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SHORT COMMUNICATION

Fingerprinting Based on Microsatellite Markers for the Identification of Rice Varieties in Chhattisgarh

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Molecular markers are useful tools for determining cultivar identity. The purpose of this study to establish fingerprints of four rice varieties in Chhattisgarh. Microsatellite combines several features of an ultimate molecular marker and they are being used increasingly in various plant genetic studies and applications. The varieties were evaluated utilizing 115 microsatellite markers distributed over the whole rice genome. A total of 160 alleles were detected using 24 microsatellite primer-pairs, and the number of alleles/marker ranged from 2 to 4 with an average of 2.95 alleles/locus. The result suggested that a relatively rapid and small number of microsatellite markers could be used for the identification of rice varieties.

Key Words: DNA Fingerprinting, Markers Rice, Microsatellite, Variety identification

Rice, *Oryza sativa* (2n = 24) belonging to the family *Graminae* and subfamily *Oryzoidea* is the staple food for one-third of the world's population and occupies almost one-fifth of the total land area covered under cereals. It is grown under diverse cultural conditions and over wide geographical range. Most of the world's rice is cultivated and consumed in Asia, which constitutes more than half of the global population. High-quality seeds and elite cultivars play a crucial role in its production. However, since new cultivars normally arise from hybridizations between members of an elite group of genetically similar parents, the amount of genetic variability among newly developed cultivars is likely to become even smaller (Rahman *et al*., 2009), which makes it more difficult to distinguish one cultivar from other by visual observation, but keeping track of many commercial inbreeds, hybrid varieties and landraces makes the task dauntingly inefficient. Fingerprinting with molecular markers allows precise, objective and rapid cultivar identification, which has been proved to be an efficient tool for crop germplasm characterization, collection and management. Simple sequence repeat (SSR) markers have been widely used for genetic analysis and cultivar identification because of their abundance, co-dominance inheritance, high polymorphism, reproducibility, and ease of assay by polymerase chain reaction (PCR) (Kuleung *et al*., 2004; Xie *et al*., 2011). Therefore, it has been applied widely in the identification, registration of plant variety, and in monitoring of the seed purity and the authenticity with high accuracy, high reliability and low cost. The usefulness of DNA fingerprinting for cultivar identification in rice was first demonstrated by Dallas (1988). Establishing the identity of crop varieties has assumed greater importance for protecting plant breeders and farmers rights in the post-CBD scenario particularly in the developing countries. This investigation is conducted to fingerprint the four main commercial rice cultivar under cultivation now in Chhattisgarh region based on SSR markers.

The nucleus seed of four released rice varieties from Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur, namely, Karmamasuri, Danteshwari, Samleshwari and Mahamaya were taken for the present study for developing DNA fingerprinting.

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DNA of five plants from nucleus seed of four varieties was used for molecular studies. DNA was isolated by mini prep method (Doyel and Doyel 1990) quantified using nanodrop (*NANODROP 2000c,* Thermo Fisher Scientific, Wilmington, USA) and diluted appropriately to working solution of 20 ng/µl.

For the development of DNA based fingerprinting of each of four varieties, 115 rice microsatellite primer pairs (http://www.gramene.org/) were used in the analysis. PCR reactions were performed in a volume of 20 µl. Amplification protocol consisted of an initial denaturation at 95°C for 5 min, followed by 34 cycles of 94°C for 1 m, annealing for 1 m at 55°C, 72°C for 1 min and a final extension step of 72°C for 7 min. was carried out with the established program in thermal cycler (AB applied Biosystem/Verti 96 well thermal cycler). After PCR amplification the amplicons were electrophoresed in five percent polyacrylamide gels for 1 hours at 120 volts. The gel was then stained with ethidium bromide (Sambrook *et al.,* 1989) and visualised in a gel documentation system (make Bio-RAD/gel dox^{TM} XR+, Bio-Rad Laboratories, Inc., USA)

The size (in nucleotides) of the most intensely amplified band for each microsatellite marker was determined based on its migration relative to the molecular weight (mw) size markers, 50bp DNA ladder (GENEI Pvt. Ltd. Bangalore, India) and the polymorphism information content (PIC) was calculated according to formula

$$PIC = 1-\Sigma P_i^2$$

Where P_i is the allele frequency for the i-th allele (Nei, 1973).

$$\text{Per cent polymorphism} = \frac{\text{Number of polymorphic bands}}{\text{Total number of bands}} \times 100$$

Molecular marker data were recorded from gels upon band size classification and expressed in binary code. Cluster analysis was conducted on similarity estimates, by using Unweighted pair group method on arithmetic averages (UPGMA). The comparison of similarity matrices of different markers was made by MAXCOMP module of NTSYSpc.

A total of 115 microsatellite primer-pairs distributed across the genome throughout the 12 chromosomes were used for fingerprinting of four rice varieties, out of which only 24 SSR primers were found to be polymorphic. A total of 71 alleles were obtained using these 24 polymorphic SSR primers with an average of 2.95 alleles per primer. The number of alleles amplified for each primer ranged from two to four. The PIC for

Table 1. Size and frequency of alleles at twenty four microsatellite loci in four rice varieties released in Chhattisgarh

Locus	Number of alleles	Allele size (bp)	Allele frequency	Gene diversity (PIC = 1-ΣX_i^2)	Location on chromosome	Primer sequence (5' to 3')
RM 55	3	240	0.25	0.625	3	TCCCGGTTATTTTAAGGCG
		245	0.25			CCGTCGCCGTAGTAGAGAAG
		300	0.50			
RM 169	4	170	0.25	0.75	5	TCCCGTTGCCGTTCATCCCTCC
		175	0.25			TGGCTGGCTCCGTGGGTAGCTG
		195	0.25			
		305	0.25			
RM 164	4	253	0.25	0.75	5	GCAGCCCTAATGCTACAATTCTTC
		260	0.25			TCTTGCCCGTCACTGCAGATATCC
		275	0.25			
		290	0.25			
RM 204	3	105	0.50	0.625	6	GTGACTGACTTGGTCATAGGG
		110	0.25			GCTAGCCATGCTCTCGTACC
		120	0.25			
RM 211	2	150	0.75	0.375	2	CCGATCTCATCAACCAACTG
		152	0.25			CTTCACGAGGATCTCAAAGG
RM 85	3	100	0.25	0.625	3	GCACAAGGTGAGCAGTCC
		115	0.25			CCAAAGATGAAACCTGGATTG
		125	0.50			
RM 418	3	310	0.50	0.625	7	GAGCACATATGCCACGTACG
		320	0.25			TCGCGTATCGTCATGCATAG
		340	0.25			

Locus	Number of alleles	Allele size (bp)	Allele frequency	Gene diversity (PIC = 1-ΣX_i^2)	Location on chromosome	Primer sequence (5' to 3')
RM 13	4	135	0.25	0.75	5	GGTGGCATTCGATTCCAG
		140	0.25			TCCAACATGGCAAGAGAGAG
		145	0.25			
		160	0.25			
RM 1	2	80	80	0.375	1	GCGAAAACACAATGCAAAAA
		120	120			GCGTTGGTTGGACCTGAC
RM 206	3	130	0.25	0.625	11	CCCATGCGTTTAACTATTCT
		155	0.50			CGTTCCATCGATCCGTATGG
		160	0.25			
RM 72	4	170	0.25	0.75	8	GCATCGGTCCTAACTAAGGG
		185	0.25			CCGGCGATAAAACAATGAG
		195	0.25			
			0.25			
RM 332	3	170	0.25	0.625	11	GCGAAGGCGAAGGTGAAG
		185	0.25			CATGAGTGATCTCACTCACCC
		195	0.50			
RM 19	4	230	0.25	0.75	12	CAAAAACAGAGCAGATGAC
		245	0.25			CTCAAGATGGACGCCAAGA
		250	0.25			
		253	0.25			
RM 247	2	145	0.50	0.375	12	CATATGGTTTTGACAAAGCG
		150	0.50			TAGTGCCGATCGATGTAACG
RM 21	3	140	0.25	0.625	11	ACAGTATTCCGTAGGCACGG
		155	0.25			GCTCCATGAGGGTGGTAGAG
		175	0.50			
RM 202	3	120	0.5	0.625	11	CCAGCAAGCATGTCAATGTA
		125	0.25			CAGATTGGAGATGAAGTCCTCC
		130	0.25			
RM 254	2	115	0.25	0.375		
		125	0.75			
RM 474	3	230	0.50	0.625	10	TATGAGCTGGTGAGCAATGG
		250	0.25			AAGATGTACGGGTGGCATTC
		255	0.25			
RM 432	4	175	0.25	0.75	7	TTC TGT CTC ACG CTG GAT TG
		180	0.25			AGC TGC GTA CGT GAT GAA TG
		190	0.25			
		350	0.25			
RM 484	3	148	0.25	0.625	10	TGCTGCCCTCTCTCTCTCTC
		155	0.25			TCTCCCTCCTCACCATTGTC
		160	0.50			
RM 152	2	140	0.25	0.375	8	CCGTAGACCTTCTTGAAGTAG
		155	0.75			GAAACCACCACACCTCACC G
RM 144	3	225	0.25	0.625	11	GCTAGAGGAGATCAGATGGTAGTGCATG
		245	0.5			TGCCCTGGCGCAAATTTGATCC
		255	0.25			
RM 303	3	200	0.5	0.625	4	GCATGGCCAAATATTAAAGG
		205	0.25			GGTTGGAAATAGAAGTTCGGT
		210	0.25			
RM 108	4	70	0.25	0.75	9	CGTGCACCACCACCACCACCAC
		75	0.25			TCTCTTGCGCGCACACTGGCAC
		80	0.25			
		90	0.25			

these primers ranged from 0.37 to 0.75 (Table 1). Of the twenty four microsatellite markers seven microsatellite markers *viz.,* RM169, RM164, RM13, RM19, RM 108, RM 72 and RM 432 differentiated all the four varieties at least with a single marker allele difference.

Allele sizing technologies are well established and can be readily used to size microsatellite alleles from any organism (Song *et al.,* 1999). SSR genotypic data from a number of loci have the potential to provide unique allelic profiles or DNA fingerprints for precisely establishing genotypic identity. Comparisons between SSR band positions against each marker in this study are shown in Table 2. The band patterns corresponding to individual variety help to recognize the variety in question. When one primer would not distinguish individual variety from others, another primer should be considered

Table 2. Analysis of band positions due to microsatellite primers for four rice varieties

Varieties	Band Positions Due to Primers (bp)																			
	RM 55			RM 169				RM 164				RM 204			RM 211	
	A	B	C	A	B	C	D	A	B	C	D	A	B	C	A	B
KARMAMASURI		245				195				275				120		152
DANTESHWARI	240			170				253				105			150	
SAMLESHWARI			300		175				260			105			150	
MAHAMAYA			300				305				290		110		150	

Varieties	Band Positions Due to Primers (bp)																					
	RM 85			RM 418			RM 13				RM 1		RM 206			RM 72				RM 332		
	A	B	C	A	B	C	A	B	C	D	A	B	A	B	C	A	B	C	D	A	B	C
KM	100					340			145		80		130			98				170		
DN		115		310			135					120			160				160			195
SM			125	310				140				120		155			145				185	
MH	100				320					160		120		155				150				195

Varieties	Band Positions Due to Primers (bp)																					
	RM 21			RM 202			RM 19				RM 247		RM 474			RM432				RM 484		
	A	B	C	A	B	C	A	B	C	D	A	B	A	B	C	A	B	C	D	A	B	C
KM		155				135				253	145		230						350		155	
DN	140			120				245				150		250		175				148		
SM			175	120			230				145				255		180					160
MH			175		125				250			150	230					190				160

Varieties	Band Positions Due to Primers (bp)																		
	RM 144			RM 303			RM 254		RM 152		RM108			
	A	B	C	A	B	C	A	B	A	B	A	B	C	D
KM	225				205			125		155	70			
DN		245		200			115		140					90
SM		245				210		125		155			80	
MH			255	200				125		155		75		

and sometimes, combination of more than one primer should be taken into account. Thus, additional primer or set of primers might be needed to test or identify all the expected varieties. In the present study four rice varieties were discriminated successfully and it shows 100 per cent polymorphism by the seven SSR markers namely, RM169, RM164, RM13, RM19, RM 108, RM72 and RM 432, respectively. Twenty-four primers on an average produced 96 bands across the four varieties and out of which, 71 were polymorphic accounted for high level of per cent polymorphism (73.95%). Microsatellite DNA markers based on simple sequence repeats (SSRs) as amplified by PCR have been used successfully as tools for varietal identification (Yang *et al*., 1994; Rongwen *et al*., 1995). DNA based molecular markers have proven to be a powerful tool in identification of genetic variation and in the elucidation of genetic relationships within and among species, characterized by abundance and untouched by environmental influence (Powell *et al*., 1996). Several workers have also demonstrated the usefulness of SSR markers in determining the relationship between closely related genotypes (Ravi *et al*., 2003; Ganeshram *et al*., 2007).

Cluster I consisted of variety namely Karmamahsuri having 40.5 percent whereas, Cluster II consisted of Danteshwari, Samleshwari and Mahamaya having 50.7 percent similarity among them. Cluster II again partitioned into two sub-clusters in which one sub-cluster had Danteshwari and Samleshwari with 58 percent similarity, whereas another sub cluster had Mahamaya with 50.7 percent similarity with Danteshwari and Samleshwari. Thus, microsatellite markers provided more definitive separation of clusters, indicating a higher level of efficiency for determining the relationship between closely related genotypes.

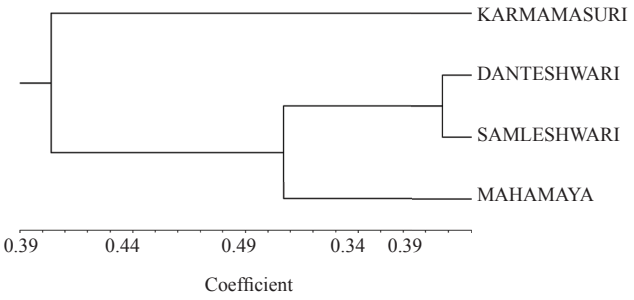

Fig. 1. Dendrogram of rice varieties based on microsatellite markers

In this study, an elementary DNA fingerprinting database of the four main commercial rice cultivars under cultivation in Chhattisgarh region was built using 24 SSR primer-pairs, which could be expanded as the number of additional cultivars and molecular markers (systems) increase. Dendrogram of SSR markers divided the four cultivars into two major clusters. It suggests that SSR markers were efficient to generate locus specific allelic information which can serve as molecular IDs for four rice varieties namely, Karmamasuri, Danteshwari, Samleshwari and Mahamaya.

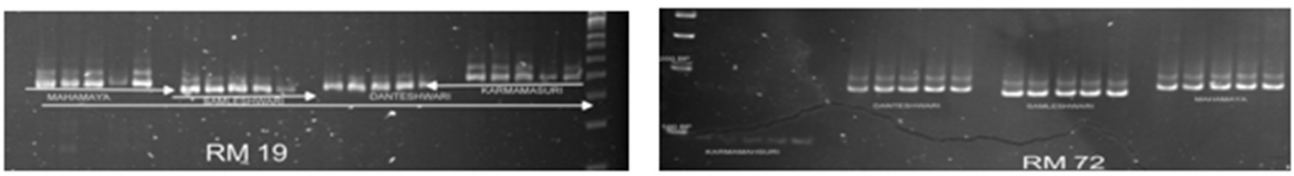

Fig. 2. DNA profile of four rice varieties obtained through microsatellite markers

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