Performance of Rice Landraces Under Salt Stress at the Reproductive Stage Using SSR Markers

ABSTRACT

Rice is the staple food crop of half of the world population and salinity is the most significant causes of rice yield reduction. The aim of this study was to screen 24 rice genotypes including 20 landraces to find out potential germplasm source for salt tolerance breeding program. Screening was performed at reproductive stage by evaluating the yield and yield attributes in sustained water bath maintaining the salinity level at 8 dS/m. Three microsatellite markers linked with salt tolerance quantitative trait loci *viz.* RM234, RM134 and RM9 were selected in response to salinity in rice landraces. At the reproductive stage, four landraces *viz.* Kute Patnai, Kashrail, Bazra Muri and Tal Mugur were identified as salt tolerant on the basis of phenotypic evaluation but SSR based marker, eight rice genotypes *viz* Binadhan-8, Patnai, KutePatnai, BazraMuri, Tal Mugur, Pokkali, Kashrail and FL 378 were found as tolerant. Combined assessment of morphological and SSR marker, four genotypes were considered as true salt tolerant lines. These identified landraces could be a potential germplasm sources for future salt tolerance rice breeding program.

Keywords: *Rice, landrace, salt tolerant, microsatellite marker*

Introduction

Rice (*Oryza sativa* L.) is an important crop that feeds more than half of the world's population. Asian farmers contribute about 92 % of the total world's rice production [1]. But it is very sensitive to salinity stress and is currently listed as the most salt sensitive cereal crop with a threshold of 3 dS/m for most cultivated varieties [2]. Salinity is most important abiotic stress that directly regulates the plant growth and development [3-5]. It affects all the growth stages of rice from seedling to maturation [6] but reproductive stage is more sensitive for grain yield production [7]. Rice yield in salt-affected land is significantly reduced with an estimation of 30–50% yield losses annually [8]. Due to natural salinity and human interferences, the arable land is continuously transforming into saline that is expected to have overwhelming global effects, resulting in up to 50% land loss by 2050 [9,10].

In Bangladesh, 11.37 million hectares of land produces 34.53 million tons of rice [11] and about 1.8 million ha of coastal land is affected by different degrees of salinity. Most of the southern districts of the country are under saline zones which cover an area of 25-30% of the total cultivable land [12]. The population of Bangladesh is still growing by two million every year and may increase by another 30 million over the next 20 years. Thus, Bangladesh will require about 27.26 million tons of more rice for the year 2020 (http://www.knowledgebank-brri.org/riceinban.php). To increase the production it needs development of salt tolerant variety and utilization of salt affected areas. Methods for salinity tolerance screening are important for the success of a breeding program. As improving yield of plants undergoing salinity stress is one of the main targets of plant breeding, salinity tolerance screening based on agronomical parameters such as growth, yield and yield components has become the method of choice by labs worldwide [13-16].

A number of genomic tools, such as molecular markers can greatly improve the efficiency of breeding programs. The use of molecular markers has been increasing considerably because of their reliability and helps in deciding the distinctiveness of species [17]. Among the molecular marker technologies, microsatellite or simple sequence repeats (SSRs) are widely used in rice genetic studies because of their availability, widespread distribution in the genome, high allelic diversity and have been found to be efficient in identification of rice cultivars [18-20]. Microsatellite or SSR markers are proved to be ideal for making genetic maps [19,21] assisting selection [20] and have been applied to analyze diversity [22,23]. SSR markers are playing important role to identify genes and quantitative trait loci [24,25] that can be helpful for plant breeders to develop new cultivars. Landraces are currently being used as preferred potential donors of salt tolerance traits because of their local adaptation. Due to genetic similarities between cultivated rice species, the transfer of useful genes from one to another is possible. The presence of markers tightly linked to resistance genes will allow selection and maintenance of the desirable resistant genotypes in breeding process [26,27]. Thus, the evaluation of rice landraces could provide valuable information for genetic improvement of salt tolerant rice variety.

The objective of this study was to identify the salt tolerant geneotypes based on phenotype and molecular markers linked to the salt tolerance which can be used further for marker assisted selection in rice breeding program.

Materials and methods

Plant Materials

A total of 24 rice lines including 20 landraces, 2 BINA developed high yielding varieties and 2 advanced lines were collected from the germplasm center of Bangladesh Institute of Nuclear Agriculture (BINA). BINA developed salt tolerant variety Binadhan-8 was used as tolerant control and HYV Binadhan-7 as susceptible control.

Plant growth condition and phenotypic evaluation under Salinity

IRRI standard protocol [13] was followed to assess the genotypes for their tolerance to salinity in sustained water bath. Completely randomized design (CRD) with three replications was followed for experimental design. Both Normal and salinized setups were maintained. The seeds were kept in the convention oven for 5 days at 50˚C for breaking the seed dormancy. The oven treated seeds were soaked with tap water for 24 hours for pre-germination. The pre-germinated seeds were sown on the soil surface in perforated pots (3/4 seeds/pot) which were kept in the tray with water. After 2 weeks, the seedlings were thinned to two per pot and the water level was raised up to 1 cm above the soil surface. The water level was maintained daily and the plants were protected from pests and diseases. After 3 weeks of sowing the pots were salinized at EC 8 dS/m by dissolving crude salt and EC was monitored in every week till maturity. Data were recorded on plant height (cm), days to flowering, number of effective tillers/plant, number of field grains and unfilled grains, percent fertility and grain yield (g).

Percent fertility was calculated using the following formula.

% fertility = {(No. of filled grains/ (No. of filled grains+ No. of unfilled grains)} x100

Percent reduction was calculated using the following formula**:**

% reduction= {(traits in normal - traits in saline)/Traits in normal} x100

DNA extraction, PCR amplification and molecular marker analysis

Modified CTAB mini prep method was followed for genomic DNA extraction from 25-day-old seedling leaf sample [28]. Ten primers were surveyed and among them three primers showed polymorphism and clear bands (Table 1). 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 analysis was performed according to our previous study by Akter et al. [29] with little modifications. 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 30 second. annealing at 55^{o}C for 30 second and polymerization at 72^{o}C for 1min., and a final extension of 7 min. at 72^{o}C. Primer pairs were optimized for PCR to amplify microsatellite loci. Parental varieties were used to identify SSR polymorphism associated with the salt tolerance gene. Finally, we used the three polymorphic SSR markers (Table 1) for genotyping the 24 rice landraces. The amplified PCR products were separated in a 2.5 % agarose gel and then stained in 0.1 g/ml ethidium bromide containing water. Banding patterns were visualized with ultraviolet gel documentation system. The banding patterns of 24 genotypes were scored by comparing with tolerant and susceptible controls and similar banding pattern with Binadhan-8 were considered as tolerant and Binadhan-7 as salt susceptible.

Table 1. The sequence and size of the microsatellite markers used for screening salt tolerant rice lines

Primer name	Expected PCR product size (bp)	Primer sequence		Annealing Temp.(^{o}C)
RM234	156	For.	ACAGTATCCAAGGCCCTGG	55
		Rev.	CACGTGAGACAAAGACGGAG	
RM134	93	For.	ACAAGGCCGCGAGAGGATTCCG	55
		Rev.	GCTCTCCGGTGGCTCCGATTGG	
RM9	136	For.	GGTGCCATTGTCGTCCTC	55
		Rev.	ACGGCCCTCATCACCTTC	

Results and discussion

Phenotypic performance of rice landraces at reproductive stage

A wide range of phenotypic variation was observed at reproductive stage among the rice germplasms under 8 dS/m salinity stress. Normal plant growth and development was observed in normal setup but in salinized setup growth and development was retarded. Different adverse symptoms, such cracked and dried leaves, stunted plant growth and early flowering & maturity were observed in saline condition. Rice genotypes showed significant difference in reduction of plant height, panicle length and number of filled grains.

The percentage of plant height reduction ranged from 6.55 to 29.24 and highest reduction rate was observed in Volanath (29.24%) followed by Rupessor (28.59%), Binadhan-7 (27.42%) and Koicha binni (26.88%). On the other hand, Pokkali (6.55%) followed by Binadhan-8 (6.61%), Kashrail (7.54%), FL-378 (8.17%), Tal Mugur (8.84%), Bazra Muri (8.96%), FL-478 (9.43%), Kute Patnai (10.63%), Nona Bokra (10.74%), Jamai naru (12.44%) and Patnai (12.77%) showed comparatively lower reduction rate (Table 2). This reduction may be due to the inhibition of cell division or cell enlargement for salt stress. Reduction in plant height due to salt stress was also reported by Rubel *et al.* [30], Bhowmik *et al.* [31] and Choi *et al.* [32]. Percent reduction in panicle length was ranged from 6.88 to 22.61. Considering the panicle length, Volanath (22.61%), Binadhan- 7 (21.91%), Rupessor (21.35%) and Koicha Binni (21.56%) showed heigher reduction. Besides, Kashrail (6.88%), Pokkali (7.11%), Binadhan-8 (7.20%), FL-478 (7.43%) Patnai (7.69%), FL-378 (8.19%), Bazra Muri (8.72%), Nona Bokra (8.99%), Kute Patnai (9.13%), Tal Mugur (9.40%) and Jamai Naru (9.60%) showed lower reduction in panicle length (Table 2).

Table 2. Effects of salinization (EC 8dS/m) on plant height, panicle length and number of filled grains at reproductive stage of the rice germplasm grown in sustained water bath at BINA

SL No.	Genotypes	Plant height (cm)			Panicle Length (cm)			No. of filled grains/ panicle		
		Non-salinized (mean)	Salinized (mean)	% Reduction	Non-salinized (mean)	Salinized (mean)	% Reduction	Non-salinized (mean)	Salinized (mean)	% Reduction
1.	Jamai Naru	144.40	122.40	15.24	19.80	17.90	9.60	89.30	39.20	56.10
2.	Patnai	134.70	117.50	12.77	20.80	19.20	7.69	112.10	81.20	27.56
3.	Kute Patnai	136.40	121.90	10.63	20.80	18.90	9.13	102.70	58.30	43.23
4.	Holde Gotal	125.50	105.50	15.94	22.63	20.03	11.49	99.20	47.30	52.32
5.	Bashful Balam	138.60	111.70	19.41	22.90	20.10	12.23	122.20	64.10	59.56
6.	Bazra Muri	129.40	117.80	8.96	19.50	17.80	8.72	78.10	51.20	34.44
7.	Ghunshi	141.10	116.40	17.51	21.10	18.50	12.32	88.20	44.80	60.54
8.	Chinikani	123.20	100.30	18.59	18.60	15.40	17.20	101.30	41.20	59.33
9.	Binadhan 7	100.30	72.80	27.42	17.80	13.90	21.91	99.70	27.80	72.12
10.	Volanath	139.20	98.50	29.24	23.00	17.80	22.61	122.20	28.90	76.35
11.	Rupessor	147.60	105.40	28.59	21.87	17.20	21.35	146.90	44.20	69.91
12.	Kalo Mota	138.50	118.90	14.15	23.17	20.40	11.96	116.30	48.40	58.38
13.	Nona Kochi	141.50	118.00	16.61	23.50	21.00	10.64	106.20	46.60	56.12
14.	Tal Mugur	123.30	112.40	8.84	23.40	21.20	9.40	104.10	57.20	45.05
15.	Ghigoj	146.33	115.50	21.07	23.40	19.20	17.95	114.20	57.40	49.78
16.	Fulkainja	138.00	105.40	23.62	17.50	13.89	20.63	99.70	37.60	62.29
17.	Koicha binni	138.40	101.20	26.88	21.80	17.10	21.56	114.60	35.60	68.94
18.	Nona Bokhra	131.30	117.20	10.74	22.03	20.05	8.99	98.80	53.70	45.65
19.	Binadhan 8	87.70	81.90	6.61	21.12	19.60	7.20	131.20	74.60	43.14
20.	FL 378	83.20	76.40	8.17	21.13	19.40	8.19	135.40	75.20	44.46
21.	Kashrail	131.30	121.40	7.54	21.23	19.77	6.88	112.30	67.70	39.72
22.	Jolkumri	134.00	116.20	13.28	22.30	19.80	11.21	133.20	69.60	47.00
23.	Pokkaly	131.20	122.60	6.55	23.48	21.81	7.11	120.20	74.90	37.69
24.	FL 478	85.90	77.80	9.43	20.20	18.70	7.43	103.50	53.90	47.92

Considering the number of filled grains per panicle, Volanath (76.35%), Rupessor (69.91%), Binadhan-7 (72.12%) and Koicha Binni (68.94%) showed higher reduction and Patnai (27.56%), Bazra Muri (34.44%), Pokkali (37.69%), Kashrail (39.32%), Binadhan-8 (43.14%), Kute Patnai (43.23%), FL-378 (44.46%), Tal Mugur (45.05%) and FL-478 (47.92%) showed lower reduction in filled grains per panicle (Table 2).

Under salt stress condition, about 80 unfilled grains panicle^{-1} was found in Volanath, Rupessor, Koicha Binni, and Holde Gotal whereas Kashrail, Pokkali, Binadhan-8, FL-478, Patnai, FL-378, Bazra Muri, Kute Patnai, Tal Mugur and Nonabokra produced less than 50 unfilled grains per panicle (Table 3). But under normal growth condition, the range of unfilled grain was found about 15 to 35 per panicle except Binadhan-7 and Bashful Balam.

Considering the effective tiller plant^{-1} Bashful Balam, Chinikani, Volanath, Rupessor and Fulkainja showed higher (>30) reduction. But Kashrail, Pokkali, Nona Bokra, Kute Patnai, Patnai, Bazra Muri, Kalo mota, Binadhan-8 and Kashrail showed lower reduction (< 20) (Table 3).

Under salinized condition, the rice genotypes Binadhan-8, Kashrail, Pokkali, FL-478, Nona Bokra, Kute Patnai, Tal Mugur, Patnai, FL-378 and Bazra Muri showed higher fertility (> 60%) and Rupessor, Koicha Binni, Volanath, Jamainaru, Ghunshi and Holde Gotal showed lower fertility (< 45%) (Table 4). All the genotypes showed more than 70% fertility under normal condition.

Under normal condition all the genotypes produced about 10 g or more yield plant^{-1}. But under salt stress all the genotypes produced less than 10 g yield plant^{-1} proved that yield has been reduced due to salt stress in all tested lines. Jamai Naru, Kute Patnai, Holde Gotal, Bazra Muri, Kalo Mota, Tal Mugur, Binadhan-8, FL-378, Kashrail and Pokkali produced more than 8 g yield plant^{-1} and Ghunshi, Volanath, Binadhan-7, Rupessor and Jolkumri produced less than 5 g yield plant^{-1} (Table 4). This result supported by Asch *et al.* [33] who worked with 80 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 much variation in grain yield under salinity stress.

Table 3. Mean values of number unfilled grain/plant, effective tiller/plant, days to flowering of studied rice germplasm under salinized (EC 8dS/m) and non-salinized condition at reproductive stage

SL No.	Genotypes	No. of unfilled grain		No. of effective tiller/plant			Days to flowering	
		Non-salinized	Salinized	Non-silanized	Salinized	% Reduction	Non-salinized	Salinized
1.	Jamai Naru	25	74.23	12	9	25.00	133	123
2.	Patnai	30	42.78	10	8	20.00	118	115
3.	Kute Patnai	33	36.45	12	11	8.33	108	105
4.	Holde Gotal	26	91.45	11	8	27.27	114	108
5.	Bashful Balam	70	78.4	11	6	45.45	113	107
6.	Bazra Muri	18	28.34	12	10	16.67	126	123
7.	Ghunshi	22	54.68	7	5	28.57	128	123
8.	Chinikani	20	51.09	10	7	30.00	116	111
9.	Binadhan 7	45	69.2	9	6	33.33	106	101
10.	Volanath	25	101.6	11	7	36.36	126	121
11.	Rupessor	30	99.1	12	8	33.33	103	97
12.	Kalo Mota	17	68.3	11	9	18.18	131	127
13.	Nona Kochi	30	54.3	11	9	27.27	128	124
14.	Tal Mugur	29	44.34	10	8	20.00	92	89
15.	Ghigoj	38	56.34	7	5	28.57	108	105
16.	Fulkainja	25	67.45	12	8	33.33	98	92
17.	Koicha binni	42	88.45	11	8	27.27	96	90
18.	Nona Bokhra	28	41.23	10	9	10.00	103	99
19.	Binadhan 8	30	48.98	12	10	16.67	91	88
20.	FL 378	28	43.8	13	9	25.00	93	89
21.	Kashrail	31	46.7	9	8	11.11	94	91
22.	Jolkumri	32	54.3	10	8	20.00	93	90
23.	Pokkaly	26	35.78	13	11	15.38	96	93
24.	FL 478	25	41.45	14	11	27.27	95	92

SSR marker survey for salt tolerance rice genotypes

In this experiment, initially ten primers namely, RM314, RM140, RM1594, RM9, RM407, RM510, RM51, RM121, RM134 & RM234 were used for polymorphism survey of twenty four rice landraces. Of them, three SSR markers *viz.*, RM19, RM134 and RM234 showed highly polymorphism and that were selected to evaluate 24 rice germplasms for salt tolerance. According to the phenotypic performance, Binadhan-8 was considered as tolerant and Binadhan-7 was considered as susceptible. The genotypes having similar banding pattern to Binadhan-8 were considered as tolerant and similar to Binadhan-7 were considered as salt susceptible (Table 5).

Table.4 Fertility (%), yield/plant of rice landraces under salnized (EC 8dS/m) and non-salinized condition at reproductive stage

SL No.	Genotypes	Fertility (%)		Yield/plant (g)	
		Non-salinized	Salinized	Non-salinized	Salinized
1.	Jamai Naru	78.13	45.99	10.34	8.45
2.	Patnai	78.89	60.16	16.95	7.36
3.	Kute Patnai	79.18	69.88	18.97	8.34
4.	Holde Gotal	79.23	43.81	17.34	8.87
5.	Bashful Balam	72.89	56.08	16.19	6.19
6.	Bazra Muri	81.27	64.28	13.99	9.95
7.	Ghunshi	80.04	47.16	11.75	4.77
8.	Chinikani	83.51	56.07	9.80	5.83
9.	Binadhan -7	68.90	57.61	6.32	2.34

SL No.	Genotypes	Fertility (%)		Yield/plant (g)	
		Non-salinized	Salinized	Non-salinized	Salinized
10.	Volanath	81.78	44.68	15.34	4.23
11.	Rupessor	83.04	50.35	13.67	4.89
12.	Kalo Mota	87.25	51.46	18.72	8.38
13.	Nona Kochi	77.97	56.53	19.17	5.12
14.	Tal Mugur	78.21	51.54	17.34	8.05
15.	Ghigoj	77.93	61.87	16.42	5.06
16.	Fulkainja	79.95	47.73	11.41	5.59
17.	Koicha binni	58.89	43.98	17.35	5.27
18.	Nona Bokhra	77.92	64.25	13.35	7.96
19.	Binadhan -8	81.39	64.62	19.38	8.11
20.	FL 378	69.29	58.70	15.61	8.13
21.	Kashrail	70.06	61.79	15.86	8.97
22.	Jolkumri	82.44	65.61	10.92	4.67
23.	Pokkali	82.22	73.43	14.43	9.33
24.	FL 478	69.70	55.90	14.08	6.96

Table 5. Genotypic performance of twenty four rice germplasm using SSR markers

Genotypes	Salt tolerance with SSR markers		
	RM9	RM134	RM234
Binadhan-8, Patnai, KutePatnai, BazraMuri, Tal Mugur, Pokkali, Kashrail and FL 378	T	T	T

Binadhan-7, Bashful, Balam, Volanath, Rupessor, Nona Kochi and Koichabinni	S	S	S
HoldeGotal, KaloMota, Nona Bokra and FL- 478	S	T	S
Ghunshi	T	S	T
Chinikani	T	S	S
Ghigoj	T	T	S
Fulkainja and Jolkumri	S	S	T
Jamai naru	S	T	T

Where, S=Susceptible and T=Tolerant

As compared to Binadhan-8, genotypes Patnai, Kute Patnai, Chinikani, Tal Mugur, Ghigoj, Bazra Muri, Ghunshi, Kashrail, Pokkali and FL-378 were found tolerant when samples were amplified with RM9 because they positioned in the same level of Binadhan-8. In the same reaction, Holde Gotal, Bashful Balam, Volanath, Rupessor and FL 478 were found susceptible as they positioned in the same level of Binadhan-7 (Fig. 1).

In case of RM134 primers, BazraMuri, Patnai, Kute Patnai, Holde Gotal, Nona Bokra, Kashrail, Pokkali and FL 378 were found tolerant and Volanath, Rupessor, and Jolkumri were identified as susceptible (Fig. 2). Regarding to RM234 primers, KutePatnai, BazraMuri, Tal Mugur, Kashrail, Pokkali and FL-478 were identified as tolerant. Patnai, Ghunshi, Chinikani, Volanath Nona Bokra and Rupessor were found susceptible (Fig. 3). These three primers (RM109, RM7134 and RM234) showed polymorphisms in studied genotypes because they showed different banding pattern and discriminate tolerant genotypes from susceptible in relation to Binadhan-8 (tolerant) and Binadhan-7 (susceptible). These markers were reported as highly polymorphic for tagging salt tolerant genes [19,21].

But the rice genotypes, Kute Patnai, Bazra Muri, Kashrail, Tal Mugur, FL-378, and Pokkali were found as tolerant and Bashful Balam, Nona Kuchi, Rupessor, Volanath and Koichabinni were found as susceptible in all the tested markers. Based on Phenotypic observation, Binadhan-8, Kute Patnai, Kashrail, FL-378, Tal Mugur, Bazra Muri were found as tolerant while Binadhan-7, Rupessor, Koicha Binni, Volanath were found as susceptible. This phenotypic observations support the genotypic findings for identification of salt tolerant rice genotypes.

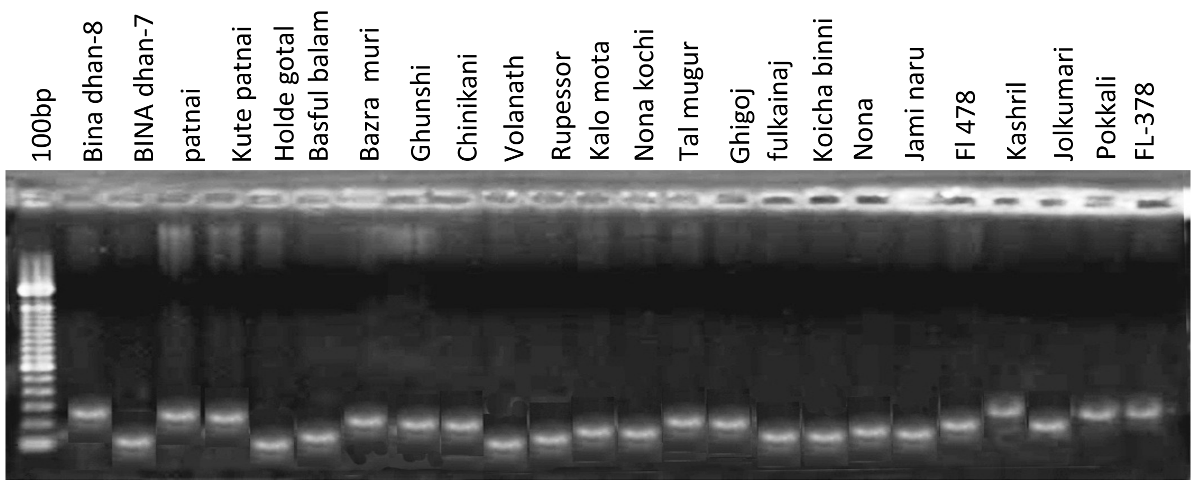

Fig. 1 . Banding profiles of 24 rice germplasm using RM9 primer

100bp Ladder
BINA dhan-8
BINA dhan-7
patnai
Kute patnai
Holde gotal
Basful balam
Bazra muri
Ghunshi
Chinikani
Volanath
Rupessor
Kalo mota
Nona kochi
Tal mugur
Ghigoj
fulkainaj
Koicha binni
Nona bokhra
Jami naru
Fl 478
kashril
Jolkumari
Pokkali
FL-378

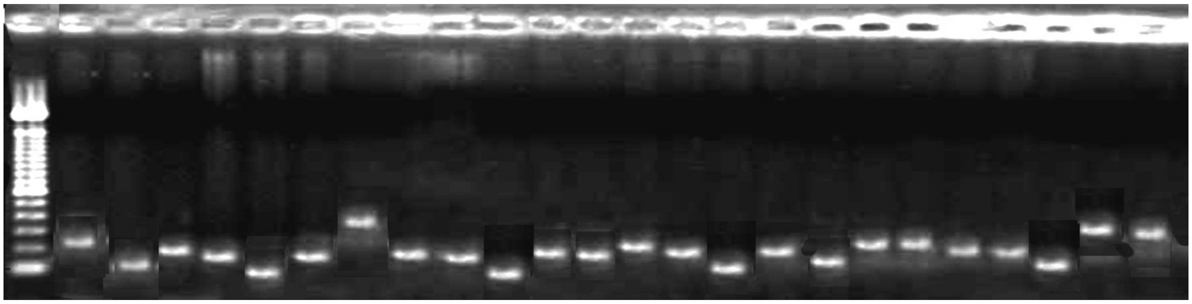

Fig. 2 Banding profiles of 24 rice germplasm using primer RM134

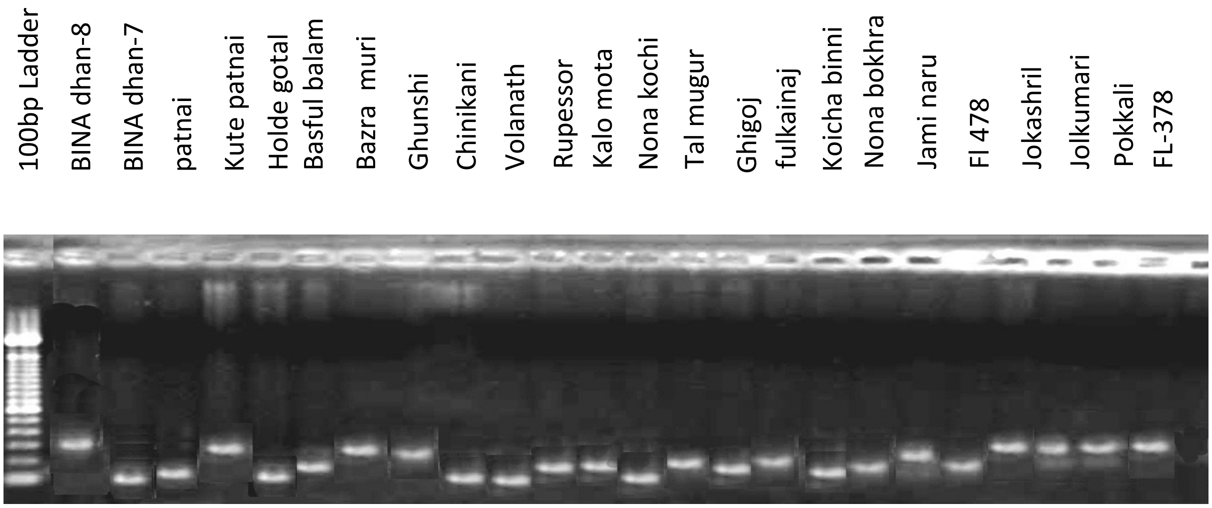

Fig. 3 Banding profiles of 24 rice germplasm using primer RM234

REFERENCES

1. Mitin A. 2009. Documentation of selected adapted strategies to climate change in rice cultivation. East Asia Rice Working Group, pp. 25-28.
2. USDA. 2013. *Bibliography on Salt Tolerance. Fibres, Grains and Special Crops*. Riverside, CA: George E. Brown, Jr. Salinity Lab. US Department Agriculture, Agriculture Research Service.
3. Galvani A. 2007. The challenge of the food sufficiency through salt tolerant crops. Rev. Env. Sci. Biotechnol., 6: 3-16.
4. Lauchli A, Grattan SR. 2007. Plant growth and development under salinity stress. In: *Advances in Molecular Breeding Toward Drought and Salt TolerantCrops*, (Eds.): M.A. Jenks, P.M. Hasegawa and S.M. Jain. Springer, Dordrecht, Netherlands.
5. Arshad, M, Saqib M, Akhtar J, Asghar M. 2012. Effect of calcium on the salt tolerance of different wheat (*Triticum aestivum* L.) genotypes. Pak. J. Agri. Sci., 49: 497-504.

6. Manneh B. 2004. Genetic, physiological and modeling approaches towards tolerance to salinity and low nitrogen supply in rice (*Oryza sativa L.*). PhD Thesis, Wageningen University, Wageningen, The Netherlands, pp. 260.
7. Yokoi S, Bressan RA, Hassegawa PM (2002) Salt Stress tolerance of Plant. JIRCUS Working Report pp. 25-33.
8. Eynard A, Lal R, Wiebe K. 2005. Crop response in salt-affected soils. J. Sustain.Agric. 27: 5-50.
9. Saha P, Chatterjee P, Biswas AK. 2010. NaCl pretreatment alleviates salt stress by enhancement of antioxidant defense and osmolyte accumulation in mungbean (*Vigna radiata* L. Wilczek). Indian J. Exp. Biol., 48:593-600.
10. Hasanuzzaman M, Nathan K, Fujita M. 2013. Plant response to salt stress and role of exogenous protectants to mitigate salt-induced damages. In: Ahmad, P., Azooz, M.M., Prasad, M.N.V. (Eds.), Ecophysiology and responses of plants under salt stress. Springer, New York, pp. 25-87.
11. BBS. 2014. Agriculture Wing. Bangladesh Bureau of statistics, Ministry of planning, Government of the People's Republic of Bangladesh, Dhaka.
12. SRDI. 2012. Saline soils of Bangladesh. Soil Resources and Development Institute. Ministry of Agriculture, Farmgate, Dhaka-1215.
13. Gregorio GB, Senadhira D, Mendoza RD. 1997. Screening rice for salinity tolerance. IRRI discussion paper series no. 22. Manila (Philippines): International Rice Research Institute. pp. 1-30.
14. Zeng L, Shannon MC, Grieve CM. 2002. Evaluation of salt tolerance in rice genotypes by multiple agronomic parameters. Euphytica, 127: 235-245.
15. Lee, IS, Kim DS, Lee SJ, Song HS, Lim YP, Lee YI. 2003. Selection and characterizations of radiation-induced salinity tolerant lines in rice. Breed. Sci. 53:313-318.
16. El-Hendawy SE, Ruan Y, Hu Y, Schmidhalter U. 2009. A Comparison of screening criteria for salt tolerance in wheat under field and controlled environmental conditions. J. Agronomy Crop Sci. 195, 356-367.
17. Mani P, Bastin J, Arun kumar R, Ahmed ABA. 2010. RAPDanalysis of genetic variation of four important rice varieties using two OPR primers. ARPN J. Agric. Biol. Sc., 5: 25-31.
18. Garland SH, Lewin L, Abedinia M, Henry R, Blakeney A. 1999. The useof microsatellite polymorphism for the identification of Australian breeding lines of rice (Oryza sativa L). Euphytica, 108:53-63.
19. Islam MM. 2004. Mapping salinity tolerance genes in rice (*Oryza Satiya L.*) at reproductive stage. Ph. D. Dissertation. University of the Philippines Los Banos, College, Laguna, Philippines. p. 150.

20. Bhuiyan MAR. 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.
21. Niones JM. 2004. Fine mapping of the salinity tolerance gene on chromosome 1 of rice (*Oryza sativa L.*) using near-isogenic lines. M.S. Dissertation. University of the Philippines Los Banos, Laguna, Phil. P 78.
22. Cho YG, Ishii T, Temnykh S, Chen X, et al. (2000). Diversity of microsatellites derived from genomic libraries and GenBank sequences in rice (*Oryza sativa* L.). Theor. Appl. Genet., 100: 713-722.
23. Harrington S. 2000. A survey of genetic diversity of eight AA genome species of *Oryza* using microsatellite markers. MS thesis Cornell University, Ithaca.
24. Bres-Patry C, Loreux M, Clément G, Bangratz M, et al. (2001). Heredity and genetic mapping of domestication-related traits in temperate japonica weedy rice. Theor. Appl. Genet., 102: 118-126.
25. Moncada P, Martinez CP, Borrero J, Châtel M, et al. 2001. Quantitative trait loci for yield and yield components in an *Oryza sativa* x *Oryza rufipogon* BC2F2 population evaluated in an upland environment. Theor. Appl. Genet. 102: 41-52.
26. Hittalmani S, Foolad MR, Mew T, Rodriguez RL, et al. 1995. Development of PCR based markers to identify rice blast resistance gene, Pi-2(t), in a segregation population. Theor. Appl. Genet. 91: 9-14.
27. Naqvi NI, Chattoo BB. 1996. Development of a sequence characterized amplified region (SCAR) based indirect selection method for a dominant blast-resistance gene in rice. Genome, 39: 26-30.
28. IRRI. 1997. International Rice Research Institute. Annual Report for 1997. Los Banos, Laguna, Philippines. P. 308.
29. Akter MB, Kim B, Lee Y, Koh E, Koh H-J. 2014. Fine mapping and candidate gene analysis of a new mutant gene for panicle apical abortion in rice. Euphytica, 197:387-398.
30. Rubel MH, Hassan L, Islam MM, Robin AHK, Alam MJ. 2014. Evaluation of rice genotypes under salt stress at the seedling and reproductive stages using phenotypic and molecular markers. Pak. J. Bot., 46(2): 423-432.
31. Bhowmik SK, Islam MM, Emon RM, Begum SN, Sultana S. 2007. Identification of salt tolerant rice cultivars via phenotypic and marker-assisted procedures. Pak. J. Biol. Sci., 10 (24): 4449-4454.
32. Choi WY, Lee KS, Ko JC, Choi SY, Choi DH. 2003. Critical saline concentration of soil and water for rice cultivation on a reclaimed saline soil. Korean J. Crop Sci., 48: 238-242.
33. Asch F, Dingkuhn M, Wittstock C, Doerffling K. 1998. Sodium and Potassium uptake of rice panicles as affected by salinity and season in relation to yield and yield components. J. Plant Soil., 207: 133-145.