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Molecular Marker Based Genetic Diversity Analysis in Rice Genotypes (*Oryza sativa* L.) using SSR Markers

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ABSTRACT

Assessment of genetic diversity is important in plant breeding if there is to be improvement by selection. The role of a broad genetic base and systematically characterized germplasm in the crop improvement of cultivated plants has been well recognized. Genetic variability studies are important in selection of parents for hybridization as sound crop improvement depends upon the magnitude of variability in the base population. In the present investigation five rice genotypes were evaluated for genetic diversity. Upon PCR amplification the alleles were separated on Agarose Gel Electrophoresis system. Initial polymorphism detection was conducted using twenty primer pairs distributed on five rice chromosomes. A total of 65 alleles were detected with an average of 3.25 alleles per locus. The polymorphism information content (PIC) reflections of alleles diversity frequency among the varieties, which is ranged from 0.215 to 0.791, with an average of 0.493. RM 260 was found as the best marker for identification of genotypes as revealed by PIC values. The highly informative markers identified in this study could be utilized in further studies for comparative mapping and marker assisted selection for drought tolerance.

Key words: *Genetic Diversity, SSR, Oryza sativa, Molecular markers*

INTRODUCTION

Rice is the staple food and about half of the world population depends on rice for their survival. It is cultivated in 114 a country across the globe, but 90 per cent of world's rice is grown in Asia. India has the largest area under rice among the rice growing countries in the world and ranks second in production after China. Rice is grown in almost all the states of India, but major rice producing states fall in the regions of middle and lower gangetic plains, as well as the coastal lowlands of peninsular India. Being a leading producer of rice, there is a need of more production per unit area to fulfil the needs of ever growing population. In order to meet this, hybridization is a prime consideration for which the diversified genotypic assessment has to be done.

Genetic diversity is a pre-requisite for any crop improvement program as it helps in estimating and establishing of genetic relationship in germplasm collection, identifying diverse parental combinations to create segregating progenies with maximum genetic variability and superior recombinations for further selection and introgressing desirable genes from diverse germplasm[12,29].

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Which is commonly measured by genetic distance or genetic similarity, both of which imply that there are either differences or similarities at the genetic level[31]. Molecular Marker based Genetic Diversity Analysis (MMGDA) also has potential for assessing changes in genetic diversity over time and space[10].

Molecular markers have proven to be powerful tools in the assessment of genetic variation and in the elucidation of genetic relationships within and among species. The recent development of DNA markers has provided new opportunities for the genetic improvement of rice cultivars[5]. Satellite loci also known as simple sequence repeats (SSRs) are the most commonly used molecular markers because they are highly informative, mostly monolocus, codominant, easily analyzed and cost effective[11]. SSR markers are able to detect high level of allelic diversity and they have been extensively used to identify genetic variation among rice subspecies[19].

Microsatellites are PCR-based markers that are efficient and cost-effective to use. Compared with other markers, they are abundant, co-dominant, highly reproducible and interspersed throughout the genome[23,28]. In particular, microsatellite markers have been widely applied in rice genetic studies as they are able to detect high levels of allelic diversity[16]. These markers can detect a significantly higher degree of polymorphism in rice[19,20] which becomes ideal for studies on genetic diversity and intensive genetic mapping[7]. They have been used for characterizing genetic diversity in several crop species including sorghum[8,27], maize[25], cotton[15] and wheat[24].

In rice, SSRs have been used to assess the genetic diversity of both wild and cultivated species[4,18,26]. Rice microsatellites also have a demonstrated utility for gene-tagging and marker-assisted selection[6] and are polymorphic between[1,23] and within rice varieties[21]. These studies showed that SSR markers are efficient in detecting genetic polymorphisms and discriminating among genotypes. The important advantages of microsatellites are that they are usually single locus and because of the high mutation rate, are often multi-allelic. Microsatellite or simple sequence repeat (SSR) markers are considered to be appropriate for assessment of genetic diversity and variety identification because of their ability to detect large numbers of discrete alleles repeatedly, accurately and efficiently. The aims of this study was to investigate the genetic diversity among five rice genotypes using SSR markers.

MATERIAL AND METHODS

The present experiment was conducted during *kharif*-2015 (at Department of Plant Biotechnology, University of Agricultural Sciences, Bengaluru, India).

Plant materials and experimental conditions

The experimental material for this investigation consists of five rice genotypes, which are grown at field of aerobic rice laboratory, Department of Plant Biotechnology, University of Agricultural Sciences, Bangalore, India in Randomized Complete Block Design (RCBD) with three replications. The studied genotypes details are presented in Table 1.

Table 1: Name, origin, type, pedigree and some features of the studied genotypes

Sl. No	Genotype	Origin	Type	Parentage
1	ARB 6	UASB, India	Indica	Bddha X IR 64
2	Moroberekan	Republic of Guinea	Tropical japonica	IR8-24-6 (M307H5)
3	AM 65	UASB, India	Japonica	Azucena X moroberekan
4	AM 72	UASB, India	Japonica	Azucena X moroberekan
5	MTU1001	India	Japonica	Vajram x MTU 7014

Isolation of genomic DNA

Genomic DNA was isolated from young leaves of 21 days old plants using CTAB method as per the protocol described by Doyle and Doyle[9].

DNA quality estimation

The genomic DNA was quantified spectrophotometrically both at 260 nm and 280 nm wavelengths. The absorbance at 260 nm allows the calculation of DNA concentration in the sample. An OD of 1 at 260 nm corresponds to 50 µg of double stranded DNA. A pure sample of DNA shows the ratio of OD 260/280 as 1.8. Ratios less than 1.8 indicate contamination in the isolation either with phenol or with proteins. The values higher than this indicate the presence of RNA in the isolation.

Normalization of the DNA concentration was done to bring all the DNA concentrations to a relatively equal level (25ng/µl) by appropriate dilutions for PCR reaction. Dilution was done with double distilled sterile water.

SSR markers

Total of twenty simple sequence repeat (SSR) markers were used for studying molecular diversity. The details of SSR primers used are presented in Table 2.

Table 2: Details of the microsatellite primers used in present study

Sl. No	Name	Product size (bp)	Forward primer (5'---->3')	Reverse primer (5'--->3')	Repeat motif
1	RM1261	167	GTCCATGCCCAAGACACAAC	GTTACATCATGGGTGACCCC	(AG)16
2	RM319	134	ATCAAGGTACCTAGACCACCAC	TCCTGGTGCAGCTATGTCTG	(GT)10
3	RM212	136	CCACTTTCAGCTACTACCAG	CACCCATTTGTCTCTCATTATG	(CT)24
4	RM263	199	CCCAGGCTAGCTCATGAACC	GCTACGTTTGAGCTACCACG	(CT)34
5	RM324	175	CTGATTCCACACACTTGTGC	GATTCCACGTCAGGATCTTC	(CAT)21
6	RM279	174	GCGGGAGAGGGATCTCCT	GGCTAGGAGTTAACCTCGCG	(GA)16
7	RM315	133	GAGGTACTTCCTCCGTTTCAC	AGTCAGCTCACTGTGCAGTG	(AT)4(GT)10
8	RM72	166	CCGGCGATAAAACAATGAG	GCATCGGTCCTAACTAAGGG	(TAT)5C(ATT)15
9	RM248	102	TCCTTGTGAAATCTGGTCCC	GTAGCCTAGCATGGTGCATG	(CT)25
10	RM219	202	CGTCGGATGATGTAAAGCCT	CATATCGGCATTCGCCTG	(CT)17
11	RM260	111	ACTCCACTATGACCCAGAG	GAACAATCCCTTCTACGATCG	(CT)34
13	RM511	130	CTTCGATCCGGTGACGAC	AACGAAAGCGAAGCTGTCTC	(GAC)7
14	RM543	98	CTGCTGCAGACTCTACTGCG	AAATATTACCCATCCCCCCC	(GCG)10
15	RM279	174	GCGGGAGAGGGATCTCCT	GGCTAGGAGTTAACCTCGCG	(GA)16
16	RM321	200	CCAACACTGCCACTCTGTTC	GAGGATGGACACCTTGATCG	(CAT)5
17	RM566	239	ACCCAACTACGATCAGCTCG	CTCCAGGAACACGCTCTTTC	(AG)15
18	RM1153	114	ACCAACGCCAAAAGCTACTG	TACTCGCCCTGCATGAGC	(AG)13
19	RM246	116	GAGCTCCATCAGCCATTCAG	CTGAGTGCTGCTGCGACT	(CT)20
20	RM525	131	GGCCCGTCCAAGAAATATTG	CGGTGAGACAGAATCCTTACG	(AAG)12

PCR amplification

PCR amplification reactions were done in 10 µl reaction mixtures, containing 2 µl of template DNA, 0.5 µl of each forward and reverse primer, 4 µl of PCR master mix and 3 µl ddH_2O. Thermal cycler was used with the following PCR profile: an initial denaturation step at 94°C for 5 min, followed by 35 cycles

of denaturation at 94°C for 1 min, annealing at 55°C for 30 seconds and primer elongation at 72°C for 1 min and then a final extension at 72°C for 10 min. Amplified products were stored at -20°C until further use.

The SSR amplification products were separated on 2.5 % agarose gel supplemented with ethidium bromide. The TAE 1X was used as a running buffer and 100 bp DNA ladder was used to estimate the molecular size of the amplified fragments. Electrophoresis was conducted at 60 Volts for 2 hours. Gels were then visualized and photographed using Alpha Innotech gel documentation instrument.

Data analysis

Polymorphic information content (PIC) values were calculated for each SSR locus based on Anderson *et al*.[2]. The amplified bands were scored for each SSR primer pairs based on the presence or absence of bands, generating a binary data matrix of 1 and 0 for each marker system. These binary data matrix was then utilized to generate genetic similarity data among the different rice genotypes. Both matrices were then analyzed using the NTSYS pc statistical package version 2.2. The data matrices were used to calculate genetic similarity based on Jaccard's similarity coefficients.

RESULTS AND DISCUSSION

Molecular diversity in rice is done by using SSR markers. It is the complimentary sequences of DNA which lie close to the particular gene or QTL. So by annealing the primer we can amplify our target region close to the gene.

Polymorphism level of among rice cultivars was evaluated by calculating allelic number and PIC values for each of the twenty SSR loci evaluated. A total of 65 alleles were detected at the loci of twenty microsatellite markers across five rice genotypes. The results revealed that all the primers showed distinct polymorphisms among the cultivars studied indicating the robust nature of microsatellites in revealing polymorphism. Among the polymorphic markers, 6 produced two alleles each, 7 produced three alleles each, 4 generated four alleles each, 2 produced five alleles each and only one produced six alleles (table 3).

The number of alleles per locus ranged from 2 (RM319, RM 212, RM 324 *etc*.,) to 6 alleles (RM260) with an average of 3.25 alleles across the twenty loci. The Value is comparable to 1-8 allele per SSR locus with an average number of alleles of 4.58 per locus for various classes of microsatellite[26].

The amplicon size of all five genotypes for each marker alleles varied from 103-130 bp produced by RM260 and 215 – 262 bp produced by RM566. Of the 65 alleles scored all of 65 were found to be polymorphic. Maximum number of polymorphic alleles (6) was obtained with the marker RM260, while the minimum numbers of polymorphic alleles (2) was obtained by using RM319, RM212, RM324, RM315, RM543 and RM321.

PIC value

The polymorphic information content (PIC) was employed for each locus to assess the information of each marker and its discriminatory ability and it is a reflection of allele diversity and frequency among varieties. PIC values ranged from 0.215 to 0.791 with an average of 0.493 (table 3). The highest PIC value 0.791 was obtained for RM260 followed by respectively RM219 (0.74), and RM72 (0.70). PIC value revealed that RM260 was considered as best marker for five test genotypes. The PIC value observed, are comparable to three previous estimates of microsatellite analysis in rice via 0.34-0.88 [30], 0.20-0.90 with an average of 0.56 [13]. Figure 1 showed gel pictures of amplified fragment using primer designed for the SSR marker RM219 and RM263.

Table 3. Number of alleles, highest frequency allele and Polymorphism Information Content (PIC) Values found among five rice germplasms for twenty SSR markers

SL. No	Primer name	Chromosome number	Amplicon size range (bp)	Allele number	Annealing temperature	PIC value
1	RM1261	12	166 - 189	4	50	0.641
2	RM319	1	140 - 144	2	55	0.370
3	RM212	1	122 - 125	2	55	0.325
4	RM263	2	161 - 178	3	55	0.502
5	RM324	2	135 - 154	2	55	0.370
6	RM279	2	150 - 162	3	55	0.407
7	RM315	1	140 - 144	2	55	0.325
8	RM72	8	151 - 184	5	55	0.700
9	RM248	7	118 - 124	3	55	0.502
10	RM219	9	186- 216	5	55	0.740
11	RM260	12	103 - 130	6	55	0.791
12	RM511	12	129 - 135	3	55	0.407
13	RM543	1	114 - 121	2	55	0.215
14	RM279	2	150 - 162	3	55	0.407
15	RM321	9	196 - 200	2	55	0.325
16	RM566	9	215 - 262	4	55	0.641
17	RM1153	4	119 - 140	3	55	0.407
18	RM246	1	114 - 125	3	50	0.580
19	RM525	2	122 - 152	4	55	0.641
20	RM7	3	159 - 172	4	55	0.57

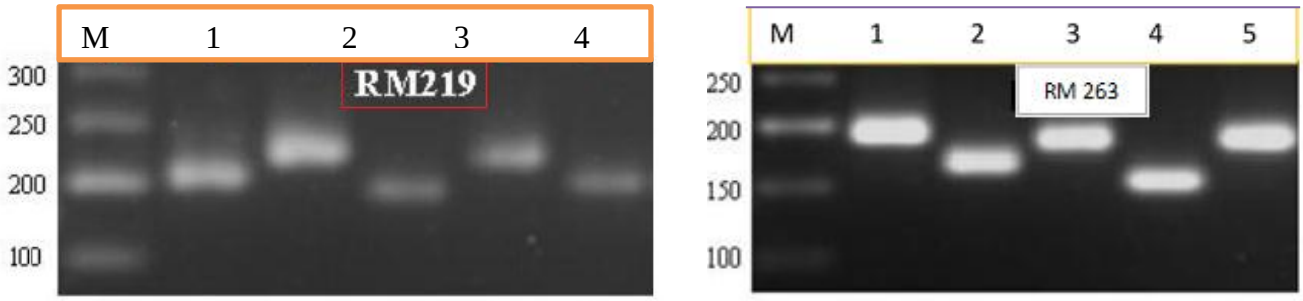

Fig. 1: Agarose gel electrophoresis of PCR amplified fragments for the highest polymorphic SSR markers RM219, and RM263, M is 100 bp DNA ladder; 1-ARB 6; 2-Moroberekan; 3-AM 65; 4-AM 72; 5-MTU1001.

Many studies have been reported on the assessment of genetic diversity in a relatively large set of cultivated germplasm. This include diversity analysis of high yielding cultivars[19], aromatic rice[17], indigenous aromatic rice[14] and even lowland rice[3,32] using various molecular fingerprinting techniques like RFLP, RAPD, SSR, AFLP etc. The informative primers would prove useful in marker-assisted selection, linkage mapping and gene tagging for specialty traits. In the conclusion, SSR markers based molecular fingerprinting could serve as a sound basis in the identification of genetically distant accessions as well as sorting of duplicate germplasm of morphologically close accessions.

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