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MOLECULAR CHARACTERIZATION AND GENETIC DIVERSITY STUDY IN F_3 POPULATION OF RICE

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ABSTRACT

Random Amplified Polymorphic DNA (RAPD) assay was initiated for molecular genetic analysis among 13 F_3 rice lines and their parents. Four out of 15 decamer random primers were used to amplify genomic DNA and the primers yielded a total of 41 RAPD markers of which 37 were considered as polymorphic with a mean of 9.25 bands per primer. The percentage of polymorphic loci was 90.24. The highest percentage of polymorphic loci (14.63) and gene diversity (0.0714) was observed in 05-6 F_3 line and the lowest polymorphic loci (0.00) and gene diversity (0.00) was found in 05-12 and 05-15 F_3 lines. So, relatively high level of genetic variation was found in 05-6 F_3 line and it was genetically more diverse compared to others. The average co-efficient of gene differentiation (G_{ST}) and gene flow (N_m) values across all the loci were 0.8689 and 0.0755, respectively. The UPGMA dendrogram based on the Nei's genetic distance differentiated the rice genotypes into two main clusters: PNR-519, 05-19, 05-14, 05-12 and 05-17 grouped in cluster 1. On the other hand, Baradhan, 05-9, 05-13, 05-11, 05-5, 05-6, 05-1, 05-4, 05-15 and 05-25 were grouped in cluster 2. The highest genetic distance (0.586) was found between 05-4 and 05-17 F_3 lines and they remain in different cluster.

Key words : Genetic diversity, RAPD, Rice

INTRODUCTION

Rice (*Oryza sativa* L.), belongs to the family Gramineae, is one of the most important cereal crops in the world. The production and consumption of rice are concentrated in Asia where more than 90% of the rice is produced. It is considered as the most important staple food crop in Bangladesh (Bhuiyan *et al.,* 2002). Rice varieties of Bangladesh have been developed traditionally by selection, hybridization and back crossing with locally adapted high-yielding lines. The number of parental lines used in the breeding programme is however quite small, resulting in a narrow genetic base. Genetic uniformity in crops can be undesirable in terms of vulnerability of the crop to epidemics and environmental disasters. Assessing the level of genetic variation among the cultivated rice varieties has been of great interest to plant breeders. Traditional

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techniques, based on morphological and cytological characters, breeding behaviour and ecological distribution have been used to assess the genetic variability and relationships among rice cultivars (Sato, 1986) which takes 8-12 years to develop a variety through conventional breeding approach. In recent years, DNA polymorphism assays and molecular marker have been used for genetic mapping, genome fingerprinting, and for determining genetic relatedness and for marker assisted selection (MAS). Genetic diversity is commonly measured by genetic distance or genetic similarity both of which imply that there are either differences or similarities at the genetic level (Weir, 1990). In this study the genetic diversity among 15 rice genotypes was investigated using RAPD markers.

MATERIALS AND METHODS

Fifteen rice genotypes including thirteen pedigree lines and their parents were collected from Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. Total DNA was isolated from young leaves of 25-day old seedlings following the mini preparation modified CTAB method. Fifteen primers of random sequence were screened on two parents to evaluate their suitability for amplifying DNA sequences, which could be scored accurately. A final subset of 4 primers (OPA-02, OPB-10, OPB18 and OPB06) exhibiting good quality banding patterns and sufficient variability were selected for further analysis. To perform the amplification of RAPD, a single oligonucleotide of arbitrary DNA sequence was mixed with genomic DNA in the presence of a thermostable DNA polymerase and suitable buffer, and then subjected to temperature cycling conditions typical to the polymerase chain reaction. Each 10 µl reaction contained 1 µl extracted genomic DNA, 1 µl Taq polymerase buffer, 1 µl dNTPs, 2.5 µl primer, 0.2 µl ampli Taq DNA polymerase and 4.3 µl sterile deionized water. PCR was initiated by the following temperature profile: 3 min at 94°C for initial denaturation, 41 cycles 1 min at 94°C (denaturation), 1 min at 34°C (annealing), extension 2 min at 72°C and last cycle, a final extension of 7 min at 72°C was added. The amplified product was separated electrophoretically in 1.5% agarose gel with 20 bp DNA ladder. After electrophoresis, the gel was taken out carefully from the gel chamber and transferred in a prepared ethidium bromide solution for staining then placed on the UV transilluminator in the dark chamber of the Image Documentation System. The image was visualized on the monitor and the photographs were saved.

RAPD data analysis

Since RAPD markers are dominant, we assumed that each band represented the phenotype at a single allelic locus. The size of the amplification products was estimated by comparing the distance traveled by each amplified fragment with that of the known sized fragments of molecular weight marker (20 bp DNA ladder). All distinct bands or fragments (RAPD markers) were thereby given identification numbers according to their position on gel and scored visually on the basis of their presence (1) or absence (0), separately for each individual and each primer. The scores obtained using all primers in the RAPD analysis were then conducted to create a single data matrix. This was used to

estimate polymorphic loci, Nei's (1973), gene diversity, population differentiation, (G_{ST}), gene flow (N_m), genetic distance (D) and constructing a UPGMA (Unweighted Pair Group Method with Arithmetic Means) (Sneath and Sokal, 1973) dendrogram among populations using the POPGENE (Version 1.31) and TREEVIEW computer program (Sneath and Sokal, 1973; Yeh *et al.*, 1999).

RESULTS AND DISCUSSION

Fifteen primers were initially surveyed for their ability to produce polymorphic patterns. Out of 15, four primers viz. 0PA02, OPB06, 0PB10 and OPB18 gave reproducible and distinct polymorphic amplified products.

Selected four primers generated 41 bands. Out of the 41 bands, 37 bands (90.24%) were polymorphic and 4 bands (9.76%) were monomorphic (Table 1). Similar result (90% polymorphic bands) was also reported by Ravi *et al*. (2003) in rice (*Oryza* spp.) using the same DNA marker. The primer OPA02 produced the highest number of polymorphic bands (12). Thus it showed a higher level of polymorphism. On the other hand, the primer OPB10 and OPB06 generated least number of polymorphic bands 7 and 8, respectively.

The banding patterns of different rice genotypes using primers OPA02, OPB06, OPB10 and OPB18 are shown in Fig. 1(a-d).

Table 1. RAPD primers with corresponding bands scored with polymorphic bands observed in 13 F_3 rice lines and their parents

Primer codes	Sequences (5/-3/)	Number of bands	No. of polymorphic bands	Polymorphic loci (%)
OPA02	CTCTGGAGAC	13	12	
OPB06	TGCTCTGCCC	8	8	90.24
OPB10	TGCTCTGCCC	8	7	
OPB18	TCCACAGCAG	12	10	
Total		41	37	
Average		10.25	9.25	

The primer OPA02 produced maximum number of bands (13), whereas OPB06 and OPB10 generated the least number of bands (8). The primer OPA02 amplified maximum number of polymorphic bands (12). The four primers generated 10.25 scorable bands and 9.25 polymorphic RAPD markers per primer. This was a high level of polymorphism detected by the arbitrary primers compared to the previous report in other RAPD studies on rice, i.e. 4.5 polymorphic bands per primer in Australian rice (Ko *et al.*, 1994), 5.4 in Indian aromatic rice (Choudhury *et al.*, 2001).

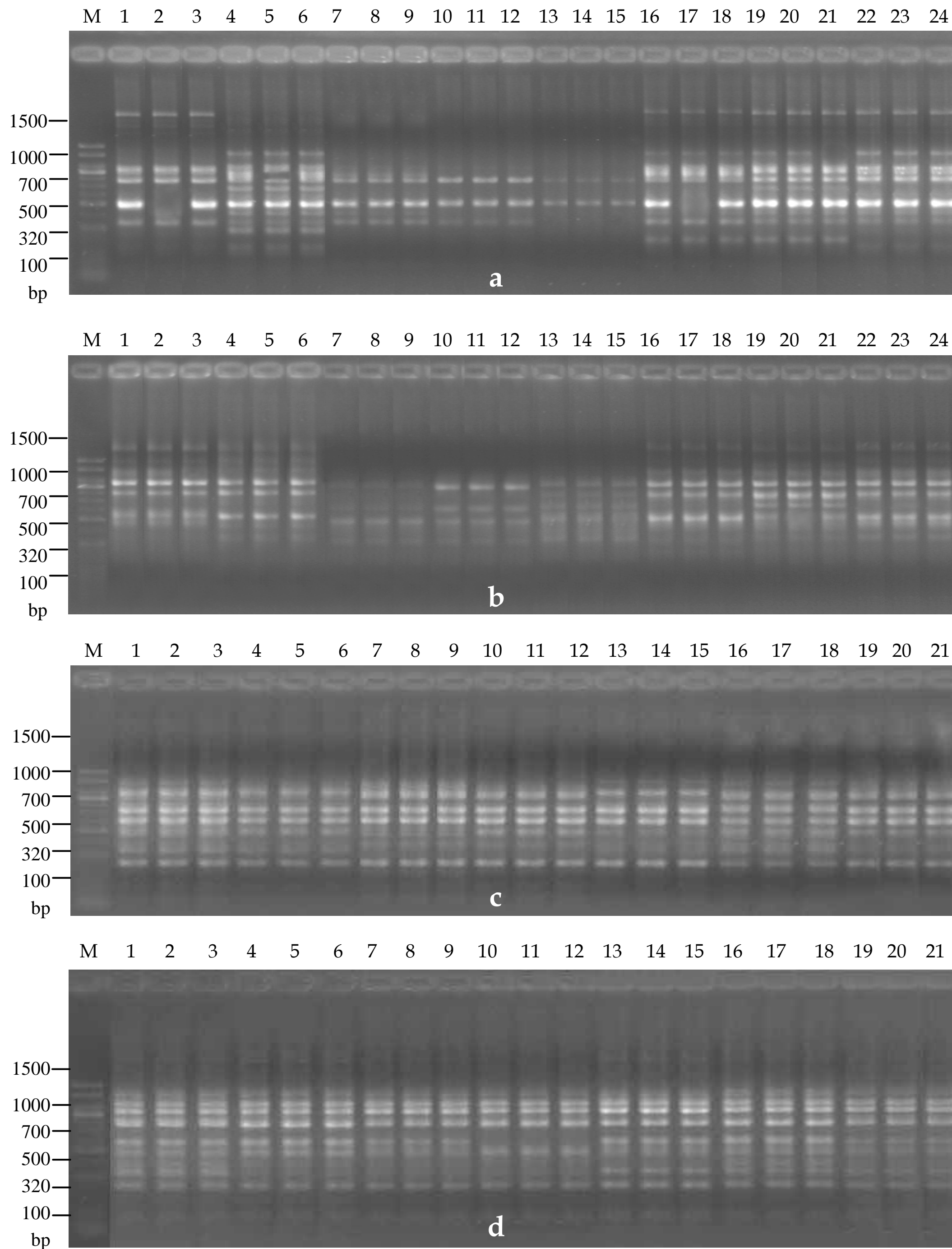

Fig. 1(a-d). RAPD profiles of some selected rice lines using primers (a) OPA2, (b) OPB6, (c) OPB10 and (d) OPB18. Lane 1-3: 05-15, Lane 4-6: 05-9, Lane 7-9: 05-13, Lane 10-12: 05-19, Lane 13-15: 05-25, Lane 16-18: 05-4, Lane19-21: 05-1. M: Molecular weight marker (20 bp DNA ladder).

In the present study the percentage of polymorphic loci was 90.24. A diverse level of polymorphism in rice genotypes were reported by Rahman *et al.* (2007), 53.85%, Qian *et al.* (2006), 83.5%, Shivapriya *et al.* (2006), 74.1%, Vibha *et al.* (2005), 94.36%. The highest polymorphic loci (14.63%) was found in the 05-6 line which gave 6 polymorphic bands and the lowest polymorphic loci (0.00%) was found in the 05-12 and 05-15 lines which performed zero polymorphic bands (Table 2).

Table 2. Estimation of genetic variation. Percentage of polymorphic loci, Nei's gene diversity (h), and Shannon's information index (I) obtained from studied 13 F_3 rice lines and their parents

Genotype	Number of polymorphic loci	Percentage of polymorphic loci	Gene diversity (h)	Shannon's information index (I)
05-1	4	9.76	0.0476	0.0665
05-4	4	9.76	0.0476	0.0665
05-6	6	14.63	0.0714	0.0997
05-9	5	12.20	0.0595	0.0831
05-5	3	7.32	0.0357	0.0498
05-11	4	9.76	0.0476	0.0665
05-12	0	0.00	0.0000	0.0000
05-13	2	4.88	0.0238	0.0332
05-14	3	7.32	0.0357	0.0498
05-15	0	0.00	0.0000	0.0000
05-17	2	4.88	0.0238	0.0332
05-19	2	4.88	0.0238	0.0332
05-25	4	9.76	0.0476	0.0665
PNR519	3	7.32	0.0357	0.0498
Baradhan	5	12.20	0.0595	0.0831
Total			0.5593	0.7809

The 05-6 pedigree line showed the highest level of gene diversity (0.0714) than other genotypes. The 05-12 and 05-15 lines showed the lowest (0.0000) gene diversity. Gene diversity across all genotypes for all loci was 0.5593. The 05-6 line showed the highest 'I' value (0.0997) and the 05-12 and 05-15 lines showed the lowest 'I' value (0.0000). Shannon information index (I) of all rice genotypes was 0.7809. Since 05-6 line exhibited higher percentage of polymorphic loci, gene diversity and Shannon's information index suggested higher polymorphism.

For above consideration this 05-6 line could be utilized in breeding programme. The number and proportion of polymorphic loci was found to be the highest in 05-6 line,

which was 6 and 14.63%, respectively and the lowest in 05-12 and 05-15 lines which were 0.00. The number and proportion of polymorphic loci were studied by Rahman *et al.,* 2007 (highest 7 and 26.92%, lowest 0 and 0%).

The average estimated gene flow (Nm) was 0.0755 and co-efficient of gene differentiation (G_{ST}) was 0.8689 across all loci. Average H_T was 0.2844 and H_S was 0.0373 across all primers. RAPD marker OPA02-11 revealed low level of differentiation (G_{ST}), (0.4059) in the studied genotypes.

Average gene diversity (h) and Shannon's Information index (I) across all primer against genotypes for all loci were found 0.2844 and 0.4342, respectively. High level of gene diversity value and Shannon's Information index was found in locus OPA02-5 (0.4997 and 0.6928). Lowest level of gene diversity value 0.000 and Shannon's Information index (0.000) were found in locus OPA02-6, OPB10-2, OPB18-5 and OPB18-12. Over all gene diversity across all populations for all the loci were observed 0.208 (Rahman *et al.,* 2007).

The highest Nei's genetic distance (0.586) was found between 05-4 and 05-17 pair lines than other pair combination. The lowest genetic distance (0.082) was revealed between 05-25 and 05-15 F_3 lines. As these F_3 lines agronomic performances were very similar like plant height, days to flowering, days to maturity. Considering the genetic distance values, the result indicated that the genotypes were genetically different from each other which could be used in breeding programmed to have potential genetic gains.

Thirteen F_3 rice lines and their parents were used to make dendrogram based on Nei's (1972) genetic distance using UPGMA were grouped into two main clusters (1 and 2): PNR-519, 05-19, 05-14, 05-12 and 05-17 grouped in cluster 1. On the other hand Baradhan, 05-9, 05-13, 05-11, 05-5, 05-6, 05-1, 05-4, 05-15 and 05-25 were grouped in cluster 2. (Fig. 2). The cluster 1 divided into two sub-clusters. The parent PNR-519 alone grouped in one sub-cluster while 05-19, 05-14, 05-12 and 05-17 F_3 lines were grouped in another sub-cluster where 05-12 and 05-17 lines were grouped in a sub-sub-cluster. The cluster 2 grouped in two sub-clusters; (1 and 2). Baradhan, 05-9, 05-13, 05-11, 05-5, 05-6 and 05-1 lines were grouped in sub-cluster 1 while 05-4, 05-15, and 05-25 lines were grouped in sub-cluster 2. In sub-cluster 1 the parent Baradhan alone grouped in sub-sub-cluster 1 where 05-9, 05-13, 05-11, 05-5, 05-6 and 05-1 lines were grouped in sub-sub-cluster 2. In sub-cluster 2 the line 05-4 alone grouped in sub-sub-cluster 1 on the other hand the lines 05-15 and 05-25 were grouped in sub-sub-cluster 2.

The results from the current study indicate that RAPDs offer a reliable and effective means of assessing genetic variation and thus provide an alternative avenue for predicting performance in rice. From the present study it has also proved that DNA fingerprinting is a useful tool for varietal security to show possession or origin of plant lines.

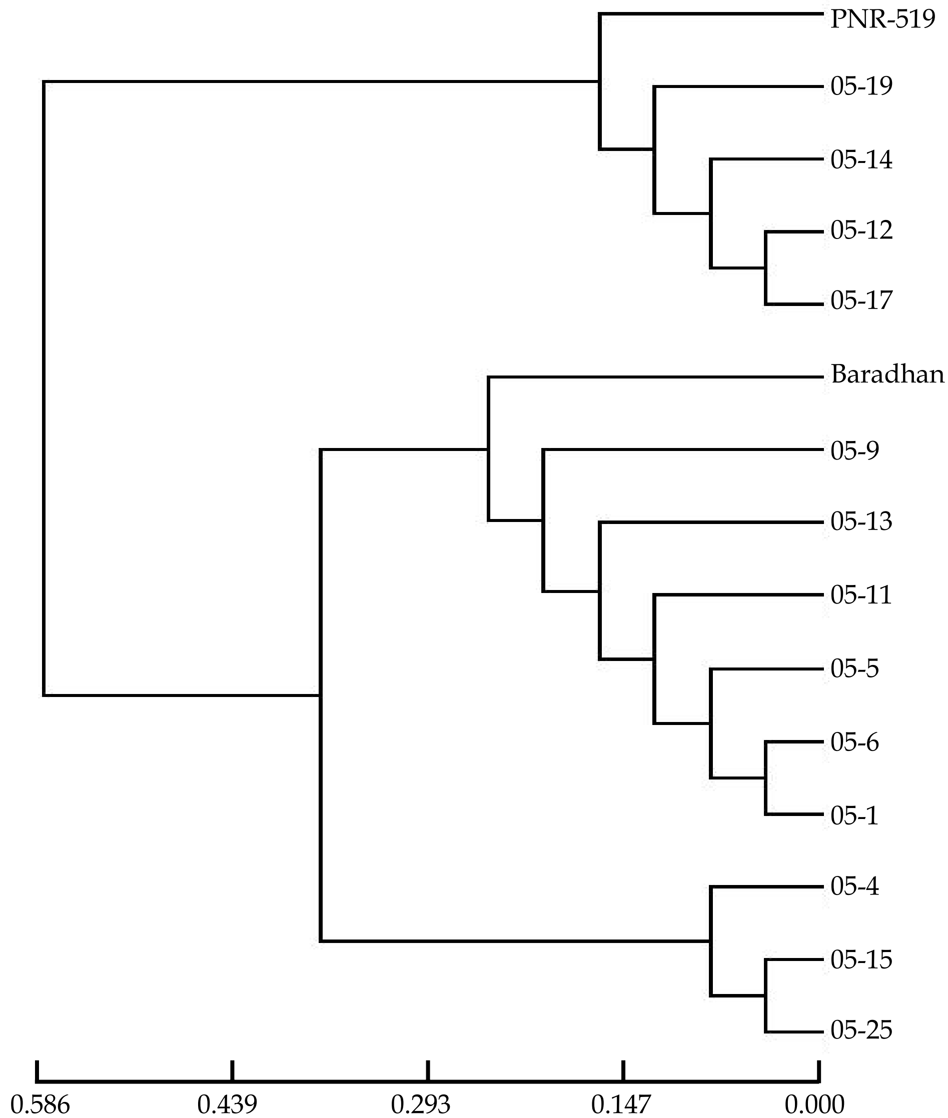

Fig. 2. UPGMA dendrogram based on Nei's (1972) original measures of genetic distance, summarizing the data on differentiation between 13 F_3 rice lines and their parents according to RAPD analysis

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