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SSR marker-based molecular characterization and genetic diversity analysis of aromatic landreces of rice (*Oryza sativa* L.)

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ABSTRACT

Molecular characterization of the genotypes gives precise information about the extent of genetic diversity which helps in the development of an appropriate breeding program. In the present study, a total of 24 SSR markers were used across 12 elite aromatic rice genotypes for their characterization and discrimination. Among these 24 markers 9 microsatellite markers were showed polymorphism. The number of alleles per locus ranged from 2 alleles (RM510, RM244, and RM277) to 6 alleles (RM 163), with an average of 3.33 alleles across 9 loci obtained in the study. The polymorphic information content values ranged from 0.14 (RM510) to 0.71 (RM163) in all 9 loci with an average of 0.48. RM163 was found the best marker for the identification of 12 genotypes as revealed by PIC values. The frequency of most common allele at each locus ranged from 41% (RM163, RM590, and RM413) to 91% (RM510). The pair-wise genetic dissimilarity co-efficient indicated that the highest genetic distance was obtained between Basmati PNR 346 and Deepa; Basmati PNR 346 and Patnai-23; Dolargura and Sugandha; Bhogganijia and Sugandha; and finally between Dolargura and Chinikani (88.89%). Opchaya, Basmati PNR 346 and Sugandha had close similarity among them but showed wide dissimilarity with other genotypes. Being grouped into distant clusters Dolargura and Opchaya could be utilized as potential parents for the improvement of fine grain aromatic rice varieties. Genotypes Deepa and Patnai-23 (having zero dissimilarity) might have possessed somewhat similar genetic background and more markers are needed to discriminate them. The microsatellite marker based molecular fingerprinting could serve as a sound basis in the identification of genetically distant accessions as well as in the duplicate sorting of the morphologically close accessions.

Key words: molecular characterization, genetic diversity, aromatic rice landraces, *Oryza sativa*, SSR

Introduction

Plant uniformity, which can be resulted by the use of modern plant breeding techniques, can produce plants, which are more efficient by means of different goals including enhanced resistance under stress, however much more research must be performed to indicate the most optimized methods that can be used for the production of efficient plants. This is of significance for the production of food for the world increasing population (Fu & Somers, 2009; Khodadadi et al., 2011). Accordingly, the increased attention to the production of resistant plant species for prolonged food

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production under different conditions indicates the necessity of performing breeding experiments (Martin et al., 2008; Khodadadi et al., 2011). One of the important approaches to rice breeding is hybridization and subsequent selection. Parents' choice is the first step in plant breeding program through hybridization. In order to benefit transgressive segregation, genetic distance between parents is necessary. Asian rice (*Oryza sativa* L.) has been cultivated for an estimated 10,000 years (Liu et al., 2007) and currently feeds more than one third of the world's population.

In Bangladesh, rice occupies about 70% of the total cropped area of about 13.9 million hectares. Approximately 11% of the world's arable land is cultivated annually with rice (Cantrell & Hettel, 2004), ranking next after wheat. Different cultures have preferences for different types of rice. 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). Scented rice is popular in Asia, and has gained wider acceptance in Europe and USA because of their aroma, flavor and texture. The rice world market ranks aromatic rice at the top. For the 4-5 million tons of aromatic rice worth 2-2.5 billion US Dollars.

Aromatic rice (*Oryza sativa* L.) is known for its characteristic fragrance when cooked. Cultivation of fine as well as aromatic rice has been gaining popularity in Bangladesh over the recent years, because of its huge demand both for internal consumption and export (Das & Baqui, 2000). Despite the generally favorable agro-climatic conditions, area of aromatic rice is less than 2% of the national rice acreage of Bangladesh. More than four thousand landraces of rice are adopted in different parts of Bangladesh. Only some of these are unique for quality traits including fineness, aroma, taste and protein contents (Kaul et al., 1982). Aromatic rice varieties have occupied about 12.5% of the total transplant aman rice cultivation (BBS, 2005). Production of aromatic rice in Bangladesh is becoming popular due to its high prices and export potentiality (Dutta et al., 2002). It is also preferred by some consumers despite their price and yield. Farmers' net income was increased by 23% with the adoption of modern varieties (Shrestha et al., 2002).

Landrace refers to domesticated plants adapted to the natural and cultural environment in which they live (or originated) and, in some cases, work. Landraces have been shown to be excellent sources of genes for novel alleles (McCouch et al., 1997; Hoisington et al., 1999; Jackson 1999; Loresto et al., 2000). 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 Interantional Rice Research Institute (IRRI) for longer storage (Jackson, 1999). About 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).

Growth and development of agricultural resources is mostly depending on genetic diversity among different crop plants and it is estimated that not even 15% of the potential diversity has utilized. This implies that thousands of valuable allelic variations of traits of economic significance remain unutilized (Hossain et al., 2007). Therefore, landraces of distinct genetic structure are a good promise for the future rice crop improvement. Thus, identification of genotypes and their inter-relationships is vital. Development of new biotechnological techniques provides increased support to evaluate genetic variation in both phenotypic and genotypic levels. 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).

Simple sequence repeat is an important tool for genetic variation identification of germplasm (Powell et al., 1996; Ma et al., 2011). SSR marker have some merits such a quickness, simplicity, rich polymorphism and stability, thus being widely applied in genetic diversity analysis, molecular map construction and gene mapping (Zhang et al., 2007; Ma et al., 2011), construction of fingerprints (Xiao et al., 2006; Ma et al., 2011), genetic purity test (Peng et al 2003; Ma et al., 2011), analysis of germplasm diversity (Zhou et al 2003; Jin et al 2010; Ma et al., 2011) utilization of heterosis, especially in identification of species with closer genetic relationship. A total of 18,828 Class 1 di-, tri- and tetra-nucleotide SSRs, representing 47 distinctive motif families, were identified and annotated on the rice genome. An abundance of microsatellite markers is now available through the published high-density linkage map; there was an average of 51 hypervariable SSRs per Mb, with the highest density of markers occurring on chromosome 3 (55.8 $SSRMb^{-1}$) and the lowest occurring on chromosome 4 (41.0 $SSRMb^{-1}$) (IRGSP 2005).

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In the present study, twelve aromatic landraces of rice were analyzed for genetic variation using SSR markers. Specially, the objective of the study was DNA fingerprinting and genetic diversity analysis of aromatic landraces to measure the extent of genotypic differences, genetic relationship and to assist in broadening the germplasm base of future aromatic rice breeding programs.

Materials and Methods

Germplasm collection and genomic DNA extraction

A total of 12 rice genotypes were evaluated in this study including Jamaisohagi, Sugandha, Darsail, Chinikani, Bhogganijia, Dolargura, Depa, Patnai-23, Opchaya, Holdijorun, Jingasail and Basmati PNR 346. All seeds those collected from the Genetic Resource Center (GRC), Bangladesh Rice Research Institute (BRRI) were germinated at aseptic condition by keeping them at 30ºC for 1 day and raised in pots in a net house. At 3 weeks of age, about 2 cm of leafs from each plant was harvested and bulked for each genotype. Total genomic DNA was extracted from the bulked leaf samples by following a miniprep DNA extraction protocol, which did not require liquid nitrogen and required only a small amount of tissue samples (Zheng et al., 1995). The quality of DNA was also checked by DNA quantification using a Thermo Scientific NanoDrop™ 1000 spectrophotometer (Thermo Fisher Scientific, USA). All chemicals used for DNA extraction were purchased from Sigma-Aldrich, Germany.

SSR markers and PCR amplification

Twenty four SSR primer pairs (Promega Corporation, USA) were selected on the basis of the published rice microsatellite framework map for the genetic diversity analysis of the 12 aromatic rice cultivars in Bangladesh. Primers that showed polymorphic banding patterns were selected whereas primers that showed monomorphic banding patterns were excluded. Finally, 9 microsatellite primers with a distinct chromosome number were used for final polymerase chain reaction (PCR) amplification. Information regarding the original source, repeat motifs, primer sequences, expected length, chromosomal localizations and repeat types of the SSRs can be found in the Web database (http://www.gramene.org). Prior to DNA amplification, a PCR cocktail was prepared containing all required components. All reagents were purchased from Sigma-Aldrich. PCR amplification reactions were done in 10 µl reaction mixtures, containing 3 µl of diluted template DNA, 0.5 µl of each forward and reverse primer, 0.25 µl of 10 mM dNTPs, 1.5 µl of 10x buffer, 0.2 µl of *Taq* polymerase, 1.8 µl of $MgCl_2$ and 2.25 µl of ddH_2O. An DNA thermal cycler (Model: ALS 1296, BioRad, USA and G-STORM, GSI, England, Serial no: GT-11620) was used along with the following PCR profile: an initial denaturation step 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 seconds each and then a final extension at 72°C for 5 min. Amplified products were stored at -20°C until further use.

Electrophoretic separation and visualization of amplified products

Prior to electrophoresis, each PCR product was mixed with gel loading dye (bromophenol blue, xylene cyanol and sucrose) and electrophoresis was carried out in a mini vertical electrophoresis tank (CBS Scientific Co Inc., CA. USA), run on 8% polyacrylamide gels in TBE buffer. Four microliters of the sample were loaded in each well and run at 80 V for 90 minutes. The gel, after electrophoresis, was stained with ethidium bromide for 30-35 min, kept in dark, and then scanned using an UVPRO (Uvipro Platinum, EU) gel documentation unit linked to a PC. The reproducibility of amplification products was confirmed twice for each primer.

SSR data analysis

The size of most intensely amplified fragments was determined by comparing the migration distance of amplified fragments relative to the molecular weight of known size markers, 50 base pairs (bp) DNA ladder using Alpha-Ease FC 5.0 software (Alpha Innotech, USA). The number of alleles per locus, major allele frequency, gene diversity and PIC values were calculated using PowerMarker version 3.25 (Liu & Muse, 2005). All the genotypes were scored for the presence and absence of the SSR bands throughout all 12 genotypes and the data were exported to binary data for the presence (1) or absence (0) or as a missing observation for further 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 & Edwards, 1967) in PowerMarker with tree viewed using Treeview software.

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Results

Overall allelic diversity

The Twenty primers were used across 12 elite aromatic rice genotypes for their characterization and discrimination of aromatic rice landraces (ARLs). Among these 24 primer pairs, 9 primers well spread on chromosome 5, 6, 8, 10, 11 and 12 were identified as polymorphic whereas primers with monomorphic banding patterns were excluded. A total of 27 alleles were detected at the loci of 9 microsatellite markers across 12 aromatic rice genotypes. The number of alleles per locus generated by each marker varied from 2 to 6 alleles, with an average of 3.3 alleles per locus. The highest number of alleles (6.0) was detected in the locus RM163 and the lowest number of alleles (2.0) was detected on each of locus RM 510, RM 244, and RM 277. On average, 56% of the 12 rice accessions shared a common major allele at any given locus ranging from 41% (RM163, RM590, and RM413) to 91% (RM510) common allele at each locus. A moderate level of diversity exists among 9 loci studied across 12 rice accessions, ranged from 0.15 to 0.75 with an average of 0.54.

Figure 1 shows a gel image of amplified fragments produced by primer RM247 and RM590. RM163 on chromosome 5 detected 6 alleles followed by RM247 (5 alleles), RM224 (4 alleles) and RM590 and RM413 (3 alleles). This suggests that these markers could be potentially used for molecular characterization of aromatic rice germplasm from various sources. However, there were a number of markers which produced only few alleles. Three markers produced three 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.

PIC value

SSR markers were highly informative and polymorphic as evident from its PIC value. The polymorphism information content (PIC) value is a measure of polymorphism among varieties for a marker locus used in linkage analysis. The PIC value of each marker, which can be evaluated on the basis of its alleles, varied greatly for all tested SSR loci - from 0.14 to 0.71 with an average of 0.48 (Table 1). The highest PIC value 0.71 was obtained for RM163 followed respectively by RM247 (0.64), RM590 (0.59), RM244 and RM277 (0.54) and RM256 (0.50).

Genetic distance-based analysis

SSR Genetic distance refers to the genetic divergence among populations, which can be measured by a variety of parameters in relation to the frequency of a particular trait. The UPGMA-based dendrogram was obtained from the binary data deduced from the DNA profiles of the samples analyzed where the genotypes that are derivatives of genetically similar types clustered together.

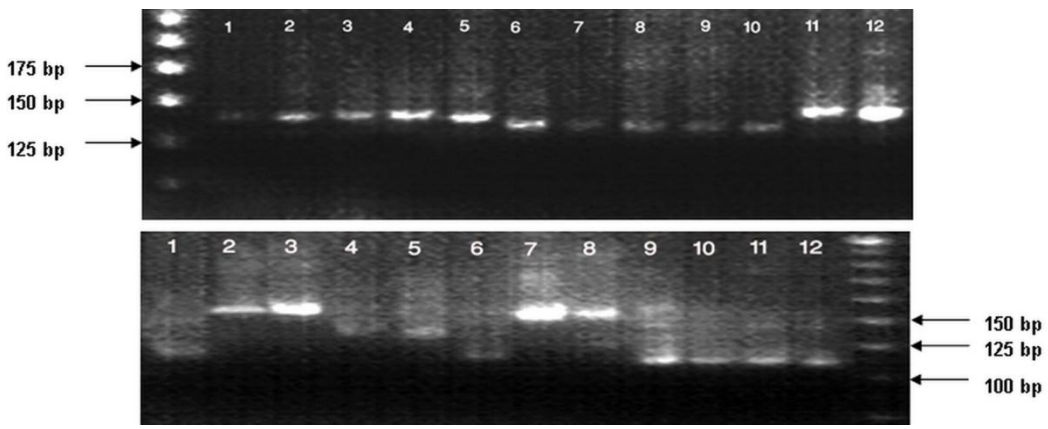

Figure 1. *Separation of alleles on 8% polyacrylamide gel followed by staining with ethidium bromide and visualized under UV light. PCR products were amplified with rice SSR primers RM247 (upper block) and RM590 (lower block). The lane marked with band sizes is the ladder marker. All were 25 base pair (bp). 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 represent rice landraces Jamaisohagi, Sugandha, Darsail, Chinikani, Dolargura, Bhogganijia, Deepa, Patnai-23, Opchaya, Holdijorun, Jingasail, and Basmati PNR 346.*

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Table 1. *List of 9 microsatellite markers with their chromosome locations, number of alleles per locus, highest frequency allele, diversity and polymorphism information content (PIC) values found among 12 rice genotypes.*

Marker	Chr.	RM*	NA	Freq.	Diversity	PIC
RM163	5	(GGAGA)4(GA)11C(GA)20	6	0.4167	0.7500	0.7193
RM510	6	(GA)15	2	0.9167	0.1528	0.1411
RM256	8	(CT)21	3	0.5833	0.5694	0.5045
RM244	10	(CT)4(CG)3C(CT)6	2	0.5833	0.4861	0.3680
RM590	10	(TCT)10	3	0.4167	0.6250	0.5454
RM224	11	(AAG)8(AG)13	4	0.5000	0.6528	0.5994
RM277	12	(GA)11	2	0.7500	0.3750	0.3047
RM413	5	(AG)11	3	0.4167	0.6250	0.5454
RM247	12	(CT)16	5	0.5000	0.6806	0.6427
Mean				**0.5648**	**0.5463**	**0.4856**

Legend: 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).

Using 63% similarity as the threshold for UPGMA clustering, we observed five major genetic clusters (Figure 2). Group I contained most of the aromatic rice accessions (4 rice genotypes) used in this study which had two separate additional sub-clusters within it. For instance, Jamaisohagi - Bhogganijia and Deepa - Patnai 23 formed separate groups within the cluster I where Jamaisohagi and Bhogganijia showed 22% dissimilarity, but Deepa and Patnai-23 showed 100% similarity. Doalrgura alone was grouped in a single cluster, cluster II. Another 3 aromatic rice genotypes (Sugandha, Opcahya and Basmati PNR 346) formed a single cluster (III). Here, cluster IV consisted of Holdijorun and Jingasail, which showed 33% dissimilarites between them. The remaining two, Darsail and Chinikani, formed a single cluster which showed 11.11% dissimilarities between them. The genetic similarity analysis with the construction of neighbor-joining tree (Figure 3) using the neighbor-joining data agreed with UPGMA clustering revealed five groups in the 12 genotypes based on the alleles detected by 9 SSR markers.

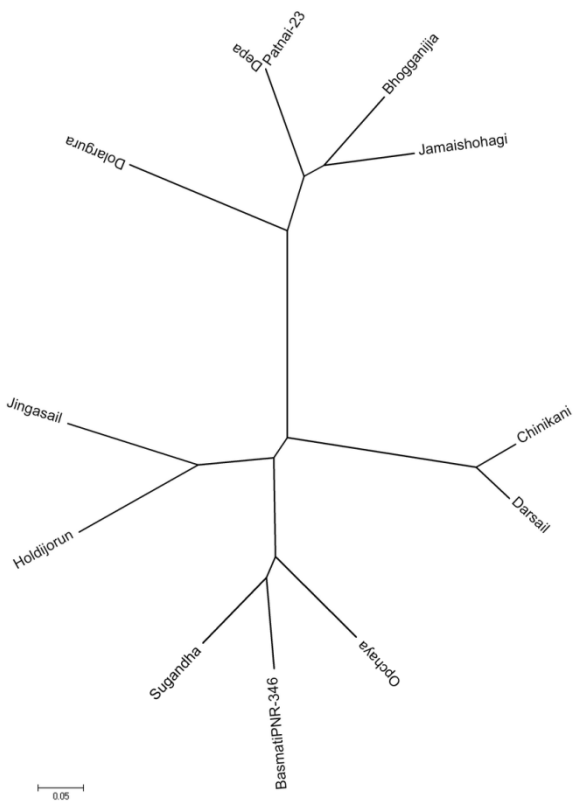

Figure 2. *An unrooted neighbor-joining tree showing the genetic relationships between the 12 rice accessions based on 9 microsatellite markers. The five major groups are found based on relatedness between themselves.*

Pairwise genetic dissimilarity

A dissimilarity matrix was used to determine the level of relatedness among the cultivars studied. The pairwise genetic dissimilarity indices (Table 2) indicated that the highest genetic dissimilarity was between Basmati PNR 346 and Deepa (88.89%), Bhogganijia and Sugandha (88.89%), Basmati PNR 346 and Patnai-23 (88.89%), Dolargura and Sugandha (88.89%), Deepa and Darsail (88.89%), Darsail and Patnai-23 (88.89%), Deepa and Chinikani (88.89%) as well as between Dolargura and Chinikani (88.89%).

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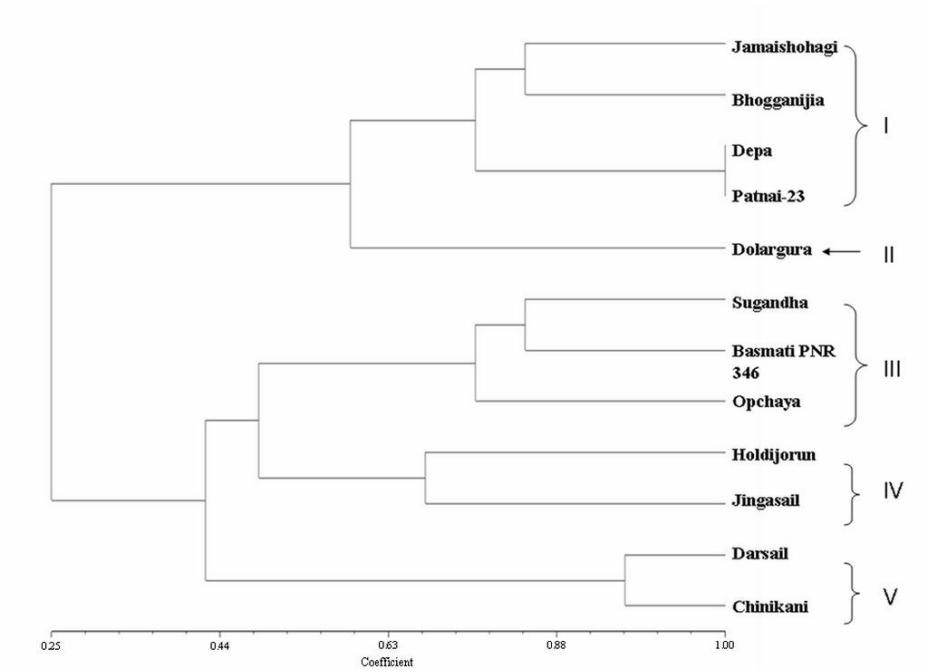

Figure 3. *An UPGMA cluster dendrogram showing the genetic relationships among 12 ARLs based on 9 SSR markers.*

These pairs were followed by Jamaisohagi and Sugandha (77.78%), Deepa and Sugandha (77.78%), Dolargura and Jingasail (77.78%), Patnai-23 and Sugandha (77.78%), Doalrgura and Darsail (77.78%), Bhogganijia and Darsail (77.78%), Basmati PNR 346 and Dolargura (77.78%), Basmati PNR 346 and Bhogganijia (77.78%), Chinikani and Bhogganijia (77.78%), Deepa, Patnai-23 and Opchaya (77.78%) and declining thereafter. Both, Deepa and Patnai-23, were found in duplicate (i.e., 100% similarity).

Table 2. *Pairwise genetic distance indices among 12 aromatic rice landraces (ARLs) obtained from microsatellite marker analysis.*

ECOTYPES	**ARL1**	**ARL10**	**ARL11**	**ARL12**	**ARL2**	**ARL3**	**ARL4**	**ARL5**	**ARL6**	**ARL7**	**ARL8**	**ARL9**
ARL1	0.0000											
ARL10	0.7778	0.0000										
ARL11	0.6667	0.5556	0.0000									
ARL12	0.6667	0.6667	0.1111	0.0000								
ARL2	0.4444	0.8889	0.7778	0.8889	0.0000							
ARL3	0.2222	0.8889	0.7778	0.7778	0.3333	0.0000						
ARL4	0.3333	0.7778	0.8889	0.8889	0.4444	0.2222	0.0000					
ARL5	0.3333	0.7778	0.8889	0.8889	0.4444	0.2222	0.0000	0.0000				
ARL6	0.6667	0.3333	0.4444	0.5556	0.6667	0.6667	0.7778	0.7778	0.0000			
ARL7	0.5556	0.6667	0.6667	0.6667	0.6667	0.4444	0.5556	0.5556	0.4444	0.0000		
ARL8	0.6667	0.5556	0.6667	0.5556	0.7778	0.6667	0.7778	0.7778	0.4444	0.3333	0.0000	
ARL9	0.6667	0.2222	0.4444	0.5556	0.7778	0.7778	0.8889	0.8889	0.2222	0.5556	0.4444	0.0000

Legend: ARL1 = Jamaisohagi; ARL2 = Dolargura; ARL3 = Bhogganijia; ARL4 = Deepa; ARL5 = Patnai-23; ARL6 = Opchaya; ARL7 = Holdijorun; ARL8 = Jingasail; ARL9 = Basmati PNR 346; ARL10 = Sugandha; ARL11 = Darsail; ARL12 = Chinikani.

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The lowest genetic dissimilarity among rice landraces was between Darsail and Chinikani (11%), followed by Bhogganijia and Jamai sohagi (22%), Bhogganijia and Deepa (22%), Bhogganijia and Patnai-23 (22%); Basmati PNR 346 and Sugandha (22%), Opchaya and Basmati PNR 346 (22%). Unsurprisingly, genetic dissimilarity between the aromatic rice cultivars was comparatively high. Thus, SSR markers provide adequate power of resolution to discriminate between aromatic varieties and it could serve as a potential tool in the identification and characterization of genetically distant cultivars from various sources.

Discussion

Genetic diversity assessment of the traditional rice varieties landraces is essential component in germplasm characterization and conservation to identify potential parents. Morphological and seed traits have long been the means of studying taxonomy and variability among plant species. Microsatellites are among the most widely used DNA marker for many purposes such as diversity, genome mapping, varietal identification, etc. (Teixeira da Silva, 2005). Unlike the morphological and biochemical markers, molecular markers are not stressed by environmental factors and growth practices (Ovesna et al., 2002). The use of these markers to investigate genotypic variations among different cultivars was previously reported by some researchers (Singh et al., 2004; Joshi & Behera, 2006).

The present investigation addresses the utilization of 9 microsatellite markers to reveal genetic polymorphism and ensures unambiguous identification of 12 ARLs. The mean allele (3.3 alleles) obtained in our study was comparable with the result reported by Etemad et al. (2012) detecting 3.57 alleles per SSR locus, who used 26 rice (*Oryza sativa*, L.) accessions, consisting of 13 Iranian and 13 Malaysian cultivars was investigated using SSR markers distributed across the rice genome. In another study, Hossain et al. (2012) found an average of 3.8 alleles per locus in rice using Bangladeshi ARLs. 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 a different set of rice germplasm. 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 mean value obtained from our study is somewhat lower than the results observed in previous diversity studies, having 1-8 alleles with an average of 4.58 alleles for various classes of microsatellite (Siwach et al., 2004) and also 3 to 9 alleles, with an average of 4.53 alleles per locus for 30 microsatellite markers (Hossain et al., 2007).

The number of alleles detected in the present study was lower than the average number of alleles reported by Xu et al. (2004), Jain et al. (2004), Jayamani et al. (2007), Zeng et al. (2007) and Prathepha et al. (2012) who reported an average of 11.9, 7.8, 14.6, 7.7 and 11.85 alleles per locus using US rice genetic resources, Indian quality rice germplasm, a diverse collection of Portuguese rice, rice landraces from China and wild rice (*Oryza rufipogon)* from Northeastern Thailand and Laos respectively. Similar results were observed in some earlier reports by Pervaiz et al. (2010), Upadhyay et al. (2011) and Rahman et al. (2012) who found an average of 4.4, 4.35 and 4.18 alleles per locus. Pervaiz 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 (3.33 alleles per locus). Such variability exist in the number of alleles detected per locus might be due to the diverse germplasm used and selection of SSR primers with scorable alleles.

The number of bands produced across 12 rice genotypes by different anchored SSR motifs is consistent with published reports on microsatellite frequency in their genome. From Table 1 could be seen that there were no correlations between the number of allele detected and the number of SSR repeats present in a particular locus. For example, the number of allele detected did not show any correlation with containing (CT) motifs varying from $(CT)_{16}$ to $(CT)_{21}$. Majority of SSR primers used in this study had dinucleotide repeats (GA and CT). The perfect dinucleotide repeat motif (GA) has been reported to display high level of variation among the rice genotypes (Temnykh et al., 2000).

In this study, loci with perfect dinucleotide motifs detected lower number of alleles per locus (mean 3; n=5) than those with other perfect trinucleotide and a mixture of compound di-, tri-, and pentanucleotide SSR motifs (mean 3.75; n=4) considered. RM163 (chr. 5) had a mixture of di-,

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and pentanucleotide $(GGAGA)_4(GA)_{11}C(GA)_{20}$ motifs and detected maximum (6 alleles) number of alleles. Markers with the highest number of discernable alleles could be the best markers for molecular characterization and diversity analysis. In the present study, the level of polymorphism determined by the PIC value (mean= 0.48) is consistent with the reported PIC value in previous works (Lu et al., 2005; Wong et al., 2009; Hossain et al., 2012). According to the early reports on the PIC values ranged from a low of 0.24 to a high of 0.92 and averaged 0.61 (Jain et al., 2004), 0.19 to 0.90 with an average of 0.75 (Borba et al., 2009), which is markedly higher than the result in our study. Upadhyay et al. (2011) also reported the average PIC value of 0.78. These result revealed that markers RM163 would be best in screening 12 rice genotypes followed by RM247, RM493 and so on. Thus, the PIC value indicates that all these primers were highly informative and capable of distinguishing between genotypes.

The genetic dissimilarity between the aromatic rice cultivars was also determined using a dissimilarity matrix. Generally, modern rice cultivars share a relatively narrow genetic background, when compared to the unexplored vast variability existing in rice landraces worldwide. For example, the pedigree of maximum IRRI rice varieties can be traced back to few Indian landraces such as Kitchili Samba, Vellaikar, Tadukan, Thekkan and Eravaipandi (Khush & Virk, 2005). Therefore, it is highly necessary not only to conserve landrace genotypes, but also to reveal the gene-pool of rice landraces and unlock valuable genes for breeding purposes (Rabbani et al., 2008).

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, which can be used in future breeding programs. With the aid of microsatellite makers and clustering data, different distantly related rice genotypes may be combined by intercrossing genotypes, for instance, aromatic rice genotypes with non-aromatic rice genotypes from different clusters to get hybrid varieties with highest heterosis. Many studies have also reported significantly greater allelic diversity of microsatellite markers than other molecular markers (McCouch et al., 2001). Marker-aided backcrossing (MAB), enabled by advances in genomics and molecular mapping in recent years, is more precise, time-saving, and cost-effective way to develop rice varieties that can withstand these abiotic stresses than conventional breeding.

In summary, the present study revealed a wide variation among the germplasms. The result indicated that the SSR markers are neutral and co-dominant and could be a powerful tool to assess the genetic variability of the cultivars. The information about the genetic diversity will be very useful for proper identification and selection of appropriate parents for breeding programs, including gene mapping, and ultimately for emphasizing the importance of marker-assisted selection (MAS) in aromatic rice improvement worldwide.

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