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DNA FINGERPRINTING AND GENETIC DIVERSITIES IN SOME BANGLADESHI AUS RICE (*Oryza sativa* L.) GENOTYPES

M.M. Islam[*], H.A. Begum, M.S. Ali, M. Kamruzzaman S. Hoque and M.I. Hoque

Bangladesh Institute of Nuclear Agriculture (BINA), Bangladesh Agricultural University (BAU) campus, Mymensingh-2202, Bangladesh

ABSTRACT

The allelic diversity and relationship among 120 Aus rice landraces were determined through DNA fingerprinting using microsatellite (SSR) markers. A total of 85 SSR markers were used to characterize and discriminate all tested Aus rice genotypes, 45 of which were polymorphic for different chromosome numbers. The number of alleles per locus varied from 6 alleles (RM484 and RM541) to 30 alleles (RM519) with an average of 13 alleles per locus. The polymorphic information content (PIC) values varied ranged from 0.5211 (RM536) to 0.9369 (RM519) with an average 0.8217. The highest PIC value (0.9369) was obtained for RM519 followed by RM286 (0.9357). The genetic distance-based results seen in the unrooted neighbor-joining tree clustering revealed nine genetic groups. Being grouped into distant clusters and with highest genetic distance, eleven genotypes viz., Atithi dhan, Kadar chap, Pankiraj, Japanese-7, Jamri saity, Logi jota, Joba, Lada moni, Manik Mondal-2, Boilum and Brmulka-2 could be selected as potential parents for crop improvement for their distinctive characters. Panchash and Parija had closest distance in the SSR based CS-Chord distance (0.000) might have same genetic background. The highest genetic dissimilarity (1.000) was found among the nineteen Aus genotypes combinations followed by the second highest (0.9778) among 94 Aus rice combinations. Whereas lowest genetic dissimilarity was found between Kala and Kalo Hizli (0.1778) followed by Holat and Holae (0.2667). This information will be useful in the selection of diverse parents, background selection during backcross breeding programs and assist in broadening germplasm-based rice breeding programs in the near future.

Keywords: Aus rice, genetic diversity, microsatellite markers, DNA fingerprinting

[*] Corresponding author email: mislambrri73@gmail.com

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INTRODUCTION

Rice (*Oryza sativa*) is one of the most important food crops and a primary source of food for more than half of the world's population (Khush, 2005). According to the United Nations (UN) estimates, the current world population 6.1 billion is expected to reach 8.0 billion by 2025. Most of this increase (93%) will take place in the developing world. Global rice production must reach 800 million tones of paddy rice to meet projected demand in 2025 (Peng et al., 1999) which is about 200 million tones more than rice production in 2006. This additional rice must come mainly from irrigated land in Asia, because improving rice yield in most rainfed regions is constrained by drought, flooding and poor soil quality (Cassman, 1999). 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.

For the study of genetic diversity, the plant scientists have used generally morphological, physiological as well as chemical features of plant. The number of scoreable morphological characters is varying as compared to the biological active genes. 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. Several types of molecular markers viz., RFLP, RAPD, AFLP, microsatellites and SNP have been developed. PCR-based markers such as microsatellites are co-dominant, hyper variable, abundant and well distributed throughout the rice genome (Temnykh et al., 2001). Microsatellites have shown great promise in genetic diversity, 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 (McCouch et al., 2002; IRGSP 2005) or public database. A study was conducted on 234 rice landraces in Plant breeding division, Cornell University and they identified five distinct groups corresponding to *indica, aus, aromatic, temperate japonica and tropical japonica* rice (Amanda et al., 2004). They also have very high diversity with 98% of loci polymorphic in Aus groups. Despite of their drought tolerance and early maturity, the group has received less attention compared to *indica* and *japonica* group.

There are four distinct ecotypes of rice-Boro, Aus, Transplanted aman and Deep water aman in Bangladesh. Bangladesh has a good source of indigenous rice cultivars. About 4000 T. Aman, 2500 Boro and 1500 Aus landraces are present in BRRI rice germplasm gene bank. Only a few decades ago large numbers of farmers

were growing local cultivars as their main crop. Those cultivars have good adaptation but are poor yielder. Actually cultivation of these landraces was gradually replaced by high yielding varieties during last twenty years. These landraces adapted in different parts of this country, some of which have very nice quality, fineness, aroma, taste and high protein contents (Dutta et al., 1998). After establishment of BRRI, DNA fingerprinting has been done only for a small number of local germplasm. Indigenous crop landraces were characterized at both molecular and phenotypic level 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. 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 (Ananda and Rawat, 1984; De et al., 1988). That's why, the present investigation has been undertaken in order to find out the genetic diversities among Aus genotypes at the molecular level.

MATERIALS AND METHODS

Plant materials and genomic DNA isolation

One hundred and twenty genotypes, including twelve BRRI released Aus genotypes were used in this study (Table 1). Genomic DNA was isolated from young leaves from 21 days old plants with minor modification of CTAB method. The concentration of extracted DNA was estimated by DNA confirmation test by (1.5%) agarose gel electrophoresis with lambda DNA (50ng/µl).

Table 1. List of one hundred and twenty Aus rice genotypes

SL#	Name of Genotypes	Acc No.	Origin	Collection	SL #	Name of Genotypes	Acc No.	Origin	Collection
1	Ajab Bett	1546	Chittagong	BRRI	61	Kheri Jamri	4029	Kushtia	BRRI
2	Agun Ban	1770	Jessore	BRRI	62	Khamar Mundu	4040	Meharpur	BRRI
3	Atithi dhan	4568	Dhaka	BRRI	63	Kaika	4041	Meharpur	BRRI
4	Aalo Sate	4752	Feni	BRRI	64	Kadar Chap	4042	Meharpur	BRRI
5	Begun Bahar	651	Comilla	BRRI	65	Laksmi lofa	1211	Faridpur	BRRI
6	Boilum	1205	Faridpur	BRRI	66	Lada Moni	1286	Kushtia	BRRI
7	Barmulka-2	1212	Faridpur	BRRI	67	Lagi jota	1768	Jessore	BRRI
8	Benaful	1529	Dinajpur	BRRI	68	Manik Modu	1323	Kushtia	BRRI
9	Benaful	1773	Jessore	BRRI	69	Mary satia	1626	Comilla	BRRI
10	Bathuri	1550	Chittagong	BRRI	70	Manik Mondal-1	1692	Faridpur	BRRI
11	Baisamugur	1696	Faridpur	BRRI	71	Manik Mondol-2	1765	Jessore	BRRI
12	Baismuguria	1701	Faridpur	BRRI	72	Madhu Mala	1737	Khulna	BRRI
13	Bawoi	1721	Khulna	BRRI	73	Maraka Migichak	2316	Dhaka	BRRI

SL#	Name of Genotypes	Acc No.	Origin	Collection	SL #	Name of Genotypes	Acc No.	Origin	Collection
14	Beri	1751	Khulna	BRRI	74	Mazra	4019	Faridpur	BRRI
15	Beni muri	1767	Jessore	BRRI	75	Magi Sarsa	4033	Jessore	BRRI
16	BR319-1-HR-12	3843	Dhaka	BRRI	76	Moush Doll	4036	Jessore	BRRI
17	Bora dhan	4020	Kustia	BRRI	77	Morich Boti	4043	Meharpur	BRRI
18	Baisha Muri	4026	Faridpur	BRRI	78	Mi-Mandi	4573	Dhaka	BRRI
19	Bar Pa	4027	Jhenaidhah	BRRI	79	Mi-mandisarang	4574	Dhaka	BRRI
20	Balion	4032	Faridpur	BRRI	80	Matia	4596	Noakhali	BRRI
21	Bil Kalae	4038	Jessore	BRRI	81	Nayan Tara	654	Comilla	BRRI
22	Balam	4045	Kustia	BRRI	82	Nusha Ratoi	4046	Khulna	BRRI
23	Bhatkarari	4551	Dhaka	BRRI	83	Nordi	4751	Jessore	BRRI
24	Boailla	4559	Dhaka	BRRI	84	Porangi 7	1216	Faridpur	BRRI
25	Borga Dhan	4565	Dhaka	BRRI	85	Parangi	1689	Faridpur	BRRI
26	Bali Bokri	4587	Dhaka	BRRI	86	Paik jota	1528	Dinajpur	BRRI
27	Chenri	808	Sylhet	BRRI	87	Pankirajs	1700	Faridpur	BRRI
28	Chamka	1549	Chittagong	BRRI	88	Pipre Sail	1723	Khulna	BRRI
29	Chiknal	1642	Noakhali	BRRI	89	Panburi	1730	Khulna	BRRI
30	Chitri	2081	Dhaka	BRRI	90	Padma Moni	1782	Jessore	BRRI
31	Chapila	4571	Dhaka	BRRI	91	Padha Moidu	4039	Meharpur	BRRI
32	Chakulya	4575	Dhaka	BRRI	92	Panchash	4566	Dhaka	BRRI
33	Darial	649	Comilla	BRRI	93	Parija	4588	Feni	BRRI
34	Goreswar	953	Faridpur	BRRI	94	Ratol	1772	Jessore	BRRI
35	Gutle	1774	Jessore	BRRI	95	Rathail	4047	Bagarhat	BRRI
36	Hidi 2	1289	Kushtia	BRRI	96	Saita	1681	Faridpur	BRRI
37	Holat	1551	Chittagong	BRRI	97	Sribalium	1699	Faridpur	BRRI
38	Holae	1656	Dhaka	BRRI	98	Soloi	1720	Khulna	BRRI
39	Haita saita	1691	Faridpur	BRRI	99	Sodai Soru	1725	Khulna	BRRI
40	Honuman jota	1739	Khulna	BRRI	100	Saribail	1756	Jessore	BRRI
41	Hijoli Aus	4048	Pabna	BRRI	101	Soda	1762	Jessore	BRRI
42	Haji Sail	4564	Dhaka	BRRI	102	Sail bogi	2077	Dhaka	BRRI
43	Hati Bajor	4766	Bagerhat	BRRI	103	Tarabali	811	Sylhet	BRRI
44	IR19746-28-2-2	3821	Dhaka	BRRI	104	Tepakain	1532	Dinajpur	BRRI
45	Jamri saity	1317	Kushtia	BRRI	105	Tapa sail	1752	Khulna	BRRI
46	Jamurus	1525	Dinajpur	BRRI	106	Tusha	4567	Dhaka	BRRI
47	Jagli	1761	Jessore	BRRI	107	Udobali	4572	Dhaka	BRRI
48	Japanese #7	3611	Japanese	BRRI	108	Zamir Saita	4044	Meharpur	BRRI
49	Japanese #3	3617	Japanese	BRRI	109	BR1(chandina)	MV*	-	BRRI
50	Joba	4030	Kushtia	BRRI	110	BR2 (Mala)	MV	-	BRRI
51	Korcha Muri	948	Khulna	BRRI	111	BR3(Biplob)	MV	-	BRRI
52	Khushni	952	Khulna	BRRI	112	BR6	MV	-	BRRI

SL#	Name of Genotypes	Acc No.	Origin	Collection	SL #	Name of Genotypes	Acc No.	Origin	Collection
53	Katar	1632	Chittagong	BRRI	113	BR7 (Brri Balam)	MV	-	BRRI
54	Kali Bori	1633	Jessore	BRRI	114	BR8 (Aasa)	MV	-	BRRI
55	Kamani sail	1662	Chitta. H. T	BRRI	115	BR9 (Sufala)	MV	-	BRRI
56	Kali Boro	1707	Faridpur	BRRI	116	BR12 (Mayana)	MV	-	BRRI
57	Koblerash	1728	Khulna	BRRI	117	BR15 (Mohinye)	MV	-	BRRI
58	Korcha	1755	Jessore	BRRI	118	BR16 (Shahi Balam)	MV	-	BRRI
59	Kala	4023	Jessore	BRRI	119	BR20 (Nizamy)	MV	-	BRRI
60	Kalo Hizli	4025	Kushtia	BRRI	120	BR21 (Niamat)	MV	-	BRRI

* = Modern BRRI released Aus Variety; # = Sl No. 7-94 local Aus landraces

SSR primers analysis

A total of 45 primers were selected (Table 2) to detect polymorphic DNA alleles for discriminating the tested Aus rice genotypes. Each PCR was carried out in a 10 µl reaction volume containing 1 µl of $MgCl_2$ free 10X PCR buffer with $(NH_4)_2SO_4$, 1.2 µl of 25 mM $MgCl_2$, 0.2 µl of 10 mM dNTPs, 0.2 µl of 5 U/µl Taq DNA polymerase, 0.5 µl of 10 µM forward and reverse primers and 3 µl (10ng) of DNA using a 96 well thermal cycler. The mixture was overlaid with one drop (3 µl) of mineral oil to prevent evaporation. The temperature profile used for PCR amplification comprised 94° C for 5 minutes (initial denaturation) followed by 35 cycles of 94° C for 1 minute (denaturation), 55° C for 1 minute (annealing), 72° C for 2 minutes (extension) with a final extension for 7 minutes at 72° C at the end of 35 cycles. The annealing temperatures were adjusted based on the specific requirements of each primer combination. The PCR products were mixed with gel loading dye (bromophenol blue, xylene cyanol and sucrose) and electrophoresed in 8% polyacrylamide gel using vertical poly acrylamide gels for high throughput manual genotyping. Three-Four µl of amplification products were resolved by running gel in 1X TBE buffer for 1.5 hrs to 2.5 hrs depending upon the allele size at around 90 volts and 500 mA electricity. The gels were stained in 1 µg/ml ethidium bromide and were documented using UVPRO (Uvipro Platinum, EU) gel documentation unit.

Data analysis

Size for each amplified allele was measured in base pair using Alpha-EaseFC 5.0 software. The summary statistics including the number of alleles per locus, major allele frequency, gene diversity, Polymorphism Information Content (PIC) values were determined using Power Marker version 3.25 (Liu and Muse, 2005). The allele frequency data from Power Marker was used to export in binary format (allele

presence=1 and allele absence=o) for analysis with NTSYS-pc version 2.1 (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 clustering method as implemented in NTSYS-pc.

Table 2. Selected primers, their sequence and chromosome number

Primer code	Chr No	Position (cM)	Product Size(bp)	Forward primer sequence (5' to 3')	Reverse primer sequence (5' to 3')
RM1	1	29.7	113	GCGAAAACACAATGCAAAAA	GCGTTGGTTGGACCTGAC
RM283	1	31.4	151	GTCTACATGTACCCTTGTTGGG	CGGCATGAGAGTCTGTGATG
RM237	1	115.2	130	CAAATCCCGACTGCTGTCC	TGGGAAGAGAGCACTACAGC
RM259	1	54.2	162	TGGAGTTTGAGAGGAGGG	CTTGTTGCATGGTGCCATGT
RM431	1	178.3	251	TCCTGCGAACTGAAGAGTTG	AGAGCAAAACCCTGGTTCAC
RM452	2	58.4	209	CTGATCGAGAGCGTTAAGGG	GGGATCAAACCACGTTTCTG
RM154	2	4.8	183	ACCCTCTCCGCCTCGCCTCCTC	CTCCTCCTCCTGCGACCGCTCC
RM327	2	67.45	213	CTACTCCTCTGTCCCTCCTCTC	CCAGCTAGACACAATCGAGC
RM514	3	216.4	259	AGATTGATCTCCCATTCCCC	CACGAGCATATTACTAGTGG
RM489	3	29.2	271	ACTTGAGACGATCGGACACC	TCACCCATGGATGTTGTCAG
RM85	3	231	107	CCAAAGATGAAACCTGGATTG	GCACAAGGTGAGCAGTCC
RM307	4	0	174	GTACTACCGACCTACCGTTCAC	CTGCTATGCATGAACTGCTC
RM252	4	99	216	TTCGCTGACGTGATAGGTTG	ATGACTTGATCCCGAGAACG
RM119	4	76.1	166	CATCCCCCTGCTGCTGCTGCTG	CGCCGGATGTGTGGGACTAGCG
RM178	5	118.8	117	CAGTGGGCGAGCATAGGAG	ATCCTTTTCTCCCTCTCTCG
RM413	5	26.7	79	GGCGATTCTTGGATGAAGAG	TCCCCACCAATCTTGTCTTC
RM169	5	57.9	167	TGGCTGGCTCCGTGGGTAGCTG	TCCCGTTGCCGTTCATCCCTCC
RM153	5	0-2.3	201	ACCAACGCCAAAAGCTACTG	TACTCGCCCTGCATGAGC
RM122	5	6.4	227	GAGTCGATGTAATGTCATCAGTGC	GAAGGAGGTATCGCTTTGTTGGAC
RM161	5	96.9	187	AAACTGTTTTACCCCTGGCC	ATCCCCTTCTGCGGTAAAAC
RM541	6	75.5	158	TATAACCGACCTCAGTGCCC	CCTTACTCCCATGCCATGAG
RM204	6	25.1	169	GTGACTGACTTGGTCATAGGG	GCTAGCCATGCTCTCGTACC
RM217	6	28.6	133	ATCGCAGCAATGCCTCGT	GGGTGTGAACAAAGACAC
RM11	7	47	140	TCTCCTCTTCCCCCGATC	ATAGCGGGCGAGGCTTAG
RM18	7	90.4	157	TTCCCTCTCATGAGCTCCAT	GAGTGCCTGGCGCTGTAC
RM134	7	73.2	93	ACAAGGCCGCGAGAGGATTCCG	GCTCTCCGGTGGCTCCGATTGG
RM25	8	52.2	146	GGAAAGAATGATCTTTTCATGG	CTACCATCAAAACCAATGTTC
RM44	8	60.9	99	ACGGGCAATCCGAACAACC	TCGGGAAAACCTACCCTACC
RM105	9	32.1	134	GTCGTCGACCCATCGGAGCCAC	TGGTCGAGGTGGGGATCGGGTC
RM215	9	99.4	148	CAAAATGGAGCAGCAAGAGC	TGAGCACCTCCTTCTCTGTAG
RM219	9	11.7	202	CGTCGGATGATGTAAAGCCT	CATATCGGCATTCGCCTG
RM171	10	58.1	328	AACGCGAGGACACGTACTTAC	ACGAGATACGTACGCCTTTG
RM147	10	99.8	97	TACGGCTTCGGCGGCTGATTCC	CCCCCGAATCCCATCGAAACCC
RM484	10	97.3	299	TCTCCCTCCTCACCATTGTC	TGCTGCCCTCTCTCTCTCTC
RM216	10	17.6	146	GCATGGCCGATGGTAAAG	TGTATAAAACCACACGGCCA
RM536	11	55.1	243	TCTCTCCTCTTGTTTGGCTC	ACACACCAACACGACCACAC

Primer code	Chr No	Position (cM)	Product Size(bp)	Forward primer sequence (5' to 3')	Reverse primer sequence (5' to 3')
RM209	11	73.9	134	ATATGAGTTGCTGTCGTGCG	CAACTTGCATCCTCCCCTCC
RM167	11	73.9	128	GATCCAGCGTGAGGAACACGT	AGTCCGACCACAAGGTGCGTTGTC
RM206	11	102.9	147	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG
RM286	11	0	110	GGCTTCATCTTTGGCGAC	CCGGATTCACGAGATAAACTC
RM144	11	123.2	237	TGCCCTGGCGCAAATTTGATCC	GCTAGAGGAGATCAGATGGTAGTGC ATG
RM287	11	68.6	118	TTCCCTGTTAAGAGAGAAATC	GTGTATTTGGTGAAAGCAAC
RM20	12	0	234	ATCTTGTCCCTGCAGGTCAT	GAAACAGAGGCACATTTCATTG
RM519	12	62.6	122	AGAGAGCCCCTAAATTTCCG	AGGTACGCTCACCTGTGGAC
RM277	12	57.2	124	CGGTCAAATCATCACCTGAC	CAAGGCTTGCAAGGGAAG

RESULTS

Overall microsatellite diversity

One hundred and twenty Aus genotypes were assessed for genetic variability using 45 polymorphic Microsatellite DNA markers. A total of 228 alleles were detected at the loci of 45 microsatellite markers across the Aus rice genotypes.

Table 3. Data on the number of alleles, allele size range, highest frequency allele and polymorphism information content (PIC)

Marker	Chr No	Position (cM)	Motif*	Allele no.	Size range (bp)	Highest frequency allele		PIC Value
						Size (bp)	Freq (%)	
RM1	1	29.7	(GA)26	19	70-121	79	16	0.9052
RM283	1	31.4	(GA)18	13	146-170	155	33	0.8172
RM237	1	115.2	(CT)18	7	136-150	140	36	0.7146
RM259	1	54.2	(CT)17	16	152-175	168	23	0.8672
RM431	1	178.3	(AG)16	17	235-270	262	13	0.9154
RM452	2	58.4	(GTC)9	12	185-217	190, 197	17	0.8760
RM154	2	4.8	(GA)21	25	160-220	190	15	0.9161
RM327	2	67.45	(CAT)11(CTT)5	14	193-216	215	16	0.8890
RM514	3	216.4	(AC)12	9	245-262	245, 252	18	0.8475
RM489	3	29.2	(ATA)8	18	236-271	254	18	0.8753
RM85	3	231	(TGG)5(TCT)12	7	89-117	107	35	0.7062
RM307	4	0	(AT)14(GT)21	18	129-186	148	23	0.8880
RM252	4	99	(CT)19	16	198-245	203	21	0.8829
RM119	4	76.1	(GTC)6	10	160-174	160	18	0.8508
RM178	5	118.8	(GA)5(AG)8	9	117-124	124	33	0.7682
RM413	5	26.7	(AG)11	14	70-101	82	19	0.8705
RM169	5	57.9	(GA)12	19	163-219	204	12	0.9211
RM153	5	0-2.3	(GAA)9	25	177-230	218	14	0.9332

Marker	Chr No	Position (cM)	Motif*	Allele no.	Size range (bp)	Highest frequency allele		PIC Value
						Size (bp)	Freq (%)	
RM122	5	6.4	(GA)7A(GA)2A(GA)11	8	223-238	227	28	0.8032
RM161	5	96.9	(AG)20	8	165-186	172	46	0.6917
RM541	6	75.5	(TC)16	6	158-170	168	28	0.7671
RM204	6	25.1	(CT)44	16	106-155	115	22	0.8639
RM217	6	28.6	(CT)20	11	124-155	134,137	23	0.8080
RM11	7	47	(GA)17	8	123-147	124	48	0.6227
RM18	7	90.4	(GA)4AA(GA)(AG)16	11	153-171	160	32	0.7831
RM134	7	73.2	(CCA)7	7	82-94	93	21	0.8272
RM25	8	52.2	(GA)18	14	124-158	135	29	0.8477
RM44	8	60.9	(GA)16	14	104-120	111	13	0.8907
RM105	9	32.1	(CCT)6	13	130-144	135	21	0.8539
RM215	9	99.4	(CT)16	11	144-168	147	25	0.8120
RM219	9	11.7	(CT)17	14	195-230	210	17	0.8834
RM171	10	58.1	(GATG)5	18	289-334	317	15	0.8907
RM147	10	99.8	(TTCC)5(GGT)5	7	93-99	95	37	0.7036
RM484	10	97.3	(AT)9	6	290-319	299	48	0.6233
RM216	10	17.6	(CT)18	12	122-147	128	37	0.7112
RM536	11	55.1	(CT)16	9	238-252	250	62	0.5211
RM209	11	73.9	(CT)18	17	122-160	154	18	0.8779
RM167	11	73.9	(CT)18	10	124-159	147	28	0.8056
RM206	11	102.9	(CT)21	14	126-171	132	24	0.8478
RM286	11	0	(GA)16	25	96-130	117	13	0.9357
RM144	11	123.2	(ATT)11	24	214-261	241	15	0.9218
RM287	11	68.6	(GA)21	14	96-119	103	28	0.8382
RM20	12	0	(ATT)14	9	155-191	165	25	0.8078
RM519	12	62.6	(AAG)8	30	117-150	129	10	0.9369
RM277	12	57.2	(GA)11	7	116-124	124	37	0.6556
				13			25	0.8217

* Motif of the SSR and number of repeats as previously published (http://www.gramene.org)

The highest amplicon size was produced by RM171 (334 bp) and the lowest by RM1 (70 bp). The number of alleles per locus ranged from 6 alleles (RM484 and RM541) to 30 alleles (RM519), with an average of 13 alleles across the 45 loci (Table 3). The frequency of the most common allele at each locus ranged from 10% (RM519) to 62% (RM536). On average, 25% of the 120 Aus rice genotypes shared a common major allele at any given locus. The polymorphic information content (PIC)

values were ranged 0.5211 (RM536) to 0.9369 (RM519) with an average 0.8217. The highest PIC value (0.9369) was obtained for RM519 followed by RM286 (0.9357), RM153 (0.9332), RM144 (0.9218) and RM169 (0.9211), respectively. PIC value revealed that RM519 and RM286 were considered as the best marker for 120 Aus genotypes. The gel pictures of figure 1 showed amplified fragment using primer designed for the SSR marker RM519 for all 120 genotypes.

Table 4. 100% dissimilarity of the nineteen Aus genotypes

Sl #	Combinations
1	IR19746-28-2-2 × Tapa sail
2	Holae × Sail bogi
3	IR19746-28-2-2XUdobali
4	IR19746-28-2-2 × Zamir Saita
5	Jamri saity × BR6
6	Boilum × BR6
7	Barmulka-2 × Soda
8	Mi-Mandi × Baisamugur
9	Baismuguria X Lagi jota
10	Beni muri × Lakhi Lata
11	Bar Pa × Noroi
12	Balam × Noroi
13	Bil Kalae × Padha Moidu
14	Bil Kalae × Panchash
15	Balam × Padha Moidu
16	Balam × Panchash
17	Darial × Parija
18	Holae × Parija
19	Hati Bajor × Sodai Soru

Genetic distance-based analysis

An unrooted neighbor-joining tree showing the genetic relationships among 120 Aus rice genotypes of Bangladesh was constructed based on the alleles detected by 45 microsatellite markers. The genetic distance-based results seen in the unrooted neighbor-joining tree revealed nine groups in the 120 genotypes (Figure 2). Aus genotypes of BRRI released modern varieties were clustered in the same group in the cluster IX. All Aus landraces were distributed into different cluster but Panchash (sl no.92, acc. no. 4039) were not found in any cluster, it may be duplicate with Parija (sl

no. 93, acc. no.4566). Cluster number VIII contain highest number of genotypes (23) and cluster no IV contain only one genotypes, it was Jamri saity. Furthermore, the two Aus landraces viz., Madhu Mala (73) and Khushni (52) were clustered in the same group (cluster II). Three Aus landraces (Hati Bajor, Haji Sail, and IR19746-28-2-2) were clustered distinctly in the same group (cluster VII). Cluster III and Cluster V contains same number of genotypes (21) on the other hand cluster I and cluster VI contain 17 and 19 number of genotypes, respectively. Genetic dissimilarity co-efficient was recognized between every two genotypes based on DNA profile. The highest genetic dissimilarity (1.000) was found among the nineteen Aus genotypes combinations (Table 4.) Followed by the second highest (0.9778) ninety four Aus rice combinations (Table 5). Whereas lowest (0.1778) genetic dissimilarity was found Kala and Kalo Hizli.

Table 5. 98% dissimilarity found in 98 combinations of Aus genotypes

Sl #	Combinations	Sl #	Combinations
1	Bathuri × BR6	48	Bil Kalae × Mary satia
2	Bathuri × BR12 (Mayana)	49	Boailla × Lagi jota
3	Baisamugur X Soda	50	Beri × Mazra
4	Soda × BR2 (Mala)	51	Moush Doll × Bawoi
5	Beni muri × BR319-1-HR-12	52	Mazra × Morich Boti
6	Beni muri × Bora dhan	53	Mazra × Mi-mandisarang
7	Beni muri × Agun Ban	54	Mazra × Beri
8	Beni muri × Bil Kalae	55	Mi-Mandi × Nordi
9	Beni muri × Borga Dhan	56	Mi-Mandi × BR319-1-HR-12
10	Beni muri × Bali Bokri	57	Mi-Mandi × Bar Pa
11	Beni muri × Soda	58	Mary satia × Bil Kalae
12	BR319-1-HR-12 × BR1(chandina)	59	Noroi × Bil Kalae
13	Borga Dhan × Zamir Saita	60	Lagi jota × Boailla
14	Chamka × Panchash	61	Noroi × Boailla
15	Chamka × BR1(chandina)	62	Nordi × Boailla
16	Chamka × BR2 (Mala)	63	Padha Moidu × BR15 (Mohinye)
17	Boailla × BR6	64	Panchash × BR15 (Mohinye)
18	Bhatkarari × BR7 (Brri Balam)	65	Sodai Soru × BR15 (Mohinye)
19	Atithi dhan × Soda	66	Beni muri × Padma Moni
20	Gutle × Soda	67	Beni muri × Rathail
21	Darial × Sail bogi	68	BR319-1-HR-12 × Pankliiras
22	Holat × Sail bogi	69	Bora dhan × Padma Moni

Sl #	Combinations	Sl #	Combinations
23	Darial × Tapa sail	70	Bora dhan × Sodai Soru
24	Gutle × Tapa sail	71	Balion × Padha Moidu
25	Benaful × Tarabali	72	Balion × Panchash
26	Holat× Zamir Saita	73	Boailla × Parija
27	Hijoli Aus × Zamir Saita	74	Boailla × Rathail
28	Hati Bajor × Tapa sail	75	Khushni× Chitri
29	Hati Bajor × Zamir Saita	76	Bali Bokri × Mary satia
30	Jamri saity × Zamir Saita	77	Pankliiras × Borga Dhan
31	Jagli × Soda	78	Borga Dhan X Rathail
32	Kali Bori × Soda	79	Chakulya × Parangi
33	Katar × BR8 (Aasa)	80	Darial × Pankliiras
34	Baisamugur × Kalo Hizli	81	Ratol × Rathail
35	Baisamugur × Kheri Jamri	82	Ratol × Darial
36	Lagi jota × Baisamugur	83	Barmulka-2 × Japanese #3
37	Manik Mondol-2 × BR9 (Sufala),	84	Benaful × Joba
38	Padha Moidu × Panchash	85	Panburi × Japanese #3
39	Padha Moidu × Tepakain	86	Soloi, × Haji Sail
40	Bil Kalae × Balam	87	Soloi × Hati Bajor
41	Bil Kalae × Boailla	88	Soloi × Japanese #7
42	Bil Kalae × BR16 (Sahya Balam)	89	Benaful × Moush Doll
43	Jamri saity × BR15 (Mohinye)	90	Morich Boti × Benaful
44	Jamurus × BR16 (Sahya Balam)	91	Morich Boti × Benaful
45	Japanese #3 × Baismuguria	92	Benaful × Matia
46	Khamar Mundu × Baismuguria	93	Benaful × Porangi 7
47	Bawoi × Laksmi lofa	94	Benaful × Parangi

DISCUSSIONS

In crop improvement breeding program these genetically diverse genotypes could be chosen as parents for crossing program to create genetic variability and produce transgressive segregants. It was also recognized that two Aus landraces (Panchash and Parija) were sorted out as exactly same genotypes in this analysis (zero dissimilarity) might possess same genetic background. Hence, microsatellite marker based molecular fingerprinting could serve as a potential basis in the identification of genetically distant accessions as well as in duplicate sorting of the morphologically close accessions.

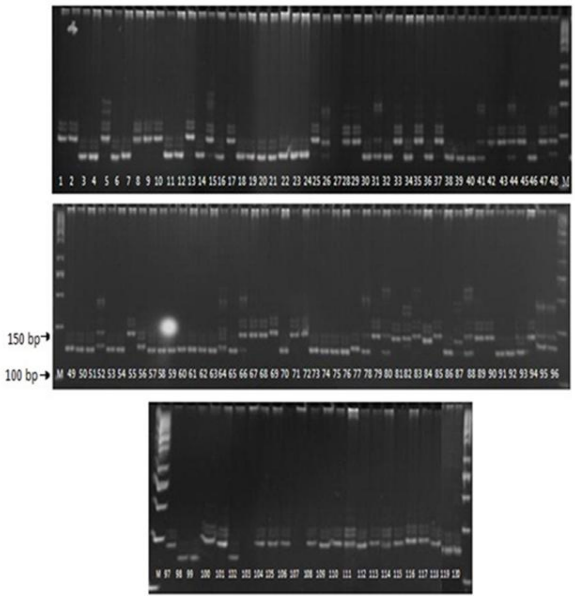

Figure 1: DNA profile of 120 Aus genotypes (108 landraces and 12 BRRI released Aus variety) with the SSR marker RM519

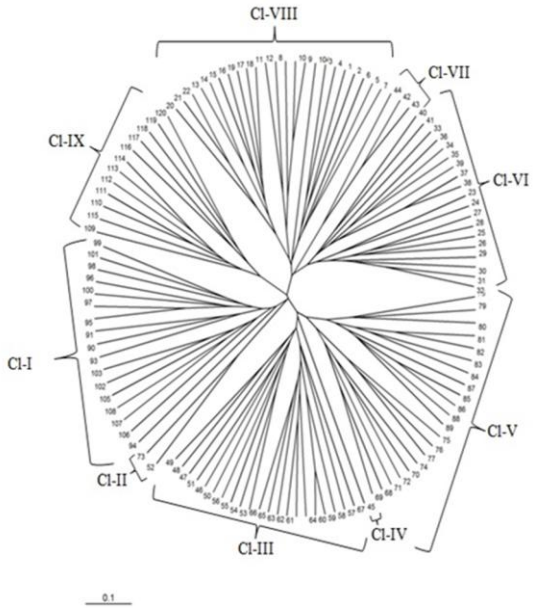

Figure 2: An unrooted neighbor-joining tree showing the genetic relationships among 120 Aus landraces based on the alleles detected by 45 microsatellite markers

In contrast, DNA-based molecular markers have proven to be powerful tools in the assessment of genetic variation and in the elucidation of genetic relationships within and among species, characterized by abundance and untouched by environmental influence (Powell et al. 1996). Ravi et al. (2003) also generated unique SSR profiles in rice by using a few primers that covered all 12 chromosomes. In the present investigation, SSR marker loci generated by 45 primers were used to assess the genetic diversity among 120 Aus rice genotypes. The SSR primers generated 228 alleles with the number of alleles per locus varying from 6 to 30. Similar number of microsatellite markers was previously used as subset for genetic diversity analysis of *O. sativa* (Garris et al.; 2005 Chakrabarthia and Naravaneni, 2006). The average number of alleles per locus was 13.0, indicating a greater magnitude of diversity among the plant materials included in this investigation. This value is comparable to 4 alleles (RM484) to 31 alleles (RM474), with an average of 13.0 alleles across the 30 loci (Thomson et al., 2007). The polymorphic information content (PIC) values were ranged 0.5211 (RM536) to 0.9369 (RM519) with an average 0.8217. The PIC values observed, are comparable to three previous estimates of microsatellite analysis in rice viz., o.67-0.88 (Gohain et al., 2006), 0.20-.90 with an average 0.560 (Jain et al., 2003). Many studies have also reported significant differences in allelic diversity among various microsatellite loci (Ravi et al., 2003). The alleles revealed by markers showed a high degree of polymorphism. The mean PIC value observed in this study was higher than the PIC value of 0.578 recorded by Ravi et al. (2003) in an earlier study among rice cultivars, landraces and wild relatives. The findings indicated that the genotypes used in the present study were more diversed due to differences in origin, ecotype and speciation. Panaud et al. (1996) studied using SSR markers in rice, described similarly high genetic similarity among landraces of common geographic origin and low similarity among landraces of diverse geographic origins.

The efficient use of SSR markers to discriminate between *Oryza* species with various genomes was also demonstrated by Cai and Morishima (2002). The multi allelic nature of SSR markers has the unambiguous advantage of discriminating between the genotypes more precisely. The Unrooted neighbor-joining tree cluster analysis of the SSR-based genetic similarity matrix resulted in the classification of Aus genotypes into separate clusters. Moreover, varietal profiling based on SSR markers will be more reliable as compared to profiling based on other markers, since SSR markers detect finer levels of variations among closely related lines. Cluster I was obtained as largest constellation and included 23 genotypes.

CONCLUSION

The allelic diversity revealed by 45 SSR primers was sufficient enough to distinguish among the tested Aus rice genotypes. The allelic variation was lower within the genotypes group than the other genotypes, indicating the possibility to exploit distant relatives to broaden the genetic base of rice.

REFERENCES

Amanda, J.G., Tai T.H., Coburn, J., Kresovich, S. and McCouch, S. 2004. Genetic Structure and Diversity in *Oryza sativa* L. *Genetics*, 169: 1631-1638.

Amanda, I.J. and Rawat, D.S. 1984. Genetic diversity, combining ability and heterosis in brown mustard. *Indian Journal of Genetics and Plant Breeding*, 44: 226-234.

Cassman, K.G. 1999. Ecological intensification of cereal production systems: Yield potential, Soil quality, and precision agriculture. Proceedings of the National Academy of Sciences (USA), 96: 5952-5959.

Cai, H. and Morishima, H. 2002. Diversity of Rice Varieties and Cropping Systems in Bangladesh Deep water Areas. JARO, 34(4): 225-231.

Chakravarthi, B.K. and Naravaneni, R. 2006. SSR marker based DNA fingerprinting and diversity study in rice (*Oryza sativa*. L). *African Journal of Biotechnology*, 5 (9): 684-688

De, R.N., Setharam, R., Sinha, M.K. and Banarjee, S.P. 1988. Genetic divergence in rice. *Indian Journal of Genetics and Plant Breeding*, 48: 189-194

Dutta, R. K., Lahiri, B. P., Baset and Mian, M. A. 1998. Characterization of some aromatic and fine rice cultivars in relation to their phsico-chemical quality of grains. *Indian Journal of plant physiology*., 3(1): 61-64

Garris, A.J., Tai, T.H. Coburn, J., Kresovich, S. and Mcouch, H. 2005. Genetic Structure and Diversity in *Oryza sativa* L. *Genetics*, 169: 1631-1638

Gohain, B., Talukdar, A. and Modi, M.K. 2006. Mining of allele(s) for aroma in local aromatic rice (joha) germplasm of Assam using SSR marker. Abstracts. 26th International Rice Conference-2, International Rice Congress 2006 October 9-13, 2006, New Delhi, India. P: 74

IRGSP, 2005. The map-based sequence of the rice genome. *Nature*, 436: 793-800

Khush, G.S. 2005. What it will take to feed 5.0 billion rice consumers in 2030. Plant Molecular Biology, 59: 1-6

Liu, K. and Muse, S.V. 2005. Power Marker: Integrated analysis environment for genetic marker data. *Bioinformatics*, 21: 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., Fjellstorm, R., Declerck, G., Schneider, D., Cartinhour, S., Ware, D. and Stein, L. 2002. Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). *DNA Research*, *9*: 199-207

Panaud, O., Chen, X. and McCouch, S. R. 1996. Development of microsatellites markers and characterization of simple sequence length polymorphism (SSLP) in rice (O. sativa L.). *Molecular Genetics and Genomics*, 252:597-607

Peng, S., Cassman, K.G., Virmani, S.S., Sheehy, J. and Khush, G.S. 1999. Yield potential trends of tropical rice since release of IR8 and the challenge of increasing rice yield potential. *Crop Science*, 39: 1552-1559

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

Ravi, M., Geethanjali, S., Sameeyafarheen, F. and Maheswaran, M. 2003. Molecular Marker based Genetic Diversity Analysis in Rice (*Oryza sativa* L.) using RAPD and SSR markers. Molecular Marker based Genetic Diversity Analysis in Rice (*Oryza sativa* L.) using RAPD and SSR markers. *Euphytica*, 133: 243-252

Rohlf, F. 2002. NTSYS-pc: Numerical taxonomy and multivariate analysis system, 2.2 edn. Department of Ecology and Evaluation, State University of NY, Stony Brook.

Temnykh, S., Clerck, W.D., Carthinour, S., Lukashova, A., lipovich, L. and McCouch, S.R. 2001. Computational and experimental analysis microsatellites in rice (*Oryza sativa* L.): frequency, length variation, transpose associations and genetic marker potential. *Genome Research*, 11: 1441-1552

Thomson, M.J., Septiningsih, E.M., Suwardjo, F., Santoso, T.J., Silitonga, T.S. and McCouch, S. R. 2007. Genetic diversity analysis of traditional and improved Indonesian rice (Oryza sativa L.) germplasm using microsatellite markers. *Theoretical and Applied Genetics*, 114(3): 559-568