SAARC J. Agric., 21(2): 1-14 (2023) *DOI: https://doi.org/10.3329/sja.v21i2.68567*

MOLECULAR DIVERSITY ANALYSIS OF SOME SELECTED BRRI DEVELOPED RICE VARIETIES USING SSR MARKERS

M.Z. Islam[1*], M.A. Siddique[2], M.F.R.K. Prince[3]
E.S.M.H. Rashid[2] and M. Khalequzzaman[4]

[1]Genetic Resources and Seed Division, Bangladesh Rice Research Institute, Regional Station, Gopalganj
[2]Genetic Resources and Seed Division, Bangladesh Rice Research Institute, Gazipur
[3]NATP-2, Genetic Resources and Seed Division, Bangladesh Rice Research Institute, Gazipur
[4]Director (Research), Bangladesh Rice Research Institute, Gazipur

ABSTRACT

Molecular characterization of 26 modern rice varieties of Bangladesh was performed using 52 simple sequence repeat markers (SSRs) to estimate the genetic diversity and to reveal genetic relationships among rice cultivars. A total of 156 alleles were detected where the number of alleles per locus generated by each marker varied from 2 to 7, with an average of 3. The band size for a given microsatellite locus ranged from 79 to 278 bp. The polymorphism information content (PIC) for the SSR loci ranged from 0.08 to 0.79, with an average of 0.35. RM566 was the best marker for identification and variability assessment of varieties as revealed by PIC values. A UPGMA dendrogram generated using the NTSYS-pc revealed four clusters with a similarity coefficient of 0.55, whereas phylogenetic cluster analysis of the SSR data based on Nei-genetic distance divided the varieties into three groups. Two- and three-dimensional graphical views of principal coordinate analysis showed the spatial distribution of the varieties and revealed that BRRI dhan56, BRRI dhan51, BRRI dhan52, BRRI dhan61, BRRI dhan62 and BRRI dhan66 were found far away from the centroid of the cluster. The findings of this study are helpful for varietal identification, background selection during backcross breeding and for selecting the suitable genetically diverse parents for crossing programs.

Keywords: Cluster analysis, molecular diversity, rice, SSR markers, varietal identification

INTRODUCTION

Rice is the first major grain crop and is the main source of energy of Bangladeshi people. It contributes about 46% of the protein and 62% of the calories to the average

* Corresponding author: zahid.grs@gmail.com

Received: 02.09.2023 *Accepted: 20.10.2023*

person's daily diet (HIES, 2010). Additionally, it guarantees the nation's political stability and gives its citizens a sense of food security.

Bangladesh Rice Research Institute (BRRI) has released 113 high yielding rice varieties including eight hybrid varieties that have high yield potential, several biotic and abiotic stress tolerances and premium quality for varied rice ecosystems around the country (http://www.brri.gov.bd). These varieties are cultivated in about 80 percent of the total rice areas and contribute almost 91 percent of total rice production of the country (BRRI, 2022). As a result, the gap between technology and farmer has been bridged, allowing Bangladesh to finally become rice self-sufficient.

In Bangladesh, variety identification and DNA fingerprinting of rice through SSR marker have been started few years back but they are very discrete and fragmentary. DNA fingerprinting at the molecular level is crucial to safeguarding rice cultivars against biopiracy. Till 2013, Biotechnology division of BRRI accomplished DNA fingerprinting of BR1 to BRRI dhan-50 rice varieties. In 2016/17, the Genetic Resources and Seed Division (GRSD) conducted DNA fingerprinting ranging from BRRI dhan-51 to BRRI dhan-73, and three hybrid varieties. However, assessment of genetic diversity and molecular characterization among elite rice varieties of BRRI is important for varietal identification. The use of DNA markers has been suggested for precise and reliable characterization and discrimination of rice genotypes (Khalequzzaman et al., 2022). For genetic variability assessment, DNA markers are extensively used, because these are not affected by environmental factors. Microsatellites (SSRs) are the marker of choice because of their advantages over other markers. The SSRs are most suitable for rice because of their reproducibility, multiallelic nature, hypervariability, codominant inheritance, relative abundance, and genome-wide coverage (Powell et al., 1996; Das et al., 2013).The SSR markers are particularly suitable for evaluating genetic diversity and relationships among plant species, populations, or individuals (Choudhury et al., 2013; Roy et al., 2016), studying morpho-molecular divergence of restorer lines for hybrid rice (Islam et al., 2019); marker-assisted selection breeding (Rani and Adilakshmi 2011); cultivar identification; hybrid purity analysis and gene mapping studies (Sarao et al., 2010).

Information regarding genetic variability at molecular level could be used to help, identify and develop genetically unique germplasm that complements existing cultivars (Rahman et al., 2012; Babu et al., 2014). Diversity of modern BRRI rice varieties of Bangladesh has great importance for food security that seeks to assess the genetic diversity among these varieties. The study was, therefore, conducted to evaluate genetic variation among some modern BRRI developed rice varieties at DNA level using SSR markers. The objectives of this research were to (i) assess the genetic variation and diversity of 26 elite BRRI rice varieties, (ii) determine the genetic relationships among these varieties for breeding purposes, and (iii) characterize these rice varieties at molecular level.

MATERIALS AND METHODS

Plant materials and genomic DNA extraction

The experiment was conducted in the Molecular Laboratory of Genetic Resources and Seed Division (GRSD) of BRRI during 2016–2017. Twenty-six modern rice varieties developed by BRRI were used in this investigation as shown in Table 1. Seeds were germinated in germination chamber at 30°C temperature. Three days after germination, germinated seeds were sown in earthen pots. The pots were then kept in a net house. Genomic DNA was extracted from young leaves of 20-day-old seedlings following the Mini scale method described by Zheng et al. (1995).

Table 1. List of BRRI developed rice varieties with special characters used for the study.

Sl. No.	Variety	Code name	Growing Season	Special characters	Sl. No.	Variety	Code name	Growing Season	Special characters
1	BRRI dhan51	V1	T. Aman	Submergence tolerant	14	BRRI dhan64	V14	Boro	Zinc enriched (25 mg/kg)
2	BRRI dhan52	V2	T. Aman	Submergence tolerant	15	BRRI dhan65	V15	Aus	Drought tolerant
3	BRRI dhan53	V3	T. Aman	Tolerant to 8 dS/m salinity at seedling and reproductive stages	16	BRRI dhan66	V16	T. Aman	Drought tolerant
4	BRRI dhan54	V4	T. Aman	Tolerant to 8 dS/m salinity at seedling and reproductive stages	17	BRRI dhan67	V17	Boro	Salt tolerant
5	BRRI dhan55	V5	Boro/Aus	Moderately tolerant to salt, drought and cold	18	BRRI dhan68	V18	Boro	Green leaf at maturity
6	BRRI dhan56	V6	T. Aman	Drought tolerant	19	BRRI dhan69	V19	Boro	Flag leaf erect
7	BRRI dhan57	V7	T. Aman	Drought escaping	20	BRRI dhan70	V20	T. Aman	Aromatic
8	BRRI dhan58	V8	Boro	Five-day earlier than BRRI dhan29	21	BRRI dhan71	V21	T. Aman	Drought tolerant
9	BRRI dhan59	V9	Boro	Flag leaf erect and deep green, non-lodging	22	BRRI dhan72	V22	T. Aman	Zinc enriched (25 mg/kg)
10	BRRI dhan60	V10	Boro	Early maturing, extra-long grain	23	BRRI dhan73	V23	T. Aman	Salt tolerant
11	BRRI dhan61	V11	Boro	Salt tolerant	24	BRRI Hybrid dhan2	V24	Boro	Short duration
12	BRRI dhan62	V12	T. Aman	Moderately zinc enriched (19 mg/kg), high protein (9%) and early maturing	25	BRRI Hybrid dhan3	V25	Boro	Short duration
13	BRRI dhan63	V13	Boro	Premium quality rice	26	BRRI Hybrid dhan4	V26	T. Aman	Short duration

SSR Markers and PCR amplification

From the prior rice research (McCouch et al., 2002; Islam et al., 2018a), sixty pairs of primers were used, with some of them being chosen at random. Detailed information of the primers is obtained from the website (www.gramene.org/ markers/microsat). Markers that showed monomorphic banding patterns was excluded. Finally, 52 polymorphic SSR markers were selected for diversity analysis. The total PCR reaction was performed using the procedure established by Islam et al. (2018b).

Electrophoresis and visualization of amplified products

The 10 μL of each PCR product with 2 μL of a loading dye was analysed using 8% polyacrylamide gel electrophoresis in 1 × TBE buffer at 75 V for about 2.0–2.5 h depending upon the allele size. The gels were stained with ethidium bromide solution (0.5 mg/mL) for 25 min and exposed to UV light using the molecular Imager gel documentation unit (XR System, Uvitec Cambridge, France) for visualization.

Data analysis

The size of the band for each marker was calculated by AlphaEaseFC-4.0 software. The summary statistics, including the number of alleles, major allele size and frequency, gene diversity and polymorphism information content (PIC) value were determined using Power Marker version 3.25 (Liu and Muse, 2005). Binary format (allele presence= "1" and allele absence = "0") for allele frequency was prepared using Power Marker software and used for dendrogram construction by NTSYS-pc version 2.2 (Rohlf, 2002). A similarity matrix was calculated with the SimQual subprogram using the Dice coefficient, followed by cluster analysis with the SAHN subprogram using the UPGMA (unweighted pair group method using arithmetic mean) clustering method as implemented in NTSYS-pc. The similarity matrix was also used for principal coordinate analysis (PCoA) with the DCenter, Eigen, Output, and MXPlot subprograms in NTSYS-pc. Again, software MEGA 5.1 was applied to make the neighbor joining tree (Tamura et al., 2011; Hall, 2013).

RESULTS AND DISCUSSION

SSR markers and allelic diversity

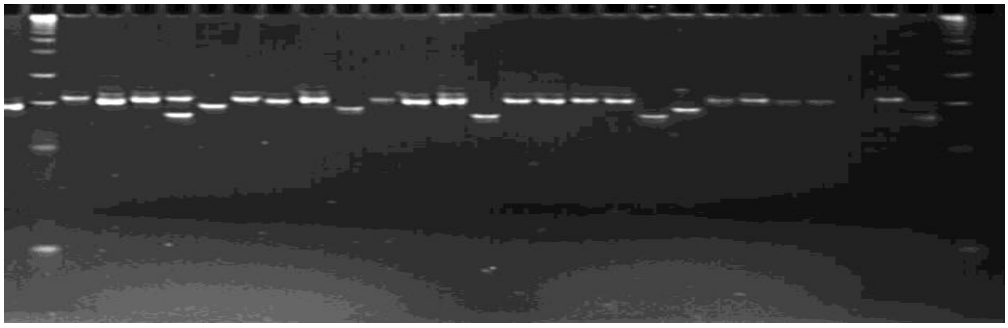
In this study, 60 SSR markers distributed 12 chromosomes of rice were genotyped in 26 BRRI developed rice varieties. Among the 60 SSR markers, 52 (86.67%) were polymorphic while 8 markers revealed mono-morphic patterns and excluded from the study. A total of 156 alleles were detected for the 52 polymorphic primers (Table 2). The number of alleles per locus generated by each marker ranged from 2 to 7, with an average number of alleles identified per locus was 3.00. The gene diversity varied from 0.09 to 0.82, with an average of 0.39, whereas the size of alleles ranged from 79 to 278 bp. The polymorphism information content (PIC) for the SSR loci ranged from 0.08 (RM5, RM487, RM178, RM214, RM542, RM500) to 0.79 (RM566), with an average of 0.35. RM566 had the highest PIC value (0.79) and the highest number

of alleles (7) revealed the highest level of polymorphism. PIC value revealed that RM566 was supposed to be the best marker for characterizing the BRRI developed 26 rice varieties. Again, the second highest PIC value (0.73) was obtained for RM1337, followed by RM591, RM5473, RM21 and RM304. Total 14 highly informative markers (PIC>0.50), 19 informative markers (0.50>PIC>0.25) and 19 slightly informative markers (PIC<0.25) were obtained which might be effectively used for genetic diversity and relationships study of rice. The variability in allele number per locus, the frequency of the most common allele at each locus, ranged from 22.23% (RM566) to 96.15% (RM487, RM214). On average, 70.25% of the 26 rice varieties shared a common major allele at any given locus (Table 2). The DNA profile of 26 rice varieties with SSR marker (RM224) was depicted in Fig.1.

Table 2. Number of alleles, allele size range, highest frequency allele, gene diversity and polymorphism information content (PIC) found among 26 varieties for 52 microsatellite markers

Marker	Chr. No.	Position (cM)	Motif	Allele No.	Size range (bp)	Size (bp)	Frequency (%)	Gene diversity	PIC
RM1	1	29.7	(GA)26	4	115-184	115	60.00	0.57	0.51
RM5	1	94.9	GA)14	2	104-112	113	95.83	0.09	0.08
RM312	1	71.6	(ATTT)4(GT)9	3	98-104	97	52.00	0.60	0.53
RM145	2	49.8	-	2	166-177	166	80.77	0.31	0.26
RM262	2	103.3	(CT)16	2	146-157	154	76.92	0.36	0.29
RM16	3	131.5	(TCG)5(GA)16	4	178-226	181	76.92	0.38	0.35
RM487	3	127.9	(AC)10	2	171-182	176	96.15	0.09	0.08
RM282	3	100.6	(GA)15	2	132-138	136	88.46	0.20	0.18
RM489	3	29.2	(ATA)8	3	266-290	271	53.85	0.59	0.52
RM503	3	153.9	(CA)59(TA)26	3	250-263	268	92.31	0.15	0.14
RM293	3	193.4	(GT)20	2	195-201	201	76.92	0.36	0.29
RM554	3	106.0	(GA)14	2	240-245	245	84.62	0.26	0.23
RM518	4	25.5	(TC)15	2	160-167	167	65.38	0.45	0.35
RM537	4	8.5	(CCG)9	2	214-222	222	84.62	0.26	0.22
RM551	4	8.5	(AG)18	3	178-190	190	84.62	0.27	0.25
RM567	4	153.6	(GA)21	2	271-283	271	88.46	0.20	0.18
RM5473	4	107.65	(TC)20	5	131-161	131	33.33	0.73	0.68
RM178	5	118.8	(GA)5(AG)8	2	115-120	115	96.15	0.09	0.08
RM334	5	141.8	(CTT)20	4	167-182	167	52.17	0.63	0.57
RM413	5	26.7	(AG)11	4	79-109	79	80.77	0.33	0.31
RM3	6	74.3	(GA)2GG(GA)25	2	127-145	145	76.92	0.36	0.29
RM510	6	20.8	(GA)15	2	217-223	223	69.23	0.43	0.34

Marker	Chr. No.	Position (cM)	Motif	Allele No.	Size range (bp)	Size (bp)	Frequency (%)	Gene diversity	PIC
RM70	7	64.66	(ATT)33	3	152-185	185	92.31	0.14	0.13
RM214	7	34.7	(CT)14	2	113-133	113	96.15	0.09	0.08
RM320	7	62.5	(AT)11GTAT(GT)13	4	180-221	180	41.67	0.70	0.65
RM505	7	78.6	(CT)12	3	207-217	207	88.46	0.21	0.20
RM542	7	34.7	(CT)22	2	96-102	102	96.15	0.09	0.08
RM478	7	93.8	(AG)12	2	200-207	207	76.92	0.36	0.29
RM500	7	36.1	(AAG)9	2	252-260	260	96.15	0.09	0.08
RM533	7	39.3	(CT)16	2	240-254	254	84.62	0.26	0.23
RM342	8	78.4	(CAT)12	4	126-146	146	73.08	0.44	0.41
RM447	8	124.6	(CTT)8	3	103-134	103	73.08	0.41	0.35
RM215	9	99.4	(CT)16	2	140-145	145	73.08	0.39	0.32
RM205	9	114.7	(CT)25	3	115-125	115	84.62	0.27	0.25
RM316	9	1.8	(GT)8-(TG)9 (TTTG)4 (TG)4	2	189-196	189	69.23	0.43	0.34
RM566	9	47.7	(AG)15	7	225-265	225	22.23	0.82	0.79
RM285	9	1.8	(GA)12	3	140152	152	80.00	0.34	0.31
RM460	9	45.6	(AT)11	2	234-278	278	84.62	0.26	0.23
RM269	10	69.6	(GA)17	2	170-176	176	88.46	0.20	0.18
RM304	10	73.0	(GT)2(AT)10(GT)33	4	133-172	172	38.46	0.68	0.62
RM474	10	-	(AT)13	4	223-255	255	60.87	0.56	0.51
RM496	10	113.0	(TC)14	2	245-252	252	84.62	0.26	0.23
RM591	10	118.3	(AC)10	6	237-269	269	34.62	0.75	0.72
RM21	11	85.7	(GA)18	4	130-163	163	41.67	0.69	0.63
RM206	11	102.9	(CT)21	4	125-158	158	50.00	0.59	0.51
RM224	11	120.1	(AAG)8(AG)13	4	130-150	150	73.08	0.43	0.40
RM287	11	68.6	(GA)21	4	98-117	117	73.91	0.43	0.40
RM536	11	55.1	(CT)16	2	128-135	135	88.00	0.21	0.19
RM20	12	-	(ATT)14	3	136-148	148	69.23	0.47	0.43
RM1337	12	-	(AG)21	6	163-211	211	39.13	0.76	0.73
RM5364	12	-	(TC)13	3	115-148	148	46.15	0.63	0.55
RM7102	12	-	(AGAT)8	3	115-148	148	50.00	0.54	0.43
Total				156					
Range				2-7		79-278	22.23-96.15	0.09-0.82	0.08-0.79
Average				3.00			70.25	0.39	0.35



L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 L

Figure 1. DNA Profile of 26 BRRI Rice varieties with RM224

Genetic distance-based analysis

Genetic distance is the degree of gene or genomic difference between species or populations. An unrooted neighbor-joining tree constructed showing the genetic relationships among 26 BRRI rice varieties that formed three groups based on the alleles identified by 52 SSR markers (Fig. 2). Cluster I contained four varieties (BRRI dhan59, BRRI dhan61, BRRI dhan69, BRRI dhan70), which themselves were close to each other. The cluster II had the highest number of varieties (14). Again, cluster III is made up of BRRI dhan51, BRRI dhan53, BRRI dhan54, BRRI dhan55, BRRI dhan57, BRRI dhan60, BRRI dhan62 and BRRI dhan65. The genetic similarity analysis using UPGMA clustering method partially agreed with the neighbor-joining tree data.The dendogram based on Dice similarity index and UPGMA method (Fig. 3) was obtained from the binary data that was deduced from the DNA profiles of the samples analyzed. Four distinct clusters were created from the analysis of the pooled SSR marker data at the similarity coefficient of 0.55. Cluster I consisted of 21 rice varieties, while clusters II had only one variety (BRRI dhan52). BRRI dhan52 is a submergence tolerant variety which is grown mainly Kishoreganj, Pabna, Gopalganj and Habiganj district of Bangladesh. Clusters III and IV had three (BRRI dhan63, BRRI dhan64, BRRI dhan68) and one (BRRI hybrid dhan4) varieties, respectively (f. 3).

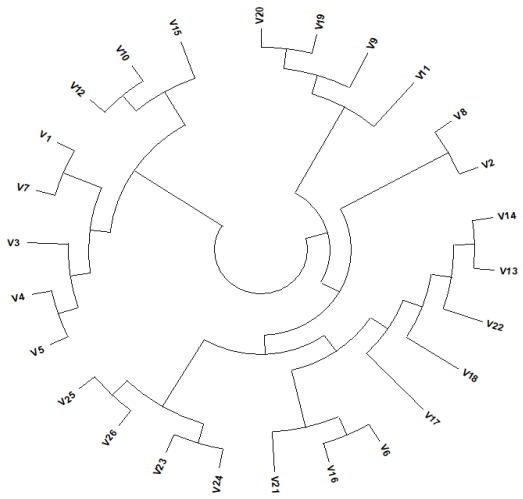


Figure 2. An unrooted neighbor-joining tree showing the genetic relationships among 26

BRRI rice varieties based on the alleles detected by 52 SSR markers. (Legend: V1= BRRI dhan51, V2= BRRI dhan52, V3= BRRI dhan53, V4= BRRI dhan54, V5= BRRI dhan55, V6= BRRI dhan56, V7=BRRI dhan57, V8=BRRI dhan58, V9=BRRI dhan59, V10=BRRI dhan60, V11=BRRI dhan61, V12=BRRI dhan62, V13=BRRI dhan63, V14=BRRI dhan64, V15= BRRI dhan65, V16= BRRI dhan66, V17= BRRI dhan67, V18=BRRI dhan68, V19= BRRI dhan69, V20= BRRI dhan70, V21=BRRI dhan71, V22=BRRI dhan72, V23=BRRI dhan73, V24=BRRI Hybrid dhan2, V25=BRRI Hybrid dhan3, V26=BRRI Hybrid dhan4)

The values of pair-wise comparisons of Nei' s genetic distances (D) between varieties were computed from combined data for the 52 primers, ranged from 0.12 to 0.58, whereas average genetic distance was 0.39 indicating a moderate range of genetic variation among the varieties (Table 3). The pair-wise genetic dissimilarity coefficients indicated that the highest genetic dissimilarity was found between BRRI dhan56 and BRRI Hybrid dhan4 (58.00%) followed by BRRI dhan60 and BRRI dhan64 (56.00%), BRRI dhan52 and BRRI dhan68 (54.00%), BRRI dhan57 and BRRI dhan58 (52.95%), BRRI dhan65 and BRRI dhan68 (52.94%), BRRI dhan64 and BRRI dhan67 (52.00%), BRRI dhan57 and BRRI hybrid dhan4 (51.03%) and so on. The lowest genetic dissimilarity among rice varieties was between BRRI dhan60 and BRRI dhan62 (12.00%), BRRI Hybrid dhan2 and BRRI Hybrid dhan3 (17.02%), BRRI dhan69 and BRRI dhan70 (18.00%), BRRI dhan58 and BRRI Hybrid dhan3 (22.45%), BRRI dhan73 and BRRI Hybrid dhan2 (22.92%) and BRRI dhan53 and BRRI dhan55 (24.00%). It may be noted that none of the BRRI varieties were found in duplicate (i.e., 100% similarity).

Table 3. Genetic dissimilarity between pair of BRRI varieties obtained from SSR data analysis

Varieties	V1	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19	V2	V20
V1	0.0000												
V10	0.3673	0.0000											
V11	0.4000	0.3333	0.0000										
V12	0.3469	0.1200	0.2745	0.0000									
V13	0.4400	0.4510	0.4231	0.4706	0.0000								
V14	0.4800	0.5600	0.4902	0.5000	0.3529	0.0000							
V15	0.4800	0.2941	0.3654	0.2941	0.4038	0.4314	0.0000						
V16	0.4583	0.4082	0.3800	0.3878	0.4800	0.4490	0.3400	0.0000					
V17	0.4286	0.3600	0.3922	0.3922	0.3922	0.5200	0.3725	0.4082	0.0000				
V18	0.4898	0.4400	0.4510	0.4800	0.3922	0.4400	0.5294	0.4082	0.3600	0.0000			
V19	0.3600	0.3333	0.2885	0.3529	0.3077	0.3922	0.3077	0.3600	0.3333	0.3922	0.0000		
V2	0.4490	0.4200	0.3922	0.4000	0.4118	0.4800	0.4706	0.4898	0.5000	0.5400	0.4314	0.0000	
V20	0.3878	0.3469	0.3000	0.3469	0.4000	0.4490	0.4000	0.3542	0.3673	0.4082	0.1800	0.4694	0.0000
V21	0.0000												
V22	0.3400	0.0000											
V23	0.3878	0.3265	0.0000										
V24	0.3125	0.3469	0.2292	0.0000									
V25	0.2857	0.2857	0.3125	0.1702	0.0000								
V26	0.4694	0.5102	0.4583	0.2766	0.2500	0.0000							
V3	0.3200	0.3800	0.3878	0.3125	0.2653	0.4082	0.0000						
V4	0.4118	0.3137	0.3600	0.3673	0.3200	0.5000	0.2941	0.0000					
V5	0.4000	0.4600	0.4082	0.3958	0.4000	0.5102	0.2400	0.3137	0.0000				
V6	0.3922	0.4314	0.4400	0.4490	0.4800	0.5800	0.4314	0.5192	0.5098	0.0000			
V7	0.4600	0.4200	0.4200	0.3958	0.3469	0.5103	0.3000	0.3333	0.4200	0.5295	0.0000		
V8	0.3800	0.2400	0.3265	0.2708	0.2245	0.4082	0.3137	0.2941	0.3800	0.4314	0.3200	0.000	
V9	0.5000	0.3800	0.3469	0.4167	0.3878	0.4490	0.3000	0.3529	0.3800	0.4902	0.4600	0.3400	0.0000

Legend: V1= BRRI dhan51, V2= BRRI dhan52, V3= BRRI dhan53, V4= BRRI dhan54, V5= BRRI dhan55, V6= BRRI dhan56, V7=BRRI dhan57, V8=BRRI dhan58, V9=BRRI dhan59, V10=BRRI dhan60, V11=BRRI dhan61, V12=BRRI dhan62, V13=BRRI dhan63, V14=BRRI dhan64, V15= BRRI dhan65, V16= BRRI dhan66, V17= BRRI dhan67, V18=BRRI dhan68, V19= BRRI dhan69, V20= BRRI dhan70, V21=BRRI dhan71, V22=BRRI dhan72, V23=BRRI dhan73, V24=BRRI Hybrid dhan2, V25=BRRI Hybrid dhan3, V26=BRRI Hybrid dhan4.

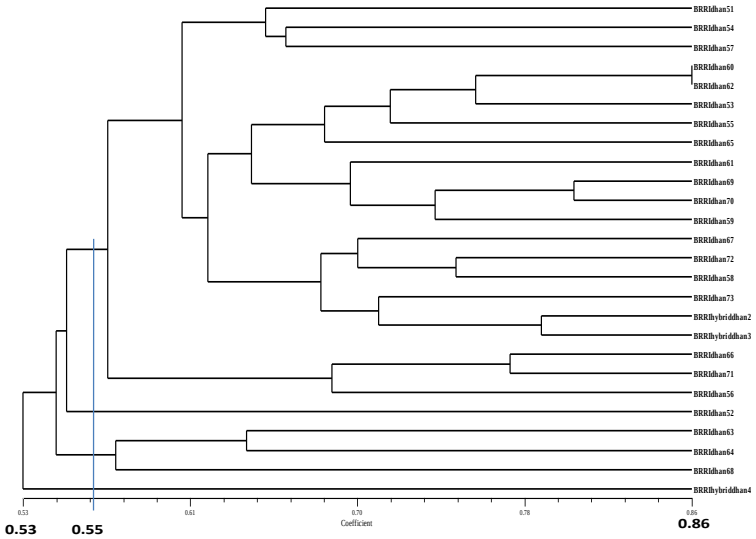


Figure 3. An UPGMA cluster dendrogram showing the genetic relationships between 26 rice varieties of Bangladesh based on the alleles detected by 52 SSR markers.

Principal coordinate analysis

The two dimensional graphical views of principal coordinate analysis showed the spatial distribution of the BRRI rice varieties. The rice varieties namely BRRI dhan56, BRRI dhan51, BRRI dhan52, BRRI dhan61, BRRI dhan62 and BRRI dhan66 were to be found far away from the centroid of the cluster and the rest of the varieties were close to the centroid (Fig. 4). Genotypes close to the centroid suggest similar characteristics, while genotypes distant from the centroid suggest different traits (Islam et al., 2018c).

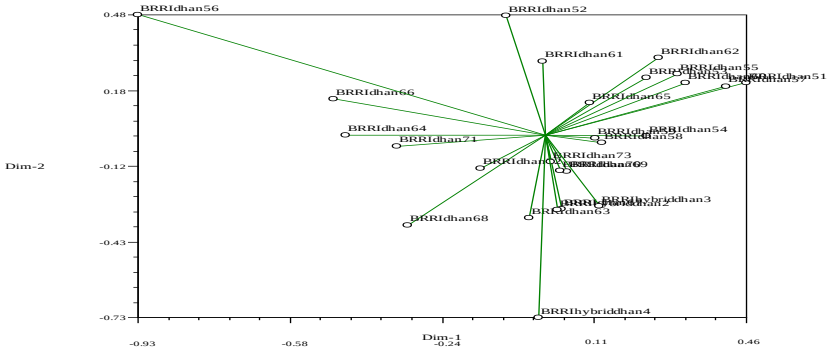


Figure 4. Two-dimensional view of principal coordinate analysis (PCoA) with 52 SSR markers over 26 BRRI rice varieties.

Molecular characterization has been earlier documented in rice genotypes (Salgotra et al., 2015; Ahmed et al., 2016; Siddique et al., 2016a, b, c; Islam et al., 2021; Vabna et al., 2021). In the present study, 156 alleles were detected among 26 studied rice varieties with an average number of 3 alleles per locus and an average PIC of 0.35. The genetic diversity observed in the present study is similar to earlier studies (Shah et al., 2013), they detected 2.75 alleles per locus and an average PIC value of 0.38 among 40 rice accessions of Pakistan. Similarly, SSR diversity was also observed in a study with 36 polymorphic SSRs in which they detected 2.22 alleles per locus and an average PIC value of 0.25 in 375 Indian rice varieties collected from different regions of India (Singh et al., 2013). The result differs with the result of Siddique et al. (2016a and 2016b) where average PIC value of 0.90 and 0.81 for 96 T. Aman and 20 GI rice cultivars, respectively.

The UPGMA dendogram formed four clusters which is similar to other studies. Khalequzzaman et al. (2022) studied diversity and population structure of 48 Boro rice landraces showed four major groups. Pachauri et al. (2013) made the UPGMA dendogram based on molecular marker analysis and clustered the 41 genotypes into four major clusters. Similarly, Das et al. (2013) found four groups among a set of 26 rice cultivars.

Though the genetic divergence was not very high, but these studied varieties showed considerable genetic diversity. Narrow genetic base of modern rice varieties are available from different countries like Latin America (Herrera et al., 2008; Rabelo et al., 2015), Japan (Wanga et al., 2014), USA (Xu et al., 2004) and Korea (Song et al., 2002). This might be due to the varietal selection pressure and adaptation capability of the specific variety in the specific climatic conditions.

CONCLUSION

This study showed that the tested samples possessed a considerable level of genetic variation. The PIC values for the microsatellite loci varied from 0.08 to 0.79 with a mean of 0.35. Comparatively higher genetic distance was observed between BRRI dhan56 and BRRI Hybrid dhan4 genotypes pair followed by BRRI dhan60 and BRRI dhan64 genotypes pair than the other combinations. The identified informative (0.50>PIC>0.25) and highly informative (PIC>0.50) SSR markers could be effectively used in background selections during backcross breeding.

ACKNOWLEDGMENT

The authors are grateful for the financial support provided by the Bangladesh Rice Research Institute (BRRI) through the Ministry of Agriculture, Bangladesh.

REFERENCES

Ahmed, M.S.U., Khalequzzaman, M., Bashar, M.K. and Shamsuddin, A.K.M. (2016). Agromorphological, physico-chemical and molecular characterization of rice germplasm with similar names of Bangladesh. *Rice Science*, 23(4): 211-218.

Babu, BK., Meena, V., Agarwal, V. and Agrawal, P.K. (2014). Population structure and genetic diversity analysis of Indian and exotic rice (*Oryza sativa* L.) accessions using SSR markers. *Molecular Biology Reports*, 41(7):4328 - 4339.

BRRI. (2022). Modern rice cultivation, 24th edition, p.112

Choudhury, B., Khan, M.L. and Dayanandan, S. (2013). Genetic structure and diversity of indigenous rice varieties (*Oryza sativa* L.) in Eastern Himalayan region of Northeast India. *Springer Plus*, 2: 228-237.

Das, B., Sengupta, S., Parida, S.K., Roy, B., Ghosh, M. and Prasad, M. (2013). Genetic diversity and population structure of rice landraces from eastern and north eastern states of India. *BMC Genetics*, 14 (1):71.

Hall, B.G. (2013). Building phylogenetic trees from molecular data with MEGA. *Molecular Biology and Evolution*, 30:1229-1235.

Herrera, T.G., Duque, D.P., Almeida, I.P., Núñez, G.T., Pieters, A.J., Martinez, C.P. and Tohme, J.M. (2008). Assessment of genetic diversity in Venezuelan rice cultivars using simple sequence repeats markers. *Electronic Journal of Biotechnolgy***,** 11: 1-15.

Islam, M.Z., Khalequzzaman, M., Prince, M.F.R.K., Siddique, M.A., Rashid, E.S.M.H., Ahmed, M.S.U., Pittendrigh, B.R. and Ali, M. P. (2018a). Diversity and population structure of red rice germplasm in Bangladesh. *PLoS ONE*, 5: e0196096.

Islam, M.Z., Khalequzzaman, M., Bashar, M.K., Ivy, N.A., Mian, M.A.K., Pittendrigh, B.R., Haque, M.M. and Ali, M.P. (2018b). Variability assessment of aromatic rice germplasm by pheno-genomic traits and population structure analysis. *Scientific Reports*, 8:9911.

Islam, M.Z., Khalequzzaman, M., Bashar, M.K., Ivy, N.A., Haque, M.M., Mian, M.A.K. and Tomita, M. (2018c). Agro-morphological characterization of Bangladeshi aromatic rice (*Oryza sativa* L.) germplasm based on qualitative traits. *Bangladesh Rice Journal*, 22 (2): 41-5

Islam, M.Z., Siddique, M.A., Akter, N., Prince, M.F.R.K., Islam, M.R., Anisuzzaman, M. and Mian, M.A.K. (2019). Morpho-molecular divergence of restorer lines for hybrid rice (*Oryza sativa* L.) development. *Cereal Research Communication*, 47(3): 531-540.

Islam, M.Z., Khalequzzaman, M., Chakrabarty, T., Akter, N., Khan, M.F.R., Bhuiya, A. and Siddique, M.A. (2021). Genetic diversity and population structure of similar named aromatic rice (*Oryza sativa* L.) landraces of Bangladesh. *SAARC Journal of Agriculture*, 19(2): 57-71.

Khalequzzaman, M., Islam, M.Z., Prince, F.R.K., Rashid, E.S.M.H. and Siddique, A. (2022) Genetic diversity and population structure of Boro rice landraces of Bangladesh. *Journal of Rice Research*, 10: 305.

Khush, G.S. (2005). What it will take to feed 5.0 billion rice consumers in 2030. *Plant Molecular Biology*.

Liu, K. and Muse, S.V. (2005). Power Marker: an integrated analysis environment for genetic marker analysis. *Bioinformatics*, 21(9): 2128-2129.

McCouch, S.R., Teytelman, L., Xu, Y., Lobos, K.B., Clare, K., Walton, M., Fu, B., Maghirang, R., Li, Z., Xing, Y., Zhang, Q., Kono, I., Yano, M., Fjellstrom, R., DeClerk, G., Schneider, D., Cartinhour, S., Wari, D. and Stein, L. (2002). Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). *DNA Research*, 9: 199-207.

Pachauri, V., Taneja, N., Vikram, P., Singh, N.K. and Singh, S. (2013). Molecular and morphological characterization of Indian farmers rice varieties (*Oryza sativa* L.). *Australian Journal of Crop Science*, 7: 923-932.

Powell, W., Machray, G.C. and Provan, J. (1996). Polymorphism revealed by simple sequence repeats. *Trends in Plant Science*, 1:215-222.

Rabelo, H.O., Guimarães, J.F.R., Pinheiro, J.B. and Silva, E.F. (2015). Genetic base of Brazilian irrigated rice cultivars. *Crop Breeding and Applied Biotechnology*, 15: 146-153.

Rahman, M., Rasul, G., Hossain, M.A., Iftekharuddaula, K.M. and Hasegawa, H. (2012). Molecular characterization and genetic diversity analysis of rice (*Oryza sativa* L.) using SSR markers. *Journal of Crop Improvement*, 26(2): 244-257.

Rani, M.G. and Adilakshmi, D. (2011). Genetic analysis of Blast resistance in rice with simple sequence repeats (SSR). *Journal of Crop Improvement*, 25: 232-238.

Rohlf, F. (2002). NTSYS-pc: numerical taxonomy and multivariate analysis system, 2.1. Department of Ecology and Evolution, State University of NY, Stony Brook.

Roy, S., Marndi, B.C., Mawkhlieng, B., Banerjee, A., Yadav, R.M., Misra, A.K. and Bansal, A.K. (2016). Genetic diversity and structure in hill rice (Oryza sativa L.) landraces collected from the North-Eastern Himalayas of India. *BMC Genetics*, 17: 107.

Salgotra, R.K., Gupta, B.B., Bhat, J.A. and Sharma, S. (2015). Genetic diversity and population structure of basmati rice (*Oryza sativa* L.) germplasm collected from north western himalayas using trait linked SSR markers. *Plos One*, 10(7): e0131858.

Sarao, N.K., Vikal, Y., Singh, K., Joshi, M.A. and Sharma, R.C. (2010). SSR marker-based DNA fingerprinting and cultivar identification of rice (*Oryza sativa* L.) in Punjab state of India. *Plant Genetic Resources: Characterization and Utilization*, 8: 42-44.

Shah, S.M., Naveed, S.A. and Arif, M. (2013). Genetic diversity in basmati and non-basmati rice varieties based on microsatellite markers. *Pakistan Journal of Botany*, 45: 423-431.

Siddique, M.A., Khalequzzaman, M., Islam, M.M., Fatema, K. and Latif, M.A. (2016a). Molecular characterization and genetic diversity in geographical indication (GI) rice (*Oryza sativa* L.) cultivars of Bangladesh. *Brazilian Journal Botany*, 39(2):631-640.

Siddique, M.A., Khalequzzaman, M., Fatema, K., Islam, M.Z., Islam, M.M. and Chowdhury, M.A.Z. (2016b). Molecular characterization and genetic diversity of Aman rice (*Oryza sativa* L.) landraces in Bangladesh. *Bangladesh Rice Journal*, 20(2):1–11.

Siddique, M.A., Khalequzzaman, M., Islam, M. Z, Rashid, E.S.M.H., Baktiar, M.H.K. and Chowdhury, M.A.Z. (2016c). Genetic diversity in Aus rice genotypes using ILP markers. *Bangladesh Rice Journal*, 20(2):13-19.

Singh, N., Choudhury, D.R., Singh, A.K., Kumar, S., Srinivasan, K. and Tyagi, R.K. (2013). Comparison of SSR and SNP markers in estimation of genetic diversity and population structure of Indian rice varieties. *Plos One*, 8(12): e84136.

Song, M.T., Lee, J.H., Cho, Y.S., Jeon, Y.H., Lee, S.B., Ku, J.H., Choi, S.H. and Hwang, H.G. (2002). Narrow genetic background of Korean rice germplasm as revealed by DNA fingerprinting with SSR markers and their pedigree information. *Korean Journal of Genetics*. 24: 397-403.

Tamura, K., Peterson, D., Peterson, N., Steche, G., Nei, M. and Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, 28: 2731-2739.

Vabna, F.A., Islam, M.Z., Prince, M.F.R.K. and Hoque, M.E. (2021). Molecular diversity analysis in Boro rice (*Oryza sativa* L.) landraces using SSR markers. *Asian Journal of Biology*, 12(1): 36-48.

Wanga, J., Jianga, T., Zoub, D., Zhao, H., Li, Q., Liub, H. and Zhoud, C. (2014). Genetic diversity and genetic relationships of Japonica rice varieties in Northeast Asia based on SSR markers. *Biotechnol Biotechnol Equip*, 28: 230-237.

Xu, Y., Beachell, H. and McCouch, S.R. (2004). A marker-based approach to broadening the genetic base of rice in USA. Crop Science, 44: 1947-1959.

Zheng, K., Huan, N., Bennet, J. and Khus, G.S. (1995). PCR-based marker assisted selection in rice breeding. IRRI, Manila, Philippines. pp. 16-18.