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Molecular Diversity Analysis in Boro Rice (*Oryza sativa* L.) Landraces using SSR Markers

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Authors' contributions

This work was carried out in collaboration among all authors. Authors FAV, MEH and MZI designed the study and wrote the draft of the manuscript. Authors FAV and MFRKP performed the research work. Author MZI performed the statistical analysis. Authors MEH and MZI contributed to revising it critically. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of the study was to determine the genetic diversity of twenty four Boro rice landraces using rice genome specific twelve well known SSR markers.
Study Design: Genomic DNA extraction, PCR amplification, Polyacrylamide gel electrophoresis (PAGE) and data analysis-these steps were followed to perform the research work. Data was analysed with the help of following software; POWERMAKER version 3.25, AlphaEaseFC (Alpha Innotech Corporation) version 4.0. UPGMA dendrogram was constructed using MEGA 5.1 software.
Place and Duration of Study: The study was conducted at the Genetic Resources and Seed Division (GRSD), Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur, Bangladesh during the period of November 2017 to March 2018.
Methodology: Simple Sequence Repeat (SSR) markers were used to assay 24 landraces of Boro rice collected from the Gene Bank of Bangladesh Rice Research Institute (BRRI).
Results: A total fifty four (54) alleles were detected, out of which forty five (45) polymorphic alleles were identified. The Polymorphic Information Content (PIC) of SSR markers ranged from 0.08

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(RM447) to 0.84 (RM206) with an average value of PIC = 0.49. Gene diversity ranges from 0.08 (RM447) to 0.86 (RM206) with an average value of 0.52. The RM206 marker can be considered as the best marker among the studied markers for 24 rice landraces. Dendrogram based on Nei's genetic distance using Unweighted Pair Group Method of Arithmetic Mean (UPGMA) indicated the segregation of 24 genotypes into three main clusters.

Conclusion: The result revealed that SSR markers are very effective tools in the study of genetic diversity and genetic relationships and this result can be conveniently used for further molecular diversity analysis of rice genotypes to identify diverse parent for the development of high yielding variety in rice.

Keywords: Molecular diversity; Rice; Landrace; SSR marker.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is a self-pollinated cereal crop belonging to the family Gramineae having chromosome number 2n=24 [1]. It is a monocot which is normally grown as an annual plant. The Himalayan foothills including parts of Bangladesh are considered to be the secondary center of diversity of the genus *Oryza* [2]. Rice (*Oryza sativa* L.) is one of the most important staple food crops for more than 3.5 billion people [3]. Rice accounts for 50 % of agricultural income in Asia and supplies nutrition almost 80% of the planet. Rice being staple food constitutes over 90% of the food grain production in Bangladesh. It provides 75% of the calories and 55% of the proteins in the average daily diet for the people of the country [4]. In 2020, Bangladesh has ranked 3rd position in rice production in the world [5]. Rice provides more than 80% of the food requirements for the common people of Bangladesh [6]. Rice is cultivated here throughout the year as Aus, Aman and Boro. Boro rice is generally cultivated during November–May in waterlogged, low-lying or medium lands with irrigation. It is the most important and single largest crop in Bangladesh in respect of volume of production that meet up the national demand. Until now, Bangladesh Rice Research Institute (BRRI) has collected and preserved a Gene bank of about 8700 varieties/landraces/cultivars/wild relative's /genotypes from indigenous and exotic sources. Out of these, more than 8600 germplasm have been registered in BRRI Gene bank. DNA profiling and genetic diversity estimation is necessary for proper utilization of these genotypes. Polymorphism and DNA fingerprinting will be helpful for selection of diverge genotypes for further rice improvement program. Hence, out of those collected gene pool, 24 different Boro rice landraces have been used to identify their molecular diversity in this present study.

Genetic diversity measured through morphological differences of quantitative important traits has some disadvantages in terms of time, space, cost involved and environmental factors. Molecular characterization by using DNA marker gives more precise, convenient and reliable results for genetic variability assessment. DNA markers are extensively used because of their advantages of other markers as they are technically simple, time saving, highly informative and require small amount of DNA and independence from effects related to environmental conditions and also the physiological stage of plant. Among the PCR-based markers, the SSR markers have proved to be very effective tools in the study of genetic diversity and genetic relationships within and among the species. The SSR markers are highly polymorphic, highly transferable, abundant in eukaryotic organisms and well distributed throughout the genome. They are easily amplified by PCR reactions using DNA nucleotide primers, the unique sequences flanking the repeat motifs [7]. SSR markers have been widely applied in the genetic diversity analysis, genotypic identification and population structure estimation in several rice genetic studies [8-9]. SSR markers even in less number can give a better genetic diversity spectrum due to their multi allelic and highly polymorphic nature [10]. Assessment of genetic diversity in any crop is basically important for improving heterotic germplasm selection over the existing ones. Local varieties of rice have evolved from their wild progenitors under both natural and human selection, resulting in a high level of genetic diversity [11]. Therefore, research emphasis has been given on genetic diversity through microsatellite DNA markers in local Boro rice landraces. The main objectives of this research were: to assess the polymorphism and molecular diversity of 24 Boro rice landraces using SSR markers, to establish dendrogram for classifying genotypes in different groups based on their

genetic distances as well as to determine the genetic relationship among the Boro rice germplasm.

2. MATERIALS AND METHODS

2.1 Plant Materials and Sample Collection

A total of 24 Boro rice *(Oryza sativa* L.) germplasm accessions were used in this experiment (Table 1). It was collected from the Gene Bank of Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur. The experiment was conducted at the Genetic Resources and Seed Division (GRSD), Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur -1701 during the period of November 2017 to March 2018.

2.2 Genomic DNA Extraction for SSR Analysis

About 3 cm long leaf tips were collected as sample from young green leaves of the rice plants at 10-15 DAT (Days after transplanting). The microfuge tubes (1.5 ml) containing the leaf samples were kept in poly bags and placed in the chamber of -80°C freezer. The leaf samples were crushed immediately for DNA extraction.

For genomic DNA extraction, the leaves were washed thoroughly by running tap water followed by de-ionized water. Then, the leaves were sterilized by ethanol and dried on tissue paper. Total genomic DNA was isolated using a quick modified CTAB DNA extraction method developed by Ferdous et al. [12]. DNA samples were stored at -20^0 C refrigerator.

2.3 SSR Markers and Polymerase Chain Reaction (PCR) Amplification

Rice genome specific 12 well known SSR primers were selected and synthesized for diversity analysis (Table 2). PCR analysis was performed in 10 µl reaction sample containing 3 µl of DNA template, 4.5 µl of Go Taq G2 Green Master Mix (Promega), 1.5 µl of Nuclease-Free Water and 0.5 µl each of 10 µM forward and reverse primers. GeneAtlas G (Astec, Japan) 96-well thermal cycler was used for DNA amplification and twelve-channel pipette was used for transferring DNA from dilution plate to PCR plate. The mixture was overlaid with 10 µl of mineral oil to prevent evaporation. The PCR plate was wrapped with adhesive film. After initial denaturation for five minutes at 94°C, each cycle comprised 30 sec denaturation at 95°C, 30 sec annealing at 55°C and 25 sec extension with a final extension for 5 min at 72°C at the end of 32 cycles.

2.4 Polyacrylamide Gel Electrophoresis (PAGE)

Three micro liters of PCR products with SSR markers were subjected to electrophoresis using Polyacrylamide gel at 100 volt for different time settings according to EPS (Expected Product Size) to check the DNA quantification and PCR amplification. The concentration of gels used for PAGE was 8%. To prepare eight percent of PAGE gel the following chemicals were used: Sterile nanopure H_2O, 10X TBE buffer, 40% Acrylamide, 10% APS and TEMED.

2.5 Staining and Visualization of DNA Banding Patterns

The acrylamide gel was removed and transferred in the SYBR Safe staining solution (0.5 mg/ml) for around 20 minutes. The stained gels were put in the exposure cabinet of the gel documentation system (Molecular Imager Gel Doc XR System, BIO-RAD, Korea). The gel was viewed in the computer monitor by exposing it first to white light. The gel was exposed to UV light for taking photograph (gel image) and saving as a JPEG file.

2.6 Data Analysis

The summary statistics including the number of alleles per locus , major allele frequency, gene diversity and Polymorphism Information Content (PIC) values were destined using POWERMAKER version 3.25 [13] software. AlphaEaseFC (Alpha Innotech Corporation) version 4.0 software was used for estimating molecular weight for each microsatellite products, in base pairs. Polymorphic information content (PIC) values were computed with the following formula [14]: $PIC_i = 1-\sum^n_{j=1} p^2_{ij}$ .Genetic diversity was also assessed and the phylogeny trees were drawn using MEGA 5.1 [15-16] based on Nei's [17] genetic distance.

3. RESULTS

The study of genetic diversity in any breeding population is very essential and known as the backbone of any crop improvement program. It helps in the development of crop that is suitable

and adaptable to rapid climate change through the introduction of foreign genes [18-19]. Twenty four Boro rice landraces were successfully amplified with the 12 microsatellite marker pairs (Table 3) each referred to as loci and DNA bands as alleles.

Table 1. List of rice landraces (*Oryza sativa* L.) with their accession number

Sl. No.	Name	Accession No.	Sl. No.	Name	Accession No.
1.	Kalo Boro	7663	13.	Tepi Boro Dhan (Kalo)	8058
2.	Asami Boro	7667	14.	Dhali Boro 7/2	2243
3.	Pabdafor	7668	15.	Black Rice	8096
4.	Amania	7669	16.	Dhali Boro 74/3	2244
5.	Khaiya Boro	7671	17.	Boro Habj 1	8100
6.	Rata	7676	18.	Vawailia	8103
7.	Lakhai Boruni	7677	19.	Kali Boro	8104
8.	Sada Boro	7679	20.	Jira	NC
9.	Sona Biron	941	21.	Dhali Boro 87/1	2245
10.	Rajkumar	8052	22.	Dhali Boro 94	2246
11.	Dol Boro	8053	23.	Dhali Boro 104/1	2247
12.	Boro Dhan (Indian)	8055	24.	Dhali Boro 105/2	2248

Table 2. List of twelve SSR markers used for diversity analysis

Primer Name	Location in Chromosome	Forward primer (5′–3′)	Reverse primer (5′–3′)
RM1	1	GCGAAAACACAATGCAAAAA	GCGTTGGTTGGACCTGAC
RM206	11	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG
RM207	2	CCATTCGTGAGAAGATCTGA	CACCTCATCCTCGTAACGCC
RM253	6	TCCTTCAAGAGTGCAAAACC	GCATTGTCATGTCGAAGCC
RM304	10	TCAAACCGGCACATATAAGAC	GATAGGGAGCTGAAGGAGAG
RM252	4	TTCGCTGACGTGATAGGTTG	ATGACTTGATCCCGAGAACG
RM1337	12	GTGCAATGCTGAGGAGTATC	CTGAGAATCTGGAGTGCTTG
RM320	7	CAACGTGATCGAGGATAGATC	GGATTTGCTTACCACAGCTC
RM205	9	CTGGTTCTGTATGGGAGCAG	CTGGCCCTTCACGTTTCAGTG
RM447	8	CCCTTGTGCTGTCTCCTCTC	ACGGGCTTCTTCTCCTTCTC
RM3646	3	ACTAGAGCACCCTCGCTGAG	CTCAGCCACCCCATCAAC
RM413	5	GGCGATTCTTGGATGAAGAG	TCCCCACCAATCTTGTCTTC

Table 3. Number of alleles, no. of polymorphic alleles, allele size range, allele frequency, gene diversity and Polymorphism information content (PIC) found among 12 microsatellite markers across 24 Boro rice landraces

Marker	Total no. of allele	No. of polymorphic allele	Size range (bp)	Allele frequency (%)	Gene diversity	PIC
RM1	6	5	80-175	75	0.41	0.37
RM206	8	8	130-280	16.67	0.86	0.84
RM207	5	5	140-190	80.34	0.28	0.24
RM253	7	7	125-200	45.83	0.64	0.57
RM304	4	3	140-200	29.17	0.82	0.80
RM252	2	1	200-240	62.5	0.57	0.53
RM1337	6	4	180-300	33.33	0.81	0.78
RM320	1	0	200	91.67	0.16	0.15
RM205	7	6	175-300	66.67	0.47	0.39
RM447	3	2	110-180	95.83	0.08	0.08
RM3646	2	1	150-162	70.83	0.45	0.41
RM413	3	3	70-110	45.83	0.70	0.66
Total	54	45	-	-	6.25	5.82
Average	4.5	--	-	--	0.52	0.49

3.1 DNA amplification, Allele Size and Polymorphic Allele through SSR Marker

In this study, twelve (12) SSR markers were screened, out of which 11 SSR markers were found to be polymorphic which were suitable for diversity analysis (Table 3). Only, the SSR primer RM320 amplified 200 bp of DNA fragment which indicated a monomorphic banding pattern, however, other 11 markers indicated polymorphic banding pattern for all the individuals. A total of 54 alleles were detected for the 12 SSR loci, with an average number of alleles/locus of 4.5 and a range between 1 to 8 no. of alleles. A total 45 polymorphic alleles were detected among the experimental genotypes. The length of the DNA fragments varies within a range of 70 bp to 300 bp. The highest, eight fragment of DNA amplification were noticed by SSR primer RM206 and the size of the amplification ranged from 130 to 280 bp (Table 3, Plate 1). Obviously, eight types of band indicate a highly polymorphic banding pattern (Plate 1). Plate 2 and Plate 3 were representing the gel image of amplified fragments produced by primer RM207, RM1337 respectively. However, the frequency of the major allele ranged between 16.67% (RM206) to 95.83% (RM447).

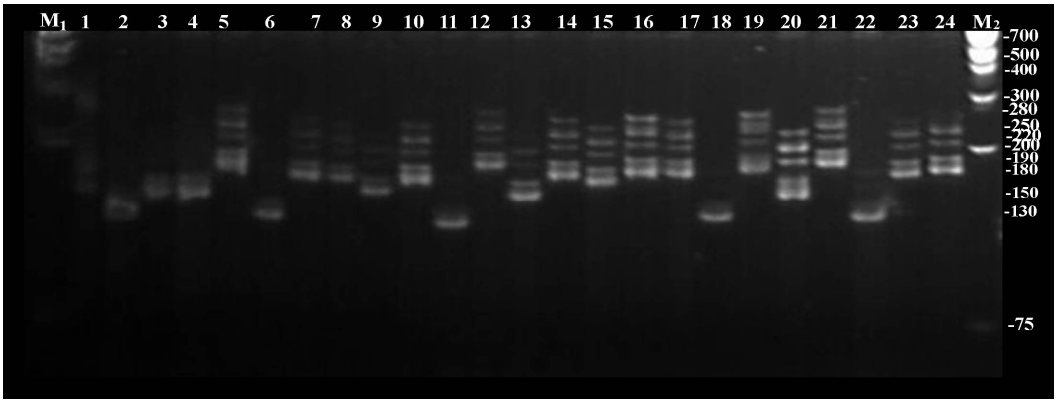

Plate 1. SSR profile of twenty four Boro rice landraces using primer RM206

M1 and M2 = Molecular marker (Thermo Scientific GeneRuler 1 kb Plus DNA Ladder). Lane: 1. Kalo Boro, 2. Asami Boro, 3. Pabdafor, 4. Amania, 5. Khaiya Boro, 6. Rata,7. Lakhai Boruni, 8. Sada Boro, 9. Sona Biron, 10. Rajkumar, 11. Dol Boro, 12. Boro Dhan (Indian), 13. Tepi Boro Dhan (Kalo), 14. Dhali Boro 7/2, 15. Black Rice, 16. Dhali Boro 74/3, 17. Boro Habj 1, 18. Vawailia, 19. Kali Boro, 20. Jira, 21. Dhali Boro 87/1, 22. Dhali Boro 94, 23. Dhali Boro 104/1, 24. Dhali Boro 105/2

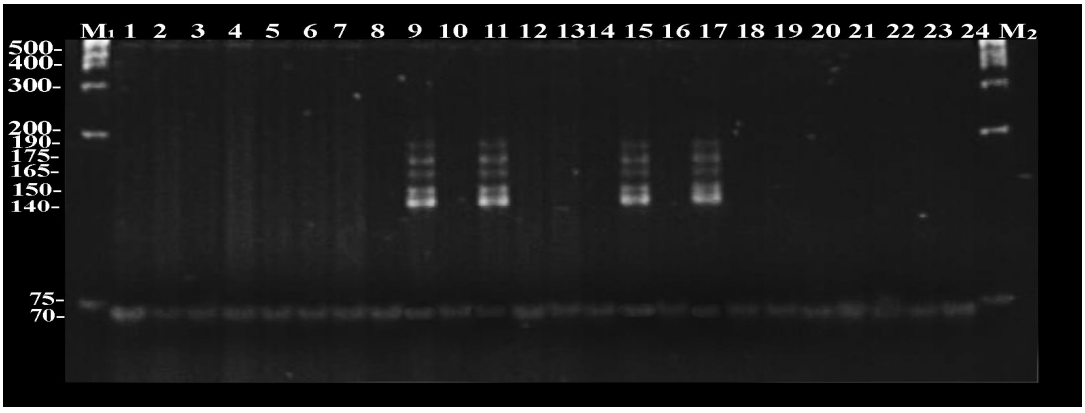

Plate 2. SSR profile of twenty four Boro rice landraces using primer RM207

M1 and M2 = Molecular marker (Thermo Scientific GeneRuler 1 kb Plus DNA Ladder). Lane: 1. Kalo Boro, 2. Asami Boro, 3. Pabdafor, 4. Amania, 5. Khaiya Boro, 6. Rata,7. Lakhai Boruni, 8. Sada Boro, 9. Sona Biron, 10. Rajkumar, 11. Dol Boro, 12. Boro Dhan (Indian), 13. Tepi Boro Dhan (Kalo), 14. Dhali Boro 7/2, 15. Black Rice, 16. Dhali Boro 74/3, 17. Boro Habj 1, 18. Vawailia, 19. Kali Boro, 20. Jira, 21. Dhali Boro 87/1, 22. Dhali Boro 94, 23. Dhali Boro 104/1, 24. Dhali Boro 105/2.

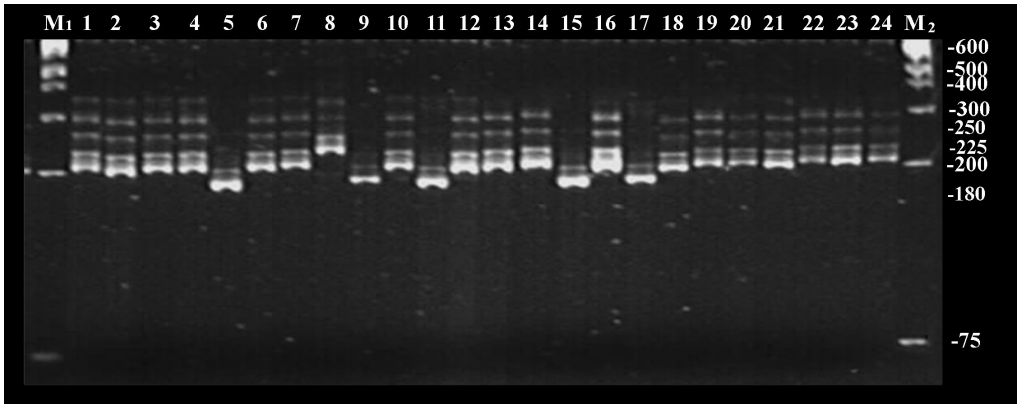

Plate 3. SSR profile of twenty four Boro rice landraces using primer RM1337

M1 and M2 = Molecular marker (Thermo Scientific GeneRuler 1 kb Plus DNA Ladder). Lane: 1. Kalo Boro, 2. Asami Boro, 3. Pabdafor, 4. Amania, 5. Khaiya Boro, 6. Rata,7. Lakhai Boruni, 8. Sada Boro, 9. Sona Biron, 10. Rajkumar, 11. Dol Boro, 12. Boro Dhan (Indian), 13. Tepi Boro Dhan (Kalo), 14. Dhali Boro 7/2, 15. Black Rice, 16. Dhali Boro 74/3, 17. Boro Habj 1, 18. Vawailia, 19. Kali Boro, 20. Jira, 21. Dhali Boro 87/1, 22. Dhali Boro 94, 23. Dhali Boro 104/1, 24. Dhali Boro 105/2

3.2 Gene Diversity and Polymorphism Information Content (PIC)

Gene diversity and Polymorphism Information Content (PIC) value of experimental landraces are presented in Table 3. Gene diversity ranged from 0.08 to 0.86 with an average of 0.52. Primer RM206 showed the highest gene diversity (0.86) followed by RM304 (0.82), RM1337 (0.81), RM413 (0.70), RM253 (0.64), RM252 (0.57), RM205 (0.47), RM3646 (0.45), RM1 (0.41), RM207 (0.28), RM320 (0.16) and RM447 showed the lowest gene diversity (0.08) (Table 3). Total gene diversity obtained 6.25 with an average 0.52. Polymorphic Information Content (PIC) value for 12 SSR markers ranged from 0.08 to 0.84 and the average PIC value was 0.49. The polymorphism information content (PIC) value is a reflection of allelic diversity and frequency among the landraces. The highest PIC value was obtained from RM206 (0.84) and the lowest PIC value 0.08 was showed by primer RM447. From the PIC value, it is clear that RM206 might be considered as the best marker for the studied landraces. RM447 can be considered as the least powerful marker due to its lowest PIC value. The results indicated that the studied rice landraces revealed high degree of heterozygosis which is really noticeable.

3.3 Nei's Genetic Distance Based Analysis

The value of pair-wise comparisons of Nei's (1983) genetic distance among 24 relatives of rice landraces were computed from combined data for the 12 primers, ranged from 0.1667 to 0.9167 with an average of 0.5417 (Table 4). Comparatively higher genetic distance (0.9167) was observed between a numbers of landraces. Among them Sona Biron (G9) showed highest genetic dissimilarity with maximum number of landraces. Also it was observed in Kalo Boro vs. Lakhai Boruni vs. Sona Biron vs. Dhali Boro 104/1; Pabdafor vs. Boro Dhan (Indian); Lakhai Boruni vs. Boro Dhan (Indian); Sona Biron vs. Boro Dhan (Indian); Boro Dhan (Indian) vs. Dhali Boro 104/1. On the contrary, some pairs showed the lowest genetic distance (0.1667) which indicated genetically much more closeness among them. However, the highest genetic distance between the landraces indicated that genetically they are dissimilar and also highly diverse.

3.4 UPGMA Clustering and PCA Analysis

When UPGMA dendrogram of the 24 landraces were constructed on the basis of the Nei's genetic distance calculation, they were clustered into 3 main groups (I, II, III) and six sub-groups (I-1, I-2, II-1, II-2, III-1, III-2)(Fig. 1). G12, G19, G21, G23 were grouped in cluster I; G5, G7, G8, G9, G10, G11, G14, G15, G16, G17, G18, G24 were grouped in cluster II and G1, G2, G3, G4, G6, G13, G20, G22 were grouped in cluster III. The dendrogram revealed that the landraces that derivatives of genetically similar type were clustered together. ("G" indicated the Boro rice genotypes). The three-

dimensional graphical views of PCA showed the spatial distribution of the considered genotypes along three principal axes (Fig. 2). The landraces Sona Biron (G9), Dol Boro (G11), Black Rice (G15), Boro Habj 1 (G17) were found far away from centroid of the cluster and the rest of the landraces were distributed more or less around the centroid (Fig. 2). The results indicated that the genotypes that were placed far away from the centroid were more genetically diverse. On the other hand, the genotypes that were placed near the centroid occupied more or less similar genetic background.

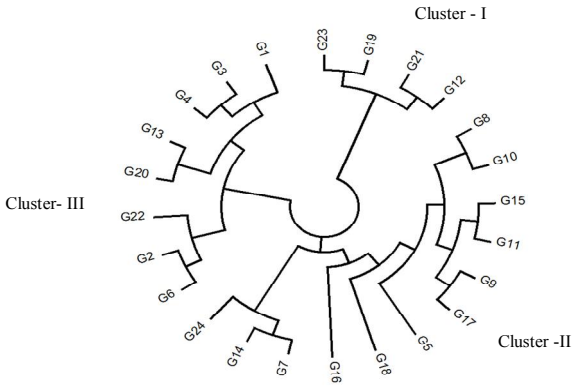

Fig. 1. UPGMA cluster analysis of 24 landraces of Boro rice based on SSR markers polymorphism using MEGA software. G1 (Kalo Boro), G2 (Asami Boro), G3 (Pabdafor), G4 (Amania), G5 (Khaiya Boro), G6 (Rata), G7 (Lakhai Boruni), G8 (Sada Boro), G9 (Sona Biron), G10 (Rajkumar), G11 (Dol Boro), G12 (Boro Dhan, Indian), G13 (Tepi Boro Dhan, Kalo), G14 (Dhali Boro 7/2), G15 (Black Rice), G16 (Dhali Boro 74/3), G17 (Boro Habj 1), G18 (Vawailia), G19 (Kali Boro), G20 (Jira), G21 (Dhali Boro 87/1), G22 (Dhali Boro 94), G23 (Dhali Boro 104/1), G24 (Dhali Boro 105/2).

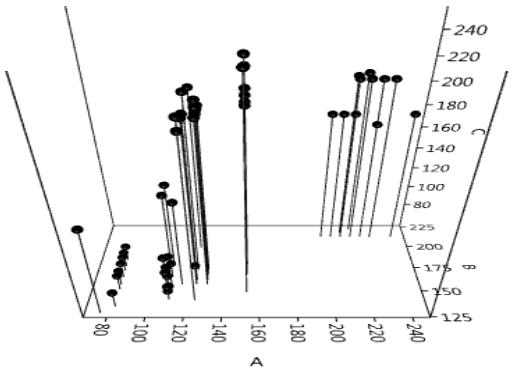

Fig. 2. Three-dimensional view of principal coordinate analysis (PCA) with 12 microsatellite markers over 24 landraces. G1 (Kalo Boro), G2 (Asami Boro), G3 (Pabdafor), G4 (Amania), G5 (KhaiyaBoro), G6 (Rata), G7 (Lakhai Boruni), G8 (Sada Boro), G9 (Sona Biron), G10 (Rajkumar), G11 (Dol Boro), G12 (Boro Dhan, Indian), G13 (Tepi Boro Dhan, Kalo), G14 (Dhali Boro 7/2), G15 (Black Rice), G16 (Dhali Boro 74/3), G17 (Boro Habj 1), G18 (Vawailia), G19 (Kali Boro), G20 (Jira), G21 (Dhali Boro 87/1), G22 (Dhali Boro 94), G23 (Dhali Boro 104/1), G24 (Dhali Boro 105/2)

Table 4. Summary of Nei's genetic distance (below diagonal) values among 24 Boro rice genotypes

Genotypes	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
G1	0.0000											
G2	0.7500	0.0000										
G3	0.8333	0.6667	0.0000									
G4	0.5833	0.6667	0.8333	0.0000								
G5	0.5000	0.6667	0.8333	0.2500	0.0000							
G6	0.4167	0.6667	0.7500	0.2500	0.3333	0.0000						
G7	0.9167	0.5000	0.4167	0.8333	0.8333	0.8333	0.0000					
G8	0.5833	0.7500	0.7500	0.3333	0.5000	0.3333	0.7500	0.0000				
G9	0.9167	0.5000	0.5000	0.8333	0.8333	0.8333	0.5000	0.7500	0.0000			
G10	0.6667	0.7500	0.6667	0.5833	0.5000	0.5833	0.5833	0.4167	0.7500	0.0000		
G11	0.5000	0.6667	0.7500	0.1667	0.2500	0.1667	0.8333	0.3333	0.7500	0.5833	0.0000	
G12	0.5000	0.7500	0.9167	0.3333	0.5000	0.5000	0.9167	0.4167	0.9167	0.5833	0.4167	0.0000
G13	0.5000	0.7500	0.8333	0.2500	0.1667	0.4167	0.8333	0.4167	0.8333	0.5000	0.3333	0.4167
G14	0.5833	0.6667	0.8333	0.0833	0.2500	0.2500	0.8333	0.4167	0.7500	0.5833	0.1667	0.4167
G15	0.5833	0.7500	0.8333	0.2500	0.2500	0.3333	0.8333	0.4167	0.8333	0.5000	0.2500	0.3333
G16	0.5000	0.5833	0.7500	0.1667	0.2500	0.1667	0.7500	0.4167	0.8333	0.5833	0.0833	0.4167
G17	0.5000	0.7500	0.8333	0.3333	0.3333	0.1667	0.8333	0.3333	0.8333	0.5833	0.3333	0.5000
G18	0.3333	0.6667	0.8333	0.4167	0.2500	0.5000	0.8333	0.5833	0.8333	0.5833	0.4167	0.4167
G19	0.4167	0.7500	0.8333	0.3333	0.1667	0.4167	0.8333	0.5000	0.8333	0.5000	0.3333	0.5000
G20	0.5833	0.6667	0.5000	0.4167	0.5000	0.4167	0.5833	0.5833	0.6667	0.5000	0.4167	0.6667
G21	0.5000	0.7500	0.8333	0.2500	0.4167	0.4167	0.7500	0.4167	0.8333	0.5833	0.3333	0.2500
G22	0.4167	0.6667	0.7500	0.2500	0.3333	0.0000	0.8333	0.3333	0.8333	0.5833	0.1667	0.5000
G23	0.9167	0.4167	0.7500	0.7500	0.7500	0.7500	0.4167	0.7500	0.5833	0.5833	0.7500	0.9167
G24	0.7500	0.6667	0.5000	0.7500	0.7500	0.7500	0.5833	0.8333	0.3333	0.7500	0.7500	0.8333

Legend: G1 (Kalo Boro), G2 (Asami Boro), G3 (Pabdafor), G4 (Amania), G5 (Khaiya Boro), G6 (Rata), G7 (Lakhai Boruni), G8 (Sada Boro), G9 (Sona Biron), G10 (Rajkumar), G11 (Dol Boro), G12 (Boro Dhan, Indian), G13 (Tepi Boro Dhan, Kalo), G14 (Dhali Boro 7/2), G15 (Black Rice), G16 (Dhali Boro 74/3), G17 (Boro Habj 1), G18 (Vawailia), G19 (Kali Boro), G20 (Jira), G21 (Dhali Boro 87/1), G22 (Dhali Boro 94), G23 (Dhali Boro 104/1), G24 (Dhali Boro 105/2).

Table 4. (Continued) Summary of Nei's genetic distance (below diagonal) values among 24 Boro rice genotypes

Genotypes	G13	G14	G15	G16	G17	G18	G19	G20	G21	G22	G23	G24
G1												
G2												
G3												
G4												
G5												
G6												
G7												
G8												
G9												
G10												
G11												
G12												
G13	0.0000											
G14	0.3333	0.0000										
G15	0.2500	0.2500	0.0000									
G16	0.3333	0.1667	0.2500	0.0000								
G17	0.3333	0.3333	0.1667	0.3333	0.0000							
G18	0.2500	0.4167	0.4167	0.4167	0.5000	0.0000						
G19	0.1667	0.3333	0.3333	0.3333	0.4167	0.0833	0.0000					
G20	0.5000	0.4167	0.5000	0.4167	0.5000	0.5833	0.5000	0.0000				
G21	0.3333	0.3333	0.2500	0.3333	0.4167	0.3333	0.3333	0.5833	0.0000			
G22	0.4167	0.2500	0.3333	0.1667	0.1667	0.5000	0.4167	0.4167	0.4167	0.0000		
G23	0.7500	0.7500	0.7500	0.6667	0.7500	0.8333	0.7500	0.5833	0.8333	0.7500	0.0000	
G24	0.7500	0.7500	0.7500	0.7500	0.7500	0.7500	0.7500	0.5833	0.7500	0.7500	0.7500	0.0000

Legend: G1 (Kalo Boro), G2 (Asami Boro), G3 (Pabdafor), G4 (Amania), G5 (Khaiya Boro), G6 (Rata), G7 (Lakhai Boruni), G8 (Sada Boro), G9 (Sona Biron), G10 (Rajkumar), G11 (Dol Boro), G12 (Boro Dhan, Indian), G13 (Tepi Boro Dhan, Kalo), G14 (Dhali Boro 7/2), G15 (Black Rice), G16 (Dhali Boro 74/3), G17 (Boro Habj 1), G18 (Vawailia), G19 (Kali Boro), G20 (Jira), G21 (Dhali Boro 87/1), G22 (Dhali Boro 94), G23 (Dhali Boro 104/1), G24 (Dhali Boro 105/2)

4. DISCUSSION

Assessment of genetic diversity is very important in molecular plant breeding program, so that it can be used to predict potential genetic gains from the respective genetic resources. In this study, each marker can distinguish most of the landraces and can assess the genetic diversity of numerous gene resources clearly. From the PCR result, it is found that the selected 12 microsatellite markers were resulted high polymorphism and had excellent repeatability. A total 54 alleles were detected, with an average number of alleles/locus of 4.5 and a range between 1 to 8 no. of alleles (Table 3). Our findings were comparable to the results obtained from Ahmed et al. [20]. In their experiment, the number of alleles detected by microsatellite markers varies from 3 to 14 with an average of 7.8 alleles per locus. A total 45 unique alleles were detected that could be used for identification, molecular characterization, and DNA fingerprinting of these landraces. Similar type of study was conducted by Rahman and his associates where they detected 57 unique alleles at 24 SSR loci [21]. Twenty two unique alleles were detected from fifty red rice germplasm [22] which also resembles our findings. They suggested that the diverse germplasm and polymorphic trait-linked SSR markers of red rice were suitable for the detection of economically desirable trait loci/genes for use in future molecular breeding programs. Mehrzad and co-workers [23] reported 5.86 polymorphic alleles per SSR locus in rice varieties which were mostly consistent with the present study. The frequency of the major allele ranges between 16.67% (RM206) to 95.83% (RM447), which is comparable with Thomson et al. [24] , Sajib et al. [25], Siddique et al. [26] and Becerra et al. [27].

The PIC values found in the present study varies significantly among the tested loci and similar results are observed in previous fingerprinting and diversity studies in rice. For instance, Rashid et al. [28], Rana et al. [29] and Donde et al. [30] have found PIC values 0.595 to 0.797 with an average of 0.6970, 0.492 to 0.745 with an average of 0.608 and 0 to 1.00 with an average of 0.66 per locus respectively in their different studies. RM206 was the best marker for identification and diversity estimation of the studied Boro rice landraces revealed by PIC values, however, a similar observation is reported by Siddique et al. [31] where RM163 was the best marker for Aman rice landraces. The observed PIC value indicates the presence of high genetic diversity among the studied rice accessions and due to their possessing of higher PIC values these SSR markers could be used for future rice development program through molecular characterization and genetic diversity analysis.

Our present study confirms that a high level of gene diversity was obtained which is higher than the findings of Aljumaili et al. [32], where gene diversity was reported within range from 0.05 (RM172) to 0.98 (RM1) with a mean of 0.36 and Singh et al**.** [33], where gene diversity ranged from 0.04 (HvSSR06-16) to 0.66 (HvSSR03-37) with an average of 0.33. Gene diversity computed that varies from 0.16 (RM17616) to 0.75 (RM287) with the average of 0.52 which is similar to our average value, evaluated by Nachimuthu et al. [34].

In this study, Unweighted Pair Group Method of Arithmetic Mean (UPGMA) clustering system generated three main distinct clusters of the considered landraces with six sub-groups. The landraces grouped in the same cluster due to lower genetic distance and the other landraces having more genetic dissimilarity grouped in another cluster due to higher genetic distance. It is clear from the experiment (from both cluster analysis and PCA analysis) that G12 (Boro Dhan, Indian), G21 (Dhali Boro 87/1), G19 (Kali Boro), G23 (Dhali Boro 104/1) were more different from most of the landraces. So they can be easily recommended for the use of future rice improvement program.

Analysis of genetic diversity of 31 Aus rice landraces [35] with 36 SSR markers where UPGMA-cluster-analysis based on genetic distance coefficients clearly separated all the landraces into five main distinct clusters. Islam et al. [36] also determined the grouping of rice restorer lines into five clusters, in another study.

5. CONCLUSION

The present study reveals a wide variation among the landraces. So, it can be concluded that SSR markers are the powerful tools to detect genetic variation and genetic relationship within and among different rice landraces. These markers are unmasking new genes for the improvement of crop varieties, assessment of genetic diversity, genome mapping, fingerprinting, determine the genetic structure, gene tagging and for marker-assisted selection (MAS). The database of DNA profiles evolved

through the selected microsatellite markers can be used for assessing the characteristics of rice genetic resources. The genetic resources under study have not been identified by the microsatellite markers yet, although there have been many recent reports of new SSR markers in other rice genotypes/landraces/varieties. The results revealed that molecular characterization and genetic diversity analysis of these Boro rice landraces can bring some useful implications in breeding as well as other research program concerning rice variety development, which will ultimately enrich the treasury of Boro rice in Bangladesh as well as other countries.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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