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Microsatellite-Based DNA Fingerprinting and Genetic Analysis of Some Selected Aus Rice (*Oryza sativa* L.) Genotypes

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Abstract

The allelic diversity and molecular characterization of 30 Aus genotypes were done through DNA fingerprinting using microsatellite (SSR) markers. All the 30 amplified products have polymorphic bands giving 176 alleles. The number of alleles per locus ranged from 4 (RM536) to 20 (RM209), with an average of 9.4889 alleles across 45 loci. A total of 156 rare alleles were detected on 45 loci, whereas 31 null alleles were detected on 30 loci. The Polymorphism Information Contents (PIC) value lied between 0.5511 (RM134) to 0.9199 (RM209). Most robust marker was found to be RM209 since it provided the highest PIC value (0.9199), followed by RM144 (0.912) and RM153 (0.9070). Pair-wise genetic dissimilarity co-efficient showed that the lowest genetic dissimilarity value (0.11) was found between BRRI dhan42 and BRRI dhan43 and the highest genetic dissimilarity value (1.00) was found among all local landraces. The Unweighted Pair Group Method with Arithmetic mean (UPGMA) dendrogram revealed 6 clusters. Cluster I contain seven local landraces: Tusha, Tapa Sail, Udobali, Zamir Saita, Soda, Sail Bogi and Tarabali. Cluster II contain BR1, BR2, BR3, BR6, BR7, BR8, BR9. Cluster III has BR12, BR15, BR16, BR20, BR21. Tepakain is in cluster IV. Cluster V contains BRRI dhan27, BRRI dhan42, BRRI dhan43, BRRI dhan48, BR24, BR26 and cluster VI with Sada Bogi, Usha, Sada Aus and Saita. Most Aus landraces is recognized to have broad genetic base. Thus, these landraces can be used for future breeding program or new genes can be incorporated into the landraces to broaden the genetic base.

Keywords: DNA fingerprinting; Genetic analysis; Landraces; Polymorphism Information Contents (PIC); Aus rice

Introduction

Rice is one of the most important cereal crops which grow in all growing seasons of Bangladesh. It grows in all the three crop growing seasons of the year and occupies about 77% (11.42Mha) of the total cropped area of about 14.94 million hectares [1]. According to the United Nations (UN) estimates, the current world population 6.1 billion is expected to reach 8.0 billion by 2025. Bangladesh is already under pressure both from huge and increasing demands for food, and from problems of agricultural land and water resources depletion. Bangladesh needs to increase the rice yield in order to meet the growing demand for food emanating from population growth.

Large variations in morphological, biochemical traits and DNA polymorphism exist in rice in Asia, its center of origin, with sub-centers of diversity in China and Indian subcontinent where both indica and japonica subspecies have been found to grow with abundant variation [2,3]. There is wide genetic variability available within existing varieties of rice and wild relatives providing wide scope for future crop improvement [4,5]. As the number of rice cultivars increases, the ability to distinguish them on the basis of morphological and biochemical traits becomes more difficult mostly due to genotype-environment interaction. Any developed or derived cultivar requires clarity from its precursor for identity and protection. Both breeders and farmers tend to select among variations in their fields in order to maintain the purity of the varieties or screen for a new type. For the study of genetic diversity, the plant scientists have used generally morphological, physiological as well as molecular characterization of plant. Moreover, in most cases, plant genomes have large amount of repetitive DNA which are not expressed and do not contribute to the physiological or morphological appearance of plants. In the case of very closely related plant varieties, there are very few morphological differences, which as a matter of fact do not represent the true genetic differences at DNA level. So, there is always a need to study polymorphism at DNA level, which can be an indicative of genetic diversity [6]. It is thus apparent that the use of molecular genetic markers would provide one solution to the problem of providing unique DNA profiles for the protection of new rice cultivars. With the development of a wide range of molecular techniques, marker-assisted breeding is now used to enhance traditional breeding programs to improve crops [7]. These include Restriction Fragment Length Polymorphism (RFLP), simple sequence repeats (SSR), random amplification of polymorphic DNA (RAPD) and the Amplified Fragment Length Polymorphism (AFLP). PCR-based markers such as microsatellites are co-dominant, hyper variable, abundant and well distributed throughout the rice genome [8]. Microsatellites have shown great promise in genetic diversity,

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Table 1: List of rice genotypes used in the experiment.

Serial No.	Name of the Genotypes	Acc. No.	Origin	Serial No.	Name of the Genotypes	Acc. No.	Origin
1	Soda	1762	Jessore	16	BR6	MV	BRRI
2	Sada Bogi	1965	Jessore	17	BR7 (BRRI Balam)	MV	BRRI
3	Sail Bogi	2077	Dhaka	18	BR8 (Asha)	MV	BRRI
4	Sada Aus	2135	Dhaka	19	BR9 (Sufala)	MV	BRRI
5	Saita (sada)	3547	Faridpur	20	BR12 (Mayana)	MV	BRRI
6	Tarabali	811	Sylhet	21	BR15 (Mohinye)	MV	BRRI
7	Tepakain	1532	Dinajpur	22	BR16 (Shahi Balam)	MV	BRRI
8	Tapa Sail	1752	Khulna	23	BR20 (Nizami)	MV	BRRI
9	Tusha	4567	Dhaka	24	BR21 (Niamat)	MV	BRRI
10	Usha	4570	Dhaka	25	BR24 (Rahmat)	MV	BRRI
11	Udobali	4572	Dhaka	26	BR26 (Sraboni)	MV	BRRI
12	Zamir Saita	4044	Meherpur	27	BRRI dhan27	MV	BRRI
13	BR1 (Chandina)	MV	BRRI	28	BRRI dhan42	MV	BRRI
14	BR2 (Mala)	MV	BRRI	29	BRRI dhan43	MV	BRRI
15	BR3 (Biplob)	MV	BRRI	30	BRRI dhan48	MV	BRRI

genome mapping, gene tagging and Marker-Assisted Selection (MAS) because they are technically simple, time saving, highly informative and require small amount of DNA. Abundance of microsatellite markers is now available through the published high-density linkage map [9,10] or public database.

Bangladesh has a good source of indigenous rice cultivars comprising Boro, Aman and Aus landraces. Those landraces have good adaptation having poor yield. Actually, cultivation of these landraces was gradually replaced by high yielding varieties during the last 20 years. These landraces adapted in different parts of the country, some of which have very nice quality, fineness, aroma, taste and high protein contents [11]. Indigenous crop landraces were characterized at both molecular and phenotypic levels by many countries. Such types of characterization have been done for keeping the crop identity and searching for new genes for further crop improvement. But information on the genetic diversity of local landraces particularly for Aus rice is very scanty in Bangladesh. Precise information on the extent of genetic diversity among population is crucial in any crop improvement program, as selection of plants based on genetic diversity has become successful in several crops. Thus, the research was undertaken to make a DNA fingerprint and genetic relationship among Aus rice genotypes by analyzing the genetic diversity using SSR markers.

Materials and Methods

Plant materials

Thirty genotypes, including eighteen BRRI released Aus varieties were used in this study (Table 1). The experiment was conducted at the experimental field and laboratory of Biotechnology Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh.

DNA extraction, purification and confirmation

Genomic DNA was extracted from 21-25 days old leaves using the mini preparation modified Cetyl Trimethyl Ammonium Bromide (CTAB) method. DNA confirmation was done by using 0.8% agarose gel electrophoresis.

Documentation of the DNA samples

After electrophoresis, the gel was taken out carefully from the gel chamber and transferred in a prepared ethidium bromide solution for staining. Staining was done for 20 minutes and then placed on the UV transilluminator in the dark chamber of the Image Documentation System (Uvipro Platinum, EU). The UV light of the system was then switched on. The image was visualized on the monitor and the photograph was saved in the Gel Doc computer.

Parental Survey and Primer Selection

Primers were evaluated based on intensity of bands, consistency within individual, presence of smearing and potentiality for population discrimination. In this experiment forty-five primers viz. RM1, RM283, RM237, RM259, RM431, RM452, RM154, RM327, RM514, RM489, RM85, RM307, RM252, RM119, RM178, RM413, RM169, RM153, RM122, RM161, RM541, RM204, RM217, RM11, RM18, RM134, RM25, RM44, RM 105, RM215, RM219, RM171, RM147, RM484, RM216, RM536, RM209, RM167, RM206, RM286, RM144, RM287, RM20, RM519 and RM277 were used for parental surveys and selected.

Allele scoring

The size (in nucleotide base pairs) of the amplified band for each microsatellite marker was determined based on its migration relative to a molecular weight size marker (20bp DNA Ladder) with the help of Alpha Ease FC 4.0 software.

Analysis of SSR Data

The summary statistics including the number of alleles per locus, major allele frequency, gene diversity and Polymorphism Information Content (PIC) values were determined using POWER MARKER version 3.23 [12], a genetic analysis software. Molecular weights for microsatellite products, in base-pairs, were estimated with Alpha Ease FC4.0 software. The individual fragments were assigned as alleles of the appropriate microsatellite loci. Polymorphism

Table 2(a): Selected primers, product size, their sequences (forward and reverse), Repeat motifs and annealing temperatures.

Name of Primer	Product Size (bp)	Primer Sequence	Repeat Motif	Annealing Temp. (°C)
RM1	113	GCGAAAACACAATGCAAAAA	(GA)26	55
		GCGTTGGTTGGACCTGAC		
RM283	151	GTCTACATGTACCCTTGTTGGG	(GA)18	55
		CGGCATGAGAGTCTGTGATG		
RM237	130	CAAATCCCGACTGCTGTCC	(CT)18	55
		TGGGAAGAGAGCACTACAGC		
RM259	162	TGGAGTTTGAGAGGAGGG	(CT)17	55
		CTTGTTGCATGGTGCCATGT		
RM431	251	TCCTGCGAACTGAAGAGTTG	(AG)16	55
		AGAGCAAAACCCTGGTTCAC		
RM452	209	CTGATCGAGAGCGTTAAGGG	(GTC)9	55
		GGGATCAAACCACGTTTCTG		
RM154	183	ACCCTCTCCGCCTCGCCTCCTC	(GA)21	61
		CTCCTCCTCCTGCGACCGCTCC		
RM327	213	CTACTCCTCTGTCCCTCCTCTC	(CAT)11(CTT)5	55
		CCAGCTAGACACAATCGAGC		
RM514	259	AGATTGATCTCCCATTCCCC	(AC)12	55
		CACGAGCATATTACTAGTGG		
RM489	271	ACTTGAGACGATCGGACACC	(ATA)8	55
		TCACCCATGGATGTTGTCAG		
RM85	107	CCAAAGATGAAACCTGGATTG	(TGG)5 (TCT)12	55
		GCACAAGGTGAGCAGTCC		
RM307	174	GTACTACCGACCTACCGTTCAC	(AT)14(GT)21	55
		CTGCTATGCATGAACTGCTC		
RM252	216	TTCGCTGACGTGATAGGTTG	(CT)19	55
		ATGACTTGATCCCGAGAACG		
RM119	166	CATCCCCCTGCTGCTGCTGCTG	(GTC)6	67
		CGCCGGATGTGTGGGACTAGCG		
RM178	117	TCGCGTGAAAGATAAGCGGCGC	(GA)5 (AG)8	67
		GATCACCGTTCCCTCCGCCTGC		
RM413	79	GGCGATTCTTGGATGAAGAG	(AG)11	55
		TCCCCACCAATCTTGTCTTC		
RM169	167	TGGCTGGCTCCGTGGGTAGCTG	(GA)12	67
		TCCCGTTGCCGTTCATCCCTCC		
RM153	201	ACCAACGCCAAAAGCTACTG	(GAA)9	55
		TACTCGCCCTGCATGAGC		
RM122	227	GAGTCGATGTAATGTCATCAGTGC	(GA)7A (GA)2A (GA)11	55
		GAAGGAGGTATCGCTTTGTTGGAC		
RM161	187	TGCAGATGAGAAGCGGCGCCTC	(AG)20	61
		TGTGTCATCAGACGGCGCTCCG		
RM541	158	TATAACCGACCTCAGTGCCC	(TC)16	55
		CCTTACTCCCATGCCATGAG		
RM204	169	GTGACTGACTTGGTCATAGGG	(CT)44	55
		GCTAGCCATGCTCTCGTACC		
RM217	133	ATCGCAGCAATGCCTCGT	(CT)20	55
		GGGTGTGAACAAAGACAC		

Table 2(b): Selected primers, product size, their sequences (forward and reverse), repeat motifs and annealing temperatures.

Name of Primer	Product Size (bp)	Primer Sequence	Repeat Motif	Annealing Temp. (°C)
RM11	140	TCTCCTCTTCCCCCGATC	(GA)17	55
		ATAGCGGGCGAGGCTTAG		
RM18	157	TTCCCTCTCATGAGCTCCAT	(GA)4AA(GA)(AG)16	55
		GAGTGCCTGGCGCTGTAC		
RM134	93	ACAAGGCCGCGAGAGGATTCCG	(CCA)7	55
		GCTCTCCGGTGGCTCCGATTGG		
RM25	146	GGAAAGAATGATCTTTTCATGG	(GA)18	55
		CTACCATCAAAACCAATGTTC		
RM44	99	ACGGGCAATCCGAACAACC	(GA)16	55
		TCGGGAAAACCTACCCTACC		
RM 105	134	GTCGTCGACCCATCGGAGCCAC	(CCT)6	55
		TGGTCGAGGTGGGGATCGGGTC		
RM215	148	CAAAATGGAGCAGCAAGAGC	(CT)16	55
		TGAGCACCTCCTTCTCTGTAG		
RM219	202	CGTCGGATGATGTAAAGCCT	(CT)17	55
		CATATCGGCATTCGCCTG		
RM171	290	AACGCGAGGACACGTACTTAC	(GATG)5	55
		ACGAGATACGTACGCCTTTG		
RM147	97	TACGGCTTCGGCGGCTGATTCC	(TTCC)5 (GGT)5	55
		CCCCCGAATCCCATCGAAACCC		
RM484	299	TCTCCCTCCTCACCATTGTC	(AT)9	55
		TGCTGCCCTCTCTCTCTCTC		
RM216	146	GCATGGCCGATGGTAAAG	(CT)18	55
		TGTATAAAACCACACGGCCA		
RM536	243	TCTCTCCTCTTGTTTGGCTC	(CT)16	55
		ACACACCAACACGACCACAC		
RM209	134	ATATGAGTTGCTGTCGTGCG	(CT)18	55
		CAACTTGCATCCTCCCCTCC		
RM167	128	GATCCAGCGTGAGGAACACGT	(CT)18	55
		AGTCCGACCACAAGGTGCGTTGTC		
RM206	147	CCCATGCGTTTAACTATTCT	(CT)21	55
		CGTTCCATCGATCCGTATGG		
RM286	110	GGCTTCATCTTTGGCGAC	(GA)16	55
		CCGGATTCACGAGATAAACTC		
RM144	237	TGCCCTGGCGCAAATTTGATCC	(ATT)11	55
		GCTAGAGGAGATCAGATGGTAGTGCATG		
RM287	118	TTCCCTGTTAAGAGAGAAATC	(GA)21	55
		GTGTATTTGGTGAAAGCAAC		
RM20	234	ATCTTGTCCCTGCAGGTCAT	(ATT)14	55
		GAAACAGAGGCACATTTCATTG		
RM519	122	AGAGAGCCCCTAAATTTCCG	(AAG)8	55
		AGGTACGCTCACCTGTGGAC		
RM277	124	CGGTCAAATCATCACCTGAC	(GA)11	55
		CAAGGCTTGCAAGGGAAG		

Information Content (PIC) values were described by Anderson [13] for self-pollinated species.

Results and Discussion

The analysis of genetic diversity is very important factor for rice improvement that can be obtained through DNA fingerprinting techniques, which is capable of exhibiting large number of loci for extensive variability.

Forty-five SSR primers (Table 2a&b) were used to analyze the DNA fingerprint and genetic relationship in 30 genotypes of rice this study. In earlier studies, Chakravarthi and Naravaneni, [4] used 30, Islam et al., [14] used 85 and Ahmed et. al., [15] used 12 microsatellite makers as subset for genetic diversity analysis of *O. sativa*.

In this study, a total of 176 alleles of 45 SSR markers were detected among the 30 rice genotypes. The average number of alleles per locus was 9.4889 with a range of 4 (RM536) to 20 (RM209) (Table 2b). Similarly, Ahmed et al., [15] found a range of 3 alleles (RM234, RM277) to 15 alleles (RM493) with an average of 8.91 and Siddique et. al., [16] found 3 alleles (RM118) to 18 alleles (RM44) with an average of 9.88. Thomson et al., [17] also found the similar range of total number allele in a study from 4 alleles (RM484) to 31 alleles (RM474), with an average of 13.0 alleles across the 30 loci. Comparing microsatellite markers with the different repeat motifs, CT repeats (Table 2) had the largest number of alleles (20). These results of the study reflected that the primers RM209 (20), RM144 (17), RM307 (15), RM (15) are ideal for distinguishing the 30 genotypes as they generate more alleles (Table 2b). Yang et al., [18] also found up to 25 alleles for 10 microsatellite markers among 238 accessions of Indica and Japonica cultivars and landraces.

An allele observed in less than 5% of the 30 rice genotypes were considered to be rare. Rare alleles were observed at all of the SSR loci with an average of 3.467 rare alleles per locus and a total of 156 across all the loci (Table 3). Similarly, Ahmed et. al., [15] observed average of 3.63 rare alleles per locus and a total of 40 across all the

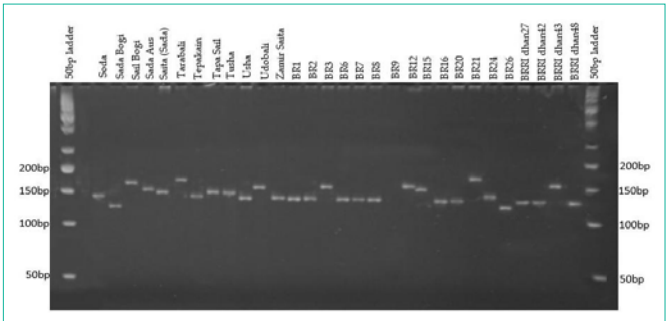

Figure 1: SSR profile of 30 rice genotypes using primer RM209.

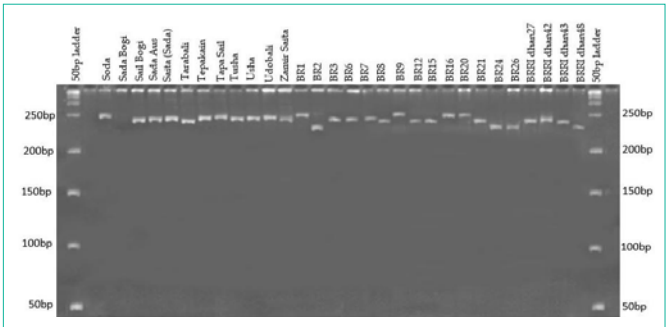

Figure 2: SSR profile of 30 rice genotypes using primer RM536.

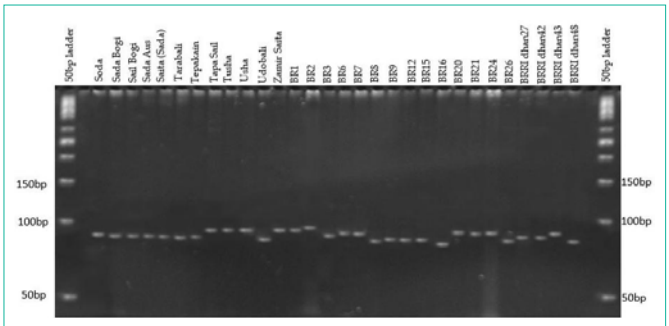

Figure 3: SSR profile of 30 rice genotypes using primer RM489.

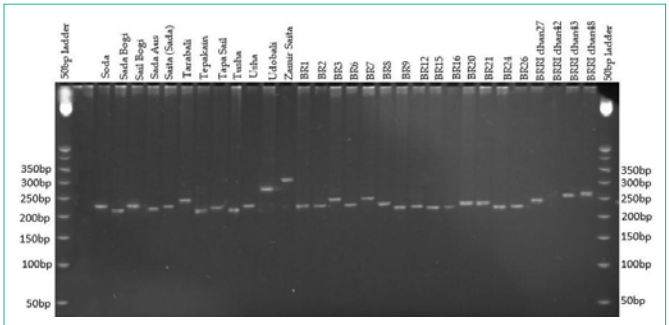

Figure 4: SSR profile of 30 rice genotypes using primer RM134.

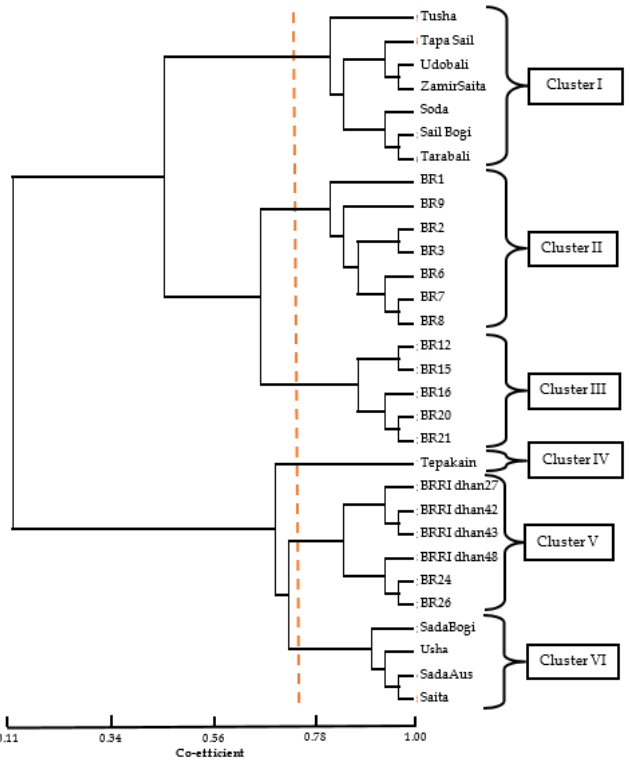

Figure 5: UPGMA dendrogram based on Nei's (1983) genetic distance differentiation among 30 rice genotypes. Arrow line indicates the scale of genetic distance (0.11-1.00).

Table 3: Data on chromosome numbers, number of alleles, allele ranges, difference of allele ranges, number of rare alleles, number of null alleles and Polymorphism Information Content (PIC) found among 30 rice genotypes for 45 microsatellite (SSR) markers.

Locus	Chr. No.	No. of Alleles	Allele Ranges	Difference	Rare Alleles	Null Alleles	PIC
RM1	1	9	80-115	35	2	0	0.8441
RM283	1	8	147-170	23	4	0	0.71
RM237	1	8	126-150	24	3	1	0.7823
RM259	1	12	152-175	23	3	0	0.8765
RM431	1	11	249-270	21	4	1	0.8156
RM452	2	8	190-217	27	1	1	0.8125
RM154	2	10	160-195	35	4	1	0.8019
RM327	2	11	202-218	16	5	1	0.8382
RM514	3	9	245-266	21	1	1	0.837
RM489	3	10	237-311	74	8	1	0.666
RM85	3	9	89-117	28	2	1	0.8177
RM307	4	15	119-173	54	7	1	0.8995
RM252	4	13	196-245	49	6	0	0.8694
RM119	4	7	160-173	13	2	1	0.7838
RM178	5	5	115-124	9	1	1	0.6047
RM413	5	11	71-101	30	4	0	0.8437
RM169	5	8	163-204	41	5	0	0.7195
RM153	5	15	177-203	26	6	1	0.907
RM122	5	8	211-238	27	1	0	0.8119
RM161	5	7	165-186	21	2	0	0.727
RM541	6	8	165-176	11	1	1	0.8202
RM204	6	8	106-144	38	3	1	0.7133
RM217	6	6	124-140	16	0	0	0.777
RM11	7	7	135-147	12	1	0	0.7222
RM18	7	9	153-171	18	4	1	0.7967
RM134	7	6	82-94	8	2	0	0.5511
RM25	8	8	128-158	30	2	1	0.7736
RM44	8	8	104-114	10	0	0	0.8388
RM105	9	11	130-142	12	4	1	0.8348
RM215	9	9	142-154	12	3	1	0.8359
RM219	9	9	195-224	29	2	0	0.7857
RM171	10	13	300-334	34	7	1	0.8637
RM147	10	6	93-99	6	1	1	0.736
RM484	10	5	293-319	26	0	1	0.6957
RM216	10	7	127-147	20	2	1	0.7063
RM536	11	4	238-250	12	0	1	0.5832
RM209	11	20	124-160	36	15	1	0.9199
RM167	11	11	121-159	38	4	1	0.8213
RM206	11	10	128-164	36	2	1	0.8381
RM286	11	14	96-129	33	6	1	0.871
RM144	11	17	214-256	42	11	1	0.912
RM287	11	10	96-119	23	4	1	0.8423
RM20	12	7	155-191	36	2	0	0.6841
RM519	12	15	116-149	33	9	0	0.8919
RM277	12	5	120-127	7	0	1	0.6121
Mean	-	**9.489**	-	**26.1111**	**3.467**	**0.667**	**0.787**

Table 4: Data on sample size, major alleles, availability, gene diversity and heterozygosity found among 30 rice genotypes for 45 microsatellite (SSR) markers.

Locus	Sample Size	Major Allele		Availability	Gene Diversity	Heterozygosity
		Size (bp)	Frequency (%)			
RM1	30	114	20	1	0.86	0
RM283	30	147	40	1	0.7444	0
RM237	30	140	26.67	1	0.8089	0
RM259	30	162	20	1	0.8867	0
RM431	30	262	33.33	1	0.8311	0
RM452	30	214	23.33	1	0.8333	0
RM154	30	190	30	1	0.8222	0
RM327	30	202	26.67	1	0.8533	0
RM514	30	245	23.33	1	0.8533	0
RM489	30	248	46.67	1	0.7022	0
RM85	30	97	30	1	0.8356	0
RM307	30	134	16.67	1	0.9067	0
RM252	30	203	23.33	1	0.88	0
RM119	30	161	23.33	1	0.8111	0
RM178	30	124	53.33	1	0.6467	0
RM413	30	101	26.67	1	0.8578	0
RM169	30	204	33.33	1	0.7578	0
RM153	30	201	16.67	1	0.9133	0
RM122	30	237	30	1	0.8311	0
RM161	30	173	40	1	0.7578	0
RM541	30	168	23.33	1	0.84	0
RM204	30	111	40	1	0.7467	0
RM217	30	134	30	1	0.8044	0
RM11	30	124	43.33	1	0.7489	0
RM18	30	160	26.67	1	0.82	0
RM134	30	88	60	1	0.5889	0
RM25	30	150	30	1	0.8	0
RM44	30	106	20	1	0.8556	0
RM105	30	138	30	1	0.8489	0
RM215	30	144	20	1	0.8533	0
RM219	30	204	30	1	0.8089	0
RM171	30	333	20	1	0.8756	0
RM147	30	96	33.33	1	0.7711	0
RM484	30	293	36.67	1	0.7378	0
RM216	30	128	43.33	1	0.7378	0
RM536	30	250	53.33	1	0.6333	0
RM209	30	158	16.67	1	0.9244	0
RM167	30	159	30	1	0.8378	0
RM206	30	161	26.67	1	0.8533	0
RM286	30	99	26.67	1	0.88	0
RM144	30	228	13.33	1	0.9178	0
RM287	30	119	23.33	1	0.8578	0
RM20	30	191	43.33	1	0.7222	0
RM519	30	131	16.67	1	0.9	0
RM277	30	120	50	1	0.66	0
Mean	**30**	**167.822**	**30.4422**	**1**	**0.8093**	**0**

Table 5: Nei's (1983) coefficient of genetic distance for SSR markers.

OTU	1	10	11	12	13	14	15	16	17	18	19	2	20	21	22	23	24	25	26	27	28	29	3	30	4	5	6	7	8	9
1	0																													
10	0.8	0																												
11	0.71	0.91	0																											
12	0.78	0.89	0.56	0																										
13	0.89	0.91	0.82	0.82	0																									
14	0.98	0.89	0.89	0.87	0.58	0																								
15	0.96	0.98	0.93	0.84	0.67	0.42	0																							
16	0.87	0.89	0.84	0.82	0.6	0.58	0.53	0																						
17	0.93	0.91	0.89	0.89	0.69	0.6	0.58	0.49	0																					
18	0.93	0.89	0.84	0.84	0.73	0.53	0.58	0.56	0.4	0																				
19	0.89	0.93	0.91	0.89	0.67	0.73	0.53	0.53	0.6	0.6	0																			
2	0.93	0.87	0.96	0.96	0.91	0.93	0.98	0.93	0.93	0.91	0.98	0																		
20	0.91	0.87	0.89	0.91	0.69	0.71	0.69	0.6	0.58	0.58	0.64	0.89	0																	
21	0.87	0.87	0.93	0.87	0.78	0.8	0.78	0.69	0.67	0.67	0.78	0.87	0.58	0																
22	0.8	0.91	0.84	0.93	0.8	0.84	0.82	0.69	0.69	0.62	0.82	0.87	0.69	0.58	0															
23	0.93	0.91	0.82	0.91	0.8	0.8	0.82	0.76	0.69	0.78	0.78	0.93	0.67	0.62	0.62	0														
24	0.87	0.96	0.89	0.89	0.82	0.82	0.78	0.78	0.78	0.8	0.78	0.91	0.71	0.73	0.58	0.53	0													
25	0.98	0.93	0.96	0.98	0.82	0.93	0.93	0.82	0.89	0.87	0.89	0.84	0.93	0.91	0.87	0.91	0.91	0												
26	**1**	0.96	0.93	0.96	0.87	0.98	0.96	0.89	0.93	0.93	0.93	0.84	0.93	0.93	0.89	0.89	0.87	0.27	0											
27	0.98	0.89	0.93	0.98	0.91	0.93	0.91	0.87	0.93	0.89	0.93	0.84	0.84	0.91	0.82	0.87	0.82	0.44	0.44	0										
28	0.98	0.96	0.89	0.96	0.91	0.91	0.91	0.93	0.96	0.89	0.96	0.87	0.89	0.96	0.87	0.89	0.82	0.47	0.49	0.4	0									
29	0.98	0.98	0.89	0.96	0.93	0.93	0.93	0.93	0.96	0.91	0.98	0.84	0.91	0.96	0.87	0.89	0.84	0.47	0.49	0.47	**0.11**	0								
3	0.58	0.82	0.71	0.76	0.82	0.87	0.91	0.87	0.89	0.89	0.84	0.93	0.93	0.89	0.89	0.84	0.87	0.98	0.98	**1**	0.98	0.96	0							
30	**1**	0.96	0.93	0.93	0.91	0.93	0.91	0.89	0.91	0.91	0.96	0.8	0.93	0.93	0.89	0.87	0.87	0.44	0.42	0.53	0.47	0.4	0.98	0						
4	0.91	0.78	0.89	0.91	0.89	0.89	0.98	0.93	0.93	0.93	0.96	0.76	0.87	0.96	0.93	0.89	0.93	0.82	0.8	0.84	0.84	0.87	0.91	0.82	0					
5	0.87	0.67	0.87	0.93	0.89	0.91	0.98	0.91	0.91	0.93	0.98	0.84	0.87	0.91	0.91	0.89	0.96	0.87	0.84	0.78	0.89	0.93	0.93	0.89	0.56	0				
6	0.69	0.82	0.64	0.71	0.87	0.91	0.91	0.87	0.87	0.91	0.87	0.93	0.89	0.87	0.84	0.84	0.84	0.96	0.93	0.96	0.96	0.96	0.49	0.96	0.89	0.87	0			
7	0.96	0.87	0.93	0.91	0.93	0.93	0.93	0.96	0.87	0.91	0.91	0.84	0.89	0.91	0.87	0.89	0.89	0.93	0.91	0.96	0.96	0.96	0.96	0.91	0.91	0.89	0.93	0		
8	0.69	0.84	0.62	0.6	0.82	0.89	0.93	0.84	0.84	0.8	0.84	0.91	0.82	0.84	0.8	0.87	0.84	0.96	0.93	0.96	0.96	0.96	0.6	0.96	0.89	0.89	0.64	0.91	0	
9	0.73	0.91	0.71	0.76	0.82	0.8	0.84	0.84	0.82	0.82	0.8	0.98	0.78	0.8	0.91	0.82	0.84	0.96	0.93	0.96	0.91	0.91	0.69	0.93	0.93	0.96	0.67	0.91	0.71	0

1 = Soda, 2 = Sada Bogi, 3 = Sail Bogi, 4 = Sada Aus, 5 = Saita (Sada), 6 = Tarabali, 7 = Tepakain, 8 = Tapa Sail, 9 = Tusha, 10 = Usha, 11 = Udobali, 12 = Zamir Saita, 13 = BR1 (Chandina), 14 = BR2 (Mala), 15 = BR3 (Biplob), 16 = BR6, 17 = BR7 (BRRI Balam), 18 = BR8 (Asha), 19 = BR9 (Sufala), 20 = BR12 (Mayana), 21 = BR15 (Mohinye), 22 = BR16 (Shahi Balam), 23 = BR20 (Nizami), 24 = BR21 (Niamat), 25 = BR24 (Rahmat), 26 = BR26 (Sraboni), 27 = BRRI dhan27, 28 = BRRI dhan42, 29 = BRRI dhan43, 30 = BRRI dhan48

loci. This result shows some dissimilarities with the average of 7 rare alleles [19]. In general, markers detecting a greater number of alleles per locus detected rarer alleles. Marker RM209 detected the highest number of alleles (20) and rare alleles (15) simultaneously. Totally absent of allele indicates null allele. In this experiment the average value of null allele is 0.6667. Among the 45 markers, 30 markers have one (1) null allele and the rest 15 markers have none (Table 3).

Polymorphism Information Content value reflects allele diversity and frequency among varieties. PIC value of each marker can be evaluated on the basis of its alleles. According to the measure of the informative nature of microsatellites, the PIC values ranged from 0.5511 (RM134) to 0.9199 (RM209) with a mean PIC value of 0.7866 (Table 3). The PIC values observed, are comparable to two previous estimates of microsatellite analysis in rice viz., 0.65- 0.91 [20], 0.59-0.90 [21], 0.08-.90 with an average 0.69 [15]. The higher PIC values in RM209 and RM144 revealed that RM209 and RM144 are the best markers for distinguishing 30 Aus landraces. PIC values also showed a significant, positive correlation with the number of alleles and allele size range for microsatellites evaluated in this study. However, lower PIC values were found in rest of the markers which may be the result of closely related genotypes and higher PIC values might be the result of diverse genotypes [22]. 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 [8,23].

Major allele is defined as the allele with the highest frequency and also known as most common allele at each locus. The size of the different major alleles at different loci ranges from 111bp (RM204) to 333bp (RM171) (Table 3). On average, 30.44% of the 30 rice

genotypes shared a common major allele ranging from 40% (RM204) to 20% (RM171) common allele at each locus. The observations show similarity with some previous studies [4,15,21].

According to Nei's [24], the highest level of gene diversity value (0.9244) was observed in loci RM209 and the lowest level of gene diversity value (0.6333) was observed in loci RM536 with a mean diversity of 0.8093 (Table 4). It was observed that marker detecting the lower number of alleles showed lower gene diversity than those, which detected higher number of alleles, which revealed higher gene diversity. The maximum number of repeats within the SSRs was also positively correlated with the genetic diversity.

Genetic similarities were calculated from the data of Nei's [24] coefficient (Table 5). The similarly matrix was used to determine the level of relatedness among the studied genotypes. Pair-wise estimates of similarity ranged from 0.11 to 1.00 with other similarities of 0.27, 0.40, 0.42, 0.44, 0.47, 0.49, 0.53, 0.56, 0.58, 0.60, 0.62, 0.6444, 0.67, 0.69, 0.71, 0.73, 0.76, 0.78, 0.80, 0.82, 0.84, 0.87, 0.89, 0.91, 0.93, 0.96 and 0.98. The lowest genetic distance (0.11) was observed in BRRI dhan42 *vs.* BRRI dhan43. The highest genetic distance of 1.00 was observed between three pairs: Soda *vs.* BRRI dhan26, Soda *vs.* BRRI dhan48 and in Sail Bogi *vs.* BRRI dhan27. Similar results were observed by Ahmed et al., [15] but Hossain et al., [25] observed highest similarity of 0.81 and lowest of 0.21, which is slightly different with this study.

Dendrogram based on Nei's [24] genetic distance using Unweighted Pair Group Method of Arithmetic Means (UPGMA) indicated differentiation of the 30 rice genotypes (by 45 markers). All 30 rice genotypes could be easily distinguished. The dendrogram obtained using the UPGMA method revealed cultivars that were genetically similar and thus clustered together and explained the relationship among the test rice varieties. The UPGMA cluster analysis led to the grouping of the 30 genotypes in six major clusters at genetic similarity level of 0.71 to 0.82 (Figure 5). Earlier observation also showed six clusters at genetic similarity level of 0.43 to -0.58 [15] and 0.40 [25]. The above results suggest that the local varieties in cluster I share common ancestors whereas, the local varieties in cluster VI are close relatives. It shows the modern varieties BR1, BR2, BR3, BR6, BR7, BR8, BR9, BR12, BR15, BR16, BR20 and BR21 are closely related to cluster I varieties at co-efficient 0.45. On the other hand, BR24, BR26, BRRI dhan27, BRRI dhan42, BRRI dhan43, BRRI dhan48 show relation to the local variety Tepakain at co-efficient 0.67 which suggests they have been developed from Tepakain. Interestingly, all the high yielding rice varieties clustered in the same group and BRRI released varieties clustered in the same group, showed the maximum divergence from the other clusters. In a previous study, 29 rice genotypes were reported to have been grouped into two distinct clusters with the aid of 20 SSR markers [26].

Conclusion

Genetic information gathered here provides unique DNA profiles for Bangladeshi Aus landraces, which will serve as a strong weapon to protect our breeders IPR. Moreover, these data will help the breeders to select parents for future breeding programs as the identification and utilization of diverse genetic resources is a prerequisite for plant improvement. Here it is found that most of the Aus landraces is recognized to have broad genetic base. Thus, it is recommended to use these landraces for future breeding program to include new and untouched landraces in order to incorporate new genes for broadening genetic base.

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