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

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ABSTRACT

Salinity is the most significant causes of rice yield reduction in many rice-growing areas of the world. The aim of this study was to screen 24 rice genotypes including 20 landraces to find the potential germplasm source for salt tolerance breeding program. Screening was performed at reproductive stage based on 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 used for investigation of salt tolerant 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. Besides, eight rice genotypes *viz* Binadhan-8, Patnai, KutePatnai, Bazra Muri, Tal Mugur, Pokkali, Kashrail and FL 378 were found salt tolerant using SSR marker. Considering both assessment, four rice genotypes *viz.* Kute Patnai, Kashrail, Bazra Muri and Tal Mugur were selected as true salt tolerant lines. Therefore, these identified landraces could be a potential germplasm sources for future salt tolerance rice breeding program.

Keywords: Rice germplasm, salinity, yield, microsatellite marker

Rice (*Oryza sativa* L.) is an important food 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 rice is very sensitive to salinity stress and currently listed as the most salt sensitive cereal crop with a threshold of 3 dS/m for most cultivated varieties [2]. Salinity is a major constraint to rice production that directly regulates the plant growth and development [3-5]. It affects all the growth stages of rice from germination through maturation [6] but early seedling and reproductive stages are most sensitive to salt stress [7, 34]. 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). The increase of rice growing areas and the adoption of salt tolerant varieties are important factors that contribute in more rice production. Methods for salinity tolerance screening are also important for the success of a breeding program. To improve the rice yield under saline condition is one of the main targets of plant breeding and screening for salinity tolerance based on agronomical parameters such as growth, yield and yield components is becoming a popular method worldwide [13-16].

The use of molecular markers has been increasing considerably in breeding programs because of their reliability which helps in deciding the distinctiveness of genotypes [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 [18-23], efficient for identification of genes and quantitative trait loci in different rice cultivars [24, 25]. 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 genotypes, the transfer of useful genes from one to another is possible. The presence of markers tightly linked to salt tolerance genes will allow selection and maintenance of the desirable tolerant genotypes in breeding process [26, 27]. Thus, the evaluation of rice landraces could provide valuable sources for genetic improvement of salt tolerant rice variety.

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

2. MATERIALS AND METHODS

2.1 Plant Materials

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A total of 24 rice lines including 20 landraces, two high yielding varieties developed by BINA (Bangladesh Institute of Nuclear Agriculture) and two advanced lines were used in this study (Table 1). BINA developed salt tolerant variety Binadhan-8 was used as tolerant and HYV Binadhan-7 as susceptible.

2.2 Phenotypic evaluation under the Saline condition

The genotypes were evaluated for their tolerance to salinity under sustained water bath condition following IRRI standard protocol for salinity screening at the reproductive stage [13]. Completely randomized design (CRD) with three replications was followed for experimental design. Both normal and salinized setups were maintained. At first, pot was prepared by inserting a cloth bag inside the pot and then it was filled up with fertilized soil followed by 50 N, 25 P and 25 K mg kg⁻¹ of soil. Initially, the soil level was about 1 cm above the topmost circle of holes and the pots with leveled soil were placed in a plastic tray which serves as water bath. Then the plastic tray was filled up with no saline tap water having EC 0.2 dS/m measured by EC meter. The soil was watered and then left for absorbing water to settle down. To maintain the accurate soil level, additional soil was added after two days. The seeds were kept in the conventional oven for 5 days at 50°C for breaking the seed dormancy during the soil settlement time. Then the oven treated seeds were soaked with tap water for 24 hours for pre-germination. The pre-germinated seeds were sown (3/4 seeds/pot) on the soil surface of the perforated pot. After 2 weeks, thinning was carried out to maintain two seedlings per pot and then water level was raised up to 1 cm above the soil surface. The experimental pots were observed on daily basis to the rice plants from pests and diseases and maintain the water level. After 3 weeks of seed sowing, the pots were salinized at EC 8 dS/m by dissolving crude salt and monitored in every week using EC till maturity. In our country, salinity level varies between 6-8 dS/m at reproductive stage of rice [19]. So, we screened our studied genotypes at EC 8 dS/m. 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). The following formula was used for calculating the percent fertility and reduction:

- 92 Percent fertility= {(No. of filled grains/ (No. of filled grains+ No. of unfilled grains)} x100
- 93 Percent (%) reduction = {(traits in normal traits in saline)/Traits in normal} x100

2.3 DNA extraction, PCR amplification and molecular marker analysis

Modified CTAB mini prep method was used for genomic DNA extraction from leaf sample of 25 days old seedling [28]. 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, 7.5 µl ddH₂O and 1.0 µl of each template DNA samples. PCR analysis was performed according to previous study by Akter et al. [29] with little modifications. PCR profile was maintained as initial denaturation at 94°C for 5 min., followed by 34 cycles of denaturation at 94°C for 30 second, annealing at 55°C for 30 second and extension at 72°C for 1min., and a final extension of 7 min. at 72°C. Ten primers were surveyed and among them three primers showed polymorphism between the parents (Table 2). Finally, three polymorphic SSR markers (Table 2) were used 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.

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3. RESULTS AND DISCUSSION

3.1 Phenotypic performance of rice landraces at reproductive stage

- 110 A wide range of significant phenotypic variation was observed at reproductive stage among the rice
- 111 genotypes under 8 dS/m salinity stress. Normal growth and development was observed in control but
- some confrontational symptoms were found in salinized condition like cracked and dried leaves,
- 113 stunted plant growth and early flowering & maturity. 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
- 117 binni (26.88%). On the other hand, Pokkali (6.55%) followed by Binadhan-8 (6.61%), Kashrail (7.54%),
- 118 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). Therefore, the reduction might be occurred due to salt stress during growth
- and development. The similar results were also reported by Rubel et al. [30], Bhowmik et al. [31] and
- 122 Choi et al. [32].
- Percent reduction in panicle length was ranged from 6.88 to 22.61. Considering the panicle length,
- 124 Volanath (22.61%), Binadhan- 7 (21.91%), Rupessor (21.35%) and Koicha Binni (21.56%) showed
- 125 heigher reduction. Besides, Kashrail (6.88%), Pokkali (7.11%), Binadhan-8 (7.20%), FL-478 (7.43%)
- 126 Patnai (7.69%), FL-378 (8.19%), Bazra Muri (8.72%), Nona Bokra (8.99%), Kute Patnai (9.13%), Tal
- 127 Mugur (9.40%) and Jamai Naru (9.60%) displayed lower reduction in panicle length (Table 3).
- 128 Considering the number of filled grains per panicle, Volanath (76.35%), Rupessor (69.91%), Binadhan-
- 129 7 (72.12%) and Koicha Binni (68.94%) showed higher reduction and Patnai (27.56%), Bazra Muri
- 130 (34.44%), Pokkali (37.69%), Kashrail (39.32%), Binadhan-8 (43.14%), Kute Patnai (43.23%), FL-378
- 131 (44.46%), Tal Mugur (45.05%) and FL-478 (47.92%) exhibited lower reduction in filled grains per panicle
- 132 (Table 3).
- 133 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
- 135 Muri, Kute Patnai, Tal Mugur and Nonabokra produced less than 50 unfilled grains per panicle (Table
- 136 4). But under normal growth condition, the range of unfilled grain was found about 15 to 35 per
- 137 panicle except Binadhan-7 and Bashful Balam.
- 138 Considering the effective tiller plant Bashful Balam, Chinikani, Volanath, Rupessor and Fulkainja showed higher
- 139 (>30) reduction. But Kashrail, Pokkali, Nona Bokra, Kute Patnai, Patnai, Bazra Muri, Kalo Mota,
- 140 Binadhan-8 and Kashrail showed lower reduction (< 20) (Table 4).
- 141 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 exposed lower fertility (< 45%) (Table 5). All the
- genotypes exhibited more than 70% fertility at control.

- 145 Under normal growth condition all the genotypes produced about 10 g or more yield plant⁻¹ but less than
- 146 10 g yield plant⁻¹ in salinized condition revealed that grain yield production was reduced due to salt stress.
- 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 (Table 4). The same result was reported by Asch et al. [33]
- 150 where 80 rice cultivars were used. This result suggests that the salt tolerant cultivars are different
- 151 from susceptible in up taking salt and yield production.

3.2 SSR marker survey for salt tolerance rice genotypes

- 153 In this experiment, initially ten primers namely, RM314, RM140, RM1594, RM9, RM407, RM510,
- RM51, RM121, RM134 & RM234 were screened for polymorphism survey using twenty four rice
- 155 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.
- 158 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).
- 160 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 the DNA samples were amplified with
- 162 RM9 as produced the band in the same level of Binadhan-8. Besides, Holde Gotal, Bashful Balam,
- 163 Volanath, Rupessor and FL 478 were found susceptible comparing with Binadhan-7 (Fig. 1). Previously,
- 164 RM9 marker was also used for identification of salinity tolerance rice genotypes [35].
- 165 In case of RM134 primers, BazraMuri, Patnai, Kute Patnai, Holde Gotal, Nona Bokra, Kashrail, Pokkali
- and FL 378 were found as tolerant and Volanath, Rupessor, and Jolkumri were identified as susceptible
- 167 (Fig. 2). Regarding to RM234 primers, KutePatnai, BazraMuri, Tal Mugur, Kashrail, Pokkali and FL-478
- 168 were identified as tolerant. Patnai, Ghunshi, Chinikani, Volanath Nona Bokra and Rupessor were found
- 169 susceptible (Fig. 3). Recently, the screening of rice genotypes was done using Binadhan-8 rice variety
- for salt tolerance using RM234 markers [36].
- 171 The results revealed that all the primer pairs detected polymorphism among the rice genotypes. The
- microsatellite loci were also multiallelic (nine to twelve allele per locus with a mean of 11.33/locus)
- and the alleles were co-dominant suggesting their relative superiority in detecting DNA polymorphism
- 174 over some other markers with different allele size. These markers were also reported as highly
- polymorphic for tagging salt tolerant genes [19,21]. So, the studied three markers might be useful for
- identifying salt tolerance rice but it should be confirmed for further use.

4. CONCLUSION

- 179 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.
- 182 The selected salt tolerant landraces can be used further in rice breeding program to develop salt tolerant high yielding
- 183 varieties.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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AUTHORS' CONTRIBUTIONS

- 192 This work was carried out in collaboration between all authors. Authors MAI and MMI designed the
- 193 study, wrote the protocol and did the statistical analysis. Authors MHR, SNB and MBA managed the
- 194 literature searches, wrote the first draft of the manuscript and final proof submission. All authors read
- and approved the final manuscript.

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Table 1. List of rice genotypes used in the experiment

SI. No. Genotypes		Туре	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

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

304 rice lines

Primer	Expected		Primer sequence	Annealing Temperature (°C	
name	PCR product size (bp)		·		
RM234	156	For.	ACAGTATCCAAGGCCCTGG	55	
		Rev.	CACGTGAGACAAAGACGGAG		
RM134	93	For.	ACAAGGCCGCGAGAGGATTCCG	55	
1411104	00	Rev.	GCTCTCCGGTGGCTCCGATTGG		
RM9	136	For.	GGTGCCATTGTCGTCCTC	55	
TAIVIO	100	Rev.	ACGGCCCTCATCACCTTC	- 33	

Table 3. 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

	Genotypes	Plant height (cm)			Panicle Length (cm)			No. of filled grains/ panicle		
SL No.		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 Bokra	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 _(.05)	3.51	3.1		0.96	1.06		3.01	1.94	

Table 4. 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
	Jamai Naru	25	74.23	12	9	25.00	133	123
1	Patnai	30	42.78	10	8	20.00	118	115
2	Kute Patnai	33	36.45	12	11	8.33	108	105
3	Holde Gotal	26	91.45	11	8	27.27	114	108
4	Bashful Balam	70	78.4	11	6	45.45	113	107
5	Bazra Muri	18	28.34	12	10	16.67	126	123
6	Ghunshi	22	54.68	7	5	28.57	128	123
7	Chinikani	20	51.09	10	7	30.00	116	111
8	Binadhan 7	45	69.2	9	6	33.33	106	101
9	Volanath	25	101.6	11	7	36.36	126	121
10	Rupessor	30	99.1	12	8	33.33	103	97
11	Kalo Mota	17	68.3	11	9	18.18	131	127
12	Nona Kochi	30	54.3	11	9	27.27	128	124
13	Tal Mugur	29	44.34	10	8	20.00	92	89
14	Ghigoj	38	56.34	7	5	28.57	108	105
15	Fulkainja	25	67.45	12	8	33.33	98	92
16	Koicha binni	42	88.45	11	8	27.27	96	90
17	Nona Bokhra	28	41.23	10	9	10.00	103	99
18	Binadhan 8	30	48.98	12	10	16.67	91	88
19	FL 378	28	43.8	13	9	25.00	93	89
20	Kashrail	31	46.7	9	8	11.11	94	91
21	Jolkumri	32	54.3	10	8	20.00	93	90
22	Pokkaly	26	35.78	13	11	15.38	96	93
23	FL 478	25	41.45	14	11	27.27	95	92
24	LSD _(.05)	1.35	2.2		0.34	0.95		

Table 5. Fertility (%), yield/plant of rice landraces under salnized (EC 8dS/m) and non-salinized condition at reproductive stage

SL No.	Genotypes	Fertilit	y (%)	Yield/plant (g)		
0		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	
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 _(.05)	1.82	1.22	0.69	0.53	

323 Table 6. Genotypic performance of twenty four rice germplasm using SSR markers

Genotypes	Salt tolerance with SSR markers			
Genotypes	RM9	RM134	RM234	
Binadhan-8, Patnai, KutePatnai, BazraMuri, Tal Mugur, Pokkali, Kashrail and FL 378	Т	Т	Т	
Binadhan-7, Bashful, Balam, Volanath, Rupessor, Nona Kochi and Koichabinni	S	S	S	
Holde_Gotal, Kalo_Mota, Nona Bokra and FL- 478	S	Т	S	
Ghunshi	Т	S	Т	
Chinikani	Т	S	S	
Ghigoj	Т	Т	S	
Fulkainja and Jolkumri	S	S	Т	
Jamai naru	S	Т	Т	

Where, S=Susceptible and T=Tolerant

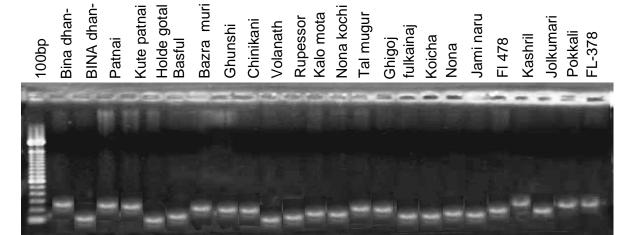


Fig.1. Banding profiles of 24 rice germplasm using RM9 primer

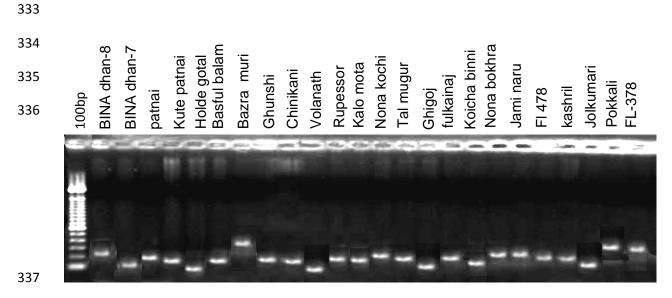


Fig. 2 Banding profiles of 24 rice germplasm using primer RM134

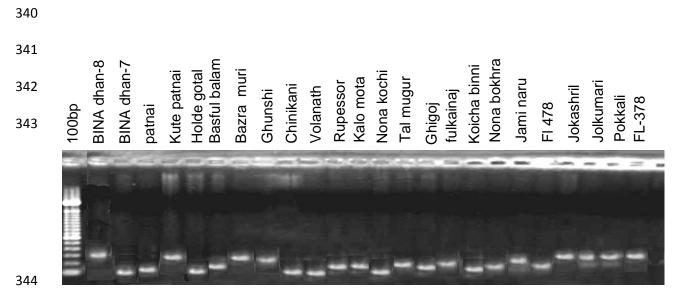


Fig. 3 Banding profiles of 24 rice germplasm using primer RM234