# 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 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 selected in response to salinity 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 using SSR marker. Considering combined assessment, four rice genotypes *viz.* Kute Patnai, Kashrail, Bazra Muri and Tal Mugur were selected 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*

## 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]. The increase of rice growing areas and the adoption of drought tolerant varieties are important factors that contribute in more rice production. 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 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 early seedling and reproductive stages are most sensitive for grain yield. [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). To increase the production it needs development of salt tolerant variety and utilization of salt affected areas. 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 and 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] and have been found to be efficient for identification of genes and quantitative trait loci in different rice cultivars [24, 25] that can be helpful for plant breeders to develop new varieties. 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 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 rice landraces based on phenotype and molecular markers evaluation 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, 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 control.

**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 |

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. The fertilizer was applied 50 N, 25 P and 25 K mg $kg^{-1}$ of soil respectively. Initially, the soil level was about 1 cm above the topmost circle of holes. 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 the 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 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). The following formula was used for calculating the percent fertility and reduction:

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

Percent (%) 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 leaf sample of 25 days old seedling [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, 7.5 μl dd$H_2O$ 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 $94^{o}$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 extension 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, three polymorphic SSR markers were used (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.($^{o}$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 significant phenotypic variation was observed at reproductive stage among the rice genotypes under 8 dS/m salinity stress. Normal growth and development was observed in control but some confrontational symptoms were found in salinized condition such cracked and dried leaves, 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 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). 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 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%) displayed lower reduction in panicle length (Table 3).

**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

| 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 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 | |

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%) exhibited lower reduction in filled grains per panicle (Table 3).

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 4). 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 4).

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.

Under normal growth condition all the genotypes produced about 10 g or more yield plant$^{-1}$ but less than 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$^{-1}$ (Table 4). The same result was reported by Asch *et al.* [33] where 80 rice cultivars were used. This result suggests that the salt tolerant cultivars are different from susceptible in up taking salt and yield production.

**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 |
| 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 |
| | $LSD_{(.05)}$ | 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 screened for polymorphism survey using 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$_{(.05)}$ | 1.82 | 1.22 | 0.69 | 0.53 |

**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 |
| Holde Gotal, Kalo Mota, 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 the DNA samples were amplified with RM9 as produced the band in the same level of Binadhan-8. Besides, Holde Gotal, Bashful Balam, Volanath, Rupessor and FL 478 were found susceptible comparing with Binadhan-7 (Fig. 1). Previously, RM9 marker was also used for identification of salinity tolerance rice genotypes [35].

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 (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). Recently, the screening of rice genotypes was done using Binadhan-8 rice variety for salt tolerance using RM234 markers [36]. These three primers (RM9, RM134 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). 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 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.

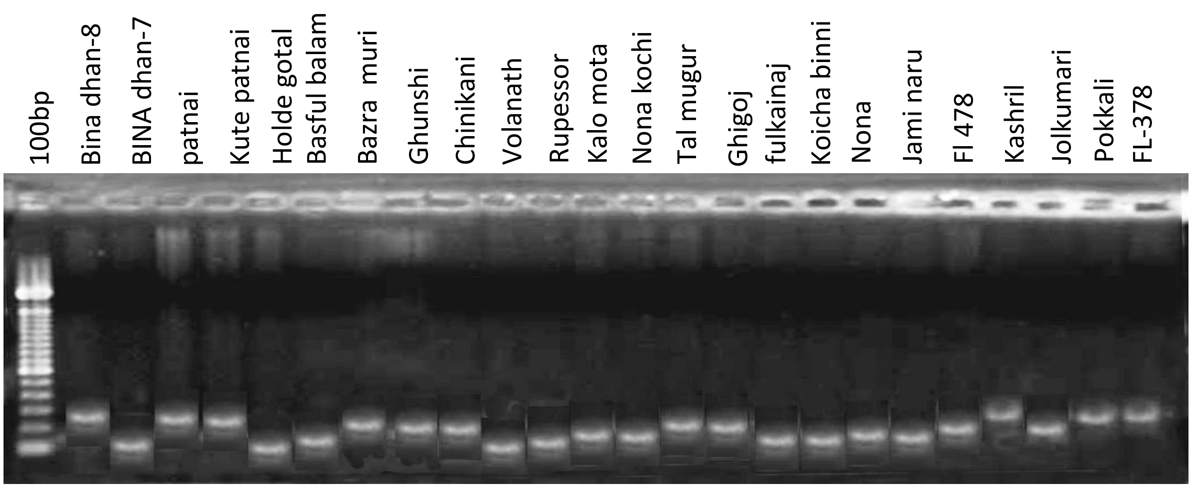


Fig. 1 . Banding profiles of 24 rice germplasm using RM9 primer

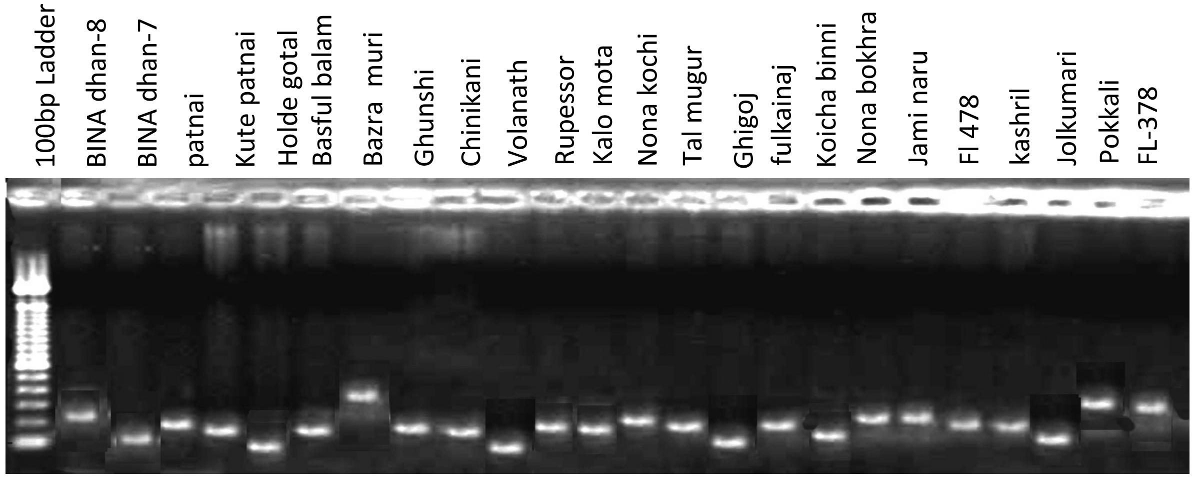


Fig. 2 Banding profiles of 24 rice germplasm using primer RM134

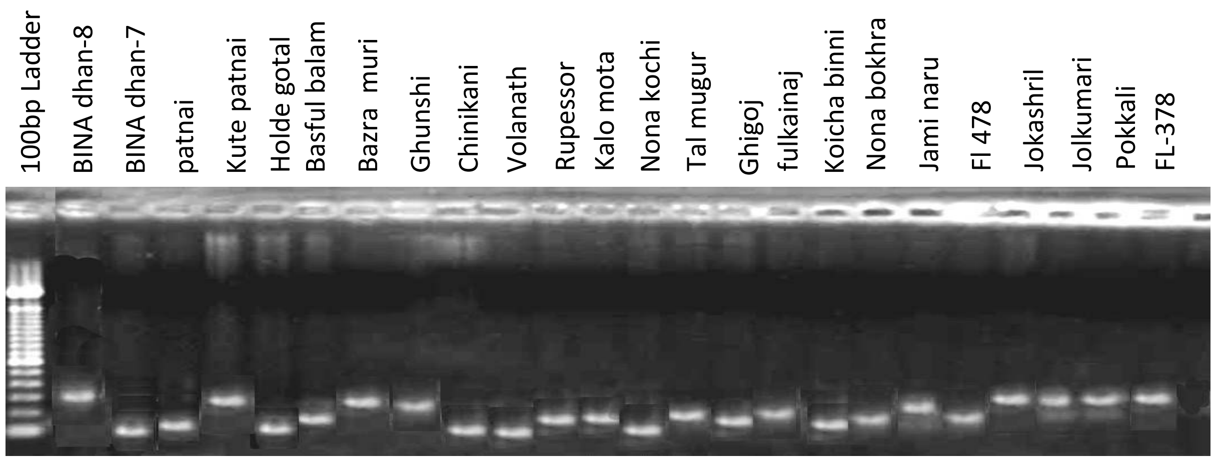


Fig. 3 Banding profiles of 24 rice germplasm using primer RM234

## 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.

## 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. 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.

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.
34. Singh RK, Gregorio GB, Jain RK. 2007. QTL mapping for salinity tolerance in rice. Physiol. Mol. Biol. Plants., 13: 87-99.
35. Sudharani M, Reddy PR, Reddy GH. 2013. Identification of genetic diversity in rice (*oryza sativa* L) Genotypes using microsatellite markers for salinity tolerance. Inter. J. Sci. Innov. Discov., 3 (1): 22-30.
36. Emon RM, Gregorio GB, Nevame AYM, Islam MM, Islam M, Ye-Yang F. 2015. Morpho-Genetic Screening of the Promising Rice Genotypes under Salinity Stress. J. Agril. Sci., 7(5): 94-111.