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Screening of Aromatic Rice Cultivars for Bacterial Leaf Blight (BLB) Disease Resistance Gene *Xa*5 through Molecular Marker

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Authors' contributions

This work was carried out in collaboration between all authors. Author MSI designed the study, followed up the practical work and wrote the first draft of the manuscript. Author MM managed the literature searches and wrote the final version of the manuscript. Author SRK performed the practical work. Authors KMN and MMI managed the analyses of the study and followed up the steps of the search. All authors read and approved the final manuscript.

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ABSTRACT

Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most common devastating diseases of rice (*Oryza sativa*) all around the world. Thus, present study has been conducted to screen 12 aromatic rice cultivars (Atashail, Basmati, Kalizira, Uknimodhu, Zira katari, BR5, BR14, Bina dhan9, BRRI dhan34, BRRI dhan37, BRRI dhan38 and BRRI dhan50) for BLB resistant gene *xa*5. The genotypes were analyzed using two genetic markers (RM 122 and RM 390) by Polymerase Chain Reaction (PCR). Where, BR 14 that was resistant to BLB was used as control. Both primers generated different banding patterns. Primer RM 122 produced 6 bands whereas primer RM 390 produced 10 bands, respectively. The highest level of gene diversity value

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(0.8889) was observed in locus RM 390 and the lowest level of gene diversity value (0.7361) was observed in locus RM122 with a mean diversity of 0.8125. The PIC values ranged from a low of 0.7007 (RM 122) to a high of 0.8785 (RM 390) with an average of 0.7896. Using the linked primer RM 122, the bands of *xa5* gene were standardized by the amplified DNAs. The DNA band of 246 bp was considered as resistance line and the DNA band of 230 bp was considered as a susceptible line. Similarly, in case of primer RM 390, 70 bp was considered as a resistance line and 112 bp was considered as susceptible line. Basmati, BRRI dhan50, Kalizira Atasail, Bina dhan9, Uknimodhu, BR 34, BR 37 and Zira Katari all of them found as partial resistant to BLB diseases. However, BRRI dhan38 was found as partial or complete susceptible cultivar.

Keywords: BLB; Oryza sativa; aromatic; cultivar; resistance; susceptible.

1. INTRODUCTION

Approximately 90% of the world's rice is grown in Asia. Rice constitutes a staple food for the people of Bangladesh [1,2]. Rice provides 75% of calories and 55% of proteins in average of the daily diet. It contains all the 18 amino acids and vitamins except vitamin-C and A [3,4]. Aromatic rice has high demand in the international market for its quality attributes such as a distinct pleasant aroma, fluffy texture of cooked rice and high volume expansion during cooking [5]. Productivity and quality of aromatic rice depend on the environmental conditions. Unfortunately, this crop has been standing before many kinds of threat like biotic stresses including diseases, pathogens [6]. BLB disease reduces quantity and quality of rice caused by the rod-shaped bacterium *Xanthomonas oryzae pv*. *Oryzae* is one of the oldest known diseases that was first noticed by the farmers of Japan in 1884 [7,8]. The disease incurring severe and can cause up to 50-80% crop yield reduction [9].

Scientists have already been reported major 29 genes for resistance to BLB disease where only 11 were identified. [10,11,12] Among these genes 14 are dominant (xa1, xa2, xa3, xa4, xa7, xa10, xa11, xa12, xa14, xa16, xa17, xa18, xa21 and xa22) and 6 recessive (xa5, xa8, xa13, xa15, xa19 and xa20) [13]. The resistant genes xa5, xa8, xa13, xa24, xa26 and xa28 occur naturally, whereas three xa15, xa19 and xa20, have been created by mutagenesis [14,15]. BLB resistance genes of rice cultivars are distributed in almost the entire Asian region, especially xa5 gene showed area specificity in Bangladesh and Nepal and the percentage of cultivars with xa5 was 25.9% and 13.3%, respectively. Due to having large effects of the environment and the little narrow sense heritability of BLB resistance it is so tricky task to select the right plant for BLB resistant through conventional methods.

Molecular markers viz. Restriction Fragment Length Polymorphism (RFLP), Randomly Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSRs), Amplified Fragment Length Polymorphism (AFLP) and Single Nucleotide Polymorphisms (SNPs) offer hope for addressing these concerns, permits the fast identification of individuals that contain gene (s) for BLB resistance. These genetic markers are found to be more reliable to assess the variability and diversity at molecular level [16]. Among these markers, Simple Sequence Repeats are neutral, co-dominant, a powerful tool to assess the genetic variability of cultivars and can be used as in the molecular aided selection (MAS) breeding program [3,17,18].

2. MATERIALS AND METHODS

2.1 Cultivars and Isolation of DNA

Twelve rice cultivars viz. Atashail, Basmati, Kalizira, Uknimodhu, Zira katari, BR5, BR14, Bina dhan9, BRRI dhan34, BRRI dhan37, BRRI dhan38 and BRRI dhan50 were collected from BRRI, BINA and different districts of Bangladesh. Where, BR 14 was used as control for Bacterial Leaf Blight disease resistance gene xa5. Seed germination was performed and germinated seeds were sown in pots. Juvenile and actively growing fresh leaf tissues were collected for the isolation of genomic DNA. Total genomic DNA was isolated from rice leaves following Phenol: Chloroform: Isoamyl alcohol purification and ethanol precipitation method [19].

2.2 PCR Analysis

The polymorphism survey of 12 rice cultivars were carried out using two microsatellite markers RM122 and RM390. 5X Buffer, dNTPs, Primers, Taq DNA polymerase, ddH2O, MgCl2 were used to prepare PCR cocktail. The total volume of

PCR cocktail for this study was 8.0 µl per sample. The PCR products were used in polyacrylamide gel electrophoresis by using DNA 100 bp and 25 bp marker (Table 1) and visualized under UV light.

2.3 Analysis of Microsatellite Data

The DNA polymorphisms were detected according to band presence and absence**.** Bands of microsatellite markers which were not clearly identified were considered as non-scorable. A single genotypic data matrix was constructed for all loci. Genetic diversity values of 12 rice cultivars for 2 primers are given according to Nei's distance [20]. Allele frequencies were calculated directly from the observed genotypes. Allelic variations and fit to Hardy-Weinberg proportions were estimated by the software POPGENE (version 1.31) by a chi-square (χ^2) test [21] with 1000 simulated samples. Molecular weight for each amplified allele was measured in base pair using Alpha Ease FC 5.0 software. The allele frequency data from Power Marker Version 3.25 [22] was used to export the data in binary format (allele presence =1 and allele absence = 0) for analysis with NTSYS-PC Version 2.2 [23].

Polymorphisms information content (PIC) value of a marker was calculated according to a simplified version after Andersons [24].

$$PIC_i = 1 - \sum X_i 2\, J$$

Where,

Xij is the frequency of the *j*th allele for the *i*th marker.

The summary statistics including the number of alleles per locus, major allele frequency, gene diversity, Polymorphism Information Content (PIC) values were determined using Power Marker Version 3.25 [26].

Expected heterozygosity *(He)* and observed heterozygosity (*Ho*) were also calculated after (Nei, 1972) using the following formula and with the help of POPGENE (version 1.31) [21] computer package program:

$$he = 2n\,(1 - \sum xi^2/N)/\,(2n - 1)$$

Where,

he ~ is the expected heterozygosity of each locus
n is the number of sampled individuals
xi is the frequency of i-th allele at each locus and
N is the number of loci examined.

Thus, the average heterozygosity (*He*) was calculated as

$$H_e = \sum h_e/r$$

Where, r is the number of loci examined [25].

Nei's genetic distance value was computed using the formula as described in the POPGENE (Version 1.31) software user manual [21]. Genetic distance values (D) [20] were calculated as

$$D = - \ln Jxy/\sum JxJy$$

Where,

$Jx = \sum Xi^2/r$ in population X
$Jy = \sum Yi^2/r$ in population Y
$Jxy = \sum XiYi$, Xi and Yi are the frequency of the i-th allele of a given locus in the two populations of chilli germplasms compared and r = the number of allele frequencies for all possible pairs of populations.

Table 1. Summary of SSR marker used for the study

Primer name	Molecular weight	Sequence	Chromosome locus	Anneling temp (^{0}C)
RM122	7464 (Rev.)	5'GAAGGAGGTATGCCTTTGTTG GAC	5	55
	7408 (For.)	5'GAGTCGATGTAATGTCATCAG TGC		
RM390	6204(Rev.)	5'CGTCAATGGGGTAGGTCTTG	5	55
	6305(For.)	5'GGAGGCCAAGGAAGAGGTAG		

For the unrooted phylogenic tree, genetic distance was calculated using the 'C.S. Churd' distance followed by phylogeny reconstruction using neighbor-joining as implemented in Power Marker with tree viewed using the TREE VIEW. The allele frequency dated from Power marker was used to export the data in binary format (allele presence = 1 and allele absence = 0) for analysis with NTSYS-PC Version 2.2. [22] A similarity matrix was calculated with Simqual Subprogram using the Dice coefficient, followed by cluster analysis with the SAHN Subprogram using the UPGMA clustering methods implemented in NTSYS-PC was used to construct a dendogram showing relationship among the rice varieties. The similarity matrix was also used for principal coordinate analysis (PCA) with Dcenter, Eigen, Output and MX Plat subprograms in computer program Numerical Taxonomy and Multivariate analysis system (NTSYS-PC).

3. RESULTS AND DISCUSSION

Two SSR markers generated a higher level of DNA polymorphism for the studying samples. The variation found at molecular level is presented and briefly discussed here.

3.1 Allelic and Loci Variation within the Genotypes

In the present study, 12 cultivars of rice were analyzed using two SSR primer pairs (RM122 and RM390). Polyacrylamide gel electrophoresis (PAGE) (Figs. 1-2) was used to analyze amplified microsatellite loci and the microsatellite loci were also multi-allelic (six to ten alleles per locus with a mean of 8/locus in the present study).

3.2 Number of Alleles per Locus and Allele Size Range

Using two SSR markers, a total of 16 alleles were detected among the 12 rice cultivars. The average number of allele per locus was 8, with a range of 6 (RM122) and to as many as 10 of RM390 which is given in Table 2. Thus, RM390 presented the smallest allele size range (70-112 bp) and had 10 alleles in a locus, while RM122 had the largest allele size range (231-246 bp) and a total of 6 alleles in Table 2.

3.3 Gene Diversity

The highest level of gene diversity value (0.8889) was observed in locusRM390 and the lowest level of gene diversity value (0.7361) was observed in locusRM122 with a mean diversity of 0.8125 in Table 2.

3.4 PIC Values

In the measured microsatellites loci, the Polymorphism Information Content (PIC) values were found ranged from a low of 0.7007 (RM122) to as a high of 0.8785 (RM390) and average of 0.7896 (Table 2). These values showed a significant, positive correlation with the number of alleles and allele size range for microsatellites evaluated in this study (Table 3).

3.5 Major Allele

Major allele is defined as the allele with the highest frequency and also known as most common allele at each locus. The frequency of the most common allele at each locus ranged from 16.6% (RM390) to 47% (RM122) with a mean frequency of 29 (Table 4). The average number of sample size was 12 with an average number of observations 12. The size of the different major alleles at different loci ranges from 234 bp (RM122) to 109 bp (RM390).

3.6 Heterozygosity for All Microsatellite Loci of 12 Rice Cultivars

The expected heterozygosity was 0.9127 for RM390 and 0.7273 for RM122. However, the heterozygosity was observed zero for both primers (Table 5).

3.7 Deviation from Hardy-Weinberg Proportion

In this study, there were no significant deviations from Hardy-Weinberg Equilibrium (HWE) for all microsatellite loci (Table 6).

3.8 Gene diversity and Gene Flow in 12 Rice Cultivars According to Locus

According to Nei's (Nei's., 1972) the highest level of Shannon's information index (2.1458) was observed in locus RM390 and the lowest level of Shannon's information index (1.3667) was observed in locus RM122 (Table 7). The highest effective number of allele (8.0667) was observed in locus RM 390 and the lowest effective number of allele (3.2703) was observed in locus RM122 (Table 7). Allele frequency of two SSR markers in all 12 rice cultivars showed in table 9 and Average gene flow for all microsatellite loci in 12 rice cultivars is 0.000 (Table 8).

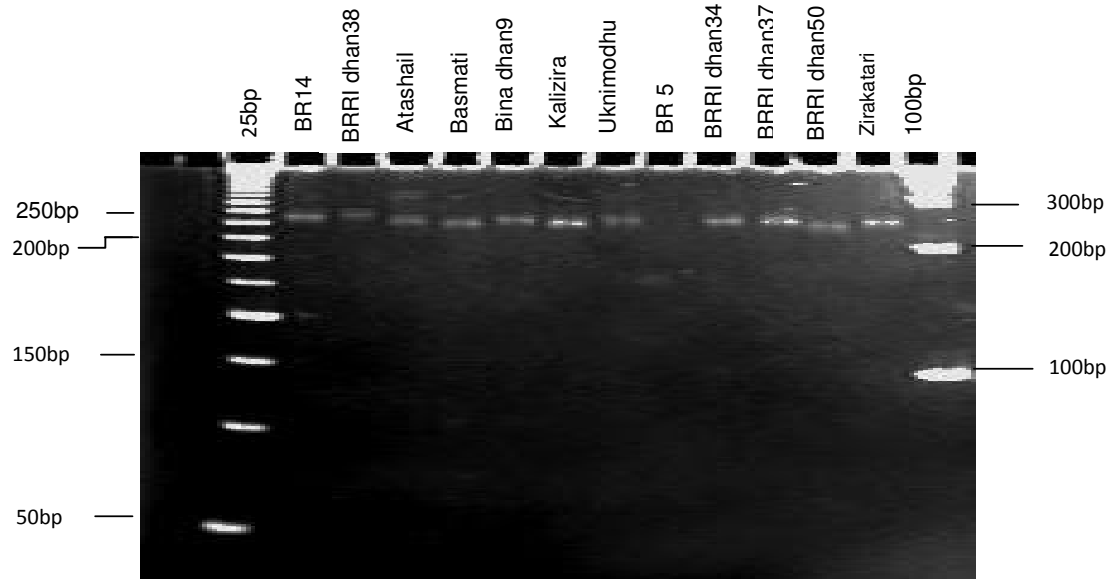

Fig. 1. Microsatellite profiles of 12 rice cultivars using RM122 marker

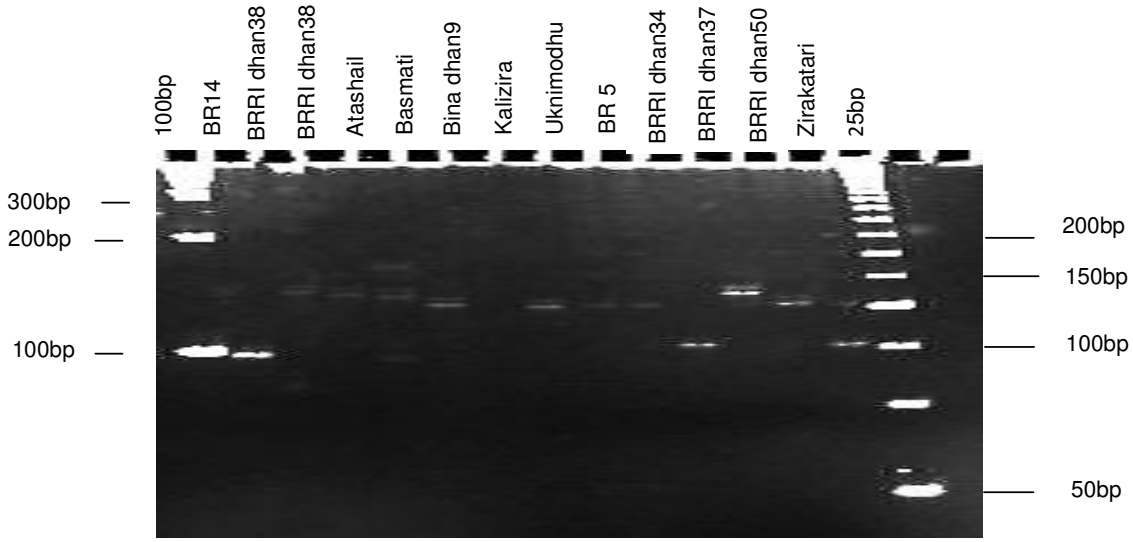

Fig. 2. Microsatellite profiles of 12 rice cultivars using RM 390 marker

Table 2. The microsatellite markers used for the chromosome location of the loci, the number of alleles, gene diversity and the diversity index of 12 rice cultivars

Primer name	Size range (bp)	No. of alleles	Gene diversity	Mean diversity	Diversity index PIC = 1-$\sum xi^2$	Average PIC value
RM122	231-246	6	0.7361	0.8125	0.7007	0.7896
RM390	70-112	10	0.8889		0.8785	

Table 3. Size, frequency, variance and standard deviation of alleles at two markers loci of 12 rice cultivars

SL. No.	Locus allele	Size	Frequency	Variance	SD
		0	0.0833	0.00636574	0.0798
		231	0.2500	0.01562500	0.1250
		234	0.4167	0.02025463	0.1423
1.	RM122	237	0.0833	0.00636574	0.0798
		243	0.0833	0.00636574	0.0798
		246	0.0833	0.00636574	0.0798
		0	0.0833	0.00636574	0.0798
		100	0.0833	0.00636574	0.0798
		101	0.1667	0.01157407	0.1076
		102	0.0833	0.00636574	0.0798
2.	RM390	103	0.0833	0.00636574	0.0798
		109	0.1667	0.01157407	0.1076
		110	0.0833	0.00636574	0.0798
		112	0.0833	0.00636574	0.0798
		70	0.0833	0.00636574	0.0798
		75	0.0833	0.00636574	0.0798

Table 4. Data on sample size, number of observation, major alleles (size and frequencies) and polymorphism information content (PIC) found among 12 rice cultivars for 2 SSR markers

Locus	Sample size	No of observation	Major alleles		PIC
			Size (bp)	Frequency (%)	
RM122	12	12	234	41.6	0.7007
RM390	12	12	109	16.6	0.8785
Mean	**12**	**12**	**212.73**	**29**	**0.7896**

Table 5. Heterozygosity for all microsatellite loci of 12 rice cultivars

Microsatellite loci	N	H_o	H_e	Polymorphism
RM122	6	0.000	0.7273	100
RM390	10	0.000	0.9127	100

3.9 Detection of BLB Resistant and Susceptible Rice Cultivars

The amplified DNA bands of xa5 gene were standardized by comparing with the study of Naveed conducted with only one primer RM 122. In that study, the amplified DNAs of IRBB-5 (having *xa*5) and IR-24 (without xa5) which were used as control for BLB resistance as well as for susceptible line. IRBB-5 showed the band of 240 bp of whereas IR-24 showed the DNA band of about 230 bp. [22] The polymorphic bands pattern of PCR products of our study is showed in Table 10.

BR 14 (resistant to gene *xa*5) showed the DNA band of 243 bp that was nearly the same to the band of IRBB-5 using the same marker RM122. So, the BR 14 is completely resistant to BLB diseases. Atashail, Basmati and Bina dhan9 showed the DNA band of 231 bp which were partially same with the band of IRBB-5. So, these are may be partial resistant to BLB diseases. Kalizira, Uknimodhu, BRRI dhan37, BRRI dhan50 and Zirakatari showed the DNA band near about 234 bp so they also are partial resistant to bacterial blight disease. BRRI dhan37 showed the band of 237 bp and considered as partial resistant and partial susceptible to bacterial blight disease. However, BRRI dhan38 showed the band of 246 and considered as complete susceptible to BLB diseases.

On the other hand, in case of primer RM 390; BR 14 showed the band of 70 bp and BRRI dhan38 showed the band of 112 bp. So, Uknimodhu considered as partially susceptible to BLB disease because it showed the band of 100 bp. Besides, BR 5 and BRRI dhan34 showed the

band of 101 bp, Zirakatari showed 102 bp, Bina dhan-9 showed 103 bp, Atashail and basmati showed 109 bp and BRRI dhan30 showed the DNA band of 110 bp all of them considered as partial susceptible to BLB disease as they showed the DNA band nearly same to the band of IRBB-5. So, these may be partially susceptible cultivar. Only BRRI dhan37 showed the band of 75 bp and considered as partially resistant to BLB disease.

Table 6. Deviation from Hardy-Weinberg expectations in 12 rice cultivars according to locus

Locus name	Degree of fredom	Chi-square (χ^2)	G-square
RM122	10	76.266667	35.350620
RM390	36	164.000000	58.190595

Table 7. Genetic variation statistics for all loci

Locus name	Na	ne	I
RM122	5.0000	3.2700	1.3667
RM390	9.0000	8.0667	2.1458
Mean	**7.0000**	**5.6658**	**1.7563**
S.D	**2.8284**	**3.3916**	**0.5509**

na = observed number of alleles;ne = effective number of alleles ;I = Shannon's information index
S.D = Standard deviation

Table 8. F-Statistics and gene flow for all microsatellite loci of 12 rice cultivars

Locus name	Fst	Nm*
RM122	1.00	0.00
RM390	1.00	0.00
Mean	**1.00**	**0.00**

**NM=Gene flow estimated from Fst = 0.25(1-Fst)/Fst*

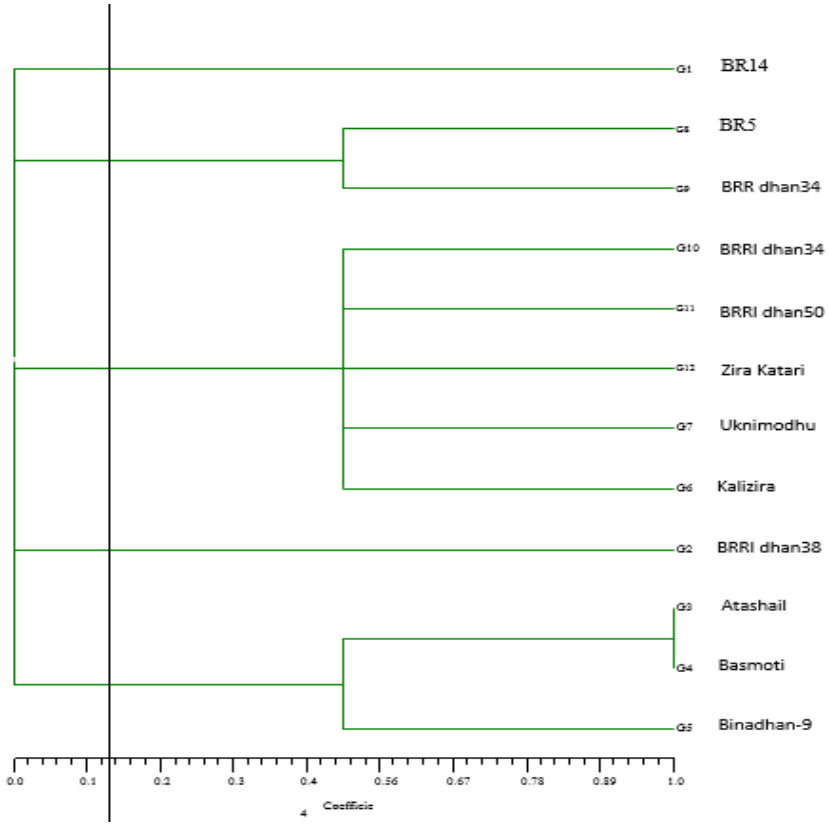

Fig. 3. UPGMA dendrogram based on Nei's (1972) genetic distance summarizing the data on differentiation among 12 rice cultivars according to SSR analysis
Here, G= gene flow

Table 9. Allele frequency of two SSR markers in all 12 rice cultivars

Marker	Allele	BR14	BRRI dhan37	BRRI dhan50	Zirakatari	BRRI dhan38	Atashail	Basmati	Bina dhan9	Kalizira	Uknimodhu	BR5	BRRI dhan34
RM122	0	0	0	0	0	0	0	0	0	0	0	0	0
RM122	231	0	0	0	0	0	1	1	1	0	0	0	0
RM122	234	0	1	1	1	0	0	0	0	1	1	0	0
RM122	237	0	0	0	0	0	0	0	0	0	0	0	1
RM122	243	1	1	0	0	0	0	0	0	0	0	0	0
RM122	246	0	0	0	0	1	0	0	0	0	0	0	0
RM390	0	0	0	0	0	0	0	0	0	1	0	0	0
RM390	100	0	0	0	0	0	0	0	0	0	1	0	0
RM390	101	0	0	0	0	0	0	0	0	0	0	1	1
RM390	102	0	0	0	1	0	0	0	0	0	0	0	0
RM390	103	0	0	0	0	0	0	0	1	0	0	0	0
RM390	109	0	0	0	0	0	1	1	0	0	0	0	0
RM390	110	0	0	1	0	0	0	0	0	0	0	0	0
RM390	112	0	0	0	0	1	0	0	0	0	0	0	0
RM390	70	1	0	0	0	0	0	0	0	0	0	0	0
RM390	75	0	1	0	0	0	0	0	0	0	0	0	0

Table 10. Detection of BLB resistance and susceptible rice cultivars using the allele (bp)

Rice cultivars	Allele (bp) DNA Band	BLB complete resistant cultivars	BLB partial resistant cultivars	BLB partial susceptible cultivars	BLB complete susceptible cultivars
BR14	243	+	-	-	-
	70	+	-	-	-
BRRI dhan38	246	-	-	+	+
	112	-	-	+	+
Atasail	231	-	+	-	-
	109	-	-	+	+
Basmati	231	-	+	-	-
	109	-	-	+	-
BINA dhan9	231	-	+	-	-
	103		-	+	-
Kalizira	234	-	+	+	-
	0	-	-	-	-
Uknimodhu	234	-	+	-	-
	100	-	-	+	-
BR5	0	-	-	-	-
	101	-	-	+	-
BR34	237	-	+	-	-
	101	-	-	+	-
BR37	234	-	+	-	-
	75	-	+	-	-
BRRI dhan50	234	-	+	-	-
	110			+	
Zirakatari	234	-	+	-	-
	102	-	-	+	-

(+) indicates presence of BLB resistance/ susceptible traits due to presence/absence of xa5 gene; (-) indicates absence of BLB resistance/ susceptible traits due to presence/absence of xa5 gene

Table 11. Summary of Nei's (1972) genetic distance (below diagonal) values for different cultivars pairs of rice

Cultivars	BR14	BRRI dhan37	BRRI dhan50	Zirakatari	BRRI dhan38	Atashail	Basmati	Bina dhan9	Kalizira	Uknimodhu	BR5	BRRI dhan34
BR14												
BRRI dhan37	0.000	5.000										
BRRI dhan50	0.000	5.000										
Zirakatari	0.000	5.000	5.000									
BRRI dhan38	0.000	0.000	0.000	0.000								
Atashail	0.000	0.000	0.000	0.000	0.000							
Basmati	0.000	0.000	0.000	0.000	0.000	1.000						
Binadhan9	0.000	0.000	0.000	0.000	0.000	5.000	5.000					
Kalizira	0.000	5.000	5.000	5.000	0.000	0.000	0.000	0.000				
Uknimodhu	0.000	5.000	5.000	5.000	0.000	0.000	0.000	0.000	5.000			
BR5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		
BRRI dhan34	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	5.000	

3.10 Genetic Distance

The values of pair-wise comparisons of Nei's genetic distance (GD) between cultivars were computed from combined data for the 2 primers, ranged from 0.000 to 5.000 (above Table 11). Comparatively higher genetic distance (5.000) was observed among BRRI dhan37 Vs BRRI dhan50, BRRI dhan37 Vs Zira Katari, BRRI dhan37 Vs Kalizira, BRRI dhan37 Vs uknimodhu, BRRI dhan50 Vs Zira Katari, BRRI dhan50 Vs Kalizira, BRRI dhan50 Vs Uknimodhu, Zira Katari Vs Kalizira, Zira Katari Vs Uknimodhu, Atashail Vs Binadhan-9, Basmati Vs Binadhan-9, Kalizira Vs Uknimodhu, BR5 Vs BRRI dhan34. Rest of the cultivars pair had the lowest numerical genetic distance (0.0000) value except Atashail Vs Basmati and their pair value was (1.000). The means of genetic distances between cultivars were used to evaluate the genetic diversity of different rice cultivars.

3.11 Genetic Distance Analysis Using UPGMA

The Unweighted Pair Group Method with Arithmetic Means (UPGMA) cluster tree analysis led to the grouping of the 12 rice cultivars in five major clusters, BR14 which was single cultivar formed cluster-1. BRRI dhan37 and BR5 were grouped in cluster-2. Cluster-3 was the largest cluster comprised of five rice cultivars. These are BRRI dhan37, BRRI dhan50, Zirakatari, Kalizira and Uknimodhu. Only BRRI dhan38 belonged to cluster 4 solely. Cluster-5 was divided into two sub cluster-I included Atashail and Basmatia and sub cluster-II included Binadhan-9 (Fig. 3).

4. CONCLUSION

Screening of genetic variation is the important step to keep up the developmental stability and to explore the biological potential of an organism. Atashail, Basmati, Kalizira, Uknimodhu, Zira katari, BINA dhan9, BR 5, BR 14, BR 34, BR 37, BRRI dhan38, BRRI dhan50 were analyzed using two markers (RM122 and RM390) by Polymerase Chain Reaction (PCR). BR 14 was used in this study as control for BLB resistant *xa*5 gene.

These two markers (RM122 and RM390) produced a higher level of DNA polymorphism in the present collection of rice cultivars. The average number of allele per locus was 8, with arrange of 6 (RM122) to as many as 10 (RM390). According to Nei's (1972) the highest level of gene diversity value (0.8889) was observed in locus RM390 and the lowest level of gene diversity value (0.7361) was observed in locus RM122 with a mean diversity of 0.8125. RM390 presented the smallest allele size range (70 bp) and had 10 alleles in a locus, while RM122 had the largest allele size range (246 bp) and a total of 6 alleles. The PIC values ranged from a low of 0.7007 (RM122) to a high of 0.8785 (RM390) and averaged of 0.7896. The frequency of the most common allele at each locus ranged from 16.6% (RM390) to 41.6% (RM122) with a mean frequency of 29%. The size of the different major alleles at different loci ranges from 234 bp (RM122) to 109 bp (RM390). The expected heterozygosity was 0.9177 for RM390 and 0.7273 for RM122. According to Nei's (Nei's 1972) the highest level of Shannon's information index (2.1458) was observed in loci RM390 and the lowest level of Shannon's information index (1.3667) was observed in loci RM122. The highest effective number of alleles (8.0667) was observed in loci RM 390 and the lowest effective number of alleles (3.2700) was observed in loci RM122. The higher genetic distance between them indicates that genetically they are varied compare to lower genetic distance value. Cultivar pair with a higher value is more dissimilar than a pair with a lower value. The values of pair-wise comparisons of Nei's (Nei's., 1972) genetic distance (GD) between varieties were computed from combined data for the 2 primers, ranged from 0.000 to 5.000. A dendrogram was constructed based on the Nei's genetic distance calculated from the 16 SSR alleles generated from the 12 rice cultivars. All 12 rice cultivars could be easily distinguished. The Unweighted Pair Group Method with Arithmetic Means (UPGMA) cluster tree analysis led to the grouping of the 12 rice cultivars in five major clusters, BR14 which was single cultivar formed cluster-1. BRRI dhan37 and BR5 were grouped in cluster-2. Cluster-3 was the largest cluster comprised of five rice cultivars. These are BRRI dhan37, BRRI dhan50, Zirakatari, Kalizira and Uknimodhu. Only BRRI dhan38 belonged to cluster 4 solely. Cluster-5 divide into two sub cluster-I includes Atashail and Basmati and sub cluster-II includes Binadhan-9. The results of this study may be employed as a baseline and local source of *xa*5 gene for elite molecular breeding program for the improvement of fine rice in Bangladesh.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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