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Research Articles

GENETIC DIVERSITY ANALYSIS OF RICE (*Oryzae sativa* L.) LANDRACES USING SSR MARKERS IN BANGLADESH

M.I. Hoque[1*], M.M. Islam[1&2], S.N. Begum[1], F. Yasmine[1] M.S.R. Khanom[1] and M. M. Islam[3]

[1]Plant Breeding Division
[2]Director General's office
[3]Biotechnology Division
Bangladesh Institute of Nuclear Agriculture, Mymensingh

ABSTRACT

It is necessary to assess genetic diversity and a molecular characteristic among Bangladesh's local rice cultivars. The purpose of the study was to assess the genetic diversity and molecular characterization of 83 rice landraces in Bangladesh using nine (9) SSR markers. A total of 262 allels were identified using nine (9) polymorphic markers. The highest number alleles 34 were identified by RM336 while the lowest was 13 by RM262. Polymorphism information content (PIC) value of each marker was evaluated on the basis of the number of alleles and it varied greatly for all the SSR loci tested. The PIC value ranged from 0.951 to 0.766 and marker RM336 was found to be the most appropriate marker to discriminate among the rice genotypes owing to the highest PIC value of 0.951. The allele frequency ranged from 37.35% (RM262) to 10.84% (RM585, RM336) with an average of 18.47%. The genotypes G82, G77, G68, G50, and G1; G65, G37, and G10; G71 and G11; G25 and G14; G39 and G27 have 100% genetic similarity according to the pair wise genetic similarity indexes. Genotype G10 had the least similarity (0.44 percent) to genotype G9, G16 with G17, G22 with G29, G28 with G30, and so on. The dendrogram based on UPGMA and Nei's genetic distance classified the 83 rice landraces into 5 clusters with a similarity coefficient of 0.6 Cluster 2 had maximum thirty-two genotypes followed by cluster 4. The landraces that were derivatives of genetically similar types were clustered together on the dendrogram. These landraces is showed wide genetic divergence among the constituent in it and in future it will be useful for hybridization programme in plant breeding.

Keywords: Rice landrace, Genetic diversity, SSR markers, UPGMA clustering

[*] Corresponding Author: apu.pstu@gmail.com

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INTRODUCTION

Rice (*Oryza sativa* L.) is a major cereal crop farmed only for human use, and it is the primary source of nutrition for roughly half of the world's population (Ramkumar et al., 2010). South East Asia is one of the world's rice diversity hotspots (Singh et al., 2016). Rice landraces include a large number of useful genes that rice breeders can employ to improve the crop, and genetic heterogeneity exists among rice accessions, allowing for a wide range of agricultural improvements (Singh et al., 2015).

Bangladesh is already under strain from rising food demand as well as issues with agricultural land and water scarcity. Bangladesh has to enhance rice yields to fulfill the rising food demand brought on by population expansion. The world's largest rice Genebank, at IRRI (International Rice Research Institute) in the Philippines, has about 1,27,000 rice accessions and wild relatives (http://irri.org/ourwork/research/genetic-diversity). The amount of scoreable morphological features used to quantify plant genetic diversity varies when compared to the number of biologically active genes. Furthermore, plant genomes typically contain a considerable quantity of repetitive DNA that is not expressed and does not contribute to plant physiological or morphological appearance. There are extremely few physical changes between very closely related plant kinds, which do not represent actual genetic differences at the DNA level. As a result, there is always a need to investigate polymorphism at the DNA level, which may serve as a marker of genetic variation. RFLP, RAPD, AFLP, microsatellites (SSR), and SNP are some of the molecular markers that have been produced. Simple sequence repeat (SSR) markers, are co-dominant, hyper variable, plentiful, and widely dispersed throughout the rice genome (Temnykh et al., 2001). Because microsatellites are technically easy, time saving, highly informative, and need a minimal amount of DNA, they have shown significant promise in genetic diversity, genome mapping, gene tagging, and marker-assisted selection (MAS). However, because of the influence of environmental factors, assessments based solely on plant phenotypes are not a trustworthy indicator of genetic difference. The development of PCR-based molecular marker technology has made it possible to measure genetic diversity in germplasm with very effective and reliable techniques (Chitwood et al. 2016). Microsatellite markers are currently available through a publicly accessible database or the published high-density linkage map (McCouch et al., 2002; IRGSP, 2005). A study reported that around 234 rice landraces and discovered five different groups that correlate to indica, aus, aromatic, temperate japonica, and tropical japonica rice (Amanda et al., 2004). They have a lot of variety as well, with 98 percent of loci polymorphic in the Aus group. The group has received less attention than the indica and japonica groups, despite their drought tolerance and early maturity. In Bangladesh, there are four different ecotypes of rice: Boro, Aus, Transplanted Aman, and Deep Water Aman.

Boro, Aman, and Aus landraces are among the indigenous rice varieties found in Bangladesh. Those landraces have a high level of adaptability but a low yield. During

the last 20 years, high yielding variants have gradually superseded the cultivation of these landraces. These landraces have adapted in various sections of the country, with some having exceptional quality, fineness, aroma, taste, and protein content (Dutta et al., 1998). As selection of plants based on genetic diversity has proven beneficial in various crops, precise information on the level of genetic variety among populations is critical in every crop development operation (Ananda and Rawat, 1984; De et al., 1988). As a result, the goals of this study were to evaluate the genetic richness and variation of 83 local rice genotypes, as well as to discover the genetic link between these genotypes for breeding purposes.

MATERIALS AND METHODS

Plant materials

This study was conducted at pot yard and Molecular Laboratory of Plant Breeding Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh from August to December 2020. Eighty-three rice landraces collected from different location of Bangladesh were used in this study as depicted in Table1.

Table 1. List of plant materials used in this study

Sl. No.	Germplasm name	Collection place	Collection year	Sl. No.	Source of origin	Collection place	Collection year
1	KuttimoraBirun	Sherpur	2019	42	Moyna shail	Sunamganj	2019
2	Dudh Binni	Sherpur	2019	42	Kashia Binni	Mymensingh	2018
3	Goa Mouri	Sherpur	2019	44	Chinishail-3	Sherpur	2019
4	Chaklashi	Sherpur	2019	45	Goabari	Sherpur	2019
5	Goar chara	Sherpur	2019	46	Marrygold	Sherpur	2019
6	Hashem Iri	Sherpur	2019	47	Lalkumri	Sherpur	2019
7	Lal Paijam	Sunamganj	2018	48	Porbot Jira	Sherpur	2019
8	Birui	Sherpur	2019	49	Sentu-18	Sherpur	2019
9	Shoragot Birun	Sherpur	2019	50	Sentu-19	Sherpur	2019
10	Aila gota	Sherpur	2019	51	Lalmatia	Sherpur	2019
11	BedhaBirun	Sherpur	2019	52	Faijam	Sherpur	2019
12	OjanaBirun	Sherpur	2019	53	Leda Binni	Sherpur	2019
13	Lal goarchara	Sherpur	2019	54	Hasa Shada	Mymensingh	2018
14	Kaijam	Sherpur	2019	55	Lal Chini shail	Sherpur	2019
15	Kotkoti	Sunamganj	2018	56	Peyarjat	Sherpur	2018
16	Lalcheng	Sherpur	2019	57	Shonajuri	Mymensingh	2018
17	Puti birun	Sherpur	2019	58	Mukta-10	Mymensingh	2018
18	Noli Goarchara	Sherpur	2019	59	Choto Sharnalota	Sherpur	2019
19	Chanmoni	Mymensingh	2018	60	Motamorang	Sherpur	2019

Sl. No.	Germplasm name	Collection place	Collection year	Sl. No.	Source of origin	Collection place	Collection year
20	Sentu-6	Sherpur	2019	61	Chapal	Sherpur	2019
21	Chapal-2	Sherpur	2019	62	Ful lota	Sherpur	2019
22	Lomba Ail	Sherpur	2019	63	Sentu-5	Sherpur	2018
23	Shada Paijam	Sunamganj	2018	64	Hashakalo	Mymensingh	2019
24	Birushail	Sunamganj	2018	65	Champa Mushuri	Sherpur	2018
25	Chini shail	Sherpur	2018	66	Madhobi lota	Sherpur	2018
26	Chenger muri	Sunamganj	2018	67	Sentu-16	Sherpur	2019
27	Sentushail	Sherpur	2019	68	Nagra	Sherpur	2019
28	Chinigura	Sunamganj	2018	69	Gobinda	Sherpur	2019
29	Shong Binni	Sherpur	2018	70	Sentu-9	Sherpur	2018
30	Champa mushuri	Sherpur	2019	71	Sentu-11	Sherpur	2019
31	Bashiraj	Natore	2019	72	Tulshimala	Sherpur	2018
32	Gandhishail	Sunamganj	2018	73	Sentu-17	Sherpur	2018
33	BoroAbji	Sunamganj	2018	74	Hashashada	Mymensingh	2018
34	Malai reti	Sherpur	2019	75	Laldinga	Sherpur	2018
35	Deshi-32	Sunamganj	2018	76	Goatibinni	Sherpur	2019
36	Moynashail	Sunamganj	2018	77	Sentu-18	Sherpur	2019
37	Maloti	Sunamganj	2018	78	Porabinni	Sherpur	2019
38	Chollish	Sunamganj	2018	79	Ranishail	Sherpur	2019
39	Paijam	Gopalganj	2019	80	Shonajuri	Mymensingh	2019
40	Sentu gold	Sherpur	2019	81	Bishali Binni	Sherpur	2019
41	Markabinni	Sherpur	2019	82	Kalo Birun	Sunamganj	2019
				83	Fulkainja	Sherpur	2019

Genomic DNA extraction

DNA was extracted from leaf tissues of 21 days old seedling following Cetyl Trimethyl Ammonium Bro-mide (CTAB) method (Doyle and Doyle 1987).

Primer selection and DNA amplification

To detect DNA for differentiating the tested rice landraces using nine SSR markers were used (Table 2). Before the selection of nine polymorphic markers, marker surveys were conducted with 120 SSR markers and nine markers were chosen according to their polymorphic bands (Fig. 1).

PCR analysis was carried out in a 10 µl reaction sample including 1 µl DNA template, 5 µl of master mix, 2 µl nuclease free water, and 1 µl each of forward and reverse primers (Siddique et al., 2016b) utilizing a Biometra T3 thermal cycler (Analytik Jena GmbH Co, Germany) with a single 96-well plate. After initial

denaturation for five minutes at 94°C, Each cycle consisted of one minute denaturation at 94°C, one minute annealing at 55°C, and two minutes extension at 72°C, with a final extension of seven minutes at 72°C at the end of 35 cycles (Siddique et al., 2016b) and amplified products were stored at (-)4^0C until further use. For high throughput manual genotyping, the PCR products were electrophoresed on an 8 percent polyacrylamide gel using mini vertical polyacrylamide gels (CBS Scientific Co. Inc., USA). Depending on the allele size, 2µl of amplification products were resolved by running gel in 1x TBE buffer for 2-2.5 hours at roughly 80 volts and 400 mA current. The gels were stained with ethidium bromide at a concentration of 0.5 mg/ml and recorded with the Whatman Biometra gel Documentation System (prod nr: 1603209) employed microsatellite or simple sequence repeat (SSR) markers for DNA analysis (Temnykh et al., 2001; McCouch et al., 2002)

Table 2. List of the nine simple sequence repeat (SSR) markers

Locus name	Amplicon size range (bp)	Repeat motif	Sequence	Annealing temperature (°C)
RM493	211	$(CTT)^9$	Forward :TAGCTCCAACAGGATCGACC Reverse: GTACGTAAACGCGGAAGGTG	55
RM248	102	$(CT)^{25}$	Forward :TCCTTGTGAAATCTGGTCCC Reverse: GTAGCCTAGCATGGTGCATG	55
RM262	154	$(CT)^{16}$	Forward :CATTCCGTCTCGGCTCAACT Reverse:CAGAGCAAGGTGGCTTGC	55
RM7075	155	$(ACAT)^{13}$	Forward:TATGGACTGGAGCAAACCTC Reverse:GGCACAGCACCAATGTCTC	50
RM224	157	$(AAG)^8$ $(AG)^{13}$	Forward:ATCGATCGATCTTCACGAGG Reverse:TGCTATAAAAGGCATTCGGG	55
RM551	192	$(AG)^{18}$	Forward:AGCCCAGACTAGCATGATTG Reverse:GAAGGCGAGAAGGATCACAG	55
RM585	233	$(TC)^{45}$	Forward:CAGTCTTGCTCCGTTTGTTG Reverse:CTGTGACTGACTTGGTCATAGG	55
RM3412b	211	$(TA)^{34}$	Forward:TCATGATGGATCTCTGAGGTG Reverse:GGGAGGATGCACTAATCTTTC	55
RM336	154	$(CTT)^{18}$	Forward:CTTACAGAGAAACGGCATCG Reverse:GCTGGTTTGTTTCAGGTTCG	55

Analysis of SSR data

Using the Alpha-EaseFC 5.0 program, the size of each amplified allele was measured in base pairs. Power Marker version 3.25 was used to calculate summary statistics such as the number of alleles per locus, major allele frequency, gene diversity, and polymorphism information content (PIC) values (Liu and Muse, 2005). For analysis with NTSYSpc version 2.1, the allele frequency data from Power Marker was exported in binary format (allele presence=1 and allele absence=0) (Rohlf, 2002). A similarity matrix was created using the Dice coefficient in the Simqual subprogram, followed by cluster analysis using the UPGMA (unweighted pair group technique using arithmetic mean) clustering algorithm implemented in NTSYS-pc in the SAHN subprogram.

RESULTS AND DISCUSSIONS

SSR markers are widely used for fingerprinting and diversity studies on rice cultivars and wild relatives due to its high polymorphic rates, which can be identified even at individual levels (Nei et al., 2002; Nagaraju et al., 2002).

The nine micro-satellite markers effectively amplified 83 rice landraces, with primer pairs referred to as loci and DNA bands referred to as alleles. A total of 262 alleles were detected using nine micro-satellite markers across 83 rice landraces.

The highest average band size was found for RM585 (233) followed by RM493 (211), and RM3412b (205). Among the nine SSR markers, the highest number of alleles (34) was found for RM336followed by RM248 (33) and RM585 (32). Previously, a similar number of microsatellite markers were utilized as a subset for *O. sativa* genetic diversity study (Siddique et al., 2016a). The polymorphism information content (PIC) values ranged from 0.951 (RM336) to 0.766(RM262), with an average of 0.90. PIC values of 0.34-0.88 (Thomson et al., 2007), 0.65-0.91 (Siddique et al., 2014) and 0.59-0.90 (Siddique et al., 2016b) are comparable to recent estimates of microsattelite analyses in rice. The PIC values for other markers were 0.910(RM493), 0.844 (RM7075), 0.913(RM224) and 0.926 (RM551), respectively (Table3). This study's mean PIC value was higher than Ravi et al., (2003)'s PIC value of 0.578 in an earlier study of rice cultivars, landraces, and wild relatives. This could mean that the genotypes utilized in this investigation were more varied. Using SSR markers in rice, Panaud et al. (1996) found substantial genetic similarity among landraces of common geographic origin and low genetic similarity among landraces of various geographic origins. PIC value revealed RM336 as the best marker. Because of its low PIC value, RM262 can be regarded the least potent marker. According to the findings, the examined rice landraces have a significant

degree of heterozygosis, which is very obvious. Figure 1 shows the polymorphism survey of some SSR marker of two landraces. The allele frequency ranged from 37.35% (RM262) to 10.84% (RM585, RM336) with an average of 18.47%. Gene diversity varied from 0.95 to 0.78 and their average value was 0.91, which also indicated the presence of adequate genetic diversity (Table 3). Figure 2 and 3 shows the DNA profiles of 83landraces with SSR marker RM493 and RM262 respectively.

Table 3. Allele number, size, frequency, genetic diversity and PIC of 83 rice landraces for nine micro-satellite markers

Locus name	No. of allele	Allele frequency (%)	Gene diversity	PIC
RM493	27	0.2169	0.9154	0.9106
RM248	33	0.1084	0.9531	0.9512
RM262	13	0.3735	0.7891	0.7660
RM7075	16	0.2530	0.8585	0.8443
RM224	25	0.2048	0.9186	0.9139
RM551	26	0.1205	0.9310	0.9269
RM585	32	0.1084	0.9496	0.9474
RM3412b	28	0.1687	0.9369	0.9338
RM336	34	0.1084	0.9537	0.9518
Mean	26	0.1847	0.9118	0.9051

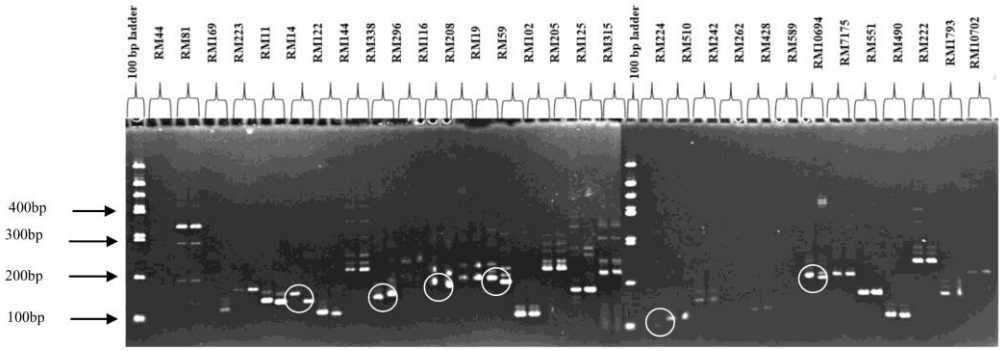

Figure 1. Partial view of gel pictures of primer survey for some primers of two rice landraces

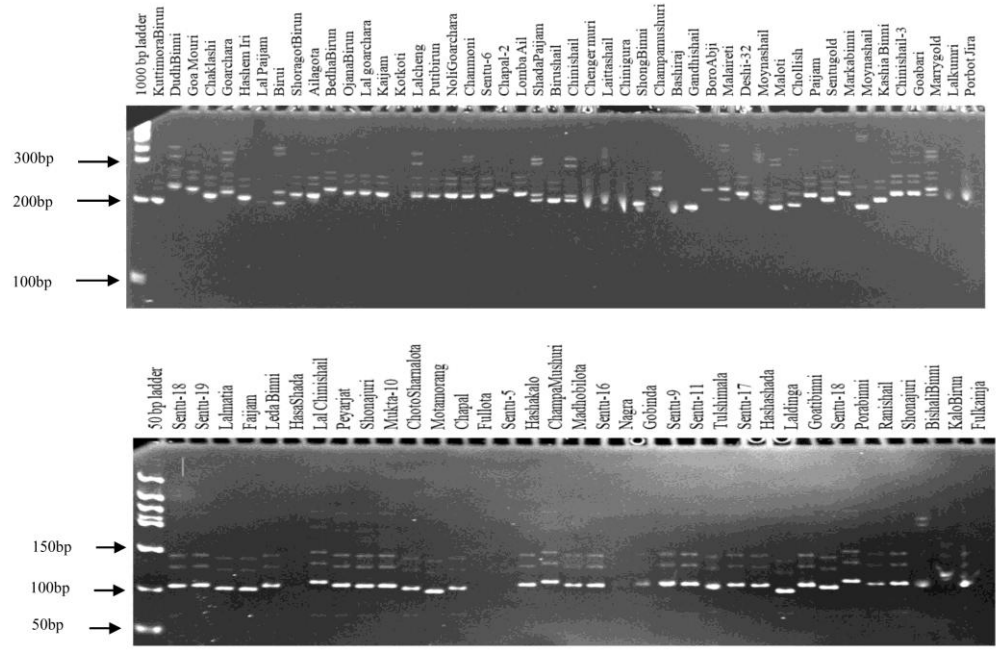

Figure 2. DNA profile of 83 rice landraces with RM493

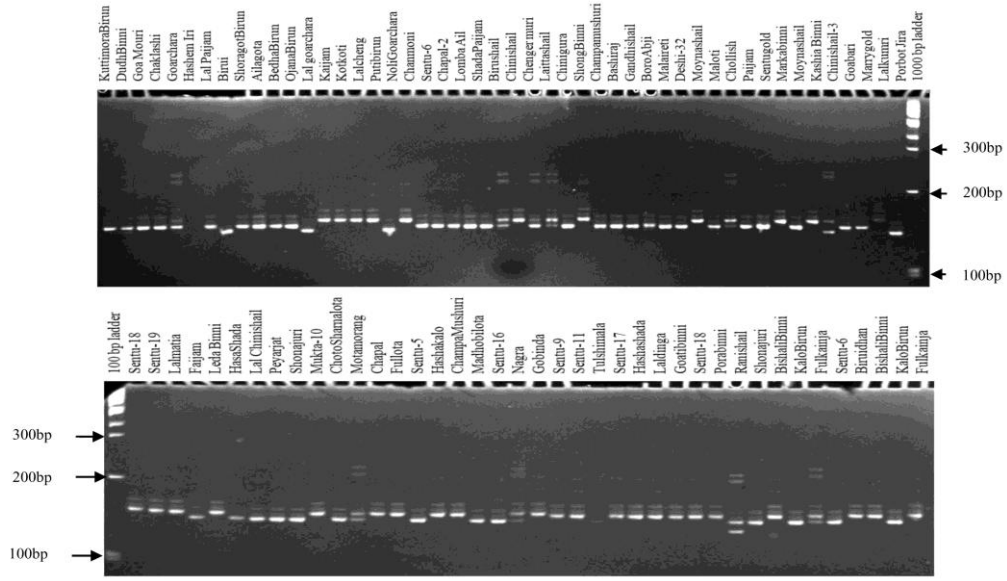

Figure 3. DNA profile of 83 rice landraces with RM262

Similarity matrix

The genetic similarity was calculated using SSR-derived data (Fig. 4). The level of relatedness among the genotypes investigated was determined using the similarly matrix. Across all 83 genotypes, pair-wise estimations of similarity varied from 0.00 to 1.00. Using molecular markers, Saini et al. (2004) discovered similar levels of similarity co-efficient among 18 basmati and non-basmati types. Using microsatellite markers, Siwach et al. (2004) discovered a higher level of similarity between basmati and non basmati long-grain indica rice types, ranging from 0.67 to 0.91. Intra-specific variation in the germplasm utilized could be one of the explanations for the high level of similarity observed in the current and earlier investigations. The genotypes G82, G77, G68, G50, and G1; G65, G37, and G10; G71 and G11; G25 and G14; G39 and G27 have 100% genetic similarity according to the pair wise genetic similarity indexes. When the examined loci were considered, genotypes that were 100 percent similar to each other were discovered to be duplicates. Deepa and Patnai-23 were also discovered as duplicates by Sajib et al. (2012). The study's findings revealed that genotype G10 had the least similarity (0.44 percent) to genotype G9, G16 with G17, G22 with G29, G28 with G30, and so on. Based on the findings of marker-assisted diversity analysis, accessions that are genetically distant (such as Lalchengand Putibirun; Shong Binni and Lomba Ail; Chiniguraand Champa mushuri; Shoragot Birunand Puti Biruin) could be chosen as parents for future breeding projects. This could promote diversity, leading to a high productivity index in terms of increased output and overall quality.

Genetic distance-based analysis

The genotypes were grouped into a dendrogram using cluster analysis (Nei, 1972). Nine markers were used to group the 83 rice landraces into five broad groups based on the dendrogram. All the genotypes could be recognized with ease. With a coefficient of 0.6, the UPGMA cluster analysis grouped the rice genotypes into five primary clusters, with similarity coefficient values ranging from 0.08 to 1.0. Cluster 2 had 31 landraces and was the largest of the five clusters, followed by cluster 4, which had 30 landraces; cluster 3 had 11 landraces; cluster 1 had 6 landraces; and cluster 5 had five landraces (Fig. 5). The landraces that were derivatives of genetically similar types were clustered together on the dendrogram. In a rice improvement breeding effort, genetically distinct landraces could be utilized as parents in a cross-breeding program to generate genetic variety and transgressive segregants.

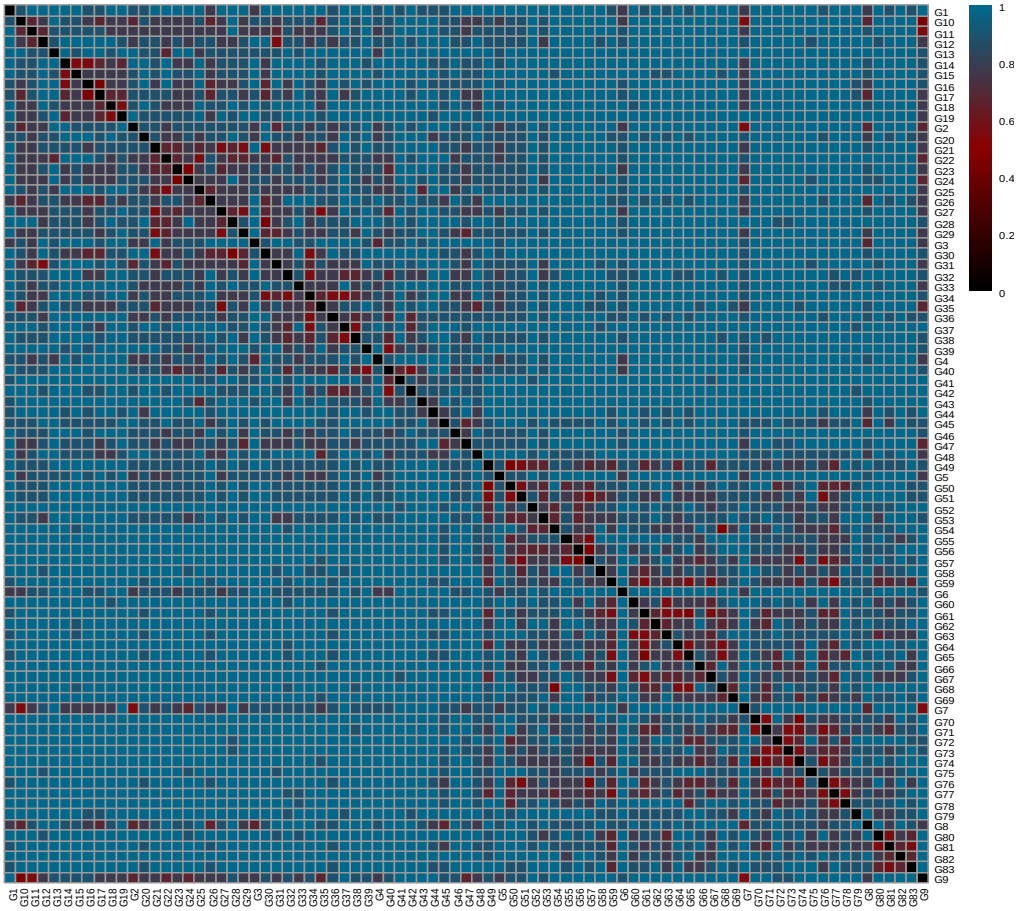

Figure 4. Nei's coefficients of similarity among 83 rice genotypes

Legend: G1-Kuttimora Birun, G2-Dudh Binni, G3-Goa Mouri, G4-Chaklashi, G5-Goar chara, G6-Hashem Iri, G7-Lal Paijam, G8-Birui, G9-Shoragot Birun, G10-Aila gota, G11-Bedha Birun, G12-Ojana Birun, G13-Lal goarchara, G14-Kaijam, G15-Kotkoti, G16-Lalcheng, G17-Puti birun, G18-Noli Goarchara, G19-Chanmoni, G20-Sentu-6, G21-Chapal-2, G22-Lomba Ail, G23-Shada Paijam, G24-Birushail, G25-Chini shail, G26-Chenger muri, G27-Laittashail, G28-Chinigura, G29-Shong Binni,G30-Champa mushuri, G31-Bashiraj, G32-Gandhishail, G33-Boro Abji, G34-Malai reti, G35-Deshi-32, G36-Moynashail, G37-Maloti, G38-Chollish, G39-Paijam, G40-Sentu gold, G41-Markabinni, G42-Moyna shail, G43-Kashia Binni, G44-Chinishail-3, G45-Goabari, G46-Marry gold, G47-Lalkumri, G48-Porbot Jira, G49-Sentu-18, G50-Sentu-19, G51-Lalmatia, G52-Faijam, G53-Leda Binni, G54-Hasa Shada, G55-Lal Chinishail, G56-Peyarjat, G57-Shonajuri, G58-Mukta-10, G59-Choto Sharnalota, G60-Motamorang, G61-Chapal, G62-Ful lota, G63-Sentu-5, G64-Hashakalo, G65-Champa Mushuri, G66-Madhobi lota, G67-Sentu-16, G68-Nagra, G69-Gobinda, G70-Sentu-9, G71-Sentu-11, G72-Tulshimala, G73-Sentu-17, G74-Hashashada, G75-Laldinga, G76-Goatibinni, G77-Sentu-18, G78-Porabinni, G79-Ranishail, G80-Shonajuri, G81-Bishali Binni, G82-Kalo Birun, G83-Fulkainja

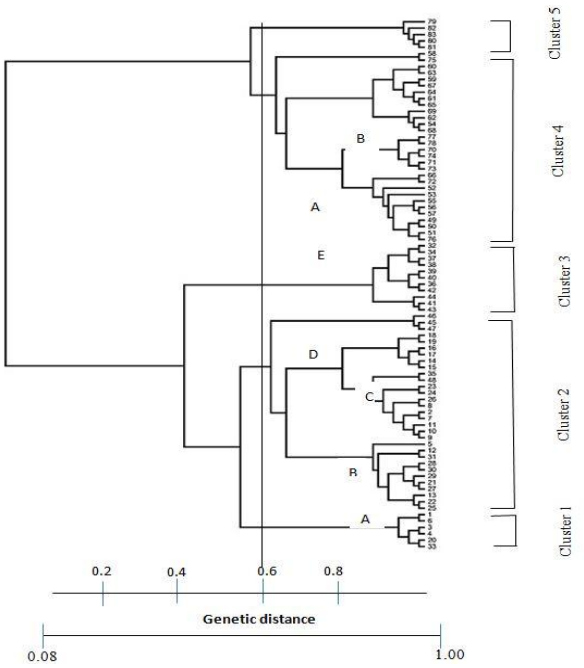

Figure 5. UPGMA cluster dendrogram showing the genetic relationships between 83 rice landraces of Bangladesh based on the alleles detected by nine micro-satellite markers.

Legend: 1-Kuttimora Birun, 2-Dudh Binni, 3-Goa Mouri, 4-Chaklashi, 5-Goar chara, 6-Hashem Iri, 7-Lal Paijam, 8-Birui, 9-Shoragot Birun, 10-Aila gota, 11-Bedha Birun, 12-Ojana Birun, 13-Lal goarchara, 14-Kaijam, 15-Kotkoti, 16-Lalcheng, 17-Puti birun, 18-Noli Goarchara, 19-Chanmoni, 20-Sentu-6, 21-Chapal-2, 22-Lomba Ail, 23-Shada Paijam, 24-Birushail, 25-Chini shail, 26-Chenger muri, 27-Laittashail, 28-Chinigura, 29-Shong Binni,30-Champa mushuri, 31-Bashiraj, 32-Gandhishail, 33-Boro Abji, 34-Malai reti, 35-Deshi-32, 36-Moynashail, 37-Maloti, 38-Chollish, 39-Paijam, 40-Sentu gold, 41-Markabinni, 42-Moyna shail, 43-Kashia Binni, 44-Chinishail-3, 45-Goabari, 46-Marry gold, 47-Lalkumri, 48-Porbot Jira, 49-Sentu-18, 50-Sentu-19, 51-Lalmatia, 52-Faijam, 53-Leda Binni, 54-Hasa Shada, 55-Lal Chinishail, 56-Peyarjat, 57-Shonajuri, 58-Mukta-10, 59-Choto Sharnalota, 60-Motamorang, 61-Chapal, 62-Ful lota, 63-Sentu-5, 64-Hashakalo, 65-Champa Mushuri, 66-Madhobi lota, 67-Sentu-16, 68-Nagra, 69-Gobinda, 70-Sentu-9, 71-Sentu-11, 72-Tulshimala, 73-Sentu-17, 74-Hashashada, 75-Laldinga, 76-Goatibinni, 77-Sentu-18, 78-Porabinni, 79-Ranishail, 80-Shonajuri, 81-Bishali Binni, 82-Kalo Birun, 83-Fulkainja

CONCLUSION

An SSR based screening of 83 rice genotypes using 09 SSR markers demonstrated a total of 262 alleles with an average of 26 alleles per locus. The highest PIC value was recorded for primer RM336 and that was the lowest for the primer RM262.Therefore, it can be concluded that RM336 was the best marker for the identification of rice genotypes followed by RM248, RM585, RM2412b, RM224, and RM493. SSR

markers used in this study were convenient and polymorphic. The genotypes G82, G77, G68, G50, and G1; G65, G37, and G10; G71 and G11; G25 and G14; G39 and G27 have 100% genetic similarity according to the pair wise genetic similarity indexes. Genotype G10 had the least similarity (0.44 percent) to genotype G9, G16 with G17, G22 with G29, G28 with G30, and so on. The dendrogram based on UPGMA and Nei's genetic distance classified the landraces into 5 clusters with a similarity coefficient of 0.6. Cluster 2 had maximum thirty-two genotypes followed by cluster 4. The landraces that were derivatives of genetically similar types were clustered together on the dendrogram. Landraces that are genetically distant such as Lalcheng and Putibirun; Shong Binni and Lomba Ail; Chinigura and Champa mushuri; Shoragot Birun and Puti Biruin could be chosen as parents for future breeding projects. This could promote diversity, leading to a high productivity index in terms of increased output and overall quality.

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