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Microsatellite based DNA fingerprinting of 28 local rice (*Oryza sativa* L.) varieties of Bangladesh

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Abstract

A total of 28 local rice (*Oryza sativa* L.) varieties of Bangladesh were selected for DNA fingerprinting with seven microsatellite DNA markers. Upon PCR amplification the alleles were separated on Agarose gel using a sequencing gel electrophoresis system. The loci were polymorphic (P_{95}) in all of the varieties. Variation was found in number of alleles, allele frequency, observed and expected heterozygosity. The primer, RM335 having motif $(CTT)_{20}$ also yielded highest number of alleles (15) and highest PIC value (0.909). Genetic differentiation (F_{st}) values were found in the ranges 0.84 to 1.00 with an average of 0.92 and gene flow (N_m) values ranged from 0.047 to 0.00 with an average of 0.02. High level genetic differentiation and low level gene flow values in 28 rice (*Oryza sativa* L.) varieties which were indicated of diversity among the varieties as most of these varieties were of landraces. Over all Nei's genetic distance value (D) ranged from nil to 2.56 among 378 varietals pairs resulting as a means of permutation combination of 28 rice varieties. The UPGMA dendrograme based on Nei's genetic distance placed the varieties into different clusters. All of the varieties were identified with at least one and/or combination of 7 primers.

Keywords: Microsatellites, DNA fingerprinting, *Oryza sativa*, Bangladesh

Introduction

Rice, *Oryza sativa* (2n = 24) belongs to the family *Gramineae* and subfamily *Oryzoidae*. Rice is the most important food crop, providing the staple food for nearly half of the global population especially in Asia, Africa, and Latin America (FAO, 2004). Rice is central to Bangladesh's economy and agriculture, providing about 75% of the calorie and 55% of the protein in the average daily diet of the people (Anonymous, 2002). Asian farmers have selected and maintained a vast variety of rice strains over thousands of years. Up till now, about 7,500 varieties have been collected and preserved in the gene bank from indigenous and exotic sources by BRRI. Out of these, nearly 5,000 varieties have been registered in the gene bank. A duplicate set of BRRI germplasm is maintained at the Genetic Resources Centre (GRC) at IRRI, for safe keeping and long-term storage as the base collection (http://banglapedia.search.com.bd/HT/G_0072.htm). Large variations of rice in respect of morphological, biochemical and DNA traits exist in Asia, as its center of origin with sub-centers of diversity more particularly in China and Indian subcontinent (Baki *et al.,* 2000; Noldin, 2000; Chen *et al.,* 2001). According to Zhimin (1999), there has been a theory that rice originated in Assam of India and Yunan of China is mostly agriculturally biased. Assam a Northeast state of India resembling Yunan province of China is on the Himalayan sub-tropic plateau. The Assam center is the closest area of Bangladesh, which is also a part of the center of origin of *Javanica* rice types with abundance of *O. rufipogon* L. and *O. nivara* L.*,* the two possible immediate ancestors of the *O. sativa* L. (Chang, 1984).

In rice, microsatellites are abundant and well distributed throughout the genome (Akagi *et al.,* 1996; McCouch *et al.,* 1997). They are valuable as genetic markers because they are codominant, detect high levels of allelic diversity, and are assayed efficiency by the Polymerase Chain Reaction (McCouch *et al.,* 1997). Microsatellites are PCR-based markers that are both technically efficient and cost-effective to use and are available for rice (Chen *et al.,* 1997; Temnykh *et al.,* 2000). Previous studies in rice have contributed to the development of several hundred microsatellite markers and a genetic map consisting of 320 SSRs (Akagi *et al.,* 1996; Panaud *et al.,* 1996; Chen *et al.,* 1997; Temnykh *et al.,* 2000. 2001). For characterization and documentation, this technique has been recently used in crop species including rice, wheat, maize, barley, rapeseed, soybean, potato and other crops by Rahman *et al.,* (2006, 2007). In Bangladesh, 9 soybean cultivars were identified by microsatellite markers, which have provided identity and might work as protection (Islam *et al.,* 2007). Thirteen maize cultivars were also characterized using microsatellite fingerprinting in combination with DUS test (Molla *et al.,* 2007). Cai and Morishima (2000) assayed sixty eight Bangladesh landraces together with 22 Asian check varieties, 8 isozyme loci by PAGE and revealed results similar to that of Glaszmann (1987).

Materials and Methods

Seeds of the local rice varieties were collected from Genetic Resource and Seed Division (GRSD) of Bangladesh Rice Research Institute (BRRI). The varieties were grown and molecular characterizations were done in the Genetic Fingerprinting Laboratory of the Department of Genetics and Plant Breeding during the period of August/2008- January/2009.

Raising of Seedlings and isolation of genomic DNA

10-12 seeds of each variety were selected randomly and dried in oven at 54°C for 48 hrs for better germination. After germination in the wet blotting paper in petridishes, the seedlings were grown in small plastic pots. Bulked DNA was isolated from 2-5 juvenile culms of 15-day-old seedlings of each of 28 rice varieties/cultivars following the protocol described by Aljanabi and Martinez (1997) and also used by Rahman *et al.*, (2007) with some modifications. Excluding usage of CTAB, the modified protocol included digestion with homogenization buffer (Solution: Tris-50 mM, EDTA-25 mM, NaCI-300 mM, 1% SDS and deionized water) at 65°C for 30 min, extraction with phenol: chloroform: isoamyl alcohol (25:24:1), precipitation with ice-cold and extra pure isopropyl alcohol and purification with absolute ethanol (Plus sodium acetate, 3M) and 70% ethanol chronologically. Finally, DNA sample of each rice variety dissolving in 30-40µl of TE buffer within 1.5 ml eppendorf tube was preserved separately at -20°C. Presence of genomic DNA was confirmed on 1% agarose gel qualitatively.

Quantification and optimization of DNA concentration

The amount of genomic DNA was quantified at 260nm spectrophotometrically (Spectronic® Genesis^{TM}). Using the absorbance reading obtained for DNA sample of each rice variety, the original DNA concentrations were determined and adjusted to 25 ng/µl.

Identification and selection of microsatellite/SSR primers

A set of seventeen microsatellite primer pairs (RM5, RM55, RM105, RM151, RM153, RM170, RM206, RM264, RM266, RM278, RM287, RM307, RM333, RM334, RM335 RM475 and RM481) distributed in the rice genome were identified from the available data-based search (http://www.gramene.org/) for rice SSR markers as described by Akagi *et al*. (1996), Panaud *et al*. (1996), Temnykh *et al*. (2000, 2001) and McCouch *et al*. (2002). From those identified primers, at first 3 to 5 primers were tested against 5 randomly selected varieties with a recommended PCR thermal profile. Based on better responsiveness in amplifying the target genomic region of template DNA, the expected PCR product sizes in base pairs was then going to check. The selected Primers were then screened against 28 varieties at a time. In this way seven primer pairs viz. RM153, RM206, RM251, RM307, RM333, RM335 and RM475 representing chromosome numbers 5, 11, 3, 4,10, 4, and 2 of rice genome (Temnykh *et al*., 2001) with clear and expected amplified product sizes were selected and used for microsatellite analysis in the present study. One (RM153) and two (RM153 and RM335) of these primers were used in the study for molecular characterization of 34 rice varieties (Rahman, *et al*., 2006) and 94 rice varieties (Rahman, *et al*., 2007) respectively.

Polymerase chain reaction (PCR)

Polymerase chain reaction were done in a volume of 10 µl containing 10x PCR Buffer, 0.25 mM each of the dNTPs. 1 µM of each of primer, 1 unit ampli Taq DNA polymerase, 50 ng template DNA and a suitable amount of sterile deionized water. Amplification was carried out in a oil free thermal cycler (Thermal cycler gradient, Eppendorf) with the following thermal profile: initial denaturation step at 94°C for 3 min. followed by 35 cycles at 95°C for 30 sec., 55°C for 30 sec. and 72°C for 1 min. and a final cycle at 72°C for 7 min. PCR was confirmed by electrophoresis on 2% agarose gel.

Electrophoretic separation and visualization of PCR products

PCR was confirmed by electrophoresis on 2% agarose gel. Prior to electrophoresis each PCR-products was prepared with loading dye (0.25% xylene cyanol, 0.25% bromophenol blue, 30% glycerol and 1 mM EDTA). Loading dye was used for monitoring the loading and the progress of electrophoresis and to increase the weight of the sample so that it stayed in the well of the gel. A molecular weight marker DNA

(100 bp) was loaded on either side of the gel. The gel was carefully placed in the electrophoresis gel chamber (Blue Marine Serva) keeping the gel horizontal and submerged in 1× TBE buffer (running buffer) and final level of buffer was ~5mm above the gel. Electrophoresis was performed at 100 V for 1 hour and 30 minutes using the power supply (EPS 301, Amersham, Pharmacia Biotech). The DNA migrate from negetive to positive electrode (black to red). The electrophoresis was stopped after the bromophenol blue dye had reached three-fourths of the gel length.PCR-products were electrophoresed on a 2% Agarose gel. Electrophoresis was done using the SequiGen GT Sequencing Gell (BIO-RAD Laboratories, Hercules, CA) electrophoresis system. Before running the gel, the power supply which connects with gel chamber was set for 2hrs and 40 minutes, 100V and 50W. For documentation, the gel was taken out from the electrophoresis chamber and placed on the high performance ultraviolet transilluminator (UVP, BioDoc-It^{TM} System) at the wave-length of 302 nm to observe the quality of the genomic DNA. Then the gel was examined and was photographed by using a Gel Cam Polaroid camera and finally saved in computer.

Scoring and analysis of microsatellite data

Microsatellite DNA profiles of all rice varieties against seven primers are shown in Fig. 1. Photograph from Gel Cam Polaroid camera was printed on 20cm X 25 cm graph paper. The bands representing particular alleles at the microsatellite loci were scored manually and designated the bands as A, B, C, etc. from the top to the bottom of the gel by four experienced scientists individually. The genotypes of different individuals were hypothetically scored as AA, BB, CC, etc. for homozygous or as AB, AC, BC etc. for heterozygous. A single genotypic data matrix was constructed for all loci. Polymorphism Information Content (PIC) was computed by deducting sum of square values for all the frequencies of different alleles produced by a single marker locus from one using the formula: $PIC= 1- \Sigma Xi^2$, where, Xi is the frequency of the i-th allele of a particular locus. PIC provides an estimate of the discriminatory power of a marker by taking into account, not only the number of alleles that are expressed, but also the relative frequencies of those alleles. PIC values range from 0 (monomorphic) to 1 (very high discriminative, with many alleles in equal frequencies). The software DNA FRAG version 3.03 was used to estimate allelic length (Nash, 1991). Expected (He) and observed heterozygosity (Ho) were also calculated as per Nei (1972) formula and with the help of POPGENE (version 1.31) (Yeh *et al.,* 1999) computer package program. Estimation of Nei's genetic distance values (D) (Nei, 1972) and construction of UPGMA (Unweighted Pair Group Method of Arithmetic Means) dendrogram was constructed using the software POPGENE (Version 1.31) (Yeh *et al.,* 1999).

Results and Discussion

Unlike morphological and biochemical markers, DNA markers are unlimited in numbers and are not affected by environmental factors and /or the developmental stages of the plant (Ovesna *et al.,* 2002, Saker *et al.,* 2005). The genetic markers arise from different classes of DNA mutations such as substitution mutation, rearrangements or errors in replication of tandemly repeated DNA.

The PIC values are dependent on the genetic diversity of the cultivars chosen and this investigation had a high proportion of traditional varieties which would have the effect of increasing the PIC values. It is important to indicate that the selection by breeders have increased the frequency of the alleles or allelic combination with favorable effects at the expense of the others, eventually eliminating many of them (Cao *et al.,* 1998). The markers in the specific chromosome and the allele size along with their frequencies and PIC values have been shown in the Table 1.

Using 7 primers across 28 rice varieties, a total of 82 alleles were found in the present study of which RM333 and RM335 showed highest number of observed number of alleles (15) followed by RM307 (14), RM251 (12), RM206 (11), RM475 (9) and RM153 (6) in descending order (Table 1). RM333 and RM335 showed highest number of alleles but effective number of allele was only highest (11.042) for RM335 (Table 2). Rahman *et al*. (2006, 2008) reported 18 and 78 alleles respectively while analyzing with 3 primers (RM11; RM151 and RM153) & 5 primers (RM1; RM151; RM153; RM334 and RM335) in which PIC values were 0.670; 0.707; 0.698 & 0.862; 0.923; 0.831; 0.865 and 0.910 respectively. Yang *et al*., (1994) found up to 25 alleles for 10 microsatellite markers among 238 accessions of *Indica* and *Japonica* cultivars and landraces.

Table 1. Size and frequency of alleles and diversity index at 7 SSR loci across 28 rice *(Oryza sativa* L.) varieties

Allele	RM-153 (Chromosome 5)	RM-206 (Chromosome 11)	RM-251 (Chromosome 3)	RM-307 (Chromosome 4)	M-333 (Chromosome 10)	RM-335 (Chromosome 4)	RM-475 (Chromosome 2)	Average
Allele A	0.0357	0.0714	0.0357	0.0357	0.0714	0.0179	0.1071	
Allele B	0.0357	0.0179	0.0536	0.0179	0.0357	0.0179	0.0179	
Allele C	0.2857	0.0893	0.0179	0.0179	0.0357	0.0357	0.0714	
Allele D	0.3214	0.0714	0.0893	0.0714	0.0357	0.0714	0.1429	
Allele E	0.2143	0.1071	0.0536	0.0893	0.0357	0.0536	0.2500	
Allele F	0.1071	0.0357	0.2143	0.1250	0.0714	0.0357	0.1964	
Allele G		0.0714	0.1429	0.0357	0.0714	0.0893	0.1250	
Allele H		0.1786	0.1071	0.0357	0.0357	0.0536	0.0357	
Allele I		0.2321	0.0357	0.1250	0.2321	0.0536	0.0536	
Allele J		0.0714	0.1250	0.0714	0.0714	0.0714		
Allele K		0.0536	0.1071	0.0357	0.1071	0.1429		
Allele L			0.0179	0.1607	0.0714	0.1607		
Allele M				0.1429	0.0357	0.0893		
Allele N				0.0357	0.0536	0.0714		
Allele O					0.0357	0.0357		
Diversity Index (PIC=1-$\sum Xi^2$)	0.755	0.869	0.878	0.897	0.897	0.909	0.841	0.864
Observed number of alleles	6	11	12	14	15	15	9	11.71
Effective number of alleles [Kimura and Crow (1964)]	4.0833	7.6863	8.2094	9.7391	9.7391	11.0423	6.3226	8.117
% 0f Effectiveness	68.05	69.88	68.41	69.57	64.93	73.61	70.25	69.243
Sequence of primers (5´-3´)	F: gcctcgagcatcatcatcag	F: cccatgcgtttaactattct	F: gaatggcaatggcgctag	F: gtactaccgacctaccgttcac	F: gtacgactacgagtgtcaccaa	F: gtt cag tgt tca gtg cca cc	F: cctcacgattttcctc caac	
	R: atcaacctgcacttgcctgg	R: cgttccatcgatccgtatgg	R: atgcggttcaagattcgatc	R: ctgctatgcatgaactgctc	R: gtcttcgcgatcactcgc	R: gaa cag aga aca gag cca cc	R: acggtgggattaga ctgtgc	
Motif	$(gaa)_9$	$(ct)_{21}$	$(ct)_{29}$	$(at)_{14}(gt)_{21}$	$(tat)_{19}(ctt)_{19}$	$(CTT)_{20}$	$(tatc)_8$	

Table 2. Summary of genetic variation and heterozygosity statistics for all loci

Locus	na*	ne*	I*	Fst	Nm*	Obs_Hom	Obs_Het	Exp_Hom*	Exp_Het*	Nei**	Ave_Het
RM153	6.0000	4.0833	1.5302	1.0000	0.0000	1.0000	0.0000	0.2312	0.7688	0.7551	0.0000
RM206	11.0000	7.6863	2.2034	0.9179	0.0224	0.8571	0.1429	0.1143	0.8857	0.8699	0.0714
RM251	12.0000	8.2094	2.2577	0.8983	0.0283	0.8214	0.1786	0.1058	0.8942	0.8782	0.0893
RM307	14.0000	9.7391	2.4232	0.9204	0.0216	0.8571	0.1429	0.0864	0.9136	0.8973	0.0714
M333	15.0000	9.7391	2.5107	0.9801	0.0051	0.9643	0.0357	0.0864	0.9136	0.8973	0.0179
RM335	15.0000	11.0423	2.5399	0.8429	0.0466	0.7143	0.2857	0.0740	0.9260	0.9094	0.1429
RM475	9.0000	6.3226	1.9797	0.9152	0.0232	0.8571	0.1429	0.1429	0.8571	0.8418	0.0714
Mean	11.7143	8.1174	2.2064	0.9232	0.0208	0.8673	0.1327	0.1201	0.8799	0.8642	0.0663
St. Dev	3.3523	2.3631	0.3564	0.0482	0.0141	0.0938	0.0938	0.0540	0.0540	0.0530	0.0469

*na = Observed number of alleles, ne = Effective number of alleles, I = Shannon's Information Index and Nm = Gene flow estimated from Fst = 0.25(1 - Fst)/Fst.
* Expected homozygosity and heterozygosity were computed using Levene (1949)
** Nei's (1973) expected heterozygosity

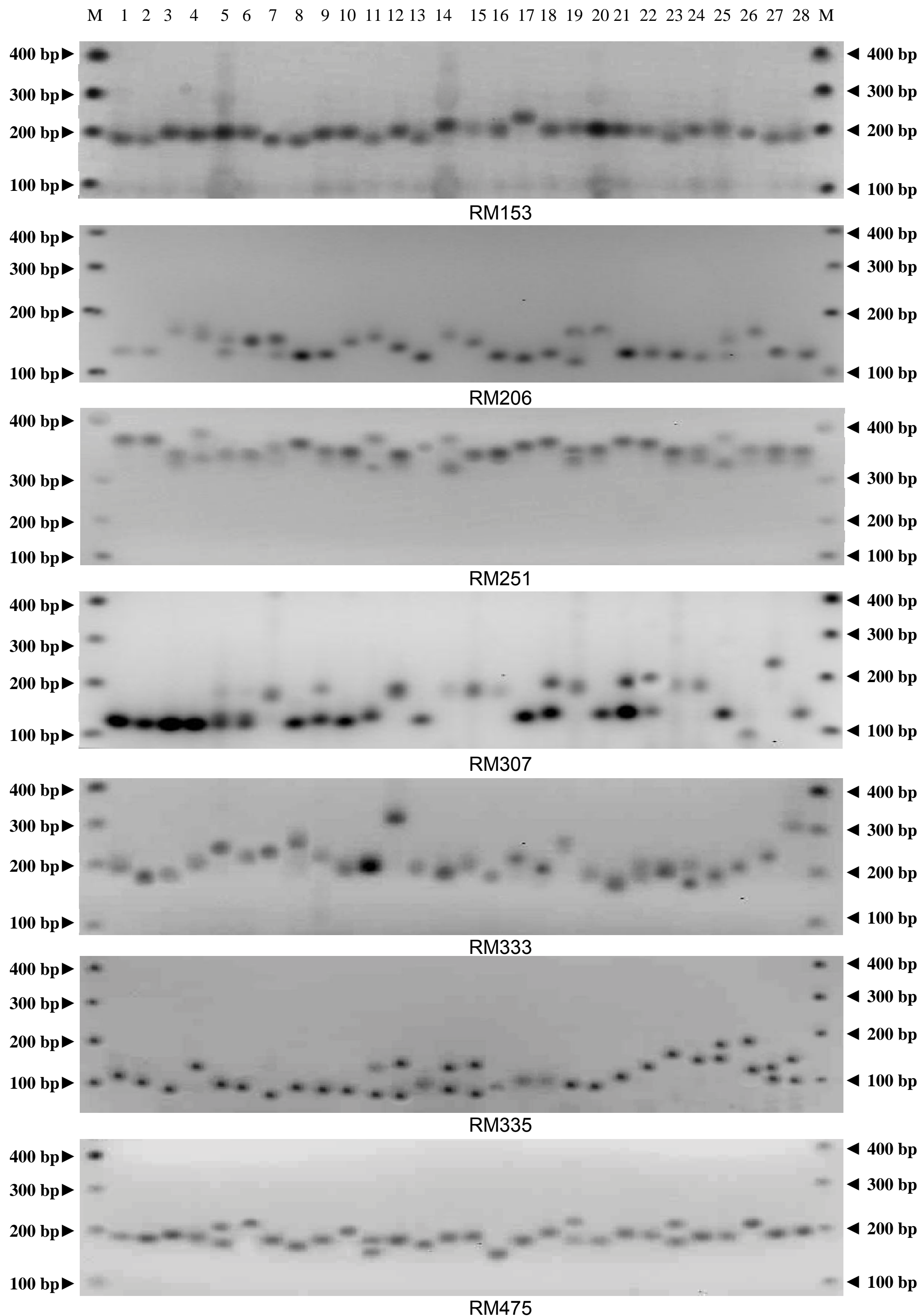

Fig. 1. A microsatellite profiles of 28 rice varieties of Bangladesh at loci, RM153, RM251, RM333, RM335 and RM475, where each consecutive lane represents a single variety. Lane, 1=Lal Amon, 2=Lau Jan, 3=Nara Aswina, 4=Buna Dhan, 5=Begun Bechi, 6=Bhasha Manik, 7=Kochu Dhola, 8=Sunga Wala, 9=Konek Chul, 10=Horinkhur Panati, 11=Ganjia, 12=Dudhsar, 13=Mathia, 14=Khirma Pat, 15=Mukut Sail, 16=Ban Kolom, 17=Safa Har (3), 18=Kal Nania, 19=Shil Pan, 20=Jabar Sail, 21=Moisha Mida, 22=Paglakushyari, 23=Pan Kaich, 24=Lal Patjat, 25=Chandda Gotok, 26=Moisha Mira, 27=Choia Mora, 28=Modhu Maloti

The PIC values for 7 primers obtained in the present study varied from 0.755 for RM153 to 0.909 for RM335, with an average PIC value of 0.864 (Table 1). Among the markers used in this study RM335 showed higher PIC values than the others. Lower PIC value may be the result of closely related genotypes and higher PIC values might be the result of diverse genotypes. The number of alleles amplified by a primer and its PIC values also depends upon the repeat number and the repeat sequence of the microsatellite sequences (Temnykh, *et al*., 2000, 2001; Yu *et al*., 2003; Ni *et al.,* 2002). Ni *et al*., (2002) showed that larger repeats and GA- repeats yield higher number of alleles and higher PIC values. Contrary to this, Temnykh *et al*., (2000) showed that (CTT), and AT-rich trinucleotide repeats amplified with higher efficiency and revealed greater polymorphism overall. RM334 and RM333 having (CTT) repeat were two most informative microsatellite markers for this set of germplasm, as they yielded 6-7 alleles and had PIC value 0.8 (Temnykh *et al*., 2001). For RM333 [(CTT)19] and RM335[(CTT)20], we observed 15 alleles and PIC value of 0.897 and 0.909, respectively which were not unusual based on repeat number and the repeat motif to that of reported by Temnykh *et al*., (2001).

Crossing over and mutation are likely to be occurred more frequently at the distant locations from the centromere position of a chromosome and therefore, enhance the possibility of forming more alleles and heterozygosity of a particular locus (Rahman *et al*., 2008). Temnykh *et al.* (2001) reported that the loci, RM153; RM206; RM251; RM307; RM333; RM335 and RM475 are located at 0.00 cM; 102.9 cM; 79.1 cM; 0.00 cM; 110.4cM; 21.5cM and 92.5cM position, respectively on chromosome 5; 11; 3; 4; 10; 4 and 2 of rice (*Oryza sativa* L.). These have been used in the present study of 28conventional rice varieties.

Genetic differentiation (Fst) values were found in the ranges of 0.84 to 1.00 with an average of 0.923 and gene flow (Nm) values ranged from 0.000 to 0.047 with an average of 0.021 (Table 2). Gao (2005) reported overall genetic differentiation and gene flow values of 0.442 and 0.316, respectively, while examined with 14 microsatellite loci in different populations of Oryza officinalis Wall. ex Watt. We observed comparatively high level of genetic differentiation and low level of gene flow values in 28 rice (0. sativa L.) varieties which are indicative of diversity among the varieties as most of the studied varieties were of land races and HYVs. In the present study, variation was found in number of alleles, allele frequency, observed and expected heterozygosity. Across 28 rice varieties, RM335 yielded highest average heterozygosity (0.142) followed by RM251 (0.089), RM206, RM307, and RM475 (0.0714) and RM153 (0.000) in descending order (Table 2). Highest heterozygosity could be explained by length, distance of RM335 on genetic map relative to centromere. The primers have been found to identify different numbers of alleles having a range between 6 in case of RM153 and 15 in case of RM335 and RM333 respectively.

Over all Nei's genetic distance value (D) ranged from nil to 2.56 among 378 varietal pairs resulting as a means of permutation combination of 28 rice varieties (Table 3). Out of 378 varietals pairs 38.89% pairs showed no genetic distance. Among the varietal pairs, 1.85% pairs were found to show highest (2.56) distance. UPGMA (The Unweighted Pair-Group Method with Arithmetic Mean) dendrogram based on Nei’s (1972) genetic distance (Table 3) indicated segregation of 28 genotypes of rice into two main clusters: In cluster 1 Lal Amon, Nara Aswina, Safa Har (3), Khirma Pat, Shil Pan, Madhu Maloti, Sunga Wala, Horinkhur Panati, Mathia, Chandda Gotok, Moisha Mira, Dudshar, Ban Kolom, Jabar Sail, Pan KaichICGV, formed sub-cluster II. Subcluster I and Subcluster II again clustering according to their genetic distance. Lau Jan, Bhasha Manik, Mukut Sail, Choia Mora, Kochu Dhola, Konen Chul, Lal Patjat, Buna Dhan, Begun bechi, Moisha Mida, Ganjia, Kal Nania and Paglakushyari grouped in cluster 2. The highest genetic distance can be explained by the fact that in one side the varieties were from the land races and on the other side the HYVs have been involved. The distance has been generated during the process of the development of HYVs. The generated distance can further be used for inclusion of gene source from the traditional varieties to more HYVs, which indicates the impact of the genetic fingerprinting and correlating the values with that of the morpho-physiological traits to find out the best performing varieties through appropriate breeding programmes.

Table 3. Summary of Nei's (1972) genetic distance values between 28 Local Rice (*Arachis hypogaea* L.) varieties for all loci

*Pop ID	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	27	28
1	**																											
2	1.25	**																										
3	0.00	1.95	**																									
4	1.22	1.91	1.50	**																								
5	1.46	1.46	1.87	0.00	**																							
6	1.95	0.00	1.25	0.00	1.18	**																						
7	2.60	0.99	0.00	0.00	2.53	0.00	**																					
8	1.95	0.85	1.95	1.91	2.56	0.00	1.50	**																				
9	2.60	0.00	1.91	0.00	1.14	1.50	0.00	1.91	**																			
10	1.95	0.00	1.95	1.91	0.95	1.25	2.60	0.00	0.99	**																		
11	1.42	1.83	0.00	0.54	0.00	0.00	2.48	0.00	0.00	1.42	**																	
12	0.00	1.91	1.91	0.00	1.14	1.91	1.87	0.00	1.47	1.50	2.08	**																
13	1.95	1.95	0.00	1.91	1.46	0.00	1.91	0.00	1.91	0.00	1.83	0.00	**															
14	1.18	0.00	1.46	1.83	0.00	2.56	0.00	2.56	2.53	0.00	3.14	1.61	0.00	**														
15	1.91	0.00	1.91	0.96	2.53	0.00	2.55	2.60	1.87	1.22	1.79	1.87	0.00	1.14	**													
16	1.95	1.95	1.95	0.00	2.56	1.95	2.60	1.95	0.00	0.00	2.52	0.00	0.00	2.56	1.91	**												
17	0.00	1.95	0.00	0.00	0.00	0.00	1.22	0.00	1.91	0.00	1.42	1.91	1.25	0.00	0.00	0.00	**											
18	1.22	1.91	1.22	0.00	1.43	1.91	2.55	1.22	1.87	1.91	0.00	1.87	0.00	1.43	0.00	1.91	1.91	**										
19	0.00	1.42	0.00	0.00	1.75	1.83	2.48	0.00	0.00	0.00	3.09	2.48	1.83	0.00	1.79	1.13	2.52	0.00	**									
20	0.00	0.00	1.95	0.00	2.56	1.95	0.00	0.00	1.22	1.95	0.00	0.00	1.95	2.56	1.91	1.25	0.00	2.60	1.83	**								
21	0.00	0.00	1.91	0.00	0.00	0.00	0.00	1.91	1.87	0.00	0.00	0.00	0.00	2.53	1.87	1.50	0.00	1.18	1.38	1.91	**							
22	1.91	0.00	0.00	1.87	0.00	0.00	0.00	0.00	1.87	0.00	0.00	0.00	1.91	1.83	0.96	1.91	0.00	0.00	1.79	1.91	1.06	**						
23	0.00	2.60	1.91	1.87	1.14	1.50	2.55	0.00	1.18	1.22	1.57	0.96	0.00	0.00	1.87	0.00	2.60	1.87	2.48	0.00	1.87	1.87	**					
24	1.91	0.00	2.60	1.47	1.83	0.00	0.00	0.00	0.00	2.60	2.48	1.87	0.00	1.83	0.96	1.91	0.00	0.00	1.38	1.91	1.87	1.18	0.96	**				
25	1.13	1.83	2.52	1.79	3.14	0.00	3.17	2.52	0.00	0.00	3.09	0.00	0.00	1.34	1.10	1.13	0.00	1.79	1.70	0.73	1.79	1.10	0.00	0.87	**			
26	0.00	0.00	1.91	1.47	1.83	1.22	0.00	0.00	1.18	0.81	1.57	1.87	0.00	0.00	1.87	0.00	0.00	1.87	2.48	1.22	0.00	0.00	0.96	2.55	0.00	**		
27	1.91	0.00	1.91	1.47	1.83	0.00	1.87	0.00	0.00	0.00	1.57	1.18	1.91	0.00	0.00	0.00	0.00	1.87	0.00	0.00	1.47	0.00	1.87	1.87	0.00	3.26	**	
28	1.50	0.00	0.00	1.87	1.83	0.00	0.00	0.00	1.87	1.91	1.79	1.18	1.91	0.00	0.00	0.00	0.00	2.55	0.00	1.91	1.87	1.87	1.18	1.47	1.57	0.00	1.18	**

*Pop ID	Variety	Pop ID	Variety	Pop ID	Variety	Pop ID	Variety
1	Lal Amon	8	Sunga wala	15	Mukut Sail	22	Paglakushyari
2	Lau Jan	9	Konek Chul	16	Bankolom	23	Pan Kaich
3	Nara Aswina	10	Horinkhur Panati	17	Safa Har (3)	24	Lal Patjat
4	Buna Dhan	11	Ganjia	18	Kal Nania	25	Chandda Gotok
5	Begun Bechi	12	Dudssar	19	Shul Pan	26	Moisha Mira
6	Bhasha Manik	13	Mathia	20	Jabar Sail	27	Choia Mora
7	Kochu Dhola	14	Khirma Pat	21	Moisha Mida	28	Modhu Maloti

Attempt was made to distinguish and identify all the varieties from one another with at least one and/or combination of 7 primers. Comparative microsatellite profiles and DNA band positions against 7 SSR primers (Table 4). Rahman *et al.* (2006 and 2008) identified 29 varieties during characterization of 34 rice varieties with 3 microsatellite markers and 93 varieties during characterization of 94 rice varieties with 5 microsatellite markers.

Table 4. Comparisons between SSR band position and selected Breeder's traits of distinctness of the rice varieties

Sl. No	Variety Name	Distinguishing primer(s)	Band positions due to primers (bp)						
			RM153	RM206	RM251	RM307	RM 333	RM335	RM475
1	Lal Amon	RM333	191	119	108	108	194	106	178
2	Lau Jan	RM333	184	119	108	104	171	88	173
3	Nara Aswina	RM333	195	150	149	104	182	84	185
4	Buna Dhan	RM251	191	145	149, 104	104	207	120	178
5	Begun Bechi	RM333/RM475	195	139, 119	140	108	242	88	200, 167
6	Bhasha Manik	RM206/RM251/RM333	195	134	145	108	212	84	205
7	Kochu Dhola	RM307/RM335	184	139, 119	126	152	228	69	173
8	Sunga Wala	RM333/RM475	184	119	113	104	296	79	161
9	Konek Chul	RM307	195	124	133	164, 108	220	79	173
10	Horinkhur Panati	RM335	195	139	133	108	207	74	190
11	Ganjia	RM251/RM335/RM475	191	145	176, 108	119	207	120, 74	173, 151
12	Dudhsar	RM206/RM333/RM335	195	129	140	164	319	125, 74	173
13	Mathia	RM307	191	114	126	113	207	88	167
14	Khirma Pat	RM153/RM251/RM335	206	150	176, 113	164	200	125, 84	178
15	Mukut Sail	RM335	200	139	149	164	207	130, 79	178
16	Ban Kolom	RM333/RM475	200	119	149	170	235	84	151
17	Safa Har (3)	RM153	222	114	126	119	220	95	173
18	Kal Nania	RM307	200	119	113	182, 124	207	95	185
19	Shil Pan	RM206/RM251/RM333/RM475	200	156, 110	154, 145	170	256	88	210, 173
20	Jabar Sail	RM153 + RM251	200	161	133	124	188	84	167
21	Moisha Mida	RM307/RM333/RM335	200	124	113	170, 129	169	100	185
22	Paglakushyari	RM251/RM307/RM335	200	124	120	195, 129	200	130	178
23	Pan Kaich	RM335/RM475	195	124	140	176	200	154	205, 173
24	Lal Patjat	RM206/RM333/RM335	200	110	140	176	207, 176	142	178
25	Chandda Gotok	RM206/RM251/RM335	200	150, 119	162, 108	124	188	175, 142	178
26	Moisha Mira	RM206/RM307/RM335	195	161	133	95	207	180, 120	205
27	Choia Mora	RM307/RM335	191	129	140	229	228	120, 100	185
28	Modhu Maloti	RM335	191	124	140	124	319	142, 106	190

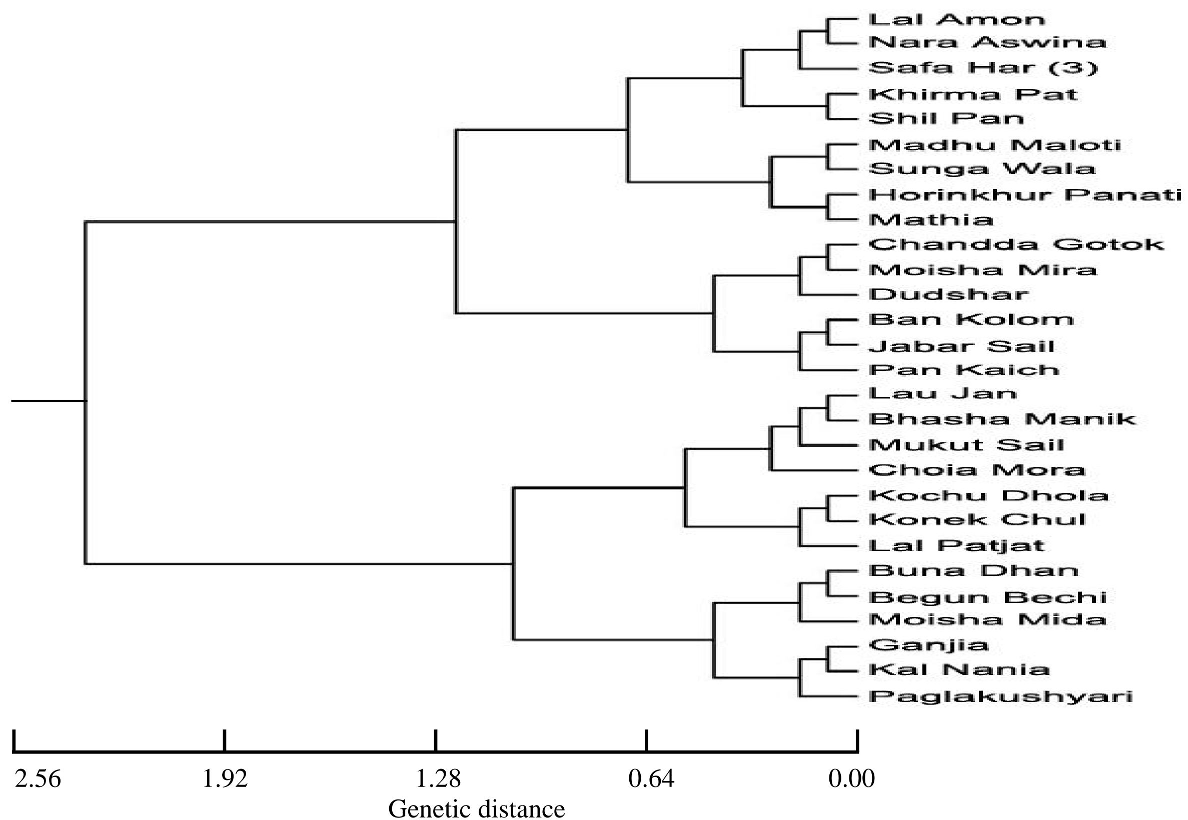

Fig. 2. UPGMA dendrogram based on Nei's (1972) genetic distance, summarizing the data on differentiation between 28 local rice varieties according to microsatellite

Conclusion

The present study showed average number of alleles of all the rice genotypes were 11.71 over the seven microsatellite loci. The coefficient of population differentiation (F_{st}) and gene flow (N_m) values across all the loci were 0.92 and 0.02 respectively. The Unweighted Pair Group Method of Arithmetic Mean (UPGMA) dendrogram based on Nei's (1972) genetic distance, resulted in two major clusters with several sub cluster. Through the present study, a total of 28 variety's specific alleles were identified with specific SSR primer. The result of the present study would be useful to know genetic variation, population structure, parentage assessment, genome mapping, Marker Assisted Selection (MAS), forensics, stock purity, etc. of different populations of the studied species before undertaking any breeding any breeding program, and also will be used as baseline information for further study. However, more extensive molecular data is needed in order to draw and conclusive remarks about the relationship between rice cultivars. Large number of samples would be necessary to determine if there are inherent differences in genetic distance between the rice cultivars. Moreover, using higher number of markers would give a clear idea about the genetic variation and genetic diversity which might be of greater interest for the plant breeders for the development of rice varieties.

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