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Molecular Characterization and Genetic Diversity of Aman Rice *(Oryza sativa* L.*)* Landraces in Bangladesh

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ABSTRACT

Assessment of genetic diversity and molecular characterization among rice landraces of Bangladesh is important for their identification. Genetic diversity of 96 Aman (rainfed, partially irrigated) rice landraces of Bangladesh were evaluated using eight SSR markers to characterize the landraces and also to establish the sovereignty of Bangladeshi rice gene pool. A total of 159 alleles were detected. The number of alleles per locus ranged from 13 (RM60, RM237) to 34 (RM163), with an average of 19.88. The polymorphism information content (PIC) which ranged from 0.86 (RM237) to 0.95 (RM163) with an average of 0.90 revealed much variation among the studied landraces. RM163 was the best marker for identification and diversity estimation of Aman rice landraces as revealed by PIC values. The allele frequency ranged from 8.33% (RM163) to 22.92% (RM60, RM125) with an average of 15.89. The UPGMA dendrogram based on Nei's genetic distance revealed seven distinct clusters with a similarity coefficient of 0.09. The two-dimensional graphical view of Principal Coordinate Analysis (PCoA) revealed that the landraces Pankaij, Lotha, Chinigura, Patjag, Chinikanai, Badshabogh, Panati, Jirasail, Joria, Dudhmoni, Jhingasail were found far away and distributed around the centroid of the cluster. The findings of this study are useful for landraces identification to assist plant breeders in selecting suitable genetically diverse parents for breeding programmes.

Key words: Allele, gene pool, polymorphism, genetic diversity, SSR markers

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 global population (Garris *et al*., 2005; Ramkumar *et al*., 2010). It has been cultivated in Asia since ancient times and for generations farmers have maintained thousands of different landraces (Jackson, 1995). 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% 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 (Anonymous, 2002). This crop provides about 75 and 55%, respectively, of the calorific and protein content of the average daily human diet.

Due to great significance and intimate association of rice in food security and local ways of life and culture, Asian farmers have selected and maintained a vast array of rice landraces over thousands of years. Scientists estimate that more than 1,40,000 rice varieties have been developed/selected/isolated in Asia. 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) located in the Philippines (http://irri.org/our-workresearch/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, 8,044 landraces have been registered.

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Research has been conducted on Bangladeshi local landrace in the world by many scientists, but research on indigenous landraces in BRRI is very limited. Only few years ago a large number of farmers grew these local cultivars as their main crop. These cultivars have good adaptation but are poor yielder. Actually, cultivation of these landraces gradually has been replaced through high yielding varieties over the last 20 years. In Bangladesh, rice is grown in three seasons namely Aus, Aman and Boro. BRRI Genebank is enriched with about 4,990, 1,670, 1,350 rice accessions for Aman, Boro and Aus season, respectively. They are adapted in different parts of the country, some of which have very nice quality, fineness, aroma, taste and high protein content (Dutta *et al*., 1998). After establishment of BRRI, characterization or DNA fingerprinting has been done only for a small number of local landraces. Many countries in the world have characterized their indigenous different crop landraces at both molecular and phenotypic level. This has been done for keeping their crop identity and for searching new genes for further crop improvement. But information about the genetic diversity of local landraces as well as Aman rice is very limited. The needs for varietal improvement for such situations are very important. Precise information about the extent of genetic diversity among population is crucial in any crop improvement programme, because selection of plants based on genetic diversity has become successful in several crops (Ananda and Rawat, 1984; De *et al*., 1988). Genetic diversity in any crop is basically important for improving heterotic crop genotypes over the existing ones. Therefore, research emphasis has been given on genetic diversity for micro-satellite DNA markers in local Aman rice landraces.

Molecular markers have successfully been applied in registration activities of cultivars (Mailer *et al*., 1994) and also identification of varieties as a part of seed and grain trade (Bligh *et al*., 1999). A powerful technique for DNA fingerprinting is the Polymerase Chain Reaction (PCR) amplification of tandem repeat sequences. This has long been known to be polymorphic and widespread in plant genomes and is referred to as Simple Sequence Repeats (SSR) or Micro-satellite polymorphism (Cregan, 1992; Morgante and Olivieri, 1993). It has been demonstrated that SSRs are highly informative, locus specific markers in many species of plants (Akkaya *et al*., 1992; Legarcrantz *et al*., 1993; Wu and Tanksley, 1993; Rahman *et al*., 2007). The user-friendly nature of SSR markers was successfully exploited in many crop species for a better understanding of the genetic diversity, domestication process and geographic divergence and distribution. SSRs are increasingly useful for integrating the genetic, physical, and sequence-based maps of rice, and they simultaneously provide breeders and geneticists with an efficient tool to link phenotypic and genotypic variation.

In rice, micro-satellites are abundant and well distributed throughout the genome (Wu and Tanksley, 1993; Akagi *et al*., 1996; McCouch *et al*., 1997). They are valuable as genetic markers because they are co-dominant in nature, detect high levels of allelic diversity and are assayed efficiently by the PCR technique (McCouch *et al*., 2002). The level of average genome-wide coverage provided by micro-satellites in rice, one marker every 6 centimorgans (Temnykh *et al*., 2000), was sufficient to be useful for assessment of hybrid seed purity and for genotype identification. Akagi *et al*. (1997) suggested that hyper-variable micro-satellites could be used to classify individual rice cultivars and to maintain the purity of rice seeds by eliminating contamination. This technique has been used for characterization and documentation in 20 crop species including rice, wheat, maize, barley, rapeseed, soybean, potato and other crops by Rahman *et al*. (2006, 2007). Reports also reveal that micro-satellites have been used for rice variety/ cultivar identification in Bangladesh (Rahman *et al*., 2006, 2007 and 2008). Based on the above reports, the present study was designed with 96 Aman rice germplasm using molecular traits for characterization and diversity analysis. The objectives of this research were to: (1) assess the genetic variation and diversity of 96 Aman rice landraces, (2) determine the genetic relationship among these landraces for breeding purposes, and (3) characterize them.

MATERIALS AND METHODS

Plant materials

Ninety-six Aman rice landraces of Bangladesh were studied (Table 1). A five gram seed from each of the entry was germinated and then sown in earthen ware pots for growth and subsequent DNA extraction.

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

Variety	BRRI accession no.	Place of collection	Variety	BRRI accession no.	Place of collection
Biruin	4887	Sherpur	Chinikanai	4356	Khulna
Kalijira	4755	Barguna	Panati	4090	Nilphamari
Topa	962	Khulna	Horibhogh	977	Khulna
Chinigura	4867	Mymensingh	Akhnisail	3446	Sylhet
Chamara	2006	Mymensingh	Ratisail	876	Sylhet
Patjagh	3647	Pabna	Gochisail	4877	Mymensingh
Aloi	4866	Mymensingh	Varisail	900	Sylhet
Sadamota	1576	Patuakhali	Laki	910	Sylhet
Lalmota	1583	Patuakhali	Gandhi biruin	5986	Sylhet
Khama	1456	Dhaka	Tolabiruin	833	Sylhet
Bajal	2044	Satkhira	Hashim	7282	Mymensingh
Dadkhani	6721	Rajbari	Jhoria	860	Sylhet
Bashful	1508	Bakerganj	Kataribhogh	4791	Dinajpur
Binni	4477	Sherpur	Jirasail	6718	Naogaon
Tulshimala	3671	Kishoreganj	Badshabhogh	3	Dhaka
Joina	5315	Bagerhat	Daudin	14	Dhaka
Parangi	7273	Faridpur	Dudsar	21	Dhaka
Horkoch	4772	Khulna	Dhepi	23	Dhaka
Bekibalam	5304	Khulna	Hatisail	31	Dhaka
Ashfol	4842	Satkhira	Jessobalam	34	Dhaka
Chapail	2018	Khulna	KhiraiJali	40	Dhaka
Chengai	3858	Barisal	Latisail	43	Dhaka
Jalpaira	5303	Khulna	Nizersail	49	Dhaka
Latma	5271	Sunamganj	Patnai	52	Dhaka
Nonakuchi	5323	Khulna	Rajasail	57	Barisal
Birpala	5320	Jessore	SR-26-B	59	Dhaka
Jamainaru	4788	Satkhira	Tilockachari	61	Dhaka
Gandhokasturi	5319	Bagerhat	Lalrodadhan	69	Manikganj
Sabrimaloti	5342	Bagerhat	Bhobanibhogh	70	Manikganj
Jotabalam	5341	Khulna	Ratasail	71	Dhaka
Patnai	4843	Satkhira	Lambosail	73	Munshiganj
Godalaki	25	Sylhet	Ropaaman	75	Manikganj
Dudlaki	911	Sylhet	Kartiksail	76	Manikganj
LalAman	44	Habiganj	Lotha	79	Dhaka
MaliaVhangor	46	Dhaka	Gangasagar	80	Munshiganj

Table 1. Continued.

Madhumala	395	Rajshahi	Pankaij	81	Dhaka
Gabura	24	Dhaka	Holidjaran	82	Manikganj
Morichful	74	Dhaka	Apchaya	83	Manikganj
Sakkorkhana	7316	Jhalakathi	Madhusail	85	Manikganj
Kumragoir	1047	Khulna	Jamalbhogh	86	Dhaka
Aloi	2092	Jamalpur	Arichadhigha	87	Manikganj
Radhunipagol	6711	Rajshahi	Jhuldhigha	88	Manikganj
Matorsail	3294	Sylhet	Boron	90	Manikganj
Sitavhogh	5317	Bagerhat	Sechi	93	Dhaka
Dudsail	1023	Khulna	Soider boron	95	Manikganj
JiJhingasail	35	Dhaka	Lantkhama	96	Manikganj
Dudhmoni	1156	Jessore	Hashfal boron	97	Manikganj
Borondhan	394	Rajshahi			
Fulkarai	924	Sylhet			

SSR markers

Eight SSR markers (Table 2) were used for diversity analysis.

Genotyping

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 µl reaction sample containing 5-25 ng of DNA template, 1.25 µl 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 µl of 25 mM $MgCl_2$, 0.25 µl of 10 mM dNTP, 0.25 µl of 5 U/µl Taq polymerase enzyme, 0.625 µl each of 10 µM 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 7 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

Table 2. List of the eight simple sequence repeat (SSR) markers.

Locus name	Chr.	Repeat motif	Forward primer	Reverse primer
RM60	3	$(AATT)_5AATCT(AATT)$	CAAGTTCACCCGCCTTCTCG	TTTCCATCATTAGCAGGCAGTAGC
RM163	5	$(GGAGA)_4$ $(GA)_{11}C$ $(GA)_{20}$	ATCCATGTGCGCCTTTATGAGGA	CGCTACCTCCTTCACTTACTAGT
RM218	3	$(TC)_{24}ACT_5$ $(GT)_{11}$	TCAAACCAAGGTCCTTCAACTGC	TTTCTTCCACCGTCCATGTATCC
RM237	1	$(CT)_{18}$	CAAATCCCGACTGCTGTCC	TGGGAAGAGAGCACTACAGC
RM259	1	$(CT)_{17}$	GAAGTGCTCCCTAAACTTGTTGC	TTATGGAGGATGGATTCGAAGG
RM125	7	$(GCT)_8$	ATCAGCAGCCATGGCAGCGACC	AGGGGATCATGTGCCGAAGGCC
RM278	9	$(GA)_{17}$	GTAGTGAGCCTAACAATAATC	TCAACTCAGCATCTCTGTCC
RM283	1	$(GA)_{18}$	GTCTACATGTACCCTTGTTGGG	CGGCATGAGAGTCTGTGATG

polyacrylamide gels for high throughput manual genotyping (CBS Scientific Co. Inc., CA, USA). 2.5 µl of amplification products were resolved by running gel in 1x TBE buffer for 2-2.5 hrs depending upon the allele size 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. Micro-satellite or simple sequence repeat (SSR) markers were used for molecular analysis (Temnykh *et al*., 2001; McCouch *et al*., 2002).

Data analysis

Molecular weight for each amplified allele was measured in base pair using Alpha-Ease 5.0 software. The summary statistics including the number of alleles per locus, major allele frequency, gene diversity, polymorphism information content (PIC) values were analysed using PowerMarker - 3.25 (Liu and Muse, 2005). For the unrooted phylogenetic tree, genetic distance was calculated using the "C.S. Chord 1967" distance measure (Cavalli-Sfoza and Edwards, 1967) followed by phylogeny reconstruction using neighbour-joining as implemented in PowerMarker using Treeview (Page, 1996). The allele frequency data were exported in binary format (allele presence="1" and allele absence = "0") for analysis with NTSYS-pc version 2.2 (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 (Un-weighted pair group method using arithmetic mean) clustering method as implemented in NTSYS-pc. The similarity matrix was also used for principal coordinate analysis (PCoA) with the DCenter, Eigen, Output, and MXPlot subprogrammes in NTSYS-pc.

RESULTS AND DISCUSSION

Overall SSR diversity

Ninety-six rice landraces were successfully amplified with the eight micro-satellite markers where primer pairs referred to as loci and DNA bands as alleles. A total of 159 alleles were detected using eight micro-satellite markers across 96 rice landraces. The highest average band size was found for RM259 (173.09) followed by RM60 (169), and RM283 (149.44). Among the eight SSR markers, the highest number of alleles (34) were found for RM163 followed by RM259 (23); RM218 (22); RM278 (19); RM283 (18); RM125 (17); RM60, RM237 (13). The polymorphism information content (PIC) values ranged from 0.86 (RM237) to 0.95 (RM163), with an average of 0.90. The allele frequency ranged from 8.33% (RM163) to 22.92% (RM60, RM125) with an average of 15.89 alleles. The PIC values for other markers were 0.92 (RM259, RM218, RM278), 0.91 (RM283), 0.88 (RM60) and 0.87 (RM125), respectively (Table 3). PIC value revealed RM163 as the best marker. Gene diversity varied from 0.87 to 0.95 and their average value was 0.91, which also indicated the presence of adequate genetic diversity (Table 3). Figure 1 shows the DNA profiles of 96 T. Aman landraces with SSR marker RM60.

Genetic distance-based analysis

The similarity matrix was constructed using Dice coefficient method. Cluster analysis was done to group the genotypes into a dendrogram. From this dendrogram, the 96 rice accessions were grouped into seven major clusters at a coefficient of 0.09 and the similarity coefficient value ranged from 0.06 to 0.75. Cluster III consisted of 25 accessions and is the biggest group among seven clusters, followed by cluster II, which contained 22 accessions; cluster VI comprised 17 accessions; cluster IV comprised 14 accessions; cluster I and V were both composed of seven accessions; cluster VII had four accessions (Fig. 2). The genetic differences between 96 accessions were the highest between most of the landraces. Cent percent dissimilarity was found among the following 20 landrace combinations:

Biruin × Khama, Kalijira × Dadkhani, Topa × Bashful, Chinigura × Binni, Chamara × Joina, Parangi × Patjagh, Sadamota × Jotabalam, Tulsimala × Birpala, Horkoch × Rajasail, Sakkorkhana × Pankaij, Bajal × Godalaki, Patnai × Gandhokasturi, Gabura × Sabrimaloti, Kataribhog × Jhingasail, Radhunipagal × Kartiksail, Badshabhog × Chinikanai, Dhudsar × Latisail, Nizersail × Jamainaru, Lalmota × Laki, Maliavhangor × Ashfol.

Table 3. Allele number, allele size, frequency, genetic diversity and PIC of 96 T. Aman rice landraces for eight micro-satellite markers.

Marker	Chr. No.	Position (cM)	Allele no.	Allele sizes (bp)	Allele freq (%)	Genetic diversity	PIC value
RM60	3	0.1	13	169	22.92	0.8861	0.8764
RM163	5	91.4	34	147.68	8.33	0.9551	0.9532
RM218	3	67.8	22	131.91	14.58	0.9264	0.9219
RM237	1	115.2	13	129	17.71	0.8717	0.8584
RM259	1	54.2	23	173.09	16.67	0.9266	0.9223
RM125	7	24.8	17	120	22.92	0.8776	0.8669
RM278	9	77.5	19	146.89	11.49	0.9262	0.9214
RM283	1	31.4	18	149.44	12.50	0.9188	0.9131
Mean					15.89	0.9111	0.9042

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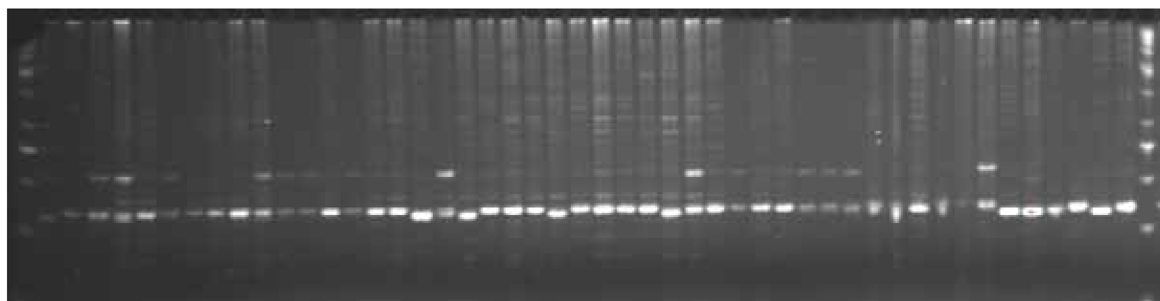

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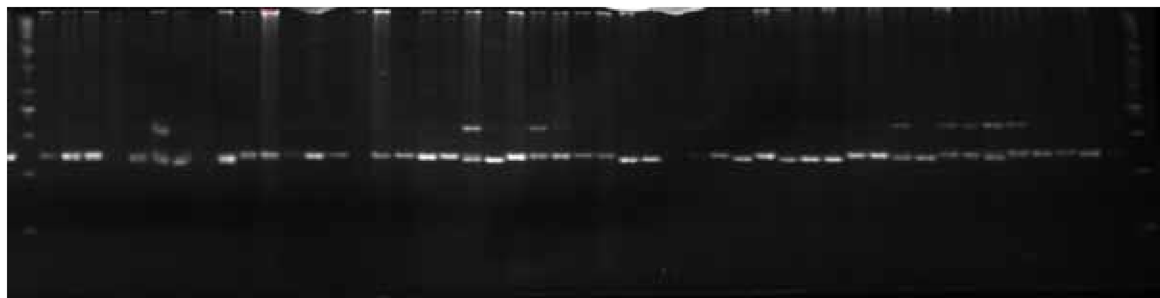

Fig. 1. DNA profile of 96 T. Aman landraces with RM60.

Legend: Lane1=Biruin, 2=Kalijira, 3=Topa, 4=Chinigura, 5=Chamara, 6=Patjagh, 7=Aloi, 8=Sadamota, 9=Lalmota, 10=Khama, 11=Bajal, 12=Dadjhani, 13=Bashful, 14=Binni, 15= Tulshimala, 16= Joina, 17= Parangi, 18=Horkoch, 19= Bekibalam, 20= Ashfol, 21=Chapail, 22=Chengai, 23=Jalpaira, 24=Latma, 25=Nonakuchi, 26=Birpala, 27=Jamainaru, 28=Gandhokasturi, 29=Sabrimalati, 30=Jatabalam , 31=Patnai, 32=Godalaki, 33=Dudlaki, 34=Lal aman, 35=Malia vhangor , 36=Madhumala, 37= Gabura, 38=Marichful, 39=Sakkorkhana, 40= Kumragori, 41=Aloi, 42=Radhunipagal, 43=Matorsail, 44=Sita vhogh, 45=Dudsail, 46=Jhingasail, 47= Dudmoni, 48=Boron dhan, 49=Fulkarai, 50=Chinikanai, 51=Panati, 52=Horovhogh, 53=Akhnisail, 54=Ratisail, 55=Gochisail, 56=Vorisail, 57=Laki, 58=Gandhi biruin, 59=Tola biruin, 60=Hashim, 61=Joria, 62=Katarivhogh, 63=Jirasail, 64=Badshavhogh, 65=Daudin, 66=Dudsar, 67=Dhepi, 68=Hatisail, 69=Jesso balam, 70=Khirai jali, 71=Latisail, 72=Nizersail, 73=Patnai-23, 74=Rajasail, 75=SR-26-B, 76=Tilocikachari, 77=Lal roda dhan, 78=Bhobanivhogh, 79=Ratasail, 80=Lambosail, 81=Ropa aman, 82=Kartiksail, 83=Lotha, 84=Gangasagar, 85=Pankaij, 86=Holid jaran, 87=Apchaya, 88=Madhusail, 89=Jamalbhogh, 90=Aricha digh, 91=Jhul digh, 92=Boron, 93=Sechi, 94=Soider boron, 95=Lani khama, 96=Hashfal boron.

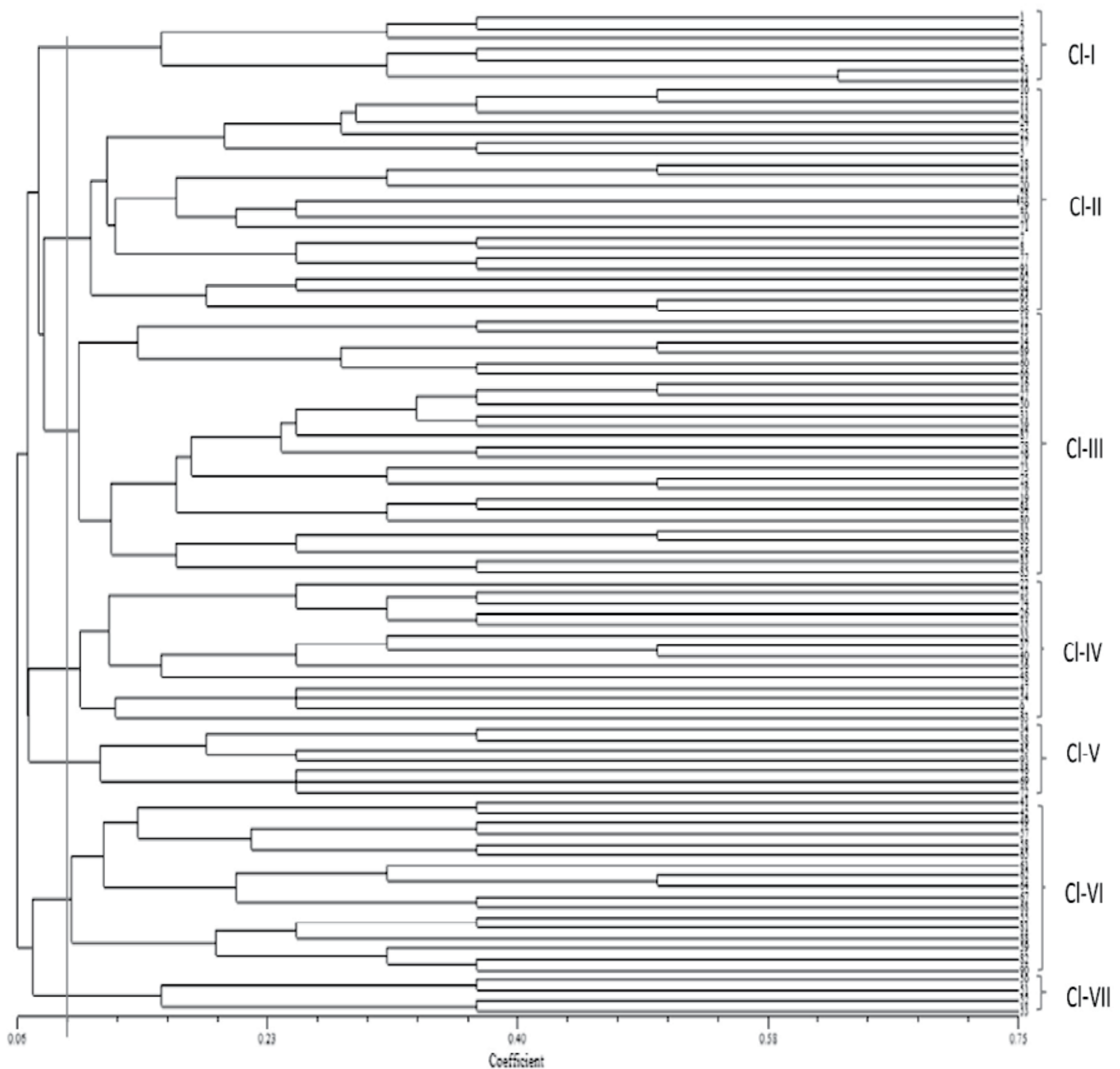

Fig. 2. An UPGMA cluster dendrogram showing the genetic relationships between 96 Aman rice landraces of Bangladesh based on the alleles detected by eight micro-satellite markers.

Legend: Lane 1=Biruin, 2=Kalijira, 3=Topa, 4=Chinigura, 5=Chamara, 6=Patjagh, 7=Aloi, 8=Sadamota, 9=Lalmota, 10=Khama, 11=Bajal, 12=Dadjhani, 13=Bashful, 14=Binni, 15= Tulshimala, 16= Joina, 17= Parangi, 18=Horkoch, 19= Bekibalam, 20= Ashfol, 21=Chapail, 22=Chengai, 23=Jalpaira, 24=Latma, 25=Nonakuchi, 26=Birpala, 27=Jamainaru, 28=Gandhokasturi, 29=Sabrimalati, 30=Jatabalam , 31=Patnai, 32=Godalaki, 33=Dudlaki, 34=Lal aman, 35=Malia vhangor , 36=Madhumala, 37= Gabura, 38=Marichful, 39=Sakkorkhana, 40= Kumragori, 41=Aloi, 42=Radhunipagal, 43=Matorsail, 44=Sita vhogh, 45=Dudsail, 46=Jhingasail, 47= Dudmoni, 48=Boron dhan, 49=Fulkarai, 50=Chinikanai, 51=Panati, 52=Horovhogh, 53=Akhnisail, 54=Ratisail, 55=Gochisail, 56=Vorisail, 57=Laki, 58=Gandhi biruin, 59=Tola biruin, 60=Hashim, 61=Joria, 62=Katarivhogh, 63=Jirasail, 64=Badshavhogh, 65=Daudin, 66=Dudsar, 67=Dhepi, 68=Hatisail, 69=Jesso balam, 70=Khirai jali, 71=Latisail, 72=Nizersail, 73=Patnai-23, 74=Rajasail, 75=SR-26-B, 76=Tilocikachari, 77=Lal roda dhan, 78=Bhobanivhogh, 79=Ratasail, 80=Lambosail, 81=Ropa aman, 82=Kartiksail, 83=Lotha, 84=Gangasagar, 85=Pankaij, 86=Holid jaran, 87=Apchaya, 88=Madhusail, 89=Jamalbhogh, 90=Aricha digh, 91=Jhul digh, 92=Boron, 93=Sechi, 94=Soider boron, 95=Lani khama, 96=Hashfal boron.

Principal coordinate analysis

The two-dimensional graphical view of principal coordinate Analysis (PCoA) showed the spatial distribution of the landraces along the two principal axes. The landraces Pankaij, Lotha, Chinigura, Patjag, Chinikanai, Badshabogh, Panati, Jirasail, Joria, Dudhmoni, Jhingasail were found far away from centroid of the cluster and the rest of the landraces were placed more or less around the centroid (Fig. 3). The results indicated that the landraces placed far away from the centroid were more genetically diverse while the landraces placed near around the centroid possessed more or less similar genetic background. However, centroid may be defined as the vector representing the middle point of the cluster, which contained at least one number for each variable. The connecting lines between each landrace and the centroid represented eigan vectors for the respective landraces.

The study of genetic diversity is a critical component of applied plant breeding for optimizing the choice of parents in a crop-breeding programme. An effective germplasm assessment provides the scientific basis for the selection of parents/donors for recombination breeding or hybrid breeding, and to breed

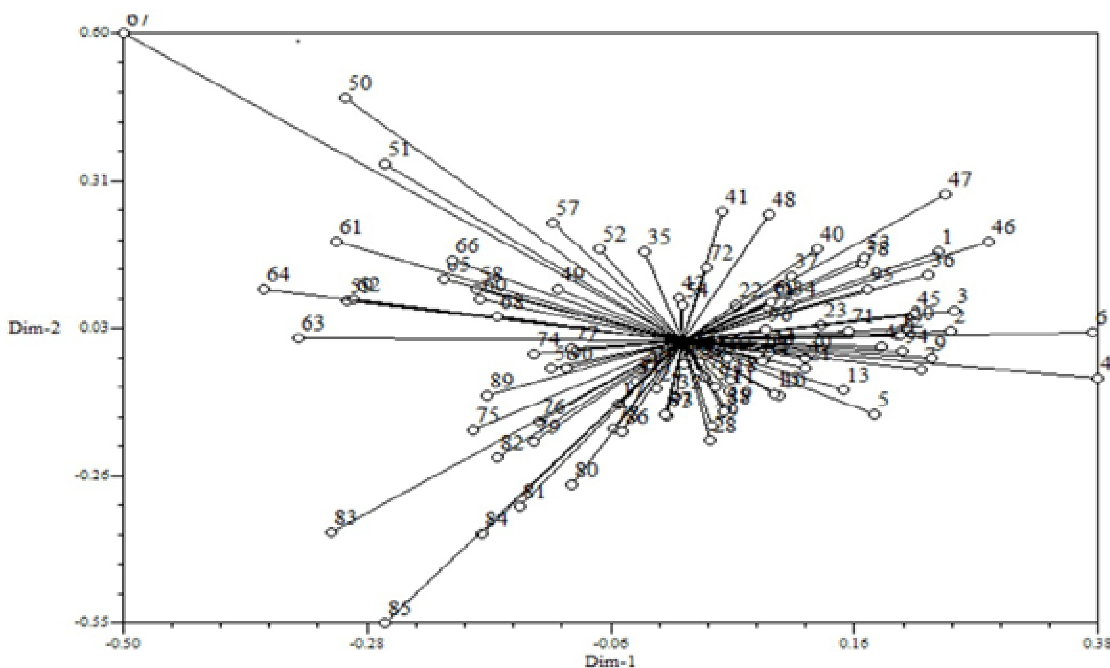

Fig. 3. Two-dimensional view of principal coordinate analysis (PCoA) with 8 micro-satellite markers over 96 Aman rice landraces.

Legend: Lane 1=Biruin, 2=Kalijira, 3=Topa, 4=Chinigura, 5=Chamara, 6=Patjagh, 7=Aloi, 8=Sada mota, 9=Lal mota, 10=Khama, 11=Bajal, 12=Dadjhani, 13=Bashful, 14=Binni, 15= Tulshimala, 16= Joina, 17= Parangi, 18=Horkoch, 19= Bekibalam, 20= Ashfol, 21=Chapail, 22=Chengai, 23=Jalpaira, 24=Latma, 25=Nonakuchi, 26=Birpala, 27=Jamainaru, 28=Gandhokasturi, 29=Sabrimalati, 30=Jatabalam , 31=Patnai, 32=Godalaki, 33=Dudlaki, 34=Lal aman, 35=Malia vhangor , 36=Madhumala, 37= Gabura, 38=Marichful, 39=Sakkorkhana, 40= Kumragori, 41=Aloi, 42=Radhunipagal, 43=Matorsail, 44=Sita vhogh, 45=Dudsail, 46=Jhingasail, 47= Dudmoni, 48=Boron dhan, 49=Fulkarai, 50=Chinikanai, 51=Panati, 52=Horovhogh, 53=Akhnisail, 54=Ratisail, 55=Gochisail, 56=Vorisail, 57=Laki, 58=Gandhi biruin, 59=Tola biruin, 60=Hashim, 61=Joria, 62=Katarivhogh, 63=Jirasail, 64=Badshavhogh, 65=Daudin, 66=Dudsar, 67=Dhepi, 68=Hatisail, 69=Jesso balam, 70=Khirai jali, 71=Latisail, 72=Nizersail, 73=Patnai-23, 74=Rajasail, 75=SR-26-B, 76=Tilocikachari, 77=Lal roda dhan, 78=Bhobanivhogh, 79=Ratasail, 80=Lambosail, 81=Ropa aman, 82=Kartiksail, 83=Lotha, 84=Gangasagar, 85=Pankaij, 86=Holid jaran, 87=Apchaya, 88=Madhusail, 89=Jamalbhogh, 90=Aricha digh, 91=Jhul digh, 92=Boron, 93=Sechi, 94=Soider boron, 95=Lani khama, 96=Hashfal boron.

for specific agro-ecological conditions and situations (Kumar *et al*., 2012). Diversity analysis at the molecular level using PCR-based markers is the efficient and rapid method of identifying the relationships and/or differences among the landraces (Schulman, 2007). Among the PCR-based markers, micro-satellites are becoming popular and suitable for large-scale analysis, both for genetic diversity and breeding research (Brown and Kresovich, 1996; Joshi *et al*., 2000). Proficient and consistent use of molecular markers such as SSR for the study of genetic diversity in any food crop requires selection and application of primers, which will give clear, distinct, reliable and sufficient information required to study the divergence that occurs within the crop (Arolu *et al*., 2012). Distinct band patterns were produced from the studied SSR markers amplified among the 96 Aman rice accessions and it was possible to reveal polymorphism from each marker.

The observed PIC values ranged from 0.858 (RM237) to 0.953 (RM163) with a mean value of 0.904. The allele frequency ranged from 8.33% (RM283) to 22.92% (RM163, RM125) with an average of 15.89. Our result is in agreement with the findings of Siddique *et al*. (2014), who estimated genetic diversity among T. Aman (rainfed lowland) rice germplasm using SSR markers. Gene diversity varied from 0.87 to 0.95 and their average value was 0.91, which also indicated the presence of adequate genetic diversity (Table 3). This average value was higher than the value recorded in rice by Zai-quan *et al*. (2012) who found 0.33 and by Siddique *et al*. (2016) who found 0.83. A better resolution of the relationship among the 96 accessions was provided by the UPGMA cluster analysis using SSR markers. Seven major groups were found at similarity coefficient levels of 0.09 where the rice accessions were widely clustered (Fig. 3) and the similarity coefficient varied from 0.06 to 0.75. It is notable that SSRs produced seven groups, but in the group of III the number of landraces was the highest (25) and the reason could be explained in this way that SSR markers might target a larger number of repeated sequences specifically in the centromeric region that might heavily influence the classification pattern (Parsons *et al*., 1997). These clustering patterns prove the acceptability and adaptability of SSR markers for the genetic diversity analysis among Aman rice germplasm. It was found that cluster analysis was profoundly supported by principal coordinate analysis (PCoA) but varied from unrooted neighbour-joining tree where six clusters were formed. The genetic diversity results that were observed under this study were supported by the consonance between cluster and PCoA analysis (Fig. 2–3).

CONCLUSION

The results obtained from this study on molecular characterization provided some useful implications for establishment of sovereignty of Bangladeshi rice gene pool. There was a high level of genetic diversity among accessions of Aman rice. In this study, it is suggested that SSR markers were effective in the detection of polymorphism in this ecosystem. To broaden the genetic base and for the improvement of Aman rice, accessions having the lowest genetic similarities could be selected as parents. Therefore, hybridization may be made between two distant populations. Considering all these criteria and results from genetic diversity analysis, accessions that are far apart based on their genetic coefficient (like Biruin and Khama; Kalijira and Dadkhani; Bashful and Topa; Binni and Chinigura; Chamara and Joina; Patjag and Porangi; Sadamota and Jotabalam; Tulshimala and Birpala; Rajasail and Horkoch; Pankaij and Sakkorkhana) could be selected as parents for further breeding programmes.

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