophyll. ND samples were not considered for statistical analysis. Values represent the mean of four replications. Same letter in a column represents insignificant difference at $P<0.05$.

Accumulation of proline is one of the main adaptive mechanisms to salt stress in plants. In addition to its role as an osmoprotectant, proline counteracts the adverse effects of salt stress by reducing cellular damage and increasing antioxidant defense systems (Hoque et al., 2007a, b, 2008; Banu et al., 2009; 2010). The protective mechanisms of proline have recently been reported in plants against various oxidative stresses (Hossain et al., 2014). In the present study, exogenous proline improved growth and yield of maize exposed to NaCl stress. Notably, 25 mM proline application remarkably produced grain yield of BARI Hybrid Maize-5 at 50 mM NaCl stress (Table 1). There are increasing evidences that salinity reduced growth and yield of a variety of plants. Conversely, improved growth of plants as well as cultured-cells by exogenous proline under salt and water stresses have been demonstrated by various authors (Ali et al., 2008; Hoque et al., 2007a; Banu et al., 2009; Abbas et al., 2012; Sobahan et al., 2012; Talat et al., 2013).

Table 3. Effect of proline on nutrient uptake (g/plant) by maize under salt stress

Treatments	BARI Hybrid Maize-5						Hybrid Maize Pacific -987					
	Straw N	Grain N	Straw P	Grain P	Straw S	Grain S	Straw N	Grain N	Straw P	Grain P	Straw S	Grain S
T_1	1.57e	1.49b	0.05c	0.09b	0.13b	0.11	2.25a	1.29ab	0.14b	0.14c	0.20b	0.11a
T_2	1.72d	1.30c	0.05c	0.07b	0.13b	0.07	1.64d	1.22ab	0.08c	0.09d	0.15c	0.07b
T_3	1.92c	1.63a	0.11ab	0.26a	0.19a	0.08	1.93bc	1.31a	0.11c	0.37b	0.15c	0.08ab
T_4	2.08b	1.14d	0.09b	0.30a	0.18a	0.08	1.99b	1.17b	0.09c	0.42a	0.19bc	0.08ab
T_5	2.25a	1.64a	0.09b	0.27a	0.21a	0.07	1.80cd	0.51c	0.17a	0.12cd	0.25a	0.03c
T_6	0.25g	ND	0.01d	ND	0.02c	ND	0.11e	ND	0.01d	ND	0.02d	ND
T_7	0.91f	1.47b	0.04c	0.27a	0.05c	0.08	0.21e	ND	0.01d	ND	0.02d	ND
T_8	0.17g	ND	0.03cd	ND	0.02c	ND	0.16e	ND	0.01d	ND	0.03d	ND
T_9	0.25g	ND	0.01d	ND	0.02c	ND	0.11e	ND	0.01d	ND	0.01d	ND
SE (±)	0.14	0.03	0.02	0.02	0.01	NS	0.15	0.05	0.01	0.02	0.02	0.01
CV (%)	10.97	2.40	22.36	8.64	12.33	5.16	13.42	4.77	18.04	10.52	13.82	6.78

"ND" indicates data not determined. ND samples were not considered for statistical analysis. Values represent the mean of four replications. Same letter in a column represents insignificant difference at $P<0.05$. Treatment details are shown in the legend of Figure 1.

Chlorophyll is one of the most important pigment components of a plant. Chlorophyll content may vary with varying salt concentration, affecting plant growth and development. Salt stress significantly decreased chlorophyll contents in both maize varieties. Proline application did not remarkably affect chlorophyll contents in BARI Hybrid Maize-5 induced by salt stress although increased chlorophyll contents in Hybrid Maize Pacific-987 were observed by proline under salt stress (Table 2). Reduction in chlorophyll content has been reported in plants in response to salt stress (Ali et al., 2004; Islam et al., 2007; Sobahan et al., 2012; Talat et al., 2013). Conversely, exogenous application of proline increased chlorophyll content in a variety of plants reported by several authors (Deivanai et al., 2011; Sobahan et al., 2012; Talat et al., 2013).

Inorganic nutrients such as N, P and S play essential roles in plant metabolism. In addition to osmotic adjustment, genotypic differences in inorganic ion uptake under salinity have implications for maintaining adequate nutrition related to salinity tolerance mechanisms. There are evidences that proline minimizes the harmful effects of various stresses on plants by affecting uptake and accumulation of inorganic nutrients (Ali et al., 2008). Salt stress reduced nutrient (NPS) uptake by both maize varieties while exogenous proline increased nutrient uptake by maize under salt stress. It is significant to note that P uptake by grain was much higher when maize was grown at salt stress with exogenous proline (Table 3). Exogenous proline has been shown to increase nutrient uptake (NPK), thereby mitigating adverse effects of water stress in maize (Ali et al., 2008). Abd El-Samad et al. (2011) have shown that proline treatments enhanced nutrient uptake in maize and broad bean plants under salt stress.

Salinity imposes ionic toxicity to plants, leading to nutrition disorder. It has been reported that salt stress causes increased uptake of Na^+ and Cl^-, and decreased uptake of essential cations particularly K^+ (Khan et al., 2003). Salt tolerance is directly associated with K contents because of its involvement in osmotic regulation and competition with Na. Proline has an added advantage under soil salinity as it may lower down Na uptake and increase K uptake, thereby protecting plants from the detrimental effects of Na. There are reports that exogenously supplied proline reduces Na^+ accumulation and increases K^+/Na^+ ratio in plants under salt stress (Ahmed et al., 2011; Abd El-Samad et al., 2011; Nounjan et al., 2012; Sobahan et al., 2012). In this study, salinity decreased K^+/Na^+ ratio in both maize varieties whereas this K^+/Na^+ ratio increased by proline application at 25 mM NaCl stress. Additionally, 25 mM proline maintained a higher grain K^+/Na^+ ratio in BARI Hybrid Maize-5 during 50 mM NaCl stress (Figure 2B). These results show that exogenous proline could contribute to the reduction of Na uptake as well as increment of K uptake during salinity stress.

Conclusions

Salt stress caused a reduction in growth and yield of both maize varieties. High salt stress drastically reduced the growth of maize. Exogenous proline significantly improved growth and yield of BARI Hybrid Maize-5 in response to salt stress. Furthermore, BARI Hybrid Maize-5 conferred tolerance to high salt stress when proline was applied exogenously. The present study suggests that exogenous application of proline ameliorates the adverse effects of salt stress on maize by increasing the K^+/Na^+ ratio and uptake of nutrients particularly P. However, the protective mechanisms of proline in plant responses to salinity remain to be elucidated. Better understanding about the biochemical and physiological mechanisms of proline is crucial for plant tolerance mechanisms to salinity.

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References

Abd El-Samad, H. M., M. A. K. Shaddaad, and N. Barakat. 2011. Improvement of plants salt tolerance by exogenous application of amino acids. *Journal of Medicinal Plants Research* 5: 5692-5699.

Ahmed, C. B., S. Magdich, B. B. Rouina, S. Sensoy, M. Boukhris, and F. B. Abdullah. 2011. Exogenous proline effects on water relations and ions contents in leaves and roots of young olive. *Amino Acids.* 40: 565-73.

Ali Q, M Ashraf, M Shahbaz, H Humera (2008). Ameliorative effect of foliar applied proline on nutrient uptake in water stressed maize (*Zea mays* L.) plants. *Pak Journal Botany* 40: 211–219.

Ali Y, Z Aslam, MY Ashraf, GR Tahir (2004). Effect of salinity on chlorophyll concentration, leaf area, yield and yield components of rice genotypes grown under saline environment. *International Journal of Environmental Science and Technology* 1: 221–225.

Ashraf M, MR Foolad (2007). Roles of glycinebetaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany* 59: 206–216.

Athar, HR, M Ashraf (2009). Strategies for crop improvement against salinity and drought stress: An overview. In: *Salinity and Water Stress*, eds. M. Ashraf, M. Ozturk and H. R. Athar, pp. 1-16. Springer Science+ Business Media B.V.

Azevedo NAD, JT Prisco, J Enéas-Filho, CEBD Abreu, E Gomes-Filho (2006). Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. *Environmental and Experimental Botany* 56 : 87–94.

Banu MNA, MA Hoque, MS Watanabe, MM Islam, M Uraji, K Mastuoka, Y Nakamura, Y Shimoishi, Y Murata (2010). Proline and glycinebetaine ameliorated NaCl stress via scavenging of hydrogen peroxide and methylglyoxal but not superoxide or nitric oxide in tobacco cultured cells. *Bioscience Biotechnology Biochemistry* 74: 2043-2049.

Banu MNA, MA Hoque, MW Sugimoto, K Matsuoka, Y Nakamura, Y Shimoishi, Y Murata (2009). Proline and glycinebetaine induce antioxidant defense gene expression and suppress cell death in cultured tobacco cells under salt stress. *Journal of Plant Physiology* 166: 146–156.

Deivanai S, R Xavier, V Vinod, K Timalata, OF Lim (2011). Role of exogenous proline in ameliorating salt stress at early stage in two rice cultivars. *Journal of Stress Physiology & Bio-chemistry.* 7: 157–174.

FAO (2008). *Land and Plant Nutrition Management Service*. Rome, FAO of UN. Available from: http://www.fao.org/ag/agl/agll /spush.

Flowers TJ, AR Yeo (1995). Breeding for salinity resistance in crop plants: where next? *Australian Journal of Plant Physiology* 22: 875–884.

Hasegawa PM, RA Bressan, JK Zhu, HJ Bohnert (2000). Plant cellular and molecular responses to high salinity. *Annual Review of Plant Physiology and Plant Molecular Biology* 51: 463–499.

Hoque MA, E Okuma, MNA Banu, Y Nakamura, Y Shimoishi, Y Murata (2007a). Exogenous proline mitigates the detrimental effects of salt stress more than exogenous betaine by increasing antioxidant enzyme activities. *Journal of Plant Physiology* 164: 553–561.

Hoque MA, MNA Banu, E Okuma, K Amako, Y Nakamura, Y Shimoishi, Y Murata (2007b). Exogenous proline and glycinebetaine increase NaCl-induced ascorbate–glutathione cycle enzyme activities, and proline improves salt tolerance more than glycinebetaine in tobacco Bright Yellow-2 suspension-cultured cells. *Journal of Plant Physiology* 164: 1457–1468.

Hoque MA, MNA Banu, Y Nakamura, Y Shimoishi, Y Murata (2008). Proline and glycinebetaine enhance antioxidant defense and methylglyoxal detoxification systems and reduce NaCl-induced damage in cultured tobacco cells. *Journal of Plant Physiology* 165: 813–824.

Hossain MA, MA Hoque, DJ Burritt, M Fujita (2014). Proline protects plants against abiotic oxidative stress: biochemical and molecular mechanisms. In: *Oxidative damage to plants*, eds. P. Ahmad, pp. 477–522. San Diego: Elsevier Inc.

IPCC (Intergovernmental Panel on Climate Change) (2007). Summary for Policymakers. In: *Climate Change 2007: Impacts, Adaptation and Vulnerability,* eds. ML Parry, OF Canziani, JP Palutikot, PJ van der Linden, and CE Hanson, IPCC Fourth Assessment Report, Cambridge: Cambridge University Press.

Islam MZ, MAB Mia, A Akter, MH Rahman (2007). Biochemical attributes of mutant rice under different saline levels. *International Journal of Sustainable Crop Production* 2: 17–21.

Jaleel CA, R Gopi, P Manivannan, R Panneerselvam (2008). Soil salinity alters the morphology in *Catharanthus roseus* and its effects on endogenous mineral constituents. *EurAsia Journal of BioScience* 2: 18-25.

Khan AA, SA Rao, T. McNeilly (2003). Assessment of salinity tolerance based upon seedling root growth response function in maize (*Zea mays* L.). *Euphetica* 131: 81-89.

Khanam M, MM Rahman, MR Islam (2001). Effect of manures and fertilizers on the growth and yield of BRRI dhan30. *Pakistan Journal of Biological Science* 4: 172-174.

Munns, R. 2002. Comparative physiology of salt and water stress. *Plant Cell and Environment* 25: 239–250.

Nounjan N, PT Nghia, P Theerakulpisut (2012). Exogenous proline and trehalose promote recovery of rice seedlings from salt-stress and differentially modulate antioxidant enzymes and expression of related genes. *Journal of Plant Physiology* 169: 596-604.

Okuma E, Y Murakami, Y Shimoishi, M Tada, Y Murata (2004). Effects of exogenous application of proline and betaine on the growth of tobacco cultured cells under saline conditions. *Soil Science and Plant Nutrition* 50: 1301–1305.

Porra RJ, WA Thompson, PE Kriedemann **(**1989). Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll II standards by atomic absorption spectroscopy. *Biochimica et Biophysica Acta* 975: 384–394.

Sobahan MA, N Akter, M Ohno, E Okuma, Y Hirai, IC Mori, Y Nakamura, Y Murata (2012). Effects of exogenous proline and glycinebetaine on the salt tolerance of rice cultivars. *Bioscience Biotechnology Biochemistry* 76: 1568–1570.

SRDI (2010). *Saline soils of Bangladesh.* SRMAF Project, Ministry of Agriculture, Dhaka, Bangladesh.

Talat A, K Nawaz, K Hussian, KH Bhatti, EH Siddiqi, A Khalid, S Anwer, MU Sharif (2013). Foliar application of proline for salt tolerance of two wheat (*Triticum aestivum* L.) cultivars. *World Applied Sciences Journal* 2013; 22: 547-554.

Zhu JK (2003). Regulation of ion homeostasis under salt stress. *Current Opinion in Plant Biology* 6: 441–445.