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SOIL AMENDMENTS WITH FARM YARD MANURE AND POULTRY MANURE CONFER TOLERANCE TO SALT STRESS IN RICE (*Oryza sativa* L.)

Md. Zulfiker Alam, Debasish Kumar Das, Md. Abul Hashem and Md. Anamul Hoque*

Department of Soil Science, Faculty of Agriculture, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

*Corresponding author: Md. Anamul Hoque; E-mail: anamul71@yahoo.com

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ABSTRACT

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Salinity causes unfavorable environment that restricts normal crop production. Organic amendments could contribute to the improvement of crop production in coastal areas. Two rice varieties viz. BRRI dhan29 (salt-sensitive) and Binadhan-8 (salt-tolerant) were grown in replicated pots to investigate the mitigation potential of salt stress in rice by organic amendments. Two doses of farm yard manure (FYM: 5 and 10 t ha⁻¹) and poultry manure (PM: 4 and 8 t ha⁻¹) were mixed with soils before transplanting. Rice plants were exposed to different concentrations of NaCl (25 and 50 mM) at active tillering stage. Salt stress caused a significant reduction in growth and yield of both rice varieties. Salt stress also decreased reproductive growth, chlorophyll contents, K⁺/Na⁺ ratio, nutrient contents and nutrient uptake in both rice varieties. Salinity caused a significant increase in intracellular proline content in BRRI dhan29 but a decrease in Binadhan-8. Organic amendments with FYM and PM resulted in an increase in growth and yield components, chlorophyll content, K⁺/Na⁺ ratio and nitrogen (N) uptake. No plants of BRRI dhan29 survived at 50 mM NaCl stress even after addition of FYM and PM. On the other hand, Binadhan-8 conferred tolerance to 50 mM NaCl stress when soils were amended with organic sources, suggesting that cultivation of Binadhan-8 might be profitable in saline affected areas with organic amendments. The present study suggests that organic amendments with FYM and PM confer tolerance to salinity in rice by increasing chlorophyll content, K⁺/Na⁺ ratio and N uptake.

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www.agroaid-bd.org/ralf, E-mail: editor.ralf@gmail.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 (FAO 2008). Out of 2.86 million ha of coastal and offshore lands in Bangladesh, about 1.056 million ha 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 varieties and inappropriate management practices. Salinity imposes both ionic toxicity and osmotic stress to plants (Zhu 2003). Salt stress disturbs cytoplasmic K^+/Na^+ homeostasis, causing an increase in Na^+ to K^+ ratio in the cytosol (Zhu 2003). Accumulation of excess Na^+ and Cl^- causes ionic imbalances that may impair the selectivity of root membranes and induce K^+ deficiency (Gadallah 1999) that are often associated with a decrease in photosynthetic electron transport activities in photosynthesis.

It has been reported that soil fertility in coastal regions of Bangladesh are quite low (Haque 2006). Appropriate management strategies and techniques with suitable genotypes having higher yield potential could contribute to the improvement of crop production in the coastal areas of Bangladesh. The best means of maintaining soil fertility, productivity and salt tolerance could be addition of organic manures (Das *et al.* 2013). Various organic amendments such as FYM, PM, compost and mulch can be used for the amelioration of saline soils. Organic amendments improve physical, chemical and biological properties of soils under saline conditions. There are evidences that soil amendments with organic manures reduce the toxic effects of salinity in various plant species (Idrees *et al.* 2004, Abou El-Magd *et al.* 2008, Leithy *et al.* 2010, Raafat and Thawrat 2011). The role of 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.

MATERIALS AND METHODS

Experimental site

The pot experiments were carried out at the net-house of the Department of Soil Science, Bangladesh Agricultural University (BAU), Mymensingh to investigate the mitigation of the adverse effects of soil salinity on the growth and yield of rice through organic amendments.

Soils and pot preparation

Soils were collected from the Soil Science Field Laboratory, BAU. Each 15 L plastic pot was filled with 8 kg soil and 5 L of water. Soil was silt loam texture having pH 6.15, electrical conductivity 0.17 dS m^{-1} , exchangeable Na $0.35 \text{ meq } 100 \text{ g}^{-1}$ soil, total N 0.11% and organic matter 1.90%.

Experimental design, crops and treatments

The experiment was laid out in a completely randomized design with four replications. Two rice varieties viz. BRRI dhan29 (salt-sensitive) and Binadhan-8 (salt-tolerant) were used as test crops. Rice seedlings were transplanted in pots. FYM and PM were mixed with soils before transplanting as per treatment. Plants were exposed to different concentrations of NaCl (0-50 mM) at active tillering stage. There were eleven treatment combinations such as control (no salt added), 25 mM NaCl, 50 mM NaCl, 25 mM NaCl + 5 t/ha FYM, 25 mM NaCl + 10 t/ha FYM, 50 mM NaCl + 5 t/ha FYM, 50 mM NaCl + 10 t/ha FYM, 25 mM NaCl + 4 t/ha PM, 25 mM NaCl + 8 t/ha PM, 50 mM NaCl + 4 t/ha PM, 50 mM NaCl + 8 t/ha PM.

Fertilization, management practices, crop harvesting and data recorded

Fertilization and other management practices were performed as and when required (BARC, 2012). The crops were harvested at full maturity. Plant growth, yield attributes and grain and straw yields were recorded.

Assay of chlorophyll content

Chlorophyll content was measured according to Porra *et al.* (1989). An aliquot of green leaf of maize 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 (5000 rpm) for 15 min and the absorbance was recorded at 645 nm and 663 nm in a spectrophotometer

Assay of intracellular proline contents

Proline content was measured according to the method of Bates *et al.* (1973). An aliquot of green leaf of rice was collected before panicle initiation and homogenized in 10 ml of 3% sulfosalicylic acid. Then the homogenate was centrifuged at 5000 rpm for 15 min (NF 800, Nuve, Turkey). Two milliliters of the supernatant were reacted with 2 ml of acid ninhydrin (1.25 g ninhydrin dissolved in 30 ml of glacial acetic acid and 20 ml of 6 M phosphoric acid) and 2 ml of glacial acetic acid for 1 hr at 100 °C and the reaction was then terminated in an ice bath. The colored reaction mixture was extracted with 4 ml of toluene and the absorbance was recorded at 520 nm. Proline content was calculated from a standard curve.

Assay of N, P, K, S and Na contents

The N, P, K and S contents were measured from grain and straw samples following standard methods as described by Khanam *et al.* (2001).

Statistical analysis

Data were analyzed statistically using analysis of variance (ANOVA) with the help of software package MSTAT-C to examine the treatment effects. The mean differences were adjudged by Duncan's Multiple Range Test (DMRT) and ranking was indicated by letters.

RESULTS AND DISCUSSION

Growth and yield of rice

Salt stress caused a significant reduction in growth and yield of both salt-sensitive and salt-tolerant rice varieties (Tables 1-3). Higher concentration of salt stress (50 mM) caused death of plant at early stage in salt-sensitive rice (cv. BRRI dhan29). Conversely, plants of salt-tolerant rice (cv. Binadhan-8) survived at high concentration of salt stress but did not produce grains. Organic amendments with both FYM and PM resulted in significant increase in growth and yield of both rice varieties under salt stress conditions. It is surprising that high dosage of either FYM or PM caused a toxic effect on growth of rice under salt stress (Tables 1-2). However, Binadhan-8 conferred tolerance to significantly higher salinity than BRRI dhan29 when soils were amended with FYM or PM. FYM performed better in increasing plant growth of both varieties than that of PM at 25 mM NaCl stress condition (Tables 1-2). Similar results were observed by several authors, suggesting that soil amendments with organic manures alleviate the toxic effects of salinity in plants (Idrees *et al.* 2004, Abou El-Magd *et al.* 2008, Leithy *et al.* 2010, Raafat and Thawrat 2011).

Chlorophyll contents

Chlorophyll is one of the most important pigment components of a plant. Photosynthesis is the harbor of the plant which occurs in green part of the plant. Due to salt stress the chlorophyll content may vary with varying salt concentration and effect on the plant growth and development. No significant variation in chlorophyll-a content in BRRI dhan29 was observed in response to salt stress whereas chlorophyll-b and total chlorophyll contents decreased significantly due to salt stress (Table 4). Salt stress caused significant decrease in chlorophyll-a, chlorophyll-b and total chlorophyll contents in Binadhan-8. On the other hand, both manures FYM and PM resulted in an increase in chlorophyll contents in both rice varieties (Table 4). Islam *et al.* (2007), after experimentation with three rice genotypes, also showed that total chlorophyll content was reduced by the salinity treatments. Ali *et al.* (2004) also found reduction in chlorophyll content by salinity in eighteen advanced rice genotypes. On the other hand, Niazi *et al.* (2002) have shown that FYM increases protein and chlorophyll contents and fresh and dry weights of plant in saline-sodic soil.

Table 1. Effects of organic manures on the growth of BRR1 dhan29 under salt stress

Treatments	Plant height (cm)	Effective tillers hill ⁻¹	Panicle length (cm)	Filled grains panicle ⁻¹	1000-grain weight (g)
Control	92.0a	20.0a	25.0b	141.0a	18.80b
25 mM NaCl	73.5d	9.0e	23.0c	119.0d	17.5c
50 mM NaCl	51.0g	-	-	-	-
25 mM NaCl + FYM (5 t ha ⁻¹)	88.5ab	17.0b	26.3ab	136.0 b	18.7b
25 mM NaCl + FYM (10 t ha ⁻¹)	89.7a	13.0d	27.0a	139.0ab	18.8b
50 mM NaCl + FYM (5 t ha ⁻¹)	48.3g	-	-	-	-
50 mM NaCl + FYM (10 t ha ⁻¹)	56.3f	-	-	-	-
25 mM NaCl + PM (4 t ha ⁻¹)	82.5c	14.0c	25.3b	128.0c	20.8a
25 mM NaCl + PM (8 t ha ⁻¹)	84.3bc	14.0c	26.5ab	128.0c	19.5b
50 mM NaCl + PM (4 t ha ⁻¹)	57.2f	-	-	-	-
50 mM NaCl + PM (8 t ha ⁻¹)	64.3e	-	-	-	-
SE(±)	5.02	2.42	4.03	20.83	3.00
CV%	4.38	6.02	7.28	4.18	5.55

(-) indicates no plants survived during the data recording; Same letter in a column represents insignificant difference at $p < 0.05$; SE = Standard errors of means, CV = Co-efficient of variation

Table 2. Effects of organic manures on the growth of Binadhan-8 under salt stress

Treatments	Plant height (cm)	Effective tillers hill ⁻¹	Panicle length (cm)	Filled grains panicle ⁻¹	1000-grain weight (g)
Control	116.0 a	22.0a	26.7ab	135.0a	24.1a
25 mM NaCl	93.4c	8.0c	24.5c	81.0f	20.2b
50 mM NaCl	55.0f	-	-	-	-
25 mM NaCl + FYM (5 t ha ⁻¹)	97.5b	14.0b	27.3a	123.0b	18.5cd
25 mM NaCl + FYM (10 t ha ⁻¹)	98.7b	14.0b	26.3ab	118.0c	18.8cd
50 mM NaCl + FYM (5 t ha ⁻¹)	68.0e	7.0c	22.5d	100.0e	17.9de
50 mM NaCl + FYM (10 t ha ⁻¹)	56.0f	-	-	-	-
25 mM NaCl + PM (4 t ha ⁻¹)	81.3d	13.0b	25.7bc	112.0d	19.2bc
25 mM NaCl + PM (8 t ha ⁻¹)	83.0d	14.0b	25.4bc	110.0d	18.3cde
50 mM NaCl + PM (4 t ha ⁻¹)	52.3fg	7.0c	22.60d	82.0f	17.2e
50 mM NaCl + PM (8 t ha ⁻¹)	48.5g	-	-	-	-
SE(±)	6.85	2.16	3.57	15.90	2.77
CV%	3.60	11.41	5.45	3.92	5.56

(-) indicates no plants survived during the data recording; Same letter in a column represents insignificant difference at $p < 0.05$; SE = Standard errors of means, CV = Co-efficient of variation

Table 3. Straw and grain yields of rice influenced by organic manures under salt stress

Treatments	BRR1 dhan29		Binadhan-8	
	Straw yield (g plant ⁻¹)	Grain yield (g plant ⁻¹)	Straw yield (g plant ⁻¹)	Grain yield (g plant ⁻¹)
Control	49.3a	45.3a	57.1a	45.0a
25 mM NaCl	15.0f	20.1e	15.5e	16.5c
50 mM NaCl	4.51h	-	2.79h	-
25 mM NaCl + FYM (5 t ha ⁻¹)	41.8b	34.9b	32.2c	30.2b
25 mM NaCl + FYM (10 t ha ⁻¹)	28.2e	28.1d	34.9b	32.2b
50 mM NaCl + FYM (5 t ha ⁻¹)	5.26gh	-	13.8ef	18.6c
50 mM NaCl + FYM (10 t ha ⁻¹)	6.24g	-	5.41g	-
25 mM NaCl + PM (4 t ha ⁻¹)	32.9c	35.9b	29.3d	32.7b
25 mM NaCl + PM (8 t ha ⁻¹)	30.0d	32.4c	29.6d	31.4b
50 mM NaCl + PM (4 t ha ⁻¹)	6.16g	-	12.7f	17.7c
50 mM NaCl + PM (8 t ha ⁻¹)	5.17gh	-	4.85g	-
SE (±)	1.32	1.69	1.98	2.60
CV (%)	5.00	5.46	5.00	4.66

(-) indicates data not detected; Same letter in a column represents insignificant difference at $p < 0.05$. SE= Standard errors of means, CV=Co-efficient of variation

Proline contents

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 (Ashraf and Foolad 2007). Proline is the most common compatible solute that occurs in a wide variety of plants. Increased levels of proline accumulated in plants correlate with enhanced salt tolerance (Ashraf and Foolad 2007). Binadhan-8 accumulated higher intracellular proline than BRR1 dhan29 (Table 5). Salinity caused a significant increase in intracellular proline content in BRR1 dhan29 but decrease in Binadhan-8. Neither FYM nor PM influenced intracellular proline contents in BRR1 dhan29 under salt stress. On the other hand, all amendments except PM (4 t ha⁻¹) did not influence intracellular proline contents in Binadhan-8 under salt stress (Table 5). Abou El-Magd *et al.* (2008) conducted pot experiments on sweet fennel variety "Dulce" with the combination of two levels of organic manure and saline water treatments, and results indicated that organic manure treatment increased proline contents of bulbs.

Nutrient uptake

Inorganic nutrients such as N, P, K and S play essential role 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. In our experiments, salinity caused a decrease in nutrient uptake (N, P and S) by both rice varieties. In some cases, an increase in N uptake was observed in response to FYM or PM under salt stress (data not shown). 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).

Table 4. Chlorophyll contents in rice influenced by organic manures under salt stress

Treatments	BRRi dhan29			Binadhan-8		
	Chl a ($\mu\text{g ml}^{-1}$)	Chl b ($\mu\text{g ml}^{-1}$)	Total Chl ($\mu\text{g ml}^{-1}$)	Chl a ($\mu\text{g ml}^{-1}$)	Chl b ($\mu\text{g ml}^{-1}$)	Total Chl ($\mu\text{g ml}^{-1}$)
Control	5.51abc	9.98a	15.5a	5.40c	8.98c	14.4c
25 mM NaCl	5.66ab	7.20c	12.9c	4.96d	6.64e	11.4e
50 mM NaCl	-	-	-	-	-	-
25 mM NaCl + FYM (5 t ha ⁻¹)	5.45bc	10.2a	15.5a	5.48c	8.75c	14.2c
25 mM NaCl + FYM (10 t ha ⁻¹)	5.90a	9.0b	14.9ab	6.97a	8.83c	15.8b
50 mM NaCl + FYM (5 t ha ⁻¹)	-	-	-	7.27a	10.7a	17.9a
50 mM NaCl + FYM (10 t ha ⁻¹)	-	-	-	-	-	-
25 mM NaCl + PM (4 t ha ⁻¹)	5.29bc	8.58b	13.9bc	5.35c	7.37d	13.0d
25 mM NaCl + PM (8 t ha ⁻¹)	5.19c	8.81b	14.0bc	5.45c	9.75b	15.2b
50 mM NaCl + PM (4 t ha ⁻¹)	-	-	-	6.38b	7.01d	13.4d
50 mM NaCl + PM (8 t ha ⁻¹)	-	-	-	-	-	-
SE (\pm)	0.879	1.43	2.29	0.86	1.25	2.09
CV (%)	9.20	7.40	11.04	6.09	5.70	5.07

(-) indicates data not detected; Same letter in a column represents insignificant difference at $p < 0.05$.
SE= Standard errors of mean, CV=Co-efficient of variation

Table 5. Proline contents and K⁺/Na⁺ ratio in rice influenced by organic manures under salt stress

Treatments	BRRi dhan29			Binadhan-8		
	Proline contents (mM)	K ⁺ /Na ⁺ (straw)	K ⁺ /Na ⁺ (grain)	Proline contents (mM)	K ⁺ /Na ⁺ (straw)	K ⁺ /Na ⁺ (grain)
Control	4.930b	1.06a	1.70a	7.83a	0.66b	2.33a
25 mM NaCl	6.32a	0.61e	1.13d	4.43c	0.37fg	1.87c
50 mM NaCl	-	0.26g	-	-	0.33g	-
25 mM NaCl + FYM (5 t ha ⁻¹)	3.46e	0.70de	1.25cd	3.28e	0.50e	2.14b
25 mM NaCl + FYM (10 t ha ⁻¹)	4.36c	0.75cd	1.29c	4.01d	0.48e	2.13b
50 mM NaCl + FYM (5 t ha ⁻¹)	-	0.33fg	-	3.50e	0.38f	1.86c
50 mM NaCl + FYM (10 t ha ⁻¹)	-	0.32fg	-	-	0.38f	-
25 mM NaCl + PM (4 t ha ⁻¹)	3.91d	0.91b	1.31c	5.08 b	0.60c	2.06b
25 mM NaCl + PM (8 t ha ⁻¹)	4.32c	0.81c	1.46b	4.51 c	0.81a	2.28a
50 mM NaCl + PM (4 t ha ⁻¹)	-	0.34fg	-	4.31cd	0.55d	1.87c
50 mM NaCl + PM (8 t ha ⁻¹)	-	0.40f	-	-	0.48e	-
SE (\pm)	0.75	.083	0.21	0.74	0.044	0.295
CV (%)	9.50	10.30	13.58	7.82	6.48	3.90

(-) indicates data not detected; Same letter in a column represents insignificant difference at $p < 0.05$.
SE= Standard errors of mean, CV=Co-efficient of variation

K⁺/Na⁺ ratio

Salt stress disturbs cytoplasmic K⁺/Na⁺ homeostasis, causing an increase in Na⁺ to K⁺ ratio in the cytosol (Zhu 2003). 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. Salt stress caused a significant decrease in K⁺/Na⁺ ratio in both straw and grain in both salt-sensitive and salt-tolerant varieties (Table 5). Organic amendments with both FYM and PM significantly increased K⁺/Na⁺ ratio in

both straw and grain at 25 mM NaCl stress. At 25 mM NaCl stress, both manures increased K^+/Na^+ ratio in straw but PM showed a significant higher ratio than FYM under 50 mM NaCl stress in BRRI dhan29. PM performed better in increasing K^+/Na^+ ratio than FYM in both varieties (Table 5). Idrees *et al.* (2004) also found that salinity decreased K^+/Na^+ ratio and it could be increased by organic matter amendment in soil. Leithy *et al.* (2010) reported that biofertilizer and compost application under saline conditions did not show any positive change in N, P, K contents except for Na%.

CONCLUSION

Soil amendments with FYM and PM increase chlorophyll content, K^+/Na^+ ratio and nitrogen uptake by rice and thereby conferring tolerance to salinity. However, rice cultivation in saline areas might be profitable with organic amendment of soils. Extensive field research work is needed in this area since organic manures like FYM and PM are more available and less expensive.

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