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Evaluation of rice genotypes for salinity tolerance at the seedling stage

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Abstract: Rice (*Oryza sativa* L.*)* is one of the main staple food crops taken throughout the world. The production of rice is harshly affected by salinity, in chief at the seedling stage. Therefore, the great task for rice breeders is to research and improve the rice varieties. It is important to identify donors, possessing genes for salt tolerance for successful breeding to develop salinity tolerant rice varieties. The morphological screening was performed with 14 advanced rice lines of International Rice Research Institute (IRRI) along with check varieties in salinized and non-salinized conditions at glasshouse of Bangladesh Institute of Nuclear Agriculture (BINA) for evaluation of salinity tolerance. The test entries were evaluated for their tolerance to salinity in a hydroponic system at seedling stage using IRRI standard protocol. Six lines viz. RC-225, RC-222, RC-291, RC-248, RC-251 and RC-193 were identified as salt tolerant. The moderately salinity tolerant germplasms were viz. RC-227, RC-229, RC-249, RC-221 and RC-292. The lines RC-217, RC-252 and RC-250 were salt susceptible. Based on the study, the identified salt tolerant lines would be used as donors and crossed with recurrent parents to develop salinity tolerant varieties to meet the needs of farmers in coastal regions.

Key words: Hydroponic system, Salinity tolerance, Rice.

Introduction

Rice (*Oryza sativa* L.), the staple food for more than half of the humankind of the world ((Dawe *et al.*, 2010), particularly the east and south-east Asian continents (Singha *et al.*, 2015). The major abiotic stresses such as high salinity, drought, submergence and cold (Sanghera *et al.*, 2011) are causing risks to food security worldwide. Salinity is the second most prevalent soil problem in rice-growing countries after drought (Mohammadi-Nejad *et al.*, 2008) and rice is considered as a salt sensitive crop in early seedling stages which limits its productivity (Todaka *et al.*, 2012; Wani and Gosal, 2011). Salt stress leads to severe inhibition of plant growth and development, membrane damages, ion imbalances due to Na^+ and Cl^- accumulation, enhanced lipid peroxidation and increased production of reactive oxygen species like superoxide radicals, hydrogen peroxide and hydroxyl radicals (Kundan *et al*., 2013). Therefore, it is important to develop salt tolerant rice varieties.

Similarly, extensive and reliable screening techniques of the breeding for salinity tolerance in rice is crucial to determine the extent of the genetic basis of such tolerance. Reliable phenotypic evaluation of cultivars include on-field mass screening and controlled environment screening using hydroponics or other artificial media (Ismail and Horie, 2017). Screening under field conditions is tough due to stress heterogeneity, presence of other soil-related stresses, and the significant influence of environmental factors such as temperature, relative humidity and solar radiation (Tavakkoli, 2011; Kopittke *et al*., 2011). Most of the work on salinity tolerance, particularly at the seedling stage, has been done by applying NaCl artificially under controlled conditions.

The present studies were designed to evaluate the genetic basis of salt tolerance of some advanced lines in a hydroponic system at seedling stage using IRRI standard protocol (IRRI, 1997) and identify the perfect donors for marker assisted selection (MAS).

Materials and Methods

The experiments in this study were conducted at the glasshouse with ambient temperature and normal light conditions at the glasshouse of Plant Breeding Division at Bangladesh Institute of Nuclear Agriculture (BINA), BAU campus, Mymensingh-2202 in Boro Season. Fourteen pedigree advance rice lines (imported from IRRI) (Linh *et al.*, 2013), some varieties from BINA were used in this study (Table 1).

Table 1. List of rice genotypes used in the salinity screening experiment

Sl. No.	Genotypes	Type and Source
1	RC-227	Advanced line, IRRI
2	RC-229	Advanced line, IRRI
3	RC-225	Advanced line, IRRI
4	RC-217	Advanced line, IRRI
5	RC-222	Advanced line, IRRI
6	RC-291	Advanced line, IRRI
7	RC-248	Advanced line, IRRI
8	RC-251	Advanced line, IRRI
9	RC-249	Advanced line, IRRI
10	RC-250	Advanced line, IRRI
11	RC-252	Advanced line, IRRI
12	RC-253	Advanced line, IRRI
13	RC-292	Advanced line, IRRI
14	RC-193	Advanced line, IRRI
15	FL-478	Salt tolerant, recombinant inbred line
16	Binadhan-7	Short duration variety, BINA
17	Binadhan-8	Salt tolerant variety, BINA
18	Binadhan-10	Salt tolerant variety, BINA
19	Binadhan-11	Submergence tolerant variety, BINA
20	Binadhan-12	Submergence tolerant variety, BINA

To conduct the screening technique at seedling stage, there were used some basic equipment and reagents (Table 2). The genotypes were evaluated for their tolerance to salinity in a hydroponic system at seedling stage using IRRI standard protocol (IRRI, 1997). Salinized and non-salinized setups with 2 replications were maintained.

Table 2. Basic equipment and reagents used for screening

Equipment		Reagents
Water	Incubator	Crude salt (NaCl)
pH meter	Seed	NaOH
EC meter	Styrofoam sheet	HCl
Balance	Magnetic stirrer	Peter fertilizer (20-20-20)
Nylon net	Plastic tray	Ferrous sulphate ($FeSO_4.7H_2O$)
Plastic containers	Tissue	-
Petri dish	Stirrer	-

For screening techniques, breaking of seed dormancy was done by heat-treated for 5 days in an incubator at 51^0C. Rice seeds were soaked in water for 24 h and then were sown in the soil (Azarin *et al.*, 2016). Then the seeds were washed and rinsed with tap water and placed on petri dishes with moistened filter papers and incubated for 48 hrs at room temperature (about 31°C) to germinate.

The setup required styrofoam seedling floats. A float is a fabrication of a rectangular styrofoam (36 cm x 31 cm x 1.25 cm) having 100 holes (10X10) with nylon net bottom and 2.5 cm thick frame pasted on top. The frame helps fit the float to a rectangular plastic tray with 12L capacity. Two pregerminated seeds were sown per hole on styrofoam seedling float with the nylon mesh at bottom (Bharathkumar *et al.*, 2015). The radicle should be inserted through the nylon mesh. The styrofoam seedling float was suspended on the tray filled with tab water. Two setups were maintained; salinized and non-salinized setup. In the non-salinized setup, the seedlings were grown in nutrient solution without salinization. In salinized setup, when seedlings were well established at 3 to 4 leaves stage (after 3 days of sowing), water was replaced with salinized nutrient solution by adding crude salt with nutrient solution. Initial salinity was at EC = 6 dS m-1. Three days later, salinity level was increased up to EC=12 dS m-1 by adding NaCl to the nutrient solution. The solution was renewed in every 8 days and the pH was maintained at 5.0 by adding either 1N NaOH or 1N HCl.

Modified standard evaluating score was used (Table 4) for rating the visual symptoms of salt toxicity. Experimental design was a Randomized Complete Block Design (RCBD) with two replications (Senanayake *et al.*, 2017). Commercially available peter professional water-soluble fertilizer (20-20-20) (Table 3) and ferrous sulphate ($FeSO_4.7H_2O$) were used as nutrient solution. To prepare nutrient solution, 1.0 g peter fertilizer and 200mg/L ferrous sulphate were mixed carefully per liter of tap water.

Table 3. Active Ingredients of Peters Professional 20-20-20

Element	Active Ingredients	Amount (%)
N	Total Nitrogen	20%
P	Available Phosphate	20%
K	Soluble Potash	20%
Mg	Magnesium	0.05%
B	Boron	0.0125%
Cu	Copper	0.0125%
Fe	Iron	0.05%
Mn	Manganese	0.025
Mo	Molybdenum	0.01%
Zn	Zinc	0.03%

The SES score (Table 4) was used to assess the visual symptoms of salt stress injury of seedlings, in which 1 indicates highly tolerant and 9 indicates highly susceptible (Thomson *et al.*, 2010).

Table 4. Modified Standard Evaluation Score (SES) of visual salt injury at seedling stage

Score	Observation	Tolerance
1	Normal growth, no leaf symptoms	Highly tolerant (HT)
3	Nearly normal growth, but leaf tips or few leaves whitish and rolled	Tolerant (T)
5	Growth severely retarded; most leaves rolled; only a few are elongating	Moderately tolerant (MT)
7	Complete cessation of growth; most leaves dry; some plants dying	Susceptible (S)
9	Almost all plants dead or drying	Highly Susceptible (HS)

Source: Gregorio *et al.* (1997)

In salinized setup the genotypes showed wide variation. The main symptoms were leaf rolling and formation of the new leaves, leaf rolling and whitening of the tips which lead to complete cessation of the growth and finally drying the leaves (Fig. 1). The initial evaluation of visual injury symptoms of fourteen advanced lines and check genotypes were completed on at 7 days after sowing (DAS), 14 DAS and 21 DAS.

Figure. 1. Salinized and non-salinized condition of the genotypes; evaluated for their tolerance to salinity in a hydroponic system at seedling stage using IRRI standard protocol (IRRI, 1997)

After final assessment via SES scoring, survived plants were counted and survival percentage was calculated and compared with the seedlings, grown at non-salinized condition. And then survival of seedlings reduced due to salt stress could be calculated. Shoot length and root length of the seedlings grown at both setup were recorded.

Results

Fourteen advanced lines from IRRI (International Rice Research Institute), six high yielding varieties from BINA and BRRI were collected for the study. These seeds were screened in hydroponic system to verify the function of *Saltol* locus tolerance. In the non-salinized condition, the seedlings were grown in nutrient solution. All genotypes were grown robustly and showed uniform green colour and height and exhibited 100% survival under non-salinized condition. The observed plant population in this condition had normal seedling growth (Fig. 2).

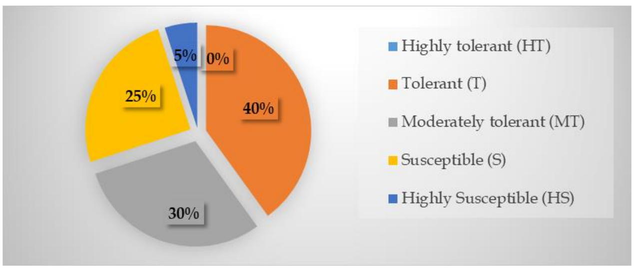

Figure. 2. Percent presentation of rice genotypes at different level of salinity tolerance at 21 DAS

In salinized condition, seedlings were allowed to grow for 3 days in tab water. After 3 days, water was replaced with salinized nutrient solution. Then seedling growth is suppressed under salinity stress and showed various degrees of responses to the salinity. The genotypes showed wide variation in phenotypes ranging from score 1 (highly tolerant) and score 9 (highly susceptible) (Table 5).

Scoring was done at three stages of plant growth viz. at 7 days after sowing (DAS), at 14 DAS and at 21 DAS (Table 7). At 7 DAS, the lines RC-251 along with check varieties FL-478 were scored 1 and identified as highly salt tolerant. The moderately salinity tolerant genotypes were scored 5 and these were RC-252, RC-292 and Binadhan-7 while rest of all genotypes were salt tolerant (3). At 14 DAS, RC-227, RC-229, RC-225, RC-222, RC-291, RC-248, RC-251, RC-221, RC-193, FL478, Binadhan-8, Binadhan-10 and Binadhan-11 were observed as salt tolerant (3) and the genotypes RC-217, RC-249, RC-252, RC-292, Binadhan-7 and Binadhan-12 were scored 5 (moderately tolerant) and only the line RC-250 was salt susceptible. Final scoring was done at 21 DAS and the lines RC-225, RC-222, RC-291, RC-248, RC-251, RC-193 along with check varieties FL-478 and Binadhan-10 were scored 3 and identified as salt tolerant. The moderately salinity tolerant genotypes were scored 5 and these were RC-227, RC-229, RC-249, RC-221, RC-292 and Binadhan-8. The other lines viz. RC-217, RC-252, Binadhan-7, Binadhan-11 and Binadhan-12 were scored 7 (salt susceptible) except the line RC-250. This line was scored 9 and identified as highly susceptible line in the experiment (Table 5).

Table 5. Standard Evaluation Score (SES) of rice genotypes for salinity tolerance

Genotypes	Standard Evaluation Score (SES)			Salinity Tolerance
	At 7 DAS	14 DAS	21 DAS	
RC-227	3	3	5	Moderately tolerant (MT)
RC-229	3	3	5	Moderately tolerant (MT)
RC-225	3	3	3	Tolerant (T)
RC-217	5	5	7	Susceptible (S)
RC-222	3	3	3	Tolerant (T)
RC-291	3	3	3	Tolerant (T)
RC-248	3	3	3	Tolerant (T)
RC-251	1	3	3	Tolerant (T)
RC-249	3	5	5	Moderately tolerant (MT)
RC-250	5	7	9	Highly susceptible (HS)
RC-252	5	5	7	Susceptible (S)
RC-221	3	3	5	Moderately tolerant (MT)
RC-292	5	5	5	Moderately tolerant (MT)
RC-193	3	3	3	Tolerant (T)
FL-478	1	3	3	Tolerant (T)
Binadhan-7	5	5	7	Susceptible (S)
Binadhan-8	3	3	5	Moderately tolerant (MT)
Binadhan-10	3	3	3	Tolerant (T)
Binadhan-11	3	3	7	Susceptible (S)
Binadhan-12	3	5	7	Susceptible (S)

At final scoring (21 DAS), only 5% genotypes showed the category of highly salt sensitive level, 25% under the sensitive level and 30% belonged to the moderately tolerant category. The highest number of genotypes (40%) showed tolerant level and scored 3 while no line (0%) was performed the score 1 (highly salt tolerant) in this experiment (Fig. 2).

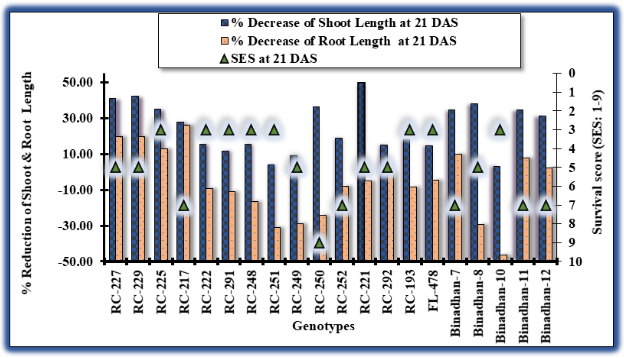

Figure 3. Performance of rice genotypes with regard to standard evaluation score (SES), reduction (%) of shoot and root length after salt stress

The genotypes were compared based on SES, % shoot and root length reduction (Figure 3). The progenies RC-251 and Binadhan-10 exhibited minimum shoot length (<5%) and root length (-47%to -36%) reduction but they were salt tolerant and scored 3. The genotypes RC-222, RC-291, RC-248, RC-249, RC-252, RC-292, RC-193 and FL-478 gave lower shoot length (9% to 20%) and root length (-32% to -9%) reduction while they performed moderately salt tolerance to salt tolerance level except RC Fourteen advanced lines from IRRI (International Rice Research Institute), six high yielding varieties from BINA and BRRI were collected for the study. These seeds were screened in hydroponic system to verify the function of *Saltol* locus tolerance. In the non-salinized condition, the seedlings were grown in nutrient solution. All genotypes were grown robustly and showed uniform green colour and height and exhibited 100% survival under non-salinized condition. The observed plant population in this condition had normal seedling growth (Figure 2). In salinized condition, seedlings were allowed to grow for 3 days in tab water. After 3 days, water was replaced with salinized nutrient solution. Then seedling growth is suppressed under salinity stress and showed various degrees of responses to the salinity. The genotypes showed wide variation in phenotypes ranging from score 1 (highly tolerant) and score 9 (highly susceptible) (Table 5).

Scoring was done at three stages of plant growth viz. at 7 days after sowing (DAS), at 14 DAS and at 21 DAS (Table 7). At 7 DAS, the lines RC-251 along with check varieties FL-478 were scored 1 and identified as highly salt tolerant. The moderately salinity tolerant genotypes were scored 5 and these were RC-252, RC-292 and Binadhan-7 while rest of all genotypes were salt tolerant (3). At 14 DAS, RC-227, RC-229, RC-225, RC-222, RC-291, RC-248, RC-251, RC-221, RC-193, FL478, Binadhan-8, Binadhan-10 and Binadhan-11 were observed as salt tolerant (3) and the genotypes RC-217, RC-249, RC-252, RC-292, Binadhan-7 and Binadhan-12 were scored 5 (moderately tolerant) and only the line RC-250 was salt susceptible. Final scoring was done at 21 DAS and the lines RC-225, RC-222, RC-291, RC-248, RC-251, RC-193 along with check varieties FL-478 and Binadhan-10 were scored 3 and identified as salt tolerant. The moderately salinity tolerant genotypes were scored 5 and these were RC-227, RC-229, RC-249, RC-221, RC-292 and Binadhan-8. The other lines viz. RC-217, RC-252, Binadhan-7, Binadhan-11 and Binadhan-12 were scored 7 (salt susceptible) except the line RC-250. This line was scored 9 and identified as highly susceptible line in the experiment (Table 5).

At final scoring (21 DAS), only 5% genotypes showed the category of highly salt sensitive level, 25% under the sensitive level and 30% belonged to the moderately tolerant category. The highest number of genotypes (40%) showed tolerant level and scored 3 while no line (0%) was performed the score 1 (highly salt tolerant) in this experiment (Figure 2).

The genotypes were compared based on SES, % shoot and root length reduction (Fig. 3). The progenies RC-251 and Binadhan-10 exhibited minimum shoot length (<5%) and root length.

-252 (susceptible line). Higher shoot length 27%-50%) and root length reduction were identified to the genotypes RC-227, RC-229, RC-225, RC-217, RC-250, RC-221, Binadhan-7, Binadhan-11 and Binadhan-12 were moderately salt tolerance to highly salt susceptible genotype. In case of the line RC-292 were observed no change in root length (Table 6) ant this was moderately salt tolerance line.

Table 6. Shoot & Root Length of seedlings at salinized and non-salinized condition

Genotypes	Shoot Length (21 DAS)			Root Length (21 DAS)			Standard Evaluation Score (SES) (21 DAS)	Salinity Tolerance
	Salinized	Non-Salinized	% decreased	Salinized	Non-Salinized	% decreased		
	Mean	Mean		Mean	Mean			
RC-227	21.75	36.75	40.82	9.25	11.50	19.57	5	Moderately tolerant (MT)
RC-229	20.50	35.50	42.25	9.25	11.50	19.57	5	Moderately tolerant (MT)
RC-225	20.75	32.00	35.16	10.00	11.50	13.04	3	Tolerant (T)
RC-217	30.50	42.25	27.81	9.25	12.50	26.00	7	Susceptible (S)
RC-222	36.75	43.50	15.52	11.75	10.75	-9.30	3	Tolerant (T)
RC-291	34.50	39.00	11.54	10.25	9.25	-10.81	3	Tolerant (T)
RC-248	26.25	31.00	15.32	10.50	9.00	-16.67	3	Tolerant (T)
RC-251	31.25	32.50	3.85	10.50	8.00	-31.25	3	Tolerant (T)
RC-249	30.00	33.00	9.09	10.00	7.75	-29.03	5	Moderately tolerant (MT)
RC-250	24.25	38.00	36.18	10.25	8.25	-24.24	9	Highly susceptible (HS)
RC-252	29.00	35.75	18.88	10.25	9.50	-7.89	7	Susceptible (S)
RC-221	20.25	40.25	49.69	10.50	10.00	-5.00	5	Moderately tolerant (MT)
RC-292	28.50	33.50	14.93	11.25	11.25	0.00	5	Moderately tolerant (MT)
RC-193	34.50	42.00	17.86	9.50	8.75	-8.57	3	Tolerant (T)
FL-478	35.00	41.00	14.63	11.00	10.50	-4.76	3	Tolerant (T)
Binadhan-7	32.00	49.00	34.69	11.25	12.50	10.00	7	Susceptible (S)
Binadhan-8	20.75	33.50	38.06	11.00	8.50	-29.41	5	Moderately tolerant (MT)
Binadhan-10	32.25	33.25	3.01	10.25	7.00	-46.43	3	Tolerant (T)
Binadhan-11	29.75	45.50	34.62	11.75	12.75	7.84	7	Susceptible (S)
Binadhan-12	31.25	45.50	31.32	12.25	12.50	2.00	7	Susceptible (S)

Discussion

The conventional methods of plant selection for salt tolerance are difficult because of the large effects of the environment. The main objective of this study was to select salt-tolerant rice genotypes for identify suitable donor parents. In the present experiment, a total of 22 genotypes were screened in hydroponic system to prove the action of *Saltol* locus tolerance. Ali *et al.* (2014) also investigated and carried out to evaluate some genotypes for assessment of their tolerance in salinity stress at seedling stage.

Observed salt tolerance in rice at seedling stage was generally reported to be in agreement with the tolerance of the genotypes (Islam *et al.*, 2012). All genotypes were grown robustly and showed uniform green colour and height and exhibited 100% survival in nutrient solution without salinization. In salinized condition, seedling growth were suppressed under salinity stress and showed various degrees of responses to the salinity (Thomson *et al.*, 2010). At 7 DAS, only two genotypes were scored 1 and identified as highly salt tolerant and 16 progenies were salt tolerant while only three genotypes were moderately salinity tolerant. At 14 DAS, 13 genotypes were observed as salt tolerant while six genotypes were moderately tolerant as well as only one line was salt susceptible. At final scoring, 21 DAS 8 genotypes identified as salt tolerant, another 6 genotypes were moderately salinity tolerant, five progenies were salt susceptible and only one line were observed as highly susceptible line in the experiment. These findings are in close agreement with the researcher Mansuri *et al.* (2012). They grouped the genotypes used SES for evaluation of cultivars for salinity tolerance during young seedling stage. They found only one genotype such as Pokkali was highly salt tolerant, ten cultivars were salt tolerant, four genotypes were moderately salt tolerant, one was salt susceptible and another one was highly salt susceptible.

The idea of using physiological criteria for screening salt tolerance has been embraced by many researchers. Bhowmik *et al.* (2007) confirmed that salt stress modified morphological traits of rice such as reduction of root, shoot length and tiller number that lead to reduction in biomass. In this study, growth of all genotypes was decreased by salinity stress in all stages, but reduction of growth in tolerant genotypes was lower than that in susceptible genotypes. In the present study, the genotypes were compared based on the agronomic traits such as percent shoot and root length reduction and others. The progenies those showed minimum or lower shoot length and root length reduction were evaluated as salt tolerant. The genotypes showing higher shoot length and root length reduction were identified as moderately salt tolerant to highly salt susceptible. Plant height was obviously reduced due to salinity stress. Similar results were also found by Purnendu *et al.* (2004) and Maiti *et al.*, (2006). Munns and Tester (2008) also reported that salinity might directly or indirectly inhibit cell division and enlargement during plant growing period. Choi *et al.* (2003) observed that the plant height decreased due to salt effect. Tolerant genotypes had lower plant height reduction than the susceptible genotypes. Present result is consistent with the result observed by Islam, (2004). He reported that total biomass of tolerant lines was reduced by 49.5% in salinized condition whereas those of susceptible lines were reduced by 64%. This result was also supported by Bhowmik *et al.* (2009) who observed rice genotypes with lowest reduction of total dry mater were tolerant to salinity.

Conclusion

The experiment was conducted to select salinity tolerant donor lines for MAS. For the experiment, a total of 14 advanced lines were collected from IRRI and screened for their tolerance to salinity with other check varieties using IRRI standard protocol. The genotypes were screened through hydroponic system with 12 dSm^{-1} of Electrical Conductivity (EC) at seedling stage. Six lines such as RC-225, RC-222, RC-291, RC-248, RC-251 and RC-193 were identified as salt tolerant and these advanced lines would be used as donors and crossed with recurrent parents to develop salinity tolerant varieties to meet the needs of farmers in coastal regions.

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