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GENETIC VARIATION AND RELATEDNESS AMONG HIGH YIELDING RICE VARIETIES (*Oryza sativa* L.) REVEALED BY RAPD MARKERS

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ABSTRACT

Genetic variation is a principal concern for the plant breeders. Genetic variation and relationship among high yielding rice varieties viz. Binadhan 4, Binadhan 5, Binadhan 6, Binasail, BRRI dhan28 and BRRI dhan29 were analyzed using four decamer random primers. Polymerase Chain Reaction (PCR) amplified 22 RAPD markers, of which 18 (81.82%) were polymorphic. The proportion of polymorphic loci and the gene diversity values were 59.09% and 0.25 for the Binadhan 4; 59.09% and 0.21 for Binadhan 6; 54.55% and 0.23 for Binasail; 54.55% and 0.19 for BRRI dhan29; 50.00% and 0.19 for Binadhan 5 and 45.45% and 0.18 for BRRI dhan28, respectively. The coefficient of gene differentiation (G_{st}) across all loci was calculated as 0.35 reflecting the existence of high level of genetic variation among the six modern rice varieties. UPGMA dendrogram based on Nei's genetic distance segregated the six high yielding rice varieties into two clusters: all four mutant varieties viz. Binadhan 4, Binadhan 5, Binadhan 6 and Binasail formed one cluster and two varieties of BRRI grown in boro season, BRRI dhan28 and BRRI dhan29 grouped together in another cluster. Among the mutants, two boro season varieties, developed from the same parent, Binadhan 5 and Binadhan 6 grouped together with genetic distance of 0.10. Therefore, RAPD offer a reliable method to evaluate genetic variation and relatedness among the high yielding rice varieties.

Keywords: Genetic variation, RAPD markers, Rice

INTRODUCTION

Rice (*Oryza sativa* L.) is the most important and extensively grown food crop leading cereal in the world including Bangladesh. Over the last two decades the entire growth in rice production has been increased mainly due to the conversion of local to high yielding varieties (HYVs) (World Bank, 1995). HYV rice is developed from local varieties through different improved breeding programs. Intra- or inter-specific genetic variation among different varieties plays a key role in the improvement of grain through the breeding programs. Therefore, knowledge of genetic variation is essential in any breeding program.

Plant genetic diversity is a key component of any agricultural production system. This genetic diversity or similarity is measured through genetic markers and is used to determine evolutionary relationship within and between species, genera or higher taxonomic

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categories (Paterson *et al.*, 1991). However, breeders tend to concentrate on specific genotypes for determination of genetic diversity which combine traits of interest and may be used as progenitors in several breeding programmes in order to introduce agronomical important traits. Molecular markers are the molecules that could be used to trace a desired gene (s) in examined genotypes. DNA markers seem to be the best candidates for efficient evaluation and selection of plant material. Unlike protein markers, DNA markers segregate as single genes and they are not affected by the environment. Genetic variation increasingly are being estimated based upon information at the DNA level by various molecular markers such as, Randomly amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLP), Simple Sequence Repeat (SSR) or Microsatellite etc. Among them, RAPD markers, generated by the polymerase chain reaction (PCR) is widely used since 1990's to assess intra-specific genetic variation at nuclear level (Welsh and McClelland, 1990; and Williams *et al.*, 1990).

The RAPD technology provides a quick and efficient screen for DNA sequence–based polymorphisms at a very large number of loci. The vast range of potential primers that can be used gives the technique great diagnostic power. Reproducible RAPD bands can be found by a careful selection of primers, optimization of PCR conditions for the target species, and replication to ensure that only the reproducible bands are scored. In rice, RAPD analysis has been used extensively for various purposes which include identification and classification of accessions (Virk *et al*., 1995), identification of hybrids (Qian *et al*., 1996), genetic diversity analysis (Mackill, 1995; Cao and Oard., 1997) and predicting quantitative variation within germplasm (Virk *et al.*, 1996). The aim of the present work is to evaluate genetic variation and relatedness of some indigenous rice germplasm by RAPD technique as it is important particularly for variety selection for breeding purpose, even in evaluation and conservation of their diverse gene pool.

MATERIALS AND METHODS

Study Materials

Six high yielding rice varieties viz. Binadhan 4, Binadhan 5, Binadhan 6, Binasail, BRRI dhan28 and BRRI dhan29 were used in the present study. The seeds of the varieties were collected from Genetic Resource Centre (GRS), Bangladesh Rice Research Institute (BRRI) and Bangladesh Institute of Nuclear Agriculture (BINA). Varieties were different in breeding strategies, production potentials, cultivation season etc. Plants were regenerated from the seeds in the laboratory of BINA. Leaf tissues of the plants were used as source of DNA in the experiment.

Extraction of Genomic DNA

Total genomic DNA was isolated from young actively growing fresh leaf tissues following SDS extraction, phenol: chloroform: isoamyl alcohol purification and ethanol precipitation method. Approximately 25 mg leaf tissues was cut into small pieces, homogenized and digested with extraction buffer [50 mM Tris-HCl, 25 mM ethylenediaminetetraacetic acid (EDTA), 300 mM NaCl and 1% sodium dodecyl sulphate (SDS)] at 65ºC for 15 min. DNA was purified by extraction with phenol: chloroform: isoamyl alcohol (25 : 24 : 1, v:v:v). DNA was precipitated using absolute alcohol in the presence of 0.3M sodium acetate and pelleted by centrifugation. The pellets were then washed with 70% ethanol, air dried and resuspended in an appropriate volume of TE buffer

(10 mM Tris-HCl, 1 mM EDTA, pH=8.0). DNA quality was checked by electrophoresis in a minigel. DNA samples were then quantified using a UV-spectrophotometer.

Primer selection

Initially, 20 decamer random primers from three kits (5 from kit A, 10 from kit B and 5 from kit C) of random sequence (Operon Technologies, USA) were screened on a sub sample of two randomly chosen individuals to test their suitability for amplification of the DNA sequences, that could be scored accurately. Primers were evaluated based on intensity of bands, consistency within individual, presence of smearing, and potential for population discrimination. A final subset of four primers exhibiting good quality banding patterns and sufficient variability were selected for further analysis.

PCR amplification

The amplification conditions were based on Williams *et al.* (1990) with some modifications. PCR reactions were performed on each DNA sample in a 10 µl reaction mix containing 1 µl of 10X Ampli *Taq* polymerase buffer, 2 µl of 10 µM primer, 1 µl of 10 mM dNTPs (Bangalore Genei, India), 1 unit of Ampli *Taq* DNA polymerase (Bangalore Genei, India), 100 ng genomic DNA and a suitable amount of sterile deionized water. DNA amplification was performed in an oil-free thermal cycler (Master Cycler Gradient, Eppendorf). The reaction mix was preheated at 94^{o}C for 3 minutes followed by 40 cycles of 1 min denaturation at 94^{o}C, 1 min annealing at 34^{o}C and elongation or extension at 72^{o}C for 2 minutes. After the last cycle, a final step of 7 minutes at 72^{o}C was added to allow complete extension of all amplified fragments. After completion of cycling program, reactions were held at 4^{o}C.

Agarose gel electrophoresis

The amplified product from each sample was separated electrophoretically on 1.4% agarose gel (Nacalai tesque, Inc., Kyoto, Japan) containing ethidium bromide in 1X TBE buffer at 120 V for 1 hour and 15 min. Two molecular weight marker DNA (Phi X 174 DNA / *Hae*III digest and / or 100 bp DNA ladder) was electrophoresed alongside the RAPD reactions. DNA bands were observed on UV-transilluminator and photographed with a Gel Cam Polaroid camera.

Data analysis

The RAPD markers were scored visually on the basis of their presence (1) or absence (0), separately for each individual and each primer. For more accuracy, band scoring was performed by two independent persons. Bands not identified by the two readers were considered as non-scorable. The scores obtained using all primers in the RAPD analysis were then pooled for constructing a single data matrix. This was used for estimating polymorphic loci, Nei's (1973) gene diversity, co-efficient of gene differentiation (G_{st}), genetic distance (D) and constructing a UPGMA (Unweighted Pair Group Method of Arithmetic Means) dendrogram using computer program POPGENE (Version 1.31) (Yeh *et al.*, 1999). The same program was also used to perform pair wise homogeneity test across different loci. The similarity index values (SI) between the RAPD profiles of any two individuals on the same gel were calculated from RAPD markers according to the following formula:

$$\text{Similarity index (SI)} = 2\, N_{xy} / N_x + N_y$$

Where, N_{xy} is the number of RAPD bands shared by individuals x and y respectively, and N_x and N_y are the number of bands in individual x and y, respectively (Lynch 1990). Within population, similarity (S_i) was calculated as the average of SI across all possible comparisons between individuals within a population. Between population similarity (S_{ij}) was calculated as the average similarity between randomly paired individuals from populations *i* and *j* (Lynch, 1991).

RESULTS AND DISCUSSION

RAPD Profile

All the four primers generated a total of 22 bands with size ranging from 200-1500 bp where, 18 bands (81.82%) were found to be polymorphic (either occurring in or absent in <95% of all individuals) and 4.5 polymorphic bands were produced per primer. Parameters of the four primers studied are presented in the Table 1. Primer OPB07 produced the highest number of polymorphic bands (9). Thus it showed a high level of polymorphism. On the other hand, the primer OPA10 generated least number of polymorphic bands. The banding patterns of different rice varieties using primers OPA10, OPB07, OPB10 are shown in Figure 1.

Intra-and inter-variety similarity indices

The values for intra-variety similarity indices (S_i) were higher (average: 79.34%) than inter-variety similarity indices (S_{ij}) (average: 76.43). The highest S_i value was found in BRRI dhan28 (85.10%) followed by that of Binadhan 5, BRRI dhan29, Binasail, and Binadhan 6 respectively (Table 2). The S_i value for Binadhan 4 was the lowest (73.95%). On the other hand, inter-variety similarity indices between BRRI dhan28 and BRRI dhan29 was found to be the highest (82.35%) and the lowest band sharing value (68.74%) was observed between Binadhan 4 and BRRI dhan29 variety combination (Table 3).

Table 1. RAPD primers with corresponding bands scored and their size ranges together with polymorphic bands observed in six high yielding rice varieties

Primer codes	Sequences (5′-3′)	Total number of bands scored	Size ranges (bp)	Number of polymorphic bands
OPA-10	GTGATCGCAG	2	260-530	1
OPB-07	GGTGACGCAG	10	250-1078	9
OPB-10	CTGCTGGGAC	4	310-1353	2
OPC-01	TTCGAGCCAG	6	200-1500	6
Total		22		18

Table 2. Estimates of genetic variation: number and proportion of polymorphic loci, intra-variety similarity indices (S_i) as well as gene diversity obtained in different high yielding rice varieties

Varieties	Number of polymorphic loci	Proportion of polymorphic loci (%)	Intra-variety similarity indices (S_i)	Gene diversity
Binadhan 4	13	59.09	73.95	0.25
Binadhan 6	13	59.09	77.19	0.21
Binasail	12	54.55	79.29	0.21
BRRI dhan29	12	54.55	80.02	0.19
Binadhan 5	11	50.00	80.84	0.19
BRRI dhan28	10	45.45	85.10	0.18

Table 3. Inter-variety similarity indices (%) (above diagonal) and pair wise genetic distances (below diagonal) in different high yielding rice varieties

Varieties	Binadhan 4	BRRI dhan28	BRRI dhan29	Binadhan 5	Binadhan 6	Binasail
Binadhan 4	***	74.25	68.74	80.36	81.25	79.85
BRRI dhan28	0.26	***	82.35	70.37	75.62	79.16
BRRI dhan29	0.37	0.04	***	69.17	75.57	76.30
Binadhan 5	0.11	0.27	0.35	***	81.92	75.14
Binadhan 6	0.11	0.20	0.24	0.10	***	76.43
Binasail	0.14	0.14	0.18	0.17	0.14	***

The higher S_i values reflect lower genetic variability within the individuals of BRRI dhan28 variety. Lower degree of similarity (S_i =73.95%) between RAPD profiles of the individuals of Binadhan 4 variety could be indicative of a higher genetic variability in that variety. Lower level of inter-variety similarity indices between Binadhan 4 vs. BRRI dhan29, Binadhan 5 vs. BRRI dhan29 and Binadhan 5 and BRRI dhan28 indicate that there exists a greater genetic distance between these populations. On the other hand, higher level of inter-variety similarity indices in BRRI dhan28 vs. BRRI dhan29; Binadhan 5 vs. Binadhan 6; Binadhan 4 vs. Binadhan 6 reflect the existence of lower level of genetic distance between these cultivar pairs.

Polymorphism in different high yielding rice varieties

The number and proportion of polymorphic loci was found to be the highest in Binadhan 4 and Binadhan 6 (Table 2). Highest level of Nei's (1973) gene diversity value (0.25) was also observed in this variety. On the other hand, minimal proportion of polymorphic loci and Nei's gene diversity values (45.45% and 0.18, respectively) were found in BRRI dhan28 (Table 2). Among the polymorphic loci, eight loci (OPA10-1, OPB07-5, OPB07-9, OPB07-10, OPC01-2, OPC01-3, OPC01-5 and OPC01-6) were found to cause significant departure from homogeneity in different variety combinations. Overall gene differentiation (G_{st}) of the studied rice varieties across all loci were 0.35.

From intra-variety similarity indices, Nei's gene diversity (1973), and proportion of polymorphic loci points of view, it is clear that Binadhan 4 was likely to be the most diversified variety among the six modern varieties whilst BRRI dhan28 was found to be the least diversified variety compared to the others.

Genetic Distance and dendrogram

Pair-wise Nei's (1972) genetic distances (*D*) ranged from 0.04 to 0.37 (Table 3). Comparatively high level of genetic distances was observed in Binadhan 4 vs. BRRI dhan29; Binadhan 5 vs. BRRI dhan29 and Binadhan 5 vs. BRRI dhan28 variety pairs. The lowest genetic distance (0.04) was found between BRRI dhan28 and BRRI dhan29 variety. The mean genetic distance among the four mutant varieties was 0.13 which is higher than the value (0.04) among the two varieties developed through hybridization breeding program. This implies that the genetic distance among the mutant varieties were found to be higher.

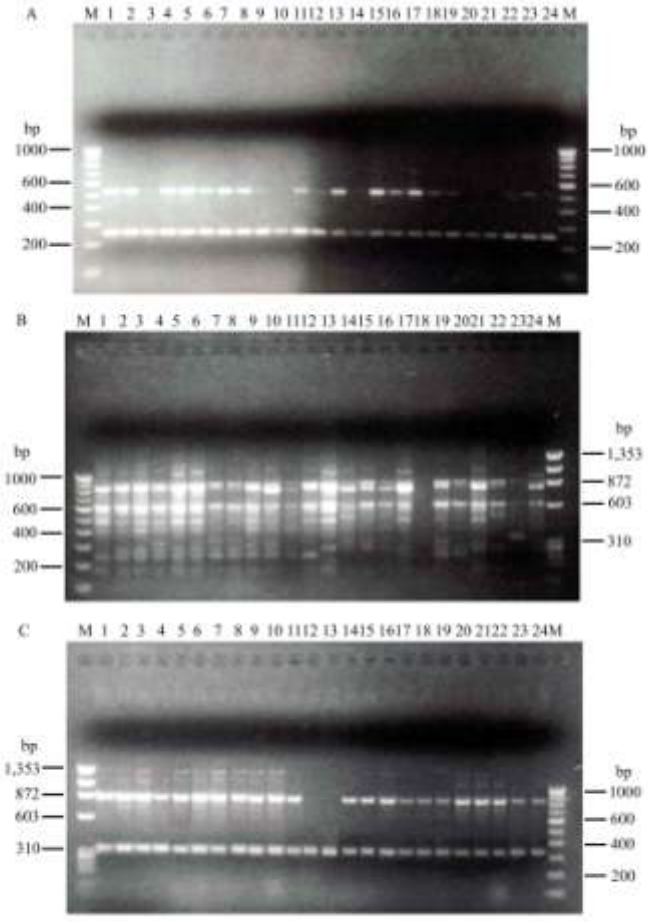

Figure 1. RAPD profiles of six high yielding rice varieties using primers OPA10 (A), OPB07 (B) and OPB10 (C), respectively. Lane 1-4: Binadhan 4; lane 5-8: Binadhan 5; lane 9-12: Binadhan 6; lane 13-16: Binasail; lane 17-21: BRRI dhan28 and lane 22-24: BRRI dhan29. Ms: Molecular weight markers (100 bp DNA ladder and Phi X 174 DNA/ Hae III digest).

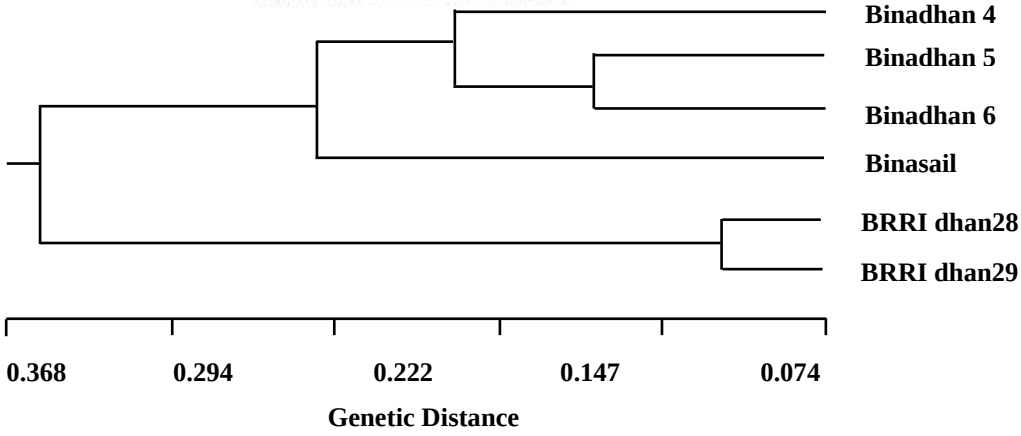

Figure 2. UPGMA dendrogram based on Nei's (1972) genetic distance summarizing the data on differentiation between six high yielding rice varieties, according to RAPD analysis

High level of mean Nei's (1972) genetic distance (D = 0.187) among the samples might be due to a diverse parental line and breeding strategy. However, the value is lower than those (mean D = 0.27) calculated for commercial U.S. rice varieties reported by Cao and Oard (1997).

The UPGMA dendrogram based on Nei's (1972) genetic distance segregates the six modern high yielding rice varieties into two groups: all four mutants, Binadhan 4, Binadhan 5, Binadhan 6 and Binasail in one cluster and two BRRI released varieties grouped together in another cluster (Figure 2). Among the mutant high yielding rice varieties, Binadhan 5 and Binadhan 6 formed a sub-cluster with lowest genetic distance (0.099). These two boro varieties developed from the same parent followed by same breeding strategy (gamma radiation in the F_2 generation of the cross between Iratom 24 and Dular). Explanation of highest genetic distance between Binadhan 5 and Binasail is that Binadhan 5 is Boro season rice while Binasail is T-Aman rice. In addition to their ecotype difference, their parental linkage also differs from each other. However, Binasail differ from the other three mutants by the year of release. Binadhan 4, Binadhan 5 and Binadhan 6 were released in 1998 while, Binasail was released in 1987. Diversity according to year of release was also reported by Khondker (2003) during studying genetic diversity of BRRI released varieties using microsatellite markers. The lowest genetic distance between BRRI dhan28 and BRRI dhan29 is consistent with the fact that these two varieties are being grown in boro season and their parental linkage information showed the involvement of the same crossing line as one of the immediate parent of these varieties. However, the highest genetic distance between Binadhan 4 and BRRI dhan29 reflects that Binadhan 4 is a T-Aman season and BRRI dhan29 is a Boro season variety.

The high level of co-efficient of gene differentiation (Gst = 0.35) among the studied rice varieties and significant (P_{95}) departure from homogeneity at a number of loci (OPA10-1, OPB07-5, OPB07-9, OPB07-10, OPC01-2, OPC01-3, OPC01-5 and OPC01-6) revealed that sufficient level of genetic variation exists among the rice varieties. Varieties having close proximity in their origin and breeding strategy are likely have less genetic distance from each other i.e., they are genetically more similar (Nei, 1978). The result of the study will be useful in the future breeding program. However, more samples and primers would be necessary to generate an appropriate genetic relationship among different rice varieties in future.

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