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PERFORMANCE OF SALINITY TOLERANCE OF F₃ RICE LINES OF BRR1 DHAN 29 × PBRC (STL-20) USING RAPD MARKERS

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ABSTRACT

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Twenty six rice lines of PBRC (salt tolerant line-20) × BRR1 dhan-29 were used to evaluate salinity tolerance at the seedling stage and tested for salt tolerance using RAPD markers. Salinity screening was done using hydroponic system at the greenhouse following IRRI standard protocol. Among the studied line, ten were moderately salinity tolerant, nine susceptible and rest of the lines highly susceptible. For assessing genetic diversity and relationship of F₃ rice lines including two parents were tested against PCR-based Random Amplified Polymorphic DNA (RAPD) technique using three arbitrary decamer primers; OPA02, OPC01, and OPC12. Selected three primers generated a total of 14 bands. Out of 14 bands, 12 bands (86.67%) were polymorphic and 2 bands (13.33%) were monomorphic. The Unweighted Pair Group Method of Arithmetic Means (UPGMA) dendrogram constructed from Nei's (1972) genetic distance produced 2 main clusters of the 28 rice genotypes. Most of the moderately tolerant lines and PBRC (STL-20) (tolerant variety) were grouped in same cluster due to lower genetic distance, while maximum susceptible along with BRR1 dhan29 (susceptible variety) showed higher genetic distance with PBRC (STL-20) and moderately tolerant lines. This result indicates that the lines which formed grouped together, they are less diversified. On the other hand the lines remain in different clusters or different groups, are much diversified. Thus RAPD perform a potentially simple, rapid and reliable method to evaluate genetic diversity and molecular characterization as well.

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INTRODUCTION

Soil salinity is considered as one of the major and widespread abiotic stresses, limiting rice production worldwide. Over 800 million hectares of land throughout the world are salt-affected by salinity (397 million ha) and this is over 6% of the world's total land area (FAO, 2005). The coastal areas of Bangladesh are the salt-affected zone over 30% of the net cultivable area (Mamun et al., 2019). In Bangladesh, salinity prone area forms one-third of the 9 million hectares of total national cultivated area (ABSPII, 2006). An increase in salinity predicts the fall of rice production by 10 % by 2050 (IPCC, 2007). Rice is the major cereal crop in Bangladesh and next to wheat in the world. It is the most important cereal crop in Asia, providing food to more than half of the world population (Ma et al., 2007). Rice ranks the highest position among the cereal crops grown in Bangladesh (Manik et al., 2016). Rice is sensitive to salinity at different stages of growth and development and reported that salinity affects germination, crop establishment, dry matter production and leaf area development, seed set rate, and sterility. The severity of these effects depends on several factors: (i) the intensity of the stress, (ii) the climatic conditions, (iii) the resistance level of the genotype (Folkard and Marco, 2000). The rice plant is relatively tolerant to salinity during germination, and then becomes very sensitive during the early seedling stage (3-leaf stage); it is again tolerant during vegetative growth, sensitive during pollination and fertilization, and finally, become increasingly more tolerant towards maturity (IRRI, 1967). Higher salinity levels cause significant reduction in the growth of parameters like leaf area, leaf length and root and shoot dry weight (Ashrafuzzaman et al., 2002). Plant selection following the conventional methods is largely affected by the environmental conditions and the low narrow-sense heritability of salt tolerance (Gregorio and Senadhira, 1993). Researchers have found that seedling stage is appropriate for the Screening of rice plants for its simple basis of selection; however, screening becomes difficult at the vegetative and reproductive stage (Gregorio et al., 1997).

The Hydroponic system is best suited for screening as it has reduced stress effects on plant and it is a very easy and fast system. Molecular markers have been used extensively in evaluating and selecting plant materials. DNA marker technology is more efficient than conventional breeding techniques and ensures the accuracy of breeding for stress-tolerant crops. In rice, Random Amplified Polymorphic DNA (RAPD) markers are widely used to study the identification of hybrids (Qian et al., 1996), genetic diversity (Yu and Nguyen, 1994; Cao and Oard, 1997) and predicting quantitative variation within germplasms (Virk et al., 1996). For identifying the salt-tolerant gene, RAPD markers are efficient and it helps plant breeders to come up with improved new cultivars. The main goal of the present study is the screening of some F₃ rice lines and their parents under salt stress (EC 12 dS/m) and Studying genetic diversity among F₃ rice lines using RAPD markers at the seedling stage.

MATERIALS AND METHODS

A total of 327 F₂ population of PBRC (SLT-20) (germplasm processes salt tolerance characteristics) × BRR dhan29 (salinity susceptible) were used, among them 118 F₂ rice lines were selected based on desired agronomic performance. Twenty six F₃ lines were selected due to better performance under salt stress in hydroponic system from 118 F₂ lines and also tested for salt tolerance using morphological and molecular markers at the seedling stage. Selected lines were grown in a hydroponic system following IRRI standard protocol for the evaluation of their salinity tolerance at seedling stages. For this evaluation nutrient solution (Yoshida et al., 1976) under controlled environment was applied at the glasshouse of BINA, Mymensingh. The (deleted) salinity level (deleted lines) was maintained by adding crude salt with nutrient solution. The solution was replaced in every 8 days with the new solution and the pH was maintained daily at 5.25. The modified standard evaluation score (SES) of IRRI was used to assess the visual symptoms of salt toxicity (Table 1). This scoring discriminates the tolerant, moderately tolerant and susceptible rice lines. Initial scoring was started at 15 days after salinization and final scoring was done at 21 days after salinization. Data were recorded on plant height (cm), days to flowering, days to maturity, number of effective tillers/plant, number of filled grains/plant, number of grains/plant and grain yield/plant (g) for phenotypic observation.

Fresh leaf tissues of 14 days old seedlings from the F₃ generation were used for DNA isolation. The DNA extraction was done using CTAB mini preparation method. The leaf samples were ground with pestle and

mortar to collect DNA maintaining strict hygiene. Then 670 µl extraction buffer and 50 µl 20% SDS were added with the leaf sample and incubated for 10 minutes at 65^o C. Hundred µl 5M NaCl was added and inverted gently to suspend the samples evenly. After that, 100 µl CTAB was mixed well with NaCl and incubated at 65^o C. Chloroform (chloroform: isoamyl alcohol = 24:1, v/v) was added at a rate of 900 µl and mixed well and centrifuged at 12000 rpm for 15 minutes. Then the supernatant was kept in a new Eppendorf tube and 600 µl ice-cold isopropanol was added. The mixture was shaken slowly and then Spun down at 12000 rpm for 15 minutes by centrifuge. The supernatant was blended and washed the pellet with 200 µl 70% ethanol. Then spun down at 12000 rpm for 5 minutes, removed the ethanol and then the pellets were allowed for air-drying for 1 hour. The pellet was then suspended in 30µl 1X TE buffer. Finally, the DNA samples were stored at -20°C. Isolated genomic DNA contains a large amount of RNA and pigments, which cause overestimation of DNA concentration during spectrophotometer reading. Therefore, the DNA samples were evaluated both qualitatively and quantitatively using agarose gel electrophoresis and spectrophotometer respectively. Ten primers were initially tested and out of 10, three primers viz. OPA02, OPC01 and OPC12 were selected on the basis of showing clear polymorphic bands. The total volume of the PCR cocktail was 8 µl per sample. 2µl genomic DNA was added with 8 µl PCR cocktail and finally, total volume was 10 µl. The PCR tubes were set on the wells of the thermocycler plate. The reaction mix was preheated at 94°C for 3 minutes followed by 40 cycles. 1-minute denaturation at 94°C and 1-minute annealing at 34°C was done. After that, polymerization was done at 72°C for 2 min and final extension by 72°C for 7 min. Completions of cycling program; reactions were held at 4°C. The amplified products were separated electrophoretically on 1.5% agarose gel. DNA bands were observed under UV light using a Gel DOC and photographed by Image Documentation System. The size of the amplification products was estimated by comparing the distance travelled by each amplified fragment with that of the known sized fragments of molecular weight marker (100 bp DNA ladder). The scores obtained using all primers in the RAPD analysis were then conducted to create a single data matrix. This was used to estimate polymorphic loci, Nei's (1973), gene diversity, population differentiation, (GST), gene flow (Nm), genetic distance (D) and constructing a UPGMA (Unweighted Pair Group Method with Arithmetic Means) dendrogram among populations using a computer program, POPGENE (Version 1.31) (Yeh et al., 1999). The same program was also used to perform the test of homogeneity in different locus between population pairs.

RESULTS AND DISCUSSION

Effects of Salt stress were first visible after two or three days of salinization. The salinity injury symptoms like rolling, tip whitening and reduction in leaf area were the first symptoms. Several symptoms of salt injury like-yellowing of leaves, drying of leave reduction in root and shoot growth, stem thickness and in many cases death of seedlings were also observed. Overall, the seedling growth was suppressed under salinity stress but seedling growth was normal in the non-salinized condition. Salinity causes premature senescence of leaves and inhibits the growth of leaves in the plants and eventually completes cessation of growth and (Bonilla et al., 2002). In salinized setup, F₃ rice lines showed wide variation. Ten lines were identified as moderately salinity tolerant and nine were identified as susceptible and rests of the lines were highly susceptible (Table 4). Islam et al., (2007) also observed wide variation in phenotypes from tolerant (score 3) to highly susceptible (score 9) lines at the seedling stage in the hydroponic system. Selected three primers generated 14 bands. Out of the 14 bands, 12 bands (86.67%) were polymorphic and 2 bands (13.33%) were monomorphic (Table 5). This proportion of polymorphism was similar compared to previous RAPD analysis in rice genotypes by Qian et al., (2006) who obtained 83.5% of polymorphic products. The three different primers generated various banding patterns, ranging from 4 (OPA2) to 5 (OPC1). The primer OPC1 and OPC12 produced the same numbers (5) of polymorphic bands. The primer OPA2 and OPC12 produced a higher level of polymorphism. On the other hand, the primer OPC1 generated a lower number of polymorphic bands (Table 4). The banding patterns of different rice genotypes using primers OPA2, OPC1 and OPC12 are shown in Figures 2, 3 and 4. The primer OPC12 not only produced a maximum number of total bands (5) but also amplified the maximum number of polymorphic bands (5). On the other hand, the primer OPA2 amplified lower number (4) of total bands and primer OPC1 produced a lower number of polymorphic bands (3).

Table 1. Modified standard evaluation score (SES) of visual salt injury at the Seedling stage

| Score | Observations | Tolerance |
|-------|---|---------------------|
| 1 | Normal growth, no leaf symptoms | Highly tolerant |
| 3 | Nearly normal growth, but leaf tips of few leaves whitish and rolled | Tolerant |
| 5 | Growth severely retarded, most leaves rolled; only a few are elongating | Moderately tolerant |
| 7 | Complete cessation of growth; most leaves dry; some plants dying | Susceptible |
| 9 | Almost all plants dead or dying | Highly Susceptible |

Table 2. Components of PCR cocktail

| SL. No. | Component | Quantity for single reaction |
|--------------|-----------------------|------------------------------|
| 1 | 10X PCR Buffer | 1 µl |
| 2 | dNTPs | 1 µl |
| 3 | Primer | 2.5 µl |
| 4 | <i>Taq</i> Polymerase | 0.2 µl |
| 5 | ddH ₂ O | 3.3 µl |
| Total | | 08.0 µl |

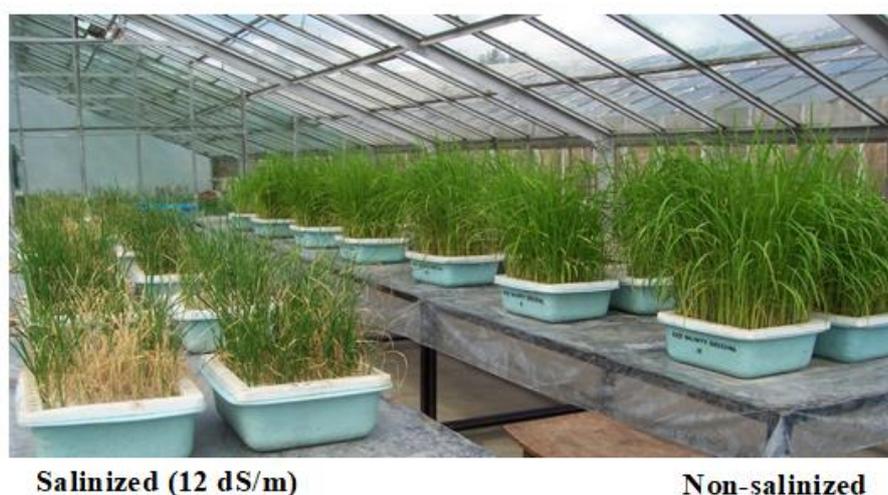
**Figure 1.** Seedlings at early growth stage in salinized and non-salinized (EC 12 dS/m) condition (21 days)

Table 3. Performance of F₃ rice lines under salinized condition (EC12dS/m) at the seedling stage grown in hydroponic system
1-9 Scale, where 1 = highly tolerant, 3 = tolerant, 5 = moderately tolerant, 7 = susceptible and 9 = highly susceptible

| Line no. | SES score | Tolerance level |
|---------------|-----------|-----------------|
| 1 | 9 | HS |
| 2 | 9 | HS |
| 3 | 7 | S |
| 4 | 7 | S |
| 5 | 5 | MT |
| 6 | 7 | S |
| 7 | 5 | MT |
| 8 | 7 | S |
| 9 | 5 | MT |
| 10 | 7 | S |
| 11 | 5 | MT |
| 12 | 5 | MT |
| 13 | 9 | HS |
| 14 | 7 | S |
| 15 | 9 | HS |
| 16 | 9 | HS |
| 17 | 9 | HS |
| 18 | 5 | MT |
| 19 | 5 | MT |
| 20 | 7 | S |
| 21 | 5 | MT |
| 22 | 7 | S |
| 23 | 5 | MT |
| 24 | 7 | S |
| 25 | 5 | MT |
| 26 | 9 | HS |
| BRR1 dhan29 | 7 | S |
| PBRC (STL-20) | 3 | T |

Table 4. RAPD primers with corresponding bands score and their size range together with polymorphic bands observed in BRR1 dhan29, PBRC (STL-20) and their 26 rice lines

| Primer code | Sequences (5'-3') | Total number of bands scored | Size ranges (bp) | Number of polymorphic bands | Polymorphic loci (%) |
|--------------|-------------------|------------------------------|------------------|-----------------------------|----------------------|
| OPA02 | TGCCGAGCTG | 4 | 400-1180 | 4 | 86.67 |
| OPC01 | TTCGAGCCAG | 5 | 200-750 | 3 | |
| OPC12 | TGTCATCCCC | 5 | 300-1000 | 5 | |
| Total | | 14 | | 12 | |

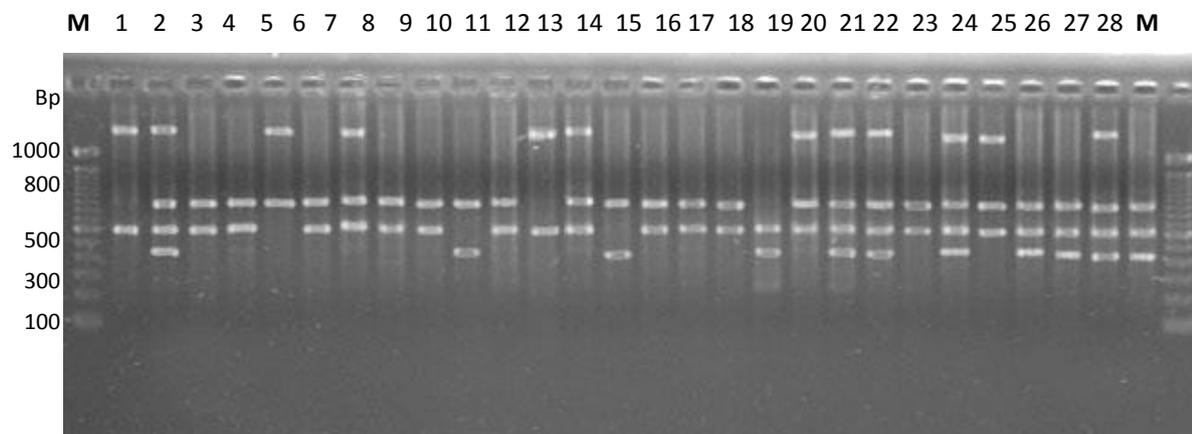


Fig. 2. RAPD profiles of 28 rice genotypes using primer OPA2. Lane 1: BRRI dhan29, 2: PBRC(STL-20), 3: Line-1, 4: Line-2, 5: Line-3, 6: Line-4, 7: Line-5, 8: Line-6, 9: Line-7, 10: Line-8, 11: Line-9, 12: Line-10, 13: Line-11, 14: Line-12, 15: Line-13, 16: Line-14, 17: Line-15, 18: Line-16, 19: Line-17, 20: Line-18, 21: Line-19, 22: Line-20, 23: Line-21, 24: Line-22, 25: Line-23, 26: Line-24, 27: Line-25, 28: Line-26, 29: Line-27, 30: Line-28 and M: Molecular weight marker (100bp DNA ladder).

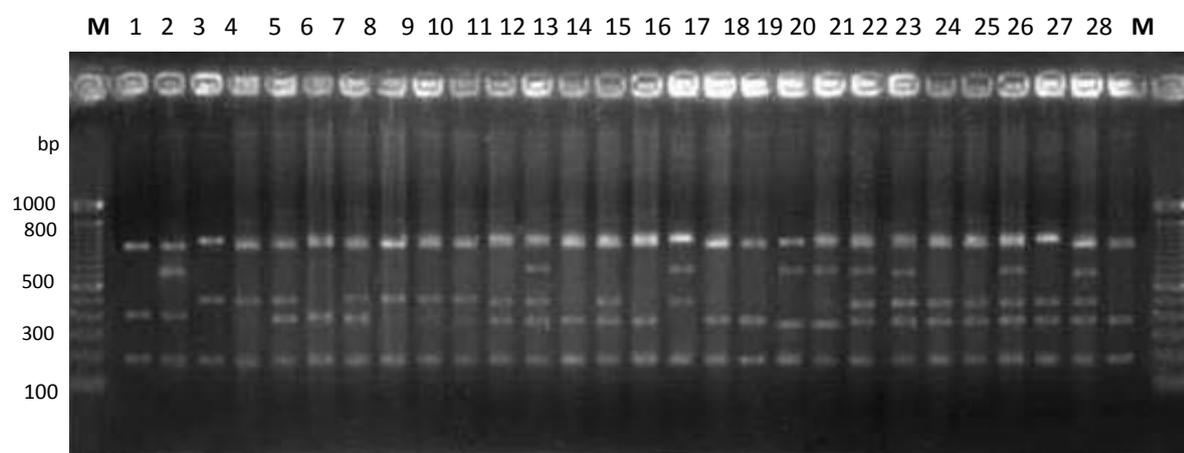


Fig. 3. RAPD profiles of 28 rice genotypes using primer OPC1 Lane 1: BRRI dhan29, 2:PBRC(STL-20), 3: Line-1, 4: Line-2, 5: Line-3, 6: Line-4, 7: Line-5, 8: Line-6, 9: Line-7, 10: Line-8, 11: Line-9, 12: Line-10, 13: Line-11, 14: Line-12, 15: Line-13, 16: Line-14, 17: Line-15, 18: Line-16, 19: Line-17, 20: Line-18, 21: Line-19, 22: Line-20, 23: Line-21, 24: Line-22, 25: Line-23, 26: Line-24, 27: Line-25, 28: Line-26, 29: Line-27, 30: Line-28 and M: Molecular weight marker (100bp DNA ladder).

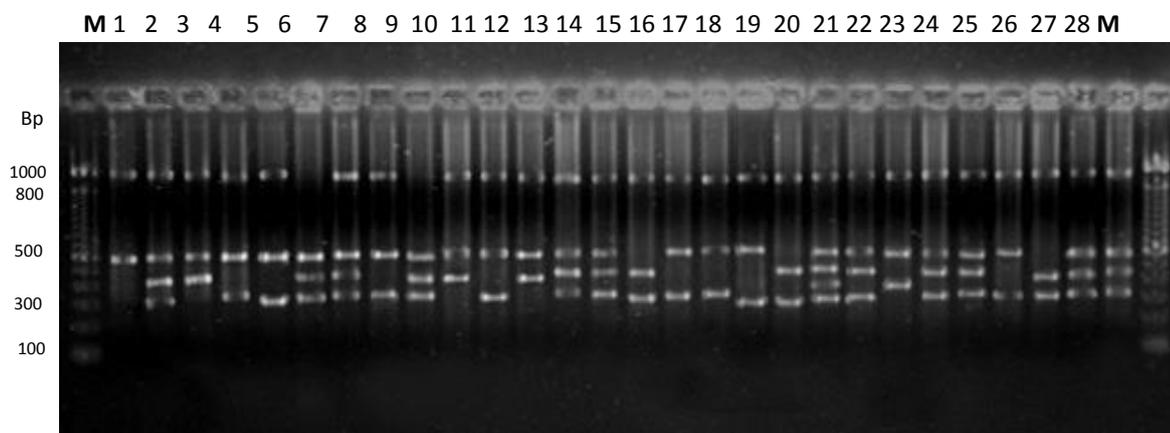


Fig. 4. RAPD profiles of 28 rice genotypes using primer OPC12. Lane 1: BRR1 dhan29, 2: PBRC(STL-20), 3: Line-1, 4: Line-2, 5: Line-3, 6: Line-4, 7: Line-5, 8: Line-6, 9: Line-7, 10: Line-8, 11: Line-9, 12: Line-10, 13: Line-11, 14: Line-12, 15: Line-13, 16: Line-14, 17: Line-15, 18: Line-16, 19: Line-17, 20: Line-18, 21: Line-19, 22: Line-20, 23: Line-21, 24: Line-22, 25: Line-23, 26: Line-24, 27: Line-25, 28: Line-26, 29: Line-27, 30: Line-28 and M: Molecular weight marker (100bp DNA ladder).

In the present study, the percentage of polymorphic loci was 86.67%. Diverse levels of polymorphism in rice genotypes were reported by Tang et al., (2002), 95.33%, Ravi et al., (2003), 90%, Valdmarr et al., (2004), 72.2%, Vibha et al., (2005), 94.36%, Qian et al., (2006), 83.5%, Shivapriya et al., (2006) 74.1%. The highest proportion of polymorphic loci (15.00%) was found in Line-5, Line-12, Line-18, Line-19 and Line-25, which gave 3 polymorphic bands and the lowest proportion of polymorphic loci (0.00%) was found in BRR1 dhan29, PBRC (STL-20), Line-1, Line-2, Line-3, Line-4, Line-7, Line-8, Line-13, Line-14, Line-16, Line-17 and Line-24 performed zero polymorphic bands. Dendrogram based on Nei's, (1972) genetic distance using unweighted Pair Group Method of Arithmetic Means (UPGMA) indicated segregation of the 28 rice genotypes into two main clusters: BRR1 dhan29, Line-2, Line-3, Line-5, Line-6, Line-7, Line-8, Line-9, Line-10, Line-13, Line-14, Line-15, Line-16, Line-20, and Line-23 in cluster 1 and Line-PBRC (STL-20), Line-11, Line-12, Line-13, Line-17, Line-18, Line-19, Line-21, Line-22, line-24, line-25 and Line-26 in cluster 2 (Fig. 5). In Cluster 1, BRR1 dhan29, and Line-16 formed sub-cluster 1 while Line-1, Line-2, Line-3, Line-4, Line-5, Line-6, Line-7, Line-8, Line-9, Line-10, Line-14, Line-15, Line-20, and Line-23 formed sub-cluster 2. Again, among the lines of sub-cluster 1, BRR1 dhan29 and Line-16 organized as sub-sub cluster 1, while Line-1, Line-2, Line-3, Line-4, Line-5, Line-6, Line-7, Line-8, Line-9, Line-10, Line-14, Line-15, Line-20, and Line-23 belongs to sub-sub cluster 2. Line-16 along with BRR1 dhan29 belonged to cluster 1 and this line is separated from other 26 lines. Under salt stress, this line was highly susceptible and showed higher genetic distance (0.8473) with tolerant parent PBRC (STL-20) and other lines. Line-25 and PBRC (STL-20) formed sub-cluster 2 and this line was moderately tolerant under salt stress. The genetic distance between Line-2 and PBRC (STL-20) was 0.2412. Moderately tolerant lines viz. Line-5, Line-12, Line-18 and Line-19 belonged to sub-sub cluster 2. The genetic distance between Line-9 and Line-6 was the lowest (0.0741) and was susceptible under salt stress. So, we can conclude that, the maximum moderately tolerant lines and PBRC (STL-20) grouped into same cluster due to lower genetic distance while majority of susceptible lines and BARI dhan29 showed higher genetic distance with moderately tolerant lines and also PBRC (STL-20).

Table 5. Summary of Nei's Genetic Identity (Above Diagonal) and Genetic Distance (Below Diagonal) of Parent BRR1 dhan29, PBRC (STL-20) and their 26 Rice Lines

| Genotype | BRR1 dhan29 | PBRC (STL-20) | Line 1 | Line 2 | Line 3 | Line 4 | Line 5 | Line 6 | Line 7 | Line 8 | Line 9 | Line 10 | Line 11 | Line 12 | Line 13 | Line 14 | Line 15 | Line 16 | Line 17 | Line 18 | Line 19 | Line 20 | Line 21 | Line 22 | Line 23 | Line 24 | Line 25 | Line 26 |
|--------------------|-------------|---------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| BRR1 dhan29 | **** | 0.6429 | 0.6429 | 0.6429 | 0.7143 | 0.6429 | 0.7143 | 0.6429 | 0.5000 | 0.5000 | 0.7143 | 0.7857 | 0.7857 | 0.5000 | 0.6429 | 0.5714 | 0.7857 | 0.7857 | 0.6429 | 0.5714 | 0.5714 | 0.6429 | 0.6429 | 0.7143 | 0.5714 | 0.5000 | 0.5714 | 0.6429 |
| PBRC STL-20 | 0.4418 | **** | 0.5714 | 0.5714 | 0.6429 | 0.7143 | 0.7857 | 0.5714 | 0.5714 | 0.5714 | 0.6429 | 0.7143 | 0.7143 | 0.5714 | 0.5714 | 0.6429 | 0.7143 | 0.7143 | 0.7143 | 0.9286 | 0.7857 | 0.7143 | 0.7143 | 0.6429 | 0.7857 | 0.5714 | 0.7857 | 0.7143 |
| Line 1 | 0.4418 | 0.5596 | **** | 0.8571 | 0.6429 | 0.7143 | 0.7857 | 0.8571 | 0.8571 | 0.8571 | 0.7857 | 0.7143 | 0.5714 | 0.5714 | 0.7857 | 0.7143 | 0.5714 | 0.4286 | 0.5000 | 0.5000 | 0.8571 | 0.5714 | 0.6429 | 0.6429 | 0.5714 | 0.5000 | 0.5714 | |
| Line 2 | 0.4418 | 0.5596 | 0.1542 | **** | 0.7857 | 0.7143 | 0.7857 | 1.0000 | 0.8571 | 0.7143 | 0.9286 | 0.5714 | 0.7143 | 0.7143 | 0.9286 | 0.8571 | 0.7143 | 0.5714 | 0.5000 | 0.6429 | 0.7143 | 0.7143 | 0.7857 | 0.7857 | 0.7143 | 0.6429 | 0.7143 | |
| Line 3 | 0.3365 | 0.4418 | 0.4418 | 0.2412 | **** | 0.6429 | 0.8571 | 0.7857 | 0.6429 | 0.6429 | 0.8571 | 0.6429 | 0.7857 | 0.7857 | 0.6429 | 0.7143 | 0.7857 | 0.6429 | 0.6429 | 0.5714 | 0.7143 | 0.6429 | 0.7857 | 0.8571 | 0.7143 | 0.6429 | 0.7143 | 0.6429 |
| Line 4 | 0.4418 | 0.3365 | 0.3365 | 0.3365 | 0.4418 | **** | 0.7857 | 0.7143 | 0.8571 | 0.5714 | 0.7857 | 0.5714 | 0.7143 | 0.5714 | 0.7143 | 0.6429 | 0.8571 | 0.7143 | 0.5714 | 0.6429 | 0.5000 | 0.7143 | 0.5714 | 0.6429 | 0.6429 | 0.5714 | 0.5000 | 0.7143 |
| Line 5 | 0.3365 | 0.2412 | 0.2412 | 0.2412 | 0.1542 | 0.2412 | **** | 0.7857 | 0.7857 | 0.6429 | 0.8571 | 0.7857 | 0.7857 | 0.6429 | 0.6429 | 0.7143 | 0.7857 | 0.6429 | 0.6429 | 0.7143 | 0.7143 | 0.7857 | 0.7857 | 0.8571 | 0.7143 | 0.6429 | 0.7143 | 0.6429 |
| Line 6 | 0.4418 | 0.5596 | 0.1542 | 0.0000 | 0.2412 | 0.3365 | 0.2412 | **** | 0.8571 | 0.7143 | 0.9286 | 0.5714 | 0.7143 | 0.7143 | 0.9286 | 0.8571 | 0.7143 | 0.5714 | 0.5000 | 0.6429 | 0.7143 | 0.7143 | 0.7857 | 0.7857 | 0.7143 | 0.6429 | 0.7143 | 0.6429 |
| Line 7 | 0.6931 | 0.5596 | 0.1542 | 0.1542 | 0.4418 | 0.1542 | 0.2412 | 0.1542 | **** | 0.7143 | 0.7857 | 0.5714 | 0.5714 | 0.5714 | 0.7857 | 0.7143 | 0.5714 | 0.4286 | 0.5000 | 0.5000 | 0.7143 | 0.5714 | 0.6429 | 0.6429 | 0.5714 | 0.5000 | 0.5714 | |
| Line 8 | 0.6931 | 0.5596 | 0.1542 | 0.3365 | 0.4418 | 0.5596 | 0.4418 | 0.3365 | 0.3365 | **** | 0.6429 | 0.5714 | 0.4286 | 0.7143 | 0.4286 | 0.6429 | 0.5714 | 0.5714 | 0.2857 | 0.5000 | 0.5000 | 0.7143 | 0.5714 | 0.5000 | 0.6429 | 0.5714 | 0.5000 | 0.5714 |
| Line 9 | 0.3365 | 0.4418 | 0.2412 | 0.0741 | 0.1542 | 0.2412 | 0.1542 | 0.0741 | 0.2412 | 0.4418 | **** | 0.6429 | 0.7857 | 0.7857 | 0.8571 | 0.9286 | 0.7857 | 0.6429 | 0.5714 | 0.7143 | 0.7857 | 0.7857 | 0.8571 | 0.8571 | 0.7857 | 0.7143 | 0.7857 | |
| Line 10 | 0.2412 | 0.3365 | 0.3365 | 0.5596 | 0.4418 | 0.5596 | 0.2412 | 0.5596 | 0.5596 | 0.5596 | 0.4418 | **** | 0.5714 | 0.4286 | 0.4286 | 0.6429 | 0.5714 | 0.5714 | 0.5714 | 0.6429 | 0.6429 | 0.8571 | 0.5714 | 0.6429 | 0.6429 | 0.4286 | 0.6429 | 0.4286 |
| Line 11 | 0.2412 | 0.3365 | 0.5596 | 0.3365 | 0.2412 | 0.3365 | 0.2412 | 0.3365 | 0.5596 | 0.8473 | 0.2412 | 0.5596 | **** | 0.7143 | 0.8571 | 0.6429 | 0.8571 | 0.7143 | 0.8571 | 0.7857 | 0.7857 | 0.5714 | 0.8571 | 0.9286 | 0.6429 | 0.7143 | 0.7857 | 0.8571 |
| Line 12 | 0.6931 | 0.5596 | 0.5596 | 0.3365 | 0.2412 | 0.5596 | 0.4418 | 0.3365 | 0.5596 | 0.3365 | 0.2412 | 0.8473 | 0.3365 | **** | 0.7143 | 0.6429 | 0.7143 | 0.7143 | 0.5714 | 0.6429 | 0.7857 | 0.5714 | 0.8571 | 0.7857 | 0.7857 | 0.8571 | 0.7857 | 0.8571 |
| Line 13 | 0.4418 | 0.5596 | 0.5596 | 0.3365 | 0.4418 | 0.3365 | 0.4418 | 0.3365 | 0.5596 | 0.8473 | 0.2412 | 0.8473 | 0.1542 | 0.3365 | **** | 0.6429 | 0.8571 | 0.7143 | 0.8571 | 0.6429 | 0.6429 | 0.5714 | 0.7143 | 0.7857 | 0.6429 | 0.8571 | 0.6429 | 0.8571 |
| Line 14 | 0.5596 | 0.4418 | 0.2412 | 0.0741 | 0.3365 | 0.4418 | 0.3365 | 0.0741 | 0.2412 | 0.4418 | 0.1542 | 0.4418 | 0.4418 | 0.4418 | **** | 0.7857 | 0.6429 | 0.6429 | 0.5714 | 0.7143 | 0.7857 | 0.6429 | 0.7143 | 0.8571 | 0.6429 | 0.7143 | 0.6429 | |
| Line 15 | 0.2412 | 0.3365 | 0.3365 | 0.1542 | 0.2412 | 0.1542 | 0.2412 | 0.1542 | 0.3365 | 0.5596 | 0.0741 | 0.5596 | 0.1542 | 0.3365 | 0.1542 | 0.2412 | **** | 0.8571 | 0.7143 | 0.6429 | 0.6429 | 0.7143 | 0.7143 | 0.7857 | 0.7857 | 0.7143 | 0.6429 | 0.8571 |
| Line 16 | 0.2412 | 0.8473 | 0.5596 | 0.3365 | 0.4418 | 0.3365 | 0.4418 | 0.3365 | 0.5596 | 0.5596 | 0.2412 | 0.5596 | 0.3365 | 0.3365 | 0.3365 | 0.4418 | 0.1542 | **** | 0.5714 | 0.6429 | 0.6429 | 0.5714 | 0.7143 | 0.6429 | 0.7857 | 0.7143 | 0.6429 | 0.8571 |
| Line 17 | 0.4418 | 0.3365 | 0.2412 | 0.5596 | 0.4418 | 0.5596 | 0.4418 | 0.5596 | 0.8473 | 1.2528 | 0.4418 | 0.5596 | 0.1542 | 0.5596 | 0.1542 | 0.4418 | 0.3365 | 0.5596 | **** | 0.7857 | 0.7857 | 0.5714 | 0.7143 | 0.7857 | 0.6429 | 0.7143 | 0.7857 | 0.7143 |
| Line 18 | 0.5596 | 0.0741 | 0.6931 | 0.6931 | 0.5596 | 0.4418 | 0.3365 | 0.6931 | 0.6931 | 0.6931 | 0.5596 | 0.4418 | 0.2412 | 0.4418 | 0.4418 | 0.5596 | 0.4418 | 0.4418 | 0.2412 | **** | 0.8571 | 0.6429 | 0.7857 | 0.7143 | 0.7143 | 0.6429 | 0.8571 | 0.7857 |
| Line 19 | 0.5596 | 0.2412 | 0.6931 | 0.4418 | 0.3365 | 0.6931 | 0.3365 | 0.4418 | 0.6931 | 0.6931 | 0.3365 | 0.4418 | 0.2412 | 0.2412 | 0.4418 | 0.3365 | 0.4418 | 0.4418 | 0.2412 | 0.1542 | **** | 0.6429 | 0.9286 | 0.8571 | 0.8571 | 0.7857 | 1.0000 | 0.7857 |
| Line 20 | 0.4418 | 0.3365 | 0.1542 | 0.3365 | 0.4418 | 0.3365 | 0.2412 | 0.3365 | 0.3365 | 0.3365 | 0.2412 | 0.1542 | 0.5596 | 0.5596 | 0.5596 | 0.2412 | 0.3365 | 0.5596 | 0.5596 | 0.4418 | 0.4418 | **** | 0.5714 | 0.6429 | 0.7857 | 0.5714 | 0.6429 | 0.5714 |
| Line 21 | 0.4418 | 0.3365 | 0.5596 | 0.3365 | 0.2412 | 0.5596 | 0.2412 | 0.3365 | 0.5596 | 0.5596 | 0.2412 | 0.5596 | 0.1542 | 0.1542 | 0.3365 | 0.4418 | 0.3365 | 0.3365 | 0.3365 | 0.2412 | 0.0741 | 0.5596 | **** | 0.9286 | 0.7857 | 0.8571 | 0.9286 | 0.8571 |
| Line 22 | 0.3365 | 0.4418 | 0.4418 | 0.2412 | 0.1542 | 0.4418 | 0.1542 | 0.2412 | 0.4418 | 0.6931 | 0.1542 | 0.4418 | 0.0741 | 0.2412 | 0.2412 | 0.3365 | 0.2412 | 0.4418 | 0.2412 | 0.3365 | 0.1542 | 0.4418 | 0.0741 | **** | 0.7143 | 0.7857 | 0.8571 | 0.7857 |
| Line 23 | 0.5596 | 0.2412 | 0.4418 | 0.2412 | 0.3365 | 0.4418 | 0.3365 | 0.2412 | 0.4418 | 0.4418 | 0.1542 | 0.4418 | 0.4418 | 0.2412 | 0.4418 | 0.1542 | 0.2412 | 0.2412 | 0.4418 | 0.3365 | 0.1542 | 0.2412 | 0.2412 | 0.3365 | **** | 0.7857 | 0.8571 | 0.7857 |
| Line 24 | 0.6931 | 0.5596 | 0.5596 | 0.3365 | 0.4418 | 0.5596 | 0.4418 | 0.3365 | 0.5596 | 0.5596 | 0.2412 | 0.8473 | 0.3365 | 0.1542 | 0.1542 | 0.4418 | 0.3365 | 0.3365 | 0.3365 | 0.4418 | 0.2412 | 0.5596 | 0.1542 | 0.2412 | 0.2412 | **** | 0.7857 | 0.8571 |
| Line 25 | 0.5596 | 0.2412 | 0.6931 | 0.4418 | 0.3365 | 0.6931 | 0.3365 | 0.4418 | 0.6931 | 0.6931 | 0.3365 | 0.4418 | 0.2412 | 0.2412 | 0.4418 | 0.3365 | 0.4418 | 0.4418 | 0.2412 | 0.1542 | 0.0000 | 0.4418 | 0.0741 | 0.1542 | 0.1542 | 0.2412 | **** | 0.7857 |
| Line 26 | 0.4418 | 0.3365 | 0.5596 | 0.3365 | 0.4418 | 0.3365 | 0.4418 | 0.3365 | 0.5596 | 0.5596 | 0.2412 | 0.8473 | 0.1542 | 0.1542 | 0.1542 | 0.4418 | 0.1542 | 0.1542 | 0.3365 | 0.2412 | 0.2412 | 0.5596 | 0.1542 | 0.2412 | 0.2412 | 0.1542 | 0.2412 | **** |

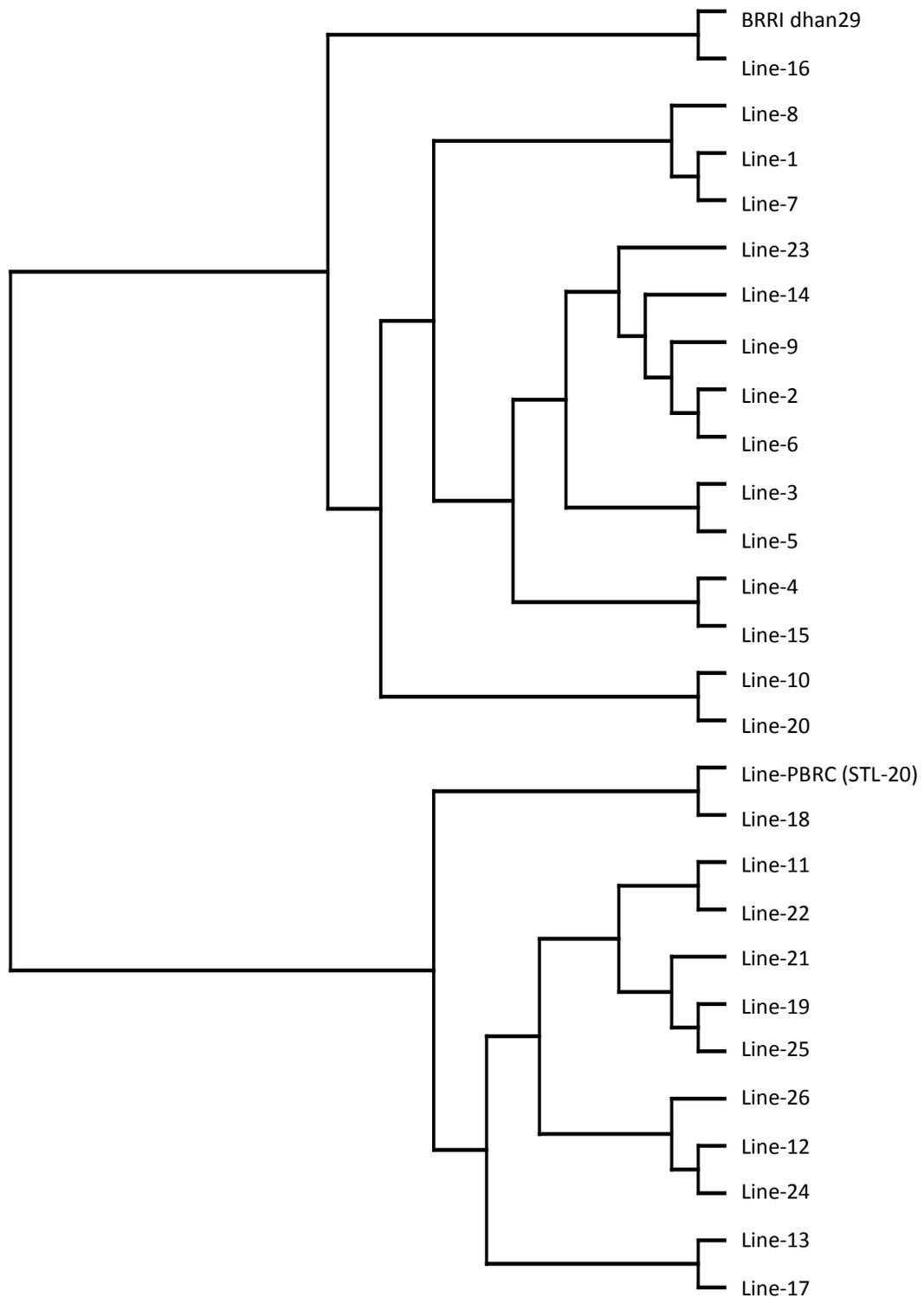


Figure 5. UPGMA dendrogram based on Nei's (1972) genetic distance summarizing the data on differentiation between 28 rice genotypes, according to RAPD analysis

CONCLUSION

From the study, it is concluded that RAPD markers can be a sensitive, simple, efficient and powerful tool for genetic diversity analysis among and within rice genotypes and effectively trace their genetic relationships. The results of this study can be used as a baseline of relationships for future diversity assessment and genetic analysis of rice varieties in Bangladesh. However, there were a few limitations in the study, a larger number of samples and a higher number of primers would be necessary to generate and construct an appropriate genetic relationship, sample identification and analysis of genetic diversity among different varieties and cultivars is widely acceptable by all concern. Using a larger number of samples and a higher number of primers could be useful in future research. Overall RAPD is a suitable technique for molecular genetic analysis and for the reasonable relationship among the germplasm studied if a large number of samples and a higher number of primers are used.

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CONFLICT OF INTEREST

There are no conflicts of research for this study.

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