Asian J. Med. Biol. Res. 2015, 1 (3), 478-486; doi: 10.3329/ajmbr.v1i3.26465

Asian Journal of
Medical and Biological Research
ISSN 2411-4472 (Print) 2412-5571 (Online)
www.ebupress.com/journal/ajmbr

Article

Mitigation of the adverse effects of soil salinity in rice using exogenous proline and organic manure

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Received: 18 November 2015/Accepted: 02 December 2015/ Published: 30 December 2015

Abstract: Salinity causes cellular damage and limits crop productivity. Accumulation of organic compound is one of the major adaptive mechanisms for salinity tolerance in plants. The main objective of the present study was to mitigate the adverse effects of soil salinity in rice through organic amendments like proline and organic manure. The field experiments were conducted at the farmer's field of Batiaghata upazilla under Khulna district with aman 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 0.84%. Rice variety BR 23 was used as a test crop. There were 15 treatment combinations with different doses of proline and organic manures. Recommended doses of N, P, K, S and Zn fertilizers were applied to all the experimental plots. Proline was applied as foliar spray at seedling and/or vegetative stages. Organic manures were added to soils during final land preparation. The experiments were laid out in a randomized complete block design with three replications. Significant reductions in growth and yield of rice were observed under saline conditions. Application of both proline and organic manures significantly increased growth, yield contributing characters, and grain and straw yields of rice under salinity conditions. There were no significant variations in growth and yield of rice due to use of different doses of proline and organic manure. Increased nutrient uptake and K^+/Na^+ ratio were observed in rice due to proline as well as organic manure application under saline conditions. The present study suggests that exogenous proline or organic manure confers tolerance to salinity in rice by increasing K^+/Na^+ ratio and nutrient uptake.

Keywords: salinity; proline; organic manure; rice

1. 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. It has been projected that more than 50% of yield reduction is the direct result of abiotic stresses (Rodriguez *et al.* 2005). Rice (*Oryza sativa* L.) is a staple food for more than half of the world's population. So it is imperative to increase rice production in different rice growing ecosystems to feed the increasing world population (Khush, 2005).

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 (FAO, 2008). Agriculture is the most important sector of Bangladesh's economy. Out of 2.86 million hectares of coastal and offshore lands, about 1.06 million hectares are affected by varying degrees of salinity (SRDI, 2010). Rice is mainly grown in the saline areas but the yield is very low due to lack of salt-tolerant high yielding variety and inappropriate management practices.

Salinity imposes both ionic toxicity and osmotic stress to plants. Salt stress disturbs cytoplasmic K^+/Na^+ homeostasis, causing an increase in Na^+ to K^+ ratio in the cytosol (Zhu, 2003). Plants have evolved a variety of adaptive mechanisms to respond to environmental stress including salt stress. One of the main adaptive mechanisms to salt stress in plants is the accumulation of compatible solute like proline. Increased levels of proline accumulated in plants correlate with improved salt tolerance (Okuma *et al*., 2004; Asharf and Foolad, 2007).

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 (Banu *et al*., 2009, 2010). Proline has been shown to scavenge free radicals and ROS. Up-regulation of the components of antioxidant defense system offered by proline protects plant against NaCl-induced oxidative damage. In cultured tobacco cells, proline suppresses cell death and improves salt tolerance by increasing the activity of enzymes and/or expression of genes involved in the antioxidant defense systems (Hoque *et al*., 2007a, b; 2008; Banu *et al*., 2009, 2010).

Soil salinization is a major process of land degradation that decreases soil fertility and crop productivity. There is a report that coastal regions of Bangladesh are quite lower in soil fertility (Haque, 2006). The best means of maintaining soil fertility, productivity and salt tolerance could be through addition of organic manures. Soil amendments with organic manure reduce the toxic effects of salinity in various plants (Idrees *et al*., 2004; Abou El-Magd, 2008; Leithy *et al*., 2010; Raafat and Tharwat, 2011). The role of organic compounds like proline and organic manures in the mitigation of salt stress in plants is yet to be elucidated in context of Bangladesh. The present study is crucial for the improvement of salinity tolerance and economic crop production in the coastal areas of southern Bangladesh. Therefore, the present study was undertaken to investigate the effect of exogenous application of proline and organic manure on the growth and yield as well as nutrient uptake of rice under saline condition.

2. Materials and Methods

2.1. Field experimentation

The field experiments were carried out at the farmer's field of Batiaghata upazilla under Khulna district to investigate the improvement of soil salinity tolerance in rice by organic manure (farmyard manure and poultry manure) and proline application. The soil of Batiaghata upazilla was extremely saline due to higher EC value (6.4 dS/m) and due to this salinity rice production shows significant reduction in yield. The experiment was conducted here so that the performance of exogenous proline and organic manure application could be assessed under field condition. The experiment was laid out in a randomized complete block design (RCBD) with three replications. Rice cultivar BR23 was used as a test crop in this study. The total numbers of plots used in this study were 45. The unit plot size was 4m×2.5m and the plots were separated from each other by ails (1m). Treatments were randomly distributed within the block.

2.2. Treatments

Soils were amended with different doses of farmyard manure (FYM) and poultry manure (PM). In another experiment, plants were treated with different concentrations of proline at seedling and vegetative stages. In experiment 1, the treatments were T_0=Control (no manure), T_1=FYM (5 t ha^{-1}), T_2=FYM (10 t ha^{-1}), T_3=PM (4 t ha^{-1}), T_4=PM (8 t ha^{-1}). In second experiment, there were ten treatments including control and those were 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, T_9 = 100 mM proline at seedling and vegetative stages.

2.3. Management practices and proline application

Recommended doses of Urea, TSP, MP, gypsum and zinc sulphate were applied as the source of N, P, K, S and Zn (BARC, 2012). Recommended doses of fertilizer were applied to all experimental plots during final land preparation. Urea was applied in three split doses; first dose was applied after ten days of transplanting, second dose was applied at active tillering stage, and third dose was applied at panicle initiation stage. Weeding and other management practices were performed as and when necessary. Birtako 10g (2g dissolved in 10 L water) was applied as plant protection measures when insect, pests and diseases were appeared. Proline solutions were sprayed over plant leaves with the help of a sprayer. For making 25 mM, 50 mM and 100 mM proline solutions, 2.88 g, 5.76 g and 11.52 g of proline powder were dissolved in 1000 ml of water and 1 ml of Tween-20 was properly mixed with it respectively. Tween-20 was used as a sticky substance which helps proline solution's droplet, maintaining a close contact with plant leaves.

2.4. Crop harvesting and data collection

Maturity of crop was determined when about 90% grains became golden yellow. The crop was harvested on 29 December, 2014 at 120 days after transplanting. Grain and straw yield as well as plant growth parameters (Plant height, number of effective tillers per hill, panicle length and 1000 grains weight) were recorded after harvesting.

2.5. Chemical analysis of the plant samples

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

2.6. Statistical Analysis

Data were analyzed statistically using analysis of variance (ANOVA) to examine the treatment effects. The mean differences were adjudged by Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984) and ranking was indicated by letters.

3. Results and Discussion

3.1. Growth and yield components

Soil salinity caused a significant decrease in different growth parameters e.g. plant height, number of effective tillers per hill, panicle length and 1000 grains weight as well as grain and straw yield of rice. Table 1 show that addition of FYM and PM to soil significantly increased different growth parameters and yield of rice cultivar (BR23) under saline conditions. It was found that application of 8 t ha^{-1} PM achieved the highest plant height among all the treatments while lowest in control. The number of effective tillers per hill, panicle length and number of grains per panicle of BR23 was also found the lowest in control (no manure) and the highest was found when the soil was amended with FYM (10 t ha^{-1}).

Lakhdar *et al.* 2008 also found that soil salinity can be reduced by organic matter amendment in soil and growth performance of crops was better with organic matter addition in soil under saline condition. Tejada *et al.* 2006 also found that organic matter application reduced salinity and increased the growth and yield components of rice. Leithy *et al.* 2010 on peanut, Abou El-Magd *et al.* 2008 on sweet funnel and Raafat *et al.* 2011 on rice have shown that organic amendments increased plant growth at salt stress. Zaki *et al.* (2009) found that organic manure increased all the vegetative growth parameters and vegetative yield under saline conditions. Amanullah (2008) showed that among the reclamation practices of coastal saline soils, FYM recorded better growth, yield parameters, yield and nutrient uptake by rice crop.

From the second experiment, proline application also significantly increased different plant parameters and grain and straw yield of BR23 (Table 2). It was found that 100 mM proline application at seedling and vegetative stage achieved the highest plant height among all the treatments. The number of effective tillers per hill, thousand grains weight and panicle length was the highest when the plants were treated with 50 mM proline at both seeding 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.

3.2. Grain and straw yields

Soil salinity caused significant reduction in grain and straw yields of rice. It is evident from the present experiment that addition of organic manure and exogenous application of proline increased both grain and straw yields of rice. From Figure 1, it was found that the highest grain yield of BR23 was found for application of PM 8 t ha^{-1} while FYM 10 t ha^{-1} and PM 4 t ha^{-1} showed same grain yield while the lowest grain yield was produced in control. The highest straw yield was found in PM 8 t ha^{-1} and the lowest was in control. The similar observations were also found by Lakhdar *et al.* 2008 and Tejada *et al.* 2006 who also concluded that soil salinity problem could be ameliorated by the addition of organic matter and yield of crops increased due to addition of organic manure and this was probably due to higher uptake of nutrients by plants. Sharma (1997) reported that application of FYM alone or in combination with inorganic amendments increased the grain yield of wheat and rice in salt affected soils. Haq *et al.* (2001) reported that combined application of gypsum and FYM produced the highest rice yield in a saline–sodic soil.

Proline application also significantly increased both grain and straw yields of rice (Figure 2). Application of 50 mM proline at seedling and vegetative stages produced the highest grain and straw yields of BR23 and the lowest was found in 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. Miah *et al.* (1992) on two rice varieties also found that salinity decreased straw yield of rice. Talat *et al*. (2013) observed that the exogenous application of proline significantly ameliorated the harmful effects of salt stress on wheat.

Table 1. Effect of FYM and PM on growth and yield component of BR23 under saline condition.

Treatments	Plant height (cm)	No. of effective tillers hill^{-1}	Panicle length (cm)	No. of grains panicle^{-1}	1000-grain weight (g)
T_0	143.0b	13.93b	21.67c	141.5d	29.27b
T_1	144.3b	16.93a	22.67bc	149.1cd	28.73b
T_2	145.0ab	18.33a	25.00a	193.5a	29.60ab
T_3	146.0ab	16.73a	24.00ab	153.5c	29.00b
T_4	147.7a	17.40a	24.67a	164.1b	30.30a
SE (±)	0.883	0.819	0.458	2.90	0.272

Same letter in a column represents insignificant difference at $p< 0.05$.
Where, T_0= Control (no manure), T_1= FYM (5 t ha^{-1}), T_2= FYM (10 t ha^{-1}), T_3= PM (4 t ha^{-1}), T_4= PM (8 t ha^{-1}).

Table 2. Effect of proline on growth and yield component of BR23 under saline condition.

Treatments	Plant height (cm)	No. of effective tillers hill^{-1}	Panicle length (cm)	No. of grains panicle^{-1}	1000-grain weight (g)
T_0	142.0b	14.40c	22.00d	163.7ab	28.21d
T_1	144.0ab	14.93bc	23.67bc	156.9b	27.96 d
T_2	143.0b	15.20bc	23.00cd	141.7c	29.96abc
T_3	143.3ab	15.03bc	23.33cd	125.3d	30.12ab
T_4	143.0b	14.20c	23.00cd	167.5a	27.63d
T_5	142.0b	12.00d	22.33cd	167.7a	29.17bcd
T_6	144.0ab	16.53a	25.33a	130.6d	31.12a
T_7	142.3b	14.87bc	22.67cd	146.1c	28.99bcd
T_8	143.3ab	15.20bc	22.67cd	121.4d	28.25cd
T_9	145.3a	15.73ab	25.00ab	145.3c	29.06bcd
SE (±)	0.654	0.357	0.476	3.24	0.524

Same letter in a column represents insignificant difference at $p< 0.05$.
Where, 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, T_9 = 100 mM proline at seedling and vegetative stages.

Table 3. Effect of organic manure on nutrient uptake and K^+/Na^+ ratio of BR-23 rice under salinity conditions.

Treatment	Total N uptake (kg/ha)	Total P uptake (kg/ha)	Total S uptake (kg/ha)	K^+/Na^+ ratio (grain)	K^+/Na^+ ratio (straw)
T_0	75.80c	15.89c	15.47c	17.24c	3.11b
T_1	82.30b	18.86b	17.27b	20.69b	3.55ab
T_2	87.95a	19.43ab	17.83b	21.43b	3.73a
T_3	87.74a	20.11a	18.11ab	24.07a	4.06a
T_4	88.54a	20.58a	19.17a	24.07a	4.03a
SE(±)	2.82	1.56	1.16	2.03	0.48

Same letter in a column represents insignificant difference at $p< 0.05$.
Where, T_0= Control (no manure), T_1= FYM (5 t ha^{-1}), T_2= FYM (10 t ha^{-1}), T_3= PM (4 t ha^{-1}), T_4= PM (8 t ha^{-1}).

Table 4. Effect of proline on nutrient uptake and K^+/Na^+ ratio of BR-23 rice under salinity conditions.

Treatment	Total N uptake (kg/ha)	Total P uptake (kg/ha)	Total S uptake (kg/ha)	K^+/Na^+ ratio (grain)	K^+/Na^+ ratio (straw)
T_0	75.58d	15.85d	15.51c	17.24d	3.11b
T_1	89.90ab	18.70c	17.88b	21.43c	3.74a
T_2	86.52c	18.36c	17.43b	23.08ab	3.82a
T_3	87.95bc	19.66bc	18.02ab	22.38bc	3.79a
T_4	86.21c	19.78bc	17.60b	22.38bc	3.82a
T_5	86.34c	19.21bc	17.45b	24.25a	3.61ab
T_6	89.18ab	20.39ab	18.63ab	22.55bc	3.68a
T_7	90.21a	21.93a	17.87b	22.25bc	3.70a
T_8	88.16ab	20.48ab	17.91b	24.25a	3.75a
T_9	90.45a	21.95a	19.07a	24.07a	3.84a
SE(±)	2.36	1.24	1.07	1.14	0.45

Same letter in a column represents insignificant difference at $p < 0.05$.

Where, 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, T_9 = 100 mM proline at seedling and vegetative stages.

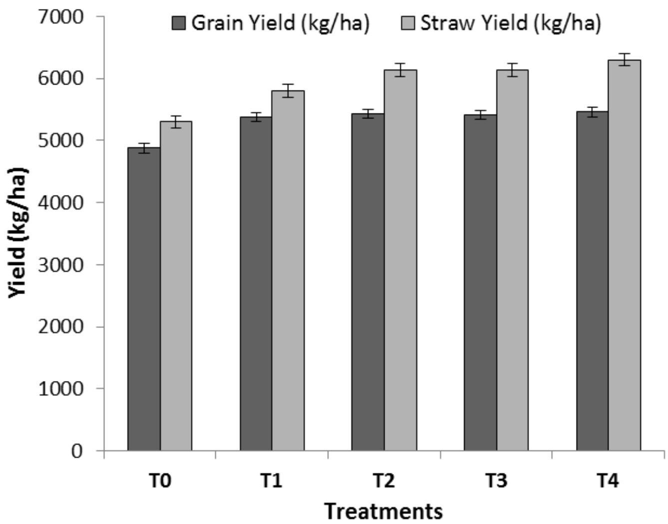

Figure 1. Effect of organic manure on the grain and straw yields of BR23 under saline condition.

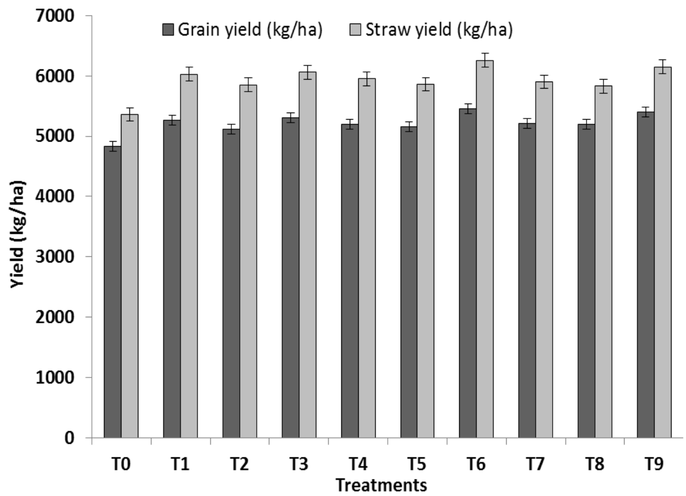

Figure 2. Effect of proline on the grain and straw yields of BR23 under saline condition.

3.3. Total nutrient uptake

We investigated whether organic manure and exogenous application of proline influenced nutrient uptake by rice under salt stress. To investigate the nutrient uptake by rice, we measured nutrient content in grain and straw of rice. Salt stress caused reductions in nutrient (NPS) uptake by grain and straw of rice. On the other hand, application of organic manure (FYM and PM) and exogenous proline increased nutrient (NPS) uptake by rice under saline conditions. Addition of organic manure resulted in an increase in nutrient uptake by rice under saline condition. From Table 3, it was found that total N (88.54 kg/ha), P (20.58 kg/ha) and S (19.17 kg/ha) uptake was the highest when soil was amended with 8 t ha^{-1} poultry manure while the lowest was found from untreated control and it was N (75.80 kg/ha), P (15.89 kg/ha) and S (15.47 kg/ha). There are evidences that organic manures reduce the adverse effects of various stresses on plants by affecting the uptake and accumulation of inorganic nutrients (Zaki *et al*. 2009. Abou El-Magd *et al.* 2008).

Exogenous application of proline also showed higher uptake of nutrients under saline condition (Table 4). The highest total N (90.45 kg/ha), P (21.95 kg/ha) and S (19.07 kg/ha) uptake was found when the plants were treated with 100 mM proline at both seedling and vegetative stages and the lowest nutrient (NPS) uptake was found in untreated control plants. 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.

3.4. 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 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 straw samples of rice. Addition of organic manure (FYM and PM) and foliar application of proline resulted in a significant increase in K^+/Na^+ ratio in rice. Organic manure application significantly increased K^+/Na^+ ratio in grain and straw of BR23 under stress condition. All rates of organic manure application increased the K^+/Na^+ ratio in grain and straw. In grain the highest K^+/Na^+ ratio was found by both in PM (4 t ha^{-1}) and PM (8t ha^{-1}) and lowest in control. In straw K^+/Na^+ ratio was observed highest at PM 4t ha^{-1} and lowest was observed in control (Table 3). Shazia *et al.* (2004) also found that salinity decreased K^+/Na^+ ratio and it could be increased by organic matter amendment in soil.

All the doses of proline increased the K^+/Na^+ ratio in grain and the same trends were found in straw (Table 4). The higher K^+/Na^+ ratio in grain was found when the plants were treated with 50 mM proline application at vegetative stage and 100 mM application at vegetative stage. A slight variation in straw K^+/Na^+ ratio was observed among the doses of proline application. Proline application at different doses showed insignificant difference in K^+/Na^+ ratio of rice straw samples but significant difference was observed between proline treated and untreated plants. The highest K^+/Na^+ ratio of rice straw was found when the plants were treated with 100 mM proline at seedling and vegetative stages. 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.

4. Conclusions

Salt stress caused a significant decrease in growth and yield of rice. Foliar application of proline and organic manures both FYM and PM significantly improved growth and yield of rice by mitigating the inhibitory effects of salinity stress. Application of poultry manure (8 t ha^{-1}) or farm yard manure (10 t ha^{-1}) showed higher yield of rice. Poultry manure showed higher yield performance than FYM application. Besides, 100 mM proline application at both seedling and vegetative stages showed better yield performance compared to other treatments. In addition to field research work, better understanding about physiological and bio-molecular mechanisms of proline will be helpful for plant breeders to develop stress-tolerant crop varieties. On the other hand, organic manures like FYM and PM are more available and less expensive.

Acknowledgements

This work was supported by a grant from the Bangladesh Agricultural Research Council, Dhaka. The authors are grateful to Professor Dr. Yoshiyuki Murata, Okayama University, Japan for the generous gift of proline

Conflict of interest

None to declare

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