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In Vitro Evaluation of Salt Tolerant Traits in Indigenous Rice Genotypes and Advanced Mutant Lines Using Different Concentration of NaCl

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Abstract:- Indigenous rice landraces i.e. Hori, Lombur, B-11 and Kalampazam collected from coastal areas of Bangladesh and two mutant lines viz. Lombur M4 and B-11 M4 were investigated in the present study for the evaluation of their salt tolerance at seedling stage. Germination percentage and other growth parameters like root length, shoot length were assessed after two weeks of exposure to five levels of salinity namely 50 mM, 100 mM, 150 mM, 200 mM and 250 mM with control. Among the rice genotype tested, Hori, Lombur, B-11 and Kalampazam showed the improved germination percentage of 66.12%, 53.33%, 51.65% and 38.66% respectively under increasing NaCl concentrations compare to salt susceptible cheek BRRI Dhan 29 which is 23.17%. Germination percentage were obtained 88.49% and 64.98% from two mutant lines Lombur M4 and B-11M4 which were found better even than the standard salt tolerant variety Pokkali is an evidence in this study. In case of shoot length, Kalampazam, Lombur M4 and B-11M4 indicated less sensitivity to increasing salt stress whereas Lombur control, B-11 control and Hori showed sharp decrease. The effect of increasing salinity on root length was found insignificant. All indigenous rice genotypes showed sharp decrease in the growth parameters studied to increasing NaCl concentration though better than the salt sensitive check but the mutant lines were found resistance to the increasing salt stress in related to these characters as in Pokkali.

***Keywords**:- Salinity Screening, NaCl, Indigenous Rice, Mutant Line, In vitro*

I. INTRODUCTION

Salinity is one of the foremost stress factors among the abiotic stresses. Salt stress brings about huge losses in worldwide agricultural productivity [01, 02]. In the world, about 400 million hectares of land are affected by high salinity environment. About 1 million hectares of land are affected by high salinity in the coastal regions in Bangladesh. Salinity hindrances almost every aspect of the physiology and biochemistry of plants and significantly reduces yield. In Bangladesh, rice production in the coastal region faced many natural complications. Coastal areas constitute about 2.5 million hectare which amounts to about 25 percent of total crop land of the country. Almost 0.84 million hectare of this land is affected by varying intensities of salinity [03]. Therefore screening of existing landraces as well as creation of new variety with improved salt tolerance traits is a major goal for a researcher. Indigenous genotypes have been indicated as the excellent sources of genes for novel alleles [04-08]. There are several rice genotypes cultivated in coastal areas like Teknaf, Borguna, Sathkhira, Khulna, Patuakhali, Bagherhat and Cox's Bazar etc. Some of these rice genotypes such as Lombur, B-11, Kalampazam and Hori are cultivated in Teknaf areas by the local people in their shrimp cultivated areas. These rice genotypes though cultivated by the local people, these have many constraints related to yield and yield contributing factors like lodging, rice quality, less tillering, prolong flowering time etc. But the most important criteria of these rice genotypes is its ability to grow in saline soil which may play a significant role in the context of continuous increasing saline areas of Bangladesh. Thus, it is important to know the level of tolerance for salinity of these varieties under study. Researchers have a great chance to look into its further improvement through some processes like advanced biotechnology, mutation breeding etc. However, in this investigation in vitro screening of salt tolerance was carried out in four rice genotypes of Hori, Lombur, Kalampazam and B-11 using MS media supplemented with different concentration of NaCl. This protocol will help an easy-to-follow procedure to select salt-tolerant rice genotypes and advance mutant lines for following field testing.

II. MATERIALS AND METHODS

Lombur, B-11, Kalampazam and Hori were collected from the Teknaf field area. Seeds of BRRI dhan-29, as a salt sensitive check, were collected from Bangladesh Rice Research Institute (BRRI), Gazipur, Bangladesh and Pokkali seeds, as a salt tolerant check, were collected from Takasaki, Japan. In addition, two advanced mutant lines, selected in PBGED experimental field, AERE, Savar, namely Lombur M4 and B-11 M4 were also tested for salt screening. The dehusked mature rice seeds were used as explant. Following surface sterilization, seeds were transferred to the laminar airflow chamber and soaked in 70% ethanol for 1-2 min, followed by 0.1% mercuric chloride (w/v) for 9-10 minutes

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and finally thoroughly washed 5-6 times with sterile distilled water. After disinfection, the seeds were inoculated on MS medium [04] supplemented with 0 mM, 50 mM, 100 mM, 200 mM and 250 mM of NaCl. Twenty seeds were inoculated per treatment. The cultures were kept in the growth chamber. The culture environment included 25°C, 60% relative humidity, and a 16 h photoperiod from white fluorescent lamps (200 µmol photons/m^2/s^{-1}). Germination percentage over control, average shoot length and root length was recorded on 14th days to observe the effect of salinity on these parameters.

Germination % over control was calculated by the following formula:

Germination % over control=

$$\frac{\text{No of germinated seed in salt treated medium rice genotype}}{\text{No of seed germinated in control medium of that rice genotype}} \times 100$$

The data were analyzed for the estimation of variance, F-value at 0.05% level by analysis of variance (ANOVA). The mean differences were adjusted with Duncan's Multiple Range Test (DMRT) using the statistical computer package program, MSTAT-C (Russell, 1986).

III. RESULTS AND DISCUSSION

Seed germination is an intricate process which plays a significant role in plant life cycle [05]. Germination of seed is strongly related with the level of salt concentration in the soil/water. As the level of salt increases, germination of seeds decreases sharply [06, 07]. Before the salt screening experiment the selected genotypes of rice landraces were cultivated at the experimental field of PBGED, IFRB, AERE, Savar, Dhaka for four season from 2015 to 2018.

A. Yield and Yield contributing factors of selected rice genotypes

All the selected rice genotypes were grown to evaluate their yield and yield attributes in Table-01. The highest yield obtained 7.93 t/ha in advanced mutant line B-11 M4 whereas 3.73 t/ha in B-11 followed by 4.27 t/ha, 4.26 t/ha, and 4.57 t/ha in Kalampazam, Hori and Lombur respectively.

Rice Genotypes	Plant height (cm)	Plant/hill (no.)	Panicle length (cm)	Filled grain/panicle (no.)	Non-filled grain/ Panicle (no.)	1000 grain weight (g)	Seed yield (t/ha)	Straw yield (t/ha)
Lombur	198.33b	6.67	26.67ab	127.33c	14.67a	29.57b	4.57bc	23.30b
Hori	155.00cd	6.33	22.00b	122.33c	9.67a	23.53cd	4.26bc	15.57c
Kalampazam	202.33b	6.33	27.33ab	225.33a	11.33a	22.13d	4.27bc	34.67a
B-11	162.22c	5.50	27.33ab	139.67bc	33.33a	25.93c	3.73c	20.43bc
Lombur M4	130.67e	8.07	28.00a	100.33c	23.33a	30.77ab	5.27b	30.17a
B-11 M4	135.00de	8.23	26.67ab	122.00c	20.00a	30.17ab	7.93a	30.20a
Pokkali	235.00a	5.50	30.33a	177.00b	18.67a	33.37a	4.36bc	16.43c
BRRI-29	100.67f	6.13	25.00ab	132.00c	33.00a	23.33cd	7.10a	16.00c
CV%	4.54	17.20	7.30	10.25	41.06	4.60	10.23	8.40

Table 1:- Genotypic effect on different yield and yield attributes in 4 selected indigenous rice genotypes with the two advanced mutant lines and check variety Pokkali and BRRIdhan-29.

In a column, the figures with similar letter (s) do not differ significantly by DMRT (Duncan's multiple range test) at $p<0.05$;
CV: Coefficient of variation; **= significant

B. Effect of Salt stress on seed germination

Result showed that there were significant differences in the germination pattern of untreated and salt treated seeds of the genotypes studied. In all the rice genotypes, maximum germination percentage for untreated seeds was 100 percent while it decreased variedly depending on the rice genotypes and salt concentration (Table 02). The germination percentage decreased severely along the increase in salt concentration in all of the 8 rice genotypes tested (fig.) which was in accordance with Siddiqi et al and Dkhil et al [06, 07]. Lima et al. (2003) also found similar kind of result who concluded that the viability of seeds under salt stress was decreased as the concentration of salt increased [08]. Suppression of seed germination happened with excess salt concentration which could be due to ion toxicity [09]. Among all of the indigenous rice genotypes in different salt concentration, highest mean germination percentage was 66.12% in Hori followed by Lombur (53.33%), B-11 (51.65%) and Kalampazam (38.66%) respectively (Table 02).

Rice Genotypes	Germination % over control							CV%	F value
	NaCl Concentrations (mM)								
	Control	50	100	150	200	250	Mean		
Lombur	100.00a	91.67a	60.00ab	31.67c	26.67c	10.00d	53.33	11.73	**
Hori	100.00a	86.50ab	79.77bc	66.27cd	51.91d	12.27e	66.12	10.05	**
Kalampazam	100.00a	34.80b	34.80b	34.80b	13.77c	13.77c	38.66	9.71	**
B-11	100.00a	100.00a	31.00bc	39.43b	27.30bc	12.17c	51.65	14.15	**
Lombur M4	100.00a	100.00a	98.33a	78.70b	73.47b	80.47b	88.49	4.87	**
B-11 M4	100.00a	84.17b	63.07c	61.23c	61.07c	20.37d	64.98	9.21	**
Pokkali	100.00a	91.40b	65.80c	79.40b	45.60d	15.10e	66.22	9.99	**
BRRI-29	100.00a	16.67b	10.67c	7.03cd	3.50de	1.67e	23.17	10.79	**

Table 2:- Effects of the different concentration of NaCl on the germination percentage over control

In a column, the figures with similar letter (s) do not differ significantly by DMRT (Duncan's multiple range test) at $p<0.05$;
CV: Coefficient of variation; **= significant

All of the indigenous rice genotypes showed better mean germination percentage than the salt sensitive check BRRI-29 (23.17%) although these revealed almost near salt tolerant characteristics compared to standard salt tolerant check Pokkali (66.22%). In this experiment, it was found that the two mutant lines revealed superior salt tolerance than any other rice genotypes tested is an evidence in this study. Lombur was irradiated with 120 Gy while B-11 was irradiated with 80 Gy of gamma from ^{60}Co 90 kci. Lombur M4 exhibited best germination percentage which was 88.49% while B-11 M4 indicated 64.98% of mean germination percentages. According to Qi, W et. al. (2014), gamma irradiation has significant role to negate the negative effect of salt stress on plant even if it is partial [10]. Gamma radiation dose of 450 Gy stimulates genetic change in plants of some sorghum varieties assessed using SSR markers while a dose of 50 Gy was shown to improve germination index and root length of Arabidopsis under salt stress. The interaction between gamma radiations induced salt tolerance response of crop plants may function at various level through the involvement of multiple attributes [11].

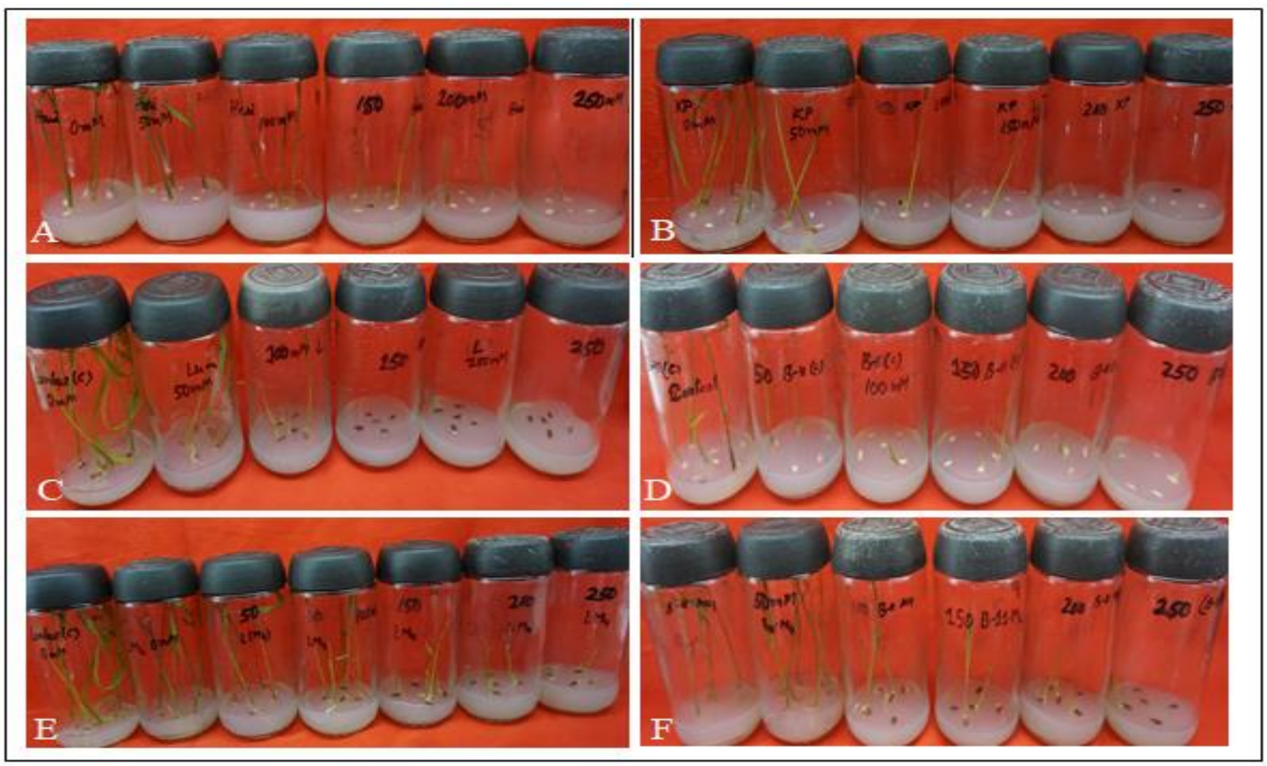

Fig 1:- *In vitro* salinity screening of 4 indigenous genotypes and 2 advanced mutant lines of rice with different concentration of NaCl (A: Hori, B: Kalampazam, C: Lombur, D: B-11, E: Lombur M4, F: B-11 M4)

C. Effect of Salt Stress on Root and Shoot Length

The root and shoots are most important parameters for checking salt stress because roots are in direct contact with soil and absorb water from soil while shoot supply it to the rest of the plant [12]. In this experiment, seedlings were grown under different salt concentrations for two weeks. Root and shoot lengths were measured after 2 weeks. Results showed that salt concentration decreased the lengths of plantlet roots and shoots significantly (Table 03 & 04). The shoot length of most of the rice genotypes studied decreased drastically with the increase in salinity (Table 03) while it showed gradual decrease in the standard salt tolerant check and two mutant lines (fig.). In all of the tested varieties revealed tolerance at 50 mM NaCl in shoot length but thereafter decreased abruptly at subsequent increase of salt stress. Mer et al. (2005) stated that plomule length in wheat, barley pea and cabbage seeds decreased with the increasing salinity [13]. Similar observation also made in this investigation.

Rice Genotypes	Shoot length of the seedlings (cm)							CV%	F value
	NaCl Concentrations (mM)								
	Control	50	100	150	200	250	Mean		
Lombur	29.67a	26.33b	7.83c	0.5d	0.90d	2.27d	11.25	14.47	**
Hori	18.50a	15.67b	9.73c	7.00d	9.30c	2.47e	10.44	9.19	**
Kalampazam	18.87a	12.57b	6.50c	4.83c	5.57c	6.47c	9.13	22.92	**
B-11	13.47a	12.17a	3.37b	4.40b	2.30b	0.50b	6.03	30.09	**
Lombur M4	19.17a	18.23ab	11.43bc	6.77cd	5.60cd	3.33d	10.76	23.43	**
B-11 M4	18.73a	17.07a	12.33b	3.93c	5.10c	0.40d	9.59	15.08	**
Pokkali	29.87a	27.50a	18.63b	10.13c	2.93d	1.33d	15.07	9.40	**
BRRI-29	9.23a	8.30a	4.47b	1.13c	1.10c	0.70c	4.16	41.36	**

Table 3:- Effects of the different concentration of NaCl on the shoot length of the seedling after 2 weeks

In a column, the figures with similar letter (s) do not differ significantly by DMRT (Duncan's multiple range test) at $p<0.05$;
CV: Coefficient of variation; **= significant

Among the wild rice genotypes, kalampazam and Hori showed higher resistant in context of shoot reduction due to salt stress than other three whereas Lombur and B-11 showed very sensitive to increasing salt stress (Table-03). But the mutant line Lombur M4 and B-11 M4 showed better results in case of shoot length reduction comparing with its respective parent. Lombur M4 showed the characteristics similar with the salt tolerant check Pokkali in case of shoot length reduction trend with the increasing NaCl concentration. In contrast to shoot length parameters against salt stress, root length of the tested 4 local rice genotypes and two advanced mutant line rice genotypes indicated less sensitivity to increasing NaCl concentration as like the salt tolerant check Pokkali (Table-04). This results is similar with the report of Cramer et al. (1985) who found that roots were less sensitive to salt than roots [14]. Only the salt sensitive check BRRI-29 revealed severe reduction in root length with the increased level of salt. The observed reduction in shoot length in salinized conditions was possible due to many reasons. This might be due to the effect of the reduced photosynthesis, decreasing turgor in expending tissue resulting from lower water potential in rooting medium and disturbances in mineral supply induced by changes in concentration of specific ions. There are in agreement with the study reported [15].

Rice Genotypes	Root length of the seedlings (cm)							CV%	F value
	NaCl Concentrations (mM)								
	Control	**50**	**100**	**150**	**200**	**250**	**Mean**		
Lombur	7.47	6.60	8.33	6.53	7.23	6.50	7.11	10.01	**
Hori	5.17c	6.00b	8.17a	6.10b	6.73b	6.10	6.38	6.10	**
Kalampazam	5.23	5.27	5.00	5.57	5.33	4.70	5.18	22.75	**
B-11	5.37	4.37	4.57	5.43	6.13	5.67	5.26	4.86	**
Lombur M4	7.27	6.33	8.00	6.30	6.97	6.27	6.86	10.84	**
B-11 M4	6.00ab	5.03c	5.27bc	5.53abc	6.37a	5.93ab	5.69	7.85	**
Pokkali	7.00a	4.70d	6.00c	6.53b	4.30e	3.80f	5.39	1.75	**
BRRI-29	7.67a	6.47ab	6.00ab	2.67bc	2.77bc	1.27c	4.47	43.93	**

Table 4:- Effects of the different concentration of NaCl on the root length of the seedling after 2 weeks

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In a column, the figures with similar letter (s) do not differ significantly by DMRT (Duncan's multiple range test) at $p<0.05$;
CV: Coefficient of variation; **= significant

IV. CONCLUSION

From this investigation screening of salt tolerant activity with the studied genotypes, it was found that all of the four collected rice genotypes namely lombur, Hori, Kalampazam and B-11 showed improved resistance to salt stress up to 100mM NaCl than the salt sensitive check varieties. Among the four selected rice genotypes, Hori and Kalampazam showed better resistance than Lombur and B-11. Two advanced mutant lines i.e. Lombur M4 and B-11 M4 obtained through mutation breeding were found upgraded in growth performance in growing salinity stress under *in vitro* condition as like the salt tolerant check variety Pokkali is evidence in this study. Therefore, this finding will be useful in the context of Bangladesh for sustainable rice production in continuously increasing saline prone areas.

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