

Genetic Diversity and Population Structure of Boro Rice Landraces of Bangladesh

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Abstract

Molecular characterization, evaluation of genetic diversity and assessment of relationship by simple sequence repeat (SSR) markers of 48 Bangladeshi Boro rice landraces was performed. Among the total 58 SSR markers, 55 markers has been distributing over 12 rice chromosomes showed clear polymorphic band patterns, and they were selected for genetic relationship assessment. A total of 228 alleles were detected with an average of 4.15 alleles per locus. The average values of gene diversity and polymorphic information content (PIC) were 0.39 and 0.36, respectively. Primer RM206 had the highest PIC value (0.78) and the highest number of alleles (10). Therefore, RM206 was detected for the highest level of polymorphism and RM206 is supposed to be the best marker for characterizing the 48 Boro rice landraces. The genetic distance-based results in the unrooted neighbor-joining (NJ) tree revealed four (4) major clusters (I, II, III and IV) and a model-based population structure analysis generated two clusters (A and B). Both neighbors joining tree analysis and the population structure analysis method showed the tested landraces as highly diverse in structure. The two and three dimensional graphical views of Principal Coordinate Analysis (PCoA) revealed that the landraces Mi-Pajang, Gopal Beshi, Borail, Madhabsail, Boro (sungu) and Jala Boro were found far away and distributed around the centroid of the cluster. This rice collection and information gained in this study will be useful for future rice breeding program.

Keywords: Rice; Genetic diversity; Molecular characterization; Simple sequence repeat

Introduction

Rice has shaped the culture, diets and economic of thousands of millions of peoples. For more than half of the humanity “rice is life”. Bangladesh has abundant diversified rice landraces from time immemorial, since rice plays an important role in the livelihood, cultures and socio-economic aspects of the people, and is also the main cereal food in Bangladesh [1]. It has been cultivated in Asia since ancient times and for generations farmers have maintained thousands of different landraces [2]. Now, 90% of world rice is produced in Asia on an area of almost 150 million hectares. Rice accounts for 50% of agricultural income in Asia and supplies almost 80% of the region’s nutrition. In Bangladesh rice engages more than 70% of the rural population and is central to agriculture and the national economy [3]. Due to great significance and intimate association of rice in food security and local ways of life and culture, Asian farmers have selected and maintained a vast array of over thousands of years. Scientists estimate that more than 1,40,000 rice varieties have been developed/selected/isolated in Asia [4]. More than 1,32,000 rice accessions and wild relatives can be found in the world’s largest Genebank for rice at IRRI (International Rice Research Institute) located in the Philippines (<https://www.irri.org/international-rice-genebank>). Until now, Bangladesh Rice Research Institute (BRRI) has collected and preserved more than 8,000 varieties/landraces/cultivars/advanced lines/wild types from indigenous and exotic sources [4]. After establishment of BRRI, characterization or DNA fingerprinting has been done only for a small number of local landraces. Many countries in the world have characterized their indigenous different crop landraces at both molecular and phenotypic level. This has been done for keeping their crop identity and for searching new genes for further crop improvement. But information about the genetic diversity of local landraces as well as Boro rice is very limited. The needs for varietal improvement for such situations are very important.

Molecular markers are the powerful tools to detect genetic variation and genetic relationship within and among species. DNA markers are used for unmasking new genes and for the improvement of crop varieties [5]. The use of DNA markers has been suggested for precise and reliable characterization and discrimination of rice genotypes [6]. For genetic variability assessment, DNA markers are extensively used because they are not affected by environmental factors. Microsatellites (SSRs) are the marker of choice because of their advantages over other markers. These markers are polymorphic, abundant in eukaryotic organisms, and well distributed throughout the genome [7,8]. The SSRs are most suitable for rice because of their reproducibility, multiallelic nature, hypervariability, codominant inheritance, relative abundance, and genome-wide coverage [9]. In addition, SSRs often have flanking regions that are highly conserved in related species, which allows the use of the same primer pairs in related genomes [10,11]. The SSR markers are particularly suitable for evaluating genetic diversity and relationships among plant species, populations, or individuals [12, 13]; studying rice landraces for either conservation or utilization [14]; marker-assisted selection breeding [15,16]; cultivar identification; and hybrid purity analysis and gene mapping studies [17,18,19,20]. Selection of parents based on genetic divergence using SSR markers has been successfully utilized in multiple crop species [21, 6].

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Assessment of genetic diversity is very important in plant breeding or in biotechnology, if selection is the basis of improvement. For the assessment or analysis of genetic diversity, molecular markers are superior to morphological, pedigree, heterosis and biochemical data [22]. Genetic diversity is generally measured by genetic distance or genetic similarity, which imply that there are either differences or similarities at the genetic level of the plant [23]. Molecular marker based Genetic Diversity Analysis (MMGDA) also has potential for assessing changes in genetic diversity over time and space [24]. As the variation among the genotypes comes from the variations in DNA sequence, therefore, variations in DNA sequence are the basis of genetic diversity analysis [25]. Though, rice genome sequence is a valuable [26], most researchers are trying to identify particular segment of DNA or gene in a definite chromosome [25]. Molecular markers are the molecules that can trace a required gene in observed genotypes [27].

Information on the genetic diversity within and among closely related crop varieties is essential for a rational use of genetic resources and is of fundamental interest to plant breeders. It contributes to monitor landraces and can also be used to predict potential genetic gains [28]. Information regarding genetic variability at the molecular level could be used to help identify and develop genetically unique landraces that complements existing cultivars [29]. The objectives of this research were to assess the genetic variation and diversity of 48 Boro rice landraces, determine the genetic relationship among these landraces for breeding purposes and characterize these boro rice landraces.

Materials and Methods

Rice materials

In total, 48 Boro rice landraces of Bangladesh were studied (Table 1). Five gram seed from each of the entry was germinated and then sown in seedbed for growth and subsequent DNA extraction.

SSR markers

A total of 55 SSR markers (Table 2) were found polymorphic and used for molecular characterization and diversity analysis.

Genomic DNA extraction and amplification

Total genomic DNA was extracted from young leaves of three-week-old plants following the quick DNA extraction protocol of Ferdous et al. [30]. PCR analysis was performed in 10µl reaction sample containing 3µl of DNA template, 4.5 µl of GoTaq G2 Green Master Mix (Promega, USA), 1.5 µl of Nuclease-Free Water, 0.5 µl each of 10 µM forward and reverse primers using a GeneAtlas G (Astec, Japan) 96-well thermal cycler. The mixture was overlaid with 10 µl of mineral oil to prevent evaporation. 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. The PCR products were analyzed by electrophoresis on 8% polyacrylamide gel with a 1 Kb plus DNA ladder (Thermo Scientific, USA) using mini vertical polyacrylamide gels for high throughput manual genotyping (CBS Scientific Co. Inc., CA, USA). 2.5 µl of amplification products were resolved by running gel in 0.5X TBE buffer for 1.5-2.5 hrs depending upon the allele size at around 100volts and 500 mA current. The gels were stained in 5 µl SYBR Safe DNA gel stain (10,000X concentration in DMSO, USA) with 200 ml 0.5X TBE buffer for 15 min and exposed to UV light using a molecular imager gel documentation unit (XR System, Vitec Cambridge, France) for visualization. Microsatellite or simple sequence repeat (SSR) markers were used for molecular analysis [31,32]. We used fifty-five well-distributed SSRs for the diversity analysis; position (cM), repeat motifs, and chromosomal positions for the SSR markers can be found in the rice genome database (Gramene Portals, 2017). Most of these markers were obtained from a panel of fifty standard SSR markers, which has been proposed by CGIAR (Consultative Group for International Agricultural Research), for rice diversity analysis [33,34].

Table 1: List of 48 Boro rice landraces.

Sl. No	Landraces	Acc. no.	Location of collection	Sl. No	Landraces	Acc. no.	Location of collection
G 1	Mi-Pajang	149	Tangail	G 25	Boro Dhan	1808	Kishorganj
G 2	Dholi Boro	180	Tangail	G 26	Boro Jagli	1809	Kishorganj
G 3	Kumri Boro	257	Mymensingh	G 27	Jagli	1810	Kishorganj
G 4	Bairagi Sail	261	Mymensingh	G 28	Deshi Boro	1815	Kishorganj
G 5	Tepi Khorch	931	Sylhet	G 29	Boro Dhan	1816	Kishorganj
G 6	Pankaich	937	Sylhet	G 30	Boro (Sunga)	1861	Dinajpur
G 7	Boro Deshi	938	Sylhet	G 31	Jala Boro	1866	Dinajpur
G 8	Gopal Beshi	939	Sylhet	G 32	Kali Boro 2/2	2189	Gazipur
G 9	Borail	940	Sylhet	G 33	Kali Boro 4/1	2190	Gazipur
G 10	Boro 6/2	2206	Gazipur	G 34	Kali Boro 26	2191	Gazipur
G 11	Kali Boro	1049	Khulna	G 35	Kali Boro 41/1	2192	Gazipur
G 12	Sonar Geye	1050	Khulna	G 36	Kali Boro 48/1	2193	Gazipur
G 13	Joya Boro	1051	Khulna	G 37	Kali Boro 80/3	2194	Gazipur
G 14	Amboro2 (Golden)	1473	Dhaka	G 38	Kali Boro 80/5	2195	Gazipur
G 15	Batti Boro	1477	Dhaka	G 39	Kali Boro 109/4	2196	Gazipur
G 16	Madhabsail	1651	Dhaka	G 40	Kali Boro 138/2	2197	Gazipur
G 17	Jagli	1704	Faridpur	G 41	Kali Boro 139/2	2198	Gazipur
G 18	Jagli	1705	Faridpur	G 42	Kali Boro 200	2199	Gazipur
G 19	Local Boro	1753	Khulna	G 43	Kali Boro 208	2200	Gazipur
G 20	Saita	1794	Kishorganj	G 44	Kali Boro 259	2201	Gazipur
G 21	Dud Saita	1795	Kishorganj	G 45	Kali Boro 266	2202	Gazipur
G 22	Bogra Boro	1804	Kishorganj	G 46	Kali Boro 576	2203	Gazipur
G 23	Deshi Boro	1805	Kishorganj	G 47	Kali Boro 600	2204	Gazipur
G 24	Jagli (DeshiBoro)	1806	Kishorganj	G 48	Kali Boro 704	2205	Gazipur

Table 2: List of 55 SSR markers.

Sl. No.	Marker Name	Chro. No.	Forward primer (5'–3')	Reverse primer (5'–3')	Annealing Temp.
1	RM1	1	GCGAAAACACAATGCAAAAA	GCGTTGGTTGGACCTGAC	55
2	RM5	1	TGCAACTTCTAGCTGCTCGA	GCATCCGATCTTGATGGG	55
3	RM212	1	CCACTTTCAGCTACTACCAG	CACCCATTTG	55
4	RM472	1	CCATGGCCTGAGAGAGAGAG	AGCTAAATGGCCATACGGTG	55
5	RM283	1	GTCTACATGTACCCTTGTGGG	CGGCATGAGAGTCTGTGATG	55
6	RM495	2	AATCCAAGGTGCAGAGATGG	CAACGATGACGAACACAACC	55
7	RM307	2	GTACTACCGACCTACCGTTCAC	CTGCTATGCATGAATGCTC	55
8	RM145	2	C CGGTAGGCGCCCTGCAGTTTC	CAAGGACCCCATCCTCGGCGTC	56
9	RM208	2	TCTGCAAGCCTTGTCTGATG	TAAGTCGATCATTGTGTGGACC	55
10	RM213	2	ATCTGTTTGCAGGGGACAAG	AGGCTAGACGATGTCGTGA	55
11	RM262	2	CATTCCGTCTCGGCTCAACT	CAGAGCAAGGTGGCTTGC	55
12	RM263	2	GCGCTGGTGAAAATGAG	GGCATCCCTCTTTGATTCCTC	55
13	RM452	2	CTGATCGAGAGCGTTAAGGG	GGGATCAAACCACGTTTCTG	55
14	RM207	2	CCATTCGTGAGAAGATCTGA	CACCTCATCCTCGTAACGCC	55
15	RM411	3	ACACCAACTCTTGCCCTGCAT	TGAAGCAAAAACATGGCTAGG	55
16	RM520	3	AGGAGCAAGAAAAGTCCCC	GCCAATGTGTGACGCAATAG	55
17	RM3646	3	ACTAGAGCACCCCTCGCTGAG	CTCAGCCACCCCATCAAC	55
18	RM338	3	CACAGGAGCAGGAGAAGAGC	GGCAAACCGATCACTCAGTC	55
19	RM16	3	CGCTAGGGCAGCATCTAAA	AACACAGCAGGTACGCGC	55
20	RM252	4	TTCGCTGACGTGATAGGTTG	ATGACTTGATCCCGAGAACG	55
21	RM273	4	GAAGCCGTGCGTGAAGTTACC	GTTTCCTACCTGATCGCGAC	55
22	RM303	4	GCATGGCCAAATATTAAGG	GGTTGGAATAGAAGTTCGGT	55
23	RM334	5	GTTTCAGTGTTCAGTGCCACC	GACTTTGATCTTTGGTGGACG	55
24	RM26	5	GAGTCGACGACGGCAGCA	CTGCGAGCGACGGTAAACA	55
25	RM289	5	TTCCATGGCACACAAGCC	CTGTGCACGAACCTCCAAG	55
26	RM413	5	GCGGATTCTTGGATGAAGAG	TCCCCACCAATCTTGTCTTC	55
27	RM190	6	C TTTGTCTATCTCAAGACAC	TTGAGATGTTCTTCCTGATG	55
28	RM170	6	TCGCGCTTCTTCTCGTCGACG	CCCCTTGCAGAGGAAGCAGCC	55
29	RM125	6	ATCAGCAGCCATGGCAGCGACC	AGGGGATCATGTGCCGAAGGCC	55
30	RM133	6	TTGGATTGTTTTGCTGGCTCGC	GGAACACGGGGTCGGAAGCGAC	61
31	RM253	6	TCCTTCAAGAGTGCAAAACC	GCATTGTATGTCGAAGCC	55
32	RM510	7	AACCGGATTAGTTTCTCGCC	TGAGGACGACGAGCAGATTC	55
33	RM320	7	CAACGTGATCGAGGATAGATC	GGATTGCTTACCACAGCTC	55
34	RM455	7	AACAACCACCACCTGTCTC	AGAAGGAAAAGGGCTCGATC	55
35	RM223	8	GAGTGAGCTTGGGCTGAAAC	GAAGGCAAGTCTTGGCACTG	55
36	RM342	8	CCATCCTCCTACTTCAATGAAG	ACTATGCAGTGGTGCACCC	55
37	RM515	8	TAGGACGACCAAAGGGTGAG	TGGCTGCTCTCTCTCTCTC	55
38	RM447	8	CCCTTGTGCTGTCTCTCTCTC	ACGGGCTTCTCTCTCTCTC	55
39	RM201	9	CTCGTTTATTACCTACAGTACC	CTACCTCCTTCTAGACCGATA	55
40	RM205	9	CTGGTTCTGTATGGGAGCAG	CTGGCCCTTACGTTTACAGTG	55
41	RM316	9	CTAGTTGGGCATACGATGGC	ACGCTTATATGTTACGTCAAC	55
42	RM228	10	CTGGCCATTAGTCTTGG	GCTTGGCGCTCTGCTTAC	55
43	RM304	10	TCAAACCGGCACATATAAGAC	GATAGGGAGCTGAAGGAGATG	55
44	RM591	10	CTAGCTAGCTGGCACCAGTG	TGGAGTCGTGTTGTAGTCG	55
45	RM484	10	TCTCCCTCCTCACCATTGTC	TGCTGCCCTCTCTCTCTCTC	55
46	RM536	11	TCTCTCCTCTTGTGGCTC	ACACACCAACACGACCACAC	55
47	RM202	11	CAGATTGGAGATGAAGTCTCC	CCAGCAAGCATGTCAATGTA	55
48	RM206	11	CCCATGCGTTTAACTATTCT	CGTCCATCGATCCGTATGG	55
49	RM209	11	ATATGAGTTGCTGTGCTGCG	CAACTGCATCCTCCCCTCC	55
50	RM144	11	TGCCCTGGCCAAATTTGATCC	GCTAGAGGAGATCAGATGGTAGGCATG	55
51	RM224	11	ATCGATCGATCTTACGAGG	TGCTATAAAAGGCATTCGGG	55
52	RM19	12	CAAAAACAGAGCAGATGAC	CTCAAGATGGACGCCAAGA	55
53	RM277	12	CGGTCAAATCATCACCTGAC	CAAGGCTTGAAGGGAAG	55
54	RM1337	12	GTGCAATGCTGAGGAGTATC	CTGAGAATCTGGAGTGCTTG	55
55	RM12	12	TGCCCTGTTATTTCTCTCTC	GGTGATCCTTCCCATTCA	55

Data analysis

The band-size for each of the markers was scored using the AlphaEaseFC 4.0 software. Using PowerMarker version 3.25 [35], summary statistics included the following: the number of alleles, the major allele size and its frequency, gene diversity, and the polymorphism information content (PIC) value. For the unrooted phylogenetic tree, the genetic distance was calculated using MEGA 6 based on Nei's unbiased pairwise [36,37]. Binary form for allele frequency was prepared using PowerMarker software and used for dendrogram construction by NTSYS-pc software [38]. The unweighted pair grouping method using arithmetic average (UPGMA) was used to determine a similarity matrix following the Dice coefficient with the SAHN subprogram. Population STRUCTURE for landraces was determined using STRUCTURE, (version 2.3.4) [39, 40]. The number of clusters (K) investigated, in this study, ranged from one to fifteen, with five replications for analysis of each K value. The model following admixture and correlated allele frequency with a 5,000 burn period and a run length of 50,000 were used for conducting model-based structure analysis. Output of analysis was collected using the STRUCTURE harvester [41] and identified 4 as the best K value based on the LnP(D) and Evanno's ΔK [42]. Principal components analysis (PCA) analysis was conducted also using the NTSYS-pc software.

Results and Discussion

SSR marker-based diversity and molecular characterization

All the 48 Boro rice landraces distributed overall 12 chromosomes of rice were genotyped with 55 simple sequence repeat (SSR) markers. All 55 markers exhibiting polymorphism.

A total of 228 alleles were identified at 55 SSR markers over 48 Boro landraces (Table 2). RM484 (296bp) produced the maximum amplicon size and RM289 (88bp) was the minimum. In the case of RM472 (286-306bp), a maximum range of band sizes was found and succeeded by RM484 (289-296bp) and RM591 (247-279 bp), respectively. The average number of alleles per locus was 4.15, ranged from 2 (RM133, RM145, RM212, RM316, RM320, RM338, RM411, RM452, RM455 and RM484) to 10 (RM206). The polymorphism information content (PIC) for the SSR loci ranged from 0.04 (RM455) to 0.78 (RM206) with an average of 0.36. Marker RM206 had the highest PIC value (0.78) and the highest number of alleles (10). SSRs which have higher PIC values have a higher number of alleles. Lower PIC value shows that the landraces under study are closely allied, whereas the higher value of PIC stipulates the higher array of materials which is the utmost need

for parents selection for hybridization as well as for the new improved variety development, accordingly. Therefore, RM206 was detected as the highest level of polymorphism and RM206 is supposed to be the best marker for characterizing the 48 Boro rice landraces. The gene diversity ranged from 0.04 to 0.81, with an average of 0.39. The frequency of the most common allele at each locus ranged from 29.17% (RM206) to 97.92% (RM455). On average, 73.83% of the 48 rice landraces shared a common major allele at any given locus. The DNA profiles of 48 Boro rice landraces with RM536 are shown in (Figure 1).

In recent past, molecular characterization, genetic diversity and population structure of Bangladeshi rice landraces have been studied by using molecular (SSR) markers [1, 36, 34, 43].

From our present study, the genetic diversity is similar to earlier studies [29], they identified 4.18 alleles per locus and an average PIC value of 0.488 among 21 rice landraces (Table 3). Also, the average PIC value 0.44 was observed among 43 Thai and 57 IRRI landraces of rice by Chakhonkaen et al. [45]. But, Ahmed et al. [1] found 350 alleles from similarly named rice landraces using 45 SSR markers and several alleles per locus ranged from 3 to 14 with an average of 7.8 which was higher than our present study. On the other hand, a lower genetic diversity was disclosed among 50 Bangladeshi red rice varieties were collected from different agro-climatic regions of Bangladesh having 3.24 alleles per locus with mean PIC value of 0.32, also reported by Islam et al. [34]. Also, a marginally lower genetic diversity was disclosed among 28 restorer lines of hybrid rice with an average of 2.67 alleles per locus and an average PIC value of 0.29 [46].

Legend: 1. Mi-Pajang, 2. Dholi Boro, 3. Kumri Boro, 4. Bairagi Sail 5. Tepi Khorch 6. Pankaich, 7. BoroDeshi, 8. Gopal Beshi, 9. Borail, 10. Boro 6/2, 11. Kali Boro, 12. Sonar Geye, 13. Joya Boro, 14. Amboro2 (Golden), 15. Batti Boro, 16. Madhabsail, 17. Jagli, 18. Jagli, 19. Local Boro, 20. Saita, 21. Dud Saita, 22. Bogra Boro, 23. Deshi Boro, 24. Jagli (DeshiBoro), 25. Boro Dhan, 26. Boro Jagli, 27. Jagli, 28. Deshi Boro, 29. BoroDhan, 30. Boro (Sunga), 31. Jala Boro, 32. Kali Boro 2/2, 33. Kali Boro 4/1, 34. Kali Boro 26, 35. Kali Boro 41/1, 36. Kali Boro 48/1, 37. Kali Boro 80/3, 38. Kali Boro 80/5, 39. Kali Boro 109/4, 40. Kali Boro 138/2, 41. Kali Boro 139/2, 42. Kali Boro 200, 43. Kali Boro 208, 44. Kali Boro 259, 45. Kali Boro 266, 46. Kali Boro 576, 47. Kali Boro 600, 48. Kali Boro 704, L= 1Kb plus Ladder.

Unique Alleles

A total of 39 unique alleles were detected at 25 microsatellite loci. Nineteen (19) of the rice landraces had unique alleles for at least one microsatellite locus. Notably, seven (7) SSR markers—RM144

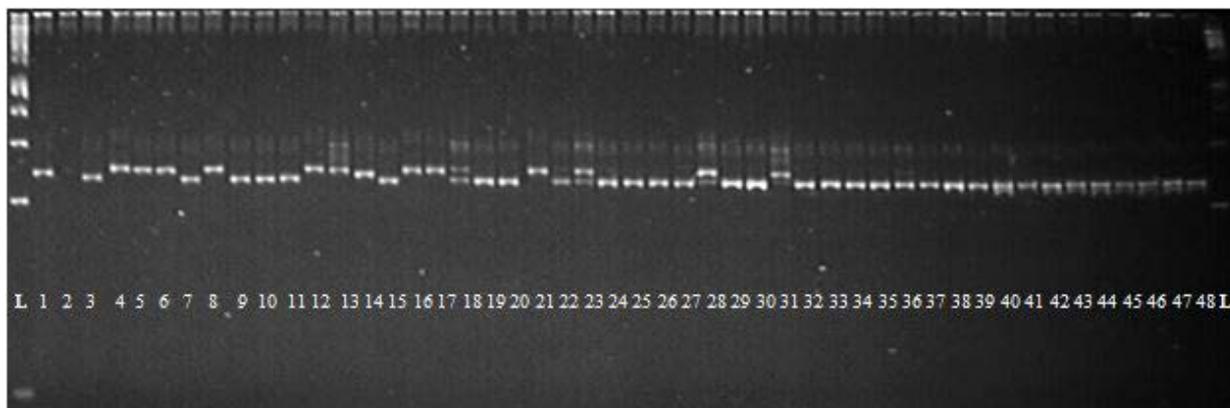


Figure 1: DNA profile of 48 Boro rice landraces with SSR marker RM536.

Table 3: Number of alleles, allele size range, frequency, gene diversity and polymorphism information content (PIC) among 48 Boro rice landraces against 55 microsatellite markers.

SL. No.	Marker	Chromosome No.	Position (cM)	Motif*	Allele No.	Unique allele	Size range (bp)	Size (bp)	Frequency (%)	Gene diversity	PIC
1	RM1	1	29.7	(GA)26	4	-	80-112	112	54.17	0.36	0.34
2	RM5	1	94.9	GA)14	4	-	111-127	111	43.75	0.63	0.56
3	RM16	3	131.5	(TCG)5(GA)16	4	1	184-240	184	85.42	0.26	0.25
4	RM12	12	109.1	(GA)21	6	2	150-215	150	72.92	0.45	0.43
5	RM19	12	20.9	(ATC)10	7	4	204-239	239	77.08	0.39	0.38
6	RM26	5	118.8	(GA)15	3	-	105-119	119	89.58	0.19	0.18
7	RM133	6	0	(CT)8	2	-	225-234	234	93.75	0.12	0.11
8	RM144	11	123.2	(ATT)11	5	1	220-247	247	54.17	0.63	0.58
9	RM145	2	49.8	-	2	-	181-192	181	95.83	0.08	0.08
10	RM170	6	2.2-7.4	(CCT)7	5	-	98-127	127	60.42	0.58	0.54
11	RM190	6	7.4	(CT)11	3	-	107-127	127	85.42	0.26	0.24
12	RM201	9	81.2	(CT)17	5	1	161-188	161	68.75	0.50	0.47
13	RM202	11	54	(CT)30	4	-	165-186	186	64.58	0.54	0.50
14	RM205	9	114.7	(CT)25	5	1	117-156	117	64.58	0.54	0.51
15	RM206	11	102.9	(CT)21	10	4	135-198	135	29.17	0.81	0.78
16	RM207	2	191.2	(CT)25	4	1	72-148	148	79.17	0.36	0.33
17	RM208	2	186.4	(CT)17	3	-	170-187	170	72.92	0.43	0.38
18	RM209	11	73.9	(CT)18	7	2	125-170	125	60.42	0.60	0.58
19	RM212	1	148.7	(CT)24	2	-	125-132	132	87.50	0.22	0.19
20	RM213	2	186.4	(CT)17	3	-	143-166	143	85.42	0.26	0.24
21	RM223	8	80.5	(CT)25	4	1	144-172	172	72.92	0.44	0.40
22	RM224	11	120.1	(AAG)8(AG)13	6	2	138-172	172	56.25	0.60	0.55
23	RM228	10	130.3	(CA)6(GA)36	4	-	108-145	145	77.08	0.38	0.36
24	RM252	4	99	(CT)19	5	1	206-230	206	75.00	0.42	0.39
25	RM253	6	37	(GA)25	4	1	125-160	160	72.92	0.44	0.41
26	RM262	2	103.3	(CT)16	5	2	145-167	145	70.83	0.47	0.44
27	RM263	2	127.5	(CT)34	4	-	192-217	199	85.42	0.26	0.25
28	RM273	4	94.4	(GA)11	3	-	127-222	222	89.58	0.19	0.18
29	RM277	12	57.2	(GA)11	3	1	124-138	124	89.58	0.19	0.18
30	RM283	1	31.4	(GA)18	3	-	159-174	159	66.67	0.49	0.43
31	RM289	5	56.7	G11(GA)16	5	-	88-146	88	39.58	0.70	0.65
32	RM304	10	73	(GT)2(AT)10(GT)33	4	-	157-174	157	45.83	0.63	0.57
33	RM316	9	1.8	(GT)8(TG)9(TTTG)4(TG)4	2	-	205-212	212	89.58	0.19	0.17
34	RM320	7	62.4	(AT)11GTAT (GT)13	2	-	225-233	233	95.83	0.08	0.08
35	RM338	3	108.4	(CTT)6	2	-	191-198	191	95.83	0.08	0.08
36	RM342	8	78.4	(CAT)12	5	3	130-169	130	81.25	0.32	0.30
37	RM411	3	127.9	(GTT)7	2	-	108-115	108	85.42	0.25	0.22
38	RM413	5	26.7	(AG)11	4	-	170-198	170	58.33	0.59	0.54
39	RM447	8	124.6	(CTT)8	5	2	106-145	106	83.33	0.30	0.28
40	RM452	2	58.4	(GTC)9	2	-	197-206	206	79.17	0.33	0.28
41	RM455	7	65.7	(TTCT)5	2	1	125-131	131	97.92	0.04	0.04
42	RM484	10	97.3	(AT)9	2	-	289-296	296	91.67	0.15	0.14
43	RM495	2	2.8	(CTG)7	3	-	159-173	159	83.33	0.29	0.26
44	RM515	8	80.5	(GA)11	6	1	211-252	211	72.92	0.45	0.43
45	RM520	3	191.6	(AG)10	3	-	244-264	244	70.83	0.45	0.40
46	RM536	11	55.1	(CT)16	3	-	236-249	249	72.92	0.41	0.36
47	RM591	10	118.3	(AC)10	5	1	247-279	247	62.50	0.56	0.53
48	RM1337	12	57.2	(GA)11	5	-	194-225	194	75.00	0.42	0.39
49	RM3646	3	6.32	(GA)14	3	-	145-158	145	85.42	0.26	0.24
50	RM303	4	116.9	[AC(AT)2]10]9 (GT)7(ATGT)6]	8	1	107-198	198	54.17	0.67	0.64
51	RM307	4	191.2	(CT)25	7	2	125-264	125	77.08	0.40	0.38
52	RM334	5	141.8	(CTT)48	7	1	156-218	156	45.83	0.72	0.68
53	RM472	1	171.6	(GA)21	3	-	286-306	286	58.33	0.57	0.50
54	RM125	7	24.8	(GCT)8	4	1	127-152	127	85.42	0.26	0.25
55	RM510	6	20.8	(GA)15	3	1	113-127	127	91.67	0.16	0.15
	Max.				10.00			296	97.92	0.81	0.78
	Min.				2.00			88	29.17	0.04	0.04
	Total				228.00	39			4060.42	21.35	19.82
	Mean				4.15				73.83	0.39	0.36

(chromosome 11, 220bp), RM205 (chromosome 9, 117bp), RM206 (chromosome 11, 152bp), RM277 (chromosome 12, 138bp), RM342 (chromosome 8, 152bp), RM515 (chromosome 8, 211bp) and RM307 (chromosome 4, 125bp) amplified specific alleles only in a slender grain rice landrace Jala Boro (Acc. No-1866). Similarly, six (6) SSR markers—RM19 (chromosome 12, 239bp), RM224 (chromosome 11, 138bp), RM262 (chromosome 2, 167bp), RM455 (chromosome 7, 125bp), RM125 (chromosome 7, 152bp) and RM510 (chromosome 6, 113bp) amplified specific alleles only in rice landrace Mi-Pajang (Acc. No-149). Also, Tepi Khorch (Acc. No-931) showed unique alleles in 4 markers and Bairagi Sail (Acc. No-261), Pankaich (Acc. No-937) and Joya Boro (Acc. No-1051) showed unique alleles in 3 markers (Table 4).

Unique alleles are precious as they can be effectively indicator of particular landraces and also for a breeding purpose. Generally, the higher number of unique alleles in landraces specifies as a source of novel alleles. The unique alleles for molecular characterization were also detected by Siwach et al. [47], Das et al. [48] and Islam et al. [34]. Siwach et al. [47] identified seven SSR markers (RM1, RM21, RM38,

RM170, RM210, RM226, and RM229) that showed unique alleles which could distinguish basmati rice varieties from non-basmati rice varieties. Also, Saini et al. [49] noticed 58 unique alleles among Basmati and non-Basmati rice varieties.

Genetic distance-based analysis

The genetic distance-based results seen in the unrooted neighbor-joining (NJ) tree revealed that the 48 Boro rice landraces were grouped into four major clusters (Figure 2). The highest numbers of landraces (37) were found in cluster I followed by clusters II (4), III (4) and the lowest in cluster IV (3).

Genetic distance is useful for assessing the diversity between species and populations within a species. The highest number of germplasm (37) was found in cluster I. Again, cluster I was further divided into 2 sub clusters (A and B). Sub cluster-A, contains 30 landraces and Sub cluster-B consisted of 7 landraces. Cluster II contains 4 landraces (Local Boro, Kali Boro 2/2, Kali Boro 4/1 and Kali Boro266) and cluster III also contains 4 boro landraces (Kali Boro 26, Kali Boro 80/5, Kali

Table 4: List of identified unique alleles along with markers for 19 Boro rice landraces.

SL. No.	Marker	Chromosome No.	Unique Allele (bp)	Landraces
1	RM16	3	184	G 30 (Boro (sungai))
2	RM12	12	183	G 6(Pankaich)
3	RM12	12	208	G 15(Batti Boro)
4	RM19	12	204	G 9 (Borail)
5	RM19	12	216	G 4 (Bairagi Sail)
6	RM19	12	227	G 2 (Dholi Boro)
7	RM19	12	239	G 1 (Mi-Pajang)
8	RM144	11	220	G 31(Jala Boro)
9	RM201	9	188	G 40 (Kali Boro 138/2)
10	RM205	9	117	G 31 (Jala Boro)
11	RM206	11	152	G 31 (Jala Boro)
12	RM206	11	171	G 32 (Kali Boro 2/2)
13	RM206	11	192	G 28 (Desi Boro)
14	RM206	11	198	G 7 (Boro Deshi)
15	RM207	2	148	G 5 (Tepi Khorch)
16	RM209	11	134	G 13 (Joya Boro)
17	RM209	11	176	G 4 (Bairagi Sail)
18	RM223	8	172	G 5 (Tepi Khorch)
19	RM224	11	138	G 1 (Mi-Pajang)
20	RM224	11	143	G 5 (Tepi Khorch)
21	RM252	4	230	G 16 (Madhabsail)
22	RM253	6	160	G 5 (Tepi Khorch)
23	RM262	2	161	G 13 (Joya Boro)
24	RM262	2	167	G 1 (Mi-Pajang)
25	RM277	12	138	G 31 (Jala Boro)
26	RM342	8	146	G 14 (Amboro 2 (golden))
27	RM342	8	152	G 31 (Jala Boro)
28	RM342	8	169	G 6 (Pankaich)
29	RM447	8	121	G 13 (Joya Boro)
30	RM447	8	145	G 18 (Jagli)
31	RM455	7	125	G 1 (Mi-Pajang)
32	RM515	8	211	G 31 (Jala Boro)
33	RM591	10	266	G 4 (Bairagi Sail)
34	RM303	4	107	G 21 (Dud Saita)
35	RM307	4	125	G 31 (Jala Boro)
36	RM307	4	147	G 6 (Pankaich)
37	RM334	5	172	G 12 (Sonar Geye)
38	RM125	7	152	G 1 (Mi-Pajang)
39	RM510	6	113	G 1 (Mi-Pajang)

Boro 259 and Kali Boro 576). The lowest number (3) of boro rice landraces was found in cluster IV (Kali Boro41/1, Kali Boro 48/1 and Kali Boro 80/3). In previous studies, Islam et al. [34] and Islam et al. [43] both found three (3) major clusters and few sub-clusters through the unrooted neighbor-joining (NJ) tree among 50 Bangladeshi red rice varieties and 113 aromatic rice landraces, respectively.

Population structure based on Model

Model-based program STRUCTURE 2.3.4 was used to find out

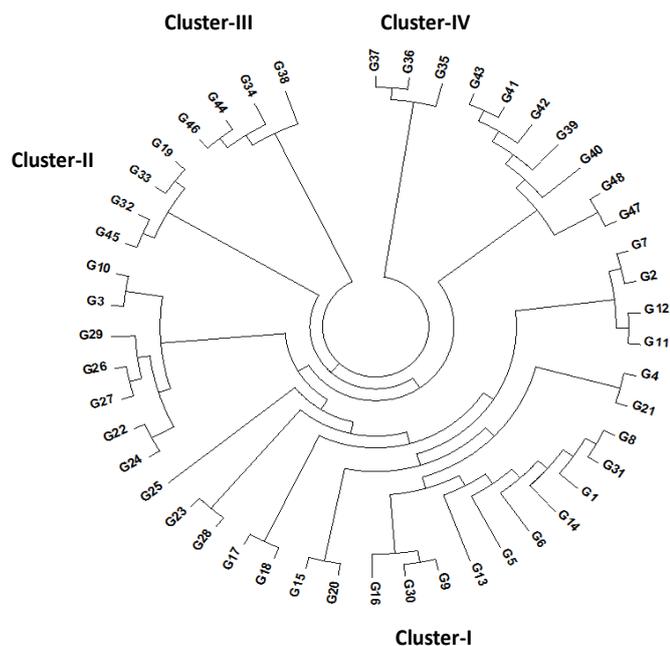


Figure 2: An unrooted neighbor-joining tree showing the genetic relationships among 48 Boro rice landraces.

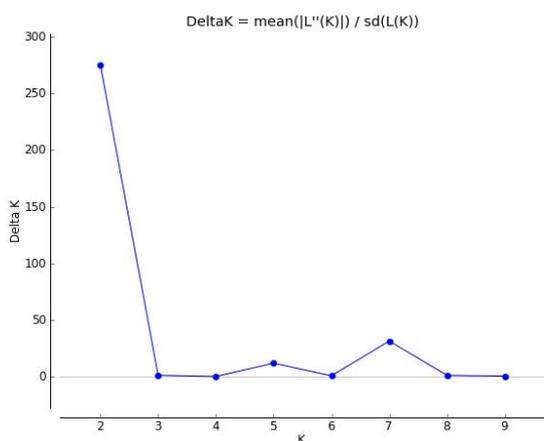


Figure 3: Estimation of population using LnP(D) derived ΔK for K from 1 to 10.

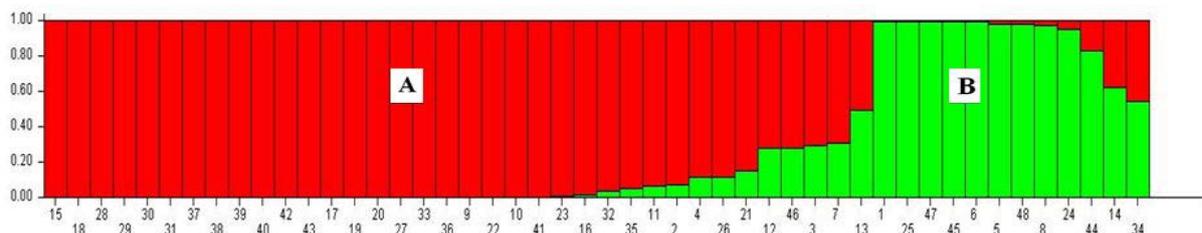


Figure 4: Assignment of 48 boro rice landraces into two populations (A and B) using STRUCTURE 2.3.4 software.

the genetic relationship among individual Boro rice landraces. By the admixture model, simulations were executed with K, range from 2 to 10 with 5 repetitions employing 48 landraces and the population structure analysis declared the log-likelihood value (ΔK) maximized to the highest value of at $K=2$ (Figure 3), demonstrating a sharp peak expressing the classification of entire landraces into two specific sub-groups, here denoted as Group A and Group B, respectively. To find out the number of pure and admixed individuals, populations were studied. The population Group A (red colour, Figure 4) and Group B (green colour, Figure 4) representing 75% (36) and 25% (12) of Boro landraces used in structure analysis, respectively. Overall, 11 (22.92%) admixed landraces were found at $K=2$. It June be noted that Group A had 36 Boro rice landraces with 28 pure and 08 admixed landraces and Group B had 12 boro rice landraces with 09 pure and 03 admixed landraces. A similar population structure of two groups was observed in previous research by Pathaichindachote et al. [50] in a collection of 167 Thai and exotic rice accessions in Thailand. Islam et al. [43] have successfully classified three groups as indica aromatic rice landraces in Bangladesh and again, Islam et al. [34] observed four groups in a collection of 50 red rice landraces in Bangladesh, which were higher than our present study. The population structure analysis of different rice diversity panels has indicated different numbers of sub-groups, from 2 to 8 [48, 51, 52].

Population structure analysis from the SSR markers grouped all the landraces into 2 groups. Besides, four groups were also constructed using SSR markers data clustering analysis. So, the most dissimilar landraces observed from this study can be used in rice breeding program. Moreover, from this study, QTLs/genes mapping can be assembled by utilize the diverse landraces and highly polymorphic SSR markers that have been determined for different physicochemical quality characters.

Principal Coordinate Analysis

The Principal Coordinate Analysis (PCoA) displayed two and three dimensional graphical views of the spatial distribution of the landraces along the two principal axes (Figures 5 and 6). The landrace namely Mi-Pajang (G 1, Acc. 149), Gopal Beshi (G 8, Acc. 939), Borail (G 9, Acc. 940), Madhabsail (G 16, Acc. 1651), Boro (sunga) (G 30, Acc. 1861) and Jala Boro (G 31, Acc. 1866) were found far away from the centroid of the cluster and the rest of the genotypes were located more or less around the centroid. The present study showed that the landraces that were located far away from the centroid were more genetically diverse, while the genotypes that were sited close to the centroid designated more or less similar genetic background. Comparable results were also stated by other authors [4, 53, 54].

Conclusion

The present study was conducted to make DNA fingerprinting and to assess the genetic relationship among these landraces using SSR markers and microsatellite markers are considered appropriate for

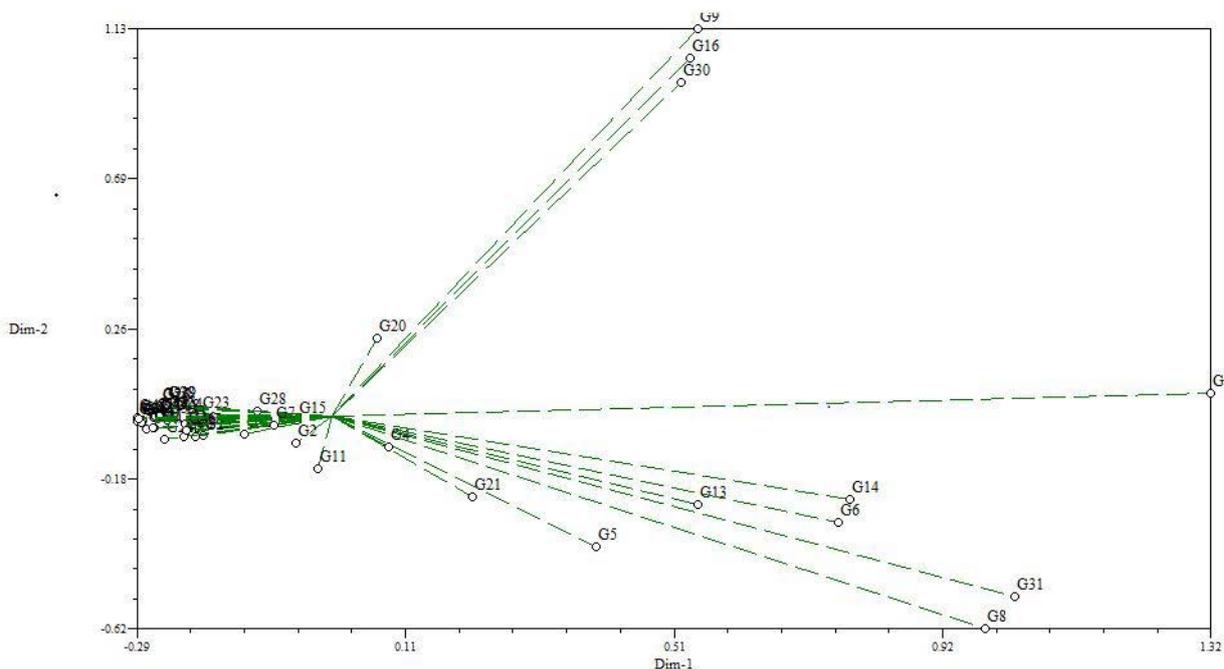


Figure 5: Two-dimensional view of principal coordinate analysis (PCoA) with 55 micro-satellite markers over 48 Boro rice landraces.

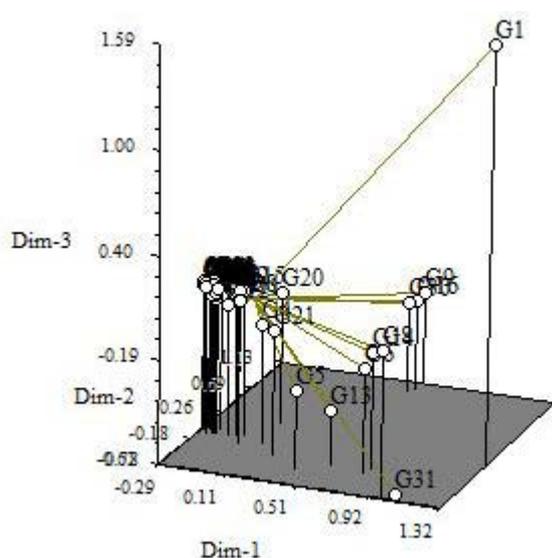


Figure 6: Three-dimensional view of principal coordinate analysis (PCoA) with 55 micro-satellite markers over 48 Boro rice landraces.

variety identification because of their ability to detect large numbers of distinct alleles frequently, exactly and efficiently. The study also confirmed the value of microsatellite loci for genetic diversity studies of rice landraces found in earlier studies. The diversity and the unique features of the Bangladeshi rice landraces collections examined in this study could be quite relevant to both domestic and global rice development.

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Conflict of Interest

The authors declare that they are no conflict of interest.

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