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GENETIC VARIATION AND RELATEDNESS FOR BLB RESISTANCE IN BRRI RICE USING RAPD MARKERS

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ABSTRACT: In this study, ten BRRI released rice germplasms were used for the purpose of genetic variation and relatedness for BLB resistance in BRRI rice using RAPD markers. Out of ten decamer primers, four were selected finally for a marker-assisted selection program. The primer OPAB02 produced maximum number of bands (10) and showed 60% polymorphic loci. The highest and lowest percentage of polymorphic loci produced by primers S1027 (66.67%) and OPA02 (25%) respectively. In this study the percentage of polymorphic loci was 55.17%. The UPGMA dendrogram based on Nei's genetic distance between different pairs was correlated with their banding pattern. The dendrogram segregated ten germplasms of rice into two main clusters; the main clusters are further divided into sub clusters. BR 10, BR 14, BR 16, BR 26, BRRIdhan29, BRRIdhan32, BRRIdhan33, BRRIdhan34 and BRRIdhan38 formed cluster I whereas BRRIdhan31 comprised the cluster II. In cluster I BR10, BR 14, BR 16, BR 26, BRRIdhan32, BRRIdhan33, BRRIdhan34 BRRIdhan38 formed sub-cluster I and BRRIdhan 29 formed sub- cluster II. Sub-cluster I further divided into two sub-sub clusters based on similar banding pattern and minimum genetic distance. BR14 and BRRIdhan 32 had same banding pattern and lower genetic distance so were in same cluster. BR10, BR16, BRRIdhan33 and BRRIdhan34 were placed in same group due to their banding similarity, intergermplasm similarity and low genetic distance. Thus, RAPD perform a potentially simple, rapid and reliable method to evaluate genetic diversity and molecular characterization as well.

KEYWORDS: Genetic variation, Relatedness, BLB resistance, BRRI rice and RAPD marker.

1 INTRODUCTION

Rice (*Oryza sativa L.) (*2n = 24) belonging to the family *Graminae* and subfamily *Oryzoidea* is the staple food for one third of the world's population, it occupies almost one-fifth of the total land area covered under cereals [1]. It is a major source of food for more than 2.7 billion people on a daily basis and is planted on about one-tenth of the earth's arable land [2]. Being a major staple food, over 85 percent of the total, or 402 million tonnes, are estimated to be destined for human consumption [3]. The total area and production of rice in Bangladesh is about 11.7 million hectares and 31.98 million metric tons, respectively [4]. In Bangladesh, it is not only the main source of carbohydrate but also provides 75% of the calories and 55% of the proteins in the average daily diet of the people.

Blight refers to a specific symptom affecting plants in response to infection by a pathogenic organism. It is simply a rapid and complete chlorosis, browning and then death of plant tissues such as leaves, branches, twigs, or floral organs. Accordingly, many diseases that primarily exhibit this symptom are called blights. Bacterial leaf blight (BLB) caused by the rod-shaped bacterium, *Xanthomonas oryzae pv. oryzae (Xoo)* is one of the most devastating diseases in rice is an important disease throughout Asia [5]. It is a vascular disease whereby *Xoo* continues to grow until the xylem vessels are clogged with

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bacterial cells and extracellular polysaccharides [6]. The use of resistant cultivars is the most promising control method against the disease.

Most breeders are interested in utilizing BLB resistant varieties, and this goal is certainly achievable providing the availability of an easy strategy to identify resistance genes. The boro rice cultivar BR 14 showed resistance to five Japanese races I (T7174), II (T7147), III (T7133), IV (H75373Y), V (H75304) of *X. oryzae pv. Oryzae* [7]. In this study BR14 was taken as BLB resistant variety. At present, biotechnology progress continuously. Identification, cloning, and functional analyses of a gene can be performed relatively rapidly. As on date, 30 major genes have been reported to confer resistance against the rice bacterial pathogen *X. oryzae pv oryzae (Xoo),* which included 21 dominant and 9 recessive R genes [8]**.** The majority of BLB resistance genes were identified in rice *O. sativa* ssp. indica and wild rice *O.longgistaminata*, *O. rufiprogon*, *O. minuta* and *O. officinalis*, while some of them were identified in *O. sativa* ssp. japonica [9]. Some resistance genes are effective only in adult plants, while others are effective at all stages of growth. Some genes confer resistance to a broad spectrum of *Xoo* races, whereas others do so against only one or a few races. This observation could be influenced by particular geographical locations [10]. Several rice resistance genes are expressed at the highest level only in the adult stage [11]. *Xa21*-mediated resistance gene was shown to be expressed since the seedling stage but the plants were found susceptible, and *Xa7* gene showed broad resistance only in adult plants. On the contrary, *xa5* gene could confer resistance at all growth stages.

Molecular markers are the molecules that could be used to trace a desired gene(s) in test genotypes. In fact, a piece of DNA or a protein can be used as a marker that is readily detected and whose inheritance can easily monitor. However, DNA markers seem to be the best candidates for efficient evaluation and selection of plant material. Recently the concept of marker aided selection has provided an advantage of molecular marker based approaches for crop improvement as compared to selection based solely on phenotype [12]. Availability of a large number of polymorphic markers enables precise classification of the cultivars and available germplasm collections. Several molecular markers viz. RFLP [13] [14], RAPD [15] [16] SSRs, ISSRs [17], AFLP [18] [19] and SNPs [20] are presently available to assess the variability and diversity at molecular level [21]. With the development of a wide range of molecular techniques, marker assisted breeding is now used to enhance traditional breeding programs to improve crops [22].

RAPD stands for random amplification of polymorphic DNA. It is a type of PCR reaction, but the segments of DNA that are amplified are random. Advantages associated with RAPDs have made them a favourite marker technique in genome mapping, the determination of phylogenetic relationships, genetic diversity, and identification of cultivars and parents in a number of plant species. Since the RAPD technique involves enzymatic amplification of target DNA by PCR using arbitrary primers, it is also called Arbitrarily Primed Polymerase Chain Reaction (AP-PCR) or DNA Amplification Fingerprinting (DAF).In this study RAPD marker was used as it is important particularly for variety selection among ten HYV rice germplasms. The specific objectives of the study were as follows:

- Screening of BRRI released rice against BLB resistance.
- To establish the genetic relationship between BLB resistant BR14 and rest of the rice germplasms at molecular level.
- Molecular genetic analysis of ten rice germplasms to observe genetic variation among them was using RAPD.

2 MATERIALS AND METHODS

The experiment was carried out at the USDA Biotechnology Laboratory, Department of Biotechnology, and central laboratory of Bangladesh Agricultural University, Mymensingh.

2.1 STUDY MATERIAL

Ten germplasms of Rice viz. BR 14, BRRIdhan 29, BR 10, BR 16, BRRIdhan 31, BRRIdhan-32, BRRIdhan 33, BRRIdhan 34, BR26 and BRRIdhan 38 were used in the study were collected from Bangladesh Rice Research Institute.

2.2 ISOLATION OF RICE GENOMIC DNA

Genomic DNA of 10 rice germplasms were collected from USDA Biotechnology laboratory, department of Biotechnology, Bangladesh Agricultural University, Mymensingh, preserved at -20^0 C.

GENOMIC DNA EXTRACTION

To extract genomic DNA, young, actively growing fresh leaves were collected from 20 day old seedlings. DNA extraction was done by using the mini preparation Modified CTAB method. Collected leaf samples were cut into 2-3 cm pieces, 670 µl extraction buffer and 40 µl 20% SDS were added. Incubated for 10 minutes at 65^0C. 100 µl 5M NaCl was added and inverted gently. 100 µl CTAB was added and was incubated for 10 minutes at 65^0C. 900 µl chloroform (chloroform: isoamylalcohol = 24:1) was added and mixed well. Spinned for 5 minutes at 12000 rpm then transferred the supernatant in new tube. 600 µl ice cold isopropanol was added into new eppendorf tubes and inverted slowly for 5 minutes. The eoppendorf tubes were centrifuged at 12000 rpm 10 minutes and decanted the supernatant. Pellets were washed with 200 µl of 70% ethanol and removed the ethanol then the samples were kept for air- drying for overnight. The pelletes were resuspended in 30µl 1X TE buffer. Extrated DNA samples checked by using 0.8% agarose gel electrophoresisand quantified by using a spectrophotometer.

REAGENTS USED FOR THE EXTRACTION OF GENOMIC DNA

i. Extraction buffer (pH = 8.0), 50 mM Tris-HCl , 25 mM EDTA (Ethylene diamine tetra acetic acid) , 300 mM NaCl and 1% SDS (Sodium Dodecyl Sulphate)

ii. Phenol: Chloroform: Isoamyl alcohol = 25: 24: 1 (v/v/v); equilibrated to pH near 8.0 with TE buffer, iii. TE (Tris-EDTA) buffer, pH = 8.0 (10 mM Tris-HCl 1 mM EDTA) iv. Sodium acetate (3 M), pH = 5.2 v. Absolute (100%) Ethanol

2.3 CONFIRMATION OF DNA PREPARATION

DNA samples of ten rice germplasms were evaluated by performing (1%) agarose gel electrophoresis.

2.3.1 PREPARATION OF AGAROSE GEL (1%) FOR ELECTROPHORESIS

REAGENTS: Agarose powder (Fisher Biotech, USA), 5X TBE Buffer (pH 8.3): Composition (For 1000 ml); Tris: 54 g (Sigma, USA), Boric Acid: 27.5g (SRL, India), EDTA: 4.65 g (Loba chemie, India)**,** 1X TBE buffer (200 ml): 20 ml 5X TBE buffer was added to 180 ml ddH_2O to make total volume 200 ml and Ethidium Bromide (SRL, India)

PROCEDURE

For preparing 1% gel, 1.5 g agarose powder (Fisher Biotech) was taken into a 500 ml Erlenmeyer flask containing 250 ml of I x TBE buffer prepared by adding 50 ml of 5 x TBE buffer in 200 ml of sterile deionized water. To prevent excessive evaporation, the top of the flask was covered with aluminium foil paper. The flask was cooked into a microwave oven for about 5 minute to dissolve agarose into TBE buffer. Then the gel was kept in room temperature for 10-15 min to cool down at tolerable level. Then the gel was poured on to the gel bed (15 x 15 x2 cm 3 in size) that was placed on a level bench and the appropriate comb was inserted and finally, the bubbles were removed. Meanwhile, the gel became completely cooled and solidified and comb was removed gently.

2.3.2 DNA SAMPLE PREPARATION AND ELECTROPHORESIS PROCEDURE

The hardened gel was transferred to the electrophoresis chamber and keeping the gel horizontal and containing sufficient 1X TBE buffer to cover the gel ~1mm. For each sample, 2 µl of water was placed on a piece of aluminum foil paper and 1 µl loading dye (0.25% xylene cyanol, 0.25% bromophenol blue, 30% glycerol and I mM EDTA) was added to it using 0.5-10 µl adjustable micropipette. Loading buffer was used for monitoring loading and the progress of the electrophoresis and to increase the density of the sample so that it was stayed in well. Finally, 3 µl extracted DNA was added to it and mixed well using same micropipette. The samples were then added slowly to allow them to sink to the bottom of the wells. The gel was placed in the gel chamber containing I x TBE buffer. The final level of the buffer was -5 mm above the gel. The electrophoresis apparatus were then connected to the Power Supply and turned on to migrated the DNA from negative to positive electrode (Black to Red). Electrophoresis was carried out at l20 v for 1.20 hour to get the dye 1/4 of the gel length. When DNA migrated sufficiently, as judged from the migration of bromophenol blue of loading buffer, the power supply was switched off. 6 µl ethidium bromides (10 mg/ml) DNA stain was added to make the DNA visible under UV transilluminator and mixed well by gentle shaking for 20 minutes.

2.3.3 DOCUMENTATION OF THE DNA SAMPLES

After electrophoresis, the gel was taken out carefully from the gel chamber and the gel gently washed in running water and placed on the UV transilluminator in the dark chamber of the image documentation system (Labortechnik. Germany). The UV light of the system was switched on; the image was viewed on the monitor, focused, acquired and saved in a floppy disc, as well as printed on thermal paper. The electrophoregram of DNA of 10 rice germplasms.

2.4 QUANTIFICATION OF DNA CONCENTRATION

One of the important variables for PCR amplification is the concentration of genomic DNA. Because different DNA extraction methods produced DNA of widely different purity, it is necessary to optimize the amount of DNA used in the RAPD -assay to achieve reproducibility and strong signal. Excessive genomic DNA may result in smears or in a lack of clearly defined bands in the gel; on the other hand, too little DNA gives nonreproducible patterns [23]. Thus, it is necessary to optimize the DNA concentration. It is best to do a series of RAPD reaction using a couple of primers and a set of serial dilutions of each genomic DNA to identify empirically the useful range of DNA concentration, for which reproducible RAPD patterns are obtained.

For quantification of DNA concentration, the spectrophotometer's (Spectronict Genesis™, New York, USA) wavelength was set at 260 nm after the spectrophotometer UV lamp was warmed up. A square cuvette was filled with 2 ml sterile distilled water and place in the cuvette chamber and the absorbance reading was adjusted to zero for standardization. The test samples were prepared by taking 2 ul of each DNA sample in the cuvette containing 2 ml sterile distilled water and through mixing by pipetting. After recording the absorbance reading, the cuvette was rinsed out with sterile water, stamped out on a paper wipe, and absorbance readings for each sample was recorded in this way and are listed in Table 1. Using the absorbance reading, the original sample concentration was determined according to the following formula:

$$\text{Absorbance} \times \frac{\text{Volume of distilled water}(\mu l)}{\text{Amount of DNA sample}\,(\mu l)} \times \text{Conversion factor}\,(0.05) \times 1000$$

Table 1. Absorbance reading and concentration of different DNA samples at 260 nm (A)

Accession No.	Absorbance	DNA Conc. (ng/ µl)
1. BR14	0.015	750
2. BRRIdhan 29	0.005	250
3. BR10	0.010	500
4. BR16	0.007	350
5. BRRIdhan31	0.014	700
6. BRRIdhan32	0.010	500
7. BBRIdhan33	0.008	400
38. BRRIdhan34	0.007	350
9. BR26	0.006	300
10. BRRIdhan38	0.006	300

2.5 AMPLIFICATION OF RAPD MARKERS BY POLYMERASE CHAIN REACTION (PCR)

To perform amplification of RAPD, a single oligonucleotide of arbitrary DNA sequence is 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 (PCR). The products of the reaction depend on the sequence and the length of the oligonucleotide, as well as the reaction conditions. The reaction involves repeated cycles, each consisting of a denaturation, a primer annealing and an elongation step. In the first step, the DNA is made single stranded by raising the temperature to 94°C (denaturation). In the second step, lowering of the temperature to about an optimal (36°C) annealing temperature, the primer binds to their target sequences on the template DNA (annealing step). In the third cycle, temperature is chosen as where the activity of the thermos table Taq DNA polymerase is optimal, i.e., usually 72°C. The polymerase then extends the 3' ends of the DNA primer hybrids towards the other primer-binding site. Since this happens at both primer-annealing sites on both the DNA strands, the target fragment is completely replicated. Repeating these three cycles 40 to 50 times results in the exponential amplification of the target between the 5' ends of the two primer binding sites. Amplification products are separated by agarose gel electrophoreses and visualized by ethidium bromide staining.

2.5.1 PRIMER SELECTION

Ten primers of random sequence (Primer code- S1027, S1069, S1089, S1136, S1155, S1184, S1320, OPA02, OPAB02, and OPA320) were screened on a sub sample of two randomly chosen individuals from ten different cultivars to evaluate their suitability for amplifying DNA sequences, which could be accurately scored. Primers were selected on the basis of band resolution intensity, presence of smearing, consistency within individuals and potential for population discrimination.. Final subset of three primers exhibiting good quality banding patterns and sufficient variability were selected for further analysis. The details of the primers are given in (Table 2).

2.5.2 REACTION MIX PREPARATION TO PERFORM PCR

Conditions for RAPD, amplification reactions were maintained essentially following [24] with some modifications. PCR reactions were performed on each DNA sample in an l0µl reaction mix containing the following reagents: Taq polymerase buffer = 1 µl, dNTPs = 1 µl, Primers = 2 µl, $MgCl_2$ = 0.6 µl, Template DNA = 1.5 µl, Taq DNA polymerase = 0.2 µl and ddH_2O = 3.7 µ.

Table 2: Parameters of random primers used in the study for screening

SL.No.	Primer code	Sequence	GC Content (%)
1	S1027	ACGAGCATGG	60
2	OPA02	TGCCGAGCTG	70
3	S1155	GAAGGCTCCC	70
4	OPAB02	GGAAACCCCT	60

DNA amplification was performed in an oil-free thermal cycler (Master Cycler Gradient, Eppendorf). The reaction mix was preheated at 94°C for 3 minutes followed 40 cycles of 1 min denaturation at 94°C, 1 min annealing at 36°C and elongation or extension at 72°C for 2 minutes. After the last cycle, a final step of 7 minutes at 72°C was added to allow complete extension of all amplified fragments. After completion of cycling program, reactions were held at 4°C.

2.5.3 ELECTROPHORESIS OF THE AMPLIFIED PRODUCTS AND DOCUMENTATION

For each sample, 2 µl loading dye was added with the PCR products.The amplified products were separated electrophoretically on 1.4 % agarose gel. The gel was prepared using 1.4g agarose powder (Fisher Biotech, New Jersey, USA), 100 ml 1 x TBE buffer. Agarose gel electrophoresis was conducted in Ix TBE buffer at 120 V for 1.30 hour. One molecular weight marker 1 kb DNA ladder size and 20bp were electrophoresed along side the RAPD reactions. After completion of gel run 10 ul ethidium bromide mixed in water, gel was kept in this mixture for 20 minutes. DNA bands were observed under UV light on a Transilluminator and photographed by image documentation system (Labortechnik, Germany).

2.6 RAPD DATA ANALYSIS

This was used to estimate polymorphic loci, Nei's [25], gene diversity, population differentiation, (G_{ST}), gene flow (N_m), genetic distance (GD) and to construct a UPGMA (Unweighted Pair Group Method with Arithmetic Means) dendrogram among populations using a computer program, POPGENE (Version 1.31) [26].

Genetic-similarity values defined as the fraction of shared bands between the RAPD profiles of any two individuals on the same gel were calculated manually. RAPD markers of the same molecular weight on the data matrix according to the following formula:

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

Where, Nxy is the number of RAPD bands shared by individuals x and y respectively, and NX and NY are the number of bands in individual x and y, respectively [27] [28] [29].

3 RESULTS

3.1 PRIMER SELECTION AND RAPD PATTERNS

Ten rice germplasms collected from BRRI (Bangladesh Rice Research Institute), were analyzed as a group and a total of 29 RAPD bands were scored (Figure 1and 2). 10 primers were initially screened for their ability to produce polymorphic patterns and 4 primers (S1027, OPA02, S1155, and OPAB02) which gave reproducible and distinct polymorphic amplified products were selected. The selected 4 primers produced comparatively maximum number of high intensity band with minimal smearing.

The four primers showing good technical resolution and sufficient variation among different cultivars produced a total of 29 RAPD markers of which the number of polymorphic loci is 16. The percentage of polymorphic loci is 55.17 (either occurring in or absent in less than 29 of all individuals). The highest numbers of bands were generated by primer OPAB02 (10) showing 60 % polymorphic loci. On the other hand the lowest numbers of bands were produced by primer OPA02 (4). The percentage of polymorphic loci was higher for primer S1027 (66.67%) whereas the percentage was lower for primer OPA02 (25%). The average scorable and polymorphic bands produced by the four primers were 7.25 and 4 respectively (Table 3). Weak bands result from low homology between the primer and the pairing site on the DNA strand. Size range of PCR amplification products scored and banding patterns of 10 Rice germplasms using primers S1027, OPA02, S1155, and OPAB02 mentioned in table 3.

3.2 FREQUENCY OF POLYMORPHIC LOCI

The DNA polymorphisms were detected according to the presence and absence of bands. Absence of bands may be caused by failure of primers to anneal a site in some individuals due to nucleotide sequence differences or by insertions or deletions in primer sites [30]. Table 4 shows overall gene frequency. Here gene frequency values less than 1.0000 (< 1.0000) were considered as polymorphic. Among 29 loci, 16 had a value less than 1.0000 thus showed polymorphism.

3.3 INTER-GERMPLASM SIMILARITY INDICES (SIJ)

The pair-wise inter-germplasm similarity indices (Sij) ranged from 70.63 to 100%. The highest similarity index (100%) was found between BR 16 Vs BRRI dhan 33 (100%), while BR14 Vs BRRI dhan 31 showed the least inter-germplasm similarity (70.63%). Among the four primers, OPA02 showed highest inter-germplasms (95.128%) similarity whereas OPAB02 showed the least value of inter-germplasm similarity index (85.863%) (Table 5).

3.4 GENETIC DIVERSITY FOR FOUR PRIMERS IN ALL SAMPLES

Genetic diversity values of 10 rice genotypes for 4 primers are given in Table 6. Average gene diversity **(**h) and Shannon's Information index (I) across all primer against genotypes for all loci was found 0.1559 and 0.2472 respectively. High level of gene diversity was found for primer S1155 whereas the gene diversity value for primer OPA02 was low. Both locus S1155-1 and OPAB02-1 showed high level of gene diversity value and Shannon's Information index (4800) and (0.6730) respectively. Lowest level of gene diversity value and Shannon's Information index (0.0000 and 0.0000) was found in locus S1027-4, S1027-5, OPA02-1, OPA02-2, OPA02-4, S1155-6, S1155-7, S1155-8, S1155-9, OPAB02-4, OPAB02-5, OPAB02-6, OPAB02-7 (Table 6).

Table 3. RAPD primers with corresponding bands scored together with observed in polymorphic bands ten Rice germplasms

Primer codes	Total number of bands scored	Number of polymorphic bands	Polymorphic Loci (%)
S1027	6	4	66.67
OPA02	4	1	25
S1155	9	5	55.56
OPAB02	10	6	60
Overall	29	16	220.68
Average	7.25	4	55.17

In the present study the percentage of polymorphic loci was 55.17%. A diverse levels of polymorphism in rice genotypes were reported by [31] 53. 85%, [32] 95.65%, [33] 95.33%, [34] 90%, [35] 72.2%, [36] 94.36%, [37] 83.5%, [38] 74.1%.

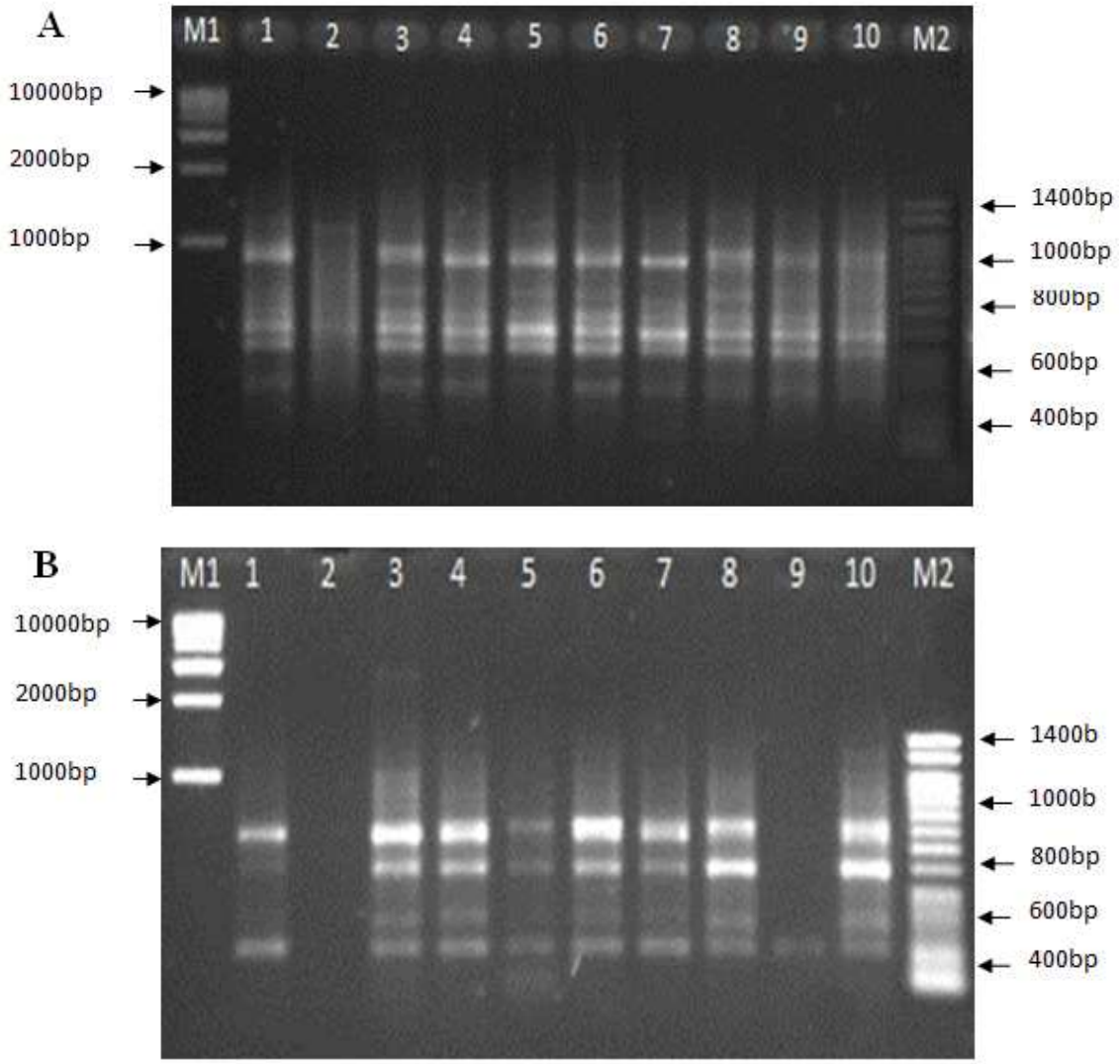

Figure 1: RAPD profile of 10 rice germplasms, Primer S1027 (A) OPA02 (B). (1-BR 14; 2-BR 29; 3- BR 10; 4-BR 16; 5- BRRIdhan31; 6-BRRIdhan32; 7- BRRIdhan33; 8-BRRIdhan34 ; 9-BR 26; 10-BRRIdhan 38; M1-1 kb DNA ladder, M2- 20bp DNA ladder).

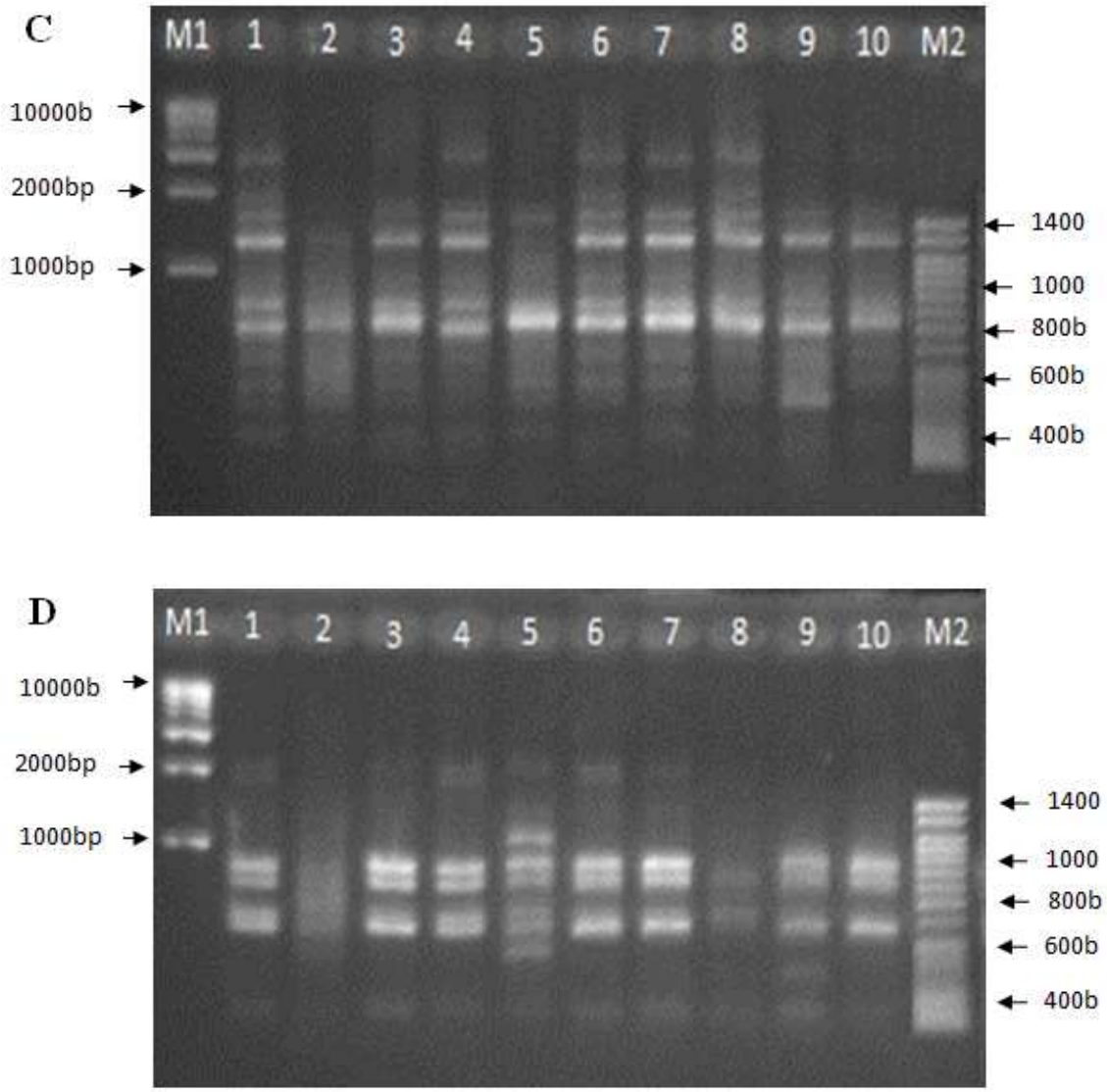

Figure 2: RAPD profile of 10 rice germplasms, Primer S1155 (C) and primer OPAB02 (D). (1-BR 14; 2-BR 29; 3- BR 10; 4-BR 16; 5-BRRIdhan31; 6- BRRIdhan32; 7- BRRIdhan33; 8- BRRIdhan34; 9-BR 26; 10-BRRIdhan 38; M1- 1kb DNA ladder, M2- 20bp DNA ladder).

Table 4. Overall Gene Frequency

Primer	**Gene Frequency**					
S1027	S1027-1 0.8000	S1027-2 0.9000	S1027-3 0.7000	S1027-4 1.0000	S1027-5 1.0000	S1027-6 0.8000
OPA02	OPA02-1 1.0000	OPA02-2 1.0000	OPA02-3 0.8000	OPA02-4 1.0000		
S1155	S1155-1 0.9000	S1155-2 0.9000	S1155-3 0.2000	S1155-4 1.0000	S1155-5 0.6000	S1155-6 0.3000
	S1155-7 1.0000	S1155-8 1.0000	S1155-9 1.0000			
OPAB02	OPAB02-1 0.6000	OPAB02-2 0.1000	OPAB02-3 0.1000	OPAB02-4 1.0000	OPAB02-5 1.0000	
	OPAB02-6 1.0000	OPAB02-7 1.0000	OPAB02-8 0.1000	OPAB02-9 0.1000	OPAB02-10 0.9000	

Table 5. Inter-germplasm similarity indices

Germplasm	Band Sharing Values (100%)				
	Primer S1027	Primer OPA02	Primer S1155	Primer OPAB02	Average
BR14 Vs BRRIdhan 29	75	85.71	62.5	80	75.8
BR14 Vs BR10	90.91	85.71	80	100	89.15
BR14 Vs BR16	91	85.71	87.5	100	91.05
BR14 Vs BRRIdhan 31	80	100	62.5	40	70.63
BR14 Vs BRRIdhan 32	91	85.71	100	100	94.17
BR14 Vs BRRIdhan 33	91	85.71	87.5	100	91.05
BR14 Vs BRRIdhan 34	91	85.71	94.11	91	90.46
BR14 Vs BR26	80	85.71	80	83.3	84.75
BR14 Vs BRRIdhan 38	89	85.71	93.3	91	89.75
BRRIdhan 29 Vs BR10	66.67	100	91	80	84.4
BRRIdhan 29 Vs BR16	66.67	100	83.33	80	82.5
BRRIdhan 29 Vs BRRIdhan 31	75	85.71	80	61.54	75.6
BRRIdhan 29 Vs BRRIdhan 32	66.67	100	71.42	80	80
BRRIdhan 29 Vs BRRIdhan 33	66.67	100	83.33	80	82.5
BRRidhan 29 Vs BRRIdhan 34	66.67	100	73	89	82.17
BRRIdhan 29 Vs BR26	75	100	91	80	86.5
BRRIdhan 29 Vs BRRIdhan 38	85.71	100	83.33	89	90
BR10-BR16	100	100	92.31	100	98.08
BR10 Vs BRRIdhan 31	91	85.71	91	46.15	78.46
BR10 Vs BRRIdhan 32	100	100	66.67	100	91.67
BR10 Vs BRdhan 33	100	100	92.31	100	98.07
BR10 Vs BRRIdhan 34	100	100	85.71	91	94.18
BR10 Vs BR26	91	100	100	83.33	93.6
BR10 Vs BRRIdhan 38	80	100	92.31	91	91
BR16 Vs BRRIdhan 31	91	85 .71	83.33	80	85
BR16 Vs BRRIdhan 32	100	100	87.5	100	97
BR16 Vs BRRIdhan 33	100	100	100	100	100
BR16 Vs BRRIdhan 34	100	100	93.33	91	96.1
BR16 Vs BR26	91	100	92.31	83.33	91.66
BR16 Vs BRRIdhan38	80	100	100	91	92.75
BRRIdhan 31 Vs BRRIdhan 32	91	85.71	71.43	80	82.035
BRRIdhan 31 Vs BRRIdhan 33	91	85.71	83.33	80	85.01
BRRIdhan 31 Vs BRRIdhan 34	91	85.71	77	71.43	81.285
BRRIdhan 31 Vs BR26	100	85.71	91	66.67	85.845
BRRIdhan 31 Vs BRRIdhan 38	89	85.71	83.33	71.43	82.3675
BRRIdhan 32 Vs BRRIdhan 33	100	100	87.5	100	96.875
BRRIdhan32 Vs BRRIdhan 34	100	100	94.18	91	96.295
BRRIdhan 32 Vs BR26	91	100	80	83.33	88.5825
BRRIdhan 32 Vs BRRIdhan 38	80	100	87.5	91	89.625
BRRIdhan 33 Vs BRRIdhan 34	100	100	93.33	91	96.0825
BRRIdhan 33 Vs BR26	91	100	92.31	83.33	91.66
BRRIdhan 33 Vs BRRIdhan 38	80	100	100	91	92.75
BRRIdhan 34 Vs BR26	91	100	92.31	91	93.5775
BRRIdhan 34 Vs BRRIdhan 38	80	100	93.33	100	93.3325
BR26 Vs BRRIdhan 38	89	100	92.31	91	93.0775
Average	87.466	95.128	86.654	85.863	88.81

Table 6: Summary of Genetic Variation Statistics for All Loci

Locus	Sample size	na*	ne*	h*	I*
S1027-1	10	2.0000	1.4706	0.3200	0.5004
S1027-2	10	2.0000	1.2195	0.1800	0.3251
S1027-3	10	2.0000	1.7241	0.4200	0.6109
S1027-4	10	1.0000	1.0000	0.0000	0.0000
S1027-5	10	1.0000	1.0000	0.0000	0.0000
S1027-6	10	2.0000	1.4706	0.3200	0.5004
OPA02-1	10	1.0000	1.0000	0.0000	0.0000
OPA02-2	10	1.0000	1.0000	0.0000	0.0000
OPA02-3	10	2.0000	1.4706	0.3200	0.5004
OPA02-4	10	1.0000	1.0000	0.0000	0.0000
S1155-1	10	2.0000	1.9231	0.4800	0.6730
S1155-2	10	2.0000	1.7241	0.4200	0.6109
S1155-3	10	2.0000	1.2195	0.1800	0.3251
S1155-4	10	2.0000	1.2195	0.1800	0.3251
S1155-5	10	2.0000	1.4706	0.3200	0.5004
S1155-6	10	1.0000	1.0000	0.0000	0.0000
S1155-7	10	1.0000	1.0000	0.0000	0.0000
S1155-8	10	1.0000	1.0000	0.0000	0.0000
S1155-9	10	1.0000	1.0000	0.0000	0.0000
OPAB02-1	10	2.0000	1.9231	0.4800	0.6730

Table 6: Summary of Genetic Variation Statistics for All Loci (continued)

Locus	Sample size	na*	ne*	h*	I*
OPAB02-2	10	2.0000	1.2195	0.1800	0.3251
OPAB02-3	10	2.0000	1.2195	0.1800	0.3251
OPAB02-4	10	1.0000	1.0000	0.0000	0.0000
OPAB02-5	10	1.0000	1.0000	0.0000	0.0000
OPAB02-6	10	1.0000	1.0000	0.0000	0.0000
OPAB02-7	10	1.0000	1.0000	0.0000	0.0000
OPAB02-8	10	2.0000	1.2195	0.1800	0.3251
OPAB02-9	10	2.0000	1.2195	0.1800	0.3251
OPAB02-10	10	2.0000	1.2195	0.1800	0.3251
Mean	10	1.5517	1.2391	0.1559	0.2472
St. Dev		0.5061	0.2907	0.1667	0.2483

** na = Observed number of alleles, * ne = Effective number of alleles, * h = Nei's (1973) gene diversity and * I = Shannon's Information index*

3.5 NEI'S (1972) GENETIC DISTANCE

The values of pair-wise comparisons of Nei's [25] genetic distance between 10 rice genotypes were computed from combined data sets for the four primers ranging from 0.0000 to 0.4299 (Table 7). The highest Nei's [25] genetic distance (0.4299) was observed in BRRIdhan 29 Vs BRRI dhan 31 and BRRIdhan 31 Vs BRRIdhan 38 and the lowest genetic distance (0.0000) was estimated in BR 16 Vs BRRIdhan 33. Furthermore, high level of genetic distance (0.3716) was found in BR 14 Vs BRRIdhan 29, BR 14 Vs BRRIdhan31, BRRIdhan29 Vs BRRIdhan32, BRRIdhan31 Vs BRRIdhan32, BRRIdhan31 Vs BRRIdhan34, BRRIdhan31 Vs BRRIdhan26 germplasms pair.

3.6 DENDROGRAM

A dendrogram based on Nei's [25] genetic distance using UPGMA segregates ten germplasms of rice into two main clusters. The main clusters are further divided into sub clusters (Figure 3). BR 10, BR 14, BR 16, BR 26, BRRIdhan29, BRRIdhan32, BRRIdhan33, BRRIdhan34 and BRRIdhan38 formed cluster I whereas BRRIdhan31 comprised the cluster II. In cluster I BR10, BR 14, BR 16, BR 26, BRRIdhan32, BRRIdhan33, BRRIdhan34 and BRRIdhan38 formed sub-cluster I and BRRIdhan 29 formed sub- cluster II. Sub-cluster I further divided into two sub-sub clusters. Sub-sub cluster I consisted of BR 10, BR14, BR16, BRRIdhan32, BRRIdhan33 and BRRIdhan34 due to similar banding pattern. BR 26 and BRRIdhan38 formed the sub- sub cluster II. Again, sub-sub cluster I grouped into two clusters. BR14 and BRRIdhan 32 had same banding pattern so they are in same group. BR10, BR16, BRRIdhan33 and BRRIdhan34 were placed in same group due to similarity in their banding pttern. In this study, the RAPD technique was found to discriminate ten rice germplasms. Germplasms having higher similarity were close in the dendogram. According to the results, BRRIdhan 31 was found more genetically diversified comparative to the other germplasms. Germplasms having high level of similarity indices showed low level of genetic distance. Inter-germplasm similarity index between BR 16 Vs BRRI Dhan 33 was 100% so genetic distance was lower between the pair (0.0000) than rest of the germplasms pair.

Table 7. Genetic distance value for different germplasms

Variety	BR14	BR29	BR10	BR16	BRRI dhan31	BRRI dhan32	BRRI dhan33	BRRI dhan34	BR26	BRRI dhan3
BR14										
BRRIdhan29	0.3716									
BR10	0.1892	0.2318								
BR16	0.1484	0.2763	0.0351							
BRRIdhan31	0.3716	0.4229	0.2318	0.2763						
BRRIdhan32	0.0715	0.3716	0.1092	0.0715	0.3716					
BRRIdhan33	0.1484	0.2763	0.0351	0.0000	0.2763	0.0715				
BRRIdhan34	0.1484	0.2763	0.1092	0.0715	0.3716	0.0715	0.0715			
BR26	0.3228	0.2763	0.1092	0.1484	0.3716	0.2318	0.1484	0.1484		
BRRIdhan38	0.1892	0.2318	0.1484	0.1092	0.4229	0 .1892	0.1092	0.1092	0.1092	

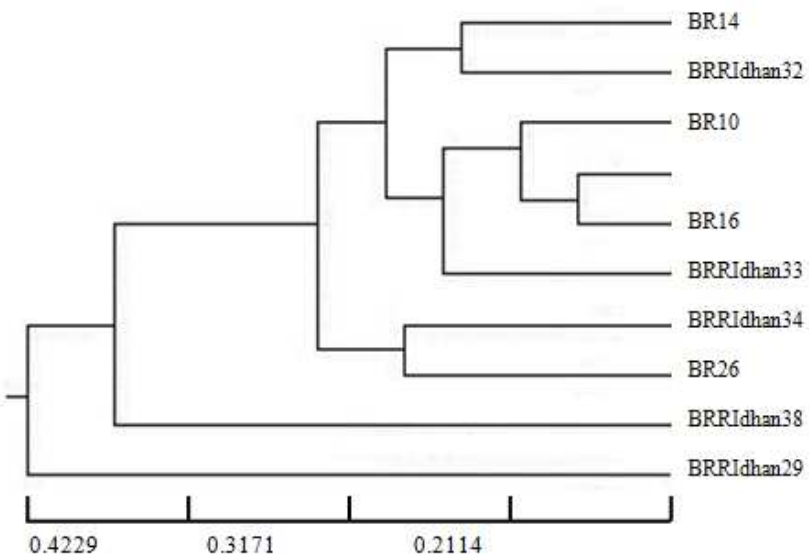

Figure 3: UPGMA Dedogram based on Nei's genetic distance, summarizing the data on differentiation among 10 Rice germplasms according to RAPD analysis.

BR14 Vs BRRI dhan 31 showed the least inter-germplasm similarity index (70.63%) had maximum genetic distance in dendogram. On the other hand, genetic similarity between BR 10 Vs BR 16 (98.08%), BR 10 Vs BRRIdhan33 (98.07%), BR 10 VS BRRI Dhan 34 (94.18%) germplasm pairs was high so genetic distance between each pair was low. Therefore, this study clearly indicates that there is a high level of genetic variation among different germplasms. Mean diversity across all population for all the loci studied was 0.1559 indicating some degree of genetic variation in rice germplasms. Genetic variation refers to the differences in the heredity constitutions of the individuals of a species and it is important in maintaining the developmental stability and biological potential of plant species.

Among BRRI released 10 rice germplasms used in this study, BR 14 is BLB resistant [7]. In this study BRRIdhan 29 is BLB susceptible variety. Genetic distance between BR 14 VS BRRIdhan 32 is (0.0715) it is the least value between BR14 VS any germplasms, again their genetic similarity is 94.17. It indicates that BRRIdhan 32 has high level similarity to BLB resistant BR14. Again their banding is also similar. It can be concluded from this study, due to close similarity between BR14 and BRRIdhan 32, BRRI dhan 32 may have resistance power to BLB. Thus it has resistance power against BLB close to BR 14. On the other hand, BR 14 VS BRRIdhan 31 had high genetic distance (0.3716) also lowest similarity. Also these two are more distant in demdogram. It can conclude that BRRIdhan 31 may possess minimal resistance against BLB.

The resistance genes from BR14 and other resistant variety will be useful in the development of durable BLB resistant rice cultivars. On the basis obtained results, we can conclude that some of the resistant materials found in this study could be used in hybridization programs for varietal improvement against the BLB. Therefore, a breeding programme should be initiated to transfer these resistance genes to the high yielding and better grain quality but BLB susceptible cultivars to control the disease effectivelty.

4 CONCLUSION

Screening of BLB resistant rice using RAPD technique from the selected rice genotypes was the main objectives of the study. Ten rice genotypes were used for screening BLB resistant at the seedling stage following IRRI standard protocol.

The genetic diversity in 10 rice genotypes were analyzed by PCR-based RAPD markers by using 4 arbitrary decamer primers (S1027, OPA02, S1155, OPAB02). Selected four primers generated a total of 29 bands. Out of the 29 bands, 16 bands (55.17) were polymorphic and 13 bands (44.83%) were monomorphic.

The four different primers generated various banding patterns, ranging from 4 (OPA02) to 10 (OPAB02). Among a total of 29 bands, four primers generated an average of 7.25 polymorphic bands.

According to Nei's [25], the genetic distance between BRRIdhan 29 Vs BRRIdhan 31, BRRIdhan31 Vs BRRIdhan 38 was highest (0.4229) and in the dendogram they were placed in different clusters. Second highest genetic distance was 0.3716 between BR14 VS BRRIdhan 29, BR14 VS BRRIdhan 31, BRRIdhan 29 Vs BRRI dhan 32, BRRIdhan 31 Vs BRRI dhan 32, BRRIdhan 31 VS BRRIdhan 34, BRRIdhan 31 VS BR 26.

On the other hand, least genetic distance was seen between BR 16 VS BRRIdhan 33(0.0000). BR 10 VS BR 16, BR 10 VS BRRIdhan 33 had minimal genetic distance (0.0351) and they were placed in same clusters. Intergermplasm similarity was highest between BR16 Vs BRRIdhan 33 (100%), again genetic variation was (0.0000) least between them. They are also very close in the dendogram.

The UPGMA dendrogram based on Nei's [25] genetic distances between different pairs were correlated with their sources of origin. The dendrogram indicates segregation of ten germplasms of rice into two main clusters; the main clusters are further divided into sub clusters. The BLB resistant BR-14 was close to the germplasm BRRIdhan 32 with the least genetic distance. Both of these two germplasms are in the same cluster.

It is concluded that some of the resistant materials found in this study would be useful for varietal improvement against the BLB. Finally, it is said that RAPD markers can be sensitive, simple, efficient and powerful tool for screening BLB resistant rice among rice genotypes and effectively trace their genetic relationships. The results of this study can be used as a base for future genetic analysis of rice varieties in Bangladesh. Besides RAPD, the use of different primers (SSR, RFLP, AFLP) etc. could be useful in future research.

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