RAPD Profile Analysis of Single and Multigrain Aman Rice (*Oryza sativa* L.) Varieties Available in Bangladesh

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Abstract

Genetic variation in 14 local Aman rice varieties of Bangladesh including single grain and multigrain rice was investigated at the DNA level by RAPD molecular typing. Fifteen random primers were initially screened against DNA from five individuals that generated highly reproducible RAPD fragments, which were then subjected to further population analysis. A total 191 RAPD fragments were generated with these primers and 153 (80.1%) were polymorphic, which indicated high level of polymorphism existed within these populations. The size of amplified fragments ranged between 450 and 6000 bp. Pair-wise distance estimated the range of 0.03 to 0.59 between single and multigrain Aman rice varieties. Results illustrated the potential of RAPD markers to distinguish improved varieties and grain specificity at DNA level.

Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops around the world, which provides the necessary daily calories and protein (15%) for more than half of the world's population (Singh et al. 2012; Tyagi et al. 2004, Maclean et al. 2002). At least 114 countries grow rice and approximately 90% of the world's rice is grown in the Asia (Salim et al. 2003, Paranthaman et al. 2009). Over the last 1000 years, rice has been the dominant crop in Bangladesh and at least 1,000 traditional varieties cultivated per landraces (Hossain and Jaim 2009). Most of the rice varieties of Bangladesh are growing in the Aman season. It is the second largest growing season of the country in respect of the quantity of production. It is notable that the area coverage of Aman is the largest as a single crop which is approximately 51% of total land area (bbs.gov.bd).

Recently a new Aman rice having a unique multigrain feature has been reported from Bangladesh (Krisoks' Weblog 2012). This unique rice has two or three grains within a seed. However, the reason behind the multigrain seed nature is still unknown due to lack of experimental reports on this aspect. The phylogenetic divergence of single- and multigrain Aman rice varieties of *Oryza sativa* L. is also not well understood. Under this circumstance, it is worth to characterize this unique rice variety and shed light on its evolutionary divergence pattern within and between other Aman rice varieties of Bangladesh. To find out the relationship and differences among the cultivars based on only morphological characters sometimes do not produce convincing conclusions. However, molecular markers provide information that helps in deciding the distinctiveness of species and their ranking according to the number of close relatives and phylogenetic position (Rahman et al. 2007). Several types of molecular markers are available for evaluating the extent of genetic variation in rice. These include RFLP (Botstein et al. 1980), RAPD (Williams et al. 1990), AFLP (Vos et al. 1995) and SSR (Tautz 1989). Of these RAPD markers are increasingly being employed in genetic research owing to its speedy process and simplicity (Williams et al. 1990). This technique always allows the examination of genomic variation without prior knowledge of DNA sequences (Hadrys et al. 1992). It is especially useful for unzipping the variations in species with low genetic variability when other techniques such as isozyme analysis fail to reveal differences among the individuals. Moreover, varietal distinctiveness and relativeness can unambiguously be estimated by RAPD fingerprinting in commercially important crops. Previously it was reported that the level of genetic differentiation between cultivars of *O. sativa* increased with geographical distance. The polymorphisms detected among the accessions are helpful in selecting genetically diverse cultivars in future breeding programme (Deepu et al. 2013). RAPD analysis also showed promise as an effective tool in estimating genetic polymorphism in different rice cultivars.

Traditional rice breeding has made significant progress towards higher yield, improved quality, greater disease resistance and other important agricultural characters in the past and even in future, it will still play an important role (Sun et al. 1990). With the growth of global population and the increasing demand for food, rice represents 27% of the dietary energy supply for the population in developing countries (Redoña and Mula 2004). To accommodate the increasing demand, new rice varieties with high yield potential and specific marker for the improvement of new variety is in much demand. To achieve this is goal, new strategic and innovative approaches are urgently needed. This study aimed at using RAPD markers to evaluate the genetic variation within a collection of local

single- and multigrain Aman rice varieties and to reveal genetic relationships among them for future use in selection, hybridization, biodiversity assessment and conservation of diverse gene pools.

Materials and Methods

A total of 14 varieties of Aman rice used in this experiment are listed in Table 1. The seeds of 11 varieties (BRRI Dhan-33, 37, 39, 40, 46, 49, 51, 53, 57, 62 and Khajur Jhupi) were released by Bangladesh Rice Research Institute (BRRI), Gazipur, Bangladesh. The rest of the three varieties are local of which Biram sundari and Double rice are multigrain (Fig. 1).

DNA extraction: Young, fresh leaves were collected from 25 days old pot grown seedlings to extract genomic DNA by cetyl trimethyl ammonium bromide (CTAB) method (Doyle and Doyle 1987). The leaves were washed in distilled water and ethanol and dried on fresh tissue paper to remove spores of microorganisms and any other source of foreign DNA. Four hundred mg leaf tissue taken in liquid nitrogen and grinded to fine powder then added 1.6 ml extraction buffer (3% CTAB; 1.4 M NaCl; 100 mM Tris-HCl, pH 8.0; 20 mM EDTA, pH 8.0 and 0.2% mercaptoethanol). The homogenous paste transferred to an Eppendorf tube (2.0 ml) and incubated at 60^0C in a water bath for 30 min. The samples were centrifuged at 13,000 rpm for 10 min at 4^0C then equal volume of chloroform : isoamyl alcohol (24 : 1) was added with the supernatants and centrifuged the tubes at 13,000 rpm for 10 minutes. The supernatant with 2/3 vol. chilled isopropanol was kept for overnight at -20^0C to precipitate the DNA. The supernatants were discarded carefully after centrifuged for 10 min at 13,000 rpm and DNA pellet was collected. The pellet was washed with 70% ice-cold ethanol and air dried. The dried DNA was dissolved in 50 µl of TE buffer and treated with RNase A for 30 min at 37°C and store at –20°C. The concentration of DNA was estimated with Nanodrop.

DNA amplification: For RAPD analysis 15 decamer primers were applied which had 60 or 70% of G + C content. Twenty five µl reaction mixture contains 2.5 µl 10 × *Taq* DNA buffer, 100 µM dNTPs, 1 µM primer, 1U *Taq* polymerase (Thermo-scientific) and 50 ng genomic DNA. PCR amplification mixture maintained at 0ºC during its preparation. The PCR amplification mixture placed in a thermal cycler (Applied Biosystems) with the following thermal cycling conditions, these were one cycle of 94ºC for 5 min, 35 cycles of 94ºC for 45 seconds, 32 or 34ºC for 30 seconds, and 72ºC for 3 min then one cycle of 72ºC for 7 min. After completion of cycling programme, the reactions were held at 4ºC.

Table 1. List of selected Aman rice varieties used in the genetic analysis.

Sl.	Name of varieties	Source
1	Khajur Chori (Acc. No. 4795)	Khulna, Sadar (BRRI)
2	Khajur Jhupi (Acc. No. 2551)	BRRI Breeding Line
3	Double Rice (Acc. No. 3905)	Tangail, Sadar (BRRI)
4	Biram Sundari	Birampur, Dinajpur (PB & B Lab. DU)
5	BRRI Dhan 33	"
6	BRRI Dhan 37	"
7	BRRI Dhan 39	"
8	BRRI Dhan 40	"
9	BRRI Dhan 46	"
10	BRRI Dhan 49	"
11	BRRI Dhan 51	"
12	BRRI Dhan 53	"
13	BRRI Dhan 57	"
14	BRRI Dhan 62	"

Fig. 1. Seeds and grains of different Aman rice varieties used in the present investigation. Identification of varieties (1 - 14) is shown in Table 1.

The amplified products were separated electrophoretically on 1% agarose gel with 1 × TAE buffer and ethidium bromide. Agarose gel electrophoresis was conducted in 1 × TAE buffer at 90 volts and 250 mA for 45 min. One molecular weight marker 1 kb plus or 1 Kb DNA ladder (GeneRuler™) was electrophoresed alongside the amplified product. Gel was photographed by using UV Transilluminator (Cleaver Scientific Ltd.).

Results and Discussion

The genetic diversity and the relationship among 14 Aman rice genotypes were evaluated by using 15 primers. Initially 20 primers were tested and out of them, 15 primers that gave rise to amplified products were selected for evaluating genetic relationship of rice cultivars (Table 1). Figs 2 and 3 are presented to present the amplification profiles generated with the primers across 14 single grain and multigrain rice varieties. A considerable level of variability was observed among different cultivars. A total of 191 reproducible and scorable amplification products were generated across 14 varieties (Table 2) and these were in the size range of 450 to 6000 bp. The number of amplification products generated by each primer varied from 5 (OPB-9) to 23 (OPB-7) with an average of 12.7 bands per primer. Out of 191 bands, 153 (80.1%) were found to be polymorphic for one or more cultivars ranging from 5 to 20 fragments per primer. Primers OPB-03 resulted in the highest percentage of polymorphic bands, while the minimum polymorphism was observed using OPA-3 primer. RAPD analyses in *O. sativa* accessions were also reported earlier (Muhammad et al. 2005, Rahman et al. 2007, Malik et al. 2008). RAPD markers also have been used to detect polymorphisms in different crops like maize 72.2% (Carvalho et al.2004), wheat 49.12% (Nimbal et al. 2009), canola 61% (Moghaieb et al. 2014) and cabbage 84. 44% (Saxena et al. 2011). In this investigation, banding pattern of primer OPB01, OPB02, OPB06, OPB07, OPB08, OPB11, OPB12, OPB14 and OPK08 were crucial for detecting and identifying polymorphism based in variety specific unique bands. Three multigrain varieties *viz.* Biram Sundari and Double rice specific unique bands were identified with primer OPB01 and OPK08 (Figs 2 and 3). The banding profiles of these multigrain containing rice varieties are clearly distinguishable from single grain containing rice variety, although they share similar pattern in banding profile within themselves.

A distance matrix based on the proportion of shared RAPD fragments was used to establish the level of divergence between and within the 14 Aman rice varieties. The Nei's distance matrix (Nei 1972) was employed to estimate the genetic distances among the varieties (Table 3). It was observed that the genetic

distances were highly variable among 14 Aman rice varieties, ranged from 0.0373 to 0.5983 as revealed by the genetic distances matrix. Deepu et al. (2013) reported 0.19 - 0.54 genetic distances within Indian cultivars and Yeasmin et al. (2013) showed 0.088 - 0.504 within BRRI varieties. Garris et al. (2005) also found overall higher gene diversity for the indica group (0.55) and lower for japonica varieties (0.47). On the contrary, Thomson et al. (2007) reported as slightly higher index of

Table 2. Analysis of reproducible amplified loci with different RAPD primers within the rice varieties.

Marker ID	Sequence (5′—3′)	Total no. of RAPD loci	No. of polymorphic RAPD loci	Percentage of polymorphism
OPA02	TGC CGA GCT G	9	6	66.67
OPA03	AGT CAG CCA C	9	5	55.56
OPB01	GTT TCG CTC C	21	17	80.95
OPB02	TGA TCC CTG G	16	15	93.75
OPB03	CAT CCC CCT G	12	12	100
OPB04	GGA CTG GAG T	9	6	66.67
OPB05	TGC GCC CTT C	9	5	55.56
OPB06	TGC TCT GCC C	15	9	60.0
OPB07	GGT GAC GCA G	23	20	86.96
OPB08	GTC CAC ACG G	12	9	75.0
OPB09	TGG GGG ACT C	5	4	80.0
OPB11	GTA GAC CCG T	12	11	91.67
OPB12	CCT TGA CGC A	11	9	81.82
OPB14	TCC GCT CTG G	17	16	94.13
OPK08	GAA CAC TGG G	11	9	81.82

gene diversity for the japonica group (0.56), while the indica group was 0.54. Relatively high distance index was observed in 'BRRI Dhan-51' *vs* 'BRRI Dhan-37', 'BRRI Dhan-53' *vs* 'BRRI Dhan-37', 'BRRI Dhan-53' *vs* 'Khajur chori', 'BRRI Dhan-57' *vs* 'BRRI Dhan-37' variety pairs comparing to the other cultivar pairs. The lowest distance index was observed in 'Biram sundari' *vs* 'Double rice' (0.03) (Table 3). Genetic distance was lowest between Biram sundari and Double rice that indicates close relatedness within multigrain varieties. This result exhibited two multigrain varieties were same or originated from the same origin or genetic variation between these varieties increased because of their geographical distance (Deepu et al. 2013). This result indicates that genotypic variation does

not necessarily reflect in phenotypic characters (Cirillo et al. 2009). Out of 12 single grain varieties breeding line Khajur Jhupi, BRRI Dhan-46 and BRRI Dhan-49 have low genetic distance with multigrain variety.
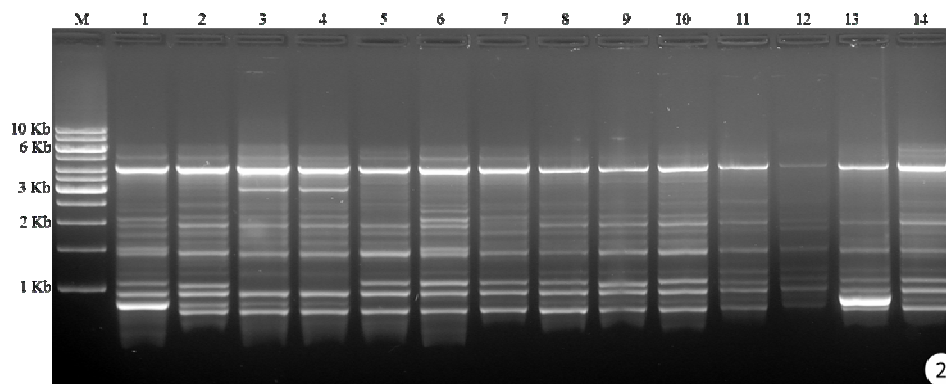

Fig. 2. RAPD profiles of the 14 Aman rice varieties with OPB-1 primer. Lane-M Ladder (1Kb) and lane 1-14, amplified DNA of (1) Khajur Chori, (2) Khajur Jhupi, (3) Double Rice, (4) Biram-sundari, (5) BRRI Dhan-33, (6) BRRI Dhan-37, (7) BRRI Dhan-39, (8) BRRI Dhan-40, (9) BRRI Dhan-46, (10) BRRI Dhan-49, (11) BRRI Dhan-51, (12) BRRI Dhan-53 (13) BRRI Dhan-57 and (14) BRRI Dhan-62, respectively.
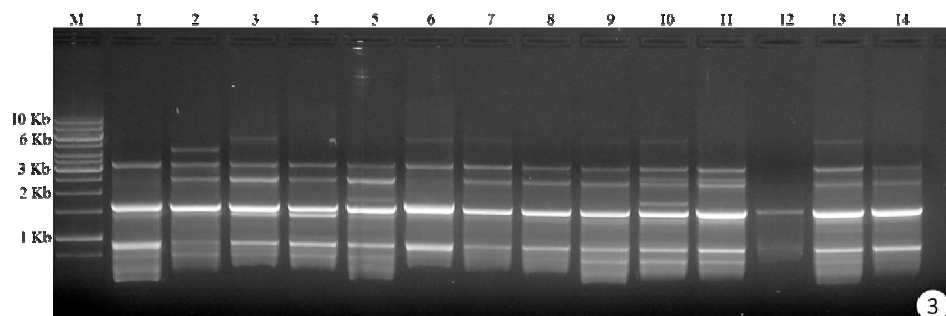

Fig. 3. Same as Fig. 2 but using OPK-8 primer.

Genetic distance obtained from RAPD data was used to create a cluster diagram. Cluster analysis based on Nei (1972) distance matrix using UPGMA grouped 14 Aman rice cultivars into 6 major clusters at the similarity coefficient of 0.44 (Fig. 4). Cluster I consisted of Khajur Chori and hybrid variety BRRI Dhan-37 indicating relatively less divergence among the cultivars of this cluster due to originating from closely related ancestors. Cluster 2 included two multigrain varieties Biram sundari, Double rice and inbred line Khajur Jhupi. In this cluster, the coefficient of distance ranged from 0.16 to 0.19 indicating high relatedness among the multigrain forming varieties. The remaining hybrid varieties that was released by BRRI were aggregated in clusters 3, 4 and 5. BRRI

Table 3. Genetic distances matrix among the Aman rice varieties (Nei's, 1972).

Rice varieties	K C	K J	D R	B S	BD-33	BD-37	BD-39	BD-40	BD-46	BD-49	BD-51	BD-53	BD-57	BD-62
Khajur Chori	0													
Khajur Jhupi	0.3847	0												
Double Rice	0.3925	0.1647	0											
Biram-sundari	0.3847	0.1960	0.0373	0										
BRRI Dhan-33	0.3771	0.2964	0.3035	0.3250	0									
BRRI Dhan-37	0.3250	0.4240	0.4320	0.4565	0.4002	0								
BRRI Dhan-39	0.3619	0.2687	0.3178	0.3396	0.1709	0.3544	0							
BRRI Dhan-40	0.3323	0.2825	0.2894	0.2964	0.2218	0.4648	0.1585	0						
BRRI Dhan-46	0.3695	0.2755	0.2284	0.2618	0.2153	0.4731	0.2024	0.1647	0					
BRRI Dhan-49	0.4320	0.2894	0.2284	0.2618	0.2153	0.4731	0.2153	0.1524	0.1224	0				
BRRI Dhan-51	0.4648	0.2755	0.2687	0.2755	0.2964	0.5983	0.2687	0.2024	0.1709	0.1834	0			
BRRI Dhan-53	0.5073	0.4160	0.4081	0.4160	0.3470	0.5888	0.3470	0.3178	0.3250	0.2964	0.3544	0		
BRRI Dhan-57	0.4081	0.2825	0.2618	0.3106	0.2618	0.5338	0.3035	0.2350	0.2153	0.2416	0.2687	0.2755	0	
BRRI Dhan-62	0.3771	0.2284	0.2350	0.2687	0.2218	0.4648	0.2350	0.2350	0.2284	0.1897	0.2416	0.2483	0.1834	0

Dhan-33, BRRI Dhan-39 and BRRI Dhan-40 were grouped under the cluster 3. Cluster 4 included BRRI Dhan-46, BRRI Dhan-49 and BRRI Dhan-52. Cluster 5 included two varieties BRRI Dhan-57 and BRRI Dhan-62. Only one variety BRRI Dhan-53 included in cluster 6 which indicated more divergence. Crossing between the genotypes with high distance coefficient will manifest high heterosis. As regards to higher quality status of 'BRRI Dhan-53' among cultivars, it is essential that to pay more attention to it in hybridization programs.
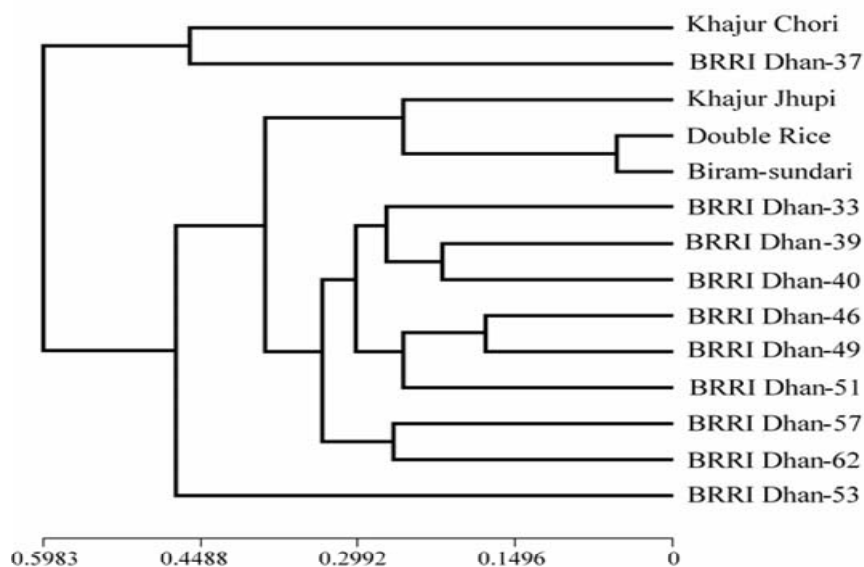

Fig. 4. UPGMA dendrogram of the unweighted pair group method with arithmetic averaging cluster analysis revealed by random amplified polymorphic DNA.

Through the present work it has been possible to find out the genetic variation and relatedness among the 14 Aman rice germplasm including single and multigrain features. Genetic variability is the key component for the successful crop improvement programme (Ravi et al. 2003). The employment of RAPD markers in genetic diversity analysis helped in grouping the genotypes. Cluster analysis based on the RAPD data revealed that although the morphology of multigrain varieties was same but they exhibited little genetic distance between them. In addition, the present study showed that cultivars in first cluster possess high degree of genetic similarity possibly due to originating from closely related ancestors. The results of the present investigation have opened up a possibility for developing a molecular genetic map that will lead to the application of marker assisted selection tools in genetic improvement of Aman rice.

Acknowledgements

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