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GENETIC DIVERSITY AMONG RICE GENOTYPES OF PAKISTAN THROUGH RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) ANALYSIS

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Abstract

Genetic diversity assessment among the 19 rice genotypes of Pakistan including 3 commercial Basmati cultivars viz., Basmati 370, Basmati 385 and Super Basmati was done using random amplified polymorphic DNA (RAPD) analysis. Out of 40 random decamer primers, 20 RAPD primers revealed polymorphism while 15 were monomorphic and no reaction was observed by using remaining 5 primers. OPI 7 generated maximum number of bands while OPZ 4 and OPZ 6 produced minimum fragments. The banding patterns obtained from the polymorphic primers were used to determine the genetic similarity coefficients which ranged from 0.42 to 0.85. Cluster analysis was performed using Unweighted Paired Group of Arithmetic Means (UPGMA) using the similarity coefficients. Cluster analysis resolved two major clusters among the 19 genotypes used. All the 3 commercial Basmati cultivars grouped together whereas the 15 rice genotypes clustered in another group. The remaining one genotype did not fall into any clusters and found to be distantly related to the rest of the genotypes. The results showed the potential of RAPD markers for genetic diversity assessment.

Introduction

Rice is the staple food for more than half of the world's population and is model plant for genomic research (Sasaki & Burr, 2000). Rice belongs to the grass family Poaceae, the genus *Oryza* having 21 wild and 2 cultivated species. It has rich genetic diversity in the form of thousands of land races and progenitor species. A study on 16 species of *Oryza* conducted by Morishima & Oka (1960), divided the *Oryza* species into three main groups 1) *O. sativa* and its relatives 2) *O. officinalis* and its relatives and 3) other more distantly related species on the basis of 42 morphological traits. Glaszmann (1987) classified the rice cultivars from different countries into 6 groups on the basis of isozyme analysis. Aromatic rice of Pakistan and India including Basmati varieties fall into group V. Intercrossing between groups show hybrid sterility as compared to intragroup crossing.

Basmati varieties and breeding lines have narrow genetic base as they are being evolved from selected Basmati lines and also very limited number of basmati breeding lines are being used repeatedly in Basmati breeding programme which only contribute good grain quality, aroma and high yield but not the resistance against biotic and abiotic stresses. For example, in Pakistan, out of 7 Basmati varieties currently under cultivation, 4 had Basmati 370 as one of the parent (Khan, 1996). Many of the Basmati rice varieties released so far are not very much resistance to many pests and diseases (Khan *et al*., 2000; Junaid *et al*., 2000; Cheema *et al*., 1998). Oka (1982) suggested the use of genetic diversity as a mechanism for slowing down the evolutionary rate of parasites to produce new races or biotypes.

Estimation of the phylogenetic relationship and knowing the genetic similarity between genotypes in germplasm of any species has several useful applications. Genetic diversity in plant varieties can be estimated by observing the phenotypes of the plants and measuring the morphological characters. Although the use of morphological and biochemical analysis provided a useful tool for genetic studies but the method has been limited by the small number of phenotypic characters and enzymatic assays. The use of molecular markers has proven its value for a variety of purposes in molecular biology. DNA fingerprinting, gene mapping and phylogenetic studies have tremendously benefited from Polymerase Chain Reaction (PCR) technology. Random Amplified Polymorphic DNA (RAPD) markers generate DNA fingerprints with a single synthetic nucleotide primer (Williams *et al.,* 1990) which could efficiently detect 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 proven useful in genotype identification and gene mapping (Robert *et al.,* 1999). 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, amylose content and length/breadth ratio (Ram *et al*., 1998). Present research work is based upon the assessment of genetic diversity among different rice genotypes including cultivated varieties of Pakistan through RAPD analysis.

Materials and Methods

Plant materials: Seeds of the 16 rice genotypes/lines obtained from Institute of agricultural Biotechnology and Genetic Research (IABGR), National Agriculture Research Centre (NARC), Islamabad, along with 3 commercial Basmati varieties viz., Basmati 370, Super Basmati and Basmati 385, were grown in pots at National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad. The accession number and the local name of all the 16 rice lines used in this study are listed in Table 1.

Table 1. Local name of 16 rice genotypes and their accession numbers.

Sr. No.	Local name	Accession #
1.	Jhona 426- 37	Pak 0244
2.	Santhi Sufaid	Pak 0253
3.	Johni 213	Pak 0255
4.	Dhan 263	Pak 0257
5.	Tiri 424- 2	Pak 0262
6.	SM3- 34	Pak 0343
7.	Kharsu 295A	Pak 0366
8.	Muskan 77	Pak 0382
9.	Sufaida 246	Pak 0425
10.	Basmati 502	Pak 0428
11.	Mutant 11- 9	Pak 0429
12.	Son 15	Pak 0457
13.	Munji Sufaid	Pak 0467
14.	JhonaDesi 185	Pak 0469
15.	Sathra 343	Pak 0474
16.	Santhi Sufaid	Pak 0479

Plant DNA extraction: Young leaves at seedling stage were harvested for the isolation of genomic DNA. Fresh leaves from 5 individuals of each line/variety were bulked together and the DNA was extracted by following the protocol of Dellaporta *et al*., (1983). The concentration of extracted genomic DNA was measured by flouremeter DyNA Quant^{TM}200 and the DNA were diluted to 10ng/uL using sterilized distilled water and stored in microfuge tubes at 4°C for further use.

Amplification and electrophoresis: Decamer random primers (Operon Technologies, Alameda, California) were used for the amplification of DNA for polymorphic survey. A total of 40 primers were used for PCR amplification. Amplification reactions were carried out in 25uL reaction volumes containing 50ng genomic DNA, 100uM each of dATP, dCTP, dGTP and dTTP, 30ng primer, 1 unit of Taq DNA Polymerase (Fermentas), 1X Taq Polymerase Buffer and 2.5mM $MgCl_2$. DNA amplification was performed in DNA Thermal Cycler (Eppendorf) programmed as follows: an initial denaturation of 5 min at 94^{o}C; 40 cycles of 94^{o}C for 1 min (denaturation), 36^{o}C for 1 min (annealing), and 72^{o}C for 2 min (extension). One additional cycle of 10 min at 72^{o}C was used for final extension. Amplification products were resolved by electrophoresis on 1.5% agarose gels run in 0.5X TAE. The amplified products were observed under UV transilluminator after stained with ethidium bromide (10ug/mL).

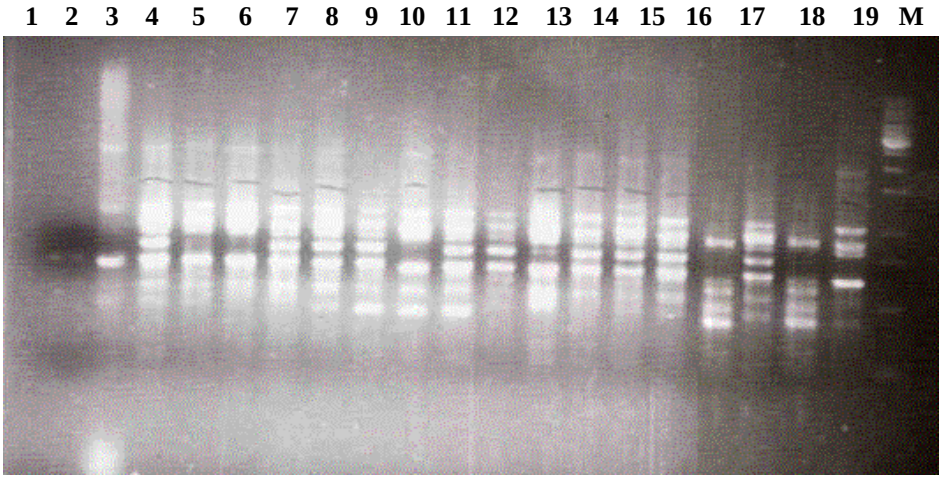
Data analysis: The amplification profile of all the used varieties for any given primer were compared with each other and presence of each band were scored as "1" and the absence of the same band of the same size in other varieties were scored as "0". In this way all the amplified profiles from all the primers used were scored and integrated together to form a data matrix for the estimation of genetic distance between the lines. The genetic similarity coefficients were calculated using Nei & Li method (1979). Cluster analysis was performed based on the similarity coefficient between genotypes using unweighted pair group of arithmetic means (UPGMA).

Results and Discussion

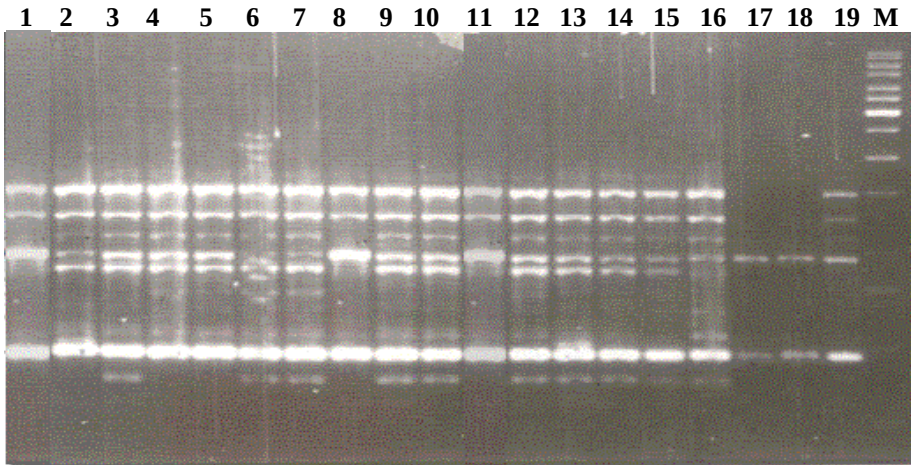
Amplification of the genomic DNA from each of the 19 genotypes using all the 40 decamer primers revealed a variety of RAPD patterns. A total of 1299 bands were amplified with an average of 67 and 93 bands with each variety and primer respectively. Out of 40 random primers used, 20 RAPD primers showed polymorphism between the genotypes while 15 were monomorphic and 5 did not show any amplification. The monomorphic bands are constant bands and cannot be used to study diversity while polymorphic bands revealed differences and can be used to examine and establish systematic relationships (Hadrys *et al*., 1992). In this study OPI 7 generated maximum number of bands (149) while OPZ 4 and OPZ6 amplified minimum number of bands (54) across all the gentoypes. The variation in the number of bands amplified by different primers influenced by variable factors such as primer structure, template quantity and less number of annealing sites in the genome (Kernodle *et al*., 1993).

Different banding patterns were obtained from different primers used (Fig. 1a,b). The 19 genotypes analyzed clustered into two distinct groups A and B, separated the non cultivated rice lines (group A) from the cultivated Basmati varieties (group B) (Fig. 2). The 3 Basmati varieties Basmati 370, Super Basmati and Basmati 385 were grouped together which indicates that they are more genetically similar with each other as compared to other rice genotypes used. 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 Basmati 385 and Super Basmati are the descendants of Basmati 370 during the course of selection, hybridization and artificial crossing (Singh, 2000). In the similar type of study conducted by Choudhary *et al*., (2001), using RAPD markers, commercial cultivars Basmati 370 and Basmati 385 were also grouped together along with other related Basmati rice varieties, whereas the other short grained aromatic rices fell into another group. However narrow genetic diversity among aromatic rice genotypes was observed.



a



b

Fig. 1 a, b: RAPD banding patterns of 19 rice genotypes/cultivars using OPS 7 and OPJ 13 respectively. Lane 1= Jhona 426-37, Lane 2 = Santhi Sufaid, Lane 3 = Johni 213, Lane 4 = Dhan 263, Lane 5 = Tiri 424- 2, Lane 6 = SM3- 34, Lane 7 = Kharsu 295A, Lane 8 = Muskan 77, Lane 9 = Sufaida 246, Lane 10 = Basmati 502, Lane 11 = Mutant 11- 9, Lane 12 = Son 15, Lane 13 = Munji Sufaid, Lane 14 = JhonaDesi 185, Lane 15 = Sathra 343, Lane 16 = Santhi Sufaid, Lane 17 = Basmati 370, Lane 18 = Super Basmati, Lane 19= Basmati 385 and Lane M = 1kb DNA ladder.

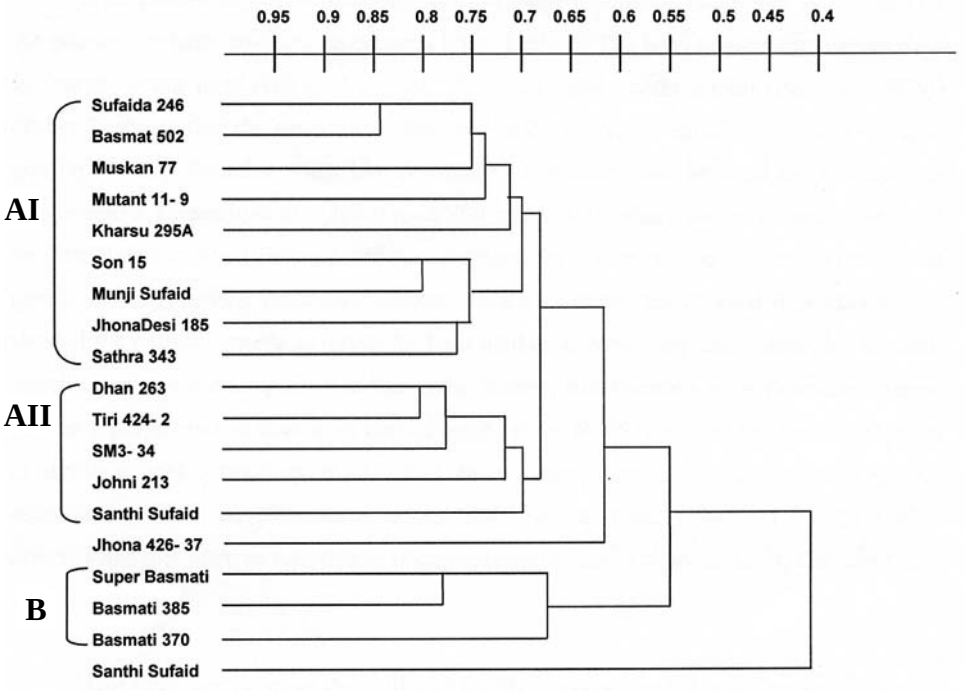


Fig. 2. Dendogram showing phylogenetic relationship among 19 rice genotypes/cultivars based on 20 RAPD primers using UPGMA method. Scale on top is Nei and Li's coefficients of similarity.

Group A comprised of 15 lines with the similarity coefficient of 0.68 (68%). The genotypes Sufaida 246, Basmati 502, Muskan 77, Mutant 11-9, Kharsu 295A, Son 15, Munji Sufaid, JhonaDesi 185 and Sathra 343 formed one cohesive subgroup (AI) distinct from the other subgroup (AII) genotypes i.e., Dhan 263, Tiri 424-2, SM3-34, Jhona 213, and Santhi Sufaid with genetic similarity estimates of 0.707. The genotype Jhona 426-37 was separated from the two subgroups, AI and AII with the genetic similarity of 68%. In subgroup AI, Sufaida 246 and Basmati 502 are the most closely associated genotypes with similarity coefficients of 0.85 (85%). Bligh *et al*., (1999) found different cluster of India and Pakistani Basmati which are separated from the Europe, Australia and United States long grained rice cultivars using SSLP markers. In this study two lines with same name Santhi Sufaid but different accession numbers were used but both did not fall to same cluster. One Santhi sufaid (Pak 0479) clustered with non-Basmati type (group AII) while other having accession number Pak 0253 did not fall in any group and observed to be more distantly related to both Basmati and non-Basmati genotypes. This indicates that both accessions could belong to different lines and/or climate conditions and would have some morphological or agronomical difference among them.

The amount of genetic diversity within rice germplasm is quite high as revealed by the genetic similarity coefficients between lines (Table 2). The level of similarity between the two most distant lines (GS 0.42 between Jhona 213 and Santhi Sufaid (Pak Acc. 0479)) (Table 2) shows that the genetic variation within the species is considerably high. If these genotypes carry desirable traits like resistance to diseases, can be used for rice cultivar improvement . Nagaraju *et al*., (2002) used SSR and ISSR markers for the

Table 2. Genetic similarity coefficients of 19 rice genotypes/cultivars based on Nei & Li coefficients.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1.	1																		
2.	0.65	1																	
3.	0.57	0.72	1																
4.	0.58	0.73	0.8	1															
5.	0.56	0.69	0.73	0.82	1														
6.	0.61	0.7	0.66	0.78	0.79	1													
7.	0.63	0.66	0.7	0.69	0.69	0.69	1												
8.	0.69	0.69	0.67	0.7	0.72	0.71	0.71	1											
9.	0.67	0.75	0.69	0.72	0.72	0.71	0.77	0.78	1										
10.	0.67	0.69	0.66	0.71	0.7	0.73	0.75	0.75	0.85	1									
11.	0.62	0.69	0.64	0.71	0.67	0.71	0.66	0.71	0.72	0.79	1								
12.	0.65	0.74	0.66	0.71	0.7	0.72	0.72	0.71	0.77	0.76	0.75	1							
13.	0.61	0.71	0.66	0.66	0.67	0.69	0.69	0.65	0.73	0.77	0.73	0.82	1						
14.	0.58	0.63	0.59	0.64	0.66	0.62	0.66	0.63	0.7	0.73	0.68	0.75	0.77	1					
15.	0.66	0.66	0.58	0.68	0.64	0.69	0.65	0.68	0.69	0.73	0.71	0.76	0.74	0.77	1				
16.	0.49	0.52	0.42	0.49	0.47	0.53	0.52	0.54	0.56	0.57	0.51	0.57	0.55	0.63	0.61	1			
17.	0.58	0.6	0.53	0.56	0.54	0.57	0.61	0.63	0.61	0.63	0.57	0.69	0.65	0.61	0.64	0.66	1		
18.	0.55	0.57	0.56	0.55	0.52	0.54	0.51	0.59	0.54	0.56	0.58	0.59	0.59	0.56	0.58	0.54	0.68	1	
19.	0.48	0.52	0.52	0.54	0.5	0.53	0.49	0.51	0.54	0.52	0.53	0.59	0.62	0.55	0.59	0.52	0.66	0.78	1

1= Jhona 426-37, 2= Santhi Sufaid, 3= Jhona 213, 4= Dhan 263, 5= Tiri-424-2, 6= SM3-34, 7= Kharsu 295A, 8= Muskan 77, 9= Sufaida 246, 10= Basmati 502, 11= Mutant 11-9, 12= Son 15, 13= Munji Sufaid, 14= Jhona Desi 185, 15= Sathra 343, 16= Santhi Sufaid, 17= Basmati 370, 18= Super Basmati, 19= Basmati 385

genetic analysis of traditional and evolved Basmati and non-Basmati rice varieties. They observed the lowest genetic diversity among traditional Basmati varieties whereas the evolved Basmati varieties showed the highest genetic diversity by both marker assays. They also found that Basmati varieties are easily distinguishable from non Basmati rice varieties by both marker assays. Similar types of studies have been carried out by using RAPD markers for the classification of aromatic rices (Choudury *et al*., 2001), for the identification and classification of rice accessions (Virk *et al*., 1995; Qian *et al*., 1996; Farooq *et al*., 1994) and for the genetic diversity and quantitative variation in rice germplasm (Yu & Nguyen, 1994; Cao & Oard, 1997).

This study demonstrate the ability of RAPD markers to reliably differentiate between different rice germplasm/landraces and commercial varieties and also represents an initial but important step in using RAPD markers as a tool for the estimation of genetic diversity of Pakistani rice cultivars. The information about genetic similarity will be helpful to avoid the chance of use of genetically similar landrace/genoytpes and will also be helpful in future breeding programme to select genetically diverse parents for basmati breeding programme as well.

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