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RESEARCH ARTICLE

Genetic diversity studies of coarse and fine rice using RAPD markers

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Abstract **The availability of a genetically diverse gene pool is vitally important in varietal development. Molecular markers are being extensively utilized to explore the genetic diversity among native and exotic germplasm. This study was designed to reveal the genetic diversity and patterns of relationships among the 20 accessions/genotypes representative of basmati and non-basmati rice from the existing rice gene pool using RAPD markers. Employing RAPD, 17 decamer oligonucleotide primers directed the amplification of 116 fragments, out of which 101 were polymorphic (87.06%) while 15 fragments were monomorphic (12.93%). Similarity coefficients had ranged from 0.47 to 0.90. The average genetic similarity was calculated 0.68 (68%). In this study, the coarse rice genotypes showed more polymorphism (85.84%) than the fine rice genotypes (61.76%). Genotypes were clustered into 8 distinct groups: A, B, C, D, E, F, G, and H but two genotypes, i.e., Shadab and Kangni-27 showed divergence from all the genotypes of the groups. Therefore, these diverse genotypes may be included in future breeding programmes.**

Keywords RAPD, diversity studies, genetic markers, fine rice, coarse rice

Introduction

Rice (*Oryza sativa* L.) is one of the most important crops that provide food for more than half of the world's population. Pakistan ranks 15th and 6th in terms of rice production and export respectively in the world. It is the third largest crop of Pakistan after wheat and cotton. Pakistan is famous for growing and exporting long grain aromatic basmati rice. It is a high valued cash crop and also a major export item. Its cultivation area is over 2.5 million hm^2 (11% of the total cropped area), accounting for 17% of the total cereals produced annually. The annual production of milled rice is about 5.5 million tones sharing 5.5% in agriculture sector and 1.1% in GDP of the country (Anonymous, 2007). Production performance of rice during 2008–2009 was 6.5 million tones (Anonymous, 2008).

In Pakistan, it has been observed that targeted breeding in rice for a small number of traits has resulted in depletion of genetic diversity of promising varieties. Assessment of genetic diversity is important to develop widely adopted cultivars. There are several ways to assess genetic diversity which include morphological, biochemical, and molecular markers. Several types of molecular markers are available for evaluating the extent of genetic variation in rice, i.e., restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), etc. RAPD is a quick and reliable method for estimation of variability among different accessions which could be utilized by the breeders for further improvement of the scented rice genotypes. In addition to technical simplicity and speed of the RAPD methodology, its level of genetic resolution is equivalent to RFLP for determining genetic relationships (Iqbal et al., 1997). The RAPD technique has several advantages such as sampling of a relatively unbiased portion of the genome, easy to use, lower cost, and use of a small amount of plant materials (Ranade et al., 2001). RAPD markers are widely used for estimating genetic diversity in many species, including wild, weedy, and cultivated rice (Yu et al., 2005). Therefore, RAPD markers were preferred for this study.

Most of the basmati genotypes genetically resemble each other. They have a narrow genetic base as they are being

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evolved from selected basmati lines, and also very limited number of basmati breeding lines are being used repeatedly in basmati breeding programs which only contributes good grain quality, aroma and high yield of rice but not the resistance against biotic and abiotic stresses. For example, out of 7 basmati varieties currently under cultivation, 4 have Basmati-370 as one of the parent. That is why many of the basmati rice varieties released so far are not very much resistant to many pests and diseases (Muhammad et al., 2005). The objective of this study was to evaluate the genetic polymorphism of coarse and fine rice germplasm because there was a strong need to broaden the gene pool of rice for the future utilization in breeding of rice varieties with superior quality, better adaptation, and higher resistance to pests and diseases in Pakistan.

Materials and methods

The genetic diversity studies of coarse and fine rice using RAPD markers were carried out at the Centre of Agricultural Biochemistry and Biotechnology (CABB), University of Agriculture, Faisalabad, Pakistan. The materials used for this study were 10 accessions/genotypes of each fine and coarse rice. Grains of these varieties were sterilized with 70% ethanol for 1 min, then with 3% sodium hypochlorite solution for 20 min, and finally washed five times with sterile water.

Grains (20 grains/dish) were incubated on two layers of filter paper (TYPE 2, ADVANTEC, Japan) moistened with 6 mL distilled water in a Petri-dish at 4ºC in the dark for 24 h to synchronize germination. The grains were then transferred to a growth chamber (KM, Kiya Co., Japan) at 35ºC for 15 days.

DNA was extracted from 15-day-old etiolated seedlings by the protocol described by Ahmadikhah (2009). DNA quality and quantity were determined by gel electrophoresis and Nano-drop (ND-1000) Spectrophotometer respectively. Three different concentrations (15, 20, and 25 ng/μL) of the DNA diluted samples were prepared to optimize the PCR reaction for the best amplification and 15 ng/μL was selected for PCR. An optimization of PCR conditions was conducted with respect to the concentration of genomic DNA, 10×PCR buffer, $MgCl_2$, dNTPs, gelatin, primer and *Taq* DNA polymerase because the reproducibility of RAPD technique is influenced by these factors and type of thermal cycler used. PCR reaction was performed in a volume of 25 μL. The reaction was carried out under the following conditions: 5 min denaturation at 95ºC, followed by 40 cycles of 1 min at 95ºC, 1 min 36ºC and 2 min at 72ºC ,with a final extension of 10 min at 72ºC.

RAPD primers and agarose gel electrophoresis

The oligo decamer RAPD primers were synthesized by Gene Link Company, UK. Thirty primers were selected on the basis of their polymorphic natures for this study. PCR products were resolved on 1.2% (w/v) agarose gel electrophoresis.

Data analysis

The fingerprints were examined under the UV Transilluminator and photographed. The Random Amplified Polymorphic DNA (RAPD) bands were counted and designated as present (1) and absent (0). Multivariate analysis was conducted to generate a similarity matrix using Popgene32 software (version 1.44) (Yeh et al., 2000) based on Nei's Unweighted Paired Group of Arithmetic Mean Averages (UPGMA) (Nei, 1987) to estimate genetic similarity and relatedness of the rice genotypes. Genetic similarities obtained from RAPD data were used to create a cluster diagram or dendrogram. Cluster analysis has the singular efficacy and ability to identify crop accessions with the highest level of similarity using the dendrogram.

Results and discussion

In this study, twenty rice genotypes were assessed for the estimation of genetic diversity by using 30 decamer oligonucleotide random amplified polymorphic DNA (RAPD) primers. Among the 30 decamer oligonucleotide primers, 17 were selected on the basis of polymorphism. Other primers produced faint bands and monomorphic banding patterns and hence they were eliminated from the analysis. The monomorphic bands are constant bands and cannot be used to study diversity while polymorphic bands reveal differences but can be used to examine and establish systematic relationships (Hadrys et al., 1992).

A total of 1230 bands were amplified by 17 primers with an average of 72.35 bands per primer. The precision of diversity analysis was improved as more bands were detected (Moser and Lee, 1994). All the selected 17 primers produced 116 fragments, out of which 101 were polymorphic (87.06%) while 15 fragments were monomorphic (12.93%). Among all the primers, J-20 produced the highest number (173) of bands (Fig. 1) followed by C-11 producing 106 bands (Fig. 2) and A-11 producing the lowest number (16). An average of 72.35 bands per primer was obtained. The maximum number of bands (81) was produced by Shadab while the minimum number of bands (46) was produced by Kangni-27. The average number of bands produced by each genotype was 61.5. Similarity coefficients ranged from 0.47 to 0.90. The average genetic similarity calculated was 0.68 (68%).

The genotypes were divided into two groups: Group-I consisted of fine rice genotypes and Group-II consisted of coarse rice genotypes. These genotypes were further clustered into 8 distinct groups: A, B, C, D, E, F, G, and H but two genotypes, i.e., Shadab and Kangni-27 showed a distinctive behavior from all the genotypes of the groups as shown in Fig. 3.

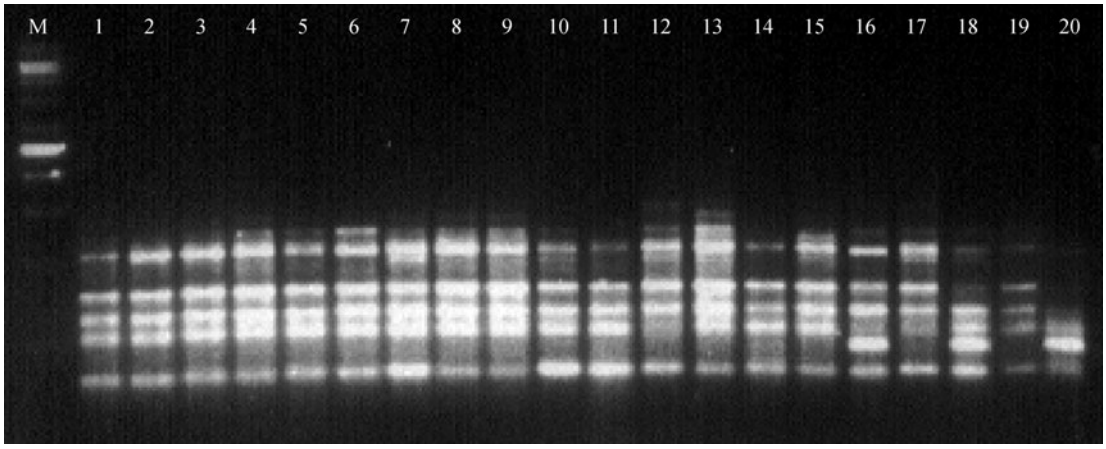

Figure 1 Amplification product of RAPD primer J-20. Note: M = 1 kb DNA ladder. Fine rice genotypes: 1 = Sugdasi-Sadagulab, 2 = Basmati-217, 3 = Punjab-Basmati, 4 = Basmati-385, 5 = Rachna-Basmati, 6 = Dhera-Dun-Basmati, 7 = Dokri-Basmati, 8 = Kashmir-Basmati, 9 = Khushboo-95, 10 = Lateefy; Coarse rice genotypes: 11 = Sathra, 12 = Shadab, 13 = Shua-92, 14 = Mahlar-346, 15 = IR-8, 16 = Kasalath, 17 = Kharai-ganga, 18 = Sonahri-Kangni, 19 = Jhona-349, 20 = Kangni-27. The same as below.

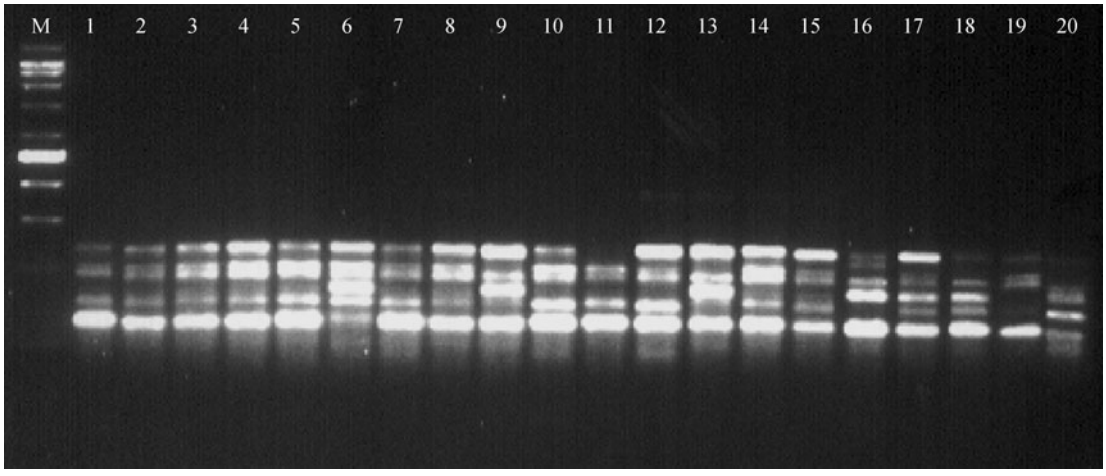

Figure 2 Amplification product of RAPD primer C-11

Fine rice genotypes

The following 10 fine rice genotypes were assessed for the estimation of genetic diversity: Sugdasi-Sadagulab, Basmati-217, Punjab-Basmati, Basmati-385, Rachna-Basmati, Dhera-Dun-Basmati, Dokri-Basmati, Kashmir-Basmati, Khushboo-95, and Lateefy.

All polymorphic primers (17) produced 102 fragments, out of which 63 were polymorphic (61.76%) while 39 fragments were monomorphic (38.23%). Fine rice genotypes were clustered into 5 distinct groups: A, B, C, D, and E. The cluster analysis placed most of the aromatic genotypes into a close relation showing a high level of genetic relatedness. Among the fine rice genotypes, Basmati-217 and Punjab-Basmati were the closest genotypes with the highest similarity of 90% followed by 88% similarity noted between Basmati-385 and Dokri-Basmati of Group-C. Dhera-Dun-Basmati and Lateefy both showed the lowest similarity (81%) among all the fine rice genotypes. The genetic similarity coefficients ranged from 0.68 to 0.90 in the fine rice genotypes (Table 1). The average genetic similarity among the aromatic rice genotypes was 0.79 (79%). The above study described that the basmati genotypes were genetically very much similar to each other and less diverse.

Coarse rice genotypes

Ten coarse rice genotypes: Sathra, Shadab, Shua-92, Mahlar-346, IR-8 in Group-I, Kasalath, Kharai-Ganga, Sonahri-Kangni, Jhona-349, and Kangni-27 were evaluated for the estimation of genetic diversity.

A total of 113 fragments were produced, out of which 97

Table 1 Similarity matrix of twenty coarse and finerice genotypes

POP ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	*****	0.8276	0.8190	0.8190	0.7672	0.7500	0.8190	0.8017	0.8276	0.8017	0.7931	0.7069	0.7931	0.7586	0.8103	0.7069	0.7586	0.7155	0.7500	0.5603
2		*****	0.9052	0.8534	0.7672	0.7328	0.7845	0.8190	0.8103	0.8017	0.7931	0.6552	0.6897	0.8276	0.8621	0.6897	0.7414	0.7155	0.7845	0.5776
3			*****	0.8621	0.7586	0.7586	0.8276	0.8276	0.8190	0.8102	0.8017	0.6810	0.6810	0.8190	0.8534	0.7155	0.7155	0.7069	0.7931	0.5862
4				*****	0.8276	0.7759	0.8793	0.8276	0.7845	0.7759	0.8190	0.7328	0.7672	0.7672	0.8017	0.7155	0.7672	0.7586	0.7759	0.5690
5					*****	0.6897	0.7931	0.8276	0.7500	0.7414	0.7845	0.6638	0.7328	0.6810	0.6983	0.6810	0.6983	0.7241	0.6897	0.6034
6						*****	0.7931	0.7759	0.7328	0.8103	0.7672	0.7328	0.7155	0.7500	0.8017	0.7500	0.7500	0.7069	0.7869	0.6034
7							*****	0.7586	0.7672	0.8448	0.7845	0.7672	0.7500	0.7500	0.8190	0.6983	0.7845	0.7586	0.7586	0.6034
8								*****	0.8190	0.8103	0.8017	0.7500	0.7845	0.7672	0.7672	0.6810	0.7328	0.6897	0.6552	0.5345
9									*****	0.7845	0.7586	0.7241	0.7586	0.7414	0.8448	0.7069	0.7414	0.6983	0.7155	0.5948
10										*****	0.8017	0.7328	0.7500	0.8017	0.8190	0.7500	0.7845	0.7759	0.7414	0.5517
11											*****	0.6379	0.7241	0.8103	0.7586	0.7069	0.7069	0.7672	0.8017	0.5948
12												*****	0.7241	0.6897	0.7586	0.6724	0.6414	0.6466	0.5776	0.5431
13													*****	0.6724	0.6724	0.6897	0.7414	0.7328	0.6638	0.5431
14														*****	0.8103	0.6897	0.7069	0.7328	0.7672	0.6293
15															*****	0.6897	0.7414	0.7155	0.7328	0.6121
16																*****	0.7931	0.7155	0.7328	0.4741
17																	*****	0.7500	0.7500	0.5259
18																		*****	0.7414	0.5690
19																			*****	0.6552
20																				*****

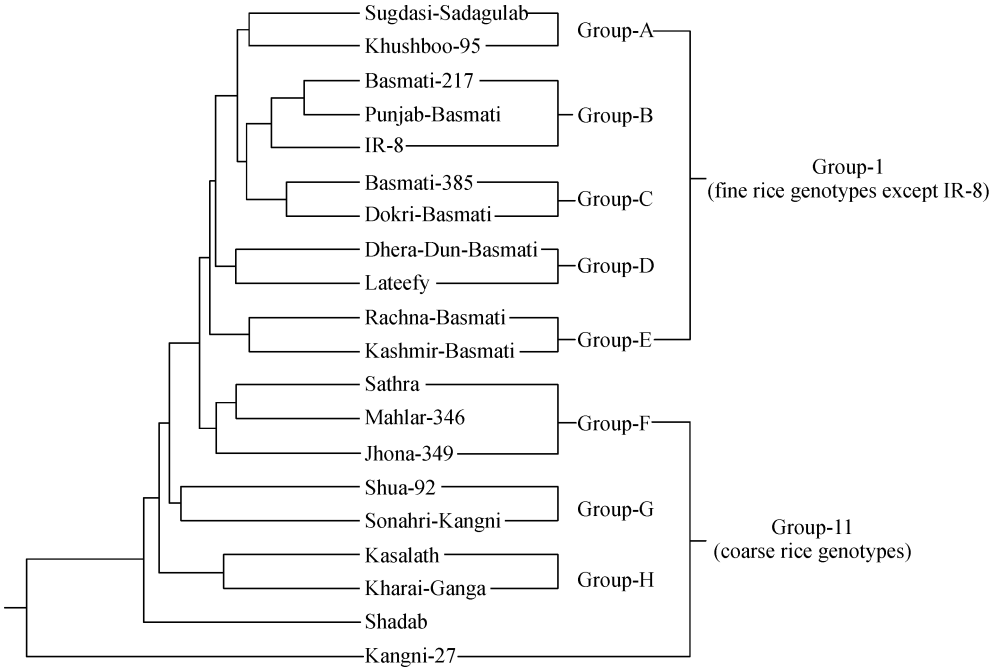

Figure 3 Dendrogram of 20 (20) rice varieties obtained from similarity matrix based on Nie's UPGMA RAPD

were polymorphic (85.84%) while 16 fragments were monomorphic (14.15%). High level of polymorphism could be that the intra-specific variation in rice is extensive. The coarse rice genotypes were clustered into 3 distinct groups: F, G, and H but two Genotypes, i.e., Shadab and Kangni-27 showed divergence from genotypes of all the other groups. The similarity coefficients ranged from 0.47 to 0.86 in coarse rice genotypes (Table 1). The average genetic similarity among the aromatic rice genotypes was 0.66 (66%). Sathra and Mahlar-346 were the closest genotypes with the highest similarity (81%) in Group-F. Kangni-27 gave the maximum diversity among all coarse rice genotypes followed by Shadab which also gave the distinct genetic diversity. Interestingly, many primers revealed characteristic fragments in these two genotypes which were not produced in any of the other genotype. Comparatively, a broad genetic diversity in coarse rice germplasm was observed.

Conclusion

In the present study, it is concluded that fine (aromatic) rice genotypes of Pakistan had a relatively narrow genetic base as compared to coarse (non-aromatic) rice genotypes. The coarse rice genotypes showed more polymorphism (85.84%) than the fine rice genotypes (61.76%). The narrow genetic base of basmati varieties evolved from selected basmati lines was due to a very limited number of basmati breeding lines that were used repeatedly in basmati breeding programs, which only contributed good quality grain, aroma and high yield but not resistance to biotic and abiotic stresses. That is why many of the basmati rice varieties released so far lacked necessary resistance to many pests and diseases. Two coarse rice genotypes, i.e., Kangni-27 and Shadab both showed considerable divergence from all other coarse as well as fine rice varieties. Therefore, these varieties should be included in the breeding program. If these genotypes carry desirable traits like resistance to diseases they would prove valuable and effective for rice cultivar improvement. The above information about genetic similarity will be helpful to avoid the chance of using genetically similar landrace/genotypes in the breeding program as well as to select genetically diverse parents for cultivar/genotype improvement in future breeding programs.

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