Genes, Genomes and Genomics ©2012 Global Science Books

Genetic Diversity Analysis of Aromatic Landraces of Rice (*Oryza sativa* L.) by Microsatellite Markers

M. M. Hossain[1] • M. M. Islam[2] • H. Hossain[2] • M. S. Ali[2] • Jaime A. Teixeira da Silva[3] • Atsushi Komamine[4] • Shamsul H. Prodhan[1*]

[1] Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet-3114, Bangladesh
[2] Biotechnology Division, Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur 1701, Bangladesh
[3] Department of Horticultural Science, Faculty of Agriculture and Graduate School of Agriculture, Kagawa University, Miki-cho, Ikenobe 2393, Kagawa-ken, 761-0795, Japan
[4] The Research Institute of Evolutionary Biology, 2-4-28 Kamiyoga, Setagaya-ku, Tokyo 150-0098, Japan

Corresponding author: * **shamsulgeb@gmail.com**

ABSTRACT

The allelic diversity and relationship among 12 elite aromatic rice cultivars were determined through DNA fingerprinting using microsatellite markers. A total of 24 SSR markers were used to characterize and discriminate all rice genotypes, 10 of which were polymorphic for different chromosome numbers. The number of alleles per locus generated by each marker varied from 2 (RM256, RM510) to 7 (RM180), with an average of 3.8 alleles per locus. The polymorphic information content (PIC) values varied widely among loci and ranged from 0.239 to 0.765 with an average of 0.508. The highest PIC value (0.765) was obtained for RM180, followed by RM207 (0.746), RM224 (0.680), RM163 (0.593), and RM566 (0.505). The genetic distance-based results seen in the unrooted neighbor-joining tree and UPGMA clustering revealed five genetic groups in which closely related genotypes were clustered together. A moderate salt-tolerant variety, Rajasail, grown mainly in coastal areas, formed a distinct monogenic cluster. Another single cluster was formed by Tilockachari, which was 54.02% dissimilar with Rajasail; the remaining aromatic land races formed distinct clusters according to their relatedness. Pairwise genetic dissimilarity was also studied to determine the level of relatedness among the cultivars in which highest genetic dissimilarity was recognized between Gondhocosturi and Lalrodha dhan (81.03%), Gondhocosturi and Rajasail (81.03%), and other pairs. 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 the near future.

Keywords: dendogram, DNA fingerprinting, polymorphic information content (PIC) value
Abbreviations: ARL, aromatic rice landrace; **BRRI**, Bangladesh Rice Research Institute; **GRC**, Genetic Resource Center; **PIC**, polymorphic information content; **SDS**, sodium dedocyl sulphate; **SSR**, simple sequence repeat; **UPGMA**, Unweighted Pair Group Method with Arithmetic Average

INTRODUCTION

Rice (*Oryza sativa* L.) occupies almost one fifth to the total land area covered by cereals and it is the principal staple food for more than 50% of the world's population (Chakravarthi *et al.* 2006; Ram *et al.* 2007). Approximately 11% of the world's arable land is cultivated annually with rice (Cantrell and Hettel 2004), ranking next after wheat. Rice is grown under diverse eco-geographical conditions in various tropical and subtropical countries like Bangladesh and other Asian countries. Despite the annual production shortfall of 2 to 4 million metric tons, rice provides more than 80% of the food requirements for the common people of Bangladesh (Jalaluddin *et al.* 2007). Rice occupies 77% of the total cropped area in Bangladesh and provides 75% of the calories and 55% of proteins of the population's average daily diet (Bhuiyan *et al.* 2002). Aromatic rice is popular in Asia and has gained wider acceptance in Europe and USA because of its aroma and texture (Hossain *et al.* 2006). Aromatic rice has seen a higher demand in recent years because of its scent and palatability for both internal consumption and export. A landrace is a geographically or ecologically distinctive autochthonous population, which is conspicuously diverse in its genetic composition both between landraces and within them and represents a unique and critical source of genetically variable traits that can serve as a resource for future rice improvement. Landraces have been shown to be excellent sources of genes for novel alleles (McCouch *et al.* 1997; Hoisington *et al.* 1999; Jackson 1999; Guevarra *et al.* 2001). More than 4000 traditional Bangladesh rice accessions or landraces have been collected and registered at a rice gene bank in the Bangladesh Rice Research Institute (BRRI) for medium-term storage and an identical set is held in trust at International Rice Research Institute (IRRI) for longer storage (Jackson 1999). Nearly 10,000 landraces are considered to exist in Bangladesh; among them, more than 4000 local landraces of rice have been adapted in different parts of the country (Kaul *et al.* 1982). Agriculture relies heavily on the genetic diversity of a crop plant and it is estimated that not even 15% of the potential diversity has been utilized. This implies that thousands of valuable allelic variations of traits of economic significance remain unutilized (Hossain *et al.* 2008). Therefore, it is highly necessary not only to conserve landrace genotypes but also to reveal the gene-pool of aromatic rice and unlock valuable genes for breeding purposes (Rabbani *et al.* 2008). Thus, identification of genotypes and their interrelationships is imperative. Genetic variation can be evaluated on both phenotypic and genotypic levels and the development of new biotechnological techniques provides increased support to achieve this goal. Molecular markers are powerful tools in the assessment of genetic variation, in the elucidation of genetic relationships within and among species and have demonstrated the potential to detect genetic diversity and to aid in the management of plant genetic resources (Virk *et al.* 2000; Song *et al.* 2003; Teixeira da Silva 2005). After the completion of rice genome sequencing in 2005 by the International Rice Genome Sequencing

Received: 9 November, 2010. Accepted: 22 May, 2011.

Original Research Paper

Project/IRGSP, the genome sequence information has served as an excellent platform to uncover single nucleotide polymorphisms (SNPs) of rice that is now more than 5 million (McNally 2006). SSR is the next most abundant marker in rice numbering to 13,000 (Romero *et al.* 2009). Microsatellite DNA markers consisting of AT repeats were found to be highly polymorphic in the rice genome and an abundance of microsatellite markers is now available through the published high-density linkage map (McCouch *et al*. 2002; IRGSP 2005). Diversity analysis for fine grain and aromatic rice landraces (ARLs) has been rarely conducted in Bangladesh. These ARLs could serve as a mine of germplasm and a new genetic pool. Great effort has been made to assess genetic variability at the molecular level among 12 ARLs using SSR markers because of their abundance in the plant genome and high level of polymorphism. Consequently, these markers would be particularly useful for developing unique DNA profiles for rice genotypes. The present study addresses the ability of microsatellite markers in revealing genetic diversity and DNA fingerprinting aromatic landraces to determine genotypic differences, seed purity, and serve as a basis for rice breeding programme, germplasm collection, management and establishment of a core collection of ARLs.

MATERIALS AND METHODS

Plant materials

Twelve elite rice genotypes of ARLs and fine rice landraces were selected for the experiment: Gondhocosturi, Gondhoraj, Hatisail, Begunbichi, Khajar, Rajasail, Basmati tap 90, Radhunipagol, Nizersail, Lalrodha dhan, Kartiksail, Tilockachari. Seeds of rice genotypes were collected from the Genetic Resource Center (GRC), BRRI for germination after dormancy breaking at 50°C for 3 days. Germination of seeds was carried out on petri dish (15 seeds of single genotype per Petri dish) with filter paper and distilled water at 28°C in dark condition. For seed germination, a minute amount of distilled water was used only to soak the filter paper under aseptic conditions and then germinated seeds were sown at specified row and column distances in pots in a glasshouse.

Genomic DNA isolation

Prior to the isolation of genomic DNA, a healthy portion of fresh youngest leaves were harvested from a single 3-week-old plant of each line grown in pots in a glasshouse. DNA extraction was carried out following a mini-prep DNA extraction protocol which did not require liquid nitrogen and required only a very small amount of tissue samples (Zheng *et al.* 1995). All chemicals used for DNA extraction were purchased from Sigma-Aldrich, Germany. Leaf tissues were cut into small pieces, homogenized and digested with extraction buffer (1 M Tris, 0.5M Na_2EDTA, 5M NaCl, 10% SDS and distilled H_2O, pH 8.0). Leaf extracts were then placed in 65º C water bath in a tube holder for 20 min and inverted at 10-min intervals and returned to the water bath each time. Following incubation, 800 µl of a mixture of chloroform and isoamyl alcohol (24: 1) was added, centrifuged for 8 min at 11,000 rpm in a microcentrifuge and 500 µl of upper aqueous layer was collected. To this supernatant, 1000 µl of cold 100% ethanol was added, mixed and centrifuged for 12 min at maximum speed (13,200 rpm). A small pellet was visible and the supernatant was decanted. The DNA pellet was washed with cold 70% ethanol and allowed to air dry. The DNA pellets were resuspended in 100 µl of 10X TE buffer and dissolved by warming in a 65°C water bath for up to 1 h (with frequent mixing or flicking of the tube). The pellet, once dissolved, was stored at -20°C. The quality of DNA was also checked by DNA quantification using a Thermo Scientific NanoDrop™ 1000 spectrophotometer (Thermo Fisher Scientific, USA).

Microsatellite markers

A total of 24 microsatellite primer pairs (Sigma-Aldrich, Germany) covering all 12 chromosomes were selected for the genetic diversity analysis of the 12 ARLs in Bangladesh. Primers that showed monomorphic banding patterns were excluded while only one primer with higher allele number was selected from primers showing a polymorphic banding pattern at the same chromosome based on number of allele detected. Finally, 10 microsatellite primers with a distinct chromosome number were used for final polymerase chain reaction (PCR) amplification. The original source, repeat motifs, primer sequences, expected length and chromosomal positions for these markers can be found in the rice genome database (http://www.gramene.org).

PCR amplification

Prior to DNA amplification, a PCR cocktail was prepared containing all required components. All reagents were purchased from Sigma-Aldrich. PCR was carried out in a DNA thermal cycler (Model: ALS1296, BioRad, USA and G- STORM, GSI, England, Serial no: GT-11620) and PCR amplification reactions were done in a total volume of 10 µl containing 1.5 µl of 10X buffer, 0.25 µl of 10 mM dNTPs, 0.5 µl of each forward and reverse primer, 2.25 µl of ddH_2O, 0.2 µl of *Taq* polymerase, 1.8 µl of $MgCl_2$ and 3 µl of diluted template DNA. For PCR amplification, the thermal cycler was set at 1 cycle for 5 min at 94°C (hot start and strand separation) followed by 34 cycles of denaturation (94°C), annealing (55°C) and primer elongation (72°C) for 30 sec each and a final extension in 1 cycle of 5 min at 72°C. Amplified products were stored at -20°C until further use. The reproducibility of amplification products was confirmed twice for each primer. PCR products were mixed with bromophenol blue gel loading dye at a 5: 3 ratio and resolved by polyacrylamide gel electrophoresis run on 8% polyacrylamide gels in TBE buffer. To each well, 4 µl of sample was loaded and run at 80 V for 90 min using a mini vertical polyacrylamide gel running apparatus for high throughput manual genotyping (model: 300 series, CBS Scientific Co Inc., CA. USA). The gels were next stained in ethidium bromide solution (50 µl in 500 ml distilled water) for 30-35 min, kept in the dark, and then scanned using an a UVPRO (Uvipro Platinum, EU) gel documentation unit linked to a PC (Windows).

SSR data analysis

The size of amplicons was estimated by comparing the migration distance of amplified fragments with that of known size fragments using 25 (bp) base pairs (Promega, Madison, USA) and 50 bp DNA ladder (Thermo Fisher Scientific, Fermentas INC., Maryland, USA). The molecular weight of distinct bands or amplified fragments was measured in bp using Alpha-Ease FC 5.0 software. Genetic diversity of cultivars by SSRs was evaluated by the number of alleles and the polymorphic information content (PIC) value, which is an estimate of the discriminatory power of a SSR marker locus. Statistics, including the number of alleles per locus, major allele frequency, gene diversity, and PIC values were calculated using PowerMarker version 3.25 (Liu and Muse 2005). SSR marker alleles were analyzed using Power Marker version 3.25 and used to export the allele frequency data to a binary format in which each SSR band was scored as present (1), absent (0), or as a missing observation for analysis with NTSYS-pc version 2.2 (Rohif 2002). NTSYS-pc was used to construct a UPGMA (unweighted pair group method with arithmetic averages) dendrogram showing the distance-based interrelationship among the genotypes. For the unrooted phylogenetic tree, genetic distance was calculated using the "C.S Chord 1967" distance (Cavalli-Sforza and Edwards 1967) in PowerMarker with tree viewed using Treeview software.

RESULTS

Overall SSR DNA marker diversity

The level of polymorphism among the rice cultivars was evaluated by calculating allele number and PIC values for each of the 10 SSR loci evaluated. In the present investigation SSR markers from all chromosomes except 4 and 12 were used to characterize and to assess genetic diversity among the 12 ARLs where, Chromosome 4 and 12 was not

Table 1 Number of alleles, allele size range, highest frequency allele and polymorphism information content (PIC) values found among 12 rice genotypes for 10 SSR markers.

Primer	Chromosome	RM	Position	NA	Freq.	Diversity	PIC
RM243	1	(CT)18	57.3 cM	4	0.6667	0.5139	0.4760
RM180	7	(ATT)10	55.8 cM	7	0.3333	0.7917	0.7648
RM207	2	(CT)25	191.2 cM	6	0.3333	0.7778	0.7456
RM163	5	(GGAGA)4 (GA)11 C(GA)20	91.4 cM	3	0.3333	0.6667	0.5926
RM510	6	(GA)15	20.8 cM	2	0.8333	0.2778	0.2392
RM256	8	(CT)21	101.5 cM	2	0.8333	0.2778	0.2392
RM566	9	(AG)15	47.7 cM	3	0.5833	0.5694	0.5045
RM590	10	(TCT)10	117.2 cM	3	0.5000	0.5694	0.4768
RM224	11	(AAG)8(AG)13	120.1 cM	5	0.4167	0.7222	0.6800
RM338	3	(CTT)6	108.4 cM	3	0.7500	0.4028	0.3633

Chr: chromosome; Freq: major allele frequency; NA: number of alleles; PIC: polymorphism information content, RM: repeat motif * Motif of the SSR markers, position and number of repeats as previously published (http://www.gramene.org).

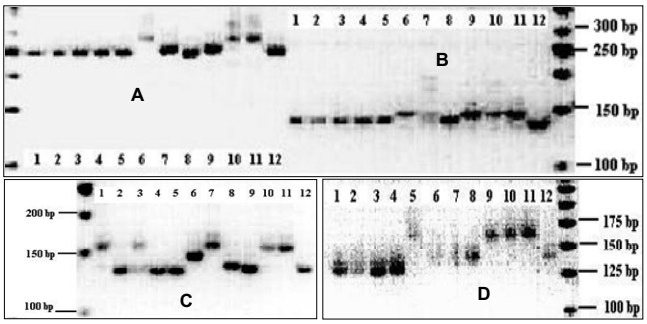

Fig. 1 Separation of alleles on 8% polyacrylamide gel followed by staining with ethidium bromide and visualized under UV light. PCR products were amplified with rice microsatellite primers (**A**) RM566 (**B**) RM590 (**C**) RM224 (**D**) RM163. The first lane of each row was the molecular marker, all are 50 base pair (bp) ladder marker except D (25 bp ladder marker) followed by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 where they represents rice landraces Gondhocosturi, Gondhoraj, Hatisail, Begunbichi, Khajar, Rajasail, Basmati tap 90, Radhunipagol, Nizersail, Lalrodha dhan, Kartiksail and Tilockachari respectively.

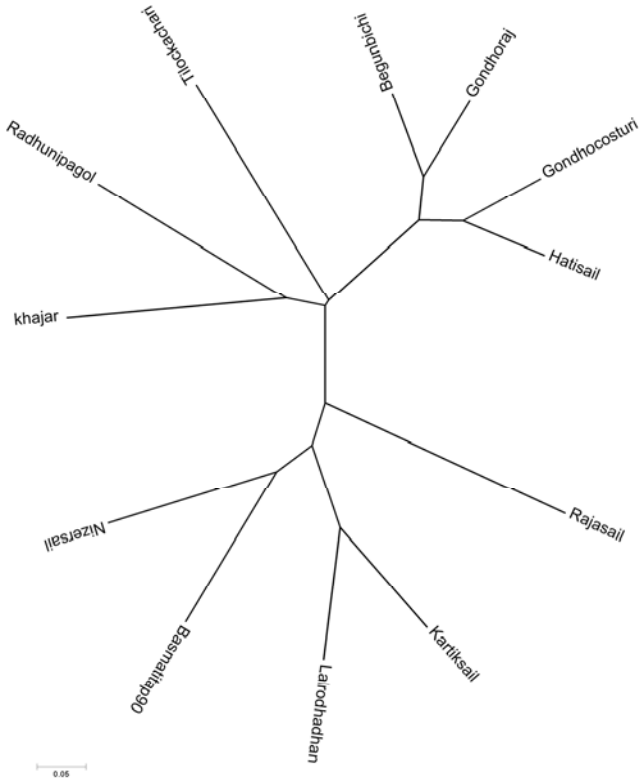

Fig. 2 An unrooted neighbor-joining tree showing the genetic relationships between the 12 rice accessions based on 10 microsatellite markers. The five major groups are found based on relatedness between themselves. Like Tilockachri, a moderate salt tolerant aromatic landrace Rajasail which mainly found in costal region form distinct single group. All other grouped separately based on their similarity and dissimilarity.

considered because of financial constrain. A total of 38 alleles were detected at 10 SSR markers in the 12 rice genotypes. Among the polymorphic markers, 2 amplified 2 alleles each, 4 markers produced 3 alleles each, only one marker produced 4 alleles while the remaining 3 markers produced 5, 6 and 7 alleles each, respectively (**Table 1**). The number of alleles per locus generated by each marker varied from 2 alleles (RM256, RM510) to 7 alleles (RM180), with an average of 3.8 alleles per locus. The variability in allele number per locus is consistent with the frequency of the most common allele at each locus, ranging from 33.33% (RM180, RM207 and RM163) to 83.33% (RM256, RM510). On average, 55.83% of the 12 rice genotypes shared a common major allele at any given locus. The genetic diversity of these 10 loci for the 12 rice landraces ranged from 0.2778 to 0.7917 with an average of 0.5570, indicating a moderate level of diversity. The highest genetic diversity (0.7917) was recorded in locus RM180 and the lowest genetic diversity (0.2778) was detected in loci RM510 and RM256 (**Table 1**). **Fig. 1** shows a gel image of amplified fragments using a primer selected RM566, RM590, RM224 and RM163. The PIC value is a reflection of allele diversity and frequency among varieties. The PIC value of each marker, which can be evaluated on the basis of its alleles, varied greatly for all the SSR loci tested, ranging from 0.239 to 0.765 with an average of 0.508. The highest PIC value (0.765) was obtained for RM180, followed by RM207 (0.746), RM224 (0.680), RM163 (0.593), and RM566 (0.505).

Genetic distance-based analysis

Genetic distance is a measure used to quantify the difference between two populations in relation to the frequency of a particular trait as well as the relationship among populations. An unrooted neighbor–joining tree (**Fig. 2**) showing the genetic-relationships among 12 aromatic landraces of Bangladesh was constructed based on the alleles detected by 10 SSR markers. The genetic distance-based results seen in the unrooted neighbor-joining tree revealed 5 groups. Four ARLs (Gondhocosturi, Hatisail, Gondhoraj, and Begunbichi) were clustered in the same group in which Gondhoraj was very close to Begunbichi, while Gondhocosturi and Hatisail were close to each other. ARL Basmati 90 was clustered with Nizersail, Lalrodha dhan and Kartiksail. A moderate salt-tolerant variety Rajasail, which is grown mainly in the coastal area, solely formed a distinct cluster. Another single cluster was formed by Tilockachari. Furthermore, the remaining two ARLs, Khajar and Radhunipagol, appeared to be close and could thus be considered as a single group. Cluster analysis was used to group these varieties and to construct a dendogram. The genetic relationship

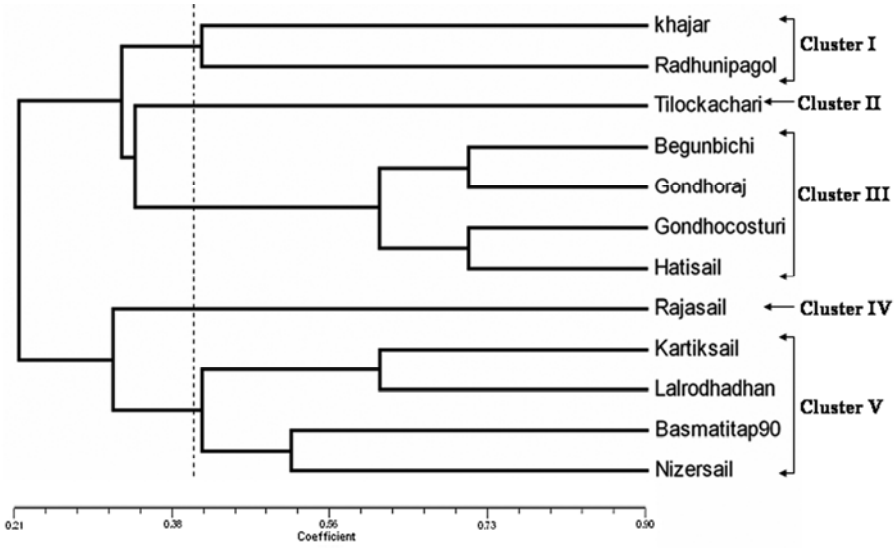

Fig. 3 An UPGMA cluster dendogram showing the genetic relationships among 12 aromatic rice landraces based on 10 SSR markers.

Table 2 Genetic dissimilarity between pair of genotypes obtained from SSR analysis.

Ecotypes	AR1	AR10	AR11	AR12	AR2	AR3	AR4	AR5	AR6	AR7	AR8	AR9
AR1	0.0000											
AR10	0.8103	0.0000										
AR11	0.7203	0.2701	0.0000									
AR12	0.6302	0.7203	0.6302	0.0000								
AR2	0.2701	0.6302	0.6302	0.5402	0.0000							
AR3	0.1801	0.6302	0.5402	0.5402	0.2701	0.0000						
AR4	0.3601	0.6302	0.5402	0.3601	0.1801	0.1801	0.0000					
AR5	0.5402	0.7203	0.6302	0.5402	0.5402	0.5402	0.4502	0.0000				
AR6	0.8103	0.4502	0.5402	0.5402	0.8103	0.7203	0.6302	0.8103	0.0000			
AR7	0.5402	0.5402	0.4502	0.5402	0.6302	0.3601	0.5402	0.6302	0.5402	0.0000		
AR8	0.5402	0.8103	0.7203	0.6302	0.5402	0.4502	0.5402	0.4502	0.8103	0.4502	0.0000	
AR9	0.7203	0.4502	0.3601	0.5402	0.5402	0.5402	0.4502	0.5402	0.6302	0.3601	0.7203	0.0000

Note: AR1 = Gondhocosturi, AR2 = Gondhoraj, AR3 = Hatisail, AR4 = Begunbichi, AR5 = Khajar, AR6 = Rajasail, AR7 = Basmati 90, AR8 = Radhunipagol, AR9 = Nizersail, AR10 = Lalrodha dhan, AR11 = Kartiksail, AR12 = Tilockachari.

among the rice cultivars was assessed by a UPGMA cluster analysis of the similarity coefficient. The UPGMA-based dendogram obtained from the binary data was deduced from the DNA profiles of the samples analyzed. The genetic similarity analysis using UPGMA clustering agreed with the neighbor-joining data. The UPGMA clustering system also generated 5 genetic clusters (**Fig. 3**) with a similarity coefficient of 44%. Here, cluster I consisted of ARLs Khajar and Radhunipagol, which showed 45.02% dissimilarity between them. Tilockachari alone was grouped in a single cluster, cluster II. Four ARLs (Gondhocosturi, Hatisail, Gondhoraj and Begunbichi) were clustered in the same group (cluster III) and also had additional sub-clusters within this cluster. For example, Tilockachari and Rajasail also formed a separate group (cluster IV) alone and showed 54.02% dissimilarity with Tilockachari. The remaining 4 ARLs (Nizersail, Lalrodha dhan, Basmati 90 and Kartiksail) formed a single cluster (V) which had two separate additional sub-clusters within it. Clusters III and V were the largest group including 4 ARLs each, cluster I comprised 2 cultivars while clusters II and IV were of equal size comprising a single cultivar each. This dendogram revealed that the genotypes that are derivatives of genetically similar types clustered together more.

Pairwise genetic distance

A dissimilarly matrix was used to determine the level of relatedness among the cultivars studied. The pair-wise genetic dissimilarity indices (**Table 2**) indicated that the highest genetic dissimilarity was between Gondhocosturi and Lalrodha dhan (81.03%), Gondhocosturi and Rajasail (81.03%), Radhunipagol and Lalrodha dhan (81.03%), Radhunipagol and Khajar (81.03%) as well as between Gondhuraj and Rajasail (81.03%). These pairs were followed by Gondhocosturi and Kartiksail (72.03%), Lalrodha dhan and Tilockachari (72.03%), Radhunipagol and Kartiksail (72.03%), Gondhocosturi and Tilockachari (63.02%), decreasing thereafter. The lowest genetic dissimilarity among rice landraces was between Gondhocosturi and Hatisail (18.01%), Hatisail and Begunbichi (18.01%) as well as Gondhoraj and Begunbichi (18.01%), followed by Gondhocosturi and Gondhuraj (27.01%), and Gondhuraj and Hatisail (27.01%). No ARLs were found in duplicate (i.e., 100% similarity). As expected, genetic dissimilarity between the ARLs was comparatively high. Hence, microsatellite markers could serve as a potential tool in the identification and characterization of genetically distant cultivars from various sources.

DISCUSSION

Assessment of genetic diversity is an essential component in germplasm characterization and conservation. Morphological and seed traits have long been the means of studying taxonomy and variability among plant species. Microsatellites are amongst the most widely used DNA marker for many purposes such as diversity, genome mapping, varietal identification, etc. (Teixeira da Silva 2005). The use of these markers to investigate genotypic variations among different rice cultivars was previously reported by some researchers (Singh *et al*. 2004; Joshi and Behera 2006). The use of these molecular techniques to investigate the genetics of Bangladeshi ARLs is limited. 10 SSR markers evenly

spread over all chromosomes (except for chromosomes 4 and 12) of rice were used in this study based on their physical position according to the "Gramene" database (http://www.gramene.org). The number of alleles detected by microsatellite markers varied from 2 to 7 with an average of 3.8 alleles per locus (**Table 1**). The numbers of alleles observed in the present study was very consistent with some earlier reports on Indian rice landraces specifically ARLs (Siwach *et al*. 2004; Neeraja *et al*. 2005). Jalaluddin *et al.* (2007) reported on the genetic diversity and DNA fingerprinting of some modern *INDICA* and *JAPONICA* rice where the number of alleles detected in the 25 rice cultivars at the five loci varied from 2 to 4 with an average 2.8. Our results were also comparable to 2.0-5.5 alleles per SSR locus for various classes of microsatellites reported by Cho *et al.* (2000), who used genomic libraries and GeneBank sequences in rice. Wong *et al.* (2009) reported the genetic relationship and diversity analysis among 8 Bario rice cultivars using 12 SSR primers, detecting a total of 31 alleles. The average number of alleles per locus was 2.6, which is markedly lower than our report. In contrast, the average number of alleles detected in the present study was lower than the average number of alleles reported by Ni *et al.* (2002), Jain *et al.* (2004), Xu *et al*. (2004), Lu *et al*. (2005), Jayamani *et al*. (2007), Thomson *et al*. (2007), Pervaiz *et al.* (2010) and Upadhyay *et al*. (2011), who reported an average of 6.8, 7.8, 11.9, 6.57, 14.6, 7.7, 13, 4.4 and 4.35 alleles per locus using rice subspecies, Indian quality rice germplasm, US rice genetic resources, traditional varieties of Brazilian rice, a diverse collection of Portuguese rice, Indonesian rice germplasm (both *indica* and *japonica*), Pakistani rice landraces and Indian rice varieties respectively. There is such big variability using SSRs in the number of alleles detected per locus and this inconsistency might be due to the diverse genotypes used and selection of SSR primers with scorable alleles. Parvaiz *et al.* (2009) used 32 SSR markers to determine the genetic diversity of 35 cultivars of Asian rice and showed a clear division of cultivars into aromatic and non-aromatic groups. In their experiment, the number of alleles detected by microsatellite markers varied from 2 to 13 with an average of 4.5 alleles per locus, which is higher than our study (3.8 alleles per locus). According to Borba *et al*. (2009), a set of 86 SSRs detected a total of 1,066 alleles across 242 accessions from the EMBRAPA Rice Core Collection (ERiCC). The average number of alleles per locus was 12.4 with a range of 3 to 32. The SSR markers involving di-nucleotide repeats amplified a relatively small number of bands as reported earlier in rice (Cho *et al.* 2000; Sainil *et al.* 2004). Similarly, microsatellites with poly (AT)n repeats represent the most abundant and polymorphic class of SSRs but are frequently associated with the Micropon family of miniature inverted-repeat transposable elements (MITEs) and are difficult to amplify (Temnykh *et al.* 2001). Markers with the highest number of discernable alleles could be the best markers for molecular characterization and diversity analysis. RM180 on chromosome 7 detected 7 alleles followed by RM207 (6 alleles), RM224 (5 alleles) and RM243 (4 alleles). This suggests that these markers could be potentially used for molecular characterization of ARL germplasm from various sources. However, there were a number of markers which produced only few alleles. Two markers produced only two alleles, and despite their ability to produce only few alleles, they were robust enough to distinguish specifically diverse genotypes or different accessions of the same genotype. The PIC value of a marker is the probability of the marker allele that can be deduced in the progeny and is a good measure of a marker's usefulness for linkage analysis. The level of polymorphism determined by the PIC values was quite high and varied (range from 0.239 to 0.765, average = 0.508) considerably among SSR loci (**Table 1**). The PIC values observed in our study were consistent with previous estimates of microsatellite marker analysis in rice by Jain *et al*. 2004 (range from 0.2 to 0.9, mean = 0.6); Siwach *et al*. 2004 (range from 0.0 to 0.78, mean = 0.62); Lu *et al*. 2005 (range from 0.028 to 0.881, mean = 0.463); Jayamani *et al*. 2007 (range from 0.179 to 0.894, mean = 0.667); Thomson *et al*. 2007 (range from 0.34 to 0.88, mean = 0.66) and Wong *et al*. 2009 (range from 0.375 to 0.656, mean = 0.52). The PIC value was lower than that previously reported by Xu *et al.* (2004) and Parvaiz *et al.* (2009), who observed an average PIC value of 0.73 and 0.603 for a world collection and Asian cultivars, respectively. According to Borba *et al.* (2009), PIC values varied greatly among markers, from 0.19 to 0.90 with an average of 0.75, which is markedly higher than the result in our study. In our study, the highest PIC value (0.765) was observed by RM180 followed by RM207 (0.746), RM224 (0.680), RM163 (0.593), and RM566 (0.505). RM180, considering its high PIC value, is supposed to be the best marker for characterizing the 12 genotypes (Islam *et al*. 2008) and, to a lesser extent, RM207, RM224, RM163 and RM566, which were also used for molecular characterization of these varieties. Molecular diversity analysis of stress tolerant rice using SSR marker was conducted by Islam *et al.* (2008), who observed RM10793 with the highest PIC value found to be the best marker for characterizing the 21 rice genotypes. The UPGMA clustering system also generated genetic clusters with a similarity coefficient of 44%. Cluster analysis based on similarity coefficients placed 12 rice genotypes into 5 major groups (**Fig. 3**). Some of the ARL clusters fell into close sub-groups. This dendogram revealed that the genotypes that are derivatives of genetically similar type clustered together more. For example, Tilockachari and a moderate salt-tolerant variety (Rajasail) which is grown mainly in coastal areas, solely formed a distinct cluster. Development of salt-tolerant germplasm, i.e. breeding lines, through crosses with salt-tolerant landraces is an intermediate step before the final transfer of salt-tolerance genes into commercial cultivars (Zeng *et al*. 2004). Identifying different salt tolerance components and pyramiding them into a salt-tolerant cultivar has been suggested (Yeo *et al*. 1986). The success in pyramiding different salt tolerance components into a cultivar can be increased using microsatellite markers because of the difficulty in identifying these tolerance components using conventional methods (Zeng *et al*. 2004). With the aid of microsatellite makers, different salt tolerance components may be combined by intercrossing genotypes from different clusters. In this study, the larger range of similarity values for cultivars revealed by microsatellite markers provides greater confidence for the assessments of genetic diversity and relationships in conjunction with pedigree records, which can be used in future breeding programs. The utilization of 10 microsatellite markers in the analysis of rice varieties revealed a high level of genetic polymorphism and these 10 markers gave sufficient information for unambiguous identification of 12 ARLs. From this study, it might be concluded that microsatellite analysis could be efficiently utilized for diversity analysis and differentiation of ARLs. Many studies have also reported significantly greater allelic diversity of microsatellite markers than other molecular markers (McCouch *et al*. 2001). In addition, marker-based identification and differentiation of ARLs could be helpful to preserve the integrity of high-quality rice varieties such as basmati and aromatic varieties, for characterization, unlocking many genes and benefiting both farmers and consumers. Molecular diversity studies are useful for characterizing vast resources of plant germplasm, especially in the context of protecting traditional plant resources maintained by individual farmers from contraventions. Markers based on polymorphism in DNA sequences are increasingly being utilized for this purpose (Weising *et al*. 2005). The results represent one of the attempts to discover a small set of microsatellite markers to discriminate ARLs from Bangladesh providing meaningful data that can be enlarged by additional rice cultivars and new microsatellite markers.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the assistance of the Biotechnology Division, Bangladesh Rice Research Institute (BRRI) in conducting this research work at the Biotechnology Laboratory and for technical support. We also gratefully appreciate the cooperation of the Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet, Bangladesh.

REFERENCES

Bhuiyan NI, Paul DNR, Jabber MA (2002) Feeding the extra millions by 2025 challenges for rice research and extension in Bangladesh. In: *National Workshop on Rice Research and Extension*, 29-31 January, 2002, BRRI, Gazipur, Bangladesh, 26 pp

Borba TCO, Brondani RPV, Rangel PHN, Brondani C (2009) Microsatellite marker mediated analysis of the EMBRAPA rice core collection genetic diversity. *Genetica* **137**, 293-304

Cantrell RP, Hettel GP (2004) New challenges and technological opportunities for rice based production systems for food security and poverty alleviation in Asia and the Pacific. In: *FAO (Food and Agriculture Organization) Rice Conference*, 12-13 February, 2004, FAO, Rome, Italy, pp 1-15

Cavalli-sforza LL, Edwards AWF (1967) Phylogenetic analysis: Model and estimation procedures. *American Journal of Human Genetics* **19**, 233-257

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

Cho YG, Ishii T, Temnykh S, Chen X, Lipovich L, McCouch SR, Park WD, Ayers N, Cartinhour S (2000) Diversity of microsatellites derived from genomic libraries and Gene Bank sequences in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* **100**, 713-722

Guevarra E, Loresto GC, Jackson MT (2001) Use of conserved rice germplasm. *Plant Genetic Resources Newsletter* **124**, 51-56

Hoisington D, Khairallah M, Reeves T, Ribaut JM, Skovmand B, Taba S, Warburton M (1999) Plant genetic resources: What can they contribute toward increased crop productivity? *Proceedings of the National Academy of Sciences USA* **96**, 5937-5943

Hossain MG, Arora RK, Mathur PN (2006) Bangladesh Agricultural Research Council, Dhaka, Bangladesh, pp 66-70

Hossain MZ (2008) Molecular characterization and genetic diversity in fine grain and aromatic landraces of rice using microsatellite markers. *Bangladesh Journal of Genetics and Plant Breeding* **20 (2)**, 1-10

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

Islam MR, Singh RK, Salam MA, Hassan L, Gregorio GB (2008) Molecular diversity of stress tolerant rice genotypes using SSR markers. *SABRAO Journal of Breeding and Genetics* **40 (2)**, 127-139

Jackson MT (1999) Managing the world's largest collection of rice genetic resources. In: Rutger JN, Robinson JF, Dilday RH (Eds) *International Symposium on Rice Germplasm Evaluation and Enhancement*, Arkansas Agricultural Experiment Station Special Report, Arkansas, USA, pp 22-28

Jain S, Jain RK, McCouch SR (2004) Genetic analysis of Indian aromatic and quality rice (*Oryza sativa* L.) germplasm using panels of fluorescently-labeled microsatellite markers. *Theoretical and Applied Genetics* **109**, 965-977

Jalaluddin M, Nakai H, Yamamoto T (2007) Genetic diversity and DNA fingerprinting of some modern *indica* and *japonica* rice. *SABRAO Journal of Breeding and Genetics* **39 (1)**, 43-52

Joshi RK, Behrea L (2006) Identification and differentiation of indigenous non-Basmati aromatic rice genotypes of India using microsatellite markers. *African journal of Biotechnology* **6**, 348-354

Jayamani P, Negrão S, Martins M, Maçãs B, Oliveira MM (2007) Genetic relatedness of Portuguese rice accessions from diverse origins as assessed by microsatellite markers. *Crop Science* **47**, 879-886

Kaul AK, Khan MRI, Munir KM (1982) Rice quality, a survey of Bangladesh germplasm. BRRI, Joydebpur, Bangladesh, pp 1-17

Liu K, Muse SV (2005) Powermarker: Integrated analysis environment for genetic marker data. *Bioinformatics* **21**, 2128-2129

Lu H, Redus MA, Coburn JR, Rutger JN, McCouch SR, Ti TH (2005) Population structure and breeding patterns of 145 US rice cultivars based on SSR marker analysis. *Crop Science* **45**, 66-76

McCouch SR, Chen X, Panaud O (1997) Microsatellite mapping and applications of SSLP's in rice genetics and breeding. *Plant Molecular Biology* **35**, 89-99

McCouch SR, Temnykh S, Lukashova A, Coburn J, Declerck G, Cartinhour S, Harrington S, Thomson M, Septiningsi E, Semon M, Moncada P, Li J (2001) Microsatellite markers in rice: Abundance, diversity and applications. In: *Rice Genetics IV*, IRRI. Manila, Philippines, pp 117-135

McNally KL, Bruskiewich R, Mackill D, Buell CR, Leach JE, Leung H (2006) Sequencing multiple and diverse rice varieties connecting whole-genome variation with phenotypes. *Plant Physiology* **141**, 26-31

Neeraja CN, Hariprasad AS, Malathi S, Siddiq EA (2005) Characterization of tall landraces of rice (*Oryza sativa* L.) using gene-derived simple sequence repeats. *Current Science* **88 (1)**, 149-152

Ni J, Colowit PM, Mackill DJ (2002) Evaluation of genetic diversity in rice subspecies using microsatellite markers. *Crop Science* **42**, 601-607

Pervaiz ZH, Rabbani MA, Pearce SR, Malik SA (2009) Determination of genetic variability of Asian rice (*Oryza sativa* L.) varieties using microsatellite markers. *African Journal of Biotechnology* **8 (21)**, 5641-5651

Pervaiz ZH, Rabbani MA, Khaliq I, Pearce SR, Malik SA (2010) Genetic diversity associated with agronomic traits using microsatellite markers in Pakistani rice landraces. *Electronic Journal of Biotechnology* **13 (3)**, 1-12

Rabbani MA, Pervaiz ZH, Masood MS (2008) Genetic diversity analysis of traditional and improved cultivars of Pakistani rice (*Oryza sativa* L.) using RAPD markers. *Electronic Journal of Biotechnology* **11**, 1-10

Ram SG, Thiruvengadam V, Vinod KK (2007) Genetic diversity among cultivars, landraces and wild relatives of rice as revealed by microsatellite markers. *Journal of Applied Genetics* **48 (4)**, 337-345

Rohif FJ (2002) NTSYS-pc: Numerical taxonomy and multivariate analysis system (Edn 2.2), Department of Ecology and Evolution, State University of NY, Stony Brook

Romero G, Adeva C, Battad II Z (2009) Genetic fingerprinting: Advancing the frontiers of crop biology research. *Philippine Science Letters* **2 (1)**, 8-13

Sainil N, Jain N, Jain S, Jain RK (2004) Assessment of genetic diversity within and among Basmati and non-Basmati rice varieties using AFLP, ISSR and SSR markers. *Euphytica* **140**, 133-146

Singh RK, Sharma RK, Singh AK, Singh VP, Singh NK, Tiwari SP, Mohapatara T (2004) Suitability of mapped sequence tagged microatellite site markers for establishing distinctness, uniformity and stability in aromatic rice. *Euphytica* **135**, 135-143

Siwach P, Jain S, Saini N, Chowdhury VK, Jain RK (2004) Allelic diversity among Basmati and non-Basmati long-grain *indica* rice varieties using microsatellite markers. *Journal of Plant Biochemistry and Biotechnology* **13**, 25-32

Song ZP, Xu X, Wang B, Chen JK, Lu BR (2003) Genetic diversity in the northernmost *Oryza rufipogon* populations estimated by SSR markers. *Theoretical and Applied Genetics* **107**, 1492-1499

Teixeira da Silva JA (2005) Molecular markers for phylogeny, breeding and ecology in agriculture. In: Thangadurai D, Pullaiah T, Tripathy L (Eds) *Genetic Resources and Biotechnology* (Vol III), Regency Publications, New Delhi, India, pp 221-256

Temnykh S, DeClerck G, Lukashova A, Lipovich L, Cartinhour S, McCouch S (2001) Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.): Frequency, length variation, transposon associations, and genetic marker potential. *Genome Research* **11**, 1441-1452

Thomson MJ, Septininsihin EM, Suwardjio F, Santoso TJ, Silintonga TS, Zeng Y, Zhang H, Li Z, Shen J, Sun J, Wang M, Liao D, Liu X, Wang X, Xiao F, Wen G (2007) Evaluation of genetic diversity of rice landraces (*Oryza sativa* L.) in Yunnan, China. *Breeding Science* **57**, 91-99

Upadhyay P, Singh VK, Neeraja CN (2011) identification of genotype specific alleles and molecular diversity assessment of popular rice (*Oryza sativa* L.) varieties of India. *International Journal of Plant Breeding and Genetics* **5 (2)**, 130-140

Virk PS, Newbury JH, Bryan GJ, Jackson MT, Ford-Lloyd BV (2000) Are mapped or anonymous markers more useful for assessing genetic diversity? *Theoretical and Applied Genetics* **100**, 607-613

Weising K, Nybom H, Wolff K, Kahl G (2005) *DNA Fingerprinting in Plants: Principles, Methods and Applications* (2nd Edn), CRC Press/Taylor and Francis Group, Boca Raton, 444 pp

Wong SC, Yiu PH, Bong STW, Lee HH, Neoh PNP, Rajan A (2009) Analysis of Sarawak Bario Rice diversity using microsatellite markers. *American Journal of Agricultural and Biological Sciences* **4 (4)**, 298-304

Xu Y, Beachell H, Mccouch SR (2004) A marker-based approach to broadening the genetic base of rice in USA. *Crop Science* **44**, 1947-1959

Yeo AR, Flowers TJ (1986) Salinity resistance in rice (*Oryza sativa* L.) and a pyramiding approach to breeding varieties for saline soils. *Australian Journal of Plant Physiology* **13**, 161-174

Zeng L, Kwon TR, Liu X, Wilson C, Grieve CM, Gregorio GB (2004) Genetic diversity analyzed by microsatellite markers among rice (*Oryza sativa* L.) genotypes with different adaptations to saline soils. *Plant Science* **166**, 1275-1285

Zheng, Huang KN, Bennet J, Khush GS (1995) PCR-based marker assisted selection in rice breeding. IRRI, Manila, pp 9-11