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Morphological and Anatomical Changes in Four Rice Varieties at Germination Phase under Different Saline Conditions

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ABSTRACT

Received date: Sep 22, 2024 Accepted date: Dec 15, 2024 Rice (Oryza sativa L.) serves as a staple food worldwide particularly in Asia but its production is significantly impacted by salinity stress especially during the germination stage. The present experiment was conducted to assess the effects of salinity on four rice varieties: Bina dhan-10, Bina dhan-8, BRRI dhan99 and BRRI dhan67. Four saline treatments (0, 40, 80 and 120 mM NaCl) were examined in seed germination at laboratory condition. The anatomical changes in saline treated samples were observed in the hand sections of root after 1% safranine staining. Results showed that BRRI dhan99 had the highest seed germination percentage (100%) under control condition (0 mM NaCl). For other varieties, seed germination percentages were considerable in control condition. The length of seedling was best for Binadhan-10 in control condition. For the other conditions, seed germination percentages, plumule length, redical length and seedling length were decreased with the increasing of NaCl concentration. The changes in root features in treated samples for all varieties were determined in the cortex and stele areas. The chemical substances depositions in the cell corners were found in cortex of NaCl solution treated roots. The stele area was larger in root sections growing treatment conditions. The metaxylem number was increased and xylem distribution prominent in case of root sections growing in different concentrations of NaCl. This study highlights the anatomical and morphological responses of rice varieties tested to different saline conditions that will be helpful for rice growers and the further higher studies.

Keywords: Anatomical responses, Morphological responses, NaCl treatment, Rice variety, Salinity, Seed germination

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1. INTRODUCTION

Rice (*Oryza sativa* L.) is the main staple food of about 3.5 billion people worldwide (Muthayya et al., 2014). Rice is primary compost of carbohydrate that makes up almost 80% of its total dry weight. It also contains about 7-8% of protein small quantity of fats and also the source of the thiamine,

niacin, riboflavin, iron, and calcium (Gul et al., 2015). Bangladesh ranks as third largest production of rice globally, reaching about 57.2 million tons. Rice is contributing about 16% to the overall gross domestic product (GDP) of the nation and about 70% to agricultural GDP (Dey, 2020). By understanding the structure and function of various parts of the rice plant, researchers and farmers can make

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informed decisions that lead to better crop management and sustainability in rice production. Elevated osmotic stress, ions toxicity (due to over accumulation of Na+ in particular), imbalance nutrition and salinity, induced oxidative damages are the principal causes to hamper plants growth under salinity (Pitman and Lauchii, 2002). El- Bassiouny and Bakheta (2001) higher Na⁺ contents may damage the membranes and organelles leading to growth diminution and unusual development prior to plant mortality. On the earth surface, there are about 380 million hectares of soil are saline prone and these are widely distributed in arid and semi-arid as well as seasonally dry coastal areas (Mansuri et al., 2012). Plant growth is adversely affected due to the excessive concentration of soluble salt in the arid and semi-arid regions (Aref and Rad, 2012). Currently 2.8 millions of rice fields are currently affected by salinity (Islam et al., 2012).

There are so many reasons behind increasing the salinity in soil. Among them, one of the major causes is that removal of the permanent deep-rooted vegetation in non-irrigated land that causes rising of water table and salts to accumulate low laying areas. Salinity causes osmotic pressure and lessen water uptake into plant roots (as less water is taken up, cell expansion growth slows down and stomata close, ultimately affecting photosynthesis). Out of the coastal cultivable saline area, about 328 (31%), 274 (26%), and 190 (18%) thousand actors of land are affected by very slight (20-40 mM NaCl), slight (41-80 mM NaCl), and moderate salinity (81-120 mM NaCl), respectively are scope to successfully crop production in Bangladesh (SRDI, 2010). According to Soil Resource Development Institute (SRDI), the last few years is affecting an average of 6200ha of fresh cultivable land every year. One research found that, among 39102 ha of cropping land in the Kolapata coastal belt, 92% of that area was identified as serenity affected in dry season production (Alam et al., 2017). In coastal Bangladesh 1% increase in salinity reduces rise yield by 0.08% (Nahar and Hamid, 2016), while rice yield loses of up to 50% are due to high salinity (Islam et al., 2021). The severity of salinity of this area increases with the desiccation of the soil.

Currently, salinity is one of the most significant risks to rice production and reduces crop yield and thus economically harmful. In addition, particularly improvements of yield potential and genetical qualities as well as development of salt tolerant rice varieties are crucial in overcoming the problem of salt stress. The selection and adoption of high-salt tolerance varieties have always been the preferred choice to improve productivity in salt affected soils. The present study was done to observe the germination performance, morphological and anatomical traits of different rice varieties under salinity stress conditions of rice seedling.

2. MATERIALS AND METHODS

The experiment was conducted at the Crop Botany lab in EXIM Bank Agricultural University Bangladesh, Chapainawabganj, Bangladesh. Among four (04) saline tolerant varieties studied here, two (02) varieties were collected from Bangladesh Institute of Nuclear Agriculture

(BINA) and rest two (02) from Bangladesh Rice Research Institute (BRRI). The varieties are Binadhan-1(V1), Binadhan-8(V2), BRRI dhan99 (V3), BRRI dhan67 (V4).

For, this experiment, Completely Randomized Design (CRD) was selected set with two factors using three replications. Here, Factor 1: Four rice varieties (Binadhan-10, Binadhan-8, BRRI dhan99, BRRI dhan67) and Factor 2: Salinity level 0, 4, 8, 12dsm⁻¹ (Deci siemens per meter)/0 mM NaCl, 40 mM NaCl, 80 mM NaCl, 120 mM NaCl.

Number of individuals was 1200 that was calculated with 4 varieties, 3 replications, 4 treatments and 25 seeds. Total number of experimental plot was 48 that considered each petridishes. The four varieties were randomly assigned to 4 petri-dishes of each petri dish contained 25 seeds. Rice seeds were treated 6 hours with fungicide (Carbendazim) and then washed with tap water. The washed seeds were soaked overnight in water and then placed in petri dish on sterilized tissue paper to permit them to develop in room temperature and the wet condition was maintained sprayed with distilled water. Germination percentage, seedling length, plumule length and radicle length, fresh weight of seedling, dry weight of seedling were deliberated as morphological parameters after 10 days of germination.

Data collection covered the period from the emergence of the shoot, distinct parameters were measured and preserved. Statistical analysis was performed using Excel and Statistics 10, incorporating ANOVA and correlation analysis.

3. RESULTS AND DISCUSSION

3.1. Effects of Salinity Stress on Plumule Length, Radicle Length and Seedling Length

The plumule length were recorded 7.40cm, 8.40cm, 5.50cm, 7.43cm for Binadhan-10, Binadhan-8, BRRI dhan99, BRRI dhan67 in control condition, respectively (Table 1). In four different conditions examined, 0mM NaCl (control) shows the highest length among the other treatment 40mM NaCl, 80mM NaCl and 120mM NaCl for all varieties tasted. The 5.50 cm, 6.57 cm, 5.33 cm and 4.67 cm seedlings length were observed in 40 mM NaCl treatment for Binadhan-10, Binadhan-8, BRRI dhan99 and BRRI dhan67 respectively. In case of 80 mM NaCl, 4.60 cm, 5.60 cm, 4.30 cm and 6.20 cm where as in 120 mM NaCl treatments, 3.33 cm, 3.33 c m, 2.47 cm and 4.47 cm for Binadhan-10, Binadhan-8, BRRI dhan99 and BRRI dhan67 were observed respectively (Table 1). The radicle length were recorded in control condition 5.27 cm, 3.67 cm, 4.88 cm and 3.27cm for Binadhan-10, Binadhan-8, BRRI dhan99, BRRI dhan67 respectively (Table 1). The 0 mM (control) NaCl shows the higher length than the other length in treatment of 40 mM NaCl, 80 mM NaCl and 120 mM NaCl for all varieties tested. The 4.73cm, 3.63 cm, 3.27 cm, 2.90 cm radical length were observed in 40 mM NaCl treatment for Binadhan-10, Binadhan-8, BRRI dhan99 and BRRI dhan67 respectively (Fig.1). In case of 80 mM NaCl, 3.03 cm, 3.07 cm, 2.23 cm and 1.50 cm for Binadhan-10, Binadhan-8, BRRI dhan99 and BRRI dhan67, respectively. In 120 mM NaCl treatments, 2.07 cm, 1.37 cm, 2.17cm, and 1.23 cm for Binadhan-10, Binadhan-8, BRRI dhan99 and BRRI

dhan67 respectively (Table 1). BRRI dhan99 showed highest radical length in treatment 3. Both the radical and plumule showed a decrease in length with the increase in NaCl concentration (Sabreen et al., 2018).

Among different conditions, 0mM NaCl (control) showed the higher result than the other treatment 40mM NaCl, 80mM NaCl and 120mM NaCl in length for all varieties. The seedling length were recorded in control condition 12.67 cm,11.48 cm,10.33 cm and 10.40 cm for Binadhan-10, Binadhan-8, BRRI dhan99, BRRI dhan67 respectively (Table 1). The seedlings length were observed 10.23 cm,10.20 cm,8.60 cm and 9.37 cm in 40 mM NaCl treatment for Binadhan-10, Binadhan-8, BRRI dhan99 and BRRI dhan67 respectively. In case of 80 mM NaCl, 7.63cm, 9.27cm, 6.53 cm and 7.70cm seedling length, on the other

hand in 120 mM NaCl treatments, 5.40cm,4.70 cm,5.63 cm and 6.67cm seedling length for Binadhan-10, Binadhan-8, BRRI dhan99 and BRRI dhan67 were measured respectively (Table 1). Zeng and Shannon (2000) reported that, similar trends in seedling growth in saline condition. It was noticed that, neutral conditions performed better results for all varieties in plumule, radicle and seedling lengths (Fig.1). The present study suggests that, the increasing NaCl salt concentrations in culture decreased lengths of plumule, radicle and seedling for all varieties of rice tested. Reduction in seedling growth and loss of stand due to salinity has been implicated as causative factors for yield losses in rice production (Heenan et al., 1988).

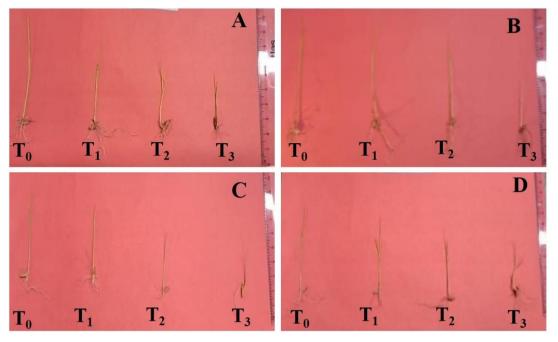


Fig.1 Shows the visualization of radical length, plumule length and seedling length comparing their growth at salinity stress with their control conditions after 10 days of culture. A) seedlings of Bina dhan-10, B) seedlings of Binadhan-8, C) seedlings of BRRI dhan99, D) seedlings of BRRI dhan67. Here, T_0 =0 mM NaCl; T_1 = 40 mM NaCl; T_2 = 80 mM NaCl; T_3 =120 mM NaCl concentration.

3.2. Effects of Salinity Stress on Germination Percentage

In the control condition, the germination percentage (%) were recorded 96%, 89.33%, 100% and 99.33% for Bina dhan-10, Bina dhan-8, BRRI dhan99 and BRRI dhan67, respectively (Fig 2). In different salinity conditions, 40 mM NaCl showed the highest percentages among the other 80 mM NaCl and 120 mM NaCl treatments for all varieties tested. It was noticed that, the lower concentration of NaCl performed the higher seed germination percentage for all varieties. The 94.67%, 77.33%, 97.33% and 96% seed germination were observed in 40 mM NaCl treatment for Bina dhan-10, Bina dhan-8, BRRI

dhan99 and BRRI dhan67, respectively. In case of 80 mM NaCl, 93.33%, 58.67%, 90.67% and 88% where as in 120 mM NaCl treatments, 86.67%, 57.33%, 82.67% and 81.33% for Bina dhan-10, Bina dhan-8, BRRI dhan99 and BRRI dhan67 respectively (Fig 2). It was suggested that, the increasing NaCl salt concentrations in culture decreased the seed germination percentages for all varieties of rice. It has been reported that, rice shows salt tolerant during germination, active tillering and toward maturity, but at the time of early vegetative stage and reproductive stage it shows susceptibility (Munns and Tester, 2008).

Table1: Comparison of rice varieties grown in saline conditions at seed germination stage

Treatments and	Plumule length	Radicle length	Seedling length	Total fresh	Total dry
varieties	(cm)	(cm)	(cm)	weight (g)	weight (g)
	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE
V_1T_0	$7.40\pm1.2b$	$5.27 \pm 0.8a$	12.67±2.1a	$0.72\pm0.06abc$	$0.53\pm0.04a$
V_1T_1	5.50±1.0e	4.73±0.4b	10.23±1.0c	0.67±0.06bcde	0.41±0.03cde
V_1T_2	$4.60\pm0.2f$	3.03±0.2e	$7.63\pm1.0g$	$0.61 \pm 0.05 defg$	0.41 ± 0.03 cde
V_1T_3	3.33±0.3h	$2.07 \pm 0.2 f$	5.40 ± 0.8 k	0.57 ± 0.03 fghi	$0.34 \pm 0.04 fgh$
V_2T_0	8.40±1.5a	3.67±0.3e	11.48±0.5b	$0.77 \pm 0.08a$	0.41 ± 0.02 cde
V_2T_1	6.57±1.1c	3.63±0.3cd	10.20±1.0c	0.72 ± 0.05 abc	$0.38 \pm 0.02 efg$
V_2T_2	$5.60\pm0.5e$	3.07±0.2cd	9.27±0.8d	0.69±0.04abcd	0.38±03efg
V_2T_3	3.33±0.8h	1.37±0.1g	4.70±0.71	$0.65 \pm 0.05 cdef$	$0.39 \pm 0.03 def$
V_3T_0	5.50±0.7e	$4.88\pm0.4ab$	10.33±0.8c	$0.60 \pm 0.04 efgh$	$0.47 \pm 0.02b$
V_3T_1	5.33±0.3e	3.27±0.2de	8.60±0.7e	$0.51 \pm 0.02ij$	0.33 ± 0.01 gh
V_3T_2	4.30f±0.6g	2.23±0.2f	6.53±0.5hi	0.43 ± 0.03 jk	0.34 ± 0.01 fgh
V_3T_3	$2.47 \pm 0.2i$	2.17±0.1e	5.63±0.7k	$0.40\pm0.03k$	0.40 ± 0.02 de
V_4T_0	7.43±1.2b	3.27±0.2de	10.40±0.5c	0.69±0.05abcd	$0.39 \pm 0.03 def$
V_4T_1	6.47±1.5cd	2.90±0.4e	9.37±0.8d	0.67±0.07bcde	$0.33 \pm 0.01 gh$
V_4T_2	6.20±1.1d	1.50±0.3g	$7.70\pm0.5g$	0.53±0.04ghi	0.31±0.02hi
V_4T_3	$4.43 \pm 0.7 fg$	1.23±0.1f	6.67±0.6h	0.53±0.02ghi	0.26±0.01i
CV%	3.65	8.70	2.33	4.55	4.48

Means followed by a similar letter (s) are not significantly different whereas, means followed by a dissimilar letter(s) are significantly different as per DMRT (5% level), CV: Co-efficient of variation. V₁, Bina dhan-10; V₂, Bina dhan-8; V₃, BRRI dhan99; V₄, BRRI dhan67; T₀, 0 mM NaCl; T₁, 40 mM NaCl; T₂, 80 mM NaCl; T₃, 120 mM NaCl.

3.3. Fresh Weight of Seedling

The total fresh weight were recorded in control condition 0.72g,0.77g,0.60gand 0.69g for Bina dhan-10,Bina dhan-8, BRRI dhan99, BRRI dhan67 respectively (Table 1) in different salinity conditions. Control (0mM) conditions of NaCl shows the highest length among the other treatment 40mM NaCl, 80mM NaCl and 120mM NaCl for all varieties tested. It was noticed that, neutral conditions performed better results for all varieties. The 0.67g, 0.72g, 0.51g and 0.67g total fresh weight were observed in 40 mM NaCl treatment for Binadhan-10, Binadhan-8, BRRI dhan99 and BRRI dhan67 respectively. NaCl0.61g,0.69g,0.43g and 0.53g fresh weight where as in 120 mM NaCl treatments, 0.57g, 0.67g, 0.40g and 0.53g fresh weight for Binadhan-10, Binadhan-8, BRRI dhan99 and BRRI dhan67 respectively (Table 1). Ueda et al. (2013) were carried out an experiment with two rice cultivars namely CFX 18 (salt-tolerant) and Juma-67 (salt-sensitive). They reported that leaf area, root-shoot fresh and dry weight, leaf water content and leaf water potential significantly decreased in Juma-67 than CFX 18 with increased salinity.

3.4. Dry Weight of Seedling

The total fresh weight were recorded 0.53g,0.41g,0.47g and 0.39g for Binadhan-10, Binadhan-8, BRRI dhan99, BRRI dhan67 in control condition ,respectively(Table 1).Among different conditions examined,0mM NaCl (control) shows the better weight than the other treatments (40mM NaCl,80mM NaCl and 120mM NaCl) for all varieties tested. It was noticed that neutral conditions performed better results for all varieties. The 0.41g, 0.38g, 0.33g and 0.33g total fresh weight were observed in 40 mM NaCl treatment for Binadhan-10,

Binadhan-8, BRRI dhan99 and BRRI dhan67 respectively. In case of 80 mM NaCl 0.41g,0.38g,0.34g and 0.31gm total fresh weight where as in 120 mM NaCl treatments, 0.34g, 0.39g,0.40g and 0.26gtotal fresh weight for Binadhan-10, Binadhan-8, BRRI dhan99 and BRRI dhan67,respectively (Table 1). Due to salinity stress in rice varieties total fresh

weight and dry weight also decreases. Salinity decreased FGP, SG, GE% and led to reduction in shoot and root length and dry weight in all varieties and the magnitude of reduction increased with increasing salinity stress (Ologundudu et al., 2014)

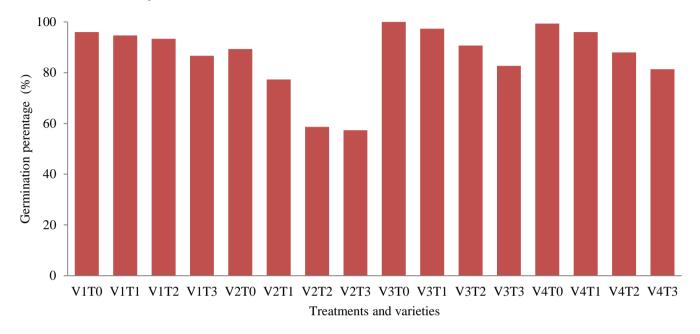


Fig 2: Percentage (%) of seed germination of four varieties of rice in salt at different conditions

3.5. Effects of Salinity Stress on Root Anatomy

Anatomical traits of root in four rice varieties were observed growing under different salinity conditions at seedling stage after 10 days of germination (Fig.3). In control condition for all varieties, epidermis forms the outermost layer followed by exodermis, cortex, pericycle, endodermis, vascular bundle (composed of xylem and phloem) and pith. The formation of those features was also observed in salinity treated roots for all the varieties tested. The changes in root features in treated samples for all varieties were determined in the cortex and stele areas. The similar results were reported in plant cells grown in salinity conditions (Bressan et al. 1990, Cachorro et al. 1995). In the cortex of NaCl solution treated roots, chemical substances depositions in the cell corners were found. Aybeke (2016) reported that phenolics, lignin and suberin deposition in cell wall was observed in rice growing under salinity conditions in the field. On the other hand, the stele area was larger in root sections growing treatment conditions. The metaxylem number was increased and xylem distribution was more prominent in case of root sections growing in different concentrations of NaCl. The xylem distribution was exact in both control and treatment conditions for all varieties studied.

4. CONCLUSION

The present study was noticed that, neutral condition performed the best results in seed germination percentages, plumule length, radicle length, seedling length, fresh weight and dry weight for all varieties. The study recommended that, the increasing NaCl salt concentrations in culture decreased the seed germination percentages for all varieties of rice. The chemical substances depositions in the cell corners were found in cortex of NaCl solution treated roots. The stele area was larger in root sections growing salt treatment conditions where the metaxylem number was increased and xylem distribution was more prominent. Though, this is very initial research, but it can help the future researchers in this field conditions. The study will enhance the understanding of rice plant responses to salinity stress during the germination phase which provides valuable insights for selecting and breeding salt-tolerant rice varieties. As well as, such efforts will contribute to sustainable agricultural practices in regions affected by soil salinity, ensuring food security and improved crop productivity.

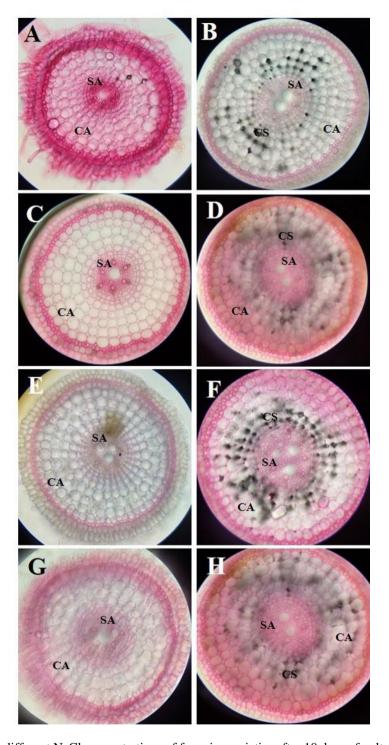


Fig.3: Cross section of root in different NaCl concentrations of four rice varieties after 10 days of culture. Binadhan-10 culture in 0mM NaCl) Binadhan-10 culture in 80mM NaCl, C) Binadhan-8 culture in 0 mM NaCl, D) Binadhan-8 culture in 80mM NaCl, E) BRRI dhan99 culture in 0 mM NaCl, F) BRRI dhan99 culture in 80mM NaCl, G) BRRI dhan67 culture in 0mM NaCl; H) BRRI dhan67 culture in80mM NaCl. All photographs were taken under 40x. (CS, chemical substances; CA, cortex area; SA, stele area)

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