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Full Length Research Paper

Molecular characterization of traditional and improved rice cultivars based on random amplified polymorphic DNAs (RAPDs) markers

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Information of genetic similarities and diversity among superior rice cultivars is needed for potential rice breeding programs and origin of plant lines. Genetic polymorphism and relationships among 35 commercial varieties and primitive cultivars of Rice (*Oryza sativa* L.) were established by using random amplified polymorphic DNAs (RAPDs) primers. Polymerase chain reaction (PCR) with 14 primers produced a total of 126 different marker bands, of which 82.5% were polymorphic. The size range of the amplified DNAs was mostly between 0.2 and 3.63 kbp. Cluster analysis using an un-weighted pair-group method with arithmetic averages (UPGMA) was used to group the cultivars. Cluster analysis placed most of the aromatic cultivars close to each other showing a high level of genetic relatedness. Lower levels of polymorphism in aromatic cultivars of rice indicated that there is a basic similarity among basmati cultivars used in this study, which is to be expected due to their same ancestors and selection for similar characteristics. The RAPD analysis offered a simple and quick method for the assessment of variability between different cultivars.

Key words: Random amplified polymorphic DNAs (RAPDs), un-weighted pair-group method with arithmetic averages (UPGMA), rice (*Oryza sativa* L.).

INTRODUCTION

Agronomically and nutritionally, rice (*Oryza sativa* L.) is one of the important cereal crops and the principal staple food in developing countries. There is extremely rich diversity in cultivated rice; however, a series of biotic and abiotic stresses continue to bound its productivity. Thus, there is an imperative need to identify diverse sources of genes that can be exploited both to make wider the existing narrow genetic base and enrich the existing varieties with preferred agronomically important traits. In Pakistan, besides its significance as a food crop, rice is the second important component of daily diet of bulk of the population after wheat. About 23% of the total foreign exchange earnings is shared by rice and thus called as “Golden Grain of Pakistan” (Shah et al., 1999).

Molecular markers have proven to be commanding tools in the evaluation of genetic variation and in the elucidation of genetic relationships within and among species. These markers allow selection for these traits to be done on the basis of a quick and easy laboratory test on a small amount plant tissue, rather than direct measurement of the character itself. The use of molecular markers as the latest tool for rice improvement will progress the efficiency of capturing desirable characters in new rice varieties. Several molecular markers, including; restriction fragment length polymorphism (RFLP) (Becker et al., 1995; Paran and Michelmore, 1993), random amplified polymorphic DNA (RAPD), (Tingey and Deltufo, 1993; Williams et al., 1990), simple sequence repeats (SSR) (Levinson and Gutman, 1987), inter simple sequence repeats (ISSRs) (Albani and Wilkinson, 1998; Blair et al., 1999), amplified fragment length polymorphism (AFLP) (Mackill et al., 1996; Thomas et al., 1995; Vos et al., 1995; Zhu et al., 1998) and single nucleotide polymorphisms (SNPs) (Vieux et al., 2002) are currently available to evaluate the variability and diversity at molecular level (Joshi et al., 2000). Information about genetic variability at molecular level could be used to help, discover and develop genetically

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Table 1. List of traditional varieties and improved cultivars of rice used in the present study.

Cultivar name	Type	Breeding Institute	Cultivar Name	Type	Breeding Institute
Basmat-370(P)	Aromatic	RRI,KSK, Lahore	Mehak	Aromatic	NIA,Tandojam, Sindh
Mushkan	Aromatic	RRI,KSK, Lahore	Azucena	Japonica	Philippines
Basmati-Pak	Aromatic	RRI,KSK, Lahore	Kasalath	Coarse	India
IR8	Coarse	RRI,KSK, Lahore	Basmati-370(I)	Aromatic	India
Basmati-198	Aromatic	RRI,KSK, Lahore	Dheradun-Basmati1	Aromatic	Nepal
Super-Basmati	Aromatic	RRI,KSK, Lahore	KDML105	Aromatic	Thailand
JP-5	Coarse	ARI, Mingora, Swat	Basmati-217	Aromatic	India
Mahlar-346	Coarse	RRI,KSK, Lahore	Dehradun-Basmati2	Aromatic	India
Purple Marker	Coarse	RRI, Dokri, Sindh	Punjab-Basmati-1	Aromatic	India
Dokri-Basmati	Aromatic	RRI, Dokri, Sindh	Pusa-Basmati-1	Aromatic	India
IR36	Coarse	IRRI, Phillipines	Chini-Sakkor	Aromatic	Bangladesh
Kinmaze	Japonica	Japan	Ranbir-Basmati	Aromatic	India
Kangni/Torh	Coarse	RRI, Dokri, Sindh	Jasmine-Scented	Aromatic	Thailand
Sonahri-Kangni	Coarse	RRI, Dokri, Sindh	Niaw-Hawn-Mali	Aromatic	Thailand
Kharai-Ganga	Coarse	RRI, Dokri, Sindh	Khao-Jao-Hawm	Aromatic	Thailand
KSK-133	Aromatic	RRI,KSK, Lahore	PK-386	Coarse	Pakistan
Shahkar	Coarse	RRI, Dokri, Sindh	TN1	Coarse	Taiwan
Shandar	Coarse	NIA,Tandojam, Sindh			

unique germplasm that compliments existing cultivars. Random amplified polymorphic DNAs (RAPDs) has been proposed as one that overcomes many limitation of RFLP analysis (William et al., 1990). Zheng et al. (1991) indicated the usefulness of RAPDs for classification of rice germplasm. Fukuoka et al. (1992) reported that RAPDs were superior to RFLPs for their technological ease. Moreover RAPD markers produce DNA fingerprints with a single synthetic nucleotide primer (Williams et al., 1990) which could identify polymorphism based on comparison throughout the genome. It does not require any prior knowledge of DNA sequence but still revealed a high level of polymorphism (Karp et al., 1997). RAPD has been proven useful in genotype identification and gene mapping (Rabbani et al., 2008; Pervaiz et al., 2010). RAPD markers have also been used to identify and tag the important genes for Basmati quality traits like aroma (Jin et al., 1995; Tragoonrung et al., 1996), cooked kernel elongation, amylase content and length/breadth ratio (Ram et al., 1998).

In the present study, RAPD markers were used for DNA profiling of traditional varieties and improved cultivars of rice and to detect genetic polymorphism within these cultivars. This study will provide information about diverse parents and also proficient strategies for the sustainable management of the genetic resources of rice crops.

MATERIAL AND METHODS

Plant material

35 commercial varieties and obsolete cultivars (Table 1) of rice from germplasm unit of IABGR, NARC were used in the present study.

Genomic DNA extraction and primer selection

Total genomic DNA was also extracted from dried seeds according to the method described by Kang et al. (1998). After isolation of DNA, its quantity was determined spectrophotometrically at a wavelength of 260 and 280 nm using NanoDrop ND-1000 Spectrophotometer. The ratio between absorbance at 260 and 280 nm (260/280) was used to estimate DNA purity. DNA of each cultivar was diluted to a working concentration of 20 ng/µl for PCR/RAPD analysis.

Initially, three cultivars, one each from aromatic, non-aromatic and japonica type was used to optimize the RAPD protocols and select the suitable primers which exhibit polymorphisms between the three cultivars. Overall, 40 arbitrary decamer oligonucleotides, belonging to kit OPA and OPB from Operon Technologies Inc. (Alameda, California, USA), were tested as single primers to identify the most promising ones for detecting polymorphism. After an initial screen, 14 primers were ultimately chosen for further use on the basis of their ability to detect the polymorphism and produce the reliable and easily scorable banding patterns in rice cultivars. Among them, three primers could not amplify the DNA from some of the cultivars used. Therefore, finally the data of 11 primers were used and compiled to look at the genetic diversity and relationship among 35 commercial varieties and primitive cultivars of Pakistani rice.

PCR-amplification and gel electrophoresis

A modified RAPD method based on Williams et al. (1990) was used with a model 9700 thermal cycler (Applied Biosystems, USA). The PCR reaction mix includes the following: 20 µl reaction mixture containing 1 × PCR buffer [10 mM Tris HCl (pH 8.3), 50 mM KCl], 1.5 mM $MgCl_2$, 200 µM each deoxynucleotide triphosphate (dNTPs), 0.4 µM of 10-mer primer (Operon Technologies Inc., Alameda, CA), 1 unit AmpliTaq Gold DNA polymerase and 20 ng of

Table 2. List of 14 random primers used for generating RAPDs in traditional and improved cultivars of rice.

S/N	Primer name	Nucleotide sequence (5'~3')	Amplified fragment	Polymorphic fragment	Fragment size (bp)
1	OPA-02	TGCCGAGCTG	9	7	400 -1850
2	OPA-03	AGTCAGCCAC	8	6	350 - 1900
3	OPA-08	GTGACGTAGG	9	7	200 - 1800
4	OPA-09	GGGTAACGCC	7	6	430 - 1440
5	OPA-10	GTGATCGCAG	7	4	390 -1200
6	OPA-11	CAATCGCCGT	6	6	350 - 900
7	OPA-13	CAGCACCCAC	10	8	400 - 1660
8	OPA-14	TCTGTGCTGG	11	10	380 - 1250
9	OPA-18	AGGTGACCGT	10	9	500 - 1680
10	OPB-08	GTCCACACGG	11	9	300 - 2400
11	OPB-12	CCTTGACGCA	8	6	660 - 2000
12	OPB-13	TTCCCCCGCT	8	7	570 - 3630
13	OPB-17	AGGGAACGAG	15	12	300 - 3400
14	OPB-18	CCACAGCAGT	7	7	680 -3150
Total			126	104	
Average			9.0	7.4	200-3630

template DNA for the amplification of rice genomic DNA. The PCR profile starts with 94°C for 5 min followed by 45 cycles of denaturation at 94°C for 1 min, annealing at 36°C for 1 min and extension at 72°C for 2 min. A final extension, 72°C for 7 min was included, followed by soaking at 4°C.

After amplification, 3 µl of gel loading dye buffer (0.02% Bromophenol blue, 0.02% xylene cyanol FF, 50% glycerol and 1% SDS) was added directly to the reaction tubes and spun for few seconds in a micro centrifuge after mixing with the entire reaction mixtures. Aliquots of 15 µl of amplification products plus loading dye were then loaded in 1.5% agarose gels for electrophoresis in 1 × TBE (10 mM Tris-Borate, 1 mM EDTA) buffer and run at 100 V for 40 min to separate the amplified products. 1 kb plus was used as a molecular size weight marker. After electrophoresis, the gels were photographed under UV light using black and white film # 667 (Polaroid, Cambridge, Mass., USA).

Data analysis

Photographs from ethidium bromide stained agarose gels were used to score the data for RAPD analysis. Each DNA fragment amplified by a given primer was treated as a unit character and the RAPD fragments were scored as present (1) or absent (0) for each of the primer-cultivar combinations. Bands were scored from the top of the gel (band number 1) to the bottom. The left lane of the gel was considered as lane 1. Since DNA samples consisted of a bulk sample of DNA extracted from 5 to 10 seeds, a low intensity for any particular fragment may be explained by the lesser representation of that specific sequence in the bulk sample of DNA. Therefore, the intensity of the bands was not taken into account and the fragments with the identical mobility were considered to be the identical fragments. Only major bands were scored and faint bands were not considered. The molecular size of the amplification products was calculated from a standard curve based on the known size of DNA fragments of a 1 kb plus molecular size weight marker. The presence and absence of the bands was scored in a binary data matrix. Pair-wise comparisons of the cultivars based on the presence or absence of unique and shared amplification products were used to generate similarity coefficients. Estimates of genetic similarity (F) were calculated between all pairs of the cultivars by the Dice algorithm. The Dice algorithm is identical to that of Nei and Li (1979) as follows:

$$\text{Similarity (F)} = 2N_{ab} / (N_a + N_b)$$

Where, Na = the number of scored fragments of individual ‘a’, Nb = the number of scored fragments detected in individual ‘b’, and Nab = the number of shared fragments between individuals ‘a’ and ‘b’.

The resulting similarity coefficients were used for cluster analysis using an un-weighted pair-group method with arithmetic averages (UPGMA) and then plotted in the form of a dendrogram. All computations were carried out using the computer program NTSYS, version 2.1 (Applied Biostatistics Inc., USA).

RESULTS AND DISCUSSION

DNA amplification and cultivar identification

Along with the 14 oligonucleotide primers used for the amplification of the rice genomic DNA, three primers performed poorly and did not amplify the DNA in some of the cultivars. Primers (Table 2) varied greatly in their ability to resolve variability among the cultivars. Some primers generated several bands, while others generated only few. A summary of the all amplified products from this study is given in Table 2. Data showed comparable number and size of amplified products to those of previous rice study. As expected from related rice group, a number of amplified fragments were monomorphic.

In total, 126 amplification products were generated using 14 primers among 35 cultivars, out of which 104 (82.5%) fragments were polymorphic. The amplification products varied from 4 (OPA-10) to 16 (OPA-13); Figure 1 shows the amplification profiles generated by the

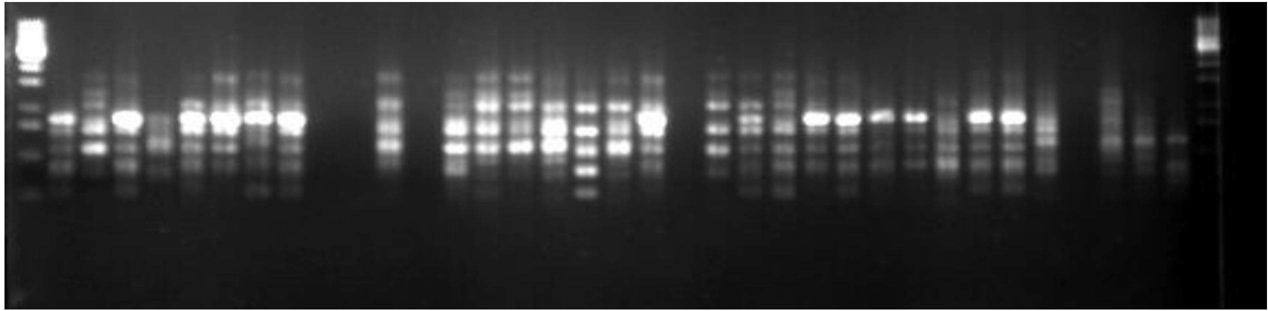

Figure 1. RAPD amplification profiles obtained with primers OPB-18 (A) and OPA-13 (B). M, Marker; 1, Basmati-370; 2, Mushkan; 3, BasmatiPak; 4, IR8; 5, Basmati-198; 6, SuperBasmati; 7, JP5; 8, Mahlar-346; 9, purple marker; 10, DokriBasmati; 11, IR36; 12, Kinmaze; 13, KangnixTorh; 14, SonahriKangni;15, KhariGanga; 16, KSK-133; 17, Shahkar; 18, Shandar; 19, Mehak; 20, Azucena; 21, Kasalath; 22, Basmati-370(I); 23, DheradunBasmati1; 24, KDML105; 25, Basmati-217; 26, DheradunBasmati2; 27, PunjabBasmati; 28, Pusa Basmati; 29, ChiniSakkor; 30, RanbirBasmati; 31, JasmineScented; 32, NiawHawnMali; 33, KhaoJaoHawm; 34, PK386; 35, TN1

primer OPA 13. The size of the amplified fragments ranged from 200 (OPA-08) to 3630 bp (OPB-13). The findings show that most of the basmati varieties resembled genetically each other. In most of the cases, aromatic rice from Punjab and Sindh exhibited similar banding patterns. The RAPDs exhibited several bands that were shared among the Basmati and fine cultivars, whereas a few bands were shared among ‘indica’ and ‘japonica’ cultivars of rice. Two japonica cultivars ‘Kinmaze’ and ‘Azucena’ shared limited number of bands with all the other cultivars, showing their more distant relationship to indica rice varieties while ‘IR8’ and ‘IR36’ had unique as well as shared fragments with the other rice varieties and cultivars from Pakistan.

Our outcomes are in accordance with those reported by other researchers (Neeraja et al., 2002; Raghunathachari et al., 2000; Thomas et al., 2001; Ravi et al., 2003; Fugang et al., 2003). One of the reasons for this high level of polymorphism could be that the intra-specific variation in rice is extensive. The other explanation could be that the primers with 60 to 70% GC content were used. Fukuoka et al. (1992) observed an increase in the number of bands with increasing GC content of the primers. They got an average of 0.8 bands primer^{-1} with 40%, 6.1 bands with 50% and 8.6 bands with 60% GC content. This close relationship has previously been reported for rice by Raghunathachari et al. (2000). Furthermore, 14 primers used in this study were pre-selected based on their performance with rice DNA in earlier studies (Song et al., 1999; Fuentes et al., 1999; Nonnatus et al., 2001; Qian et al., 2001; Neeraja et al., 2002; Santhy et al., 2003).

Similarity matrix

To establish the level of relatedness, a similarity matrix based on the proportion of shared RAPD fragments was used (Table 3). As expected, genetic distances between the aromatic cultivars were comparatively low. Pair-wise estimates of similarity for 35 varieties and cultivars ranged from 0.53 to 0.94. Our findings are also in conformity with the results of Arif et al. (2005) in which they also reported genetic similarity coefficient ranged from 0.42 to 0.85 among 19 rice genotypes of Pakistan using 40 random decamer primers; Davierwal et al. (2000) reported similarity values of 42 elite rice varieties which are commonly used in Indian breeding programs ranged from 0.59 to 0.95 with an average similarity value of 0.83, indicating narrow genetic variability in improved varieties. Two pairs of cultivars, ‘PusaBasmati’ and ‘Basnati-217’, and ‘PusaBasmati’ and ‘Dheradun-Basmati2’ were the closest genotypes with the highest similarity index of 0.94 and 0.93, respectively. Neeraja et al. (2002) also reported maximum similarity index of 0.84 between ‘Swarna’ and ‘Mahsuri’ and minimum similarity index of 0.53 between ‘Pusa1266’ and ‘Jaya’ varieties. It was followed by the similarity index of 0.88 between two cultivars ‘PusaBasmati’ and ‘DheradunBasmati2’. Minimum coefficient of similarity (0.53) was recorded between two pairs of cultivars, ‘Basmati-198’ and ‘IR8’ and ‘Azucena’ and ‘IR8’. When aromatic (Basmati-type) cultivars were compared with non-aromatic types, Basmati (aromatic) cultivars were found to be more similar to each other than to non-aromatic group. Although comparison of aromatic cultivars indicates that these were closely related, no cultivar was exactly like any other cultivar.

‘Kinmaze’ and ‘Azucena’ had the greatest dissimilarity with all the other tested varieties. The similarity coefficients of ‘Kinmaze’ with all the other indica cultivars ranged from 0.61 to 0.79, while ‘Azucena’ showed similarity coefficients of 0.53 to 0.76 with all the other genotypes used. Similarly, the similarity coefficients of ‘IR36’ and ‘PK386’ with all the other cultivars ranged from 0.57 to 0.81 and 0.56 to 0.87, respectively, while both

Table 3. Dice co-efficient of similarity based on RAPD showing the relationships between traditional and improved cultivars of rice.

Number	Cultivar	1	2	3	4	5	6	7	8	9	10	11	12
1	Basmati-370(P)	1.00											
2	Mushkan	0.79	1.00										
3	BasmatiPak	0.81	0.80	1.00									
4	IR8	0.65	0.58	0.58	1.00								
5	Basmati-198	0.80	0.80	0.84	**0.53**	1.00							
6	SuperBasmati	0.78	0.76	0.80	0.64	0.71	1.00						
7	JP5	0.73	0.67	0.66	0.63	0.64	0.66	1.00					
8	Mahlar-346	0.64	0.65	0.60	0.75	0.58	0.70	0.69	1.00				
9	PurpleMarker	0.70	0.65	0.64	0.70	0.62	0.64	0.81	0.72	1.00			
10	DokriBasmati	0.76	0.76	0.77	0.67	0.75	0.79	0.67	0.72	0.68	1.00		
11	IR36	0.67	0.68	0.63	0.71	0.57	0.65	0.62	0.81	0.66	0.66	1.00	
12	Kinmaze	0.69	0.64	0.62	0.61	0.62	0.58	0.79	0.64	0.79	0.64	0.65	1.00
13	Kangni/Torh	0.68	0.69	0.62	0.70	0.62	0.66	0.67	0.75	0.67	0.67	0.79	0.66
14	SonahriKangni	0.65	0.66	0.62	0.70	0.67	0.67	0.64	0.74	0.72	0.68	0.72	0.65
15	KharaiGanga	0.68	0.73	0.64	0.72	0.64	0.68	0.63	0.73	0.65	0.70	0.70	0.60
16	KSK133	0.66	0.67	0.64	0.73	0.64	0.66	0.65	0.78	0.72	0.70	0.70	0.64
17	Shahkar	0.67	0.65	0.62	0.84	0.58	0.72	0.60	0.80	0.66	0.69	0.76	0.60
18	Shandar	0.71	0.71	0.64	0.80	0.61	0.71	0.63	0.82	0.70	0.72	0.74	0.61
19	Mehak	0.75	0.83	0.77	0.62	0.76	0.77	0.65	0.67	0.65	0.78	0.66	0.58
20	Azucena	0.62	0.61	0.61	**0.53**	0.59	0.65	0.76	0.66	0.72	0.62	0.63	0.74
21	Kasalath	0.71	0.66	0.67	0.69	0.62	0.71	0.66	0.82	0.73	0.73	0.73	0.63
22	Basmati-370(I)	0.83	0.78	0.79	0.67	0.75	0.84	0.68	0.72	0.63	0.77	0.68	0.62
23	DheradunBasmati1	0.79	0.78	0.74	0.64	0.78	0.72	0.64	0.65	0.64	0.78	0.61	0.59
24	KDML105	0.73	0.73	0.68	0.70	0.68	0.76	0.63	0.73	0.67	0.74	0.77	0.66
25	Basmati-217	0.83	0.76	0.77	0.68	0.79	0.78	0.70	0.69	0.68	0.82	0.66	0.63
26	DheradunBasmati2	0.82	0.75	0.76	0.66	0.78	0.79	0.64	0.70	0.63	0.83	0.67	0.60
27	PunjabBasmati	0.79	0.78	0.87	0.60	0.84	0.80	0.66	0.62	0.64	0.80	0.61	0.62
28	PusaBasmati	0.83	0.75	0.79	0.67	0.77	0.77	0.74	0.67	0.72	0.79	0.64	0.64
29	ChiniSakkor	0.76	0.76	0.73	0.77	0.65	0.81	0.68	0.75	0.66	0.75	0.75	0.64
30	RanbirBasmati	0.73	0.73	0.75	0.58	0.75	0.79	0.67	0.67	0.67	0.77	0.66	0.58
31	JasmineScented	0.73	0.73	0.70	0.68	0.70	0.72	0.70	0.71	0.74	0.72	0.70	0.74
32	NiawHawnMali	0.67	0.59	0.67	0.71	0.61	0.69	0.64	0.73	0.69	0.75	0.71	0.69
33	KhaoJaoHawm	0.71	0.66	0.65	0.71	0.60	0.67	0.66	0.74	0.70	0.73	0.73	0.63
34	PK386	0.63	0.60	0.58	0.87	0.56	0.62	0.62	0.71	0.69	0.62	0.70	0.62
35	TN1	0.76	0.69	0.66	0.73	0.64	0.68	0.65	0.74	0.74	0.70	0.74	0.66

Number	Cultivar	13	14	15	16	17	18	19	20	21	22	23	24
13	Kangni/Torh	1.00											
14	SonahriKangni	0.87	1.00										
15	KharaiGanga	0.79	0.74	1.00									
16	KSK133	0.78	0.82	0.72	1.00								
17	Shahkar	0.78	0.77	0.76	0.79	1.00							
18	Shandar	0.78	0.75	0.81	0.76	0.86	1.00						
19	Mehak	0.71	0.68	0.71	0.67	0.69	0.70	1.00					
20	Azucena	0.64	0.61	0.64	0.62	0.57	0.60	0.57	1.00				
21	Kasalath	0.80	0.77	0.70	0.85	0.77	0.81	0.70	0.65	1.00			
22	Basmati-370(I)	0.67	0.72	0.67	0.74	0.74	0.72	0.78	0.60	0.73	1.00		
23	DheradunBasmati1	0.69	0.71	0.67	0.73	0.68	0.69	0.77	0.58	0.72	0.80	1.00	
24	KDML105	0.79	0.76	0.73	0.71	0.73	0.76	0.73	0.62	0.72	0.76	0.71	1.00
25	Basmati-217	0.67	0.70	0.65	0.72	0.67	0.68	0.77	**0.56**	0.71	0.84	0.87	0.72
26	DheradunBasmati2	0.68	0.69	0.68	0.73	0.70	0.69	0.77	**0.56**	0.73	0.83	0.84	0.75

Table 3. Contd.

27	PunjabBasmati	0.58	0.62	0.60	0.62	0.63	0.64	0.77	0.61	0.64	0.80	0.74	0.66
28	PusaBasmati	0.65	0.68	0.65	0.68	0.64	0.70	0.76	0.60	0.68	0.81	0.82	0.72
29	ChiniSakkor	0.75	0.69	0.77	0.73	0.82	0.78	0.75	0.59	0.75	0.80	0.74	0.77
30	RanbirBasmati	0.67	0.66	0.69	0.63	0.62	0.63	0.76	0.66	0.66	0.74	0.67	0.67
31	JasmineScented	0.79	0.76	0.71	0.70	0.69	0.74	0.69	0.60	0.74	0.70	0.69	0.85
32	NiawHawnMali	0.71	0.71	0.62	0.75	0.68	0.67	0.67	0.60	0.78	0.73	0.64	0.77
33	KhaoJaoHawm	0.74	0.67	0.68	0.72	0.72	0.73	0.66	0.61	0.79	0.70	0.67	0.72
34	PK386	0.71	0.70	0.78	0.72	0.83	0.79	0.64	0.56	0.65	0.66	0.64	0.65
35	TN1	0.78	0.73	0.78	0.74	0.75	0.82	0.67	0.60	0.77	0.68	0.71	0.76

Number	**Cultivar**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**
25	Basmati-217	1.00										
26	DheradunBasmati2	**0.93**	1.00									
27	PunjabBasmati	0.78	0.74	1.00								
28	PusaBasmati	**0.94**	0.88	0.77	1.00							
29	ChiniSakkor	0.78	0.77	0.73	0.73	1.00						
30	RanbirBasmati	0.72	0.71	0.81	0.68	0.70	1.00					
31	JasmineScented	0.74	0.73	0.64	0.76	0.73	0.63	1.00				
32	NiawHawnMali	0.71	0.70	0.69	0.69	0.70	0.69	0.81	1.00			
33	KhaoJaoHawm	0.70	0.67	0.67	0.68	0.73	0.66	0.78	0.80	1.00		
34	PK386	0.66	0.62	0.60	0.66	0.75	0.57	0.67	0.63	0.70	1.00	
35	TN1	0.72	0.69	0.63	0.72	0.76	0.65	0.78	0.69	0.75	0.75	1.00

showed 0.70 similarity index with each other.

Cluster analysis

Genetic distances obtained from RAPD data were used to create a cluster diagram. Based on analysis performed on Nei and Li's distance matrix using UPGMA; cultivars were grouped into three main clusters (Figure 2). As expected from the similarity estimates, cluster analysis placed most of the aromatic cultivars close to each other showing a high level of genetic relatedness. First cluster consisted of 15 cultivars, all belonged to the aromatic type of rice. However, the clusters formed by the aromatic cultivars were distinct from those of non-aromatic and japonica cultivars. Aromatic cultivars also showed a close association morphologically. Among the long-grains cultivars, clustering of different cultivars generally corresponded to pedigree, center of origin, grain type, and maturity. For example, 14 of the 18 aromatic cultivars were grouped in the upper portion of the dendrogram which is consistent with aromatic type as a trait that differentiates cultivars into broad categories. Similarly, Basmati-370(P), Basmati217, Dheradun-Basmati1, Basmati-Pak, PunjabBasmati and Super-basmati clustered together. These rice cultivars share Basmati-370 as one of their parents in their pedigree and show evidence of similar phenotypic traits In the findings of Arif et al. (2005), three basmati varieties 'Basmati 370' 'Basmati 385' and 'Super Basmati' grouped together and these were more genetically similar with each other as compared to other genotypes studied. It is evident from the literature based knowledge that basmati 370 is the first basmati variety released in 1933 from Kala Shah Kaku Rice Research Institute and Super-Basmati are the descendants of Basmati-370 during the course of selection, hybridization and artificial crossing (Singh et al., 2000). Khan (1996) also reported that in Pakistan, out of seven basmati varieties currently under cultivation, four had Basmati-370 as one of the parent. In the similar type of study conducted by Choudhury et al. (2001), using RAPD markers, commercial cultivars Basmati-370 and Basmati-385 were also grouped together along with related Basmati rice varieties.

16 cultivars were grouped in the second cluster. The second cluster was further divided into two sub-clusters, one containing 12 non-aromatic rice varieties and the second contained four varieties of Thai aromatic rice (Khao DawkMali 105, JasmineScented, NiawHawmMali and KhaoJaoHawm) grouped together because all belong to International Rice Research Institute, Philippine and perhaps share same ancestors. In the non-aromatic group, Shahkar and Shandar were clustered together which is reasonable because they are the mutants of either IR6 or IR8. Moreover, they display similar plant type and morphological characteristics. IR8 was also associated with this cluster. Similar results were reported by Porreca et al. (2001) who reported more closely related varieties IRAT 190 and IRAT 112; Alice and Sillaro, Asso and Bastia, and Lamone and Thaibonnet

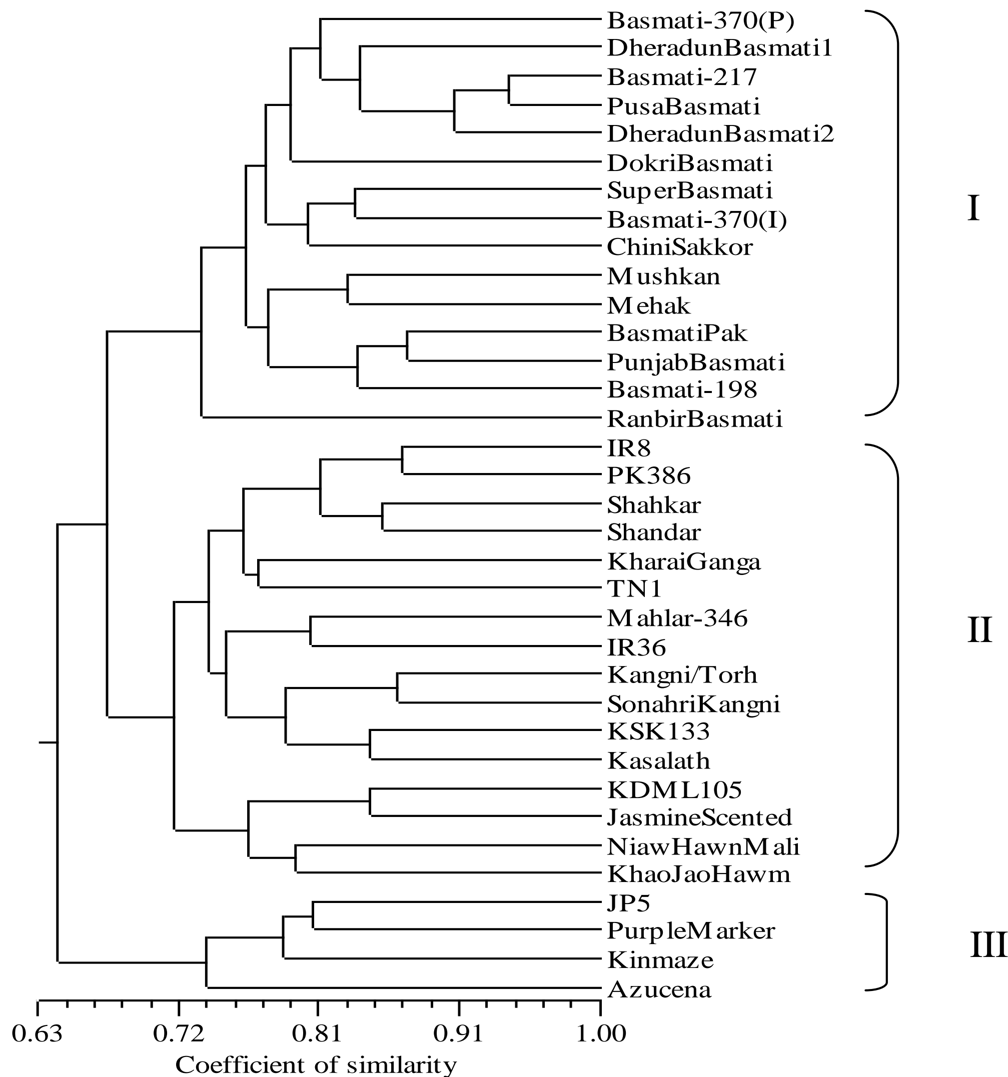

Figure 2. UPGMA based cluster analysis showing the relationship among 35 traditional and improved cultivars of Pakistani rice using RAPD analysis.

were confirmed to have at least one common parentage. This is also comparable with the findings of Arif et al. (2005). The third cluster consisted of four cultivars belonging to japonica type of rice. All japonica cultivars did not fall into any of the main groups and formed separate cluster which was more distant from all the aromatic and course varieties and cultivars. Similarly, two japonica cultivars 'Kinmaze' and 'Azucena', both showing similarity, were grouped together however they had a low level of similarity with the aromatic cultivars.

In general agreement with our RAPD-based analysis, clusters derived from morphological data were consistent with grain type. It appeared that the japonica cultivars were genetically more diverse than the indica group. Polymorphism, as revealed by RAPD markers, was generally low among the aromatic group compared with non-aromatic and japonica cultivars. Interestingly, a number of commercial varieties and obsolete cultivars originating from various parts of Pakistan form well defined distinct clusters and were grouped together in the cluster analysis, indicating an association between the RAPD patterns and the geographic origin of the varieties/ cultivars used (Figure 2). Lower levels of polymorphism in aromatic cultivars of rice indicate that there is a basic similarity among basmati cultivars used in this study, which is to be expected due to their same ancestors and selection for similar characteristics (Pervaiz et al., 2010).
This study reveals the ability of RAPD markers to reliably differentiate between different rice commercial varieties and their use as a tool for the estimation of genetic diversity among rice cultivars. The information about genetic similarity will be helpful to avoid the chance of using genetically similar landrace/genotypes and will also be helpful in future breeding programme to select genetically diverse parents for basmati breeding programme.

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