Progressive Agriculture 29 (4): 295-303, 2018 ISSN: 1017 - 8139

Progressive Agriculture
Journal homepage:http://www.banglajol.info/index.php/PA

Improvement of salinity tolerance in rice during *boro* season by proline application

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Abstract

Salinity causes cellular damage, limiting crop productivity. Accumulation of proline is one of the main adaptive mechanisms to salinity in plants. The main objective of this study was to mitigate the adverse effects of soil salinity in boro rice by exogenous application of proline. The field experiment was conducted at the farmer's field of Botiaghata, Khulna with Boro rice. The soil was silty clay loam having pH 7.2, EC 6.6 dS/m, CEC 26 meq/100 g soil and organic matter content 0.84%. Two rice varieties (salt-sensitive; BRRI dhan29 and salt-tolerant; BINA dhan-8) were used as test crops. There were ten treatment combinations with different concentrations of proline applied at seedling and/or vegetative stages. Recommended doses of N, P, K, S and Zn fertilizers were applied to the all experimental plots. The experiments were laid out in a randomized complete block design with three replications. Soil salinity caused a significant reduction in growth and yield of both salt-sensitive and salt-tolerant rice. Salt-tolerant rice produced higher grain and straw yields than salt-sensitive rice. Application of proline significantly increased growth, and grain and straw yields of boro rice under saline condition. There were no considerable variations in growth and yield of rice due to the different doses of proline. Increased nutrient uptake and K^+/Na^+ ratio in boro rice were observed due to proline application. The present study suggests that exogenous proline improves salt tolerance in rice by increasing K^+/Na^+ ratio and nutrient uptake.

Key words: Proline, salt tolerance, nutrient uptake, K^+/Na^+ ratio, rice

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Introduction

World agriculture is facing a lot of challenges like producing 70% more food for an additional 2.3 billion people by 2050 while at the same time fighting with poverty and hunger, consuming scarce natural resources more efficiently and adapting to climate change (FAO, 2009). The lower productivity in most of the cases is attributed to various abiotic stresses. As a sessile organism, plants often experience abiotic stresses like salinity, drought, high or low temperature, heavy metal toxicity, etc. which pose serious threat to the crop production (Bhatnagar-Mathur *et al.,* 2008; Ahmad and Prasad, 2012).

Salinity is one of the most brutal environmental factors limiting productivity of crop plants because most of the crop plants are sensitive to salinity (Haque *et al.*, 2014). Out of 2.86 million hectares of coastal and offshore lands, about 1.06 million hectares are affected by soil salinity (SRDI, 2010). The area under salinity is increasing with time (from 0.83 m ha to 1.056 m ha in 36 years; SRDI, 2010) due to rise in sea water level with increased global temperature. According to the IPCC (2007), crop production may fall by 10-30% by 2050 in Bangladesh due to climate change.

Salt stress induces the accumulation of reactive oxygen species (ROS) in plant cells. The excess production of ROS is toxic to plants and causes oxidative damage to cellular constituents, leading to cell death. 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 the uptake and accumulation of inorganic nutrients (Ali *et al*., 2008) and by reducing cellular damage and increasing antioxidant defense systems (Hoque *et al*., 2008; Banu *et al.,* 2009; Bhusan *et al.,* 2016).

Rice is the most important and extensively grown food crop and leading cereal in the world including Bangladesh. To meet the consequent increased food demand, crop production must be increased either by increasing arable land or by increasing yield per hectare. Increase in arable land in the densely populated country like Bangladesh is very difficult. In this situation, it is essential to explore the possible alternatives that are economically feasible.

Therefore, a well-focused approach combining the molecular, physiological, biochemical and metabolic aspects of salt tolerance is essential to develop salt-tolerant crop varieties. Several strategies have been proposed to alleviate the degree of cellular damage caused by salt stress as well as to improve salinity tolerance. Among them, exogenous application of compatible solute proline already gained considerable attention in alleviating the adverse effects of salt stress (Ashraf and Foolad, 2007; Hoque *et al.,* 2007a, b; Bhusan *et al.,* 2016). Exogenous proline enhanced the antioxidant defense systems during salinity stress conditions (Hoque *et al.,* 2007ab; 2008; Nounjan *et al.,* 2012; Bhusan *et al.,* 2016). Salinity management in salt-affected areas of Bangladesh in boro season to increase rice crop production is a great concern in recent days. Thus research should be emphasized on this issue. Exogenous application of proline would be one of the options to improve the crop productivity in salinity affected areas of Bangladesh.

Materials and Methods

***Experimental site and soils*:** The field experiments were carried out at the farmer's field of Botiaghata, Khulna belongs to the Agro-ecological Zone of the Ganges Tidal Floodplain (AEZ 13). Characteristically, the soil was silty clay loam having pH 7.2, EC 6.6 dS/m, CEC 26 meq/100 g soil and organic matter 0.84%. The experimental area is included into the tropical monsoon climate. There are three monsoon periods appear in this region. The monsoon period lasts from May to October. About 88% of the total rainfall is observed in this time. Hailstone also occurs during this time. Sometimes storms locally called Kalboishakhi are observed.

***Plant materials and treatments*:** The experiment was laid out in a randomized complete block design (RCBD) with three replications. Two rice varieties (salt-sensitive; BRRI dhan29 and salt-tolerant; BINA dhan-8) were used as test crops. There were ten treatment combinations with different concentrations of proline at seedling and vegetative stages like as T_0 = Control (no proline), T_1 = 25 mM proline at seedling stage, T_2 = 25 mM proline at vegetative stage, T_3 = 25 mM proline at seedling and vegetative stages, T_4 = 50 mM proline at seedling stage, T_5 = 50 mM proline at vegetative stage, T_6 = 50 mM proline at seedling and vegetative stages, T_7 = 100 mM proline at seedling stage, T_8 = 100 mM proline at vegetative stage, and T_9 = 100 mM proline at seedling and vegetative stages. Recommended doses of TSP, MP, gypsum, and zinc sulphate were applied to all experimental plots during final land preparation. Recommended dose of urea was applied in three splits. Thirty-day-old seedlings of two rice varieties were transplanted in the experimental plots. Three seedlings per hill were placed at a spacing of 25 cm × 20 cm. Proline solutions were sprayed over plant leaves with the help of sprayer. Tween-20 was used as a sticky substance which helps proline solution's droplet, maintaining a close contact with plant leaves. Other intercultural operations were done when necessary. Maturity of crop was determined when about 90% grains became golden yellow. The crop was harvested at full maturity i.e. BRRI dhan29 at 111 DAT (days after transplanting) and BINA dhan-8 at 126 DAT.

***Chemical analysis of plant samples*:** The representative grain and straw samples were dried in an oven at 65^0C for about 24 hours before they were ground by a grinding machine. The prepared samples were stored in paper bags and finally kept into desiccators until analysis. The N, P, K, S and Na contents from grain and straw samples were determined following standard method as 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 $P\leq0.05$ were considered significant.

Results and Discussion

***Growth and yield components of rice*:** Soil salinity caused a significant decrease in plant height of both BRRI dhan29 and BINA dhan-8. Proline application significantly increased the plant height of both rice cultivars under saline conditions. Salt stress caused a significant decrease in number of effective tillers per hill of both BRRI dhan29 and BINA dhan-8 varieties (Tables 1 and 2). It was observed that foliar application of proline resulted in an increase in number of effective tillers of both rice cultivars under salt stress condition. Panicle length of BRRI dhan29 was not affected by salt stress whereas panicle length of BINA dhan-8 was significantly decreased by salt stress (Tables 1 and 2). Application of proline also did not much affect panicle length of BRRI dhan29 under salt stress. Plants exposed with salinity drastically decreased filled grains in both BRRI dhan29 and BINA dhan-8 varieties (Tables 1 and 2). Foliar application of proline in different growth stages significantly increased filled grains in both varieties. The 1000-grain weight was decreased due to salinity in BRRI dhan29 and BINA dhan-8 cultivars (Tables 1 and 2). Proline application increased 1000-grain weight under salt stress conditions although this increase was not significant.

Islam *et al.* (2011) on hybrid rice and Miah *et al.* (1992) on two rice varieties found that plant height decreased with increasing salinity. Similar to our results, Deivanai *et al.* (2011) showed that proline application increased plant height in two Malaysian rice cultivars (MR220 and MR232). Islam *et al.* (2011) on hybrid rice found that number of productive tillers per plant decreased by the salinity of 6 and 10 dSm^{-1}. Ali *et al.* (2004) also reported that numbers of productive tillers of eighteen advanced rice genotypes were reduced by salinity at 8.5 dSm^{-1}. Zeng and Shannon (2000) observed that reduction in tiller numbers per plant was the major causes of yield loss in rice cultivar (M-202) under different salinity conditions. Similar results were found by Kibria *et al.* (2017). Talat *et al*. (2013) observed that the exogenous application of proline significantly ameliorated the harmful effects of salt stress on wheat.

***Grain and straw yields of rice*:** Plants exposed with salinity significantly decreased grain yield of both salt-sensitive (BRRI dhan29) and salt-tolerant (BINA dhan-8) rice varieties (Table 3). Foliar application of proline over plant leaves significantly increased grain yield of salt-sensitive variety under stress condition. Application of proline resulted in an increase in grain yield of salt-tolerant variety (BINA dhan-8) during salt stress. Foliar application of proline over plant leaves significantly increased straw yield in both rice variety. BINA dhan-8 produced higher grain and straw yields than BRRI dhan29 (Table 3).

Miah *et al.* (1992) demonstrated that salinity decreased grain and straw yields of two rice varieties at 2.4, 6.0 and 11.8 dSm^{-1} soil salinity condition. Plant shows significant effect on grain yield due to exogenous

Table 1. Effect of proline on growth and yield components of BRRI dhan29 under salinity conditions

Treatment	Plant height (cm)	No. of effective tillers/hill	Panicle length (cm)	No. of filled grains/panicle	1000-grain weight (gm)
T_0- Control	93.27c	14b	25.08b	98d	18.34b
T_1- 25 mM proline at seedling stage	94.80bc	16a	26.07a	115a	19.02a
T_2- 25 mM proline at vegetative stage	95.87ab	17a	25.80a	110bc	18.80ab
T_3- 25 mM proline at seedling and vegetative stages	96.20a	17a	25.32ab	110bc	18.86ab
T_4- 50 mM proline at seedling stage	97.00a	17a	25.80a	118a	19.52a
T_5- 50 mM proline at vegetative stage	96.73a	16a	25.54ab	111bc	18.94ab
T_6- 50 mM proline at seedling and vegetative stages	96.47a	16a	25.37ab	114ab	19.48a
T_7- 100mM proline at seedling stage	95.67ab	17a	25.20ab	108c	19.00a
T_8- 100 mM proline at vegetative stages	96.47a	17a	25.20b	112bc	19.26a
T_9- 100 mM proline at seedling and vegetative stages	95.40ab	16a	25.21b	112bc	19.02a
SE(±)	1.12	0.94	0.33	2.45	0.44
CV(%)	3.33	2.43	2.12	4.58	1.53

Same letter in a column represents insignificant difference at $p < 0.05$, SE = Standard errors of means, CV = Co-efficient of variation

Table 2. Effect of proline on growth and yield components of BINA dhan-8 under salinity conditions

Treatment	Plant height (cm)	No. of effective tillers/hill	Panicle length (cm)	No. of filled grains/ panicle	1000-grain weight (gm)
T_0- Control	86.06c	16c	23.76c	102d	24.00b
T_1- 25 mM proline at seedling stage	88.06b	18ab	25.20b	112c	25.45a
T_2- 25 mM proline at vegetative stage	90.67a	19a	26.90a	114bc	24.68ab
T_3- 25 mM proline at seedling and vegetative stages	91.16a	19a	27.40a	119b	24.72ab
T_4- 50 mM proline at seedling stage	92.53a	19a	26.63ab	115bc	25.38a
T_5- 50 mM proline at vegetative stage	90.53a	17bc	26.04ab	112c	25.02a
T_6- 50 mM proline at seedling and vegetative stages	90.13a	18ab	27.54a	124a	25.18a
T_7- 100 mM proline at seedling stage	90.40a	18ab	26.68ab	126a	24.53ab
T_8- 100 mM proline at vegetative stages	92.46a	18ab	26.34ab	113c	24.72ab
T_9- 100 mM proline at seedling and vegetative stages	92.33a	19a	27.06a	115bc	25.33a
SE(±)	1.32	0.99	1.13	2.25	0.45
CV(%)	3.01	2.14	3.21	4.12	4.18

Same letter in a column represents insignificant difference at $p < 0.05$, SE = Standard errors of means, CV = Co-efficient of variation.

Table 3. Effect of proline on yield of BRRI dhan29 and BINA dhan-8 under salinity conditions

Treatment	BRRI dhan29		BINA dhan-8	
	Grain yield (kg/ha)	**Straw yield (kg/ha)**	**Grain yield (kg/ha)**	**Straw yield (kg/ha)**
T_0- Control	2641c	3742c	2941c	4235c
T_1- 25 mM proline at seedling stage	3396ab	4292b	3827a	4585b
T_2- 25 mM proline at vegetative stage	3207b	4390ab	3657ab	4938a
T_3- 25 mM proline at seedling and vegetative stages	3218b	4434a	3696ab	4938a
T_4- 50 mM proline at seedling stage	3562a	4489a	3665ab	4545b
T_5- 50 mM proline at vegetative stage	3396ab	4196b	3534b	4848a
T_6- 50 mM proline at seedling and vegetative stages	3452a	4686a	3772a	4454b
T_7- 100 mM proline at seedling stage	3405ab	4588a	3619ab	4561b
T_8- 100 mM proline at vegetative stages	3313ab	4538a	3434b	4500b
T_9- 100 mM proline at seedling and vegetative stages	3396ab	4345ab	3772a	4809a
SE(±)	123	132	140	112
CV(%)	5.24	4.66	4.48	5.56

Same letter in a column represents insignificant difference at $p < 0.05$, SE = Standard errors of means, CV = Co-efficient of variation.

application of proline. Rice yield decreased at salt stress but increased due to proline application. Islam *et al.* (2011) on hybrid rice also reported that grain yields decreased by salinity of 6 and 10 dSm^{-1}. Exogenous proline has been shown to improve grain and straw yields of rice (Bhusan *et al.,* 2016).

Nutrient uptake and K^+/Na^+ ratio of rice

***Nitrogen uptake*:** The total N uptake was drastically reduced in both rice varieties under salt stress conditions (Tables 4 and 5). There were significant variations in rice N uptake with foliar application of proline. All the treatments increased N uptake over control. The N uptake was found to be highest in BRRI dhan29 at 100 mM proline application at seedling and vegetative stages. The highest N uptake by BINA dhan-8 was observed when proline was applied at 25 mM at seedling and vegetative stages. The highest N uptake in BRRI dhan29 was found at 100 mM proline application but in case of BINA dhan-8 it was found at 25 mM proline application (Tables 4 and 5).

***Phosphorus uptake*:** There were significant variations in rice P uptake due to exogenously applied proline (Tables 4 and 5). All the treatments increased P uptake over control. P uptake was found to be highest in BRRI dhan29 at 100 mM proline at seedling stage and 100 mM proline at seedling and vegetative stages. Foliar application of proline also increased P uptake by BINA dhan-8. The highest P uptake was observed when proline was applied at 25 mM at seedling and vegetative stages (Tables 4 and 5).

***Sulphur uptake*:** Salinity caused significant reductions in total S uptake by both rice varieties (Tables 4 and 5). Proline application significantly increased total S uptake in both salt-sensitive and salt-tolerant varieties. The S uptake was reduced drastically in control. All the proline treatments increased S uptake. In BRRI dhan29, the highest S uptake was found in applying 50 mM

Table 4. Effect of proline on nutrient uptake of BRRI dhan29 under salinity conditions

Treatment	**Total N uptake (kg/ha)**	**Total P uptake (kg/ha)**	**Total S uptake (kg/ha)**
T_0- Control	52.99e	12.64d	7.16c
T_1- 25 mM proline at seedling stage	80.69bc	20.92b	10.48ab
T_2- 25 mM proline at vegetative stage	71.46d	17.44c	9.60b
T_3- 25 mM proline at seedling & vegetative stages	74.93cd	18.14c	9.65b
T_4- 50 mM proline at seedling stage	83.48b	21.48ab	11.93a
T_5- 50 mM proline at vegetative stage	93.30a	19.42bc	8.73bc
T_6- 50 mM proline at seedling & vegetative stages	89.20ab	21.02ab	10.37ab
T_7- 100 mM proline at seedling stage	95.08a	23.32a	11.27a
T_8- 100 mM proline at vegetative stages	85.46b	18.84c	10.26ab
T_9- 100 mM proline at seedling & vegetative stages	96.29a	23.07a	10.10ab
SE(±)	4.32	1.76	1.12
CV(%)	2.49	2.44	4.10

Same letter in a column represents insignificant difference at $p < 0.05$, SE = Standard errors of means, CV = Co-efficient of variation.

Table 5. Effect of proline on nutrient uptake of BINA dhan-8 rice under salinity conditions

Treatment	**Total N uptake (kg/ha)**	**Total P uptake (kg/ha)**	**Total S uptake (kg/ha)**
T_0- Control	53.55c	10.67c	7.62d
T_1- 25 mM proline at seedling stage	80.10a	22.15a	10.59bc
T_2- 25 mM proline at vegetative stage	80.30a	17.23b	10.24bc
T_3- 25 mM proline at seedling & vegetative stages	83.07a	23.06a	9.62c
T_4- 50 mM proline at seedling stage	76.76ab	22.49a	11.60ab
T_5- 50 mM proline at vegetative stage	67.97b	18.26b	13.14a
T_6- 50 mM proline at seedling & vegetative stages	73.66ab	20.05ab	12.41a
T_7- 100 mM proline at seedling stage	71.14b	19.39ab	11.60ab
T_8- 100 mM proline at vegetative stages	66.96b	18.12b	11.94ab
T_9- 100 mM proline at seedling & vegetative stages	81.78a	21.85a	11.68ab
SE(±)	3.03	1.72	1.25
CV(%)	3.41	3.14	3.56

Same letter in a column represents insignificant difference at $p < 0.05$, SE = Standard errors of means, CV = Co-efficient of variation.

proline at seedling stage. In case of BINA dhan-8, S uptake was found to be highest at 50 mM proline at vegetative stage (Table 5).

K^+/Na^+ ratio

***BRRI dhan29*:** Salt stress caused a significant decrease in K^+/Na^+ ratio in both grain and straw of BRRI dhan29 (Table 6). Proline application significantly increased K^+/Na^+ ratio in both straw and grain of BRRI dhan29 under stress condition. All the doses of proline increased the K^+/Na^+ ratio in straw whereas the same condition was not found in grain. The higher grain K^+/Na^+ ratio was found in 100 mM proline at vegetative stage. A slight difference in straw K^+/Na^+ ratio were observed among the doses of proline application. However 50 mM proline at seedling and vegetative stages maintained a higher K^+/Na^+ ratio (Table 6).

Table 6. Effect of proline on K^+/Na^+ ratio of BRRI dhan29 and BINA dhan-8 under salinity conditions

Treatment	**BRRI dhan29**		**BINA dhan-8**	
	K^+/Na^+ ratio (grain)	**K^+/Na^+ ratio (straw)**	**K^+/Na^+ ratio (grain)**	**K^+/Na^+ ratio (straw)**
T_0-Control	8.38c	3.29b	11.64c	3.34c
T_1- 25 mM proline at seedling stage	14.57a	4.39a	15.00a	5.86ab
T_2- 25 mM proline at vegetative stage	12.35b	4.27a	14.61b	5.67ab
T_3- 25 mM proline at seedling & vegetative stages	14.81a	4.39a	14.57b	6.28ab
T_4- 50 mM proline at seedling stage	14.41a	4.43a	15.90ab	6.65a
T_5- 50 mM proline at vegetative stage	12.37b	4.59a	15.40ab	5.56ab
T_6- 50 mM proline at seedling & vegetative stages	15.51a	4.62a	16.50a	6.17ab
T_7- 100 mM proline at seedling stage	14.11a	4.57a	16.10a	5.09b
T_8- 100 mM proline at vegetative stages	13.41ab	4.78a	15.58ab	5.71ab
T_9- 100 mM proline at seedling & vegetative stages	14.81a	4.52a	15.75ab	5.50b
SE(±)	1.45	0.70	1.18	0.96
CV(%)	3.14	2.22	2.05	1.87

BINA dhan-8: Salt stress also caused a significant reduction in K^+/Na^+ ratio in both grain and straw of BINA dhan-8 (Table 6). Proline application significantly increased K^+/Na^+ ratio in both straw and grain in salt stress condition over control. In grain the highest K^+/Na^+ ratio was recorded in 50 mM proline at seedling and vegetative stages. In straw, K^+/Na^+ ratio was found to be highest in 50 mM proline at seedling stage.

Nutrients such as N, P, K and S play essential roles in plant metabolism. In addition to osmotic adjustment, genotypic differences in inorganic ions uptake under salinity have implications for maintaining adequate nutrition and for optimizing nutrients/elements related salinity tolerance mechanisms. There are evidences that proline minimizes the adverse effects of various stresses on plants by affecting the uptake and accumulation of inorganic nutrients (Ali *et al.,* 2008). Similar to our results, Abd El-Samad *et al.* (2011) showed that application of proline increased NPK nutrient uptake in rice plants. Soad and Shetea (2007) also showed that proline application increased NP uptake in soybean (*Glycine max* L.).

Salt tolerance is directly associated with K contents because of its involvement in osmotic regulation and competition with Na. Plant salt tolerance requires 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. K^+/Na^+ in rice grain and straw decreased due to salt stress in both rice varieties while application of proline significantly increased K^+/Na^+ in both salt-sensitive and salt-tolerant varieties. Islam *et al.* (2011) on hybrid rice at 6 dSm^{-1} and 10 dSm^{-1} salinity levels, Haq *et al.* (2009) on seven rice varieties, and Miah *et al.* (1992) on two rice varieties at 2.4, 6.0 and 11.8 dSm^{-1} salinity levels also found that salinity decreased K^+/Na^+ ratio. Nounjan *et al.* (2012) on Thai aromatic rice (cv. KDML105; salt-sensitive) also found the similar result due to exogenous application of proline.

Conclusion

Salinity is the major factor reducing crop yields in coastal areas of Bangladesh. Proline application with suitable cultivars having higher yield potential could contribute to the improvement of crop production in coastal areas of Bangladesh. Exogenous application of proline showed a significant increase in growth, and grain and straw yields accompanied with increased K^+/Na^+ ratio and nutrient uptake of both rice varieties under salinity condition. Proline confers tolerance to salinity in rice by increasing nutrient uptake, maintaining higher K^+/Na^+ ratio and probably increasing antioxidant defense systems. In addition to field research work, better understanding about the biochemical and physiological functions of proline is important to select and breed plants for plant tolerance mechanisms to salinity.

Acknowledgements

This work was supported by a grant from the Bangladesh Agricultural Research Council.

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