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Detection of genotype specific fingerprints and molecular diversity of selected Indian locals and landraces of rice (*Oryza sativa* L.) using DNA markers

M. Shivapriya and Shailaja Hittalmani[1]

Department of Genetics and Plant Breeding, University of Agricultural Sciences, GKVK, Bangalore 560 065

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A set of sixty-five local rice (*Oryza sativa* L.) accessions and three improved rice varieties were screened to identify the extent of genetic diversity present at the molecular level using RAPD markers. Sixteen primers generated 112 marker levels with 74.1% polymorphism. Dendrogram constructed based on molecular polymorphism unveiled considerable amount of diversity among the varieties. Genotype specific DNA bands were identified for selected lines. These distinct markers have the potential to be employed as genetic fingerprints for future varietal identification and classification. The use of primer duplexes resulted in generation of higher number of DNA bands compared to single primer. The study indicated that the RAPD markers provide an easy, rapid and simple technique for the preliminary assessment of genetic diversity among the local rices.

Key words: Rice, landraces, RAPD, characterization, genotype specific markers

Introduction

Rice (*Oryza sativa* L.) is one among the world's most important staple food crops. More than 90% of rice is produced and consumed in Asia, a densely populated region of the world [1]. It is the most diversified crop species due to its adaptation to a wide range of geographical, ecological and climatic regimes [2]. Thousands of years of selection by farmers, the process of dispersal during domestication, rice has evolved into a tremendously broad base for genetic diversity as reflected by number of landraces existing today [3]. These landraces provide an enormous genetic variability for the present day rice improvement program [4, 5]. Though undomesticated, unadapted germplasm and landraces are phenotypically less desirable. Breeders have long recognized their intrinsic value for the improvement of simply inherited traits including disease, pest resistance [6, 7] and other useful traits. Rich diversity in cultivated rice and their wild relatives needs to be collected, characterized, catalogued and conserved for future use.

Evaluation of genotypes for phenotypic characters based on morphological variation, supplemented with DNA characterization, helps in documentation and deployment of the available genetic variability. Study on genetic polymorphism provides scientific basis for the utilization of germplasm resources efficiently in crop improvement. Though a range of plant characters are currently available for distinguishing closely related individuals, their sensitivity to environment and scanty genome coverage hinders their further usage in breeding. DNA based molecular markers are in abundance and clearly allow the comparison of genetic material avoiding any environmental influence on gene expression. Randomly Amplified Polymorphic DNA (RAPD), a PCR based DNA marker technology, offers advantages in speed, technical simplicity, random coverage of genome and relatively higher level of polymorphism [8]. Higher annealing temperature of the primers was carried out for reproducibility of the results. In this investigation, we explore the possibility of using RAPD markers to evaluate and document the untapped genetic variability available in local rices of Karnataka State in India.

Materials and methods

Sixty-five landraces collected from different agro-ecological zones of Karnataka State, India along with three improved varieties were used in the study (Table 1). Plants were raised in a greenhouse and young leaf tissue was used for DNA extraction. Genomic DNA was isolated using modified Cetyltrimethylammonium bromide (CTAB) method [9]. Concentration and quality of the genomic DNA preparations were determined using spectrophotometer at 260nm/280nm wavelength.

Sixteen arbitrary decamer primers from Operon Technologies, U.S.A with 2 primers each from set OPAB (02 and 11) and OPBB (05 and 09), seven primers from set OPBF (01, 04, 06, 08, 13, 15 and 18) and one primer each from set OPA (06), OP AX

[1]Corresponding author E-mail: shailajah_maslab@rediffmail.com

Table 1. Local rice accessions used for DNA fingerprinting using RAPD markers

Sl. No.	Genotypes	Sl. No.	Genotypes	Sl. No.	Genotypes
1	Alugidda	23	IR-64	45	Navalisali
2	Alur sanna	24	Irylon	46	Nereguli
3	Bagya Jyothi	25	Jaddu batta	47	Onmaradinellu
4	Balle sulli	26	Jeerige sanna	48	P. Doddi
5	Beeraga	27	Kagali kirwana	49	Padmarekha
6	Betiga	28	Kareshedi	50	Puri chipiga
7	Bharani	29	Kari doddi	51	Putta batta
8	Biliakki	30	Karimurdiga	52	Sampige batta
9	Bilihasandi	31	Karthika	53	Sannavalya
10	Biliya	32	Kempasandi	54	Shakthi
11	BKB	33	Kempumadras	55	Somasali
12	Boliya	34	Kempusannakki	56	TK 107 A
13	Budda	35	Kesari	57	TK 107 B
14	Byranellu	36	Khaima	58	TT 121
15	Dabbalasele	37	Madrassanna	59	Turamuri
16	Dodda batta	38	Masale putta	60	Uralachipiga
17	Doddi	39	Mattalaga	61	Vallya
18	Dodiga	40	MM125 A	62	Varangala
19	Halugidda	41	MO-4	63	Wari sanna
20	Hillpaddy	42	MSannna	64	Warris
21	HR-12	43	Mullujeddu	65	Yedikuni
22	IET-14758	44	N.M. Batta		

(15), OPN (07), OPO (01) and OPP (18) were used for PCR amplification. The 25 µl reaction mixture contained 25 ng of template DNA, 20 ng of random decamer primer, 0.1 mM of dNTPs, 1 unit of *Taq* polymerase (M.J. Research, USA) and 1 X PCR buffer (10 mM Tris pH 8.0, 50 µm KC1, 1.8 µm $MgCl_2$ and 0.01 mg/ml gelatin). Reaction mixture for the use of paired primers contained 25 ng of template DNA, 10 ng of each random decamer primer OPP 18 and OPN 07, 0.1 mM of dNTPs, 1 unit of *Taq* polymerase 1 X PCR buffer (10 mM Tris pH 8.0, 50 µm KCl, 1.8 µm $MgCl_2$ and 0.01 mg/ml gelatin). In both the cases, DNA was amplified on a M. J. Research PTC 100 Thermal cycler programmed as follows: initial 4 min denaturation of template DNA at 94°C, and 36 cycles of denaturation for 1 min at 94°C, primer annealing for 1 min at 38°C and 2 min extension at 72°C. The final extension was at 72°C for 5 min followed by cooling to 4°C.

Amplified DNA samples were analysed by electrophoresis on 1.4% agarose gel. Gel was Stained with Ethidium bromide and visualized under UV light. DNA fragments amplified by a selected primer/ each genotype were scored as "1" for the presence of the band and "0" for the absence of the band at each level. Statistical analysis was carried out using STATISITCA package. The program used was tree clustering with raw input data. The main parameter, which guided the joining process, is Unweighted Pair Group method with Arithmetic Mean (UPGMA) and Euclidean distance was computed. The relationship among 65 rice accessions was portrayed graphically in the form of a Dendrogram.

Results and discussion

DNA polymorphism of selected rice genotypes: A varying level of polymorphism was revealed in the banding patterns across the set of 65 rice accessions. For a total of 16 primers, 112 marker levels were amplified of which 84 (74.1%) were polymorphic and 28 (25.9%) were monomorphic bands across genotypes. On an average, total number of bands generated per primer was seven of which 5.2 were polymorphic and the rest were monomorphic. The names and nucleotide sequences of primers used to generate 112 PCR products and the summary of the total number of monomorphic and polymorphic DNA fragments amplified is listed (Table 2).

Table 2. List of RAPD primers and number of PCR amplified bands generated across 65 rice genotypes

Sl. No.	Primer	Number of bands		
		Total	Monomorphic	Polymorphic
1	OPAB 02	5	0	5
2	OPAB 11	5	2	3
3	OPAF 06	6	4	2
4	OPAX 15	10	3	7
5	OPBB 05	10	1	9
6	OPBB 09	5	1	4
7	OPBF 01	7	1	6
8	OPBF 04	8	0	8
9	OPBF 06	12	1	11
10	OPBF 08	5	1	4
11	OPBF 13	7	1	6
12	OPBF 15	7	2	5
13	OPBF 18	6	5	1
14	OPN 07	5	1	4
15	OPO 01	6	4	2
16	OPP 18	8	1	7
	Total	112	28	84

Since RAPD markers are randomly scattered throughout the genome, this has made them particularly suitable to analyse population that would cover the genome to a greater extent [10]. A thorough characterization of cultivars makes it possible to study the level of diversity existing within a species and to establish an index of genetic similarities among different varieties. Our study revealed the existence of intervarietal molecular polymorphism between local rice accessions. Detection of molecular polymorphism between varieties in different crops using RAPD technique is reported by many researchers [11-18].

Dendrogram (Fig. 1) constructed based on RAPD data revealed the pattern of relatedness among 65 genotypes.

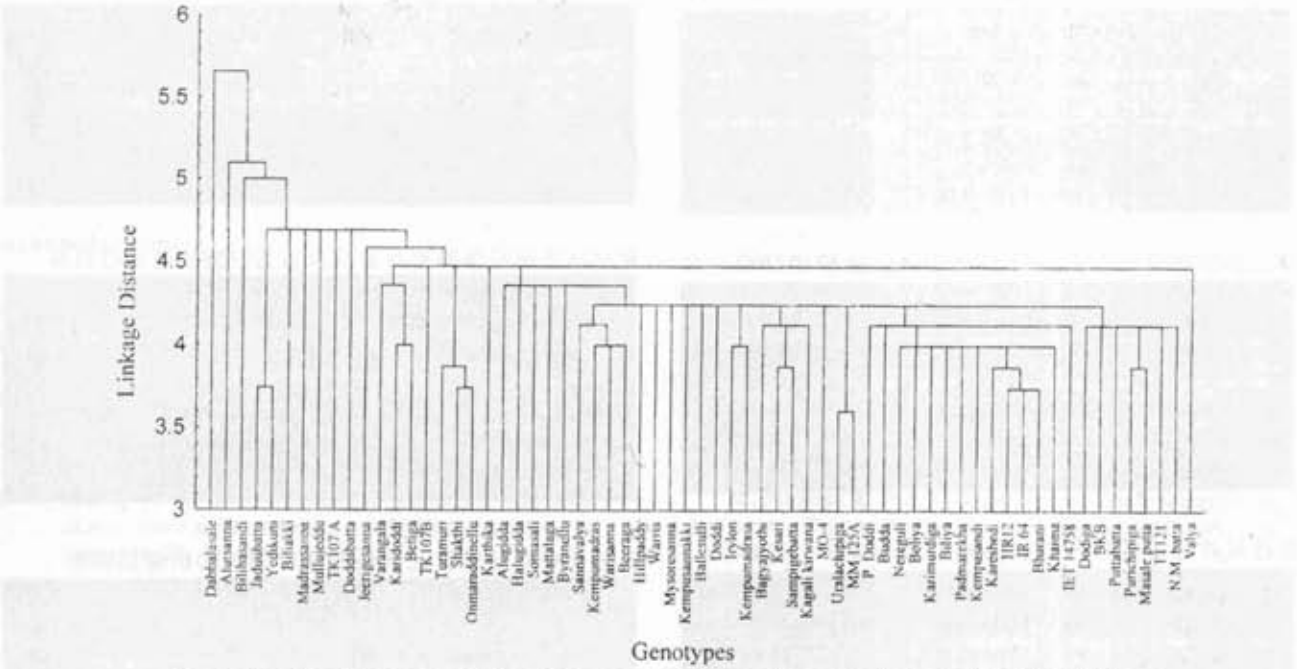

Fig. 1. Dendrogram constructed for 65 rice local genotypes based on PCR amplified RAPD data

All the genotypes were initially grouped into two clusters at similarity level of 15 per cent. Variety Dabbalasele fell out of cluster. Similarly, another genotype Alurusanna that showed genotype specific marker grouped separately when the major cluster divided at similarity level of 25 per cent. Further, at similarity level of about 30 per cent, Bilihasandi separated from the remaining genotypes. At similarity level of 42 per cent, the cluster grouped again into seven sub-clusters where two genotypes, Jaddubatta and Yedikuni clustered in one group. Varieties Biliakki, IRLON, Mullujeddu, and Doddabatta clustered in five separate clusters. Other varieties grouped into a separate cluster. The major cluster segregated into two in which variety Jeerigesanna, a scented variety fell out of cluster at similarity level of 49 per cent. This dendrogram based on molecular data showed considerable amount of genetic diversity among the varieties. Our study reflected the tremendous genetic variability available among the Indian landraces, which have not been explored till today for any crop improvement. This rich genetic diversity on which breeding efforts depend could be utilized for further studies, including screening for desirable genes.

Detection of genotype specific markers: Among the several DNA bands generated by different RAPD primers, some genotypes exhibited a unique banding pattern. DNA bands that were identified as unique to a particular accession made that genotype distinct from other accessions. Genotype Biliakki (line No. 62) showed a specific band (1.5 Kb) with primer OPBF13 (Fig. 2).

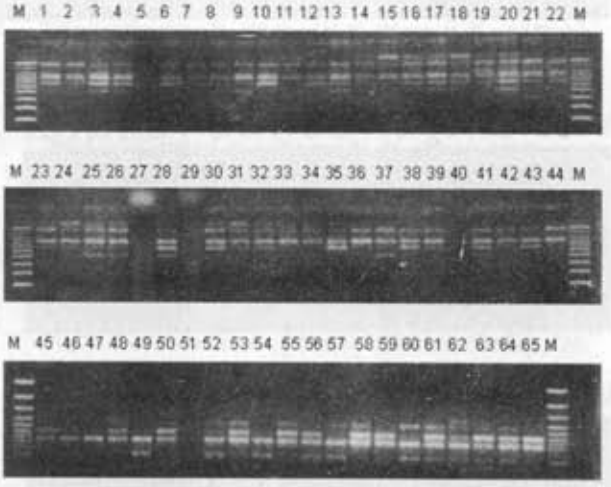

Fig. 2. DNA banding pattern of 65 genotypes for primer OPBF 13. M is 1 kb ladder and the number relates to genotypes as in Table 1. The arrow indicates the genotypes specific DNA band of size 1.5 Kb for genotype Biliakki

Genotype specific markers detected in these cultivars have the potential to be used as genetic fingerprints for future varietal identification and classification. Such markers were also detected in cotton [19], rice [20-23] and wheat (24). Fifty genotype specific DNA markers were identified during screening of cultivated and wild rice genotypes [25].

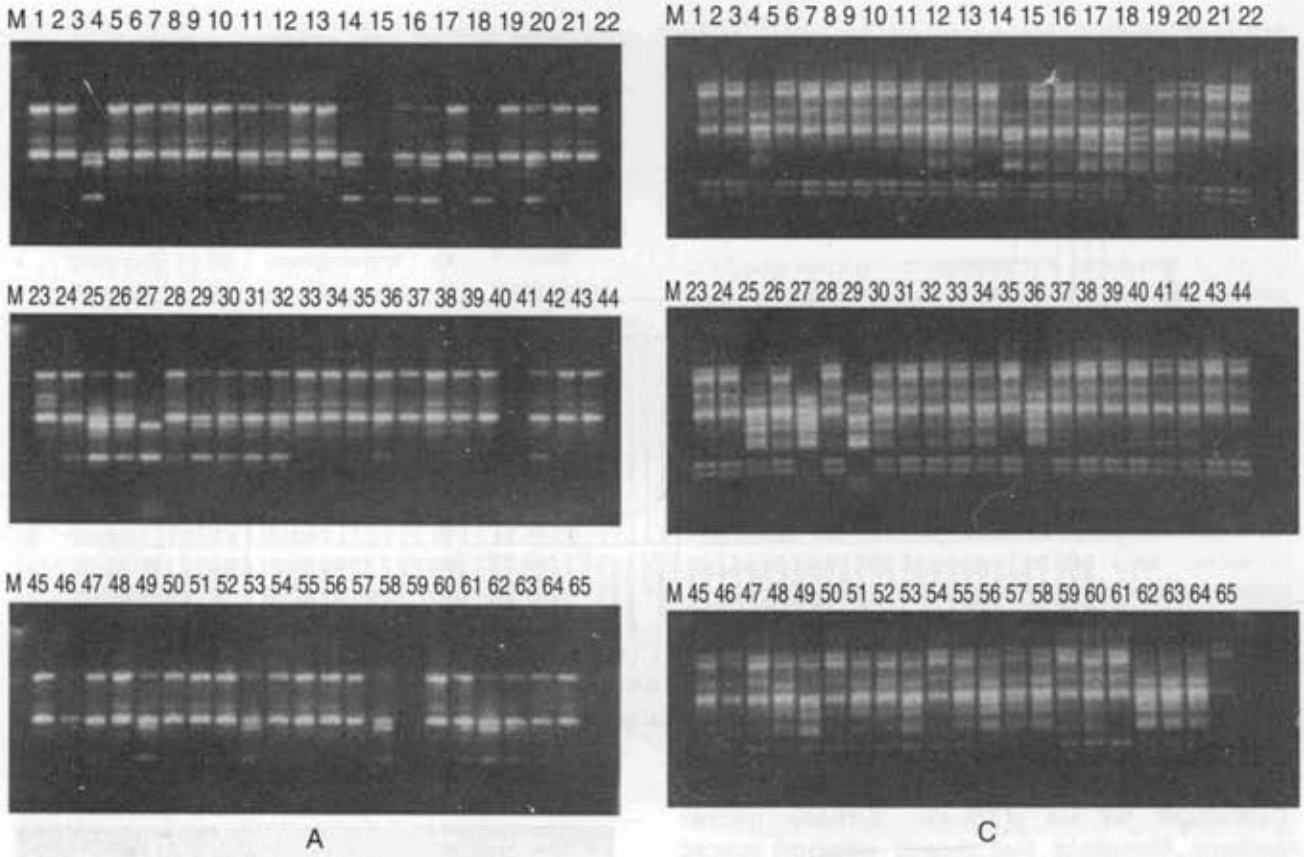

Fig. 3. Comparison of banding pattern obtained by primer OPN 7 (A), OPP 18 (B) and their combination (C) M is 1 Kb ladder. Order and genotype number is as indicated in Table 1

Polymorphism detected using conjugate RAPD primers: DNA banding pattern obtained by a single primer and a pair of conjugate primers was compared and conjugate primers yielded higher number of RAPD amplification compared to single primers (Fig. 3).

It was observed that the number of bands generated by a pair of primers, OPP 18 and OPN 07 was higher than when they were used singly (14 and 13 respectively). The presence of another random primer in the reaction increased the possibility of generating polymorphic fragments by dividing regions, which are flanked by original primer [26]. The advantage of random pairing of primer lies in increasing the variability of polymorphic fragments and also in reducing the cost of designing new primers.

Visible morphological characters are limited in number and significant interaction is present between genotype and environment. The classification based on phenotypic traits will be far more then the actual variability present. Genetic information at the DNA level could be more reliable to reflect the subtle genetic differences between accessions, as it is normally not subjected to environmental variations. Marker data also

supplements selection of genotypes based on phenotypes in a plant breeding program. Our results demonstrated that the RAPD could be one of the marker systems for cultivar identification and estimate genetic variability among the selected local rice accessions.

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