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Genetic diversity analysis of high yielding rice (*Oryza sativa*) varieties cultivated in Bangladesh

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Abstract

Investigation of genetic diversity and molecular characterization in high yielding rice varieties is important for their identification. The experiment was conducted during 2016 - 2017 to analyse the genetic diversity of fifteen high yielding rice varieties in Bangladesh by using random amplification of polymorphic DNA (RAPD) markers. Polymorphism was revealed in 12 RAPD primers out of 30, whereas no other reaction was detected on the remaining 18 primers. The 40 out of 45 bands (88.89%) polymorphics were produced by the primers and ranged from 50 to 100%. The maximum number of polymorphic bands was produced by the primer OPB-18 whereas the lowest number of polymorphic bands belonged to OPC-12. The genetic similarity coefficients were determined with the RAPD data, which ranged from 0.47 to 0.94. The unweighted paired group of arithmetic means (UPGMA) dendrogram presented the studied rice varieties into two major clusters. Moreover, the value of Nei's genetic diversity is 0.26 and the Shanon information index is 0.41. The study produced distinct positions, suggesting that the genotypes were different from each other. The results indicated that these markers could be efficient for comparing the genetic relationships, patterns of variation, and measurement of genetic distance among rice varieties. Considering all of these results, RAPD analysis is found to be an effective tool for estimating the genetic diversity of different rice varieties. The outcomes of this research may contribute to the germplasm data of rice accessions and a future breeding program of rice genotypes.

Keywords: genetic polymorphism, molecular marker, rice, similarity coefficient

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Introduction

Rice (*Oryza sativa* L.) is the main food source for more than two fifths (2.4 billion) of the world's population (Halder et al., 2016). Asia is the leading continent for the production and consumption of rice in the world. Rice occupies 11% of the world's crop field annually, and it ranks next to wheat (Chakravarthi and Naravaneni, 2006; Won et al., 2017). The conservation and characterization of high yielding varieties (HYV) rice germplasm is an important issue for breeding programme in Bangladesh. An effective management and exploitation of rice germplasm prerequisites an accurate characterization and classification. However, with an increasing global population, the demand for rice will continue to rise, which raises challenges for the breeding of high yielding rice cultivars (Zhang et al., 2013). On the other hand, several researchers reported that the most reliable method based on DNA sequence by molecular markers (Kresovich and McFerson, 1992; Mani et al., 2010).

Perspectives of crop improvement mostly rely on the genetic diversity of rice genotypes and the implementation of modern biotechnological findings. A rich diversity of rice accessions exists in Bangladesh, as it is the centre of origin of indica type rice. However, the diversity of Bangladeshi rice accessions has not been studied comprehensively at the molecular level. Whereas, many of these rice accessions are on the edge of extinction. Hence, it is essential to conduct proper conservation and characterization of existing rice varieties.

Some of the local varieties are not properly represented to conduct genetic diversity. A number of molecular markers studied Rice characteristics. Random amplified polymorphic DNA (RAPD) is a prevalent and powerful typing method based on marker technique to analyse genetic diversity at the population level and among different genotypes (Saker et al., 2018). The RAPD technique presents a higher level of polymorphism, very informative and speedy process. Enzymatic amplification of target DNA studied by PCR using arbitrary primers with the RAPD technique, also intra and inter distances of genetic markers is determined more accurately by the RAPD with a very small amount of sampling DNA (Weising et al., 1995; Islam et al., 2015).

The RAPD technique has been used extensively in the analyses of rice to estimate genetic identification and classification of accessions (Davierwala et al., 1990; Fukuoka et al., 1992; Saker et al., 2018), genetic potential and diversity (Cao and Oard, 1997), fingerprinting and duplication of accessions (Virk et al., 1995; Virk et al., 1996), and particularly important for variety evaluation and conservation existing diverse gene pool, hybridization, selection and breeding purposes (Rabbani et al., 2008). The molecular markers are vital to study the genomic variability and genetic relatedness among rice genotypes of Bangladesh (Alam et al., 2016).

Lack of information on the genetic diversity of HYV rice in Bangladesh requires using the RAPD technique. The knowledge on genetic polymorphism is essential for the conservation of existing rice germplasm and further promotion of the breeding program. Therefore, the present study is designed to assess the extent of the performance of HYV of Bangladeshi rice accessions using RAPD primers to compare genetic polymorphism between genotypes and to conduct complex analyses for rice breeding purposes.

Materials and Methods

The present study was conducted during August 2016 to May 2017 at the Molecular Genetics and Genetic Engineering Laboratory, Patuakhali Science and Technology University, Patuakhali, Bangladesh. Plant materials of fifteen HYV of rice were sampled from the Bangladesh Rice Research Institute (BRRI) to conduct this study (rice genotypes presented in Table 1). Molecular characterization of HYV of rice was done using RAPD markers employed to conduct a genetic diversity analysis on the sampled fifteen HYV of rice.

Table 1. List of fifteen rice genotypes with their source.

Sr. No.	Name	Source
1	BR2	Bangladesh Rice Research Institute
2	BR9	Bangladesh Rice Research Institute
3	BR16	Bangladesh Rice Research Institute
4	BR17	Bangladesh Rice Research Institute
5	BR18	Bangladesh Rice Research Institute
6	BR26	Bangladesh Rice Research Institute
7	BRRIdhan27	Bangladesh Rice Research Institute
8	BRRIdhan28	Bangladesh Rice Research Institute
9	BRRIdhan35	Bangladesh Rice Research Institute
10	BRRIdhan45	Bangladesh Rice Research Institute
11	BRRIdhan47	Bangladesh Rice Research Institute
12	BRRIdhan50	Bangladesh Rice Research Institute
13	BRRIdhan61	Bangladesh Rice Research Institute
14	BRRIdhan67	Bangladesh Rice Research Institute
15	BRRIdhan68	Bangladesh Rice Research Institute

Collection of leaf sample

Fresh and healthy leaf samples were taken from 25 days old seedling of each rice variety to carry out analyses of DNA through the RAPD technique. At the beginning, the selected leaf samples were cut with sterilised scissors and thoroughly washed in distilled water and then in ethanol and dried on sterile paper to eliminate spore of microorganisms and any other sources of alien DNA. The prepared leaf samples were kept in a freezer at -20℃.

DNA extraction and its quantification on agarose gel

DNA extraction was conducted from the prepared leaf samples of each variety pursuing the procedure of the cetyl trimethylammonium bromide (CTAB) method. Eight μL 2X loading dye was thoroughly mixed with 2 μL dilute DNA for each sample. Then these DNA samples (10 μL) were loaded in the 1% agarose gel. After electrophoresis, the gel was stained with ethidium bromide for at least 20 min and the bands were visualized under UV light using Gel Doc system (Bio-Rad, Hercules, USA).

Primer test

Initially, a set of 30 primers of random sequences were used to screen for amplification of the DNA sequence. After thoroughly screening, the final subset of 10 primers was selected on the bases of good quality banding patterns with sufficient variability and 9 of them were chosen for further analyses (Table 2).

Table 2. Random primers used in the present study for screening.

Primers	Sequences
Responsive primers	
OPA-02	5'- TGCCGAGCTG
OPA-04	5'- AATCGGGCTG
OPA-09	5'-GGGTAACGCC
OPB-08	5'- GTCCACACGG
OPB-12	5'- CCTTGACGCA
OPB-18	5'- CCACAGCAGT
OPC-09	5'-CTCACCGTCC
OPC-12	5'- TGTCATCCCC
OPJ-01	5'- CCCGGCATAA
OPK-06	5'- CACCTTTCCC
Non responsive primers	
OPA-14	5'- TCTGTGCTGG
OPJ-02	5'- CCCGTTGGGA
OPJ-03	5'- TCTCCGCTTG
OPJ-04	5'- CCGAACACGG
OPJ-05	5'- CTCCATGGGG
OPK-17	5'-CCCAGCTGTG
OPC-07	5'- GTCCCGACGA
OPB-13	5'- TTCCCCCGCT
OPJ-07	5'- CCTCTCGACA
OPJ-09	5'- TGAGCCTCAC
OPA-17	5'- GACCGCTTGT
OPF-01	5'- ACGGATCCTG
OPK-02	5'- GTCTCCGCAA
OPF-14	5'- TGCTGCAGGT
OPA-16	5'- AGCCAGCGAA
OPK-11	5'- AATGCCCCAG

RAPD marker analysis

The prior protocol with minor modification was employed to perform RAPD analysis (Williams et al., 1990). PCR was completed for DNA amplification. For each sample, 10x Taq buffer (B) 1.0 μL, dNTPs 1.0 μL, Taq polymerase 0.2 μL, primer forward 1.0 μL, primer reverse 1.0 μL, ddH_2O 3.8 mL were used. Two μL sample DNA was used for each reaction. Total 10 μL PCR reaction mixture was primed. Amplification of PCR was conducted using the following procedure: denaturation at 94℃ for 3 min; 40 cycles of 1 min denaturation at 94℃, 1 min annealing at 54℃, 2 min extension at 72℃ and a final extension at 72℃ for 7 min. The RAPD amplification products were separated in a horizontal 1.6% agarose gel. DNA fragments were revealed using ethidium bromide staining. The gels were stained for 20 - 25 min and were documented with a gel documentation system. The RAPD markers were analyzed for Nei's genetic distance, Shannon's information index, identity values, and the similarity index were computed from frequencies of polymorphic markers to estimate the genetic relationship between the studied 15 HYV rice varieties. The dendrogram was constructed using the software NTSYS-pc (https://ntsyspc.software.informer.com/2.2/). The unweighted paired group of arithmetic means (UPGMA) method was used to conduct cluster analysis of NTSYS-pc 2.1 software was employed to generate a dendrogram.

Results and Discussion

Molecular characterization of cultivated rice varieties in Bangladesh by using RAPD markers

Nine primers were initially screened from the 30 RAPD primers for their ability to produce polymorphic patterns. The primers OPA 02, OPA 04, OPK 06, OPB 09, OPB 08, OPB 12, OPC 12, OPB 18 and OPJ 01 were reproducible and distinct polymorphic amplified products. The other primers amplified DNA was not consistently reproducible, that is a very common feature of RAPD technique (Pervaiz et al., 2010). A total of 45 RAPD bands were scored of which 40 (88.89%) polymorphic amplification products were obtained by using these arbitrary primers (Table 3). The dissimilar numbers of bands were generated by primer OPB 18, OPB 08, OPK 06, OPA 09, OPC 12, OPC 09, and OPJ 01. Besides, the primer OPB 18, OPB 08, OPK 06, OPA 09, OPC 12, OPC 09, and OPJ 01 amplified maximum numbers of polymorphic bands (100%) while the primer OPA 04 generated the least (60%) polymorphic bands. This result is consistent with the previous finding by Skaria et al. (2011) who obtained 82.27% of polymorphic products by RAPD analysis in rice genotypes. Ravi et al. (2003) and his colleagues observed 40 cultivated rice varieties and 5 wild genotypes that produced 499 RAPD markers of which polymorphism percentage estimated to be 90.0. Pervaiz et al. (2010) studies the genetic polymorphism of 75 rice genotypes of Pakistan, a total of 145 RAPD fragments were produced by 28 decamer-primers, of which 116 (80%) were polymorphic. Polymorphic bands generated by each primer varied from 3 to 9 with a mean of 5.2 alleles per primer. The banding patterns by using primers OPK 06 are shown in Fig. 1.

Table 3. Random amplified polymorphic DNA (RAPD) primers with corresponding bands score and their size range together with polymorphic bands observed in fifteen rice genotypes.

Primer code	Sequences (5′-3′)	Total number of bands scored	Number of polymorphic bands	Proportion of polymorphic loci (%)
OPJ 01	CCCGGCATAA	5	5	100
OPA 04	AATCGGGCTG	5	3	60
OPA 09	GGGTAACGCC	6	6	100
OPB 08	GTCCACACGG	4	4	100
OPC 12	TGTCATCCCC	3	3	100
OPB 18	CCACAGCAGT	8	8	100
OPA 02	TGCCGAGCTG	5	4	80
OPB 12	CCTTGACGCA	4	2	50
OPK 06	CACCTTTCCC	5	5	100
Total		45	40	

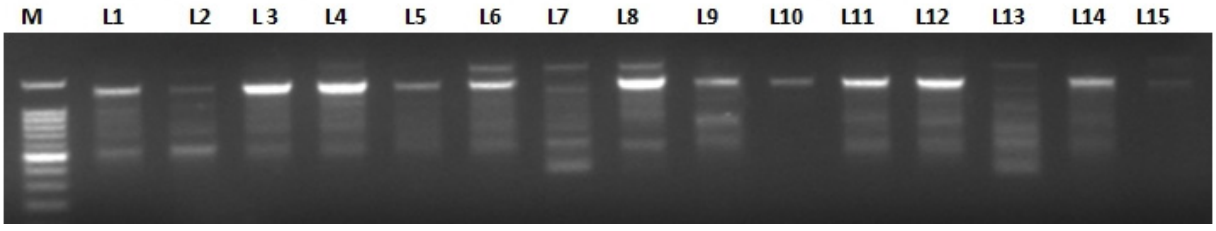

Fig. 1. Random amplified polymorphic DNA (RAPD) profiles of different rice varieties using primers OPK-06; M represent Marker (100 bp), L1 - L15 shows rice genotypes BRRI68, BRRI67, BRRI61, BRRI50, BRRI47, BRRI45, BRRI35, BRRI28, BRRI27, BR26, BR18, BR17, BR16, BR9, and BR2 respectively.

A frequency of polymorphic loci

The presence and absence of the band was a mark to find the DNA polymorphisms. The failure of primer caused to the absence of band to anneal a site in some individuals due to nucleotide sequences difference or by deletions or insertions between primer sites (Clark and Lanigan, 1993). Frequencies of polymorphic loci RAPD markers for studied 15 rice genotypes ranged from 0.5102 in OPB 08-2 up to 1.0000 in OPA 02-2, OPA 04-1, OPA 04-4, OPB 12-2, and OPB 12-3 (Table 4). Similar results were found in the studies of Koizumi et al. (2013) where the expected heterozygosity ranged from 0.031 to 0.965 and observed heterozygosity ranged from 0.031 to 1.000. Earlier, Akagi et al. (1997) reported that an identification system based on combinations of polymorphism using PCR was an effective method to identify individual genotypes and maintain the seed purity of rice cultivars.

Genetic variation

The values of different accessions of rice for across all loci were estimated by Nei's (1973) gene diversity and Shannon's information index (Table 5 and Table 6). The mean value of Nei's genetic diversity for 15 genotypes of rice accessions was estimated to be 0.26, the highest level of genetic diversity was observed with the locus OPB 18-2 and OPB 12-4 (0.4978) followed by OPJ 01-2 (0.4970), while the lowest level of genetic diversity were detected on OPA 02 -2, OPA 04-1, OPA 04-4, OPB 12-2¸ OPB 12-3 with value 0.0000.

The Shannon's information index values varied greatly among loci, ranged from 0.6909 (OPC 12-3 and OPB 18-2) to 0.0000 (OPA 02-2, OPA 04-1, OPA 04-4, OPB 12-2, OPB 12-3) with an average of 0.41 per locus. Genetic variation level was high among the studied rice genotypes according to the proportion of polymorphic loci. In the recent studies of Mukherjee et al. (2018), a higher diversity among the drought tolerant rice genotypes was found within the sub-populations (75%) compared among the populations (25%) with dissimilarity coefficient estimated to be 0.486. Jena et al. (2015) reported that genetic studies based on the characterization of the polymorphic loci of a marker are highly informative and extremely important. The authors categorized pest resistant rice genotypes through 22 gene markers into 4 major clusters with the genetic similarity rate almost 40% by using genetic diversity analysis.

Genetic differentiation and rate of migration among the subdivided population

Gene diversity in subdivided populations estimated the gene flow (N_m+) value of 0.000 according to the Nei's standard genetic distance analyses, whereas the proportion of total genetic diversity (G_{st}) was computed to be 1.0000. An expectation of average heterozygosity in sub-population (H_t) was 0.0772 according to the Hardy–Weinberg equilibrium, among the heterozygosity in sub-population of loci, H_t coefficients ranged from -0.0625 (OPA 09-5) to 0.3314 (OPJ 01-2 and OPJ 01-3) whereas the heterozygosity (H_s) was found to be constant 0.0000 (Table 7 and Table 8). In their study, Cirillo et al. (2009) found genetic differentiation among Italian rice genotypes by using RAPD technique despite low genomic variability.

Table 4. Frequencies of polymorphic random amplified polymorphic DNA (RAPD) markers in fifteen rice genotypes.

RAPD marker	Allele (gene) frequency
OPA 02-1	0.6250
OPA 02-2	1.0000
OPA 02-3	0.6250
OPA 02-4	0.7222
OPA 02-5	0.8472
OPA 04-1	1.0000
OPA 04-2	0.8756
OPA 04-3	0.8756
OPA 04-4	1.0000
OPA 04-5	0.7689
OPB 08-1	0.6089
OPB 08-2	0.5102
OPB 08-3	0.7689
OPB 08-4	0.5200
OPK 06-1	0.7551
OPK 06-2	0.6633
OPK 06-3	0.7551
OPK 06-4	0.8673
OPK 06-5	0.5408
OPB 12-1	0.8756
OPB 12-2	1.0000
OPB 12-3	1.0000
OPB 12-4	0.5022
OPC 12-1	0.8200
OPC 12-2	0.8200
OPC 12-3	0.5800
OPB 18-1	0.7689
OPB 18-2	0.5022
OPB 18-3	0.8756
OPB 18-4	0.8756
OPB 18-5	0.8756
OPB 18-6	0.8756
OPB 18-7	0.6800
OPB 18-8	0.7689
OPJ 01-1	0.5740
OPJ 01-2	0.5030
OPJ 01-3	0.5030
OPJ 01-4	0.5266
OPJ 01-5	0.5740
OPA 09-1	0.7689
OPA 09-2	0.8756
OPA 09-3	0.7689
OPA 09-4	0.5139
OPA 09-5	0.6250
OPA 09-6	0.7222

Table 5. Summary of genetic diversity statistics for all loci in fifteen rice genotypes.

RAPD marker	Genetic diversity index (h*)
OPA 02-1	0.3750
OPA 02-2	0.0000
OPA 02-3	0.3750
OPA 02-4	0.2778
OPA 02-5	0.1528
OPA 04-1	0.0000
OPA 04-2	0.1244
OPA 04-3	0.1244
OPA 04-4	0.0000
OPA 04-5	0.2311
OPB 08-1	0.3911
OPB 08-2	0.4898
OPB 08-3	0.2311
OPB 08-4	0.4800
OPK 06-1	0.2449
OPK 06-2	0.3367
OPK 06-3	0.2449
OPK 06-4	0.1327
OPK 06-5	0.4592
OPB 12-1	0.1244
OPB 12-2	0.0000
OPB 12-3	0.0000
OPB 12-4	0.4978
OPC 12-1	0.1800
OPC 12-2	0.1800
OPC 12-3	0.4200
OPB 18-1	0.2311
OPB 18-2	0.4978
OPB 18-3	0.1244
OPB 18-4	0.1244
OPB 18-5	0.1244
OPB 18-6	0.1244
OPB 18-7	o.3200
OPB 18-8	0.2311
OPJ 01-1	0.4260
OPJ 01-2	0.4970
OPJ 01-3	0.4907
OPJ 01-4	0.4734
OPJ 01-5	0.4260
OPA 09-1	0.2311
OPA 09-2	0.1244
OPA 09-3	0.2311
OPA 09-4	0.4861
OPA 09-5	0.3750
OPA 09-6	0.2778
Mean	0.2644
Standard deviation	0.1610

Table 6. Summary of information index for all loci in fifteen rice genotypes.

RAPD marker	Information index (I*)
OPA 02-1	0.3750
OPA 02-2	0.0000
OPA 02-3	0.3750
OPA 02-4	0.2778
OPA 02-5	0.1528
OPA 04-1	0.0000
OPA 04-2	0.1244
OPA 04-3	0.1244
OPA 04-4	0.0000
OPA 04-5	0.2311
OPB 08-1	0.3911
OPB 08-2	0.4898
OPB 08-3	0.2311
OPB 08-4	0.4800
OPK 06-1	0.2449
OPK 06-2	0.3367
OPK 06-3	0.4101
OPK 06-4	0.2573
OPK 06-5	0.6518
OPB 12-1	0.2449
OPB 12 -2	0.0000
OPB 12 -3	0.0000
OPB 12 -4	0.6909
OPC 12-1	0.3251
OPC 12-2	0.3251
OPC 12-3	0.6909
OPB 18-1	0.3927
OPB 18-2	0.6909
OPB 18-3	0.2449
OPB 18-4	0.2449
OPB 18-5	0.2449
OPB 18-6	0.2449
OPB 18-7	0.5004
OPB 18-8	0.3927
OPJ 01-1	0.6172
OPJ 01-2	0.6902
OPJ 01-3	0.6902
OPJ 01-4	0.6669
OPJ 01-5	0.6172
OPA 09-1	0.3927
OPA 09-2	0.2449
OPA 09-3	0.3927
OPA 09-4	0.6792
OPA 09-5	0.5623
OPA 09-6	0.4506
Mean	0.4106
Standard deviation	0.2141

Table 7. Summary of genetic variation statistics across for all loci in fifteen rice genotypes.

Locus	Sample size	H_t	H_s	G_{st}	N_m+
OPA 02-1	12	0.1875	0.0000	1.0000	0.0000
OPA 02-2	12	-0.5625	0.0000	1.0000	0.0000
OPA 02-3	12	0.0625	0.0000	1.0000	0.0000
OPA 02-4	12	-0.2014	0.0000	1.0000	0.0000
OPA 02-5	12	-0.3681	0.0000	1.0000	0.0000
OPA 04-1	15	0.0000	0.0000	****	****
OPA 04-2	15	0.1244	0.0000	1.0000	0.0000
OPA 04-3	15	0.1244	0.0000	1.0000	0.0000
OPA 04-4	15	0.0000	0.0000	****	****
OPA 04-5	15	0.2311	0.0000	1.0000	0.0000
OPB 08-1	15	0.3911	0.0000	1.0000	0.0000
OPB 08-2	14	0.4031	0.0000	1.0000	0.0000
OPB 08-3	15	0.2311	0.0000	1.0000	0.0000
OPB 08-4	15	0.4800	0.0000	1.0000	0.0000
OPK 06-1	14	0.1173	0.0000	1.0000	0.0000
OPK 06-2	14	0.2194	0.0000	1.0000	0.0000
OPK 06-3	14	0.1173	0.0000	1.0000	0.0000
OPK 06-4	14	-0.0051	0.0000	1.0000	0.0000
OPK 06-5	14	0.36622	0.0000	1.0000	0.0000
OPB 12-1	15	0.1244	0.0000	1.0000	0.0000
OPB 12-2	15	0.0000	0.0000	****	****
OPB 12-3	15	0.0000	0.0000	****	****
OPB 12-4	15	0.4978	0.0000	1.0000	0.0000
OPC 12-1	10	-0.9700	0.0000	1.0000	0.0000
OPC 12-2	10	-0.9700	0.0000	1.0000	0.0000
OPC 12-3	10	-0.5300	0.0000	1.0000	0.0000
OPB 18-1	15	0.2311	0.0000	1.0000	0.0000
OPB 18-2	15	0.4978	0.0000	1.0000	0.0000
OPB 18-3	15	0.1244	0.0000	1.0000	0.0000
OPB 18-4	15	0.1244	0.0000	1.0000	0.0000
OPB 18-5	15	0.1244	0.0000	1.0000	0.0000
OPB 18-6	15	0.1244	0.0000	1.0000	0.0000

Ht, Hardy-Weinberg average heterozygosity expected in sub-population; Hs, Hardy-Weinberg average heterozygosity obtained in sub-population; G_{st}, co-efficient of gene differentiation; Nm, estimate of gene flow from G_{st} or G_{cs}. e.g., $N_m = 0.5(1 - G_{st})/G_{st}$; ****, infinity.

Table 8. Summary of genetic variation statistics across for all loci in fifteen rice genotypes.

Locus	Sample size	H_t	H_s	G_{st}	N_m+
OPB 18-7	15	0.3200	0.0000	1.0000	0.0000
OPB 18-8	15	0.2311	0.0000	1.0000	0.0000
OPJ 01-1	13	0.1893	0.0000	1.0000	0.0000
OPJ 01-2	13	0.3314	0.0000	1.0000	0.0000
OPJ 01-3	13	0.3314	0.0000	1.0000	0.0000
OPJ 01-4	13	0.2604	0.0000	1.0000	0.0000
OPJ 01-5	13	0.1893	0.0000	1.0000	0.0000
OPA 09-1	15	0.2311	0.0000	1.0000	0.0000
OPA 09-2	15	0.1244	0.0000	1.0000	0.0000
OPA 09-3	15	0.2311	0.0000	1.0000	0.0000
OPA 09-4	15	0.1319	0.0000	1.0000	0.0000
OPA 09-5	15	-0.0625	0.0000	1.0000	0.0000
OPA 09-6	15	-0.2014	0.0000	1.0000	0.0000
Mean	14	0.0772	0.0000	1.0000	0.0000
St. Dev		0.1055	0.0000		

Ht, Hardy-Weinberg average heterozygosity expected in sub-population; Hs, Hardy-Weinberg average heterozygosity obtained in sub-population; Gst, co-efficient of gene differentiation; Nm, estimate of gene flow from Gst or Gcs. e.g., Nm = 0.5(1 - Gst)/Gst; ****, infinity. The number of polymorphic loci is: 40. The percentage of polymorphic loci is: 88.89%.

Similarity index

Jaccard's correlation coefficient (Jaccard, 1908) was effective to analyse the similarity between the rice accessions. Correlation matrices acquired from all the used primers were combined in one single matrix and the mean values ranged from 0.42 to 0.94 (Table 9). Jaccard's pair-wise similarities estimated between the rice genotypes exhibited that BRRIdhan45 and BRRIdhan28 were the closest (0.94). The highest distance was detected between the genotypes BRRIdhan68 and BRRIdhan47 (0.42). The experiments conducted by Rashid (2007) found similarity coefficient ranged from 47.83 to 97.52 of 34 rice genotypes from different origin by using RAPD technique, and interestingly, in this study the author found that the accessions belonging to Pakistan and Philippines with similarity index more than 94% and 62.0 - 93.6%, respectively were grouped in separate clusters. Pervaiz et al. (2010) found similarity coefficients ranged from 0.22 to 0.90 and 0.19 to 0.72 among the basmati and non-basmati types landraces, respectively. This study proved that RAPD markers are the reliable technique to estimate genetic diversity of rice germplasm.

Table 9. Similarity matrix of fifteen rice cultivars based on Jaccard's similarity index.

Name	Similarity index														
BR2	1.00														
BR9	0.86	1.00													
BR16	0.70	0.72	1.00												
BR17	0.64	0.65	0.70	1.00											
BR18	0.70	0.65	0.76	0.83	1.00										
BR26	0.89	0.82	0.82	0.76	0.82	1.00									
BRRI27	0.80	0.76	0.73	0.88	0.84	O.56	1.00								
BRRI28	0.76	0.68	0.76	0.80	0.88	0.86	0.84	1.00							
BRRI35	0.82	0.79	0.70	0.70	0.82	0.89	0.80	0.88	1.00						
BRRI45	0.78	0.72	0.69	0.73	0.81	0.89	0.84	0.94	0.90	1.00					
BRRI47	0.57	0.65	0.57	0.53	0.50	0.61	0.57	0.57	0.57	0.61	1.00				
BRRI50	0.83	0.76	0.77	0.80	0.83	0.88	0.84	0.93	0.83	0.86	0.57	1.00			
BRRI61	0.77	0.69	0.70	0.80	0.90	0.80	0.80	0.93	0.83	0.86	0.50	0.57	1.00		
BRRI67	0.64	0.62	0.70	0.77	0.70	0.79	0.84	0.76	0.64	0.69	0.46	0.50	0.93	1.00	
BRRI68	0.64	0.62	0.70	0.87	0.76	0.72	0.80	0.76	0.70	0.69	0.42	0.46	0.77	0.77	1.00

UPGMA dendrogram

A dendrogram was constructed by using NTSYS-pc the UPGMA. The 15 rice genotypes were divided into 2 main clusters, namely cluster 1 and cluster 2 (Fig. 2). The genotype BRRI47 was added in cluster 2. Cluster 2 i.e., BRRI dhan47 was different from other genotypes according to the similarity index. So, the genetic relatedness was not found between rice genotypes belonging to the cluster 1 and cluster 2. The following genotypes belong in cluster 1 BR2, BR9, BR16, BR17, BR18, BR26, BRRIdhan27, BRRIdhan28, BRRIdhan35, BRRIdhan45, BRRIdhan50, BRRIdhan61, BRRIdhan67, BRRIdhan68. The subsequent dividing of genotypes of the cluster 1 created sub-cluster 1 and sub-cluster 2. Sub-cluster 1 consisted of genotypes BR2 and BR9. Sub-cluster 2 formed by BR16, BR17, BRRIdhan68, BR18, BRRIdhan28, BRRIdhan45, BRRIdhan50, BRRIdhan35, BRRI dhan27, BRRIdhan67 genetic relationship was present between sub-clusters. The genotypes of sub-cluster 2 again split into two sub-sub-cluster 1 and sub-sub-cluster 2. Sub-sub-cluster 1

consisted of genotypes BR16. Sub-sub-cluster 2 formed by BR17, BRRIdhan68, BR18, BRRIdhan28, BRRIdhan45, BRRIdhan50, BRRIdhan67, BRRIdhan35, BRRIdhan27 and BRRIdhan67. Molecular characterization of the genotypic variations indicated that genetic components are an important factor to characterise genotypes belonging to different clusters, whereas geographical origin doesn't have any impact. Recently, Singh et al. (2018) came up with similar results that most of the studied rice genotypes occurred in different clusters and subclusters without any close relationships. Also, Ravi et al. (2003) speculated that natural environmental factors might have an impact on the phonological diversity of genotypes in same groups.

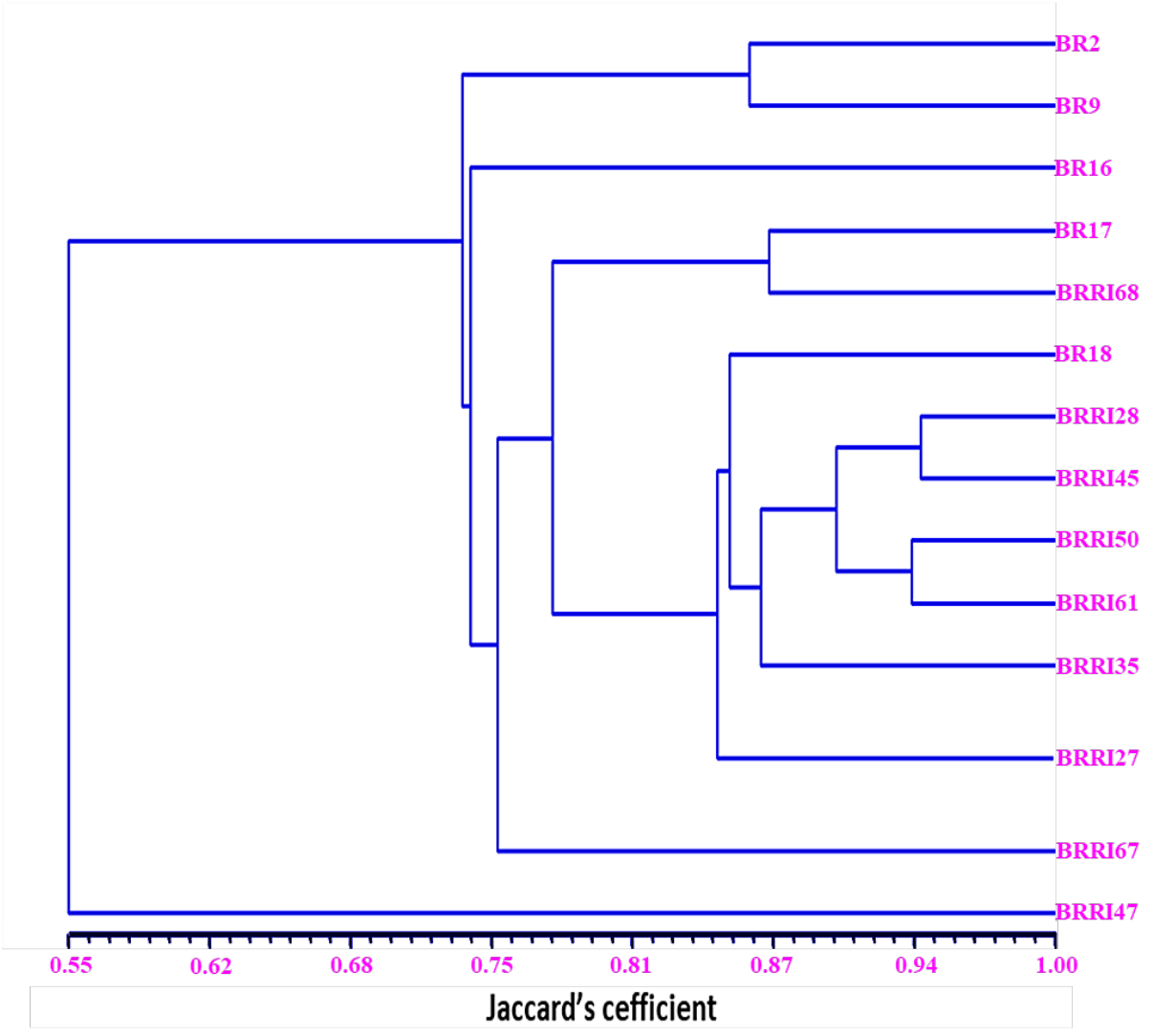

Fig. 2. Dendrogram showing genetic relationship of fifteen rice variety based on random amplified polymorphic DNA (RAPD) primers using a unweighted paired group of arithmetic means (UPGMA) method.

Conclusion

The study of molecular characterization of fifteen high yielding variety of rice showed the variation among the variety. Nei's analysis of gene diversity in subdivided populations presented the gene flow (N_m+) value of 0.000 and the proportion of total genetic diversity (G_{st}) was 1.0000. Hardy Weinberg expectation of average heterozygosity in sub-population (H_t) was 0.0772, whereas the heterozygosity (H_s) was 0.0000. From the similarity matrix, the highest similarity was observed between genotype BRRIdhan45 and BRRIdhan28 and the lowest result between BRRIdhan68 and BRRIdhan47. From the dendrogram, it was observed that each studied variety was genetically different from other varieties. Molecular characterization of genotypic variations indicated that the genetic components of genotypes are important to divide them into different clusters. Therefore, it is meaningful to conclude that for further research purposes, mainly for hybridization, genotypes should be selected from different clusters to provide maximum genetic information especially related to the yield.

Conflict of Interests

No potential conflict of interest relevant to this article was reported.

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