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DNA fingerprinting and diversity analysis in Aus genotypes using microsatellite markers

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ABSTRACT

DNA fingerprinting and genetic diversity of 94 Aus (6 BRRI released Aus variety and 88 local Aus landraces) genotypes were carried out to protect the Aus landraces from biopiracy. A total of 91 microsatellite markers were tested for screening the genotypes. Among 91 amplified products, 56% have polymorphic bands giving 195 alleles. The number of alleles per locus ranged from four (RM25 and RM147) to twenty seven (RM519), where average allele number was 9.76. The Polymorphism Information Contents (PIC) lied between 0.455 (RM5) to 0.934 (RM519). Most robust marker was found RM519 since it provided the highest PIC value (0.934). Pair-wise genetic dissimilarity co-efficient showed the lowest genetic dissimilarity was found BRRI dhan42 and BRRI dhan43 and the highest genetic dissimilarity was found local landraces each other. Here it is shown that most Aus landraces is recognized to have broad genetic base. Thus it is recommended to use these landraces for future breeding program or include new and untouched local landraces to incorporate new genes and broaden genetic base.

Key words: Aus rice, genetic diversity, microsatellite markers, DNA fingerprinting

Introduction

Rice (*Oryza sativa*) is one of the most important food crops in the world and about three billion people, nearly half the world population, depend on rice for survival. Rice occupies 77% of total cropped area in Bangladesh. It provides 75% of the calories and 55% of proteins in the average daily diet of the Bangladeshi peoples (Bhuiyan et al., 2002). Many scientists in the world research have been conducted in the Bangladeshi local landraces, but research on indigenous landraces in our research organization is very limited. Amanda et al. (2004) were conducted a study 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's. They also found that Aus group had very high diversity with 98% of loci polymorphic out of all four groups. Although the Aus groups have a historically smaller geographical distribution and receive less attention than *indica* and *japonica* rices in breeding programs, their drought tolerance and early maturity are adaptive traits that could be usefully targeted in breeding application. Bashar et al. (2000) reported that there are four distinct ecotypes of rice – Boro, Aus, transplanted Aman and Deep water aman in Bangladesh. Only few years ago a large number of farmers grow these local cultivars as their main crop. These cultivars have good adaptation but are poor yielder. Actually, cultivation of these landraces gradually replace through high yielding variety last twenty years. Bangladesh has a good source of indigenous rice cultivars. About 4000 T. Aman, 2500 Boro and 1500 Aus landraces present in BRRI rice germplasm gene bank. These landraces are adapted in different parts of the country, some of which have very nice quality, fineness, aroma, taste and high protein contains (Dutta et al. 1998).

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RESEARCH ARTICLE

Table 1. *List of the Aus genotypes*

Sl. No.	Name of genotype	Accession No.	Sl. No.	Name of genotype	Accession No.
1	BR24 (Rahmat)	MV*	48	Changdumra	6199
2	BR26 (Sraboni)	MV	49	Bateswar	66
3	BRRI dhan 27	MV	50	Kachilon(2)	185
4	BRRI dhan42	MV	51	Panki Rat	189
5	BRRI dhan43	MV	52	Agaua	191
6	BRRI dhan48	MV	53	Achar Bhog	566
7	Kolar Thor	2074#	54	Lohar Gura	812
8	Ausa Bogi	2075	55	Nuncha	942
9	Aus Dhan	2078	56	Aus Baku	1318
10	Lemma	2080	57	Rangmahal	1629
11	Bogi	2083	58	Panoik	1641
12	Ausaloi	2090	59	Irgra	1643
13	Aus Gara Binni	2091	60	Langka Biri	1645
14	Kajli	2097	61	Lal Golang	1655
15	Gori	2098	62	Mele	1671
16	Japanese IRRI	2101	63	Gorisaita	1675
17	Kali Saita	2102	64	Goyal	1680
18	Narilel Badi	2106	65	Saita	1681
19	Lemma	2107	66	Kala Manik	1682
20	Kola Bokri	2108	67	Kautukomni	1684
21	Kotak Tar	2117	68	Korchamuri	1687
22	Gyrol	2119	69	Boilam	1688
23	Prangi	2124	70	Chaita Boro	1716
24	Putiraj	2125	71	Bador jota	1717
25	Kala Kitki	2132	72	Adhakati	1718
26	Khuida Baran	2133	73	Smriri	1729
27	Sada Aus	2135	74	Benamuri	1732
28	Maraka Migichak	2316	75	Chandra Moni	1733
29	Hasha	2338	76	Madhu Mala	1737
30	Kharai Murali	3421	77	Huma Gambir	1738
31	Aus Tarabali	3434	78	Khusni	1740
32	Munshi Murali	3441	79	Fulkati	1743
33	Saita (sada)	3547	80	Kele	1744
34	Gorba	3590	81	Lakhi Kajol	1746
35	Kali Saita	3738	82	Nara Ganbio	1750
36	Meri Dhan	3740	83	Badma	1777
37	Atha Gati	4034	84	Dubraj	1781
38	Pak jota	4035	85	Gambir	1783
39	Begum Guti	4221	86	Kalo Mucha	1784
40	Balujhuri	4569	87	Ghor Bhai	1826
41	Usha	4570	88	Goria	1852
42	Begun Bitchi	4577	89	Kachilon	1958
43	Baldara	4579	90	Sada Bogi	1965
44	Kalo Chhotna	4621	91	Bafoi	2053
45	Kalo Jamri	5627	92	Chaplo	2058
46	Kalo Sate	4752	93	Boalia	2063
47	Bahoi	4753	94	Moisha Lama	2071

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

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RESEARCH ARTICLE

After establishment of BRRI only a small number of local landraces germplasm characterization or DNA fingerprinting has been done. Many countries in the world have characterized their indigenous different crop landraces at both molecular and phenotypic level. This has been done for their crop identity and search a new gene for further crop improvement. But information about the genetic diversity of local landraces as well as Aus rice is very limited. The needs for varietals improvement for such situations are very important. Precise information about the extent of genetic diversity among population is crucial in any crop improvement program, because selection of plants based on genetic diversity has become successful in several crops (Ananda & Rawat, 1984; De et al., 1988). Therefore, research emphasis has been taken on genetic diversity for microsatellite DNA Markers in BRRI released and some local Aus landraces.

Materials and Methods

Plant material and genomic DNA isolation

The ninety four genotypes, including six 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 the extracted DNA was estimated by DNA confirmation test by (1.5%) agarose gel electrophoresis with lambda DNA (50 ng/µl).

SSR primers analysis

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 (Table 2) and 3 µl (10 ng) 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 polyacrylamide gels for high throughput manual genotyping. 3-4 µl of amplification products were resolved by running gel in 1X TBE buffer for 1.5 h to 2.5 h 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.

Results and Discussion

The ninety-four Aus genotypes including six BRRI released Aus varieties were assessed for DNA fingerprinting and genetic variability study. Ninety one microsatellite markers were used initially from which fifty one primers gave polymorphism (Table 3). One hundred ninety five alleles were detected at the loci of 51 microsatellite markers across ninety four Aus genotypes. The highest amplicon size was produced by RM171 (333 bp) and the lowest by RM413 (71 bp). These fingerprinting data will identify the genotypes very easily and protect the intellectual property rights (IPR). The highest range of band sizes was found for RM171 (289-333) followed by RM484 (290-319) and RM489 (248-314) (Table 3). Results suggested that these markers may have produced more alleles. This information could be used in further molecular characterization with other local landraces. The number of alleles per locus ranged from 4 (RM25 and RM147) to 27 (RM519) with an average of 9.77 alleles across the 51 loci. The frequency of the most common allele at each locus ranged from 13.83% (RM153) to 70.21% (RM489). On an average, 36.55% of ninety four Aus genotypes shared a common major allele at any given locus. Polymorphism Information Content (PIC) values ranged from 0.4549 to 0.9338 with an average of 0.7327. The highest PIC value (0.9338) was obtained for RM519 (Figure 1) followed by RM286 (0.9001), RM153 (0.8907) and RM 252 (0.8836), respectively (Table 3). PIC values revealed that RM519 and RM286 are the best markers for distinguishing ninety four Aus landraces. Similar results were found from previous fingerprinting and diversity studies. Number of allele per locus is comparable to 1-8 allele with an average number of 4.58 for various classes of microsatellite (Siwach et al., 2004) and 2-7 as reported by Chakrabarthi & Naravaneni (2006) and also comparable to 3 alleles to 9 alleles, with an average of 4.53 alleles per locus for 30 microsatellite markers as per Hossain et al. (2007).

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RESEARCH ARTICLE

Table 2. *Selected primers, their sequence and chromosome number*

Primer code	Forward primer sequence (5 to 3)	Reverse primer sequence (5 to 3)	Chromosome No
RM1	GCGAAAACACAATGCAAAAA	GCGTTGGTTGGACCTGAC	1
RM495	AATCCAAGGTGCAGAGATGG	CAACGATGACGAACACAACC	1
RM312	GTATGCATATTTGATAAGAG	AAGTCACCGAGTTTACCTTC	1
RM283	GTCTACATGTACCCTTGTTGGG	CGGCATGAGAGTCTGTGATG	1
RM237	CAAATCCCGACTGCTGTCC	TGGGAAGAGAGCACTACAGC	1
RM5	TGCAACTTCTAGCTGCTCGA	GCATCCGATCTTGATGGG	1
RM259	TGGAGTTTGAGAGGAGGG	CTTGTTGCATGGTGCCATGT	1
RM431	TCCTGCGAACTGAAGAGTTG	AGAGCAAAACCCTGGTTCAC	1
RM452	CTGATCGAGAGCGTTAAGGG	GGGATCAAACCACGTTTCTG	2
RM154	ACCCTCTCCGCCTCGCCTCCTC	CTCCTCCTCCTGCGACCGCTCC	2
RM327	CTACTCCTCTGTCCCTCCTCTC	CCAGCTAGACACAATCGAGC	2
RM514	AGATTGATCTCCCATTCCCC	CACGAGCATATTACTAGTGG	3
RM489	ACTTGAGACGATCGGACACC	TCACCCATGGATGTTGTCAG	3
RM85	CCAAAGATGAAACCTGGATTG	GCACAAGGTGAGCAGTCC	3
RM307	GTACTACCGACCTACCGTTCAC	CTGCTATGCATGAACTGCTC	4
RM252	TTCGCTGACGTGATAGGTTG	ATGACTTGATCCCGAGAACG	4
RM119	CATCCCCCTGCTGCTGCTGCTG	CGCCGGATGTGTGGGACTAGCG	4
RM178	CAGTGGGCGAGCATAGGAG	ATCCTTTTCTCCCTCTCTCG	5
RM413	GGCGATTCTTGGATGAAGAG	TCCCCACCAATCTTGTCTTC	5
RM169	TGGCTGGCTCCGTGGGTAGCTG	TCCCGTTGCCGTTCATCCCTCC	5
RM153	ACCAACGCCAAAAGCTACTG	TACTCGCCCTGCATGAGC	5
RM122	GAGTCGATGTAATGTCATCAGT	GAAGGAGGTATCGCTTTGTTGGAC	5
RM161	AAACTGTTTTACCCCTGGCC	ATCCCCTTCTGCGGTAAAAC	5
RM133	TTGGATTGTTTTGCTGGCTCGC	GGAACACGGGGTCGGAAGCGAC	6
RM541	TATAACCGACCTCAGTGCCC	CCTTACTCCCATGCCATGAG	6
RM204	GTGACTGACTTGGTCATAGGG	GCTAGCCATGCTCTCGTACC	6
RM11	TCTCCTCTTCCCCCGATC	ATAGCGGGCGAGGCTTAG	7
RM18	TTCCCTCTCATGAGCTCCAT	GAGTGCCTGGCGCTGTAC	7
RM25	GGAAAGAATGATCTTTTCATGG	CTACCATCAAAACCAATGTTC	8
RM44	ACGGGCAATCCGAACAACC	TCGGGAAAACCTACCCTACC	8
RM284	ATCTCTGATACTCCATCCATCC	CCTGTACGTTGATCCGAAGC	8
RM408	CAACGAGCTAACTTCCGTCC	ACTGCTACTTGGGTAGCTGACC	8
RM105	GTCGTCGACCCATCGGAGCCAC	TGGTCGAGGTGGGGATCGGGTC	9
RM215	CAAAATGGAGCAGCAAGAGC	TGAGCACCTCCTTCTCTGTAG	9
RM219	CGTCGGATGATGTAAAGCCT	CATATCGGCATTCGCCTG	9
RM171	AACGCGAGGACACGTACTTAC	ACGAGATACGTACGCCTTTG	10
RM228	CTGGCCATTAGTCCTTGG	GCTTGCGGCTCTGCTTAC	10
RM147	TACGGCTTCGGCGGCTGATTCC	CCCCCGAATCCCATCGAAACCC	10
RM484	TCTCCCTCCTCACCATTGTC	TGCTGCCCTCTCTCTCTCTC	10
RM474	AAGATGTACGGGTGGCATTC	TATGAGCTGGTGAGCAATGG	10
RM216	GCATGGCCGATGGTAAAG	TGTATAAAACCACACGGCCA	10
RM536	TCTCTCCTCTTGTTTGGCTC	ACACACCAACACGACCACAC	11
RM209	ATATGAGTTGCTGTCGTGCG	CAACTTGCATCCTCCCCTCC	11
RM167	GATCCAGCGTGAGGAACACGT	AGTCCGACCACAAGGTGCGTTGTC	11
RM206	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG	11
RM286	GGCTTCATCTTTGGCGAC	CCGGATTCACGAGATAAACTC	11
RM144	TGCCCTGGCGCAAATTTGATCC	GCTAGAGGAGATCAGATGGTAGTG	11
RM287	TTCCCTGTTAAGAGAGAAATC	GTGTATTTGGTGAAAGCAAC	11
RM20	ATCTTGTCCCTGCAGGTCAT	GAAACAGAGGCACATTTCATTG	12
RM519	AGAGAGCCCCTAAATTTCCG	AGGTACGCTCACCTGTGGAC	12
RM277	CGGTCAAATCATCACCTGAC	CAAGGCTTGCAAGGGAAG	12

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RESEARCH ARTICLE

Table 3. *Data summary for 51 SSR markers across 94 Aus landraces*

Marker	Chr. No	Position (bp)	Allele No	Size range	Major Allele	Major Allele Frequency	PIC Value
RM495	1	0.21	7	148-162	159	0.4149	0.6404
RM1	1	4.63	10	80-115	80	0.3723	0.7741
RM312	1	20.69	7	100-118	107	0.6064	0.5459
RM283	1	4.88	12	146-163	150	0.4255	0.7226
RM237	1	33.29	6	126-139	132	0.2660	0.7434
RM5	1	24.13	5	110-116	114	0.6596	0.4549
RM259	1	7.44	9	152-172	170	0.3511	0.7788
RM431	1	39.22	11	235-262	249	0.2766	0.8302
RM452	2	9.50	7	195-209	200	0.3511	0.6974
RM154	2	1.08	13	165-190	172	0.2766	0.8114
RM327	2	19.49	8	202-218	217	0.2660	0.8229
RM514	3	35.22	8	245-266	255	0.4894	0.6311
RM489	3	4.31	12	248-314	248	0.7021	0.4740
RM85	3	66.76	9	89-117	93	0.3511	0.7576
RM307	4	0.00	11	113-183	136	0.3830	0.7627
RM252	4	45.21	14	194-224	203	0.1702	0.8836
RM119	4	21.22	7	160-173	166	0.2660	0.8099
RM178	5	25.08	5	115-124	122	0.5213	0.6358
RM413	5	2.19	13	71-101	82	0.3723	0.7769
RM169	5	7.47	11	162-190	173	0.2553	0.8366
RM153	5	0.67	12	185-218	194	0.1383	0.8907
RM122	5	0.29	10	211-231	223	0.2979	0.7916
RM161	5	27.89	13	162-186	165	0.2340	0.8690
RM133	6	0.00	9	216-227	226	0.3404	0.7852
RM541	6	2.72	14	166-193	176	0.2447	0.8490
RM204	6	3.17	9	106-123	115	0.3298	0.7186
RM11	7	19.25	7	122-144	124	0.6170	0.5483
RM18	7	25.65	13	140-171	164	0.3085	0.8198
RM25	8	52.2	4	125-139	139	0.6489	0.4814
RM44	8	2.88	9	104-118	111	0.4255	0.6989
RM284	8	21.08	8	133-145	143	0.2660	0.8102
RM408	8	0.12	6	118-124	120	0.2660	0.7529
RM 105	9	9.28	6	135-144	140	0.4787	0.6457
RM215	9	21.18	8	142-165	154	0.3298	0.7387
RM219	9	3.38	15	191-228	204	0.2553	0.8183
RM171	10	18.79	12	289-333	301	0.3191	0.8087
RM228	10	21.98	10	104-136	114	0.6596	0.5189
RM147	10	20.68	4	93-98	94	0.4574	0.5607
RM484	10	20.80	5	290-319	299	0.5745	0.5113
RM474	10	1.80	9	185-285	195	0.2553	0.8211
RM216	10	5.10	5	127-147	128	0.5851	0.5480
RM536	11	8.96	9	222-252	238	0.2872	0.7651
RM209	11	17.77	14	124-162	154	0.2128	0.8533
RM167	11	4.07	13	121-159	127	0.2447	0.8431
RM206	11	21.97	13	126-171	134	0.4149	0.7507
RM286	11	0.38	21	98-127	108	0.1702	0.9001
RM144	11	28.24	8	216-241	228	0.3298	0.7929
RM287	11	16.73	7	97-118	100	0.2660	0.7672
RM20	12	0.97	7	155-180	155	0.3404	0.7226
RM519	12	19.90	27	116-150	121	0.1489	0.9338
RM277	12	16.53	6	116-127	124	0.4149	0.6629
Mean			9.7647			0.3655	0.7327

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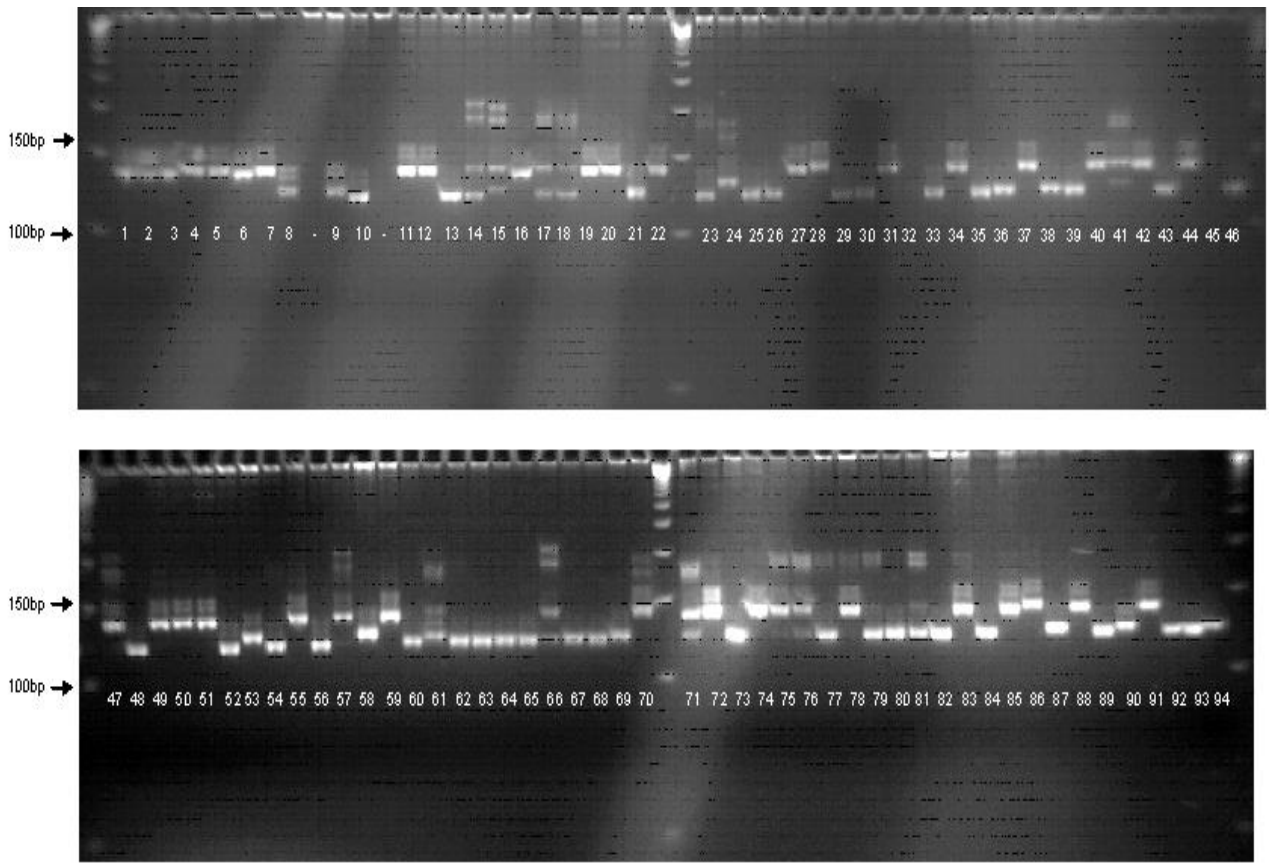

Figure 1. *DNA profile of 94 Aus genotypes (6 BRRI released and 88 local Aus) with the SSR marker RM 519.*

Legend : Lane1=BR24, 2=BR26, 3=BRRI dhan27, 4=BRRI dhan42, 5=BRRI dhan43, 6=BRRI dhan48, 7=Kolar thor , 8=Aus boga, 9=Aus dhan, 10=Lemma(1) ,11=Bogi, 12=Ausaloi, 13=Aus garra binni, 14=kajli,15=Gori, 16=Japanese IRRI, 17=Kali saita, 18=Narilel badi, 19=Lemma(2), 20=Kola bokri, 21=Kotak tar, 22=Gyrol, 23=Prangi, 24=Putiraj, 25=Kala kitki, 26=Khuida baran, 27=Sada Aus, 28=Maraka Migichak, 29=Hasha, 30=Kharai Murali, 31=Aus tarabali, 32=Munshi murali, 33=Saita(sada), 34=Gorba, 35=Kali Saita, 36=Meri dhan, 37=Atha Gati, 38=Pak jota, 39=Begum Guti, 40=Balujhuri, 41=Usha, 42=Begun Bitchi, 43=Baldara, 44=Kalo chhotna, 45=Kalo Jamri, 46=Kalo sate, 47=Bahoi, 48=Changdumra, 49=Bateswar 50=Kachilon(2), 51=Panki Rat, 52=Agaua, 53=Achar Bhog, 54=Lohar Gura, 55=Nuncha, 56=Aus Baku, 57=Rangmahal, 58=Panoik, 59=Irgra, 60=Langka biri ,61=Lal golang, 62=Mele, 63=Gorisatia, 64=Goyal, 65=Saita, 66=Kala manik, 67=Kautukomni, 68=Korchamuri, 69=Boilam, 70=Chaita Boro, 71=Bador jota, 72=Adhakati, 73=Smriri, 74=Benamuri, 75=Chandra Moni, 76=Madhu mala, 77=Huma Gambir, 78=Khusni, 79=Fulkati, 80=Kele, 81=Lakhi kajol, 82=Nara ganbio, 83=Badma, 84=Dudraj, 85=Gambir, 86=Kalo mucha, 87=Ghor Bhai, 88=Goria, 89=Kachilon, 90=Sada Bogi, 91=Bafoi, 92=Chaplo, 93=Boalia, and 94=Moisha Lama.

The Rahman et al. (2009) found an average of 6.33 alleles per locus. They included Bangladeshi high yielding varieties, local cultivars and wild rice for their study. Therefore they found higher of average allele number per locus. We can compare our frequency for most common alleles as Thomson et al. (2007) where they found 21% (RM154) to 73% (RM214). The PIC values observed, are comparable to two previous estimates of microsatellite analysis in rice viz. 0.20-0.90 with an average 0.56 (Jain et al., 2003) and 0.30-0.84 with an average of 0.58 (Hossain et al., 2007).

Pair-wise genetic dissimilarity (Table 4) coefficients were measured among the test entries. Highest genetic dissimilarity (1.000) was found among the Aus genotypes of Ausa Bogi, Aus gram binni, Monshi murali, Kachilon (2), Panki rat, Agaua, Chandra moni and Khusni, whereas lowest genetic dissimilarity was found between BRRI dhan42 and BRRI dhan43 (0.12) followed by Kautukomni and Korchamuri (0.1373). Most local Aus genotypes showed broad genetic base whereas BRRI released modern Aus variety showed narrow genetic base.

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RESEARCH ARTICLE

Table 4. *Pair -wise genetic dissimilarity coefficient (cont.)*

Genotypes	1	3	5	7	9	11	13	15	17	19	21	23	25	27	29	31
2	0.92	0.94	0.96	0.76	0.71	0.59	0.92	0.92	0.71	0.84	0.84	0.80	0.90	0.75	0.73	0.84
4	0.65	0.94	0.96	0.86	0.80	0.67	0.63	0.82	0.69	0.92	0.92	0.73	0.80	0.73	0.80	0.78
6	0.47	0.53	0.59	0.94	0.84	0.84	0.00	0.94	0.82	0.92	0.94	0.90	0.88	0.82	0.90	0.92
8	0.88	**0.98**	0.86	0.78	0.94	0.84	0.92	0.73	0.86	0.71	0.69	0.92	0.90	0.82	0.82	0.90
10	0.76	0.67	0.63	0.90	0.92	**0.98**	0.67	0.84	0.92	0.88	0.88	0.98	0.92	0.94	0.96	0.98
12	0.90	**1.00**	0.90	0.76	0.82	0.76	0.94	0.73	0.78	0.76	0.75	0.94	0.90	0.92	0.88	0.94
14	0.90	**1.00**	0.90	0.82	0.92	0.86	0.94	0.71	0.84	0.82	0.84	0.94	0.92	0.96	0.92	0.92
16	0.90	**1.00**	0.92	0.84	0.86	0.78	0.86	0.65	0.73	0.61	0.61	0.94	0.94	0.86	0.92	0.94
18	0.92	0.94	0.94	0.86	0.86	0.86	**1.00**	0.55	0.88	0.63	0.69	0.90	0.88	0.92	0.84	0.92
20	0.80	**1.00**	0.96	0.86	0.71	0.67	0.86	0.90	0.69	0.88	0.90	0.82	0.84	0.75	0.78	0.76
22	0.94	0.90	0.94	0.78	0.80	0.84	**1.00**	0.45	0.92	0.59	0.67	0.94	0.92	0.94	0.96	0.94
24	0.92	0.86	0.90	0.76	0.78	0.80	0.90	0.22	0.86	0.59	0.33	0.90	0.92	0.86	0.86	0.94
26	0.82	0.88	0.90	0.75	0.49	0.49	0.80	0.88	0.22	0.84	0.88	0.90	0.92	0.86	0.92	0.92
28	0.96	0.94	0.94	0.84	0.76	0.75	0.90	0.61	0.84	**0.14**	0.41	0.94	0.90	0.86	0.86	0.92
30	0.90	0.98	0.96	0.84	0.80	0.75	0.90	0.63	0.82	0.39	0.33	0.90	0.84	0.86	0.86	0.88
32	0.75	0.90	0.86	0.82	0.84	0.88	0.82	0.94	0.90	0.94	0.92	**0.12**	0.45	0.41	0.47	0.47
34	0.82	0.94	0.92	0.86	0.86	0.90	0.90	0.92	0.90	0.92	0.88	0.00	0.47	0.47	0.45	0.47
36	0.80	0.92	0.86	0.86	0.84	0.88	0.88	0.90	0.94	0.88	0.84	0.47	0.00	0.47	0.45	0.24
38	0.78	0.92	0.90	0.86	0.80	0.80	0.82	0.86	0.90	0.84	0.86	0.47	0.47	0.00	0.53	0.47
40	0.84	0.96	0.94	0.88	0.90	0.86	0.90	0.88	0.90	0.86	0.86	0.45	0.45	0.53	0.00	0.43
42	0.82	0.94	0.90	0.82	0.86	0.88	0.92	0.94	0.94	0.90	0.86	0.47	0.24	0.47	0.43	0.00

Legend 1= Kolar thor, 2=Japanese IRRI, 3=Munshi Murali, 4=Aus dhan 5=Kalo Jamri, 6=Aus boga, 7=Begum Guti, 8=Bahoi, 9=Kotak tar, 10=Changdumra,11=Kali Saita, 12=Kachilon(2), 13=Ausa Bogi, 14=Panki Rat, 15=Kalo Mucha, 16=Agaua, 17=Kola bokri, 18=Chandra Moni, 19=Kautukomni, 20=Aus gara binni 21=Chaita Boro, 22=Khusni, 23=BRRI dhan43, 24=Gambir, 25=BR24, 26=Lemma(2), 27=BR27, 28=Korchamuri, 29=BRRI dhan48, 30=Boilam, 31=BR26, 32=BRRI dhan42, 34=BRRI dhan43, 36=BR 24, 38=BR 27, 40=BRRI dhan48, 42=BR 26

In crop improvement program more genetic diversified Aus landraces could be chosen as parents in the crossing program to create genetic variability on various breeding purpose. In conclusion, the 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 programmes as the identification and utilization of diverse genetic resources is a prerequisite for plant improvement. Here it is shown that most Aus landraces is recognized to have broad genetic base. Thus it is recommended to use these landraces for future breeding program or include new and untouched land races to incorporate new genes and broaden genetic base.

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RESEARCH ARTICLE

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