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PHENOTYPIC STUDY OF RICE LANDRACES OF BANGLADESH FOR SALT TOLERANCE AT SEEDLING STAGE

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Abstract

The environmental stresses such as salinity (soil or water) are serious obstacles for field crops especially in the arid and semi-arid tracts of the world. In order to investigate impact of salinity stress on rice seed germination and early seedling stage conducted at glasshouse and biotechnology laboratory of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh-2200. A total number of 80 rice germplasms were used to evaluate for salinity tolerance at seedling stage following IRRI standard protocol. Hydroponic system was used to evaluate salt tolerance of these germplasms using nutrient solution and controlled environment. The modified standard evaluation system (SES) of IRRI was used to score the discriminate of the tolerant, moderately tolerant and susceptible rice lines. Scoring was done at 12th, 18th, 30th day after salinization. Among the germplasms, 12 were found as salt tolerant, 13 were found as moderately tolerant, 26 were susceptible and 29 were highly susceptible. The plant height, root length and total dry matter (TDM) weight are greatly affected by the salinity. Considering percent reduction in plant height, root length and total dry matter weight, it can be concluded that Hogla, Patnai, Jamai Naru, FL378, Bazra Muri and Jota Balam were tolerant.

Keywords: Rice, salinity, seedling.

Introduction

Rice, *Oryza sativa* (2n = 24) belonging to the family Graminae and subfamily Oryzoidea is the staple food of more than 50% of the world's population. Salinity is becoming a serious problem in several parts of the world and a widespread soil problem in rice growing countries. The saline area is three times larger than land used for agriculture (Amirjani, 2010). Soil salinity is one among the several environmental stresses causing drastic changes in the growth, physiology and metabolism of plants. Saline environment can induce a wide number of responses in plants ranging from readjustment of transport and metabolic processes to growth inhibition (Azooz, 2004). Germination percentage is not affected by salt stress, but at the same concentration of salinity, significant differences in plant height and dry weight have been reported (Momayezi *et al.,* 2009). Therefore, development of salt tolerant varieties has been considered as one of the strategies to increase rice production in saline prone coastal areas. The response of rice to salinity varies with growth stage. Several studies indicated that rice is salt tolerant during germination, becomes very sensitive during early seedling stage (2 - 3 leaf stage), gains tolerance during vegetative growth stage, again becomes sensitive during pollination and fertilization and then become increasingly more tolerant at maturity (Bhowmic and Titov, 2009). Screening of germplasms at seedling stage is readily acceptable as it is based on a simple criterion of selection; it provides rapid screening, which is difficult at vegetative and reproductive stage (Gregorio, 1997).

Rodrigues and Fernandes (2002) carried out a study on the effects of salinity of irrigation water on the germination and seedling vigor of irrigated rice (*Oryza sativa*). Germination and vigor were evaluated by adopting a completely randomized experimental design with 5 water salinity levels (Electrical Conductivity of Water, ECw: 0.5, 2.5, 4.5, 6.5 and 8.5 dS m^{-1}). Deepa *et al.* (2011) conducted an experiment on screening of rice germplasms based on traits like Spikelet sterility, Na^+: K^+ratio is being followed by breeders in salt affected soils. The objective of this study is to screen rice germplasms for salt tolerance under salinized and non-salinized conditions at the seedling stage.

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Materials and Methods

Eighty rice germplasms were used for this study which was collected from the coastal areas of Bangladesh and the experiment was conducted at glasshouse and biotechnology laboratory of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh-2200.

Method of screening of seedling for salinity tolerance

Hydroponic system (Gregorio *et al*., 1997) was used at the glasshouse to evaluate salt tolerance of the 80 rice germplasms using nutrient solution (Yoshida *et al.,* 1976). The germplasms were collected from the coastal areas of Bangladesh.

Preparation for hydroponic system

A rectangular Styrofoam with inner diameter of 18 inch having 100 holes with nylon net bottom and 1 inch thick frame pasted on top was used as seedling float. A rectangular glass fiber tray with 12L capacity and diameter of 23 inch was used to fit the floats. The holes were prepared in rows with about 1 cm diameter.

Preparation of stock solution and nutrient solution

Stock solution was prepared carefully to avoid nutrient deficiencies and mineral toxicities not attributed to salinity stress. Stock solutions were prepared in every two weeks. Macronutrients and micronutrients components for making 2.5 l stock solution are presented in Table 1 and Table 2, respectively. Nutrient solution was prepared in plastic containers. To prepare 1 l of nutrient solution, 1.25 ml of each macronutrient and 1.25 ml of micronutrient solutions were mixed with distilled water (dH_2O). The pH of the solution was measured by pH meter.

Maintenance of seedlings and salinization and data collection

Seeds were heat-treated in a convection oven set for 5 days at 50°C to break the seed dormancy. The seeds were soaked in tap water for 24 hours. The seeds were washed and rinsed with tap water and then placed on petrifies with moistened filter papers and incubated for 48 hours at 30°C for germination. Two pre-germinated seeds were sown per hole on the Styrofoam seedling float. For salinization, crude salt was dissolved with nutrient solution to reach the desired salinity level. The salinization level was measured by electrical conductivity (EC) at 12 dSm^{-1}. The pH of the solution was monitored daily and maintained around 5.25 because +1.0 deviation of culture solution pH from 5.0 makes some nutrients toxic and others deficient. The modified standard evaluation system (SES) of International Rice Research Institute (IRRI) was used to assess the visual symptoms of salt toxicity (Tables 3 and 4). Leaves, shoots and roots of individual rice plants were separately kept into the envelope and oven dried at 70°C for a week and weighed. Weight of Total Dry Matter (TDM), % reduction of Total Dry Matter and % reduction of plant height was calculated as follows:

Total dry matter = dry weight of leaves + shoots + roots

% reduction = (traits of normal - traits in saline) / Traits in normal × 100

Table 1. List of macronutrients used in preparation of stock solution

Element	Reagent	Amount of Reagent (g 2.5 l^{-1} solution)
N	Ammonium nitrate (NH_4NO_3)	228.50
P	Sodium phosphate, monobasic monohydrate ($NaH_2PO_4.H_2O$)	89.00
K	Potassium sulfate (K_2SO_4)	178.50
Ca	Calcium chloride, Dehydrated ($CaCl_2.2H_2O$)	293.40
Mg	Magnesium sulfate,7-hydrate ($MgSO_4.7H_2O$)	810.00

Source: Adapted from Yoshida and Forno *(*1976)

Table 2. List of micronutrients used in preparation of stock solution

Element	Reagent	Amount of Reagent ($g\ 2.5\ l^{-1}$ solution)
Mn	Manganouschloride,4-hydrate ($MnCl_3.4H_2O$)	3.75
Mo	Ammonium molybdate,4-hydrate [$(NH_4)_6Mo_7O_{24}.4H_2O$]	0.185
Zn	Zinc sulfate, 7-hydrate ($ZnSO_4.7H_2O$)	0.0875
B	Boric acid (H_3BO_3)	2.335
Cu	Cupric sulfate, 5-hydrate ($CuSO_4.5H_2O$)	0.078
Fe	Ferric chloride, 6-hydrate ($FeCl_3.6H_2O$) 19.25	19.25
	Citric acid, monohydrate ($C_6H_8O_7.H_2O$)	29.75

Source: Adapted from Yoshida and Forno (1976)

Table 3. Modified SES of visual salt injury at seedling Stage

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

Table 4. Method of scoring salt injury from leaf symptoms

Dead Leaves (%)	Score	Rating
0-20	1	Tolerant
21-35	2	Tolerant
36 - 50	3	Tolerant
51-70	5	Moderately Tolerant
71 -90	7	Moderately Tolerant
90-100	9	Susceptible

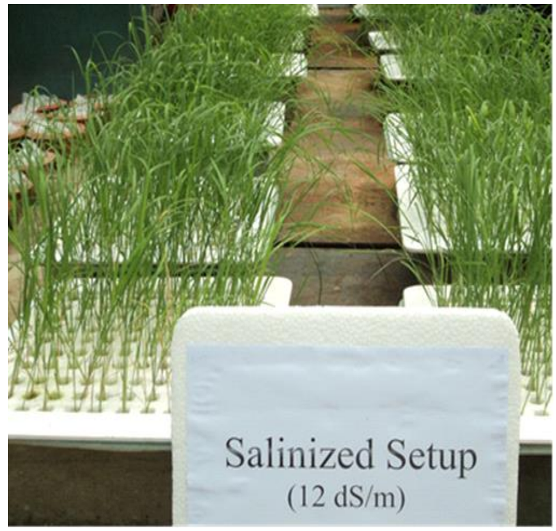
Source: Adapted from Ponnamperuma (1977)

Results and Discussion

Eighty genotypes of rice seedlings were used for screening salinity tolerance. After two or three days of salinization, salt stress symptoms were started. Seedlings grown in salinized condition showed several symptoms of salt injury like- yellowing of leaves, drying of leaves, and reduction in root growth, reduction of shoot growth and stem thickness and in many cases dying of seedlings. Some other symptoms were rolling and tip whitening. Above all of these, reduction in leaf area was the first symptom. These symptoms were also recorded by Bhuiyan (2005). On the other hand, the seedlings in the non-salinized condition showed normal growth over the salinized condition. The non-salinized and salinized setups of eighty rice germplasms are shown in Fig. 1A and 1B, respectively.

Among the 80 germplasms, according to the SES of IRRI (Gregorio *et al.*,1997), twelve were found as salt tolerant (T), which were Hogla, Jamai Naru, Dakhshail, Patnai, Kute Patnai, Holde Gotal, Bazra Muri, Ghunshi, Tal Mugur, Nona Bokhra, Kashrail, FL378. Among the rest, thirteen were found moderately tolerant (MT), twenty six were susceptible (S) and twenty nine were highly susceptible (HS). The performance of these germplasms under salinized condition is shown in Table 5 and Fig. 2.

A



B

Fig. 1.A. Setup of non-salinized conditions and B. Setup of salinized (EC level 12 dSm^{-1}) conditions of 80 rice germplasms at the seedling stage using hydroponic system

Table 5. Performance of 80 rice germplasms under salinized condition (EC 12 dSm^{-1}) grown in hydroponic system at the seedling stage

SL. No.	Name of the Variety	Score			Tolerance
		12 d/salt	18 d/salt	30 d/salt	
1	Dudh Kalam	3	5	7	S
2	Hati Bajore	3	5	7	S
3	Malagoti	1	3	7	S
4	Kuchra	3	5	7	S
5	Enghi	1	5	7	S
6	Kajol Shail	3	5	5	MT
7	**Hogla**	**1**	**3**	**3**	**T**
8	**Jamai Naru**	**1**	**3**	**3**	**T**
9	Hari	1	3	9	HS
10	**Dakh Shail**	**1**	**3**	**3**	**T**
11	Moina Moti	1	5	7	S
12	Marish Shail	3	5	7	S
13	**Patnai**	**1**	**3**	**3**	**T**
14	Bhute Shalot	1	3	5	MT
15	**Kute Patnai**	**1**	**3**	**3**	**T**
16	Mohini Shalot	3	7	9	HS
17	Moghai Balam	3	7	9	HS
18	Sada Gotal	1	5	7	S
19	Khak Shail	1	5	5	MT
20	Mohime	3	5	9	HS
21	**Holde Gotal**	**3**	**5**	**3**	**T**
22	Jota Balam	3	5	5	MT
23	Tilek Kuchi	1	5	7	S
24	Rani Shalot	3	5	7	S
25	Kathi Goccha	1	5	7	S
26	Bashful Balam	3	7	9	HS
27	**Bazra Muri**	**1**	**3**	**3**	**T**

28	Durga Bhog	1	3	7	S
29	Kumra Ghor	1	5	7	S
30	Khainol	3	5	7	S
31	**Ghunshi**	**1**	**3**	**3**	**T**
32	Chinikani	3	5	9	HS
33	Dhar Shail	1	5	7	S
34	Khejur Chori	1	5	7	S
35	Shaheb Kachi	3	7	7	S
36	Raja Shail	3	5	7	S
37	Hamai	3	5	5	MT
38	Mura Bajal	3	7	7	S
39	Lal Gotal	1	5	7	S
40	Kalmilata	3	5	7	S
41	Volanath	3	9	9	HS
42	Rupessor	3	9	9	HS
43	Sylhet Balam	3	7	7	S
44	Karengal	1	5	5	MT
45	Kalo Mota	1	5	5	MT
46	Mota Aman	1	7	9	HS
47	Ghochi	3	7	9	HS
48	Chap Shail	1	5	7	S
49	Mondeshor	1	5	5	MT
50	Nona Kochi	1	5	5	MT
51	Ghocca	3	5	7	S
52	**Tal Mugur**	**1**	**3**	**3**	**T**
53	Ghigoj	1	3	7	S
54	Tor Balam	3	5	7	S
55	Fulkainja	3	7	9	HS
56	Piarjat	3	9	9	HS
57	Koichabinni	3	7	9	HS
58	Lalbiroi 31	3	7	9	HS
59	Lalanamia	3	7	9	HS
60	Golapi	5	5	9	HS
61	Asambinni	3	7	9	HS
62	Kakuabinni	3	7	9	HS
63	**Nona Bokhra**	**1**	**3**	**3**	**T**
64	BINA 8	1	3	5	MT
65	FL 478	1	3	5	MT
66	JongliBoro	3	7	9	HS
67	**Kashrail**	**1**	**3**	**3**	**T**
68	Ledrabinni	5	7	9	HS
69	Nunnia	3	7	9	HS
70	Rotisail	3	7	9	HS
71	Gengengbinni	3	5	9	HS
72	Chinisail	3	5	9	HS
73	Jolkumri	1	3	5	MT
74	Pokkaly	1	3	5	MT
75	Ponkhiraj	3	7	9	HS
76	Mowbinni	3	7	9	HS
77	Bogi	3	9	9	HS
78	Kali Boro	3	9	9	HS
79	**FL 378**	**1**	**3**	**3**	**T**
80	Kali Boro 138/2	3	7	9	HS

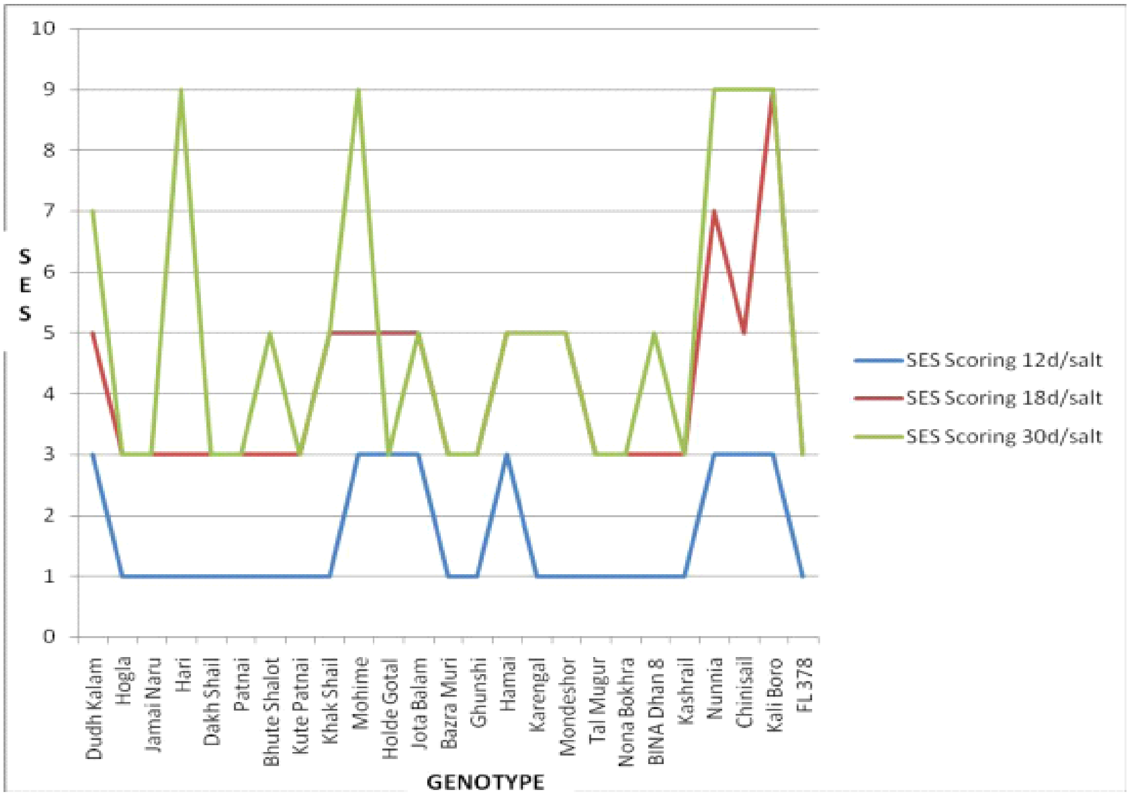


Fig. 2. Frequency distribution of 25 rice germplasms under salinized condition using SES of IRRI standard protocol

The agronomic traits of tolerant lines showed higher reduction than susceptible lines. The plant height, root length and total dry matter weight were greatly affected by the salinity. FL378 was used as a check, which was also found as a tolerant genotype at the study of Dhar *et al.* (2012). Dakh Shail (50%), Dudh Kalam (41.96%) and Hari (36.36%) showed greater plant height reduction under the salinity stress at 12 dS m^{-1} (Table 6). On the other hand, lowest plant height reduction was observed by Hogla (9.86%) followed by Patnai (12.5%), Tal Mugur (12.70%), FL378 (14.43%) and Jamai Naru (14.70%). Hamai showed highest root length reduction (37.78%) followed by Mohime (37.14%), Nona Bokhra (31.25%) and Khak Shail (29.72%). On the other hand, Kute Patnai showed zero root length reduction. Some other rice germplasms showed lower root length reduction were Jamai Naru (2.87%), Hogla (7.14%), Jota Balam (7.14%), FL 378 (11.05%) and Bazra Muri (11.63%). Morris and Dreher (2003) reported that number of roots per plant decreased with the increasing levels of salinity. Total dry matter (TDM) weight is an important trait to evaluate the performance of rice germplasms for salt tolerance. Salt stress also decreases total dry matter of rice seedlings. The highest TDM reduction was found in Dudh Kalam (43.66%) followed by Dakh Shail (39.34%), Nunnia (35.59%), Chinisail (34.72%) and Hari (31.81%). The lowest TDM reduction was found in Bazra Muri (7.02%), Jota Balam (7.54%), FL378 (7.69%), Hogla (8.16%), Jamai Naru (8.69%) and Patnai (8.92%).

Table 6. Performance of shoot length, root length and total dry matter of rice genotypes at seedling stage at hydroponic system

Sl no	Genotypes	Plant height (cm)			Root length (cm)			Total dry matter (g)		
		Non-salinized	Salinized	% Reduction	Non-salinized	Salinized	% Reduction	Non-salinized	Salinized	% Reduction
01	**DudhKalam**	56.0	32.5	41.96	17.0	13.5	20.59	0.71	0.40	43.66
02	**Hogla**	37.5	33.8	9.86	21.0	19.5	7.14	0.49	0.45	8.16
03	**JamaiNaru**	51.0	43.5	14.70	13.9	13.5	2.87	0.46	0.42	8.69
04	**Hari**	27.5	17.5	36.36	23.5	18.0	23.40	0.44	0.30	31.81
05	**DakhShail**	48.0	24.0	50.00	24.5	21.0	14.28	0.61	0.37	39.34
06	**Patnai**	52.0	45.5	12.50	21.8	19.5	10.55	0.56	0.51	8.92
07	**BhuteShalot**	50.5	40.5	19.80	19.0	15.0	21.05	0.67	0.48	28.36
08	**KutePatnai**	55.0	45.5	17.27	13.0	13.0	0	0.57	0.51	10.90
09	**KhakShail**	64.0	43.0	32.81	18.5	13.0	29.72	0.68	0.50	26.47
10	**Mohime**	41.8	33.5	19.85	17.5	11.0	37.14	0.66	0.47	28.79
11	**HoldeGotal**	60.0	38.5	35.83	18.0	14.0	22.22	0.71	0.63	11.26
12	**Jota Balam**	47.5	39.8	16.21	14.0	13.0	7.14	0.53	0.49	7.54
13	**BazraMuri**	42.5	36.0	15.29	21.5	19.0	11.63	0.57	0.53	7.02
14	**Ghunshi**	55.5	39.5	28.83	13.0	11.0	15.38	0.76	0.64	15.79
15	**Hamai**	50.5	36.5	27.72	13.5	21.7	37.78	0.62	0.50	19.35
16	**Karengal**	31.8	25.5	19.81	18.5	15.5	16.22	0.49	0.35	28.57
17	**Mondeshor**	36.0	26.5	26.38	19.5	15.0	20.51	0.50	0.36	28.00
18	**Tal Mugur**	35.3	30.8	12.70	17.5	15.8	9.71	0.44	0.39	11.36
19	**Nona Bokhra**	43.5	33.0	24.14	24.0	16.5	31.25	0.55	0.43	21.82
20	**BINA 8**	38.5	30.5	20.77	17.5	14.5	17.14	0.54	0.39	27.78
21	**Kashrail**	50.0	40.5	19.00	19.0	15.5	18.42	0.42	0.37	11.90
22	**Nunnia**	34.6	25.5	26.30	22.0	18.0	18.18	0.59	0.38	35.59
23	**Chinisail**	54.5	37.8	30.64	19.5	16.5	15.38	0.72	0.47	34.72
24	**Kali Boro**	59.5	43.5	26.89	19.0	16.0	15.79	0.79	0.54	31.64
25	**FL 378**	48.5	41.5	14.43	20.8	18.5	11.05	0.65	0.60	7.69

Conclusion

The studied germplasms showed wide range of variation of performances based on their agronomic traits viz. plant height, shoot length and total dry matter under salt stress. It was manifested from present data that germplasms, treatments and their interaction were highly significant for germination (%), root/shoot length and total dry weight. The lowest percent reduction in TDM was found in Bazra Muri, Jota Balam, FL-378, Hogla, Jamai Naru and Patnai. Considering the SES scoring as basics, the percent reduction in plant height, root length and total dry matter, it can be concluded that, Hogla, Patnai, Jamai Naru, FL-378, Bazra Muri and Jota Balam were tolerant in every criteria. Other than these germplasms, there were moderately tolerant, highly susceptible and susceptible germplasms were identified. This will be useful for developing salinity tolerant variety through conventional breeding, marker assisted selection and gene identification.

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