

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*

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]. Rice yield in salt-affected land is significantly reduced with an
27 estimation of 30–50% yield losses annually [8]. Due to natural salinity and human
28 interferences, the arable land is continuously transforming into saline that is expected to have
29 overwhelming global effects, resulting in up to 50% land loss by 2050 [9,10].

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

42 A number of genomic tools, such as molecular markers can greatly improve the efficiency of
43 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.

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

Percent reduction was calculated using the following formula:

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

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 13.0 µl reactions containing 1.5 µl 10x buffer, 0.75 µl dNTPs, 1 µl primer forward, 1 µl primer reverse, 0.25 µl taq polymerase, 8.25 µl ddH₂O 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°C for 5 min.,

93 followed by 34 cycles of denaturation at 94°C for 30 second. annealing at 55°C for 30 second
 94 and polymerization at 72°C for 1min., and a final extension of 7 min. at 72°C. Primer pairs
 95 were optimized for PCR to amplify microsatellite loci. Parental varieties were used to
 96 identify SSR polymorphism associated with the salt tolerance gene. Finally, we used the three
 97 polymorphic SSR markers (Table 1) for genotyping the 24 rice landraces. The amplified PCR
 98 products were separated in a 2.5 % agarose gel and then stained in 0.1 g/ml ethidium bromide
 99 containing water. Banding patterns were visualized with ultraviolet gel documentation
 100 system. The banding patterns of 24 genotypes were scored by comparing with tolerant and
 101 susceptible controls and similar banding pattern with Binadhan-8 were considered as tolerant
 102 and Binadhan-7 as salt susceptible.

103

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

106 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 | |

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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 |

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139 Considering the number of filled grains per panicle, Volanath (76.35%), Rupessor (69.91%),
140 Binadhan-7 (72.12%) and Koicha Binni (68.94%) showed higher reduction and Patnai
141 (27.56%), Bazra Muri (34.44%), Pokkali (37.69%), Kashrail (39.32%), Binadhan-8
142 (43.14%), Kute Patnai (43.23%), FL-378 (44.46%), Tal Mugur (45.05%) and FL-478
143 (47.92%) showed lower reduction in filled grains per panicle (Table 2).

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

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

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

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

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

166 **Table 3.** Mean values of number unfilled grain/plant, effective tiller/plant, days to flowering of studied rice germplasm under
167 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 |

168 SSR marker survey for salt tolerance rice genotypes

169 In this experiment, initially ten primers namely, RM314, RM140, RM1594, RM9, RM407,
170 RM510, RM51, RM121, RM134 & RM234 were used for polymorphism survey of twenty four
171 rice landraces. Of them, three SSR markers *viz.*, RM19, RM134 and RM234 showed highly
172 polymorphism and that were selected to evaluate 24 rice germplasms for salt tolerance.
173 According to the phenotypic performance, Binadhan-8 was considered as tolerant and Binadhan-
174 7 was considered as susceptible. The genotypes having similar banding pattern to Binadhan-8
175 were considered as tolerant and similar to Binadhan-7 were considered as salt susceptible (Table
176 5).

177
178 **Table.4 Fertility (%), yield/plant of rice landraces under salnized (EC 8dS/m) and non-**
179 **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 |

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181 **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 |

182 Where, S=Susceptible and T=Tolerant

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

188 In case of RM134 primers, BazraMuri, Patnai, Kute Patnai, Holde Gotal, Nona Bokra, Kashrail,
189 Pokkali and FL 378 were found tolerant and Volanath, Rupessor, and Jolkumri were identified as
190 susceptible (Fig. 2). Regarding to RM234 primers, KutePatnai, BazraMuri, Tal Mugur, Kashrail,
191 Pokkali and FL-478 were identified as tolerant. Patnai, Ghunshi, Chinikani, Volanath Nona Bokra
192 and Rupessor were found susceptible (Fig. 3). These three primers (RM109, RM7134 and RM234)
193 showed polymorphisms in studied genotypes because they showed different banding pattern and
194 discriminate tolerant genotypes from susceptible in relation to Binadhan-8 (tolerant) and Binadhan-7
195 (susceptible). These markers were reported as highly polymorphic for tagging salt tolerant genes
196 [19,21].

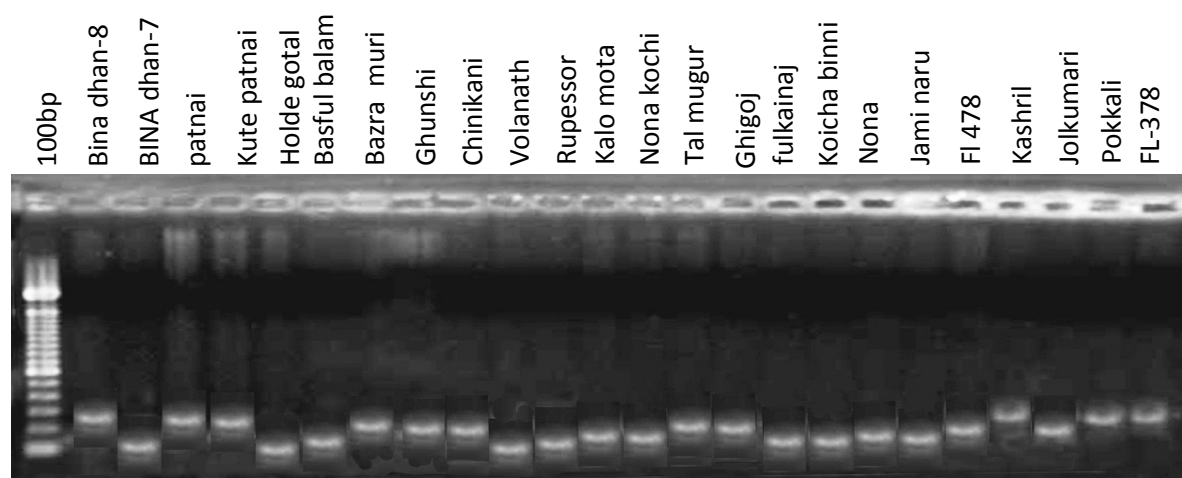
197 But the rice genotypes, Kute Patnai, Bazra Muri, Kashrail, Tal Mugur, FL-378, and Pokkali were
 198 found as tolerant and Bashful Balam, Nona Kuchi, Rupessor, Volanath and Koichabinni were found
 199 as susceptible in all the tested markers. Based on Phenotypic observation, Binadhan-8, Kute Patnai,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

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

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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
 FI 478
 kashril
 Jolkumari
 Pokkali
 FL-378

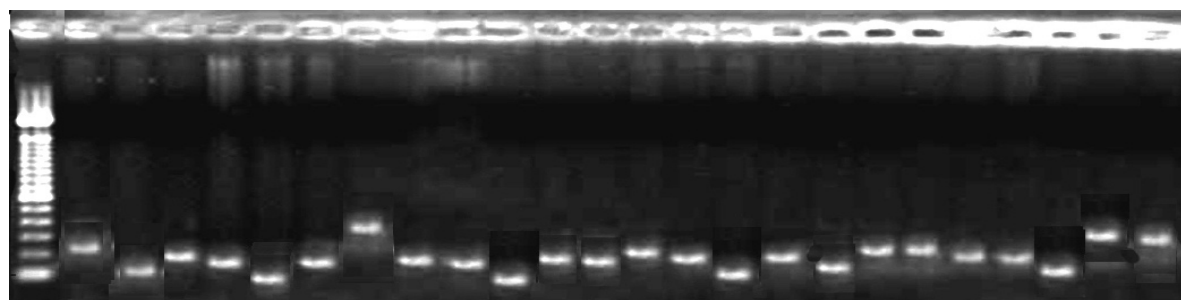


Fig. 2 Banding profiles of 24 rice germplasm using primer RM134

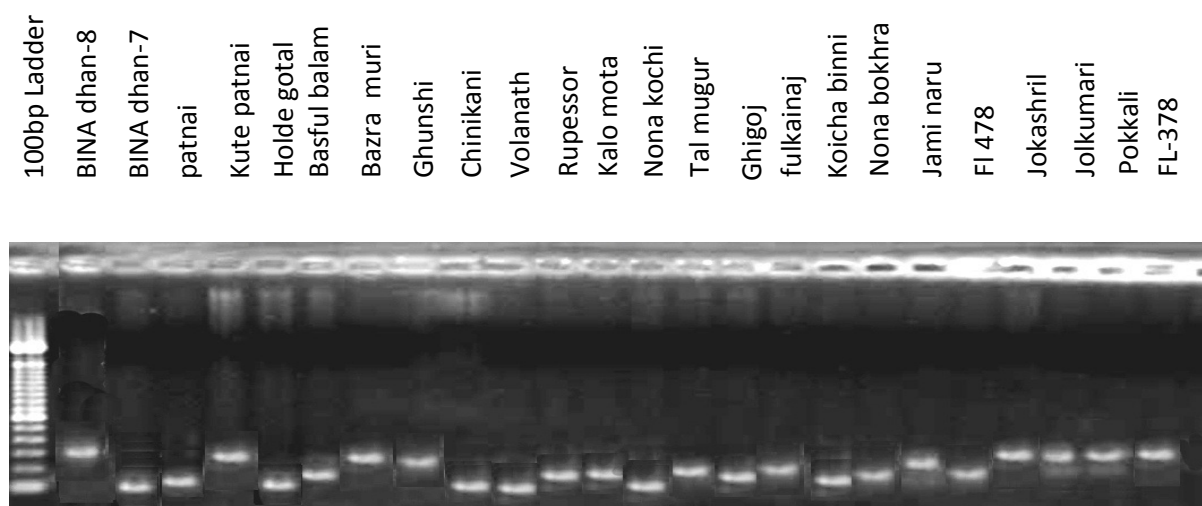


Fig. 3 Banding profiles of 24 rice germplasm using primer RM234

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