



Improvement of Salt Tolerance in Maize by Exogenous Application of Proline

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Abstract

Proline provides protection in plants against various abiotic stresses including salinity. The field experiment was conducted at the farmer's field of coastal area (Botiaghata, Khulna) to mitigate the adverse effects of salinity on growth and yield of maize by exogenous application of proline. In the experiment maize plants were treated with different concentrations of proline at seedling and/or vegetative stages. The plant growth parameters, grain and stover yields, nutrient uptake and K^+/Na^+ ratio were recorded. All the yield contributing characters were significantly increased due to exogenous application of proline at different growth stages. Among the treatments, application of 100 mM proline at seedling and vegetative stages offered the highest grain and stover yields (5.7 t ha^{-1} and 9.8 t ha^{-1} , respectively) of maize which was followed by 100 mM proline application at seedling stage where grain and stover yields were 5.7 t ha^{-1} and 9.7 t/ha , respectively. However no significant differences were found between two treatments. Total N, P and S uptake (203.66 , 23.24 and 23.14 kg ha^{-1} , respectively) were highest when the maize plants were treated with 100 mM proline at seedling and vegetative stages. The highest K^+/Na^+ ratio both in grain (12.92) and stover (5.57) was also observed in the same treatment. Therefore, it can be concluded that exogenous application of proline improves salinity tolerance in maize by increasing nutrient uptake and probably due to increasing antioxidant defense mechanisms.

Key words: K^+/Na^+ ratio, Maize growth, Nutrient uptake, Salinity

Introduction

Soil salinity is a major concern to agriculture all over the world because it affects almost all plant functions. Millions of hectares of land throughout the world are too saline to produce economic crops, and more land is becoming non-productive each year due to salinity build up. Three hectares of arable lands are lost each minute because of soil salinization. More than 6% of the world's land and one third of the world's irrigated land are significantly affected by soil salinity (FAO, 2008). Agriculture is the single 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. Maize is one of the most important and widely grown food crops and third leading cereal crop in Bangladesh. Maize yield decreases under saline condition. Hence, to meet the increasing food demand it is essential to take necessary steps to increase the maize production in coastal areas. Plants are frequently exposed to several abiotic stresses during its growth and development. Salinity imposes both ionic toxicity and osmotic stress to plants, leading to nutritional disorder and oxidative stress. Salt stress causes increased uptake of Na^+ and Cl^- , and decreased uptake of essential cations particularly K^+ (Khan *et al.*, 2003). It has been reported that salt stress causes an increase in Na^+ to K^+ ratio in the cytosol (Zhu, 2003). Exogenously supply of proline reduced Na^+ accumulation under salt stress (Ahmed *et al.*, 2011). Maize can contribute significantly towards solving food problem and thereby play its gainful role in the agro-economy of the country. But satisfactory yield cannot be obtained due to salt stress in coastal areas of southern Bangladesh. Salt stress induces the accumulation of reactive oxygen

species (ROS) including superoxide radical and hydrogen peroxide in plant cells (Banu *et al.*, 2009, 2010). The excess production of ROS is toxic to plants and causes oxidative damage to cellular constituents, leading to cell death (Banu *et al.*, 2009, 2010). Plants possess enzymatic and non-enzymatic antioxidant defense systems to protect their cells against the damaging effects of ROS. Proline has been shown to scavenge free radicals and ROS (Okuma *et al.*, 2004; Banu *et al.*, 2009, 2010). Several studies have shown that proline application effectively regulates osmotic potential and plays a vital role in sustaining plant growth under osmotic stress (Serraj and Sinclair, 2002; Ali *et al.*, 2007; Ashraf and Foolad, 2007; Hoque *et al.*, 2007). Exogenous application of proline would be one of the options to improve the crop productivity in salinity affected areas of Bangladesh. Increased levels of proline accumulated in plants correlate with improved salt tolerance (Okuma *et al.*, 2004; Ashraf and Foolad, 2007). Soil salinity management in salt-affected areas of Bangladesh in winter season to increase rice crop production, is a great concern in recent days. Thus current research should be emphasized on salinity management. Now-a-days, different salt tolerant varieties are developed for saline areas but yield potential is low. More research is necessary in order to increase crop yield in salt-affected areas. The present study is crucial for the improvement of salinity tolerance and economic crop production in the coastal areas of southern Bangladesh. Therefore, addition to management practices, exogenous application of proline is a novel approach for increasing yield of maize in coastal areas where salinity is a major concern.

Materials and Methods

Location and design of experiment

To investigate the effects of exogenous proline on growth and yield of maize, the field experiment was conducted at the farmer's field of coastal area (Botiaghata, Khulna). The experiment was laid out in Randomized Complete Block Design with three replications.

Soil

Soil texture was silty clay loam, having pH 6.71, Electrical Conductivity 4.5 dSm^{-1} , exchangeable Na $2.21 \text{ meq}/100 \text{ g}$ soil, Organic Matter content 1.55%.

Crop and variety

BARI Hybrid Maize-5 (BMH-5) was used as a test crop. Maize seeds were sown in the experimental plots maintaining appropriate spacing.

Treatment combinations

Proline at different concentrations was applied to maize plants at different growth stages. There were ten treatment combinations including control.

T₀ = Control

T₁ = 25 mM proline at seedling stage

T₂ = 25 mM proline at vegetative stage

T₃ = 25 mM proline at seedling and vegetative stages

T₄ = 50 mM proline at seedling stage

T₅ = 50 mM proline at vegetative stage

T₆ = 50 mM proline at seedling and vegetative stages

T₇ = 100 mM proline at seedling stage

T₈ = 100 mM proline at vegetative stage

T₉ = 100 mM proline at seedling and vegetative stages

Management practices and proline application

The recommended doses of urea, triple super phosphate, muriate of potash, gypsum and boric acid were applied in all the experimental plots. Irrigation was provided as and when necessary. Electrical conductivity of water used for irrigation was measured. The EC value of first and second irrigation water were 3.8 and 9.5 dS m^{-1} . Plants were treated with different concentrations of proline at seedling and/or vegetative stages. Proline containing 0.1% Tween-20 as surfactant was applied at seedling and/or vegetative stages as foliar spray at a volume of 25 mL per plant per treatment. The intercultural operations, irrigation and other management practices were done as and when necessary.

Crop harvesting and data collection

The crop was harvested at full maturity. Grain and stover yields and plant growth parameters (plant height, plant weight, cob length, cob diameter, number of grains per cob, 100 grains weight etc.) were recorded. N, P, K, S and Na contents were measured from maize grain and stover samples.

Chemical analysis of plant samples

Grain and stover samples of maize were analyzed for N, P, K, S and Na contents were determined following standard method as described by Khanam *et al.* (2001).

Statistical analysis

The data recorded on different parameters were analyzed statistically by statistical package MStatC. The significance of differences between mean values was compared by Duncan's Multiple Range Test (DMRT). Differences at $p < 0.05$ were considered significant.

Results and Discussion

Growth and yield components

Salt stress caused a significant reduction in root growth of BARI Hybrid Maize-5. Table 1 shows that different growth parameters of maize plant showed significant differences due to exogenous application of proline under saline conditions. Different growth parameters like plant height, plant weight, cob length, cob diameter, number of grains per cob etc. were found lowest when the maize plants were not treated by proline in any growth stage. Application of proline found to be effective for ameliorating the salinity problem. Plant height showed insignificant difference between untreated plants and the plants which were treated with 25 mM and 50 mM proline in different growth stages but 100 mM proline application showed significant difference in plant height. The growth parameters which were recorded found to be higher when the maize plants were treated with 100 mM proline at seedling and vegetative stages than other treatments. Plant height, cob length and diameter and number of grains per cob increased significantly due to exogenous application of 100 mM proline at both seedling and vegetative stages. Deivanai *et al.* (2011) conducted an experiment on rice to ameliorate the adverse effect of salinity and found that different growth components significantly decreased due to salinity but proline application increased the growth and yield components. Dogan (2011) also found that salinity decreased growth parameters in soybean and proline application ameliorates the adverse effect of salinity.

Grain and stover yields

Significant reductions in grain and stover yield of hybrid maize variety were observed in response to NaCl stress. Fig. 1 shows that soil salinity caused a significant decrease in the yield of maize. Significant difference was found in yield of maize

based on spraying proline at different concentrations in different growth stages. Grain yield shows a significant increase on exogenous application of proline under salt stress. Grain yield decreased under salt stress but increased when proline application was done. Application of proline significantly increased the grain and stover yields of BARI Hybrid Maize-5 under saline

conditions. The highest grain (5.7 t ha⁻¹) and stover (9.8 t ha⁻¹) yield of maize were obtained from the plots where 100 mM proline was applied at seedling and vegetative stage which was statistically identical to 100 mM proline at seedling stage and 50 mM proline at seedling and vegetative stage.

Table 1. Effect of exogenous application of proline on growth and yield components of maize under saline conditions

Treatments	Plant Height (cm)	Plant Weight (gm)	Cob length (cm)	Cob Diameter (cm)	Number of grains per cob	100-grain weight (gm)
T0	156.4 d	215 c	17.1 e	12.2 c	372 de	24.1 d
T1	157.1 d	263.3 ab	17.4 cde	12.4 bc	365 e	24.5 cd
T2	162.1 cd	254.8 ab	17.3 de	12.3 bc	368 de	24.7 bcd
T3	161.6 cd	267 ab	17.8 ab	12.5 b	371 de	26.0 abc
T4	161.5 cd	261 ab	17.4 cde	12.4 bc	398 b	24.8 bc
T5	164.3 bcd	251.7 b	17.5 cde	12.2 c	344 f	24.6 cd
T6	164.5 bcd	251.3 b	17.8 ab	12.2 c	340 f	25.0 abc
T7	164.7 bcd	266 ab	17.7 abc	12.5 b	378 c	26.1 abc
T8	168.7 abc	273.7 a	18.0 a	12.3 bc	383 cd	26.2 abc
T9	177.1 a	272 a	18.1 a	13.0 a	409 a	26.6 a
SE (±)	3.15	5.93	0.133	0.069	3.47	0.563

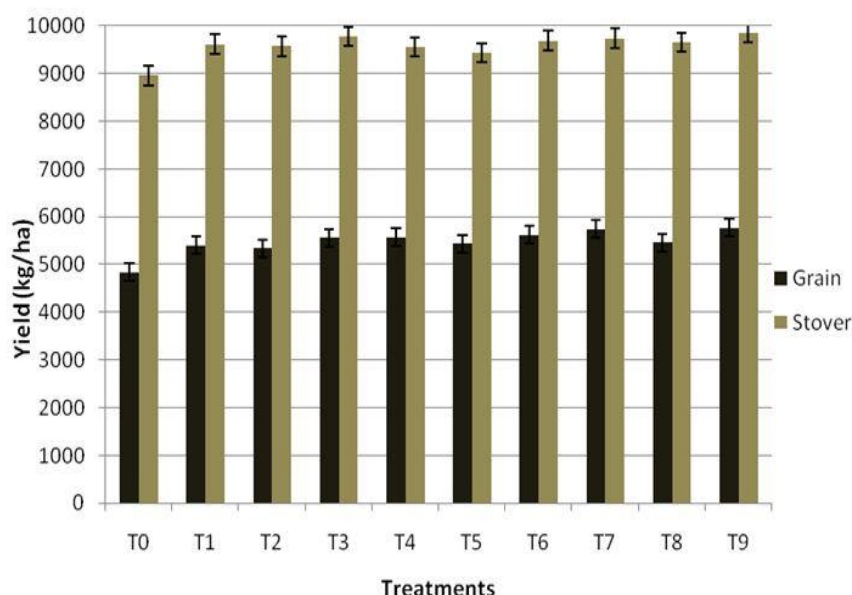


Fig. 1. Grain and stover yields of maize influenced by exogenous proline under saline conditions

The lowest grain and stover yield (4.8 t ha⁻¹ and 8.9 t ha⁻¹, respectively) of maize was recorded from untreated control. Islam *et al.* (2011) on hybrid rice also reported that grain yields decreased by the salinity. Ali *et al.* (2004) also found the similar result on grain yield of rice. Devar *et al.*, 2013 found that grain yield and stover yield significantly decrease under salt stress and proline application helps to ameliorate the salinity problem. Miah *et al.* (1992) on two rice varieties also found that salinity decreased stover yield of rice. Dogan (2011) reported that grain and stover yield reduced in maize due to salt stress.

Total nutrient uptake

We investigated whether exogenous proline influenced nutrient uptake by maize under salt stress. To investigate the nutrient uptake by maize, we measured nutrient content in maize stover and grain. Salt stress caused reductions in nutrient (NPS) uptake by stover and grain of both maize varieties. On the other hand, application of proline increased nutrient (NPS) uptake by maize under saline conditions. Exogenous proline resulted in an increase in nutrient uptake in response to salt stress. Total N (203.66 kg ha⁻¹), P (23.24 kg ha⁻¹) and S (23.14 kg ha⁻¹) uptake was significantly higher at

100 mM proline at seedling and vegetative stages while the lowest was recorded from untreated control and it was 136.54 kg ha⁻¹N, 16.6 kg ha⁻¹P and 18.7 kg ha⁻¹S (Table 2). Exogenous application of proline has been shown to increase nutrient uptake (NPS) and reduces adverse effects of salt stress in maize (Ali *et al.*, 2008). Hamed *et al.* 1994 on maize also reported that total nutrient uptake

was decreased due to salinity and increased due to exogenous application of proline. Momayezi *et al.* (2010) also found that salt stress decreased the total nutrient uptake by rice. Kumar and Sharma (1989) reported that nutrient uptake in mungbean was reduced due to salinity and it can be increased by exogenous application of proline.

Table 2. Effect of exogenous application of proline on nutrient (NPS) uptake of maize under saline conditions

Treatments	Total N uptake (kg/ha)	Total P uptake (kg/ha)	Total Suptake (kg/ha)
T0	136.54e	16.67f	18.74c
T1	179.32d	19.27de	21.26b
T2	176.30d	18.65e	21.12b
T3	194.80b	20.06cd	21.60b
T4	188.30c	19.49de	21.70b
T5	180.60d	20.61cd	21.57b
T6	196.01b	21.90bc	22.43ab
T7	195.22b	22.52ab	22.95ab
T8	183.32cd	21.85bc	21.57b
T9	203.66a	23.24a	23.14a
SE (±)	4.42	1.23	1.12

K⁺/Na⁺ ratio

Potassium (K) and Sodium (Na) are most important element for the plant to survive in salt stress. Salt tolerance is directly associated with K contents

because of its involvement in osmotic regulation and competition with Na. Plant salt tolerance requires

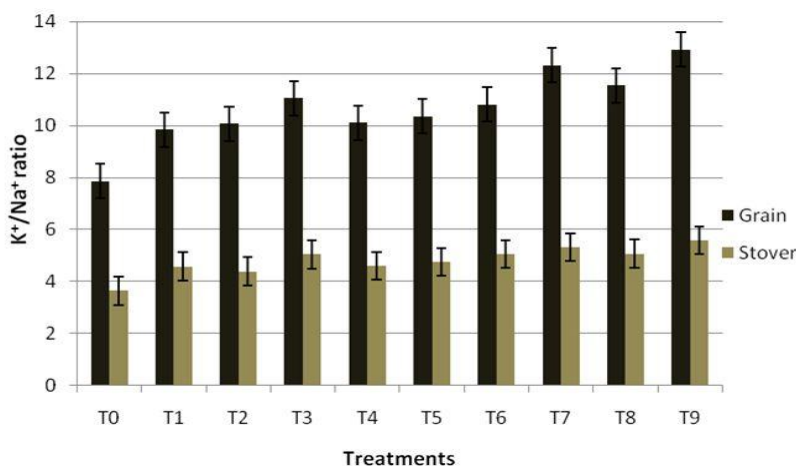


Fig. 2. K⁺/Na⁺ ratio in grain and stover of maize influenced by exogenous proline under saline conditions

not only adaptation to Na⁺ toxicity but also the acquisition of abundant K⁺ whose uptake by the plant cell is affected by high external Na⁺ concentrations. Soil salinity significantly decreased K⁺/Na⁺ ratio in both grain and stover samples. Foliar application of proline resulted in a significant increase in K⁺/Na⁺ ratio in maize plants. In grain, the highest K⁺/Na⁺ ratio (12.92) was found when the maize plants were treated with 100 mM proline at seedling and vegetative stages which was statistically identical to 100 mM proline at seedling stage and 100 mM proline at vegetative stage. In stover, the highest K⁺/Na⁺ ratio (5.57) was found at same treatment and statistically similar to T7 and T8 including T6 (50 mM proline at seedling and vegetative stages). The lowest K⁺/Na⁺ ratio (7.85

and 3.64, respectively) both in grain and stover was found in untreated plants (Fig. 2). There are evidences that exogenously supplied proline reduced Na⁺ accumulation under salt stress and increases K⁺/Na⁺ ratio (Zhu, 2003). Ahmed *et al.* (2011) also conducted an experiment on young olive and also found that exogenous application of proline increases K⁺/Na⁺ ratio significantly. Haq *et al.* (2009) on seven rice varieties, Nounjan *et al.* (2012) on Thai aromatic rice and Miah *et al.* (1992) on two rice varieties at different salinity level also found that salinity decreased K⁺/Na⁺ ratio and proline application increases the K⁺/Na⁺ ratio in rice. Devar *et al.*, 2013 conducted an experiment on maize and concluded that K⁺/Na⁺ ratio significantly decreases under salt stress.

Conclusions

This study indicates that salinity causes significant yield loss of maize and that could be minimized by application of proline during both seedling and vegetative growth stages. Crop cultivation in saline areas might be profitable with proline application. Crop plants with over accumulating proline confer tolerance to salinity.

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