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RAPD Analysis of Aromatic and Non-aromatic Rice (*Oryza sativa* L.)

S Baishya, Archana Sachdev, R P Johari and S L Mehta
Division of Biochemistry, Indian Agricultural Research Institute, New Delhi 110 012, India

Genetic variation in nine aromatic and four nonaromatic rice varieties (*Oryza sativa* L.) was investigated at the DNA level using the randomly amplified polymorphic DNA (RAPD) technique. Twenty six random primers were used to amplify DNA segments and 177 PCR products were obtained of which 98 were polymorphic. One primer did not show polymorphism. A dendrogram showing the genetic distances of 13 rice varieties was constructed based on RAPD data.

Key words : *Oryza sativa*, aromatic rice, RAPD.

Rice (*Oryza sativa* L.) is the most important staple food of over 60% of the world population. A number of different varieties of rice are grown throughout the world, but aromatic rice is highly valued throughout Asia and also has wider acceptance in Europe (1) and United States (2). By virtue of special preference among consumers, aromatic varieties get better premium than nonaromatic varieties in market. Successful breeding for aromatic rice depends mainly on effective identification of strong aroma traits. Molecular markers are now used as a tool in breeding and selection by tagging genes and manipulating useful agronomic traits. Randomly amplified polymorphic DNA (RAPD) markers have been used successfully to detect genetic variation among low land and upland rice cultivars (3), the genetic characterization and classification of *japonica* cultivars into temperate and tropical groups (4) and for analysis of genetic variability in wild rice populations (5). Qingsheng *et al* (6) investigated the feasibility of identifying molecular markers linked to the aroma genes in rice using RAPD technology. Although attempts have been made to develop molecular markers on the basis of restriction fragment length polymorphism and RAPD, so far no systematic attempts have been made at molecular level to evaluate the aromatic and nonaromatic rice varieties which could help in breeding programme. The present study has, therefore, been undertaken to study the DNA polymorphism and to develop molecular markers based on RAPD analysis of aromatic and nonaromatic rice varieties.

Materials and Methods

Nine aromatic and four nonaromatic varieties of rice were used for the present study (Table 1). Based on the grain characteristics aromatic varieties are subdivided as non-basmati aromatic and basmati aromatic.

Isolation of genomic DNA — DNA was isolated from 7-day-old etiolated seedlings following the method of Dellaporta *et al* (7). The crude DNA was purified by successive RNase (10 mg ml^{-1}) and proteinase K (100 µg ml^{-1}) treatments followed by phenol-chloroform extraction. The DNA was ethanol precipitated and dissolved in TE buffer, pH 8.0 (10mM Tris, 1 mM EDTA).

Primers — Twenty five, 10 base random primers were obtained from Operon Technologies (Alameda, California). One specific primer, *jas* 1.5 was custom synthesised from Bangalore Genei. The sequences of these primers are shown in Table 2.

Polymerase chain reaction — DNA amplification reactions were performed in a volume of 20 µl containing 2 µl of 10 x *Taq* DNA polymerase buffer, 0.2 µl of 10 mM dATP, dCTP, dGTP and dTTP, 20 ng of a single random primer, 50 ng of genomic DNA and 0.5 unit of *Taq* DNA polymerase (3 U μl^{-1}). Amplifications were performed in a mini cycle PCR machine (MJ Research, USA) programmed as follows - 1 cycle of 91°C for 1 min followed by 45 cycles of 1 min at 94°C, 1 min at 35°C and 2 min at 72°C and finally the last cycle of extension at 72°C for 5 min. The amplification products (20 µl) then were loaded on 1.5% (w/v) agarose gels for electrophoresis in 1 x TBE buffer (8). Gels were stained with ethidium bromide and photographed under UV light using polaroid 667 film.

Data analysis — An input matrix was initially produced by entering the data regarding presence or absence of

Table 1. List of varieties used in the present study

Variety	Type	Source
TTB-196-B-29-1-23-1	Non-basmati aromatic	Assam Agricultural Univ., Jorhat
TTB-196-B-43-2-4-1	Non-basmati aromatic	Assam Agricultural Univ., Jorhat
TTB-196-B-29-1-22-2	Non-basmati aromatic	Assam Agricultural Univ., Jorhat
TTB-196-B-43-2-9-1	Non-basmati aromatic	Assam Agricultural Univ., Jorhat
TTB-196-B-29-1-2-1	Non-basmati aromatic	Assam Agricultural Univ., Jorhat
TTB-196-B-29-1-15-5	Non-basmati aromatic	Assam Agricultural Univ., Jorhat
Basmati-370	Basmati aromatic	IARI, New Delhi
Pusa Basmati-1	Basmati aromatic	IARI, New Delhi
Karnal Local	Basmati aromatic	IARI, New Delhi
Jaya	Nonaromatic	IARI, New Delhi
Pusa-834	Nonaromatic	IARI, New Delhi
Lachit	Nonaromatic	Assam Agricultural Univ., Jorhat
Chilarai	Nonaromatic	Assam Agricultural Univ., Jorhat

an amplified fragment for each variety - primer combination using the editor of the NTSYS program. The routine SIMQUAL was then used for the computation of similarity coefficients using these data and Jaccard's coefficient. The resulting output was used for SAHN clustering by UPGMA (unweighted pair-group method with arithmatical averages) method. The tree display was followed for generation of the dendrogram.

Cloning and sequencing of PCR product — The characteristic band that distinguished the nonaromatic varieties from the aromatic ones, and generated by the primer *jas* 1.5, was excised and cloned into the bluescript vector (SK^-) by TA cloning. *E. coli* strain DH5α was transformed and the recombinant, named as RPS-1, was checked by digesting it with *Eco*RI and *Hind* III. The fragment generated was again checked by amplification with *jas* 1.5. The insert at *Eco*RI and *Hind* III sites on RPS-1 was sequenced by an automated DNA sequencing system with the use of T7 promoter as forward sequencing primer and T3 promoter as the reverse sequencing primer. Complete nucleotide sequence was obtained by overlapping the two sequences.

Results and Discussion

One hundred and seventy seven amplified bands were detected among 13 varieties of rice using 26 RAPD primers. Among them, 98 bands were polymorphic. One primer (OPC-07) generated a monomorphic pattern, whereas polymorphic patterns appeared with the rest of the 25 primers. Of the 26 primers, GC content of 16 primers was 60% and for the rest it was 70%.

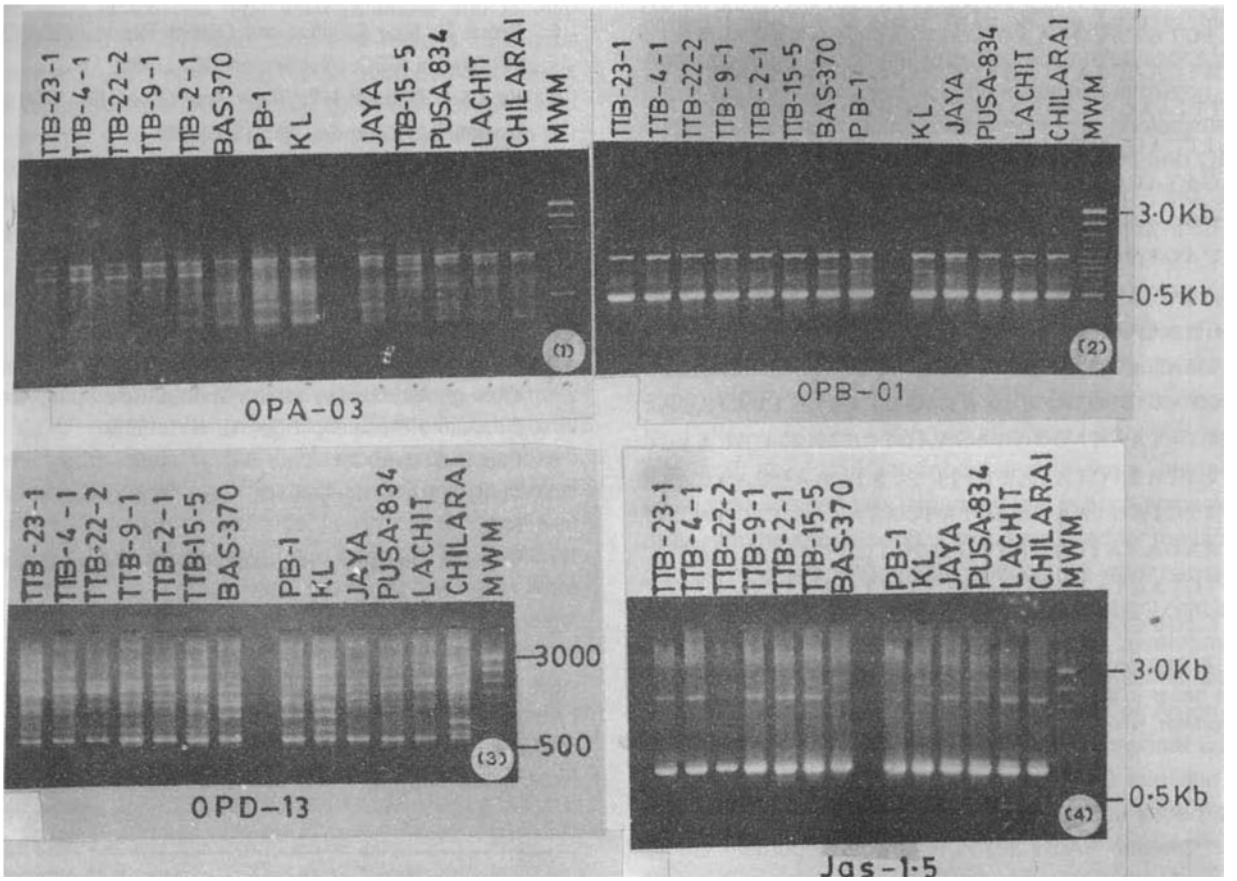
RAPD analysis was done to get fingerprints of the aromatic and non aromatic rice varieties at DNA level which could be used for identifying a particular variety and subsequent help in breeding efforts. Based on RAPD analysis molecular differentiation of the varieties could be done as follows : (a) the non-basmati aromatic varieties could be distinguished from the rest with the use of primer OPA-03 (Fig. 1), (b) characteristic bands for basmati aromatic varieties were observed with the primers OPB-01 and OPD-13 (Figs. 2 and 3), and (c) *jas* 1.5 could be used to differentiate nonaromatic varieties from aromatic ones (Fig. 4).

Considering the bands obtained using all the primers, more than 61% similarity was observed between varieties. However, among all combinations Bas-370 and Pusa-834 showed maximum dissimilarity (least similarity value, 0.611) and varieties TTB-23-1 and TTB-4-1 showed maximum similarity (0.906) between them (Table 2). Associations among the 13 varieties revealed by UPGMA cluster analysis are presented in Fig. 5. Three distinct clusters of non-basmati aromatic, basmati aromatic and nonaromatic varieties of rice were observed in the dendrogram constructed based on RAPD data. This is significant since the use of the RAPD technique for detecting genetic variation among cultivars and identifying germplasms is well established (9-11). The dendrogram shows that nonaromatic varieties are genetically closer to the non-basmati aromatic varieties as compared to aromatic basmati varieties.

Qingsheng *et al* (6) reported an amplification of a 1.5 kb fragment in the nonaromatic varieties using *jas*

Table 2. Similarity matrix for Jaccord's coefficient : range of values from 0 to 1.0, with values closer to 1.0 indicating increasing similarity

	1	2	3	4	5	6	7	8	9	10	11	12	13
	TTB-23-1	TTB-4-1	TTB-22-2	TTB-9-1	TTB-2-1	TTB-15-5	Bas-370	PB-1	KL	Jaya	Pusa-834	Lachit	Chilarai
1	1.0000												
2	0.9057	1.0000											
3	0.8625	0.8782	1.0000										
4	0.8412	0.8012	0.7719	1.0000									
5	0.8519	0.8910	0.8471	0.8155	1.0000								
6	0.8387	0.8543	0.8212	0.8125	0.8519	1.0000							
7	0.6776	0.6685	0.6141	0.7049	0.6519	0.6667	1.0000						
8	0.6927	0.7225	0.6936	0.6923	0.6954	0.6886	0.6778	1.0000					
9	0.6923	0.7022	0.6742	0.7198	0.6949	0.6765	0.8166	0.7414	1.0000				
10	0.7200	0.7310	0.7118	0.7191	0.7440	0.7329	0.6393	0.7412	0.6538	1.0000			
11	0.7321	0.7439	0.7453	0.6914	0.7362	0.7468	0.6111	0.7333	0.6724	0.7975	1.0000		
12	0.7337	0.6941	0.6946	0.7029	0.6964	0.7089	0.6404	0.6941	0.6554	0.7987	0.8158	1.0000	
13	0.7052	0.7365	0.7169	0.6760	0.7289	0.7389	0.5806	0.6959	0.6667	0.7778	0.7821	0.7722	1.0000



Figs. 1-4. RAPD patterns of rice varieties, non basmati aromatic (TTB-23-1, TTB-4-1, TTB-22-2, TTB-9-1, TTB-15-5), basmati aromatic (Basmati-370, Pusa Basmati (Jaya, Pusa - 834, Lachit, Chilaria) with different primers. (1) OPA-03, (2) OPB-01, (3) OPD-13, (4) *jas* 1.5.

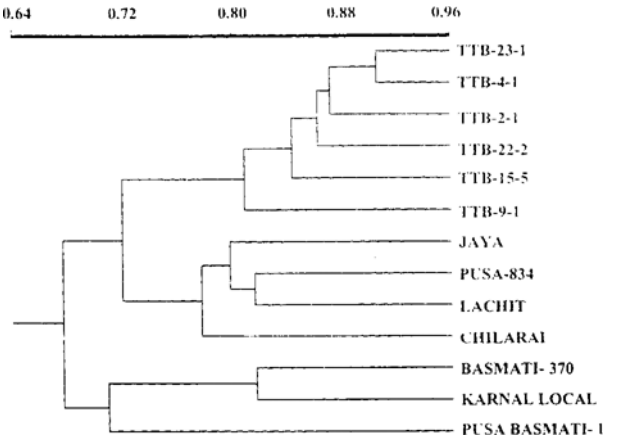


Fig. 5. Dendrogram of the rice varieties, constructed using UPGMA based on Jaccard's similarity coefficients.

*CGACGGTATCGATAAGCTTGAT*T**GAACGGACTC**
CAAGACATACTGTGTGTATGATAGGTAGGATCA
GATATTAATAGTATAGTAATTAACTATTATATT
AATTAGCTATTATATTGGTTATAGATGATTTGA
AGCTAGTGGTTAGCTATACTATTAGACTTGCTC
TAACACTAACATTCAGGATTTTATCATCCCGTGA
CATCTATACGTAAGGTGTTACGCGAAATAAGTT
CCGTCAACTATTAGACCCAAGTTGATGGCTTTTT
TTTTTAAGAAAAAAGATACCAACTTAAACTGGGA
CATTCAGAGCTCTGTAGGGAATCGTTCAGCCAT
CCGATACGTTGATCTCTGACGCTTGAAAAAAAA
GTGGAAAAAATGTACTCGAGTGGTAGTTAGCAA
ATCACTAAAATCACATTTCCAGTATCAGGAATG
AAAAGCTCAGAATGAAGATGGAAAAATTAGTGA
TTGAAAACTGTAATCACAGAATCACTCATGTGT
TCCTATAGAAGATTTCGAAAATTATTTTATTTC
TTCTATTTACATCCAATAGCTAGACTTATACAT
AATTATAAAACGGAAGAAATCCTATTGACAAGC
ACCTTATTCTATATTTTGCCATCAACGAACATT
ACTTCTAGTAAAAGCTATCATATAAGTCAACGT
CTAAGAAATGTGATTGTITGTCATCTTTCCATCC
ATTGTACTATGC**GAGTCCGTTC**A*ATCGAATTCC*
TGCAGCCCGGGGGATCCACTAGTTCTAGAGCGGC

Fig. 6. The complete nucleotide sequence of the RPS-1 insert (The bases in italics are from the vector, bases in bold are from the primer, while T and A are the overhangs in the vector).

1.5, an aroma locus linked primer. In the present study, the same primer resulted in the amplification of a 695 bp polymorphic fragment, in the nonaromatic varieties. The variation might be due to the difference in the genotypes used. The amplified band of 695 bp was cloned and sequenced. The complete nucleotide sequence of the clone RPS-1 is shown in Fig. 6. The insert was found to be AT rich (66.4 %). The homology search carried out using BLAST program (12) with database GENEBANK-UPD revealed a varying degree of the homology with a large number of different rice clones. RPS-1 showed more than 80% homology in different stretches with *Oryza sativa* genomic DNA, chromosome 8, clone : P0026F07. Since the aroma gene is located on chromosome 8 (13) the clone RPS-1 could be related to the aroma locus (6).

The study indicated that RAPD technique could be used to detect genetic variation at the level of DNA among the aromatic and non-aromatic rice cultivars.

References

1 **Berner DK & Hoff BJ,** *Crop Sci,* **26**(1986) 876.
2 **Brook D,** *Rice Situation and Outlook Report* (USDA-ERS-RS-56) US GPO, Washington DC. (1989) 11.
3 **Yu LX & Nguyen HT,** *Theor Appl Genet,* **87** (1994) 668.
4 **Mackill DJ,** *Crop Sci,* **35** (1995) 889.
5 **Buso GSC, Rangel PH & Ferreira ME,** *Mol Ecol,* **7** (1998) 107.
6 **Qingsheng J, Boqin Q, Wencho Y & Rubi L,** *Acta Agriculturae Zhejiangensis,* **7** (1995) 439.
7 **Dellaporta SL, Wood J & Hichs JB,** *Plant Mol Biol Rep,* **1** (1983) 19.
8 **Sambrook J, Fritsch EF & Maniatis T,** h *Molecular Cloning*: *A Laboratory Manual* 2nd Ed, Cold Spring Harbour Laboratory Press, Cold Spring, NY (1989).
9 **Cao D & Card JH,** *Crop Sci,* **37** (1997) 1630.
10 **Vierling RA & Nguyen HT,** *Theor Appl Genet,* **84** (1992) 835.
11 **Virk PS, Newburry HJ, Jackson MT & Ford-Lloyd BV,** *Theor Appl Genet,* **90** (1995) 1049.
12 **Altschul, Stephen F, Gish W, Miller W, Myers EW & Lipman DJ,** *J Mol Biol,* **215**(1990) 403.
13 **Ahn SN, Bollich CN & Tanksley SD,** *Theor Appl Genet,* **84** (1992) 825.