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RESEARCH PAPER

Analysis of genetic variability in scented rice varieties using RAPD marker

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Abstract : Molecular marker technology is the genetic tool for assessing genetic diversity and relationships among scented rice and other varieties of rice. In the present study, a random amplified polymorphic DNA technique was used for genetic diversity analysis among 45 genotypes of scented rice with 9 primers. A total of 57 bands were amplified by 9 pre screened polymorphic decamer primers at an average rate of 6.33 bands/primer and 83.17 were polymorphic. The data of 45 genotypes of scented rice were used to generate pair-wise matrix based on Jaccard's co-efficient. The similarity co-efficient ranged from 0.423 in between Sugandha-2 and 3231 to maximum of 0.932 in between IR106 and IR-253U. It indicated the closeness of these genotypes. The UPGMA cluster analysis showed similarity between most of the cultivars. The information of the genetic diversity will be useful for the selection of the parents for breeding rice variety.

Key Words : Scented rice, RAPD, Genetic diversity, Genotypes

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INTRODUCTION

Rice is an economically important crop and due to its good nutritive value and storage quality it is a staple food for more than one-third of the world's population (Anonymous, 2008). Rice is grown in almost all the states of India but its cultivation is mostly concentrated in river valley, deltas and low lying coastal areas of North-Eastern and Southern India (Richharia and Govindswami, 1990). The increase in supply must mainly be met by increasing crop yields through better crop, nutrient, pest and water management and the use of germplasm with a higher yield potential. Aromatic rice is one of the best variety of other rice varieties, a large number of such varieties were collectively known as Basmati (*bas* = aroma). They are highly demanded in global market due to their pleasant aroma, superfine long-slender grains with delicate curvature, remarkable linear elongation, excellent flaky soft texture on cooking (Bhasin, 2000.) On the other hand, the small and medium grain aromatic rices are being regarded as a separate class *i.e.* non-Basmati type aromatic rice (Singh *et al*., 2000).

Genetic diversity studies on rice using traditional morphological and biochemical markers are common and routinely used (Kato *et al.*, 1928; Glaszmann, 1987). Supplementing to above parameters, PCR based molecular markers are considered more suitable for analysis of genetic diversity and varietal identification since there is little effect of stage of development, environment and management practices.

A wide range of research work has been done on rice applying molecular markers *viz*., Restriction Fragment Length Polymorphism (Bostein *et al.*,1980), Random Amplified Polymorphic DNA (Ravi *et al.*, 2003; Baishya *et al.*, 2000), Simple Sequence Repeats (Bligh *et al*., 1999; Jain *et al.*, 2004), Amplified Fragment Length Polymorphism (Cho *et al.*, 1996; Saini *et al.*, 2004) and Inter Simple Sequence Repeats (Blair *et al.*,1999; Nagaraju *et al.*, 2002). All these molecular techniques have the common objective of assessing the relative diversity

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within and among the species and also to select the diverse accessions for breeding purposes.

The various molecular markers used for the plant genome analysis have their own limitations, necessitating careful evaluation of diversity analysis. Since genetic differentiations are often correlated with geographical isolation, it may be appropriate to analyze the germplasm that represents a wide range of geographical region in order to estimate the genetic diversity with in the breeding stock. Among the various molecular marker techniques available, RAPD analysis is considered to be technically simple and rapid DNA fingerprinting method. In the present study, RAPD was used to evaluate the genetic diversity and to develop DNA profile of indigenous basmati type rice germplasm representing diverse geographical locations of India.

MATERIALS AND METHODS

Plant material :

A total of 45 indigenous scented rice genotypes representing a wide range of geographical location of India were collected from Department of Plant Pathology, G.B. Pant University of Agriculture and Technology, Pantnagar. The details of cultivars used in the present study are given in Table A.

Table A : List of of rice genotypes used in the present study

Sr. No.	Name of genotypes	Sr. No.	Name of genotypes
1.	Jhumri-2	24.	Sugandha-2
2.	Tulsi-2	25.	PB-1
3.	Bindli-3298	26.	IR-162
4.	Bindli3281	27.	IR-253-U
5.	3068	28.	Bishnubhog
6.	3318	29.	Kalkatta Badshah
7.	Bhagalpur	30.	Sonasal
8.	Jawaful	31.	Swarna2
9.	3025	32.	HMT sona
10.	Jhumri-3	33.	DB-3020
11.	DP-17	34.	Chullai-1
12.	Tulsi-1	35.	Bas-370
13.	DP-20	36.	DP-14
14.	Jhumri-4	37.	3057
15.	Bishnubhog	38.	Madhuri-2
16.	3251	39.	3042
17.	3103	40.	IR106
18.	Mohan	41.	DP26
19.	3008	42.	IR102
20.	Sonam-1	43.	3084
21.	DP-12	44.	Tapovan5010-1
22.	DP-27	45.	3324
23.	DP-16		

DNA isolation :

Genomic DNA from each rice cultivar was isolated from etiolated leaf samples following (N-Cetyl) N, N, N- Trimethyl Ammonium Bromide (CTAB) method (Doyle and Doyle, 1990). The DNA was spooled out, washed twice with 70 per cent ethanol and dissolved in T.E. (10 mM Tris, 0.1 mM EDTA, pH 8.0) buffer containing 25ug/ml RNase-A, incubated at 37°C for 30 min and extracted with chloroform: isoamyl alcohol (24: 1 v/v). DNA was re-precipitated and dissolved in T.E. buffer. The quality and quantity of DNA was checked by 1per cent agarose gel electrophoresis using standard containing 100 ng/ul genomic DNA. The isolated DNA samples for PCR were diluted in T.E. (9:1) to a working concentration of approximately 50 ng/ul and stored at 4°C until PCR amplification.

Primers :

Nine pre-screened polymorphic decameric random primers (Table B) synthesized from Bangalore Genei Pvt. Ltd., India, were used for the present investigation.

Table B : List of RAPD primers and their sequences

Sr. No.	Primer code	Primer sequence (5' – 3')	GC content (%)
1.	LC-78	GTGATCGCAG	60
2.	LC-90	GTGAGGCGTC	70
3.	LC-93	GGACCCAACC	60
4.	LC-95	TGAGCGGACA	60
5.	LC-97	GTGTGCCCCA	70
6.	LC-101	GGGGTGACGA	70
7.	LC-102	CATCCGTGCT	60
8.	LC-106	GTGACATGCC	60
9.	LC-109	ACGCACAACC	60

DNA amplification and gel electrophoresis :

The amplification of target DNA was done according to the method of Williams *et al.* (1990). Amplification was achieved in eppendorf thermal cycler using 20µl reaction mixture containing 1X reaction buffer (10 mM Tris HCl, pH 8.3 and 50 mM KCl), 3.0 mM $MgCl_2$,1.5U of Taq DNA Polymerase, 10 mM each of dATP dTTP dGTP and dCTP, 30 ng of 10 nucleotide primer and approximately 50 ng of genomic DNA template. The PCR amplification condition was as follows: an initial denaturation at 94 °C for 5 min and 35 cycles at 1 min denaturation at 94 °C, 1 min annealing at 40°C and 2min polymerization at 72°C and 5 min final extension at 72 °C. PCR product were mixed with 2.5 ul of gel loading dye and electrophoresed on 1.4 per cent agarose gel in 0.5 X TBE buffer at 80 volts. The gels were stained in ethidium bromide (0.5ng/ml) and the gel image was recorded using the UV transilluminator and stored in gel documentation system (BIORAD).

Data analysis :

RAPD banding patterns were scored as present (1) or absent (0) for each primer-genotype combination. Jaccard's similarity co-efficient values for each pairwise comparison between cultivars were calculated and a similarity co-efficient matrix was constructed. This matrix was subjected to Unweighted Pair Group Method for Arithmetic Average analysis (UPGMA) to generate a dendogram. The similarity co-efficient analysis and the dendogram construction were carried out by using NTSYS-pc version 2.1 Software (Rohlf, 2000).

RESULTS AND DISCUSSION

The results of the present study as well as relevant discussions have been presented under following sub heads:

RAPD analysis :

The RAPD pattern of genomic DNA of 45 rice cultivars were analysed with respect to the distribution of the fragments, in formativeness of the markers and polymorphism for the assessment of genetic diversity present among the genotypes. All the tested primers were found to produce polymorphic amplification products. The extent of polymorphism with a primer varied between 40 per cent (primer LC-90) to 100 per cent (primer LC-93, LC-101, LC-102, LC-106 and LC-109).The reason for existence of higher percentage of polymorphism may be because of variation in the geographical distribution and seed morphology in the cultivars included in the study. A total of 57 bands were amplified using 9 decameric primers with in average of 6.33 per cent per primer. Of these, 47 bands were polymorphic showing 5.22 per cent polymorphism. The number of bands amplified fragments ranged from 150 bp (primer LC-101) to 1700 bp (primer LC-78) and an average size ranged from 280-1400 bp. A representative DNA profile for all the 45 cultivars using primer LC-102 is shown in Fig. 1. Thus all the cultivars could be distinguished from one another by the use of one or more primers. Primer LC102 produced cultivar specific band in the varieties DP-14 so this primer can be used for identification of specific cultivar (Table 1).

Genetic relationship among cultivars :

The pair-wise Jaccard's co-efficients for the genetic similarities among the 45 cultivars are presented in Table 2. The similarity co-efficient ranged from 0.42 (between Sugandha-2 and 3231) to 0.93 (between IR-106 and IR-253-U,) indicated the closeness of these genotypes. Nearly similar

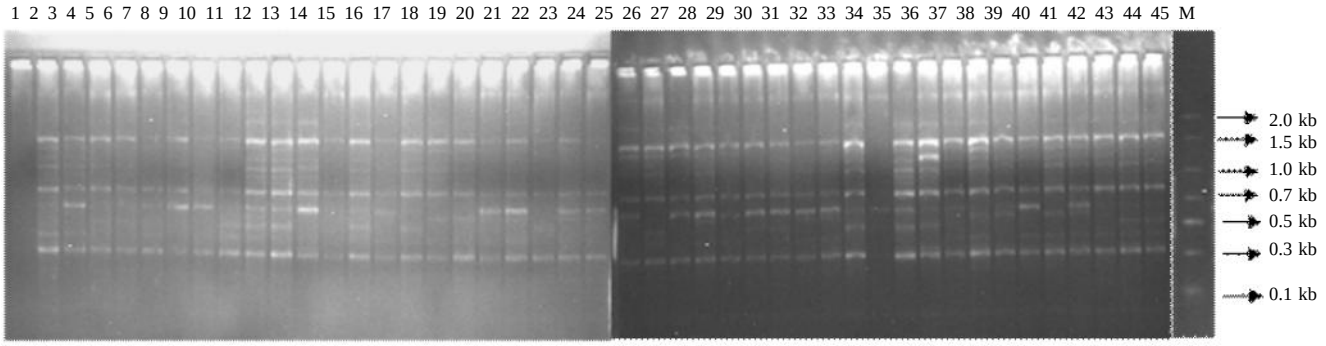

Fig.1: Amplification of 45 rice genotypes using primer LC-102 as per the genotype in sequence code in lane 1-45.

Table 1 : Details of random primers and their sequences

Sr. No.	Primer code	Primer sequence (5' to 3')	Amplified product range(bp)	Total Bands	Mono. Bands	Poly. Bands	% Poly.	Unique Band (bp)
1.	LC-78	GTGATCGCAG	400-1700	7	1	6	85.71	
2.	LC-90	GTGAGGCGTC	350-1200	5	3	2	40	
3.	LC-93	GGACCCAACC	200-1400	5	-	5	100	
4.	LC-95	TGAGCGGACA	200-1500	10	2	8	80	
5.	LC-97	GTGTGCCCCA	270-1500	7	4	3	42.85	
6.	LC-101	GGGGTGACGA	150-1400	6	-	6	100	
7.	LC-102	CATCCGTGCT	200-1500	7	-	7	100	DP-14 (1200 bp)
8.	LC-106	GTGACATGCC	350-1500	6	-	6	100	
9.	LC-109	ACGCACAACC	400-1000	4	-	4	100	
Total			-	57	10	47	-	
Average			280-1400	6.33	1.11	5.22	83.17	

Table 2 : Pairwise similarity matrix based on Jaccard's co-efficient for RAPD profile

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
Jhumri2	1.000																																													
Tulsi-2	0.660	1.000																																												
Bindli3298	0.647	0.804	1.000																																											
Bindli3281	0.680	0.844	0.867	1.000																																									
3068	0.647	0.694	0.647	0.680	1.000																																								
3318	0.702	0.612	0.667	0.633	0.667	1.000																																							
Bhagalpur	0.776	0.686	0.673	0.642	0.706	0.765	1.000																																						
Jawaful	0.660	0.673	0.725	0.660	0.692	0.647	0.717	1.000																																					
3025	0.679	0.660	0.745	0.712	0.712	0.735	0.736	0.755	1.000																																				
Jhumri3	0.848	0.647	0.635	0.635	0.635	0.653	0.692	0.679	0.731	1.000																																			
DP17	0.725	0.642	0.692	0.692	0.725	0.714	0.717	0.736	0.755	0.712	1.000																																		
Tulsi1	0.686	0.886	0.714	0.750	0.647	0.769	0.706	0.686	0.648	0.667	0.686	1.000																																	
DP20	0.556	0.660	0.714	0.647	0.647	0.702	0.673	0.725	0.816	0.635	0.725	0.647	1.000																																
Jhumri4	0.884	0.633	0.620	0.588	0.588	0.711	0.714	0.635	0.654	0.864	0.667	0.653	0.588	1.000																															
Bishnubhog	0.560	0.571	0.592	0.560	0.500	0.609	0.588	0.577	0.627	0.549	0.547	0.500	0.560	0.596	1.000																														
3231	0.612	0.529	0.612	0.580	0.491	0.667	0.673	0.596	0.647	0.600	0.627	0.549	0.580	0.617	0.587	1.000																													
3103	0.648	0.725	0.745	0.712	0.679	0.700	0.704	0.722	0.808	0.698	0.632	0.712	0.712	0.686	0.660	0.647	1.000																												
Mohan	0.667	0.712	0.731	0.698	0.667	0.686	0.722	0.774	0.759	0.717	0.679	0.698	0.698	0.673	0.647	0.667	0.827	1.000																											
3008	0.712	0.692	0.679	0.679	0.712	0.735	0.804	0.788	0.774	0.698	0.722	0.679	0.679	0.654	0.660	0.585	0.741	0.827	1.000																										
Sonam1	0.745	0.692	0.712	0.712	0.648	0.667	0.769	0.788	0.774	0.698	0.691	0.679	0.712	0.686	0.660	0.647	0.741	0.759	0.774	1.000																									
DP12	0.640	0.653	0.708	0.673	0.673	0.773	0.667	0.755	0.813	0.694	0.755	0.640	0.822	0.681	0.520	0.604	0.673	0.660	0.673	0.673	1.000																								
DP27	0.706	0.720	0.740	0.706	0.706	0.694	0.765	0.784	0.769	0.725	0.820	0.706	0.776	0.680	0.588	0.640	0.701	0.691	0.736	0.736	0.800	1.000																							
DP16	0.620	0.600	0.688	0.620	0.588	0.674	0.647	0.735	0.720	0.640	0.771	0.588	0.723	0.592	0.500	0.583	0.593	0.582	0.654	0.623	0.795	0.826	1.000																						
Sugandha2	0.519	0.560	0.549	0.491	0.646	0.500	0.577	0.596	0.585	0.538	0.627	0.519	0.612	0.551	0.490	0.423	0.555	0.545	0.585	0.585	0.540	0.608	0.583	1.000																					
PB1	0.623	0.667	0.686	0.654	0.654	0.577	0.679	0.667	0.750	0.611	0.636	0.686	0.686	0.627	0.600	0.558	0.784	0.673	0.685	0.717	0.647	0.679	0.596	0.688	1.000																				
IR102	0.679	0.660	0.679	0.648	0.679	0.700	0.736	0.661	0.843	0.705	0.755	0.648	0.745	0.680	0.596	0.585	0.741	0.696	0.741	0.679	0.740	0.769	0.720	0.680	0.717	1.000																			
IR25311	0.654	0.604	0.593	0.564	0.564	0.640	0.745	0.636	0.655	0.673	0.607	0.623	0.593	0.660	0.633	0.688	0.784	0.736	0.685	0.717	0.527	0.648	0.566	0.558	0.660	0.717	1.000																		
Bishnubhog1	0.653	0.600	0.558	0.558	0.588	0.638	0.680	0.574	0.623	0.673	0.635	0.558	0.588	0.696	0.630	0.689	0.723	0.642	0.654	0.623	0.549	0.647	0.560	0.583	0.660	0.686	0.766	1.000															
Kalkattabadshah	0.600	0.519	0.569	0.538	0.538	0.583	0.627	0.615	0.545	0.588	0.615	0.481	0.569	0.604	0.510	0.630	0.635	0.623	0.574	0.635	0.529	0.627	0.571	0.563	0.577	0.604	0.708	0.750	1.000														
Sonasal	0.667	0.618	0.667	0.667	0.636	0.593	0.691	0.709	0.727	0.685	0.709	0.607	0.667	0.611	0.556	0.635	0.759	0.745	0.727	0.792	0.600	0.691	0.611	0.574	0.673	0.727	0.804	0.573	0.720	1.000													
Swarna2	0.760	0.776	0.692	0.692	0.725	0.714	0.820	0.704	0.722	0.780	0.704	0.760	0.725	0.771	0.608	0.566	0.722	0.741	0.788	0.788	0.686	0.750	0.635	0.694	0.731	0.824	0.731	0.700	0.615	0.709	1.000												
HMTSona	0.680	0.729	0.647	0.714	0.714	0.569	0.642	0.692	0.679	0.735	0.660	0.680	0.680	0.653	0.529	0.519	0.712	0.698	0.712	0.712	0.640	0.673	0.558	0.580	0.686	0.679	0.623	0.723	0.633	0.731	0.760	1.000											
DB3020	0.700	0.714	0.700	0.700	0.667	0.688	0.725	0.648	0.667	0.686	0.712	0.700	0.667	0.640	0.519	0.633	0.698	0.655	0.667	0.698	0.660	0.760	0.640	0.600	0.673	0.765	0.673	0.573	0.620	0.685	0.780	0.667	1.000										
Chullai1	0.612	0.560	0.580	0.580	0.519	0.596	0.608	0.596	0.615	0.569	0.627	0.549	0.580	0.551	0.490	0.542	0.585	0.518	0.615	0.647	0.604	0.640	0.652	0.510	0.620	0.680	0.653	0.583	0.596	0.667	0.596	0.612	0.667	1.000									
Bas370	0.661	0.643	0.691	0.691	0.632	0.589	0.684	0.702	0.719	0.679	0.732	0.661	0.691	0.607	0.554	0.571	0.753	0.737	0.719	0.750	0.625	0.714	0.607	0.630	0.727	0.782	0.727	0.536	0.648	0.800	0.732	0.691	0.774	0.692	1.000								
DP14	0.611	0.623	0.673	0.673	0.776	0.627	0.667	0.685	0.704	0.600	0.820	0.611	0.740	0.556	0.473	0.608	0.643	0.691	0.673	0.673	0.667	0.731	0.647	0.673	0.679	0.673	0.618	0.647	0.694	0.755	0.685	0.673	0.692	0.577	0.745	1.000							
3057	0.667	0.750	0.700	0.735	0.735	0.688	0.725	0.648	0.667	0.654	0.745	0.700	0.700	0.608	0.491	0.569	0.698	0.685	0.698	0.667	0.660	0.796	0.640	0.600	0.673	0.731	0.673	0.573	0.688	0.750	0.780	0.771	0.792	0.633	0.709	0.760	1.000						
Madhuri2	0.667	0.787	0.735	0.771	0.700	0.620	0.692	0.679	0.698	0.654	0.648	0.735	0.700	0.608	0.519	0.600	0.765	0.717	0.698	0.667	0.660	0.725	0.640	0.538	0.706	0.698	0.673	0.708	0.653	0.750	0.745	0.848	0.755	0.667	0.709	0.725	0.830	1.000					
3042	0.698	0.712	0.698	0.731	0.667	0.593	0.691	0.709	0.727	0.717	0.709	0.667	0.667	0.642	0.556	0.604	0.759	0.745	0.727	0.696	0.630	0.722	0.611	0.635	0.704	0.759	0.736	0.740	0.654	0.811	0.741	0.837	0.750	0.667	0.833	0.722	0.784	0.857	1.000				
IR106	0.694	0.577	0.566	0.537	0.509	0.580	0.686	0.611	0.600	0.680	0.582	0.627	0.537	0.702	0.604	0.660	0.725	0.679	0.630	0.692	0.500	0.623	0.538	0.529	0.635	0.660	0.932	0.702	0.681	0.780	0.706	0.596	0.647	0.625	0.673	0.564	0.647	0.647	0.712	1.000			
DP26	0.604	0.647	0.667	0.604	0.700	0.620	0.725	0.679	0.698	0.623	0.780	0.667	0.735	0.577	0.491	0.600	0.635	0.655	0.667	0.607	0.660	0.760	0.708	0.667	0.706	0.731	0.673	0.573	0.653	0.685	0.712	0.635	0.686	0.600	0.679	0.833	0.755	0.720	0.685	0.615	1.000		
IR102	0.740	0.654	0.673	0.642	0.642	0.660	0.698	0.655	0.769	0.760	0.750	0.673	0.673	0.750	0.588	0.577	0.704	0.661	0.704	0.673	0.700	0.731	0.680	0.673	0.712	0.917	0.679	0.547	0.596	0.722	0.820	0.673	0.760	0.673	0.745	0.636	0.725	0.692	0.755	0.720	0.660	1.000	
3084	0.635	0.556	0.635	0.604	0.574	0.528	0.630	0.679	0.636	0.654	0.648	0.574	0.604	0.577	0.436	0.600	0.635	0.685	0.636	0.667	0.596	0.692	0.640	0.538	0.642	0.636	0.673	0.577	0.688	0.784	0.648	0.635	0.654	0.600	0.741	0.692	0.720	0.686	0.717	0.680	0.654	0.660	1.000
Tapovan5010-1	0.588	0.538	0.558	0.558	0.688	0.571	0.647	0.574	0.654	0.577	0.735	0.528	0.620	0.529	0.442	0.520	0.564	0.611	0.623	0.593	0.580	0.680	0.592	0.617	0.627	0.654	0.566	0.592	0.638	0.706	0.635	0.588	0.608	0.551	0.636	0.826	0.745	0.640	0.642	0.538	0.708	0.647	0.708	1.000
3324	0.686	0.700	0.720	0.686	0.654	0.640	0.712	0.636	0.685	0.706	0.698	0.686	0.686	0.660	0.481	0.588	0.717	0.704	0.685	0.655	0.647	0.780	0.660	0.588	0.692	0.784	0.692	0.660	0.673	0.769	0.765	0.686	0.8 3	0.688	0.792	0.745	0.851	0.813	0.769	0.667	0.740	0.780	0.776	0.766	1.000

level of polymorphism and genetic similarity was observed by earlier workers on RAPD profile of scented rice (Singh *et al.*, 2000; Kar, 2003).

Cluster analysis :

The cluster analysis band on the similarity co-efficient using (UPGMA) clearly distinguished all the 45 accession into 2 groups (Fig. 2). The first cluster which includes Bishnubhog was separated from the remaining accessions of second cluster with approximately 56 per cent similarity. Second cluster further subdivided into subclasses containing 43 groups in which one sub cluster containing single genotype Sugandha-2 was separated from the remaining genotypes with approximately 58 per cent at similarity.

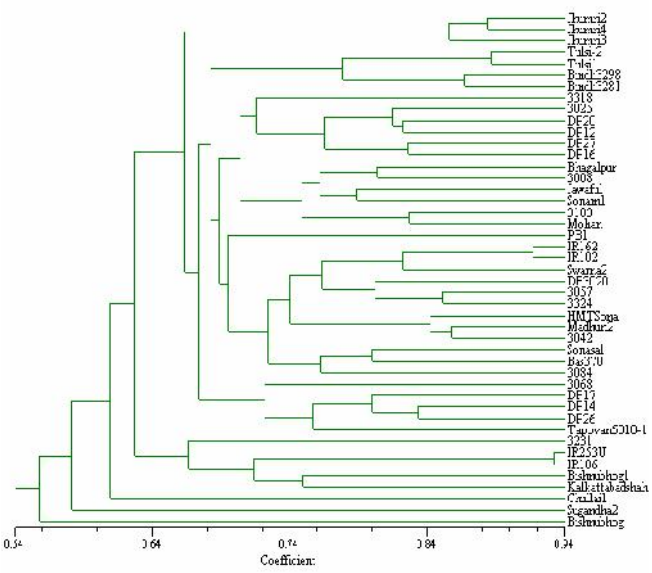

Fig. 2: UPGMA dendrogram showing clustering of 45 genotypes of scented rice based on 9 RAPD primers. The abbreviated names represent individual genotype and correspond to the ones listed in Table 1.

The varieties IR-106 and IR 253U are grouped together with maximum genotype similarity (0.93) followed by IR106 and IR162 with similarity co-efficient (0.91). There were no duplicate accessions found in the present study.

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