

DNA Fingerprinting and Genetic Diversity in Aus Rice (*Oryza sativa* L.) Landraces of Bangladesh

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ABSTRACT

The allelic diversity and relationships among 48 Aus rice landraces were determined through DNA fingerprinting using microsatellite (SSR) markers. A total of 14 SSR markers for different chromosomes were used to characterize and differentiate the studied rice genotypes. The number of alleles per locus varied from three alleles (RM118) to 18 alleles (RM44) with an average of 9.88. The polymorphic information content (PIC) varied widely among the loci and ranged from 0.3725 (RM107) to 0.9146 (RM519) with an average of 0.7248. The genetic distance-based results found in the UPGMA clustering system revealed six genetic groups with a similarity coefficient of 0.35. Chakila and Shitki saitta had closest distance in the SSR based genetic distance might have same genetic background. Based on genetic coefficient, the diverse landraces Kasalot, Balam, Pankhiraj, Dular, Hashikalmi, Galong, Panbira, Marichbati, Pidi and Surjomoni could be selected as potential parents for varietal improvement programme. The findings of this study should be useful for varietal identification and could be useful for plant breeders in selecting suitable genetically diverse parents for the crossing programme.

Key words: Aus rice, DNA fingerprinting, genetic diversity, SSR markers, UPGMA clustering

INTRODUCTION

Rice (*Oryza sativa* L.) 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). It has been cultivated in Asia since ancient times and for generations farmers have maintained thousands of different landraces (Jackson, 1995). 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. More than 1,27,000 rice accessions and wild relatives can be found in the world's largest Genebank for rice at IRRI (International Rice Research Institute) based in the Philippines (<http://irri.org/our-work/research/genetic-diversity>). Until now, Bangladesh Rice Research Institute (BRRI) has collected and preserved more than 8,000 varieties/landraces/cultivars/wild types from indigenous and exotic sources in the genebank. Out of them, 8,200 have been registered.

For the assessment of plant genetic diversity, 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 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 could be an indicator of genetic diversity. Several types of molecular markers *viz* RFLP, RAPD, AFLP, microsatellites (SSR) and SNP have been developed. PCR-based markers such as simple sequence repeat (SSR) 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

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informative and require small amount of DNA. Microsatellite markers are 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 Aus group. Despite 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 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 (Dutta *et al.*, 1998). After establishment of BRRI, DNA fingerprinting has been done only of a small number of local landraces. Indigenous rice landraces were characterized at both molecular and phenotypic level by many countries. They have been done for their crop identity and searched for new genes for further crop improvement. However, 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 programme, as selection of plants based on genetic diversity has become successful in several crops (Ananda and Rawat, 1984; De *et al.*, 1988). Hence, the objectives of this research were to (1) assess the genetic variation and diversity of 48 Aus rice genotypes, (2) determine the genetic relationship among these genotypes for breeding purposes, and (3) characterize these rice genotypes at molecular

level (DNA fingerprinting) that would help in the establishment of varietal rights.

MATERIALS AND METHODS

Plant materials

Forty-eight Aus rice landraces were used in this study (Table 1). A five gram seed from each of the entry was germinated and then sown in earthen pots for growth and subsequent DNA extraction.

SSR markers

Fourteen SSR markers were selected (Table 2) to detect DNA for discriminating the tested Aus rice landraces.

Genotyping protocol

Total genomic DNA was extracted from young leaves of three-week-old plants following the simple and modified protocol of Zheng *et al.*, 1995. PCR analysis was performed in 12.5 μ l reaction sample containing 5-25 ng of DNA template, 1.25 μ l of $MgCl_2$ free 10X PCR buffer (100 mM Tris-HCl pH 9.0 at 25°C, 500 mM KCl, 0.1% Triton® X-100 and H_2O), 1.5 μ l of 25 mM $MgCl_2$, 0.25 μ l of 10mM dNTP, 0.25 μ l of 5 U/ μ l Taq polymerase enzyme, 0.625 μ l each of 10 μ M forward and reverse primers using a MJ Research single 96-well thermal cycler. The mixture was overlaid with one drop of mineral oil to prevent evaporation. After initial denaturation for five minutes at 94°C, each cycle comprised one min denaturation at 94°C, one min annealing at 55°C, and two min extension at 72°C with a final extension for seven min at 72°C at the end of 35 cycles. The PCR products were mixed with bromophenol blue gel loading dye and were analyzed by electrophoresis on 8% polyacrylamide gel using mini vertical polyacrylamide gels for high throughput manual genotyping (CBS Scientific Co. Inc., CA, USA). 2.5 μ l of amplification products were resolved by running gel in 1×TBE buffer for 2-2.5 hrs depending upon the allele size

Table 1. Rice landraces used in the present study with their provenance.

Landrace	BRRRI accession no.	Place of collection	Landrace	BRRRI accession no.	Place of collection
Kasalot	3283	Sylhet	Panbira	50	Dhaka
Balam	4945	Khulna	Hasha kumira	63	Manikganj
Kamranga	4926	Rangamati	Dhala saita	64	Manikganj
Surjomoni	3550	Barisal	Loroi	65	Dhaka
Gopalbogh	2110	Narsingdi	Boteswar	66	Dhaka
Haitta	3232	Tangail	Chakila	152	Tangail
Goarchara	953	Khulna	Shitki saita	154	Tangail
Chengri	808	Sylhet	Baila bokri	155	Tangail
Saita	1052	Jessore	Kataktara (2)	183	Mymensingh
Company	7270	Khagrachhari	Bolorum	188	Mymensingh
Galon	743	Chittagong	Pankiraj	189	Mymensingh
Kamarang	7263	Khagrachhari	Hanpa	190	Mymensingh
Maloti	7269	Khagrachhari	Agaua	191	Mymensingh
Lal binni	753	Chittagong	Chakulia	193	Mymensingh
Suri dhan	7264	Khagrachhari	Shala dumra	263	Rangpur
Galong	7265	Khagrachhari	Sukhti	264	Rangpur
Sada galon	4923	Rangamati	Faisha manja	265	Rangpur
Sili	7251	Bandarban	Inda	266	Rangpur
Mongthong	7249	Bandarban	Thubri	267	Rangpur
Kilong	7257	Bandarban	Bolun	268	Rangpur
Dharial	18	Dhaka	Phul dumra	269	Rangpur
Dular	22	Dhaka	Kola dama	271	Rangpur
Hashikalmi	30	Dhaka	Turki	7423	Khagrachhari
Marichbati	47	Dhaka	Pidi	7770	Bandarban

Table 2. List of the 14 SSR markers.

Locus name	Chr. no.	Repeat motif	Forward primer	Reverse primer
RM6	2	(AG)16	GTCCCTCCACCCAATTC	TCGTCTACTGTTGGCTGCAC
RM204	6	(CT)44	GTGACTGACTTGTCATAGGG	GCTAGCCATGCTCTCGTACC
RM241	4	(CT)31	GAGCCAAATAAGATCGCTGA	TGCAAGCAGCAGATTTAGTG
RM279	2	(GA)16	CCTCTCACTCACGTGGACTCTCC	CCTCACCTAGGCTTTGATATGC
RM519	12	(AAG)8	AGAGAGCCCCTAAATTTCCG	AGGTACGCTCACCTGTGGAC
RM286	11	(GA)16	GGCTTCATCTTTGGCGAC	CCGATTACAGAGATAAACTC
RM44	8	(GA)16	ACGGGCAATCGGAACAACC	TCGGGAAAACCTACCCTACC
RM147	10	(TTCC)5(GGI)5	TACGGCTTCGGCGGCTGATTC	CCCCGAATCCCATCGAAACCC
RM107	9	(GA)7	AGATCGAAGCATCGCGCCGAG	ACTGCGTCTCTGGGTTCCCGG
RM118	7	(GA)8	CCAATCGGAGCCACCGGAGAGC	CACATCTCCAGCGACGCCGAG
RM10	7	(GA)15	TTGTCAAGAGGAGGCATCG	CAGAATGGGAAATGGGTCC
RM161	5	(AG)20	TGCAGATGAGAAGCGGCGCCTC	TGTGTCATCAGACGGCGCTCCG
RM1	1	(GA)26	GCGAAAACACAATGCAAAAA	GCGTTGGTTGGACCTGAC
RM85	3	(TGG)5(TCT)12	CCAAAGATGAAACCTGGATTG	GCACAAGGTGAGCAGTCC

Chr.=Chromosome.

at around 75 volts and 180 mA current. The gels were stained in 0.5 mg/ml ethidium bromide and were documented using UVPRO (Uvipro Platinum, EU) gel documentation unit. Microsatellite or simple sequence repeat (SSR) markers were used for DNA analysis (Temnykh *et al.*, 2001; McCouch *et al.*, 2002).

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 PowerMarker 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=0) for analysis with NTSYS-pc version 2.1 (Rohlf, 2002). A similarity matrix was calculated with the Simqual subprogramme using the Dice coefficient, followed by cluster analysis with the SAHN subprogramme using the UPGMA (unweighted pair group method using arithmetic mean) clustering method as implemented in NTSYS-pc.

RESULTS AND DISCUSSION

Overall SSR diversity

Forty-eight Aus landraces were successfully amplified with the 14 SSR marker pairs where marker pairs referred to as loci and DNA bands as alleles. A total of 138 alleles were detected using 14 microsatellite markers across 48 rice landraces. The number of alleles per locus ranged from three alleles (RM118) to 18 alleles (RM44), with an average of 9.88 (Table 3). PIC values ranged from 0.3725 (RM107) to 0.9146 (RM519) with an average of 0.7248. The PIC values for other markers were 0.9135 (RM286), 0.9115 (RM44), 0.8620 (RM147), 0.8439 (RM6), 0.8247 (RM279), 0.7961 (RM241), 0.7371 (RM85), 0.7252 (RM204), 0.6867 (RM1), 0.6251 (RM10), 0.5301 (RM161) and 0.4038 (RM118) respectively. The

allele frequency ranged from 12.50% (RM519, RM286, RM44) to 77.08% (RM107) with an average of 35.12 (Table 3). PIC value revealed RM519 as the best marker. Figure 1 shows the DNA profiles of 48 Aus rice landraces with SSR marker RM519.

Genetic distance-based analysis

The SSR marker data were subjected to cluster analysis using NTSYS programme. The similarity matrix was constructed using Dice coefficient method. Cluster analysis was done to group the genotypes into a dendrogram. From this dendrogram, the 48 rice landraces were grouped into six major clusters at a coefficient of 0.35 and the similarity coefficient value ranged from 0.10 to 0.86. Cluster I consisted of 18 accessions and is the biggest group among six clusters, followed by cluster IV which contained 11 accessions; cluster V comprised nine accessions; cluster II comprised six accessions; cluster III and VI were both composed of two accessions (Fig. 2).

In rice improvement breeding programme the genetically diverse landraces could be chosen as parents for crossing programme to create genetic variability and can be used as transgressive segregants. It was found that two Aus landraces (Chakila and Shitki saita) were sorted out as exactly same genotypes in this analysis 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.

In the present investigation, the SSR markers generated 138 alleles with the number of alleles per locus varying from 3 to 18. Similar number of microsatellite markers previously used as subset for genetic diversity analysis of *O. sativa* (Siddique *et al.*, 2016a). The average number of alleles per locus was 9.88, indicating a greater magnitude of diversity among the rice landraces. This value is comparable to five alleles (RM275) to 15 alleles (RM180), with an average of 9.7 across the 30 loci (Siddique *et al.*, 2016b). The PIC values

Table 3. Allele number, allele size, allele frequency and PIC of 48 Aus rice landraces for 14 SSR markers.

Marker	Chr. no.	Position (cM)	Allele no.	Allele size range (bp)	Allele freq (%)	PIC value
RM6	2	29.57	12	142-178	27.08	0.8439
RM204	6	24.04	8	106-118	39.58	0.7252
RM241	4	26.82	12	101-127	33.33	0.7961
RM279	2	13.2	11	119-136	27.08	0.8247
RM519	12	19.90	16	117-145	12.50	0.9146
RM286	11	0.38	15	95-123	12.50	0.9135
RM44	8	2.88	18	97-122	12.50	0.9115
RM147	10	20.68	14	87-109	27.08	0.8620
RM107	9	20.06	6	165-187	77.08	0.3725
RM118	7	28.01	3	163-166	52.08	0.4038
RM10	7	6.50	4	156-148	43.75	0.6251
RM161	5	27.89	4	163-189	43.75	0.5301
RM1	1	4.63	7	80-90	41.67	0.6867
RM85	3	66.76	8	89-117	41.67	0.7371
Mean			10		35.12	0.7248

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

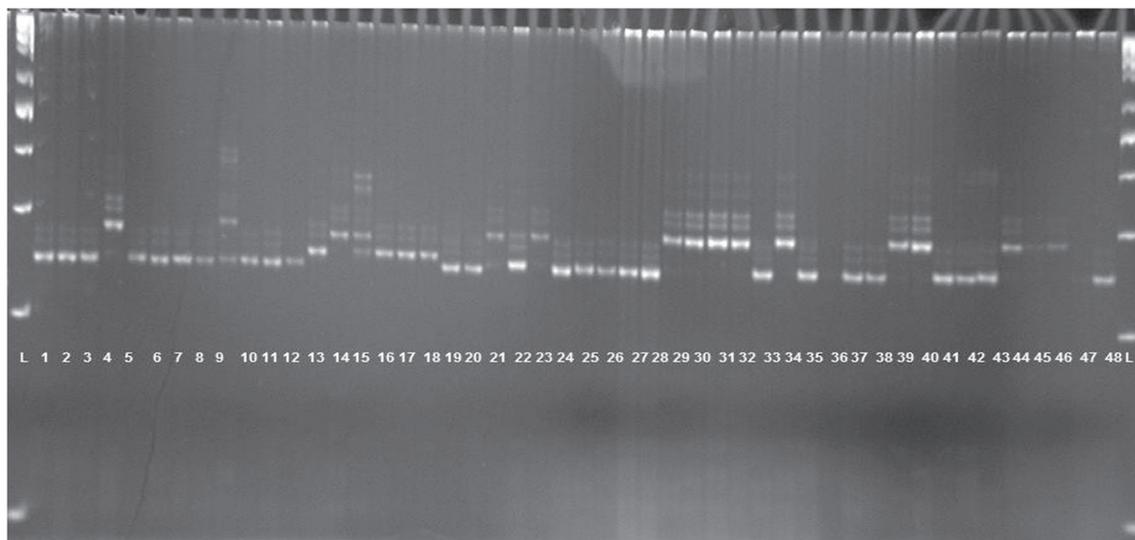


Fig. 1. DNA profile of 48 Aus rice landraces with RM519.

Legend: Lane 1=Kasalat, 2=Balam, 3=Kamranga, 4=Surjomoni, 5=Gopalbogh, 6=Haitta, 7=Goarchara, 8=Chengri, 9=Saita, 10=Company, 11=Kamarang, 12=Maloti, 13=Lalbinni, 14=Suridhan, 15=Galong, 16=Sada galon, 17=Sili, 18=Mongthong, 19=Kilong, 20=Dharial, 21=Dular, 22=Hashikalmi, 23=Marichbati, 24=Panbira, 25=Hasha kumira, 26=Dhala saita, 27=Loroi, 28=Boteswar, 29=Chakila, 30=Shitki saita, 31=Baila bokri, 32=Kataktara (2), 33=Bolorum, 34=Pankiraj, 35=Hanpa, 36=Agaua, 37=Chakulia, 38=Shala dumra, 39=Sukhti, 40=Faisha manja, 41=Inda, 42=Thubri, 43=Bohum, 44=Phul durma, 45=Kola dama, 46=Bangal bakri, 47=Turki, 48=Pidi.

ranged from 0.37 (RM107) to 0.91 (RM519, RM286, RM44) with an average of 0.7248. The PIC values observed, are comparable to previous estimates of microsatellite analysis in

rice *viz.* 0.34-0.88 (Thomson *et al.*, 2007), 0.65-0.91 (Siddique *et al.*, 2014), 0.59-0.90 (Siddique *et al.*, 2016b). The alleles revealed by markers showed a high degree of polymorphism. The

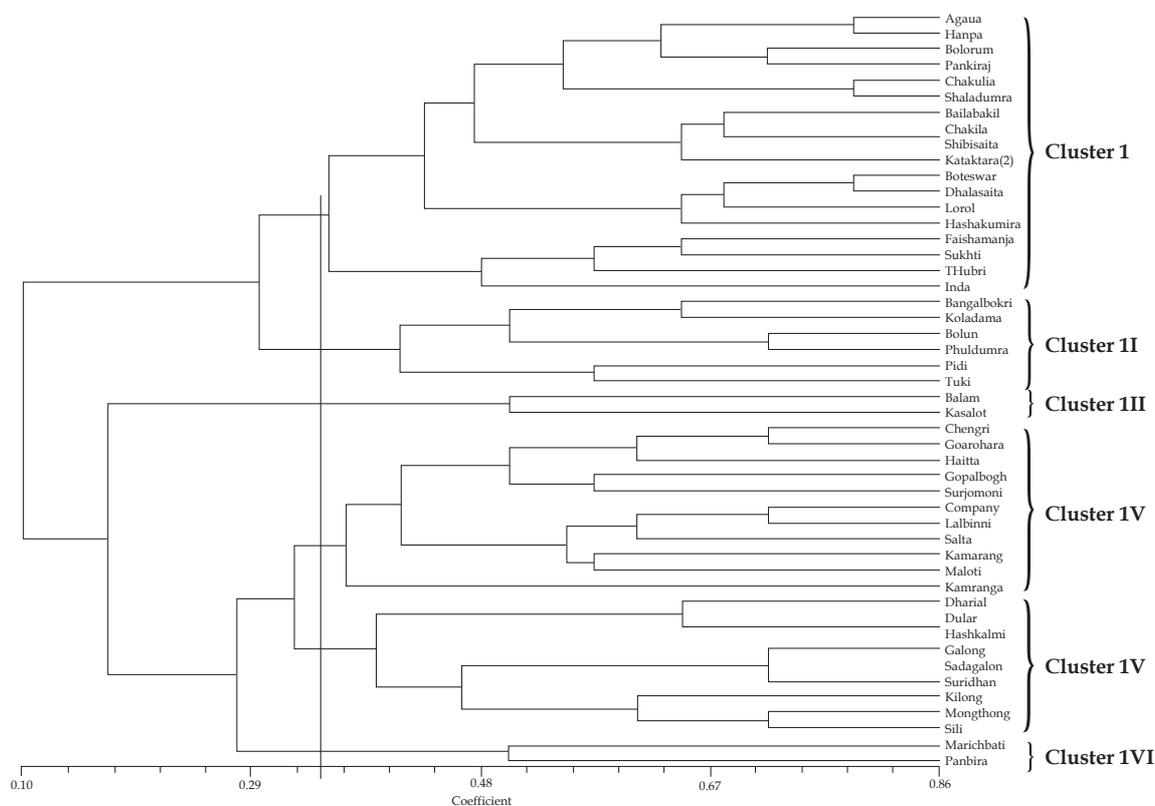


Fig. 2. An UPGMA eluster dendrogram showing the genetic relationship among 48 Aus landraces based on the alleles detected by 14 SSR markers.

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. This could indicate that the genotypes used in the present study were more diverse. Panaud *et al.*, (1996) described high genetic similarity among landraces of common geographic origin and low similarity among landraces of diverse geographic origins using SSR markers in rice.

CONCLUSIONS

The results obtained from this study provided some useful implications for establishment of Intellectual Property Rights (IPR) issues of

Bangladeshi rice landraces. There was a high level of genetic diversity among accessions of Aus rice landraces, suggesting that SSR markers were effective in the detection of polymorphism in this ecosystem. To broaden the genetic base and for the improvement of Aus rice, landraces having the lowest genetic similarities could be selected as parents. Therefore, hybridization should be made between two distant populations. Considering all these criteria and results, the diverse landraces Kasalot, Balam, Pankhiraj, Dular, Hashikalmi, Galong, Panbira, Marichbati, Pidi and Surjomoni could be selected as parents for further breeding programmes. This will bring about greater diversity, which will lead to a high productive index in terms of increase in yield and overall quality.

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