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GENETIC DIVERSITY INAUS RICE LANDRACES OF BANGLADESH USING SSR MARKERS

M. Khalequzzaman, M. Z. Islam, M. A. Siddique, M. F. R. K. Prince, E. S. M. H. Rashid and M. S. Ahamed

Genetics Resources and Seed Division
Bangladesh Rice Research Institute
Gazipur 1701, Bangladesh

Abstract

Assessment of genetic diversity is essential for germplasm characterization, utilization and conservation. Genetic diversity of 31 Aus rice landraces of Bangladesh was assessed using 36 SSR (simple sequence repeats) markers. A total of 141 alleles were detectedand the number of alleles per locus ranged from two (RM1216, RM145, RM282, RM293, RM567and RM496) to 10 alleles (RM304), with an average of 3.92. The gene diversity varied from 0.06 (RM145) to 0.80 (RM304) with an average of 0.54 and the PIC values ranged from 0.06 (RM145) to 0.78 (RM304), with an average of 0.48.PIC value revealed that RM304 was the best marker for characterizing the studied Aus rice genotypes. The dendrogram from unweighted pair-group method with arithmetic average clustering of markers classified the genotypes into five major groups with a coefficient of 0.49. Two and three dimensional graphical views of Principal Coordinate Analysis (PCA) revealed that the genotypesHashikalmi, Chaina, Puitraaijang, Saithsail, Kuchmuch, Kalodhan, Ausdhan and Itcriewere found far away from the centroid of the cluster and can be selected as parents for further breeding programs.The results provided some useful implications for establishment of sovereignty of Bangladeshi rice gene pool. This information will provide maximum selection of diverse parents, background selection during backcross breeding programs and assist in broadening germplasm-based rice breeding programs in future.

Keywords: Aus rice, landraces, cluster analysis, SSR, yield.

INTRODUCTION

Rice (*Oryza sativa* L.) is an important cereal crop grown exclusively for human consumption that is the staple food for about 50 % of the world population (Garris*et al.*, 2005; Ramkumar*et al.*, 2010). Now, 90 % of world rice is produced in Asia on an area of almost 150 million hectares. Rice accounts for 50 % of agricultural income in Asia and supplies almost 80 percent of the region's nutrition. In Bangladesh, rice engages more than 70 % of the rural population and is central to agriculture and the national economy. At present, rice alone constitutes about 93% of the total food grains produced annually in the country (BER, 2013). It provides about 62% of the calorie and 46% of the protein in the average daily diet of the people (HIES, 2010). Rice genetic resource is the primary material for rice breeding and makes a concrete contribution to global wealth creation and food security (Zhang *et al.*, 2011). Rice landraces contain greater genetic diversity than elite cultivars (Londo*et al.*, 2006). However, Bangladesh had abundant rice

germplasm from time immemorial. But the ongoing rapid changes in agricultural practices that favoragronomically improved varieties have become a serious threat for the persistence of indigenous rice varieties in Bangladesh. Besides, various interventions of rice habitat are occurring due to over population. Thus, conservation and management strategies are urgently needed to prevent further loss of genetic diversity inherent to indigenous rice varieties in the country. Therefore, rice landraces need to exploit in hybridization programme for maintaining total rice gene diversity.

Exploring diversity in the landrace collection and characterization is very essential for identifying new genes and further improvement of the germplasm (Thomson *et al*., 2007). In Bangladesh, variety identification and diversity analysis has been done mostly through morphological descriptors and limited at molecular level. Due to stage specific expression of characters and influence of environment; morphological diversity estimates are less reliable. Moreover, at times there may be little morphological diversity among cultivars with related pedigrees. Uses of molecular markers are considered best for analysis of genetic diversity and varietal identification since there is no effect of stage of development, environment or management practices. Availability of a large number of polymorphic markers enables precise classification of the cultivars and germplasm collections.

Molecular markers are powerful tools for evaluation of genetic diversity within and among varieties. Among various PCR-based markers, microsatellites (SSRs) are more appropriate and successfully used for assessing genetic diversity among closely related rice cultivars compared to other molecular markers; because it can be simply amplified by PCR reaction, abundant, highly informative, mostly mono locus, co-dominant, easily analyzed, cost effective and it can identify higher degree polymorphism in rice (Siddique*et al*., 2016). Microsatellites are abundant and well distributed throughout the genome in rice (Akagi*et al*., 1996; McCouch*et al*., 1997). The present study was, therefore, undertaken to investigate the genetic diversity of 31 Aus rice landraces using SSR markers.

MATERIAL AND METHODS

a) Rice materials

Thirty oneAusrice genotypes (Table 1) were studied in the Molecular Laboratory of Genetic Resources and Seed Division of BRRI during 2015 for diversity analysis. Five grams seeds from each of the entry was germinated and then sown in the earthen pots.

b) SSR Markers

Thirty six SSR primers distributing across the 12 chromosomes and covering the whole genome were used for diversity analysis.

c) Genomic DNA isolation and PCR amplification

DNA was extracted from young leaves of 14-day-old plants following the Miniscale method (Zheng*et al.,* 1995).The total PCR reaction volume was 10 µL, composed of 3.0 µL genomic DNA, 1.0 µL of 10 × PCR buffer ($MgCl_2$ free), 1.35 µL of 25 mmol/L $MgCl_2$, 0.2 µL of 10 mmol/L dNTPs, 0.5 µL of 10 µmol/L forward and reverse primers, 0.02 µL of 5 U/µL*Taq*DNA polymerase and 3.43 µL sterile deionized water. The temperature profile was an initial denaturation step for 5 min at 94 °C, followed by 35 cycles of denaturation (94 °C) for 45 s, annealing (55 °C or 61 °C) for 45 s and primer elongation (72 °C) for 1.3 min and then a final extension at 72 °C for 7 min.

d) Electrophoresis and visualization of amplified products

The 10 μL of PCR product with 2 μL of a loading dye was analyzed using 8% polyacrylamide gel electrophoresis in 1 × TBE buffer at 75 V for about 2.0–2.5 h depending upon the allele size. The gels were stained with ethidium bromide solution (0.5 mg/mL) for 25 min and exposed to UV light using the molecular Imager gel documentation unit (XR System, Uvitec Cambridge, France) for visualization.

e) Allele scoring and data analysis

The size of the band for each marker was scored by AlphaEaseFC 4.0 software. The summary statistics, including the number of alleles, major allele size and frequency, gene diversity and polymorphism information content (PIC) value, were determined using PowerMarker version 3.25 (Liu and Muse, 2005). For the unrooted phylogenic tree, genetic distance was calculated using the "C.S. Chord 1967" distance (Cavalli-Sfoza and Edwards, 1967) followed by phylogeny reconstruction using neighbor-joining as implemented in PowerMarker with tree viewed using Treeview (Page, 1996). The allele frequency data from Powermarker was used to export the data in binary format (allele presence="1" and allele absence = "0") for analysis with NTSYS-pc version 2.2 (Rohlf, 2002). Similarity matrix was calculated with the help of Simqual Subprogram using the Dice coefficient followed by cluster analysis with the SAHN subprogram using the UPGMA (Un-weighted pair group method using arithmetic average) clustering method as implemented in NTSYS-pc. The similarity matrix was used for principal coordinate analysis (PCA) with the DCenter, Eigen, Output, and MXPlot subprograms in NTSYS-pc.

Table 1. List of Aus rice landraces with place of collections

Sl. No.	Variety	Collection District	Sl. No.	Variety	Collection District
1	Begun bichi	Kushtia	17	Raitul	Barguna
2	Hashikalmi	Kushtia	18	Kuchmuch	Barguna
3	Kalodhan	Kushtia	19	Puitraaijang	Barguna
4	Ausdhan	Kushtia	20	Boula	Barguna
5	Dighabawalia	Kushtia	21	Chaina	Barguna
6	Hanuman jata	Kushtia	22	Saithsail	Barguna
7	Itcrie	Meherpur	23	Sadeyaus	Barguna
8	V-2	Meherpur	24	Mallika	Barguna
9	V-3	Meherpur	25	Aduballi	Barguna
10	V-4	Meherpur	26	Bardhanaus	Barguna
11	Parangi	Rajbari	27	Kaloaus	Barguna
12	Sandamioni	Rajbari	28	Bardhanaus M-74-1	Barguna
13	Jaymori	Rajbari	29	H-171	Barguna
14	Kalohizli	Rajbari	30	H-12	Barguna
15	Minikit	Barguna	31	Kadidet	Barguna
16	Parangi	Barguna	--	--	--

RESULTS AND DISCUSSION

Genetic diversity study is of prime important in conservation of endangered species and utilization of appropriate plant resources from diverse germplasm. Successful breeding

for crop development programmes depends on genetic variability that arises from genetic diversity (Rana and Bhat, 2004). Lack of genetic variability may limit breeding progress and gain from selection. So, knowledge of the genetic diversity of any germplasm collection provides a basis for improvement of crops and development of superior cultivars. This study was investigated the allelic diversity existing among a collection of 31 Aus rice landraces from different district of Bangladesh using 36 SSR markers. SSR markers have been widely applied in the genetic diversity analysis, molecular characterization, genotypic identification and population structure estimation in several rice genetic studies including basmati rice (Das *et al*., 2013; Choudhury*et al*., 2013; Allhgholipour*et al*., 2014; Singh *et al*., 2013; Shah *et al*., 2013 and Yadav*et al*., 2013). In the present study, 141 alleles were detected from 36 SSR (simple sequence repeats) markers across 31 Aus rice genotypes (Table2). 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; Thomson *et al.*, 2007).Islam (2014) also detected 140 alleles with an average of 3.11 among 113 aromatic rice accessions by using SSRs. The highest amplicon size was produced by RM474 (269 bp) and the lowest by RM413 (69 bp). The highest range of band sizes was found in RM591 (252-314 bp) followed by RM454 (213-269bp) and RM496 (250-264bp), respectively. The number of alleles per locus ranged from two alleles (RM1216, RM145, RM282, RM293, RM567, RM496) to 10 alleles (RM304), with an average of 3.92. This allele is comparable to 2-9 allele per SSR locus with an average of 4.69 for various classes of microsattelite (Behera *etal.*, 2012) and 2-7 alleles per locus as reported by Chakrabarthia and Naravaneni (2006).Our result is not fully agreement with the findings of Siddique *et al*, 2014, who estimated genetic diversity among T. Aman (rainfed lowland) rice germplasm collections using SSR markers.The gene diversity varied from 0.06 (RM145) to 0.80 (RM304) with an average of 0.54 and the PIC value ranged from 0.06(RM145) to 0.78 (RM304), with an average of 0.48. In general, higher PIC values were observed for SSRs having higher number of alleles. Lower PIC value indicates that the genotypes under study are closely related types, while the higher value of PIC indicates higher diversity of materials, which is better for development of new varieties. Primer RM304 had the highest PIC value (0.78) and the highest number of alleles (10) and it detected the highest level of polymorphism. Therefore, it confirmed that RM304 was the best marker for characterizing the studied Aus rice genotypes.The observed PIC values are similar to previous estimates of microsatellite analysis in rice of 0.04–0.59 (Shah *et al*., 2013) and 0.05–0.67 (Islam, 2014).The frequency of the most common allele at each locus ranged from 32.26% (RM474, RM334)) to 96.77% (RM145). On average, 57.55% of the31 rice genotypes shared a common major allele at any given locus.Jain *et al*.(2004) observed that 53.6% of 69 rice genotypes shared common alleles at any locus. Thomson *et al*.(2009) indicated that on an average, 62% of the 190 rice accessions of Indonesia shared a common major allele at any given of SSR locus. Similar results were also observed by others (Saini *et al*., 2004; Lu *et al*., 2005; Jayamani *et al*., 2007). The DNA profiles of 31 Aus rice with SSR marker RM3330 is shown in Fig. 1.

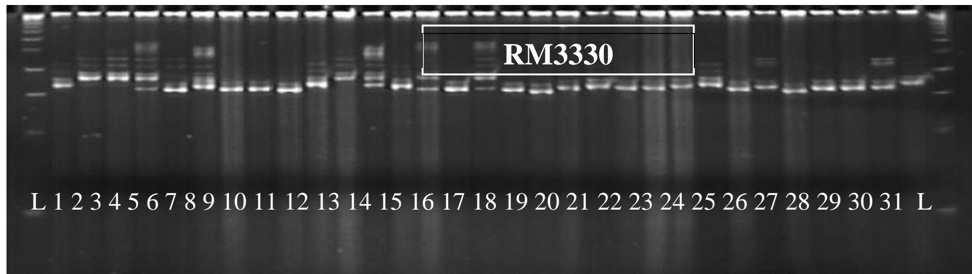
Genetic distance-based analysis

The UPGMA-based dendrogram, which was obtained from the binary data deduced from the DNA profiles of the samples analyzed, added a new dimension to the genetic similarity perspective. Five distinct groups were formed from the analysis of the pooled SSR marker data at a similarity coefficient of 0.49. The cluster analysis showed high genetic variation among the rice cultivars studied, with similarity coefficient value ranging from 0.33 to 0.86 in SSR (Fig. 2).

Table 2. Allele number, size and frequency, genetic diversity and PIC among 31 Aus rice genotypes for 36 SSR markers.

SSR Marker	Chro. No.	Position (cM)	Motif*	Allele					
				No.	Size range (bp)	Size (bp)	Freq. (%)	Gene diversity	PIC
RM 1	1	29.7	(GA)26	5	132-166	132	36.67	0.72	0.68
RM 151	1	36.2	(TA)23	6	180-259	180	53.57	0.62	0.57
RM 246	1	115.2	(CT)20	4	100-122	122	38.71	0.72	0.67
RM 1216	1	132.0	(AG)14	2	73-80	80	54.84	0.50	0.37
RM 145	2	49.8	-	2	192-196	192	96.77	0.06	0.06
RM 16	3	131.5	(TCG)5(GA)16	3	158-175	175	35.48	0.66	0.59
RM 282	3	100.6	(GA)15	2	134-139	139	80.65	0.31	0.26
RM 487	3	127.9	(AC)10	3	172-180	172	74.19	0.41	0.37
RM 293	3	193.4	(GT)20	2	209-215	209	83.87	0.27	0.23
RM 554	3	100.6	(GA)14	3	250-261	260	64.52	0.52	0.46
RM 503	3	153.9	(CA)59(TA)26	3	162-248	248	90.32	0.18	0.17
RM 518	4	25.5	(TC)15	4	164-180	180	46.67	0.68	0.63
RM 551	4	8.51	(AG)18	5	181-207	181	53.85	0.59	0.51
RM 567	4	153.6	(GA)21	2	214-222	222	61.29	0.47	0.36
RM 334	5	141.8	(CTT)20	5	173-195	173	32.26	0.75	0.71
RM 413	5	26.7	(AG)11	5	69-102	69	45.16	0.66	0.59
RM 527	6	61.2	(GA)17	3	214-241	241	61.29	0.50	0.40
RM 3330	6	61.6	(CT)15	3	134-148	148	70.97	0.44	0.39
RM 320	7	62.5	(AT)11GTAT(GT)13	4	186-234	186	38.71	0.69	0.63
RM 542	7	34.7	(CT)22	3	95-151	151	56.67	0.52	0.41
RM 72	8	60.9	(TAT)5C(ATT)15	3	122-165	165	87.10	0.23	0.22
RM 5647	8	-	(AAG)16	5	100-149	149	38.71	0.75	0.71
RM 464	9	3.3	(AT)21	3	226-240	240	84.00	0.28	0.26
RM 285	9	1.8	(GA)12	3	143-152	152	60.00	0.50	0.41
RM 304	10	73.0	(GT)2(AT)10(GT)33	10	146-214	146	38.71	0.80	0.78
RM 474	10	-	(AT)13	6	213-269	269	32.26	0.76	0.72
RM 496	10	113.0	(TC)14	2	250-264	264	54.84	0.50	0.37
RM 591	10	118.3	(AC)10	7	252-314	252	48.39	0.70	0.66
RM 21	11	85.7	(GA)18	4	133-163	163	80.65	0.34	0.32
RM 206	11	102.9	(CT)21	5	130-166	166	48.39	0.68	0.63
RM 224	11	120.1	(AAG)8(AG)13	4	135-164	164	70.97	0.45	0.41
RM 20	12	-	(ATT)14	3	154-181	154	51.61	0.57	0.49
RM 1337	12	-	(AG)21	3	162-183	183	61.29	0.55	0.49
RM 5364	12	-	TC)13	4	122-143	143	41.94	0.66	0.59
RM 7102	12	-	(AGAT)8	5	172-194	172	58.06	0.57	0.51
RM491	12	34.3	(AT)14	5	164-384	164	38.46	0.73	0.69
Total				141			2071.84	19.33	17.32
Mean				3.92			57.55	0.54	0.48

*http://www.gramene.org.



Legend: Lane 1=Begun bichi, 2=Hashikalmi, 3=Kalodhan, 4=Ausdhan, 5=Dighabawalia, 6=Hanuman jata, 7=Itcrie, 8=V-2, 9=V-3, 10=V-4, 11=Parangi, 12=Sandamioni, 13=Jaymori, 14=Kalohizli, 15= Minikit, 16= Parangi, 17= Raitul, 18=Kuchmuch, 19= Puitraaijang, 20= Boula, 21=Chaina, 22=Saithsail, 23=Sadeyaus, 24=Mallika, 25=Aduballi, 26=Bardhanaus, 27=Kaloaus, 28=Bardhanaus M-74-1, 29=H-171, 30=H-12 , 31=Kadidet.

Fig 1. DNA Profile of 31 Aus rice landraces with SSR marker RM3330.

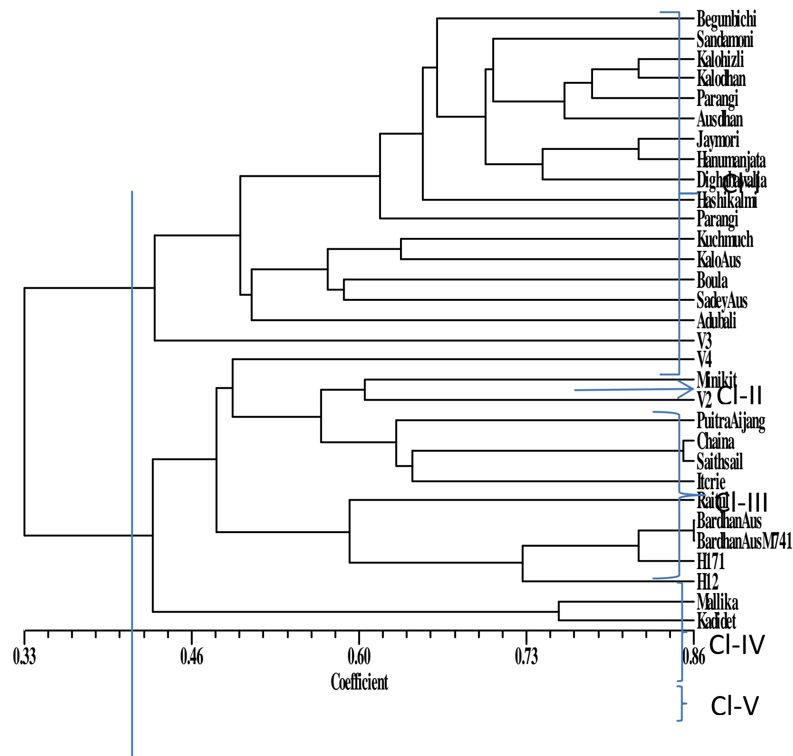


Fig. 2. A UPGMA cluster dendrogram showing the genetic relationships among 31 Aus rice genotypes based on alleles detected by 36 microsatellite markers.

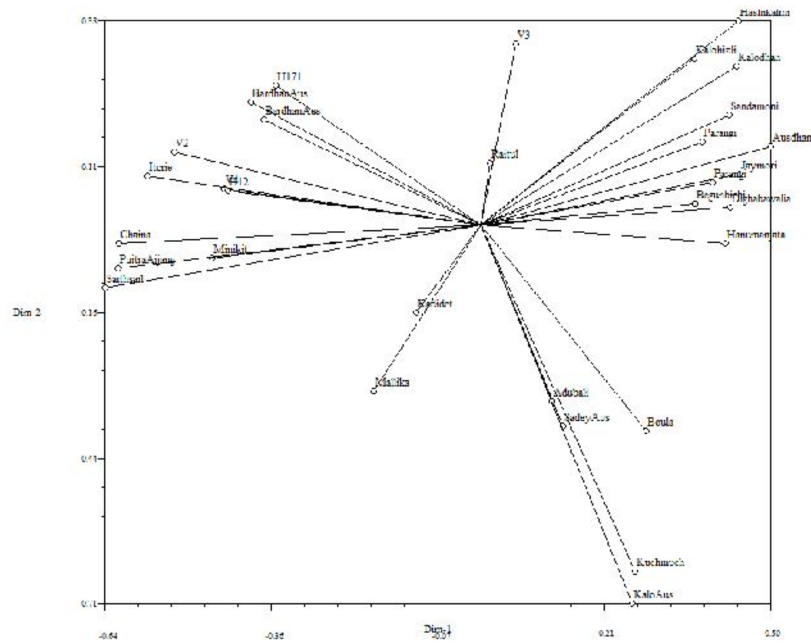


Fig.3. Two-dimensional view of principal coordinate analysis (PCoA) with 36microsatellite markers over 31 Aus rice genotypes.

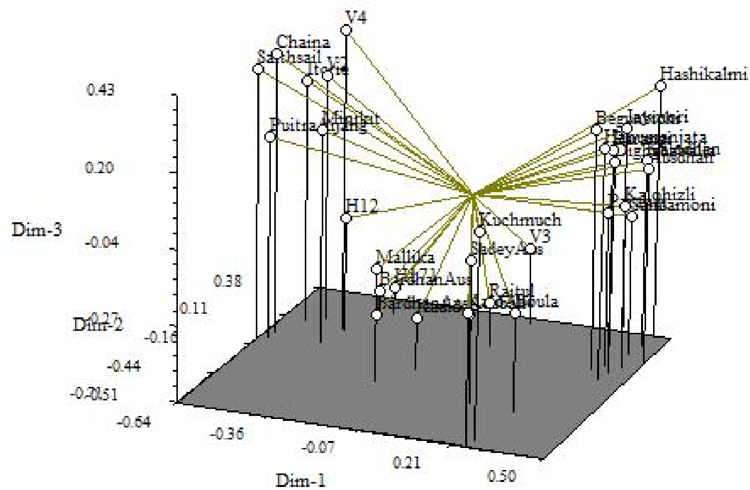


Fig.4. Three-dimensional view of principal coordinate analysis (PCoA) with 36 microsatellite markers over 31 Aus rice genotypes.

Principal Coordinate Analysis

The two and three-dimensional graphical view of Principal Coordinate Analysis (PCoA) showed the spatial distribution of the genotypes along the two and three principal axes. The genotypes Hashikalmi, Chaina, Puitraijang, Saithsail, Kuchmuch,

Kalodhan, Ausdhan and Itcriewere found far away from centroid of the cluster and the rest of the genotypes were placed more or less around the centroid (Fig. 3 and 4). The results indicated that the genotypes were placed far away from the centroid were more genetically diverse while the genotypes were placed near around the centroid possessed more or less similar genetic background. However, centroid might define as the vector representing the middle point of the cluster that contained at least one number for each variable. The connecting lines between the each genotype and the centroid represented eigan vectors for the respective genotypes.

CONCLUSIONS

The results obtained from this study on molecular characterization provided some useful implications for establishment of sovereignty of Bangladeshi rice gene pool. Genetic diversity in any crop is important for improving heterotic crop genotypes over the existing ones. There was a high level of genetic diversity among Aus rice in this study, suggesting that SSR markers were very effective in the detection of polymorphism in this ecosystem. To broaden the genetic base and improvement of Aus rice, landraces having the lowest genetic similarities could be selected as parents. Therefore, hybridization should be made between two distant populations. Based on distance matrix, the genotypesHashikalmi, Chaina, Puitraaijang, Saithsail, Kuchmuch, Kalodhan, Ausdhan and Itcrie can be selected as parents for further breeding programs.The findings of this study should be useful for varietal identification and could help in providing guides for assisting rice breeders in selecting suitable genetically diverse parents for the crossing program.

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