

# 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 markers, 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, landraces, salt tolerant, microsatellite marker*

## 19 **Introduction**

20 Rice (*Oryza sativa* L.) is an important crop that feeds more than half of the world's  
21 population. Asian farmers contribute about 92 % of the total world's rice production [1]. But  
22 it is very sensitive to salinity stress and is currently listed as the most salt sensitive cereal  
23 crop with a threshold of 3 dS/m for most cultivated varieties [2]. Salinity is most important  
24 abiotic stress that directly regulates the plant growth and development [3-5]. It affects all the  
25 growth stages of rice from seedling to maturation [6] but reproductive stage is more sensitive  
26 for grain yield production [7] and also reported the most sensitive stages are the early  
27 seedling and reproductive (panicle initiation, anthesis and fertilization) [34]. Rice yield in  
28 salt-affected land is significantly reduced with an estimation of 30–50% yield losses annually  
29 [8]. Due to natural salinity and human interferences, the arable land is continuously  
30 transforming into saline that is expected to have overwhelming global effects, resulting in up  
31 to 50% land loss by 2050 [9,10].

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

44 A number of genomic tools, such as molecular markers can greatly improve the efficiency of  
45 breeding programs. The use of molecular markers has been increasing considerably because  
46 of their reliability and helps in deciding the distinctiveness of species [17]. Among the  
47 molecular marker technologies, microsatellite or simple sequence repeats (SSRs) are widely  
48 used in rice genetic studies because of their availability, widespread distribution in the  
49 genome, high allelic diversity and have been found to be efficient in identification of rice  
50 cultivars [18-20]. Microsatellite or SSR markers are proved to be ideal for making genetic  
51 maps [19, 21] assisting selection [20] and have been applied to analyze diversity [22,23].  
52 SSR markers are playing important role to identify genes and quantitative trait loci [24,25]  
53 that can be helpful for plant breeders to develop new cultivars. Landraces are currently being  
54 used as preferred potential donors of salt tolerance traits because of their local adaptation.  
55 Due to genetic similarities between cultivated rice species, the transfer of useful genes from  
56 one to another is possible. The presence of markers tightly linked to resistance genes will  
57 allow selection and maintenance of the desirable resistant genotypes in breeding process [26,  
58 27]. Thus, the evaluation of rice landraces could provide valuable information for genetic  
59 improvement of salt tolerant rice variety.

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

## 63 **Materials and methods**

### 64 **Plant Materials**

65 A total of 24 rice lines including 20 landraces, 2 BINA developed high yielding varieties and  
66 2 advanced lines were used in the study (Table 1). BINA developed salt tolerant variety  
67 Binadhan-8 was used as tolerant control and HYV Binadhan-7 as susceptible control.

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71 **Table 1.** List of rice genotypes used in the experiment

Sl. No.	Genotypes	Type	Source of collection
1.	Jamai Naru	Bangladeshi Landrace	BINA
2.	Patnai	Bangladeshi Landrace	BINA
3.	Kute Patnai	Bangladeshi Landrace	BINA
4.	Holde Gotal	Bangladeshi Landrace	BINA
5.	Bashful Balam	Bangladeshi Landrace	BINA
6.	Bazra Muri	Bangladeshi Landrace	BINA
7.	Ghunshi	Bangladeshi Landrace	BINA
8.	Chinikani	Bangladeshi Landrace	BINA
9.	Binadhan 7	HYV	BINA
10.	Volanath	Bangladeshi Landrace	BINA
11.	Rupessor	Bangladeshi Landrace	BINA
12.	Kalo Mota	Bangladeshi Landrace	BINA
13.	Nona Kochi	Bangladeshi Landrace	BINA
14.	Tal Mugur	Bangladeshi Landrace	BINA
15.	Ghigoj	Bangladeshi Landrace	BINA
16.	Fulkainja	Bangladeshi Landrace	BINA
17.	Koicha binni	Bangladeshi Landrace	BINA
18.	Nona Bokhra	Indian local cultivar	IRRI
19.	Binadhan 8	Salt tolerant HYV	BINA
20.	FL 378	Salt tolerant exotic line	IRRI
21.	Kashrail	Bangladeshi Landrace	BINA
22.	Jolkumri	Bangladeshi Landrace	BINA
23.	Pokkali	Indian local cultivar	IRRI
24.	FL 478	Salt tolerant exotic line	IRRI

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73 Phenotypic evaluation under the Saline condition

74 The genotypes were evaluated for their tolerance to salinity under sustained water bath  
75 following IRRI standard protocol for salinity screening at the vegetative and reproductive  
76 stages [13]. Completely randomized design (CRD) with three replications was followed for  
77 experimental design. Both Normal and salinized setups were maintained. At first, pot was  
78 prepared by inserting a cloth bag inside the pot and then it was filled up with fertilized soil.  
79 The fertilizer was used 50 N, 25 P and 25 K mg kg<sup>-1</sup> of soil respectively. Initially, the soil  
80 level was about 1 cm above the topmost circle of holes. The pots with leveled soil were

81 placed in the plastic tray filled up with ordinary tap water. This serves as water bath. The  
 82 water level was same as the soil level. The soil was then started to settle down as it absorbs  
 83 water. To obtain the correct soil level extra soil was added after two days. During this soil  
 84 settlement process the seeds were kept in the conventional oven for 5 days at 50°C for  
 85 breaking the seed dormancy. The oven treated seeds were soaked with tap water for 24 hours  
 86 for pre-germination. The pre-germinated seeds were sown (3/4 seeds/pot) on the soil surface  
 87 of the perforated pot. After 2 weeks, the seedlings were thinned to two per pot and the water  
 88 level was raised up to 1 cm above the soil surface. The water level was maintained daily and  
 89 the plants were protected from pests and diseases. After 3 weeks of sowing the pots were  
 90 salinized at EC 8 dS/m by dissolving crude salt and EC was monitored in every week till  
 91 maturity. Data were recorded on plant height (cm), days to flowering, number of effective  
 92 tillers/plant, number of field grains and unfilled grains, percent fertility and grain yield (g).

93 The following formula was used for calculating the Percent fertility and reduction:

94  $\text{Percent fertility} = \{(\text{No. of filled grains} / (\text{No. of filled grains} + \text{No. of unfilled grains})) \times 100$

95  $\text{Percent (\% ) reduction} = \{(\text{traits in normal} - \text{traits in saline}) / \text{Traits in normal}\} \times 100$

96 DNA extraction, PCR amplification and molecular marker analysis

97 Modified CTAB mini prep method was followed for genomic DNA extraction from 25-day-  
 98 old seedling leaf sample [28]. Ten primers were surveyed and among them three primers  
 99 showed polymorphism and clear bands (Table 1). Each PCR reaction carried out with 13.0µl  
 100 reactions containing 1.5 µl 10x buffer, 0.75 µl dNTPs, 1µl primer forward, 1µl primer  
 101 reverse, 0.25 µl taq polymerase, 7.5 µl ddH<sub>2</sub>O and 1.0 µl of each template DNA samples.  
 102 PCR analysis was performed according to our previous study by Akter et al. [29] with little  
 103 modifications. PCR profile was maintained as initial denaturation at 94°C for 5 min.,  
 104 followed by 34 cycles of denaturation at 94°C for 30 second, annealing at 55°C for 30 second  
 105 and extension at 72°C for 1min., and a final extension of 7 min. at 72°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 2) 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 2.** 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.(°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 significant 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 3). 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 3).

142 **Table 3.** Effects of salinization (EC 8dS/m) on plant height, panicle length and number of filled grains at reproductive stage of the  
143 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.	Pokkali	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
	LSD <sub>(.05)</sub>	3.51	3.1		0.96	1.06		3.01	1.94	



145 Considering the number of filled grains per panicle, Volanath (76.35%), Rupessor (69.91%),  
 146 Binadhan-7 (72.12%) and Koicha Binni (68.94%) showed higher reduction and Patnai  
 147 (27.56%), Bazra Muri (34.44%), Pokkali (37.69%), Kashrail (39.32%), Binadhan-8  
 148 (43.14%), Kute Patnai (43.23%), FL-378 (44.46%), Tal Mugur (45.05%) and FL-478  
 149 (47.92%) showed lower reduction in filled grains per panicle (Table 3).

150 Under salt stress condition, about 80 unfilled grains panicle<sup>-1</sup> was found in Volanath,  
 151 Rupessor, Koicha Binni, and Holde Gotal whereas Kashrail, Pokkali, Binadhan-8, FL-478,  
 152 Patnai, FL-378, Bazra Muri, Kute Patnai, Tal Mugur and Nonabokra produced less than 50  
 153 unfilled grains per panicle (Table 4). But under normal growth condition, the range of  
 154 unfilled grain was found about 15 to 35 per panicle except Binadhan-7 and Bashful Balam.

155 Considering the effective tiller plant<sup>-1</sup> Bashful Balam, Chinikani, Volanath, Rupessor and Fulkainja  
 156 showed higher (>30) reduction. But Kashrail, Pokkali, Nona Bokra, Kute Patnai, Patnai, Bazra  
 157 Muri, Kalo mota, Binadhan-8 and Kashrail showed lower reduction (< 20) (Table 4).

158 Under salinized condition, the rice genotypes Binadhan-8, Kashrail, Pokkali, FL-478, Nona Bokra,  
 159 Kute Patnai, Tal Mugur, Patnai, FL-378 and Bazra Muri showed higher fertility (> 60%) and  
 160 Rupessor, Koicha Binni, Volanath, Jamainaru, Ghunshi and Holde Gotal showed lower  
 161 fertility (< 45%) (Table 5). All the genotypes showed more than 70% fertility under normal  
 162 condition.

163 Under normal condition all the genotypes produced about 10 g or more yield plant<sup>-1</sup>. But under  
 164 salt stress all the genotypes produced less than 10 g yield plant<sup>-1</sup> proved that yield has been  
 165 reduced due to salt stress in all tested lines. Jamai Naru, Kute Patnai, Holde Gotal, Bazra Muri,  
 166 Kalo Mota, Tal Mugur, Binadhan-8, FL-378, Kashrail and Pokkali produced more than 8 g yield  
 167 plant<sup>-1</sup> and Ghunshi, Volanath, Binadhan-7, Rupessor and Jolkumri produced less than 5 g yield  
 168 plant<sup>-1</sup> (Table 4). This result supported by Asch *et al.* [33] who worked with 80 rice cultivars  
 169 and found that cultivars differed in their salt uptake and tolerant cultivars had lower salt

170 effect on yield and yield components than susceptible. Filled grain weight and total dry  
171 matter weight contributed much variation in grain yield under salinity stress.

172 **Table 4.** Mean values of number unfilled grain/plant, effective tiller/plant, days to flowering of studied rice germplasm under  
173 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-salinized	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
	LSD <sub>(.05)</sub>	1.35	2.2		0.34	0.95		

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 6).

**Table.5 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
	LSD <sub>(.05)</sub>	1.82	1.22	0.69	0.53

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191 **Table 6.** 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

192 Where, S=Susceptible and T=Tolerant

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

198 In case of RM134 primers, BazraMuri, Patnai, Kute Patnai, Holde Gotal, Nona Bokra, Kashrail,  
 199 Pokkali and FL 378 were found tolerant and Volanath, Rupessor, and Jolkumri were identified as  
 200 susceptible (Fig. 2). Regarding to RM234 primers, KutePatnai, BazraMuri, Tal Mugur, Kashrail,  
 201 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.

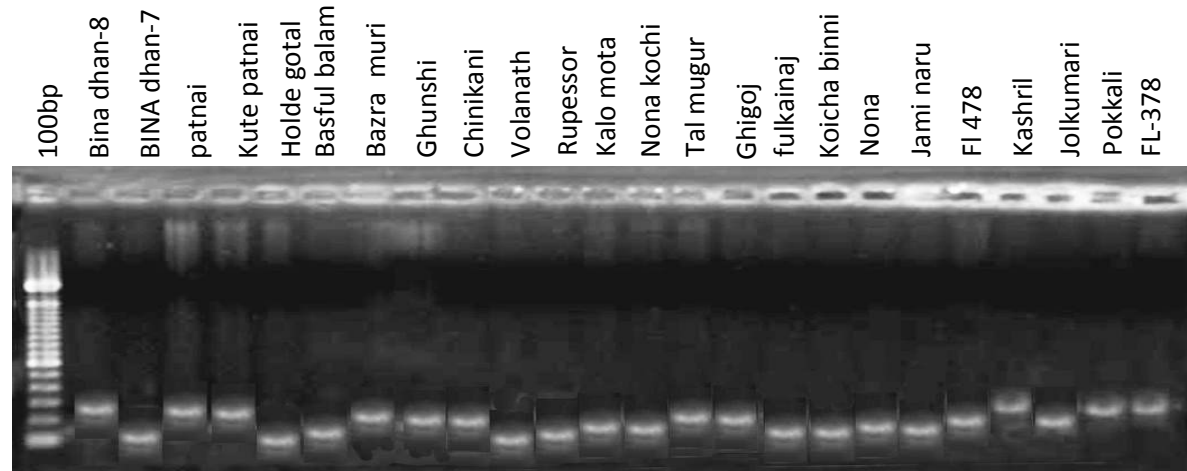


Fig. 1 . Banding profiles of 24 rice germplasm using RM9 primer

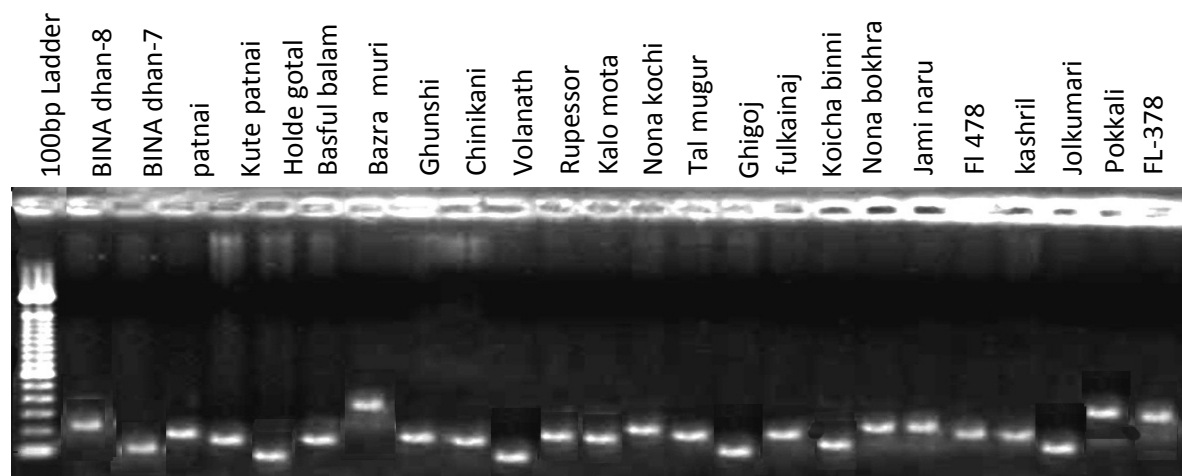


Fig. 2 Banding profiles of 24 rice germplasm using primer RM134

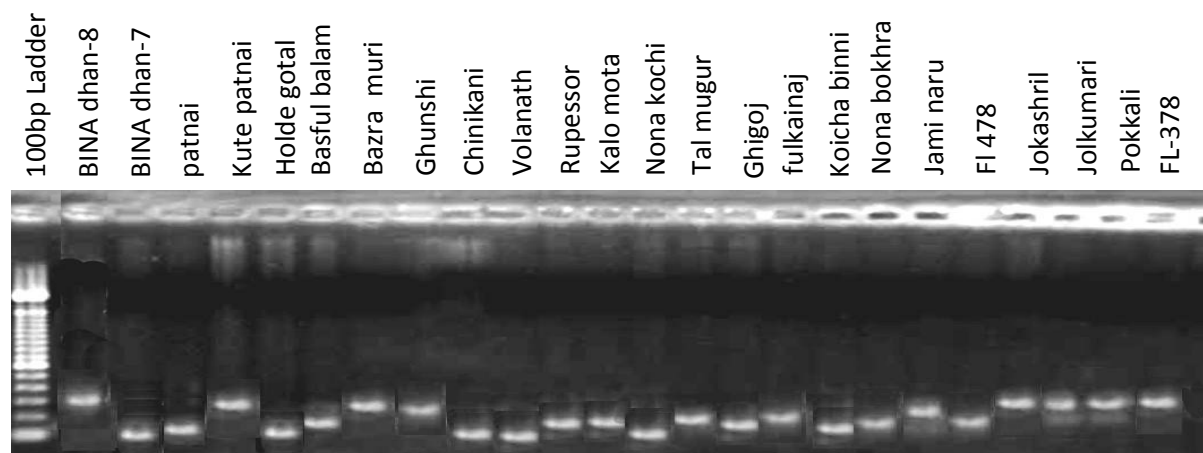


Fig. 3 Banding profiles of 24 rice germplasm using primer RM234

### Conclusion

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. The selected salt tolerant landraces can be used further in rice breeding program to develop salt tolerant high yielding varieties.



## 239 REFERENCES

- 240 1. Mitin A. 2009. Documentation of selected adapted strategies to climate change in rice  
241 cultivation. East Asia Rice Working Group, pp. 25-28.
- 242 2. USDA. 2013. *Bibliography on Salt Tolerance. Fibres, Grains and Special Crops.*  
243 Riverside, CA: George E. Brown, Jr. Salinity Lab. US Department Agriculture,  
244 Agriculture Research Service.
- 245 3. Galvani A. 2007. The challenge of the food sufficiency through salt tolerant crops. Rev.  
246 Env. Sci. Biotechnol., 6: 3-16.
- 247 4. Lauchli A, Grattan SR. 2007. Plant growth and development under salinity stress. In:  
248 *Advances in Molecular Breeding Toward Drought and Salt Tolerant Crops*, (Eds.): M.A.  
249 Jenks, P.M. Hasegawa and S.M. Jain. Springer, Dordrecht, Netherlands.
- 250 5. Arshad, M, Saqib M, Akhtar J, Asghar M. 2012. Effect of calcium on the salt tolerance of  
251 different wheat (*Triticum aestivum* L.) genotypes. Pak. J. Agri. Sci., 49: 497-504.
- 252 6. Manneh B. 2004. Genetic, physiological and modeling approaches towards tolerance to  
253 salinity and low nitrogen supply in rice (*Oryza sativa* L.). PhD Thesis, Wageningen  
254 University, Wageningen, The Netherlands, pp. 260.
- 255 7. Yokoi S, Bressan RA, Hasegawa PM (2002) Salt Stress tolerance of Plant. JIRCUS  
256 Working Report pp. 25-33.
- 257 8. Eynard A, Lal R, Wiebe K. 2005. Crop response in salt-affected soils. J. Sustain.Agric.  
258 27: 5-50.
- 259 9. Saha P, Chatterjee P, Biswas AK. 2010. NaCl pretreatment alleviates salt stress by  
260 enhancement of antioxidant defense and osmolyte accumulation in mungbean (*Vigna*  
261 *radiata* L. Wilczek). Indian J. Exp. Biol., 48:593-600.
- 262 10. Hasanuzzaman M, Nathan K, Fujita M. 2013. Plant response to salt stress and role of  
263 exogenous protectants to mitigate salt-induced damages. In: Ahmad, P., Azooz, M.M.,  
264 Prasad, M.N.V. (Eds.), *Ecophysiology and responses of plants under salt stress*. Springer,  
265 New York, pp. 25-87.
- 266 11. BBS. 2014. Agriculture Wing. Bangladesh Bureau of statistics, Ministry of planning,  
267 Government of the People's Republic of Bangladesh, Dhaka.
- 268 12. SRDI. 2012. Saline soils of Bangladesh. Soil Resources and Development Institute.  
269 Ministry of Agriculture, Farmgate, Dhaka-1215.
- 270 13. Gregorio GB, Senadhira D, Mendoza RD. 1997. Screening rice for salinity tolerance.  
271 IRRI discussion paper series no. 22. Manila (Philippines): International Rice Research  
272 Institute. pp. 1-30.
- 273 14. Zeng L, Shannon MC, Grieve CM. 2002. Evaluation of salt tolerance in rice genotypes  
274 by multiple agronomic parameters. Euphytica, 127: 235-245.
- 275 15. Lee, IS, Kim DS, Lee SJ, Song HS, Lim YP, Lee YI. 2003. Selection and  
276 characterizations of radiation-induced salinity tolerant lines in rice. Breed. Sci. 53:313-  
277 318.

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. RAPD analysis of genetic variation of four important rice varieties using two OPR primers. *ARPJ. Agric. Biol. Sc.*, 5: 25-31.
18. Garland SH, Lewin L, Abedinia M, Henry R, Blakeney A. 1999. The use of microsatellite polymorphism for the identification of Australian breeding lines of rice. *Euphytica*, 108:53-63.
19. Islam MM. 2004. Mapping salinity tolerance genes in rice 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.

- 318 30. Rubel MH, Hassan L, Islam MM, Robin AHK, Alam MJ. 2014. Evaluation of rice  
319 genotypes under salt stress at the seedling and reproductive stages using phenotypic and  
320 molecular markers. *Pak. J. Bot.*, 46(2): 423-432.
- 321 31. Bhowmik SK, Islam MM, Emon RM, Begum SN, Sultana S. 2007. Identification of salt  
322 tolerant rice cultivars via phenotypic and marker-assisted procedures. *Pak. J. Biol. Sci.*,  
323 10 (24): 4449-4454.
- 324 32. Choi WY, Lee KS, Ko JC, Choi SY, Choi DH. 2003. Critical saline concentration of soil  
325 and water for rice cultivation on a reclaimed saline soil. *Korean J. Crop Sci.*, 48: 238-242.
- 326 33. Asch F, Dingkuhn M, Wittstock C, Doerffling K. 1998. Sodium and Potassium uptake of  
327 rice panicles as affected by salinity and season in relation to yield and yield components.  
328 *J. Plant Soil.*, 207: 133-145.
- 329 34. Singh R K, Gregorio GB, Jain RK. 2007. QTL mapping for salinity tolerance in rice.  
330 *Physiol. Mol. Biol. Plants* 13: 87-99.

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