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EVALUATION OF RICE GENOTYPES UNDER SALT STRESS AT THE SEEDLING AND REPRODUCTIVE STAGES USING PHENOTYPIC AND MOLECULAR MARKERS

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Abstract

Salinity screening for 27 rice genotypes was performed at the seedling and reproductive stages respectively, in the hydroponic system and in sustained water bath. Three selected SSR markers were used to determine salinity tolerance in rice genotypes. Phenotyping of the germplasm was done at EC 12dS/m and 6dS/m at seedling and reproductive stages, respectively. Based on modified standard evaluation score for visual salt injury at seedling stage, eight genotypes were salt tolerant, four were moderately tolerant and the rest fifteen were susceptible. At the reproductive stage, six genotypes were tolerant to EC 6dS/m whereas eleven of them were susceptible. SSR based marker identified seven genotypes as tolerant but ten of them were susceptible for all three markers compared to two checks. Six genotypes were tolerant in both phenotypic and SSR screening. The indentified salt tolerant genotypes can be potential germplam sources for future breeding program.

Introduction

Rice (*Oryza sativa* L.) (2n = 24) belonging to the family *Graminae* and subfamily *Oryzoidea* is the staple food for one third of the world's population and occupies almost one-fifth of the total land area covered under cereals (Chakravarthi & Naravaneni, 2006). It is imperative to increase rice production in different rice growing ecosystems to feed the increasing world population (Khush, 2005). Approximately 11% of the world's arable land is planted annually to rice, and it ranks next to wheat (Chakravarthi & Naravaneni, 2006). This staple food ranked first position by production (10.7 million metric tons) in the year 2009-10 among all the cereals in Bangladesh (Anon., 2010).

Salinity is the second most widespread soil problem in rice growing countries after drought and is considered as a serious constraint to increase rice production worldwide (Gregorio *et al.*, 1997). It is quite well known that rice show variation for salt tolerance (Sabouri *et al*., 2009, Sabouri & Biabani, 2009, Habib *et al.*, 2013). Salinity is one of the most important abiotic stresses can directly affect on plant growth and development (Muhling & Lauchli, 2001; Galvani, 2007; Lauchli & Grattan, 2007, Arshad *et al*., 2012). In Bangladesh, the total saline area is one third of the 9 million hectares of total national cultivated area in Bangladesh (Anon., 2006). Rice is relatively tolerant to salinity at the germination stage but its panicle initiation and pollination stage are two most salinity-sensitive growth stages, which directly related to crop yield (Heenan *et al*., 1988; Khatun & Flowers, 1995; Zeng *et al*., 2001). Screening of rice genotypes at seedling stage is comparatively easier than reproductive stage and also rapid. It is very difficult at the reproductive stage (Gregorio *et al*., 1997). The conventional method of plant selection for salt tolerance is not easy because of the large effects of the environment and low narrow sense heritability of salt tolerance (Gregorio, 1997). A number of morpho-physiological growth factors are affected by NaCl stress (Salam *et al*., 2011). The identification of major gene locus for salt tolerance near a microsatellite marker can be used by plant breeders to select more efficiently and to better understand salt tolerance, at vegetative and reproductive growth stages (Saqib *et al*., 2012).

SSR or microsatellite markers are proved to be ideal for making genetic maps (Islam, 2004 & Niones, 2004), assisting selection (Bhuiyan, 2005) and studying genetic diversity in genotype. SSR markers are playing important role to identify gene for salt tolerance that can be helpful for plant breeders to develop new cultivars. The aim of the present study was to screen rice genotypes under saline and non-saline conditions and to evaluate microsatellite markers for the identification of salt tolerant genotypes at the seedling and reproductive stage.

Materials and Methods

Plant materials: A total of 27 traditional and improved rice genotypes were used in the study including one salt tolerant cultivar *viz*., BINA dhan8, nine high yielding varieties (HYVs), sixteen advanced lines and one land race (Kashrail) of Bangladesh. BINA dhan-8 is the salt tolerant variety of BINA, is used as one control (tolerant) whereas BINA dhan7 is used another control (susceptible), also developed at Bangladesh Institute of Nuclear Agriculture (BINA). All of these were collected from Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh. Three markers *viz.* RM10772, RM7075, RM296 were selected to evaluate 27 rice genotypes for salt tolerance. The genotypes having similar banding pattern to BINA dhan8 were considered as tolerant and similar to BINA dhan7 were considered as salt susceptible.

Phenotypic study of salinity tolerance at seedling stage: The genotypes were screen for salt tolerance at seedling stage in hydroponic system using IRRI standard protocol (Gregorio *et al*., 1997). Salinized and non-salinized setups with 3 replications were maintained. The evaluation was done using Yoshida *et al.*, (1976) nutrient solution at the glasshouse. The nutrient solution was salinized by adding crude salt to obtain desired EC of 12 dS/m. The modified standard evaluation system (SES) was used in rating the visual symptoms of salt toxicity (IRRI, 1997). Visual rating of salinity tolerance was done according to Table 1.This scoring discriminated the susceptible from the tolerant and the moderately tolerant genotypes. Initial and final scoring was done at 13 d and 22 d after salinization. For phenotypic observation plant height, root length and total dry matter was recorded at salinized and non-salinized conditions.

Screening of rice genotypes at the reproductive stage: The genotypes were evaluated for their tolerance to salinity under sustained water bath using IRRI standard protocol (Gregorio *et al.*, 1997). The experimental design was completely randomized design with three replications. Two setups were maintained: normal and salinized. Pre-germinated seeds of rice genotypes were sown in perforated glass fibre pots. The pots were placed in glass fibre trays with tap water. After 2 weeks, seedlings were thinned and the water level was raised to about 1 cm. The pots were salinized at EC 6 dS /m 3 weeks after sowing and EC was monitored in every week. Data were recorded for plant height (cm), days to flowering, days to maturity, number of effective tillers/plant, number of field grains, number of unfilled grains, total dry mater (g), percent fertility and grain yield (g).

Genotyping of salinity tolerant rice genotypes: Modified CTAB mini prep was used for DNA extraction for 25-day-old seedling (IRRI, 1997). Ten primers were used for this study. Among these primers, three primers were showed polymorphic and clear bands (Table 2). Each PCR reaction carried out with 13.0µl reactions containing 1.5 µl 10x buffer, 0.75 µl dNTPs, 1µl primer forward, 1µl primer reverse, 0.25 µl taq polymerase, 8.25 µl ddH_2O and 1.0 µl of each template DNA samples. PCR profile was maintained as initial denaturation at 94^{o}C for 5 min, followed by 34 cycles of denaturation at 94^{o}C for 1 min, annealing at 55^{o}C for 1 min and polymerization at 72^{o}C for 2 min; and final extension by 7 min at 72^{o}C. Then electrophoresis in 2% agarose gel was done after polymorphism in the PCR products and stained in ethidium bromide. Banding patterns were visualized with ultraviolet gel documentation system. The banding patterns of 27 germplasm were scored compared with tolerant control and susceptible control variety and similar banding pattern with BINA dhan8 were considered as tolerant and BINA dhan7 were considered as salt susceptible.

Table 1. 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 of 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 dying	Highly susceptible (HS)

Source: Gregorio *et al.,* (1997)

Table 2. The sequence and size of the microsatellite markers used for screening salt tolerant rice genotypes.

Primer name	Expected PCR product size (bp)	Primer sequence		Annealing Temp.(^{o}C)
RM10772	122	Forward	GCACACCATGCAAATCAATGC	55
		Reverse	CAGAAACCTCATCTCCACCTTCC	
RM7075	375	Forward	GCGTTGCAGCGGAATTTGTAGG	55
		Reverse	CCCTGCTTCTTCTCTCGTGCAGTCG	
RM296	154	Forward	CACATGGCACCAACCTCC	55
		Reverse	GCCAAGTCATTCACTACTCTGG	

Results and Discussion

Screening of rice genotypes for salt tolerance at the seedling stage: In salinized setup, the seedling growth is suppressed under salinity stress. The observed plant population in the non-salinized condition had normal seedling growth (Fig. 1). The varieties; BINA dhan-8, PBRC-37, PBSAL-655, BRRI dhan-47, FL-378, STL-15, AYT SL-41 and Kashrail were identified salinity tolerant. Four varieties (PBRC-30, FL-478, S-37 SL-25 and AYT SL-57) were identified as moderately tolerant. One variety (BINA dhan-7) was identified as highly susceptible and fourteen (BRRI dhan28, BINA dhan5, PYT SL-22, PYT SL-20, S-39 L-15, S-37 SL-31, S-37 SL-32, S-37 SL-37, AYT SL-1, AYT SL-3, AYT SL-7, AYT SL-23, AYT SL-32, and AYT SL-54) varieties were susceptible (Table 3). Islam *et al*., (2007) also observed wide variation in phenotypes from tolerant (score 3) to highly susceptible (score 9) lines using modified SES of IRRI standard protocol.

Rice plant showed various degrees of responses to the salinity. As expected tolerant lines were less affected by salt stress compare to susceptible lines for different agronomic traits such as plant height, root length and total dry matter (Table 4). S-37 SL-25 (59%), S-37 SL-37 (57.8%), S-37SL-32 (57.6%), AYT SL-32 (57.5%), BINAdhan-7 (57.2%), S-37 SL-31 (56.9%), and BINA dhan-5 (56.8%) had showed greater plant height reduction under the salinity stress at 12dS/m (Table 4). Maximum reduction of plant height was observed in the variety S-37

SL-25(59%). On the other hand, minimum plant height reduction was observed in Kashrail (22.6%) followed by BINA dhan-8 (28.4%), STL-15 (34.2%), FL-378 (39.2%), PYT Sl-22 (39.5%) and FL-478 (39.9%). These results indicated that plant height was reduced due to salinity stress. Similar results were also found by Purnendu *et al*., (2004) and Maiti *et al*., (2006). Munns & Tester, (2008) also reported that salinity might directly or indirectly inhibit cell division and enlargement during plant growing period. As a result, leaves and stems of the affected plants appeared stunted.

Table 3. Performance of rice genotypes under salinized condition (EC 12dS/m) grown in hydroponic system at the seedling stage.

S. No.	Genotypes	SES scoring	Tolerance
1.	PBRC-30	5	MT
2.	PBRC-37	3	T
3.	PBSAL-655	3	T
4.	BRRI dhan-47	3	T
5.	BRRI dhan-28	7	S
6.	FL-378	3	T
7.	FL-478	5	MT
8.	BINA dhan-5	3	S
9.	BINA dhan-7	9	HS
10.	BINA dhan-8	3	T
11.	PYT SL-22	7	S
12.	PYT SL-20	7	S
13.	STL-15	3	T
14.	S-39 L-15	7	S
15.	S-37 SL-25	5	MT
16.	S-37 SL-31	7	S
17.	S-37 SL-32	7	S
18.	S-37 SL-37	7	S
19.	AYT SL-1	7	S
20.	AYT SL-3	7	S
21.	AYT SL-7	7	S
22.	AYT SL-23	7	S
23.	AYT SL-32	7	S
24.	AYT SL-41	3	T
25.	AYT SL-54	7	S
26.	AYT SL-57	5	MT
27.	Kashrail	3	T

Salinity also decreased root length of the genotypes (Table 4). At seedling stage, some genotypes showed higher root length reduction *viz.* BINA dhan-7 (43.1%), S-37 SL-31 (38.2%), AYT SL-57 (35.1%), AYT SL-32 (34.6%), AYT SL-1 (34%) and PYT SL-22 (32.7%). On the other hand, S-37SL-32 had minimum root length reduction (8.5%) compared to rest 21 genotypes, among these, PBSAL-655, AYT SL-23, PYT SL-30, Kashrail, STL-15 and BINA dhan-8 are mentionable (Table 4). Maiti *et al.,* (2006) found a considerable amount of genetic variation under saline condition at the seedling stage with reference to the variables such as root length, shoot and root dry weight. Rodriguez *et al.,* (2002) reported that root length reduced due to the effect of salinity which coincided with the present study. Akbar and Yabuno (1974) also found that root length and emergence of new roots decreases significantly at salinized condition (EC 5-6dS/m).

Salt stress decreased total dry matter of rice seedlings (Table 4). Under salinity stress some genotypes showed drastical reduction of total dry matter. Among these variety BINA dhan-7, S-39 L-15, S-37SL-37, BRRI dhan-28, S-37 SL-31, BINA dhan-5, PBRC-37, BRRI dhan-47, AYT SL-54, BINA dhan-8 and S-37 SL-32 are noticeable.

The results are supported by Ali & Awan, (2004). They observed that under salinity stress some rice genotypes showed a remarkable reduction in root and shoot ratio at seedling stage. Roy *et al.,* (2002) reported that reduction of dry biomass increased with the increased of salinity level.

Screening of rice genotypes for salt tolerance at the reproductive stage: Under salt stress (EC 6 dS/m) 27 rice genotypes showed wider variation for yield and yield contributing characters. In salinized setup the genotypes had less vigorous growth whereas in non-salinized condition they had been showed vigorous growth (Fig. 2). Due to salinity, the effect on different traits at reproductive stage was severe for the rice genotypes. Rice genotypes differed from each other on the reductional effect on plant height, total dry matter and no. of filled grains. Considering plant height, the genotype PYT SL-20 had highest reduction (20.6%) where as kashrail had lowest plant height reduction (4.3%) and the rest genotypes, namely BINA dhan-7, BINA dhan-5, AYT SL-1, AYT SL-3,AYT SL-54, AYT SL-23, AYT SL-32, PYT SL-22, S-39 L-15, BRRI dhan-28, AYT SL-7, S-37 SL-32, PBRC-37, S-37 SL-25, FL-478, S-37 SL-3, STL-15, AYT SL-57, S-37 SL-37, BINA dhan-8, FL-378, BRRI dhan-47, AYT SL-41 and Kashrail showed plant height reduction (Table 5). Choi *et al.,* (2003) observed that the plant height decreased.

Considering total dry matter, the genotype PYT SL-20 had highest (61.1%) total dry matter reduction where as genotype AYT SL-1 had lowest (11.1%) total dry matter reduction and the rest genotypes, namely PYT SL-22, BINA dhan7, AYT SL-23, AYT SL-7, BINA dhan5, AYT SL-32, S-39 L-15, BRRI dhan28 and S-37 SL-31had higher reduction and the genotypes AYT SL-57, PBSAL-655, S-37 SL-37, S-37 SL-25, PBRC-37, AYT SL-3, AYT SL-1, Kashrail , FL-478, BINA dhan8, PBRC-30, STL-15, AYT SL-1and BRRI dhan47 had higher reduction (Table 5). Again, in case of number of filled grains the genotype AYT SL-41 had highest (78.7%) reduction where as PBRC-37 had lowest number of filled grains reduction and the genotypes.

BRRI dhan-28, AYT SL-23, AYT SL-7, PYT SL-20, BINA dhan-7, BINA dhan-5, AYT SL-32, S-37 SL-31, AYT SL-1 and AYT SL-54 had lower reduction AYT SL-3, FL-478, S-37 SL-25, S-37 SL-32, Kashrail, PBRC-30, S-39 L-15, BINA dhan8 , FL-378, BRRI dhan47, STL-15, PBSAL-655 and PBRC-37 had lesser (Table 5).

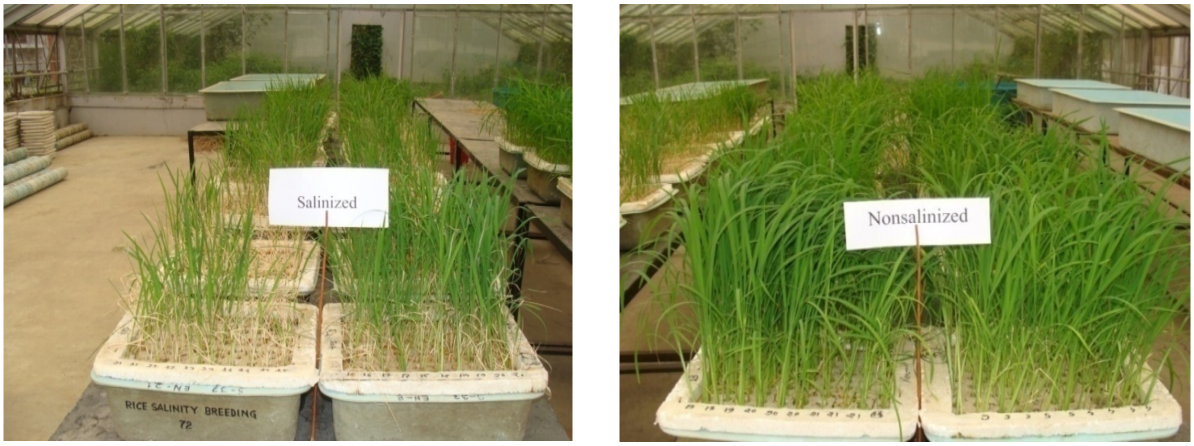

Fig. 1. Seedlings at early growth stage in salinized and non-salinized (EC 12 dS/m) condition.

Fig. 2. Performance of rice germplasm under non-salinized and salinized (EC 6 dS/m) condition at the reproductive stage.

This is because of loss of biomass production was lower in tolerant genotypes which increased the assimilation and ultimately produced the higher number of grains. Tolerant genotypes had lower reduction than the susceptible genotypes. This result was consistent with the result observed by Islam (2004) who worked with 80 recombinant inbred lines of Pokkali X IR29. 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 is supported by Bhowmik *et al.*, (2009) who worked with 11 rice genotypes and found that the genotype THDB had the lowest reduction of total dry mater where as the genotype Kaliboro had the highest reduction.

On the basis of performance at salinized condition, the genotypes BINA dhan-8, PBRC-37, Kashrail, PBSAL-655, FL-478, BRRI dhan-47, AYT SL-1 and STL-15 showed higher % fertility (>65%) and BINA dhan-7, S-37 SL-37, S-37 SL-25, PYT SL-22, AYT SL-7, AYT SL-3 and AYT SL-23 had lower % fertility (<45%) (Table 6). PBRC-37, STL-15, BINA dhan-8, AYT SL-41, Kashrail, FL-478 and BRRI dhan-47 performed higher filled grain weight **(**>3 g). On the other hand PBRC -30, BINA dhan-7, S-37 SL-37, AYT SL-23, AYT SL-57, AYT SL-32, PYT SL-30 and BRRI dhan-28 had lower filled grain weight (<1.7g). STL-15, BINA dhan-8, PBRC-37, BRRI dhan-47 and FL-478 showed better performance with respect to 1000-seed weight compared to Table 4.

PBRC-30, BINA dhan-8, STL-15, Kashrail, FL-378, AYT SL-57 and BRRI dhan-47 were tolerant on the basis of percent reduction of plant height (<8.8 cm), total dry matter (<20 g) and number of filled grain (55) and PBRC-37, PBSAL-655, FL-478, and AYT SL-41 were moderately tolerant and the rest were susceptibile on the basis of percent reduction of plant height. According to the performance of yield per plant in salinized condition, BINA dhan-8, STL-15, PBRC-37 AYT SL-41, Kashrail, FL-478 and BRRI dhan-47 were found as tolerant (>3 g) and PBRC-30, PBSAL-655, BINA dhan-7, AYT SL-1, AYT SL-3, AYT SL-7, S-37 SL-25, S-37 SL-32, PYT SL-20, BRRI dhan28, and BINA dhan5 were found to be susceptible genotype. The grain yield is reported to be decreasing with increasing salinity levels (Powar & Mehta, 1997).

This result supported by Asch *et al*., (1999) who worked with 8 rice cultivars and found that cultivars differed in their salt uptake and tolerant cultivars had lower salt effect on yield and yield components than susceptible. Filled grain weight and total dry matter weight contributed the most variation to grain yield under salinity stress and these traits were selected for tagging the salinity tolerance genes.

Table 4. Performance of the genotypes in response to plant height, root length and total dry matter at the seedling stage (30 DAS) under salinized (EC12dS/m) and non-salinized conditions.

Genotypes	Plant height (cm)			Root length (cm)			Total dry matter (g)		
	Non-salinized	Salinized	% Reduction	Non-salinized	Salinized	% Reduction	Non-salinized	Salinized	% Reduction
BINA dhan-8	52.1	37.3	28.4	18.7	15.3	18.2	25.2	17.7	29.8
BINA dhan-7	49.8	21.3	57.2	18.1	10.7	40.9	26.7	5.2	80.5
S-39 L-15	46.8	21.8	53.4	15.1	11.5	23.8	15.6	3.4	78.2
PYT SL-22	45.1	27.3	39.5	16.2	10.9	32.7	10.6	4.5	57.5
PBRC-30	40.5	21.2	47.7	17	12	29.4	16.5	10.9	33.9
PBRC- 37	41.6	19.7	52.6	15.3	10.5	31.4	13.8	11	20.3
AYT SL-7	43.1	23	46.6	14.2	10.6	25.4	12.6	6.7	46.8
AYT SL-41	42.3	21.2	49.9	11.2	8	28.6	11.7	5.9	49.6
Kashrail	57.1	44.2	22.6	12.5	10.7	16	25.4	14.2	44.1
AYT SL-23	51.2	22.2	56.6	9.9	8.7	12.1	12.3	5.7	53.7
S-37 SL-37	44.6	18.8	57.8	10.5	9.3	12	15.6	5.2	66.7
S-37 SL-25	43.2	17.7	59	13.	11.6	9.5	11.2	5.4	54.5
S-37 SL-31	42.5	18.3	56.9	13.6	8.4	38.2	13.8	5.1	63
S-37 SL-32	41	17.4	57.6	14.1	12.9	8.5	10.1	6.8	32.7
AYT SL-57	53.2	31.2	41.4	13.4	8.7	35.1	11.5	5.7	50.4
AYT SL-3	50.1	28.2	43.7	13.3	9.7	27.1	14.7	7.5	48.9
AYT SL-54	49.2	25.2	48.8	15.7	12.6	19.7	17	12.9	24.1
PYT SL -30	41.5	18.7	54.9	12.9	11.9	12.8	7.8	3.3	57.7
AYT SL-32	52.2	22.2	57.5	14.3	9.9	34.6	13.3	8.7	34.6
AYT SL-1	39.9	19.9	50.1	14.4	9.5	34	15.4	6.8	55.8
PBSAL-655	45	26.2	41.7	13	11.5	11.5	20.1	6.2	69.1
STL -15	44.4	29.2	34.2	13.7	11.5	16.1	10.7	6.2	42
FL -378	40.8	24.5	39.2	16.4	12	22.4	12.4	6.9	44.4
FL- 478	44.4	26.7	39.9	13.2	9.8	25.8	13.8	5.9	57.2
BINA dhan-5	37.3	16.1	56.8	16	10.2	36.3	17.3	6.8	60.7
BR RI dhan-47	43.2	23.3	46.1	11.8	9.6	18.6	14.5	11.3	22
BRRI dhan- 28	44.6	23.6	47.1	15.3	13.9	11.8	18.2	6.3	65.4
$Lsd_{(0.05)}$	5.4	5.9		2.9	2.9		2.2	2.4	

Table 5. Effects of salinization (EC 6dS/m) on plant height, total dry matter and number of filled grains at reproductive stage of the rice germplasm grown in sustained water bath at Bangladesh Institute of Nuclear Agriculture.

Genotypes	Plant height (cm)			Total dry matter (g)			No. of filled grains		
	Non-salinized	Salinized	% Reduction	Non-salinized	Salinized	% Reduction	Non-salinized	Salinized	% Reduction
BINA dhan-8	100	93.3	6.7	6.67	5.3	13.6	427	202.6	52.6
BINA dhan -7	92.1	73.3	18.8	7.4	3.5	39	588	155.6	73.5
S-39 L-15	93	83.1	10.6	13.2	9	31.8	360	165.1	54.1
PYT SL-22	82.1	70.4	11.8	6.4	3.7	42.2	370	103.2	72.1
PBRC -30	85	81	4.7	15.5	13.4	13.5	280	126.4	54.3
PBRC-37	97	87	10.3	13.7	11.2	18.2	297	170.9	42.5
AYT SL-7	81.3	70.7	10.5	6.5	4.1	36.9	450	107.7	76.1
AYT SL-41	83	78.5	5.9	6.3	5.6	11.1	550	117.4	78.7
Kashrail	72.4	69.3	4.3	10.3	8.6	16.5	435	196.3	54.9
AYT SL-23	81	70.2	13.5	6.7	4.1	38.8	560	129.1	76.9
S-37 SL-37	101	93.4	7.5	8	6.5	18.8	290	97.2	66.4
S-37 SL-25	102	91.5	10.3	8.2	6.7	18.3	290	120.6	58.4
S-37 SL-31	99	91.3	8.8	7.8	5.9	24.4	297	99.3	66.6
S-37 SL-32	104	93.4	10.4	10.2	8.8	13.7	290	127	56.1
AYT SL-57	98.8	91.2	7.8	10.1	7.8	22.8	420	211	49.7
AYT SL-3	95	80.1	15.7	6.2	5.1	17.7	550	222	59.7
AYT SL-54	91.3	78.9	13.6	8.2	6.9	15.9	510	187.3	63.3
PYT SL-20	82	65.1	20.6	10.8	4.2	61.1	440	109.5	75.1
AYT SL-32	81.8	70.1	11.7	6	3.9	35	617	195.1	68.4
AYT SL-1	88.6	73.4	17.2	15.3	9.9	17.4	610	205.3	66.3
PBSAL-655	93	83.3	10.4	6.2	5	19.4	249	137.1	44.9
STL-15	100	91.3	8.7	9.3	8.1	12.9	255	131.5	48.6
FL-378	84.2	78.1	6.1	7.8	6.9	11.5	260	126.5	51.3
FL-478	80.1	70.4	9.6	10.3	8.7	15.5	408	165.7	59.4
BINA dhan-5	97	80.2	17.3	9.8	6.2	36.7	301	88.7	70.5
BRRI dhan- 47	97.1	91.2	6.1	7.2	6.6	8.33	387	193.1	50.1
BRRI dhan-28	94.1	83.2	10.6	13.1	9.2	29.8	525	120.3	77.1
$Lsd_{(0.05)}$	9.4	8.9		0.76	0.64		54.7	14.6	

Table 6. Fertility (%), filled grain weight and 1000-seed weight of rice germplasm under salnized (EC 6dS/m) and non-salinized condition at reproductive stage.

	%Fertility		Yield/plant (g)		1000 seed weight	
Genotypes	**Non-salinized**	**Salinized**	**Non-salinized**	**Salinized**	**Non-salinized**	**Salinized**
BINA dhan-8	93.9	75.8	6.7	5.6	15.1	15
BINA dhan-7	88.5	33.8	7.5	1.4	12.8	12.4
S-39 L-15	79.8	56.5	5.9	2.6	14.7	13.6
PYT SL-22	86.2	44.8	5.1	2.9	13.9	12.2
PBRC-30	84.6	51.4	4.4	1.4	14.9	6.3
PBRC- 37	86.6	67.3	5.2	3.3	16.9	16.7
AYT SL-7	87	44.3	9.9	1.7	14.5	13.4
AYT SL-41	88.6	41.3	5.6	3.3	20.2	17.1
Kashrail	91	74.3	5.5	4.4	15.8	14.2
AYT SL-23	87.9	44.6	4.7	1.1	13.1	13.4
S-37 SL-37	73.4	40.9	4.9	1.5	16.9	11.9
S-37 SL-25	81.7	42.5	11.7	2.4	15	13.9
S-37 SL-31	81.4	47.2	4.5	2.1	16	15.2
S-37 SL-32	83.1	47.4	4.9	2.4	15.2	14.8
AYT SL-57	82.2	58.8	4.6	1.4	14.1	13.2
AYT SL-3	76.3	42.2	4.9	1.7	15.3	14.2
AYT SL-54	86.6	62.5	4.5	2.9	19.1	17.9
PYT SL-20	82.7	57.9	6.1	1.4	15.9	14.5
AYT SL-32	89.6	63.7	5.7	1.5	21.2	11.8
AYT SL-1	88.7	66.3	5.7	2.1	14.8	13.2
PBSAL-655	87.7	67.8	4.2	2.3	12.9	11.8
STL-15	91.1	79.2	5.1	3.7	16.7	16.3
FL -378	87.1	62.5	5.4	1.7	15.7	14.7
FL- 478	91.2	73.5	6.7	3.5	20.1	19.8
BINA dhan-5	78.9	41.5	8.2	1.7	17.4	14.3
BRRI dhan-47	91.6	66.8	6.9	3.8	16.1	16
BRRI dhan-28	86.4	58.5	7.1	1.6	15.6	11.1
$Lsd_{(0.05)}$	5.5	5.9	0.73	0.23	2.71	2.8

Genotyping evaluation of rice genotypes using SSR markers: As compared to BINA dhan-8, genotypes namely, PBRC-37, Kashrail, S-37 SL-31, AYT SL-54, PBSAL-655, STL-15, FL-378, FL-478 and BRRI dhan-47 were found tolerant when samples were amplified with RM10772 because they positioned in the same level of BINA dhan8 (Fig. 3). In the same reaction, S-39 L-15, PYT SL-20,PYT SL-22, PBRC-30, AYT SL-1, AYT SL-3, AYT SL-7, AYT SL-23,AYT SL-32, AYT SL-41, AYT SL-57, S-37 SL-37, S-37 SL-25, S-37 SL-32, BINA dhan5 and BRRI dhan28 were found susceptible as they positioned in the same level of BINA dhan7.

In case of RM7075 marker, PBRC-30, PBRC-37, AYT SL-41, Kashrail, S-37 SL-25, AYT SL-3, AYT SL-57, PBSAL-655 , STL-15, FL-378, FL-478 and BRRI dhan47 had similar band with BINA dhan8 and S-39 L-15, PYT SL-20, PYT SL-22, AYT SL-7, AYT SL-23, AYT SL-1, AYT SL-32, AYT SL-54, S-37 SL-37, S-37 SL-31, S-37 SL-32 BINA dhan5 and BRRI dhan28 had similar band with BINA dhan7 (Fig. 4).

Regarding RM296 marker, PBRC-37, PBSAL-655, STL-15, PYT SL-22, Kashrail, S-37 SL-25, AYT SL-57, AYT Sl-41, FL-378, FL-478 and BRRI dhan-47 had similar band with BINA dhan-8. PBRC-30, S-39 L-15, S-37 SL-37, S-37 SL-31, S-37 SL-32, AYT SL-1, AYT SL-3,AYT SL-7, AYT SL-23, AYT SL-32, AYT SL-54, PYT SL-20, FL-478, BINA dhan-5, AYT SL-54 and BRRI dhan-28 had similar band with BINA dhan-7 (Fig. 5).

PBRC-37, Kashrail, PBSAL-655, FL-378, FL-478, STL-15 and BRRI dhan47 had similar band with BINA dhan8 which is salt tolerant and S-39 L-15, S-37 SL-37, S-37 SL-32, BINA dhan5, AYT SL-1, AYT SL-7, AYT SL-23, AYT SL-32, PYT SL-20 and BRRI dhan28 had similar band with BINA dhan7 which is salt susceptible. There was no common variety found moderately tolerant in all the tested markers. Phenotypically, BINA dhan8, Kashrail, BRRI dhan47, FL-378, STL-15, PBRC-37 and PBSAL-655 were found as tolerant while, and BINA dhan-7, PYT SL-20, AYT SL-23, AYT Sl-32, BINA dhan-5 and BRRI dhan-28 were found as susceptible. This phenotypic observations support the genotypic findings for identification of salt tolerant rice genotypes.

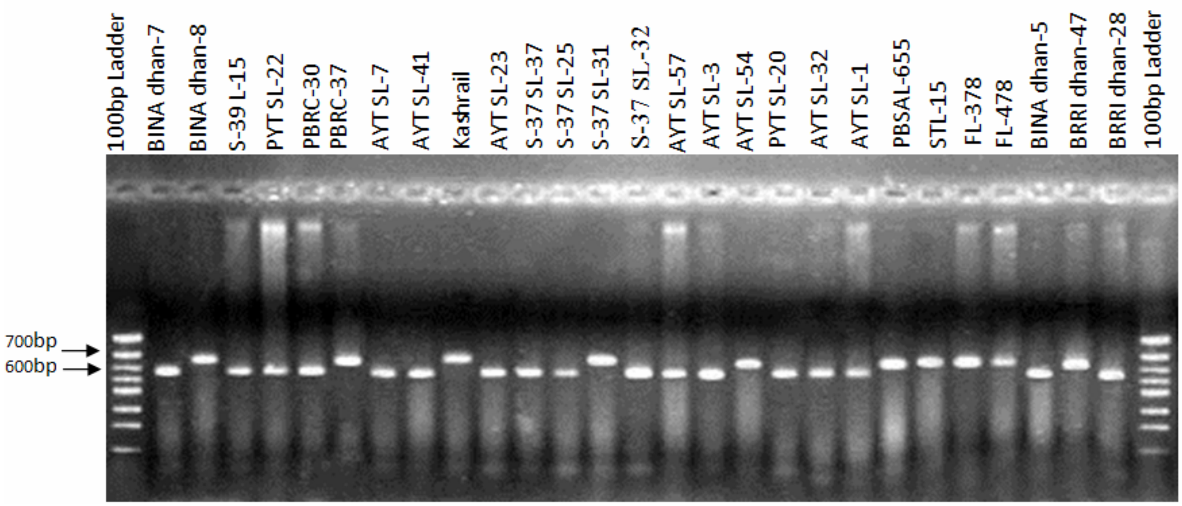

Fig. 3. Banding profiles of 27 rice genotypes using primer RM10772.

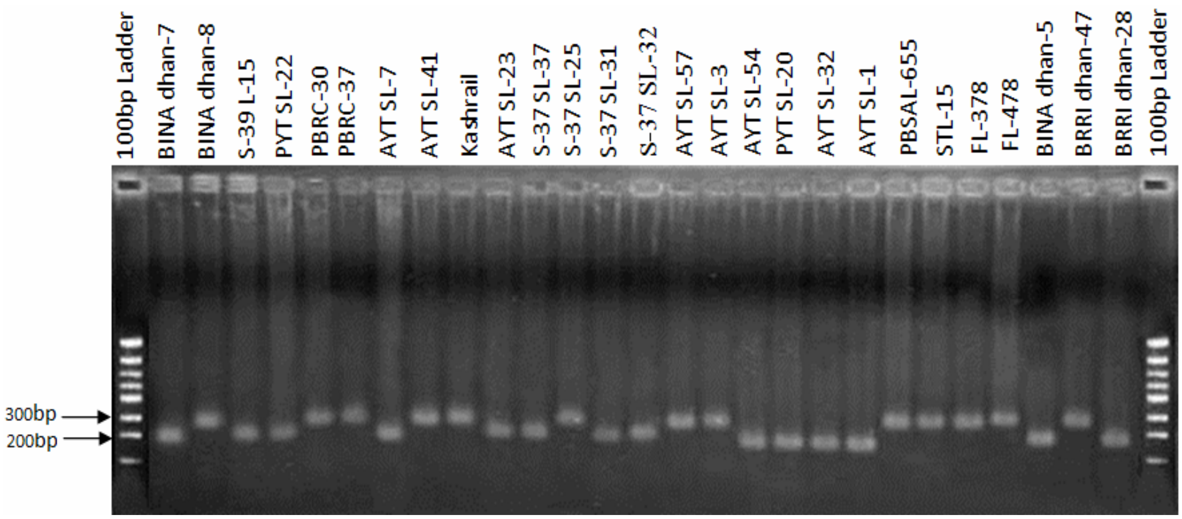

Fig. 4. Banding profiles of 27 rice genotypes using primer RM7075.

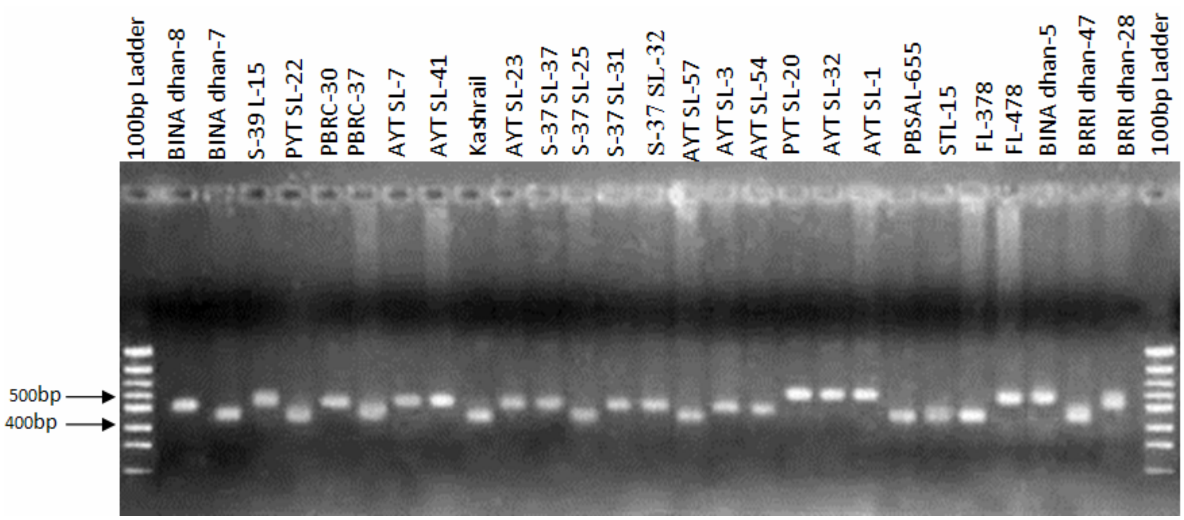

Fig. 5. Banding profiles of 27 rice genotypes using primer RM296.

These three primers (RM10772, RM7075 and RM296) showed polymorphisms in studying genotypes because they showed different banding pattern and discriminated tolerant genotypes from susceptible with relation to BINA dhan8 (tolerant) and BINA dhan7 (susceptible). Those markers were reported as highly polymorphic in IR29 x Pokkali for tagging salt tolerant genes as reported by Islam, 2004 & Niones, 2004. Chakravarthi & Naravaneni (2006) also reported that SSR primers had distinct polymorphism in rice while they studied 30 SSR primers on 15 rice genotypes.

The genotypes *viz.,* Kashrail, BRRI dhan-47, FL-378, STL-15, PBRC-37 and PBSAL-655 were identified as tolerant on the basis of phenotypic (agronomic performance) and genotypic (reaction with markers) studies. The markers *viz.,* RM10772, RM7075 and RM296 could be used in marker-assisted selection programme, quantitative trait loci (QTL) mapping, in studying genetic diversity of genotypes and also gene pyramiding of rice salinity breeding. Microsatellite marker analysis is promising to identify major gene locus for plant breeders to develop new cultivars. The selected salt tolerant rice genotypes would be further tested in saline areas to observe yield potentiality to develop high yielding and salt tolerant.

References

Akbar, M., and T. Yabuno. 1974. Breeding for saline-resistant varieties of rice. II. Comparative performance of some rice varieties to salinity during early developing stage. *Jpn. J. Breed*., 24(4): 176-181.

Ali, Y. and A.R. Awan. 2004. Influence of salinity at seedling stage and on yield and yield components of different rice lines. *Intl. J. Biol. Biotechnol*., 1(2): 175-179.

Anonymous. 2006. ABSPII. Drought tolerant rice and salinity tolerance rice. Agricultural Biotechnology Support Project II- South Asia. pp.26.

Anonymous. 2010. Agriculture Wing. Bangladesh Bureau of statistics, Ministry of planning, Government of the People's Republic of Bangladesh, Dhaka. 54.

Arshad, M., M. Saqib, J. Akhtar and M. Asghar. 2012. Effect of calcium on the salt tolerance of different wheat (*Triticum aestivum* L.) genotypes. *Pak. J. Agri. Sci*., 49: 497-504.

Asch., F., M. Dingkuhn, C. Wittstock, K. Doerffling. 1999. Sodium and potassium uptake of rice panicles as affected by salinity and season in relation to yield and yield components. *Plant and Soil*, 207(2): 133-145.

Bhuiyan, M.A.R. 2005. *Efficiency in evaluating salt tolerance in rice using phenotypic and marker assisted selection*. M.S. Dissertation, Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh, Bangladesh. pp. 96.

Bhowmik, S. K., S. Titov, M. M. Islam, A. Siddika, S. Sultana and M. D. S. Haque. 2009. Phenotypic and genotypic screening of rice genotypes at seedling stage for salt tolerance. *African J. Biotech*., 8(23): 6490-6494.

Chakravarthi, B.K. and R. Naravaneni. 2006. SSR marker based DNA fingerprinting and diversity study in rice (*Oryza sativa*. L). *African J. Biotechnol*., 5(9): 684-688.

Choi, W.Y., K.S. Lee, J.C. Ko, S.Y. Choi and D.H. Choi, 2003. Critical saline concentration of soil and water for rice cultivation on a reclaimed saline soil. *Korean J. Crop Sci*.,, 48: 238-242.

Galvani, A. 2007 .The challenge of the food sufficiency through salt tolerant crops. *Rev. Env. Sci. Biotechnol*., 6: 3-16.

Gregorio, G.B. (1997). Tagging Salinity Tolerant Genes in Rice Using Amplified Fragment Length Polymorphism (AFLP). Ph.D.dissertation. University of the Philippines Los Baños College, Laguna, Philippines, p. 118.

Gregorio, G.B., Senadhira, D. and R.D. Mendoza. 1997. Screening rice for salinity tolerance. IRRI Discussion Paper Series no. 22. Manila (Philippines): International Rice Research Institute. pp.1-30.

Habib, N., M. Ashraf and M. Shahbaz. 2013. Effect of exogenously applied nitric oxide on some key physiological attributes of rice (*Oryza sativa* L.) plants under salt stress. *Pak, J. Bot*., 45(5): 1563-1569

Heenan, D. P., Lewin, L. G. and D.W. McCaffery. 1988. Salinity tolerance in rice varieties at different growth stages. *Aust. J. Exp. Agric*., 28: 343-349.

IRRI (International Rice Research Institute) (1997). Rice Almanae. IRRIWARDA-CIAT, Los Baños, Laguna, Philippines.

Islam, M.M. (2004). Mapping salinity tolerance genes in rice (*Oryza sativa* L.) at reproductive stage. Ph.D. dissertation, University of the Philippines Los Baños College, Laguna, Philippines. pp. 1-149.

Khatun, S. and T.J. Flowers. 1995. Effects of salinity on seed set in rice. *Plant Cell Environ*., 18: 61-67.

Khush, G.S. 2005. What it will take to feed five billion rice consumers by 2030. *Plant Mol. Biol*., 59: 1-6.

Lauchli, A and S.R. Grattan. 2007. Plant growth and development under salinity stress. In: *Advances in Molecular Breeding Toward Drought and Salt Tolerant Crops*, (Eds.): M.A. Jenks, P.M. Hasegawa and S.M. Jain. Springer, Dordrecht, Netherlands.

Maiti, R.K., P. Vidyasagar, P. and P.P. Banerjee. 2006. Salinity tolerance in rice (*Oryza sativa* L.) hybrids and their parents at emergence and seedling stage. *Crop Res. Hisar*., 31(3): 427-433.

Muhling, K. H. and E. Lauchli. 2001. Physiological traits of sodium toxicity and salt tolerance. In: Horst WJ, Olfs HW, Schenk MK, Römheld V, Burkert, A, Sattelmacher B, Claassen N, Schmidhalter U, Flessa H, Schubert S, Frommer WB, Wirén NV, Goldbach H.

Munns, R. and M. Tester. 2008. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol*, .59: 651- 681.

Nguyen, T. L., S. Yanagihara and B. C. Buu. 2001. A microsatellite marker for a gene conferring salt tolerance on rice at the vegetative and reproductive stages. *SABRAO J. Breed. Genet*., 33: 11-20.

Niones, J.M. 2004. Fine mapping of the salinity tolerance gene on chromosome 1 of rice (*Oryza sativa* L.) using near-isogenic lines. MSc. Dissertation, University of the Philippines Los Baños College, Laguna, Philippines, p. 78.

Powar, S.L. and V.B. Mehta. 1997. Integrated nutrient Management for rice in coastal saline soil of high rainfall area. *Ann. Agril. Res*., 18(4): 538-540.

Purnendu, G., M.A. Mannan, P.S. Pal, M.M. Hossain and S. Parvin. 2004. Effect of salinity on some yield attributes of rice. *Pak. J. Biol. Sci*., 7(5): 760-762.

Rodríguez, A. A., A. M. Stella, M. M. Storni, G. Zulpa, and M. C. Zaccaro. 2006. Effects of cyanobacterial extracellular products and gibberellic acid on salinity tolerance in *Oryza sativa* L. Saline Systems. 2 (7):1186-1148.

Roy, S.K., S.K. Patra and K.K. Sarkar. 2002. Studies on the effect of salinity stress on rice (*Oryza sativa* L.) at seedling stage. *J. Interacademicia*, 6 (3): 254-259.

Sabouri, H. and A. Biabani. 2009. Toward the mapping of agronomic characters on a rice genetic map: Quantitative Trait Loci analysis under saline condition. *Biotechnology*, pp. 144-149.

Sabouri, H., A. M. Rezai, A. Moumeni, A. Kavousi, M. Katouzi, and A. Sabouri. 2009. QTLs mapping of physiological traits related to salt tolerance in young rice seedlings. *Biologia Plantarum* 53(4): 657-662.

Saqib, Z.A., J. Akhtar, M.A. Ul-Haq and I. Ahmad. 2012. Salt induced changes in leaf phenology of wheat plants are regulated by accumulation and distribution pattern of Na^+ ion. *Pak. J. Agri. Sci.*, 49: 141-148.

Salam, A., Z. Ali and M. Aslam. 2011. Sodium chloride tolerance in rice (*Oryza sativa* L.) At early seedling growth: genotypic variability, identification and selection. Pak. J. Bot. 43(6): 2701-2705.

Yoshida. S., D.A. Forno, J.H. Cook and K.A. Gomez. 1976. Laboratory manual for physiological studies of rice. International Rice Research Institute (IRRI), Los Baños, Laguna, Philippines. p. 61-66.

Zeng, L., M.C. Shannon and S.M. Lesch. 2001. Timing of salinity stress affects rice growth and yield components. *Agric. Water Manage*. 48: 191-206.

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