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Improving salinity tolerance in transplanted aman rice (*Oryza sativa* L.) by exogenous application of proline

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Abstract

Salinity is the major factor reducing crop yield in coastal areas of Bangladesh. Proline (Pro) application with suitable crop varieties having higher yield potential could contribute to the improvement of crop production in saline areas. The main objective of this study was to investigate the mitigation of adverse effects of salinity in *aman* rice by exogenously applied Pro. The experiment was carried out at the farmer's field of Batiaghata, Khulna. Characteristically, the soil was silty clay loam having pH 6.7, EC 4.6 dS m^{-1}, CEC 23 meq/100 g soil, organic matter 0.71%. Rice (*Oryza sativa* L.) variety BR23 was used as plant material. The experiment was laid out in a randomized complete block design with three replications. There were different treatment combinations namely control (no Pro), 25 mM Pro at seedling stage, 25 mM Pro at vegetative stage, 25 mM Pro at seedling and vegetative stages, 50 mM Pro at seedling stage, 50 mM Pro at vegetative stage, 50 mM Pro at seedling and vegetative stages, 100 mM Pro at seedling stage, 100 mM Pro at vegetative stages, and 100 mM Pro at seedling and vegetative stages. Recommended doses of N, P, K, S and Zn fertilizers were applied to the all experimental plots. Thirty-day-old seedlings were transplanted in the experimental plots. Proline solutions were sprayed over plant leaves with the help of sprayer as per treatments. Salinity caused significant reductions in growth and yield of BR23 by decreasing plant height, number of effective tillers, panicle length, filled grains $panicle^{-1}$ and 1000-grain weight. On the other hand, exogenous application of Pro showed a significant increase in growth and yield of BR23 under saline conditions. Results also revealed that growth and yield of rice did not increase proportionally with the increasing doses of Pro. Proline application resulted in significant increases in K^+/Na^+ and nutrient uptake by rice under salinity. The present study suggests that exogenous application of Pro confers tolerance to salinity in aman rice by increasing K^+/Na^+ ratio and nutrient uptake.

Introduction

Growth and yield reduction of crops are the serious issue in salinity prone areas of the world. About 20% of world's cultivated areas and nearly half of the world's irrigated lands are affected by salinity (Mali *et al.*, 2012). Salinity effects are more conspicuous in arid and semiarid regions where limited rainfall, high evapotranspiration and high temperature associated with poor water and soil management (Azevedo *et al.*, 2006; Jaleel *et al.*, 2008). Agricultural productivity is severely affected by soil salinity, and the damaging effect of salt accumulation in agricultural soils has become an important environmental concern (Jaleel *et al.*, 2007).

The world population is increasing rapidly and may reach 6 to 9.3 billion by the year 2050 whereas the crop production is decreasing rapidly because of the negative impact of various environmental stresses; therefore, it is now very important to develop stress-tolerant varieties to cope with this upcoming problem of food security. In addition, increased salinity of arable land is expected to have devastating global effects, resulting in up to 50% land losses by the middle of the twenty-first century (Mahajan and Tuteja, 2005).

Salinity causes unfavorable environment and hydrological situation that restrict the normal crop production throughout the year (Haque *et al.*, 2014). The factors which contribute significantly to the development of saline soil are tidal flooding during wet season (June-October), direct inundation by saline water, and upward or lateral movement of saline ground water during dry season (November-May). Rice is mainly grown in salinity affected areas of Bangladesh but the average yield is very low due to lack of salt-tolerant high yielding variety and inappropriate management practices. In Bangladesh, out of 2.85 million hectares of the coastal and off-shore areas, about 1.06 million hectares are affected by salinity (SRDI, 2010).

Plants possess different defense mechanisms in order to cope with stress; one of them is associated with accumulation of osmoprotectants. Proline is one of the major osmoprotectant osmolytes, which is synthesized

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by many plants in response to stress including salinity, thereby maintaining the osmotic status of the cell to ameliorate the abiotic stress (Chinnusamy *et al*., 2005; Burritt, 2012; Hossain *et al.*, 2014). Proline plays roles in scavenging free radicals, stabilizing subcellular structures, and buffering celluler redox potential under stresses (Hoque *et al.,* 2008; Banu *et al.*, 2009; Hossain *et al*., 2014). Exogenous application of Pro also provides osmoprotection and facilitates the growth of salinity-stress plants. Proline can protect cells against salinity-induced oxidative stress by up-regulating activities of various antioxidant enzyme activities (Hoque *et al.*, 2007; Bhusan *et al.,* 2016).

Exogenous application of Pro already gained considerable attention in mitigating the adverse effect of salt stress (Ashraf and Foolad, 2007; Hoque *et al.*, 2007). There are increasing evidences that Pro application effectively regulates osmotic potential and plays a vital role in sustaining plant growth under osmotic stress (Ali *et al.*, 2007; Ashraf and Foolad, 2007; Hoque *et al.*, 2007). Exogenous Pro enhanced the antioxidant enzymatic activities *viz.* ascorbate peroxidase, peroxidase and catalase during both stress and recovery period in rice (Nounjan *et al.*, 2012; Bhusan *et al.,* 2016). Little information is available on the beneficial roles of exogenous Pro in modulating salt stress tolerance in rice at various phases of plant growth. Therefore, the present study aimed to investigate the mitigation of the adverse effects of salinity in aman rice by exogenously applied Pro at both seedling and vegetative stages.

Materials and Methods

Experimental site and soils

The field experiment was carried out at the farmer's field of Batiaghata, Khulna belongs to the Agro-ecological Zone of the Ganges Tidal Floodplain (AEZ 13). Characteristically, the soil was silty clay loam having pH 6.7, EC 4.6 dS m^{-1}, CEC 23 meq/100 g soil and organic matter 0.71%. 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 with three replications. Rice variety BR23 was used as plant material. There were ten treatment combinations with different concentrations of Pro at seedling and vegetative stages like as T_0 = Control (no Pro), T_1 = 25 mM Pro at seedling stage, T_2 = 25 mM Pro at vegetative stage, T_3 = 25 mM Pro at seedling and vegetative stages, T_4 = 50 mM Pro at seedling stage, T_5 = 50 mM Pro at vegetative stage, T_6 = 50 mM Pro at seedling and vegetative stages, T_7 = 100 mM Pro at seedling stage, T_8 = 100 mM Pro at vegetative stage, and T_9 = 100 mM Pro at seedling and vegetative stages. Recommended doses of TSP, MoP, gypsum, and zinc sulphate were applied to all the experimental plots (4.0 m × 2.5 m) during final land preparation. Recommended dose of urea was applied in three splits. Thirty-day-old rice seedlings 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. The volume of the spray was 25 ml per plant at both seedling and vegetative stages. Tween-20 was used as a sticky substance which helps Pro 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.

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 $P \leq 0.05$ were considered significant.

Results and Discussion

Growth and yield components of rice

Salinity caused a significant decrease in plant height whereas Pro application significantly increased plant height (Table 1). Salinity significantly decreased effective tillers $hill^{-1}$ of BR23. All treatments except 100 mM Pro at seedling stage increased effective tillers per hill over control. It was observed that there were no significant variations in panicle length among the treatments that exposed varying concentration of Pro application such as 25 mM, 50 mM, 100 mM Pro at seedling stage, vegetative stage, and both seedling and vegetative stages. Salinity caused a drastic decrease in filled grains per panicle of BR23. Exogenous application of Pro at different growth stages significantly increased filled grains per panicle under salinity conditions. Table 1 also shows that salinity significantly reduced the 1000-grain weight of BR23. All the Pro treatments increased 1000-grain weight over control. Some of the Pro treatments contributed to the significant increase of 1000-grain weight under saline condition (Table 1).

Table 1. Effect of exogenous proline on the growth of T. Aman rice (BR23) 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	119c	12c	24a	88b	21.24d
T_1 25 mM proline at seedling stage	126abc	15a	24a	109a	22.40abcd
T_2 25 mM proline at vegetative stage	122bc	13bc	24a	112a	22.61abcd
T_3 25 mM proline at seedling and vegetative stages	125abc	14ab	24a	113a	22.33abcd
T_4 50 mM proline at seedling stage	129ab	13bc	23b	103a	23.37ab
T_5 50 mM proline at vegetative stage	128ab	13bc	24a	101a	23.52a
T_6 50 mM proline at seedling and vegetative stages	129ab	14ab	23b	107a	22.28abcd
T_7 100 mM proline at seedling stage	123abc	12c	24a	104a	22.74 abc
T_8 100 mM proline at vegetative stage	130a	14ab	24a	104a	22.01bcd
T_9 10 mM proline at seedling and vegetative stages	128ab	14ab	24a	106a	21.89cd
SE (±)	1.140	0.306	0.133	2.221	0.214
CV (%)	3.05	5.02	1.75	6.19	3.19

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

A large body of evidences reports that salinity has negative impact on growth and yield components of crops. Momayezi *et al.* (2010) showed that number of effective tillers per hill was decreased by increasing salt level in rice. Islam *et al.* (2011) on hybrid rice and Miah *et al.* (1992) on two rice varieties found that plant height decreased with increasing salinity. Papon *et al.* (2015) showed that salt stress reduced filled grains of both salt-sensitive and salt-tolerant rice varieties. On the other hand, Abbas *et al.* (2012) demostrated that exogenous application of Pro increased plant height of *Citrus sinensis* (L.) under NaCl stress. Deivanai *et al.* (2011) also showed that Pro application increased plant height in two Malaysian rice cultivars (MR220 and MR232). Papon *et al.* (2015) showed that Pro application increased number of filled grains per panicle under saline conditions. Recently it is also reported by Bhusan *et al.* (2016) that exogenous Pro application increased growth of rice.

Grain and straw yields of rice

Plant exposed with salinity significantly decreased grain yield of BR23 rice (Table 2). Foliar application of Pro over plant leaves significantly increased grain yield under salinity condition. All the treatments increased grain yield over control. Straw yield of BR23 was drastically reduced due to salinity. All the Pro treatments significantly increased straw yield over control (Table 2).

Table 2. Effect of exogenous proline on the grain and straw yields of T. Aman rice (BR23) under salinity conditions

Treatment	Grain yield (kg/ha)	Straw yield (kg/ha)
T_0 Control	4672c	5063e
T_1 25 mM proline at seedling stage	5438a	5863abc
T_2 25 mM proline at vegetative stage	4980ab	5929ab
T_3 25 mM proline at seedling and vegetative stages	5363ab	6329a
T_4 50 mM proline at seedling stage	5016ab	5929ab
T_5 50 mM proline at vegetative stage	4859bc	5130de
T_6 50 mM proline at seedling and vegetative stages	5361ab	5862abc
T_7 100 mM proline at seedling stage	4924ab	5663bcd
T_8 100 mM proline at vegetative stage	4907b	5163de
T_9 10 mM proline at seedling and vegetative stages	4941ab	5297de
SE (±)	80.188	136.486
CV (%)	5.37	5.56

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

Miah *et al.* (1992) on two rice varieties found that salinity decreased straw yield of rice at 2.4, 6.0 and 11.8 dSm^{-1} conditions. It has been demonstrated that salt stress reduced grain and straw yields of rice (Bhusan *et al.*, 2016). The protective mechanisms of Pro have been increasingly reported in the literature in plants against various stresses. It has been reported that exogenous Pro application increased grain and straw yields of rice (Papon *et al.*, 2015; Bhusan *et al.*, 2016).

Nutrient content and uptake

N content and uptake: There were significant variations in rice grain N content due to application of Pro. Most of the Pro treatments increased N content over control (Table 3). There was also significant variation in straw N content due to application of Pro. Most of the Pro treatments resulted in lower straw N content than control (Table 3). There were significant variations in rice total N uptake with application of Pro. All the treatments increased total N uptake over control (Table 3).

P content and uptake: There were significant variations in rice grain P content due to exogenously applied Pro. Most of the Pro treatments increased P content over control (Table 4). Significant variations in rice straw P content were also observed in response to Pro application. All the treatments increased P content over control (Table 4). There were significant variations in rice total P uptake with application of Pro. All the treatments increased total P uptake over control (Table 4).

Table 3. Effect of exogenous proline on nitrogen content and uptake by T. Aman rice (BR23) under salinity conditions

Treatment	N Content %		N uptake (kg/ha)		
	Grain	Straw	Grain	Straw	Total
T_0 Control	0.920e	0.53b	43.17f	26.93cd	70.10ef
T_1 25 mM proline at seedling stage	1.148bcd	0.44cd	62.43b	26.27cd	88.70bc
T_2 25 mM proline at vegetative stage	1.400a	0.42d	69.72a	24.90de	94.62a
T_3 25 mM proline at seedling and vegetative stages	1.06cde	0.42d	57.06cd	26.58cd	83.64c
T_4 50 mM proline at seedling stage	1.20bc	0.50bc	60.36bc	29.88b	90.27ab
T_5 50 mM proline at vegetative stage	0.920e	0.42d	54.90f	22.55f	76.45f
T_6 50 mM proline at seedling and vegetative stages	1.09cd	0.47bcd	58.54c	27.90bc	86.44bc
T_7 100 mM proline at seedling stage	1.26b	0.588a	62.04b	33.29a	95.33a
T_8 100 mM proline at vegetative stage	1.12bcd	0.42d	54.96d	21.68f	77.92d
T_9 10 mM proline at seedling and vegetative stages	1.03de	0.44cd	51.19e	23.73ef	74.92de
SE (±)	0.046	0.018	3.42	5.12	3.49
CV (%)	7.05	7.90	2.58	1.14	3.18

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

Table 4. Effect of exogenous proline on phosphorus content and uptake by T. Aman rice (BR23) under salinity conditions

Treatment	P Content %		P uptake (kg/ha)		
	Grain	Straw	Grain	Straw	Total
T_0 Control	0.18d	0.06c	8.40f	3.04f	11.44g
T_1 25 mM proline at seedling stage	0.26a	0.11a	14.14a	6.45a	20.59a
T_2 25 mM proline at vegetative stage	0.18d	0.09b	8.96def	5.34cd	14.30e
T_3 25 mM proline at seedling and vegetative stages	0.20c	0.09b	10.73c	5.69bc	16.42c
T_4 50 mM proline at seedling stage	0.15e	0.09b	7.52g	5.33cd	12.85f
T_5 50 mM proline at vegetative stage	0.26a	0.10ab	12.63b	5.13d	17.76b
T_6 50 mM proline at seedling and vegetative stages	0.23b	0.10ab	12.33b	5.86b	18.19b
T_7 100 mM proline at seedling stage	0.18d	0.10ab	8.86ef	5.66bc	14.52de
T_8 100 mM proline at vegetative stage	0.20c	0.090b	9.81d	4.65e	14.46de
T_9 10 mM proline at seedling and vegetative stages	0.19cd	0.11a	9.38de	5.82b	15.20d
SE (±)	7.94	10.64	4.76	4.68	3.10
CV (%)	0.011	0.0045	0.673	0.293	0.857

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

S content and uptake: Significant variation in grain S content was observed due to different treatments. Surprisingly, most of the Pro treatments did not result in increase in grain S content (Table 5). There were no significant variations in straw S content with application of Pro. Most of the treatments were found to be lower straw S content than control. Moreover, all the treatments except 25 mM Pro at seedling and vegetative stages were also found to be lower S uptake by straw than control (Table 5). There were significant variations in total S uptake due to different treatment combinations. In some cases, exogenous Pro increased total S uptake but not remarkably (Table 5).

Nutrients such as N, P, K and S play essential roles in plant metabolism. There are evidences that Pro 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 Pro increased NPK nutrient uptake in rice plants.

K^+/Na^+ ratio in grain

Potassium and sodium ratio in rice grain significantly decreased due to salinity while application of Pro significantly ameliorated K^+/Na^+ ratio. All the treatments increased K^+/Na^+ ratio over control. The

K^+/Na^+ ratio was found to be highest at 50 mM Pro application at seedling and vegetative stages (Table 6).

K^+/Na^+ ratio in straw

The K^+/Na^+ ratio in rice straw significantly decreased due to salinity but application of Pro significantly increased the K^+/Na^+ ratio. All the treatments increased K^+/Na^+ ratio over control. The K^+/Na^+ ratio was found to be highest at 25 mM Pro application at vegetative stage (Table 6).

Table 5. Effect of exogenous proline on sulphur content and uptake by T. Aman rice (BR23) under salinity conditions

Treatment	S Content %		S uptake (kg/ha)		
	Grain	Straw	Grain	Straw	Total
T_0 Control	0.09a	0.14a	4.58ab	7.29b	11.87abcd
T_1 25 mM proline at seedling stage	0.07cd	0.10e	4.02cd	6.38c	10.40ef
T_2 25 mM proline at vegetative stage	0.09a	0.12abcde	4.84ab	7.59ab	12.43ab
T_3 25 mM proline at seedling and vegetative stages	0.08bc	0.13abcd	4.50b	8.25a	12.75a
T_4 50 mM proline at seedling stage	0.07bcd	0.12bcde	3.79cd	7.38b	11.17de
T_5 50 mM proline at vegetative stage	0.08b	0.11de	4.12c	5.79c	9.91f
T_6 50 mM proline at seedling and vegetative stages	0.07d	0.12abcde	3.73d	7.52ab	11.25cde
T_7 100 mM proline at seedling stage	0.082bc	0.13abc	4.02cd	7.57ab	11.59bcd
T_8 100 mM proline at vegetative stage	0.10a	0.14ab	4.91a	7.29b	12.20abc
T_9 10 mM proline at seedling and vegetative stages	0.08bc	0.11cde	3.99cd	6.17c	10.16f
SE (±)	0.0034	0.0037	4.54	5.73	4.78
CV (%)	13.74	8.27	0.134	0.240	0.309

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

Table 6. K^+/Na^+ ratio in grain and straw of T. Aman rice (BR23) influenced by exogenous proline under salinity conditions

Treatment	K^+/Na^+ ratio	
	Grain	Straw
T_0 Control	14.37d	3.710d
T_1 25 mM proline at seedling stage	23.26ab	4.62bc
T_2 25 mM proline at vegetative stage	20.19c	6.13a
T_3 25 mM proline at seedling and vegetative stages	23.07bc	4.90b
T_4 50 mM proline at seedling stage	21.15bc	4.65bc
T_5 50 mM proline at vegetative stage	22.11bc	6.54a
T_6 50 mM proline at seedling and vegetative stages	25.96a	4.80bc
T_7 100 mM proline at seedling stage	24.04ab	5.97a
T_8 100 mM proline at vegetative stage	22.11bc	4.80bc
T_9 10 mM proline at seedling and vegetative stages	22.11bc	4.22cd
SE (±)	0.9693	0.2830
CV (%)	7.14	7.07

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

Salt stress disturbs cytoplasmic K^+/Na^+ homeostasis, causing an increase in Na^+ to K^+ ratio in the cytosol (Zhu, 2003). 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). Proline has an added advantage under soil salinity as it may lower down Na uptake by plants and increase K uptake, thereby protecting crops from the detrimental effects of Na. Kaya *et al.* (2013) showed that sodium concentrations were higher in the tissues of plants grown under saline conditions while compatible solute treatments significantly reduced sodium concentration in the plant tissues. Abd El-Samad *et al.* (2011) showed that application of Pro increased K^+/Na^+ ratio in rice plants. Nounjan *et al.* (2012) on Thai aromatic rice (cv. KDML105; salt-sensitive) also found the similar result due to exogenous application of Pro.

Conclusion

Physiological stress due to salinity is the main cause reducing crop yields in coastal saline areas of Bangladesh. Exogenous application of Pro with suitable crop varieties having higher yield potential could contribute to the improvement of crop production in saline condition. Our experiment shows that salinity reduced growth and yield of rice. On the other hand, exogenous Pro increased growth and yield contributing characters of BR-23, resulting higher grain and straw yields of rice. The increased growth and yield of rice by Pro were accompanied with higher K^+/Na^+ ratio and uptake of nutrients. It can be concluded from the present study that Pro confers tolerance to salinity in aman rice due to increasing nutrient uptake, maintaining higher K^+/Na^+ ratio and probably enhancing antioxidant defense systems. However, further studies are required to find out the beneficial roles of exogenous Pro at reproductive stage under saline conditions.

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