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Full Length Research Paper

Determination of genetic relatedness among selected rice (*Oryza sativa,* L.) cultivars using microsatellite markers

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For plant improvement programs, genetic variation information among different cultivars is very important. Genetic variation among 26 rice (*Oryza sativa,* L.) accessions, consisting of 13 Iranian and 13 Malaysian cultivars was investigated using microsatellite markers distributed across the rice genome. All the 21 selected microsatellite primers were successfully amplified by polymerase chain reaction (PCR) of which 20 (95.2%) were found to be polymorphic. A total of 75 alleles were detected at 21 microsatellite loci. The allele frequencies per locus ranged from 1 in RM338 to 5 alleles in RM307, RM161, RM334 and RM271. The mean number of alleles per locus was 3.57. Amongst these microsatellite loci, the largest polymorphism information content (PIC) value was 0.74 in RM 161, while the lowest PIC value was 0.0 in RM 338. Dendrogram was constructed based on dissimilarity values, unweighted pair group with arithmetic average (UPGMA) and it separated all the cultivars into six clusters. All these polymorphisms could be further evaluated for rice marker assisted selection and developed PCR methodology would expedite screening for large numbers of rice required for association studies.

Key words: Genetic variation, genetic dissimilarity, polymorphism information content (PIC), polymerase chain reaction (PCR).

INTRODUCTION

The increase of human population and the limited availability of agricultural resources defiantly forced the search for new resources to overcome these phenomena. Developing countries face the challenges to rapidly increase agricultural productivity and feed their growing populations based on genetic potentials. Rice is one of the most important staple crops in most part of the world from which 50 to 80% of people obtain their calories (Khush, 2003).

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Abbreviations: SSR, Simple sequence repeat; **PIC,** polymorphic information content; **PCR,** polymerase chain reaction.

Conventional breeding has played an essential role in rice cultivar improvement over the past decades. However, progress was slow and remains with several barriers such as, time-consuming in selection process and difficulties based on appropriate genotype selection. The quantitative nature of most agronomic traits has encouraged breeders to apply biotechnological techniques and molecular markers for rice breeding, which is known as molecular breeding. There are different types of molecular markers which are potentially different in cost, facilities required, reliability and detecting differences between individuals (Schlötterer, 2004; Schulman, 2007).

Molecular study of germplasm would provide information for plant improvement programs in terms of the level of genetic variation within and between species. This information would then be used to select diverse parents of the same species or to identify the most

closely related parents for inter-species crossing, to increase heterosis and to include desirable genes from more diverse backgrounds into the best germplasm (Henry, 1997). Maximum potential for genetic achievement is related to phenotypic variation which is present in the original population and maintained in the following selection cycles. Phenotypic variation is directly associated with genetic diversity even though it has interaction with environmental factors (Moose and Mumm, 2008).

The classification and quantification of genetic diversity in closely related crop germplasm has been a major objective for a long time which is needed for a balanced use of genetic resources. The study of genetic difference in breeding resources is of primary interest to plant breeders. This information is needed for the selection and monitoring of germplasm and for the prediction of possible genetic gains (Chakravarthy and Rambabu, 2006). Markers based on expressed gene products, proteins or isozymes are also influenced by the environment and reveal a low level of polymorphism and low abundance (Ravi et al., 2003). In contrast, DNA-based molecular markers have proven to be powerful tools for the evaluation of genetic variation. The explanation of genetic relationships within and among species had been characterized by many untouched environmental factors (Powell et al., 1996). Ravi et al. (2003) reported that, the unique simple sequence repeat (SSR) profiles in rice cultivars could be generated by using a few primers that covered all 12 chromosomes.

Microsatellites are tandem repeated motifs of 1 to 6 bp which have a frequent occurrence in all prokaryotic and eukaryotic genomes analyzed to date (Zane et al., 2002). The existence of microsatellites was verified by Hamada et al. (1982) in various eukaryotes and Tautz and Renz (1984) confirmed most microsatellites in plants. SSRs are present in both coding and noncoding regions and are distributed throughout the nuclear genome (Kalia et al., 2011). These can also be found in the chloroplastic (Provan et al., 2001; Chung et al., 2006) and mitochondrial (Rajendrakumar et al., 2007) genomes. SSR show high degree of length polymorphism (Zane et al., 2002) which is due to different number of repeats in the microsatellite regions; therefore, they can be easily detected by polymerase chain reaction (PCR). Microsatellites have high throughput genotyping and have proven to be an extremely valuable tool for establishment of genetic relationships (Parida et al., 2009; Kalia et al., 2011).

The objective of this study was to determine the genetic variation and relationships among selected Iranian and Malaysian rice cultivars.

MATERIALS AND METHODS

All the Iranian rice cultivar were collected from Iran Rice Research Institute, Rasht city but the Malaysia rice cultivars were from different countries like the Philippine, West Africa and West Bengal (Table 1) which were imported to Malaysia for research purposes. Henceforth, we assumed this group as Malaysian cultivars because the majority of cultivars were originated in Malaysia and obtained from the Malaysian Agriculture Research and Development Institute (MARDI). A total of 26 Iranian and Malaysian rice cultivars were selected as experimental materials, the details are described in Table 1. The seeds were raised in the soil in plastic containers (15 × 20 cm) placed in a glass house for one month.

Genomic DNA isolation

Genomic DNA was extracted from fresh leaves of rice seedlings using the method of Dellaporta et al. (1983). The quality and quantity of the extracted DNA were estimated using UV spectrophotometer in two wavelengths (260 and 280 nm). The DNA samples were diluted to 20 ng/µl concentration with TE (Tris-EDTA) for SSR analyses and kept at -20°C.

Selection of primers and PCR amplification

A total of twenty one microsatellite primer pairs (Table 2) were selected from those reported by Temnykh et al. (2000); McCouch et al. (2001).

The PCR amplification mixture was prepared in 0.2 ml micro centrifuge tubes. Each reaction mixture contains 2.5 µl of 10X PCR buffer, 1.5 mM $MgCl_2$ (25 mM), 0.2 mM of each dNTP (10 mM), 0.2 µM of each primer (forward and reverse), 100 ng of DNA, 1 unit of Taq DNA polymerase(Research Biolab) and water was added to make the final volume to 25 µl. The amplification program consisted of the following cycles: 94°C for 4 min, 30 cycles of 94°C for 45 s, 55 to 63°C (depending on the annealing temperature of primer) for 30 s and 72°C for 1 min, and a final extension at 72°C for 10 min. The reproducibility of amplified products was established twice for each of the selected primers in this study.

Electrophoresis of PCR products

The amplification products (10 µl) were mixed with 2 µl loading buffer (Promega, USA) and separated by electrophoresis on 4% metaphor agarose gel which submerged in 1X TBE buffer (10 mM tris-borate, 1 mM EDTA) at 100 V for one hour and stained in ethidium bromide (1 mg/ml). A molecular DNA ladder of 25 to 766 bp (N3233L, Sigma USA) was used to evaluate the detected bands. Ethidium bromide staining of agarose gels generally revealed a multiple number of bands (Pervaiz et al., 2009).

Data analysis

The genotypes were directly counted and scored, based on the allele size and estimated by the low molecular weight DNA ladder and allele binning were also conducted. The polymorphic information content (PIC) was employed for each locus to assess the information of each marker and its discriminatory ability. The calculation of PIC (Weir, 1996) for the *i*th marker is $PIC = 1 - \Sigma Pij(j = 1,2,..., n)$, where *Pij* is the frequency of the *j*th pattern for the *i*th marker and the summation extends over (n) patterns (Peng and Lapitan, 2005). Data analysis was carried out using POPGENE (Version 1.31) (Yeh and Boyle, 1997). To measure the genetic dissimilarity between the different genotypes within a single population, simple mismatch coefficient was conducted (Kosman and Leonard, 2005). This is identical to the normalized squared Euclidean distance (m = e2/n), where *n* is the total number of markers used for the analysis. Other softwares such as UVIDoc

Table 1. List of names and origins of the selected Iranian and Malaysian rice cultivars.

Number of cultivar	Name	Origin	Number of cultivar	Name	Origin
1	HASHEMY I	Iran	14	DULAR	West Bengal
2	SALARY	Iran	15	SALUMPIKIT	Malaysia
3	MUSA TAROM	Iran	16	IR 20	Philippines
4	KHAZAR	Iran	17	IRAT 13	Philippines
5	BINAM	Iran	18	IRAT 140	Philippines
6	DORFAK	Iran	19	MR 211	Malaysia
7	SEPID ROD	Iran	20	MR 219	Malaysia
8	DOM SIAH	Iran	21	MR 220	Malaysia
9	CHAMPA	Iran	22	MR 232	Malaysia
10	SADRY	Iran	23	MR 219 LINE 4	Malaysia
11	HASHEMY II	Iran	24	MR 219 LINE 9	Malaysia
12	KADOOS	Iran	25	MORO BEREKAN	West Africa
13	DOM SEFID	Iran	26	SIAM PILIHAN	Malaysia

Table 2. List of 21 microsatellite primer pairs with their chromosome locations, sequences (forward and reverse), repeated regions, observed sizes, number of alleles per locus and polymorphism information content (PIC) values.

Chromosome no.	Primer	Forward and reverse primer sequence (5'-3')	Repeat region	Observed size	No. of alleles	PIC
1	RM1	GCGAAAACACAATGCAAAAA GCGTTGGTTGGACCTGAC	(AG)26	88-132	4	0.671
1	RM283	GTCTACATGTACCCTTGTTGGG CGGCATGAGAGTCTGTGATG	(GA)18	152-166	2	0.349
1	RM259	TGGAGTTTGAGAGGAGGG CTTGTTGCATGGTGCCATGT	(CT)17	136-180	4	0.663
1	RM312	GTATGCATATTTGATAAGAG AAGTCACCGAGTTACCTTC	(ATTT)(GT)9	92-110	3	0.530
1	RM5	TGCAACTTCTAGCTGCTCGA GCATCCGATCTTGATGGG	(GA)14	96-126	3	0.579
1	RM237	CAAATCCCGACTGCTGTCC TGGGAAGAGAGCACTACAGC	(CT)18	110-150	3	0.522
3	RM338	CACAGGAGCAGGAGAAGAGC GGCAAACCGATCACTCAGTC	(CCT)6	183	1	0.00
3	RM55	CCGTCGCCGTAGTAGAGAAG TCCCGGTTATTTTAAGGCG	(GA)17	220-240	3	0.590
4	RM307	GTACTACCGACCTACCGTTCAC CTGCTATTGCATGAACTGCTGC	(AT)14(GT)21	124-190	5	0.706
5	RM161	TGCAGATGAGAAGCGGCGCCTC TGTGTCATCAGACGGCGCTCCG	(AG)20	132-176	5	0.745
5	RM334	GTTCAGTGTTCAGTGCCACC GACTGTTTGATCTTTGGTGGACG	(CTT)20	126-192	5	0.736

No., Number.

Table 2. Continued.

7	RM11	TCTCCTCTTCCCCCGATC ATAGCGGGCGAGGCTTAG	(GA)17	126-154	4	0.647
8	RM25	GGAAAGAATGATCCTTTTCATGG CTACCATCAAAACCAATGTTC	(GA)18	104-136	3	0.594
8	RM44	ACGGGCAATCCGAACAACC TCGGGAAAACCTACCCTACC	(GA)16	102-122	4	0.577
8	RM433	TGCGCTGAACTAAACACAGC AGACAAACCTGGCCATTCAC	(AG)13	220-246	3	0.530
9	RM316	CTAGTTGGGCATACGATGGC ACGCTTATATGTTACGTCAAC	(GT)8-(TG)9(TTTG)4(TG)4	168-210	4	0.698
9	RM105	GTCGTCGACCCATCGGAGCCAC TGGTCGAGGTGGGGATCGGGTC	(CCT)6	114-126	3	0.506
10	RM271	TCAGATCTACAATTCCATCC TCGGTGAGACCTAGAGAGCC	(GA)18	94-136	5	0.706
11	RM287	TTCCCTGTTAAGAGAGAAATC GTGTATTTGGTGAAAGCAAC	(GA)18	98-122	4	0.651
12	RM277	CGGTCAAATCATCACCTGAC CAAGGCTTGCAAGGGAAG	(GA)11	114-128	3	0.579
12	RM511	CTTCGATCCGGTGACGAC AACGAAAGCGAAGCTGTCTC	(GAC)7	129-141	4	0.648

(Version 98), PIC calculator, NTSYS and Office Excel were also employed for data analyses. The presence or absence of alleles was voted as 1 or 0, respectively. The dendrogram was constructed based on the genetic similarity values using the unweighted pair group with arithmetic average (UPGMA) method with the NTSYS computer software (Rohlf, 1993).

RESULTS

From 21 microsatellite primer pairs, 20 showed polymorphic bands (Figure 1). A total of 75 microsatellite alleles were amplified from 26 cultivars and this demonstrates the considerable variability among selected cultivars. The number of alleles per locus ranged from 1 to 5, with an average of 3.57 per locus (Figure 2).

Four microsatellite loci (RM307, RM161, RM334 and RM271) revealed 5 alleles per locus; many studies have also reported significant differences in allelic diversity among various microsatellite loci (Akagi et al., 1997; McCouch et al., 2001; Ravi et al., 2003), while only one microsatellite locus (RM338) showed monomorph pattern.

The PIC value is an evidence of allele diversity and frequency among the varieties (Pervais et al., 2009). The PIC value of each marker can also be evaluated on the basis of its alleles and diverse for all SSR loci. In this study, the PIC for the 21 microsatellite loci ranged from 0 to 0.74, with mean value of 0.578. The largest PIC value was observed for locus RM161. The results indicated that high levels of polymorphisms were detected in these rice cultivars (Table 2).

The dendrogram (Figure 3) showed six clusters that were demarcated at different dissimilarity coefficient levels. Cluster I consisted of (HashemyI and Salary) cultivars, while Clusters II comprised 10 cultivars, namely: Musa Tarom, Dom Siah, Champa, Khazar, Sadry, HashemyII, Dom Sefid, IRAT13, Dular and Salumpikit, also, in Cluster III, four cultivars (Binam, Dorfak, Sepid Rood and Kadoos) were presented. In cluster IV, IR20 cultivar was the only member and in clusters V and VI, each consisted of four and five cultivars known as (MR211, MR 219, MR220 and IRAT140) and (MR232, MR219 Line 4, MR219 Line 9, Moro Berekan and Siam Pilihan), respectively (Table 1).

Figure 1. Polymerase chain reaction (PCR) products of microsatellite primer RM1 for 26 rice cultivars. Lanes 1 to 13, Iranian cultivars; lanes 14 to 26, Malaysian cultivars (Table 1); M, DNA LADDER (25 to 766 bp).

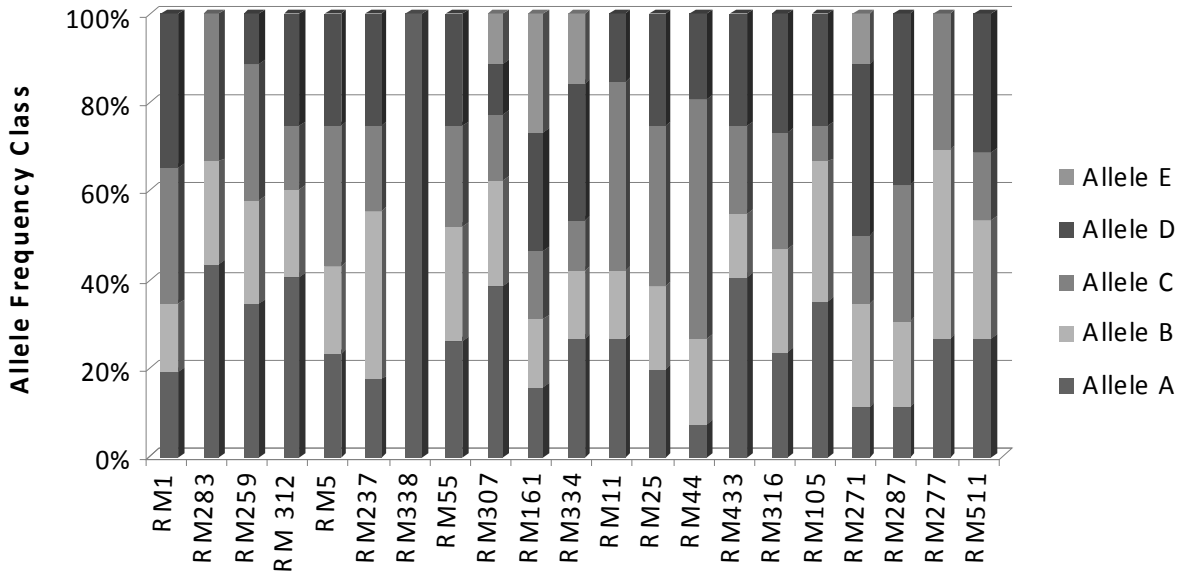

Figure 2. The frequency distribution of 75 alleles in 26 rice accessions detected by microsatellite, ranging from one to five alleles per locus.

Dissimilarity coefficiency varied from 0.188 in cluster I to 0.282 in cluster III, which consisted of Iranian cultivars. The genetic dissimilarity of cluster II which contained a mixture of Iranian and Malaysian cultivars was 0.195. Clusters IV, V and VI, consisted of Malaysian cultivars and exhibited the dissimilarity of 0.201, 0.217 and 0.326, respectively (Table 3).

DISCUSSION

The results of this study based on the number of alleles per loci were in agreements with those of Akagi et al. (1997), McCouch et al. (2001) and Ravi et al. (2003) in which there were significant differences in allelic diversity amongst a range of microsatellite loci, and was lower than those reported by Jaymani et al. (2007). This inconsistency might be due to selection of genotypes or primers with high score alleles. The microsatellite involves di-nucleotide repeat motifs particularly those with GA repeats that amplified relatively small number of bands (Pervaiiz et al., 2009). Markers with PIC values of 0.5 or higher as demonstrated in this study are greatly informative for genetic studies and useful as a marker at a specific locus (Dewoody et al., 1995). The high PIC

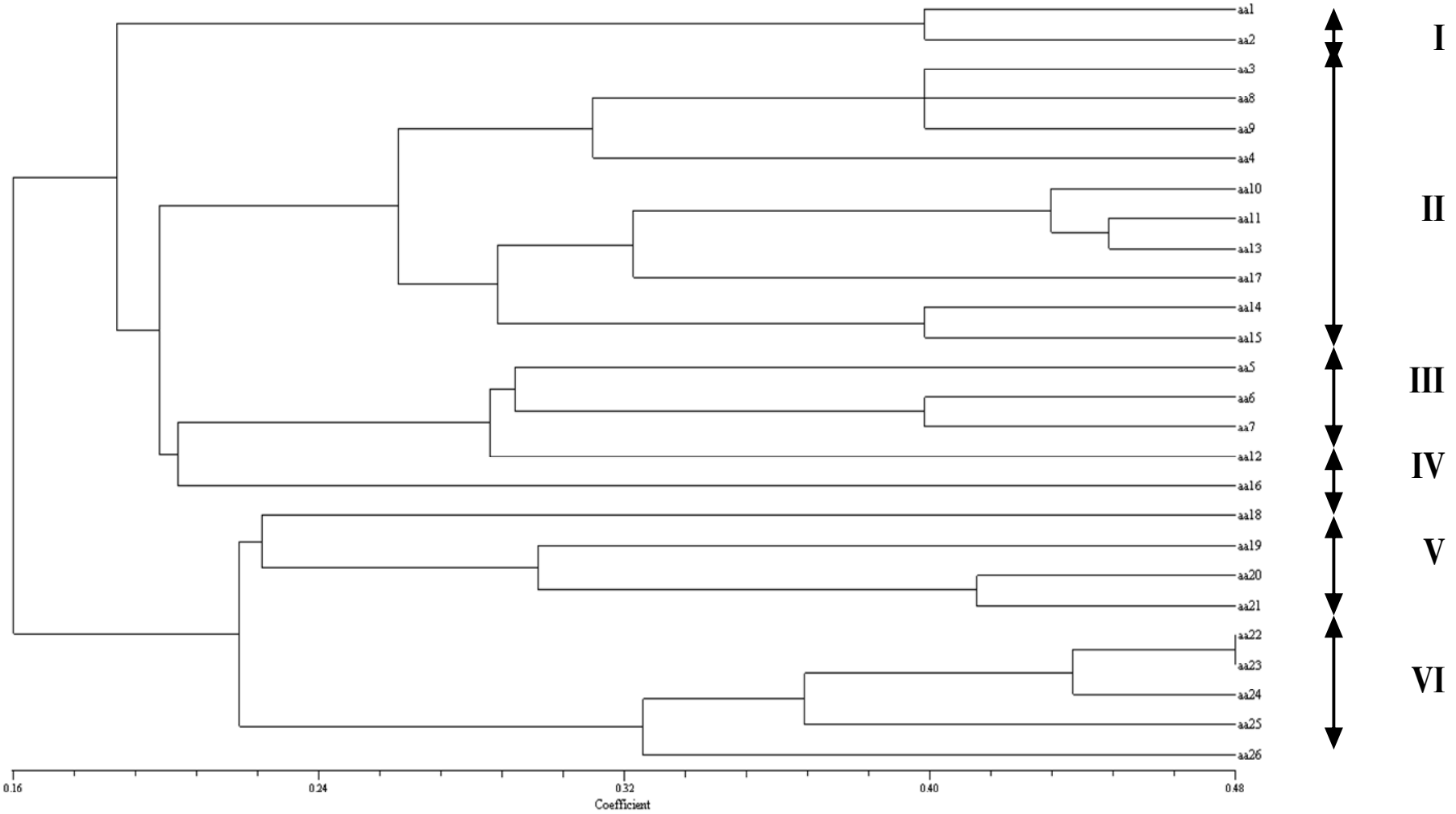

Figure 3. Dendrogram resulting from UPGMA cluster analysis of 26 rice cultivars, based on genetic dissimilarity distance, data derived from 21 microsatellite primer pairs.

values in this study maybe due to co-dominant expression and multiple alleles (Ferreira and Grattapaglia, 1998). Based on the differences among the selected rice cultivars, six distinct clusters were observed which illustrated that the selected markers were consistent as they can detect advanced levels of variations among closely related lines (Ganesh et al., 2007).

Conclusion

The rice cultivars used in the present study were diverse because of differences in their origins,

Table 3. Composition and size of clusters along with genetic dissimilarity values among rice accessions.

Cluster number	Number of genotype	Nature of origin	Genetic dissimilarity
Cluster I	2	Iran	0.188
Cluster II	10	Iran and Malaysia	0.195
Cluster III	4	Iran	0.282
Cluster IV	1	Malaysia	0.201
Cluster V	4	Malaysia	0.217
Cluster VI	5	Malaysia	0.326

ecotypes and geographical ranges, resulting in low similarity index values between them. Cultivars with lowest genetic similarity can be selected and used in breeding programs and screening for higher grain quality rice accession by back-cross program. The results suggested that microsatellite markers are reliable markers for detecting genetic differences among cultivars that originated from different geographical areas.

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REFERENCES

Akagi H, Inagaki YA, Fujimura T (1997). Highly polymorphic microsatellite of rice consist of AT repeats, and a classification of closely related cultivars with these microsatellite loci. Theor. Appl. Genet. 94: 61–67.

Chakravarthy KB, Rambabu N (2006). SSR marker based DNA fingerprinting and diversity study in rice (*Oryza sativa* L.). Afr. J. Biotechnol. 5: 684–688.

Chung AM, Staub JE, Chen JF (2006). Molecular phylogeny of Cucumis species as revealed by consensus chloroplast SSR marker length and sequence variation. Genome 49: 219–229.

Dellaporta S, Wood L, Hicks JB (1983). A plant DNA mini preparation: version II. Plant Mol. Biol. Report, 1(14):19-21.

Dewoody JA, Honeycutt RL, Skow LC (1995). Microsatellite markers in white-tailed deer. J. Hered. 86: 317–319.

Ferreira ME, Grattapaglia D (1998). Introdução ao uso de marcadores moleculares em Análise Genética. 3. ed. Embrapa, Brasília: 220.

Ganesh Ram S, Thiruvengadam V, Kurungari VK (2007). Genetic diversity among cultivars, landraces and wild relatives of rice as revealed by microsatellite markers. Theor. Appl. Genet. 48(4), 337–345.

Hamada HM, Petrino MG, Kakunaga T (1982). A novel repeated element with Z-DNAforming potential is widely found in evolutionarily diverse eukaryotic genomes. Proc Natl Acad Sci USA 79:6465–6469.

Henry R (1997). Practical applications of plant molecular biology, Chapman & Hall, London.

Jayamani P, Negrao S, Martins M, Macas B, Oiveira MM (2007). Genetic relatedness of Portuguese rice accessions from diverse origins as assessed by microsatellite markers. Crop Sci. 47: 879-886.

Kalia RK, Rai MK, Kalia S, Singh R, Dhawan AK (2011). Microsatellite markers: an overview of the recent progress in plants. Euphytica, 177:309–334.

Khush G (2003). Productivity improvements in rice. Nutri. Rev. 61: S114-S116.

Kosman E, Leonard KJ (2005). Similarity coefficients for molecular markers in studies if genetic relationships between individuals for haploid, diploid, and polyploidy species. Mol Ecol, Oxford, 14: 415-424.

McCouch SR, Temnykh A, Lukashova J, Coburn G, De-Clerck S, Cartinhour S, Harrington M, Thomson E, Septiningsih M, Semon P, Moncada M, Li JM (2001). Microsatellite markers in rice: abundance, diversity, and applications. Rice Genet. 117-135.

Moose SP, Mumm RH (2008). Molecular plant breeding as the foundation for 21st century crop improvement. Plant Physiol. 147: 969-977.

Parida SK, Kalia SK, Sunita K, Dalal V, Hemaprabha G, Selvi A, Pandit A, Singh A, Gaikwad K, Sharma TR, Srivastava PS, Singh NK, Mohapatra T (2009). Informative genomic microsatellite markers for efficient genotyping applications in sugarcane. Theor. Appl. Genet. 118:327–338.

Peng JH, Lapitan N (2005). Characterization of EST-derived microsatellites in the wheat genome and development of eSSR markers. Fund. Int. Genom, 5: 80–96.

Pervaiz ZH, Rabbani MA, Pearce SR, Malik SA (2009). Determination of genetic variability of Asian rice (*Oryza sativa* L.) varieties using microsatellite markers. Afr. J. Biotechnol. 8: 5641-5651.

Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S, Rafalski A (1996). The comparison of RFLP, RAPD, AFLP and SSR (Microsatellite) markers for germplasm analysis. Mol. Breed. 2: 225–238.

Provan J, Powell W, Hollingsworth PM (2001). Chloroplast microsatellites: new tools for studies in plant ecology and evolution. Trends Ecol. Evol. 16:142–147.

Rajendrakumar P, Biswal AK, Balachandran SM, Srinivasarao K, Sundaram RM (2007). Simple sequence repeats in organellar genomes of rice: frequency and distribution in genic and intergenic regions. Bioinfo. 23:1–4.

Ravi M, Geethanjali S, Sameeyafarheen F, Maheswaran M (2003). Molecular marker based genetic diversity analysis in rice (*Oryza sativa* L.) using RAPD and SSR markers. Euphytica, 133: 243–252.

Rohlf FJ (1993). NTSYS-pc. Numerical taxonomy and multivariate analysis system, version 2.02. Setauket (New York): Exeter Publishing.

Schlötterer C (2004). The evolution of molecular markers - just a matter of fashion. Nat. Rev. Genet. 5: 63-69.

Schulman AH (2007). Molecular markers to assess genetic diversity. Euphytica 158: 313-321.

Tautz D, Renz M (1984). Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucleic Acids Res. 12:4127–4138.

Temnykh S, Park WD, Ayres N, Cartinhour S, Hauck N, Lipovtiesich L (2000). Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). Theor. Appl. Genet. 100: 697–712.

Weir BS (1996). Genetic data analysis II, 2nd ed. Sunderland, Massachusetts, Sinauer Associates: 377.

Yeh F, Boyle T (1997). Population genetic analysis of codominant and dominant markers and quantitative traits. Bel. J. Bot. 129: 157–163.

Zane L, Bargelloni L, Patarnello T (2002). Strategies for microsatellite isolation: A Review. Mol. Ecol. 11:1–16.