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Screening of Rice Varieties for Bacterial Leaf Blight Resistance by Using SSR Markers

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Abstract

Genetic variations of seventeen rice germplasms were studied using two SSR markers (RM122 and RM390) linked to the rice bacterial leaf blight (BLB) resistance genes.The average number of allele per locus was 10, with a range of 7 (RM122) to as many as 13 (RM390). The highest level of gene diversity value (0.9135) was observed in loci RM390 and the lowest level of gene diversity value (0.7059) was observed in loci RM122 with a mean diversity of 0.8097. The PIC values ranged from a low of 0.6715 (RM122) to a high of 0.9069 (RM390) and averaged of 0.7892. A dendrogram based on genetic distance using UPGMA, indicates segregation of the 17 rice varieties in six major clusters, BR10 and Binadhan 5, which were single variety formed cluster-1 and cluster-2 respectively. BRRIdhan 38, Malshira, Binadhan 7, BRRIdhan 29, BRRIdhan 31 were grouped in cluster-3, in which sub cluster-I include BRRIdhan 38, Malshira and sub cluster-II includes Binadhan 7, BRRIdhan 29, BRRIdhan 31. Cluster-4 was the largest cluster comprised of seven rice varieties. These are Iratom 24, BRRIdhan 34, Binasail, Binadhan 4, BR 26, BR 16 and Binadhan 6. Only BRRIdhan 33 was formed cluster-5. BR 14 and BRRIdhan 32 formed cluster-6. Thus, using the same linked marker RM122, BR 14 and BR 10 might be resistant to BLB diseases. SSR markers offer a potential, simple, rapid and reliable method in marker assisted breeding.

Keywords: *Screening, BLB, Resistance, Rice varieties and SSR markers*

I. Introduction

Rice (*Oryza sativa* L.) (2n = 24) belonging to the family Poaceae is the staple food for one third of the world's population that occupies almost one-fifth of the total land area covered under cereals (Chakravarthi and Naravaneni, 2006). Over half of the world's population depends on rice as staple food. More than 90% of the world's rice is grown and consumed in Asia (Khush, 2005). In Bangladesh, rice is the principal crop sharing over 94% of the total food grain production of 26.7 million metric tons. Rice provides 75% of the calories and 55% of the proteins in the average daily diet of the people of Bangladesh (Bhuiyan, 2005).By the year 2025, 21% increase in production over that year 2000 of will be needed (Bhuiyan *et al.*, 2002). To achieve a substantial improvement in grain yield in a limited period, a 'second green revolution' based on advanced plant biotechnology and plant genomics is needed (Conway and Toenniessen, 2000).

Bacterial leaf blight (BLB) caused by the rod-shaped bacterium, Xanthomonas oryzae pv. Oryzae (Xoo) is one of the most devastating diseases in rice (*Oryza sativa* L.). Outbreaks of BLB usually occur in irrigated and rain-fed lowland ecologies throughout Asia. Leaf blight symptom can occur at all stages of rice plants. BLB disease can cause yield loss typically ranging from 20-30% but in severe cases, it can cause as high as 80% yield reduction. However, it depends on rice growth stages,

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geographic locations or seasonal conditions (Singh *et al*., 1977; Ou, 1985). Since the bacterial races vary continually influenced by the artificial and natural selection of genes resistance to bacterial blight, it is critical to explore and identify the new resistant resources to control the changeful races (Xia *et al.,* 2012).Since the chemical control is not effective, the utilization of resistant varieties carrying resistance genes have been considered to be the most effective way to control the disease (Nino-Lui *et al.,* 2006). As biotechnological development progresses identification, cloning, and functional analysis of a gene can be performed relatively rapidly. In the case of BLB resistance, 35 BLB resistance genes have been identified in cultivated rice and the wild relatives (Nino-Lui *et al.,* 2006; Singh *et al.,* 2007; Wang *et al.,* 2009).

Unlike protein markers DNA segregate as single gene and they are affected by the environment. Molecular markers based on the DNA sequence are more varied and reliable. Several molecular markers viz. RFLP, RAPD, SSRs, ISSRs, AFLP and SNPs are presently available to assess the variability and diversity at molecular level (Joshi *et al.,* 2000). With the development of a wide range of molecular techniques, marker assisted breeding is now used to enhance traditional breeding programs to improve crops (Frey *et al.,* 2004). Among various PCR based markers, SSR markers are more popular in rice because they are highly informative, mostly mono locus, co-dominant, easily analyzed and cost effective (Gracia *et al.,* 2004). Simple Sequence Repeats (SSRs) or microsatellites are most suited to routine application in breeding programs. SSRs or microsatellite markers are proved to be ideal for making genetic maps (Islam, 2004; Niones, 2004), assisting selection (Bhuiyan, 2005) and studying genetic diversity in germplasm. Microsatellite marker analysis is promising to identify major gene locus for BLB resistance that can be helpful for plant breeders to develop new cultivar.Bangladeshi rice varieties have been developed traditionally by selection, hybridization and back crossing with locally adapted high-yielding lines. The conventional methods of plant selection for BLB resistance are not easy because of the large effects of the environment and the low narrow sense heritability of BLB resistance. This hinders the development of an accurate, rapid and reliable screening technique. Individual with target gene in a segregating population can be identified with the assistance of DNA markers. Recent progress and technical advances in DNA marker technology permit the rapid and accurate identification of individuals that contain gene (s) for BLB resistance. In the present study, 17 rice genotypes were fingerprinted using 2 Simple Sequence Repeats (SSRs) primers in view to identify and tag the BLB resistance/susceptible Bangladeshi rice varieties using SSR markers linked to xa5 gene.

II. Materials and Methods

Seventeen germplasm of rice viz. BR 10, BR 14, BR 16, BR 26, BRRIdhan 29, BRRIdhan 31, BRRIdhan 32, BRRIdhan 33, BRRIdhan 34, BRRIdhan 38, Malshira (Local Farmer: Rangpur), Iratom 24, Binasail, Binadhan 4, Binadhan 5, Binadhan 6 and Binadhan 7 were used in the study. These varieties were different in their parent and also in breeding strategies. Seeds were collected from the Bangladesh Rice Research Institute (BRRI), Bangladesh Institute of Nuclear Agriculture (BINA) and different districts of Bangladesh. Seed germination was performed and germinated seeds were sown in the pot at Biotechnology field. Fresh and young leaf samples were collected from 18 day old seedlings and used as the source of genomic DNA. In order to carry out SSR analysis, youngest leaves from each of the seventeen germplasm were collected randomly from each population as the source of genomic DNA.

Collection of sample and isolation of Rice genomic DNA: Young 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. Finally DNA samples were stored at – 20^0C.The extracted genomic DNAsamples were evaluated both qualitatively and quantitatively using agarose gel electrophoresis (0.8%, 200 ml) and spectrophotometer respectively. After electrophoresis, DNA samples were documented through Image Documentation System.

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Quantification of DNA Concentration: DNA was quantified through spectrophotometer (260 nm) and absorbance readings for the other samples were taken in the same way and recorded according to the following formula:

$$\text{DNA Conc. (ng/ µl)} = \text{Absorbance} \times \frac{\text{Volume of distilled water (µl)}}{\text{Amount of DNA sample (µl)}} \times \text{C.F (0.05)} \times 1000$$

The concentration of DNA in the extracted leaf samples as quantified through absorbance reading using spectrophotometer is given in Table 1. Absorbance reading and DNA concentration is varies from 0.005-0.015 and 250-750µl respectively.

Preparation of Working Solution of DNA Samples: Before PCR, Original stock solution concentration of each DNA sample was adjusted to a unique concentration (50 ng/µl) using the following formula:

$$V_1 \times S_1 = V_2 \times S_2, \quad V1 = \frac{V2 \times S2}{S1}$$

Where, V1= Final volume of DNA solution (µl), S_1 = Final DNA concentration (ng/µl), V_2 = Initial volume of DNA solution (µl)and S_2 = Initial DNA concentration (ng/µl)

Table 01. Quantification of DNA Absorbance and Concentration of DNA

SL. No.	Name of varieties	Absorbance (at 260 nm)	DNA Concentration (ng/µl)	DNA working solution (µl)
1	BR 10	0.010	500	100
2	BR 14	0.015	750	150
3	BR 16	0.007	350	70
4	BR 26	0.006	300	60
5	BRRIdhan 29	0.005	250	50
6	BRRIdhan 31	0.014	700	140
7	BRRIdhan 32	0.010	500	100
8	BRRIdhan 33	0.008	400	80
9	BRRIdhan 34	0.007	350	70
10	BRRIdhan 38	0.006	300	60
11	Malshira	0.009	450	90
12	Iratom 24	0.011	550	110
13	Binasail	0.005	250	50
14	Binadhan 4	0.005	250	50
15	Binadhan 5	0.006	300	60
16	Binadhan 6	0.009	450	90
17	Binadhan 7	0.005	250	50

PCR Analysis for Microsatellite Markers

Primer selection: Polymorphism survey of 17 rice varieties was carried out using two microsatellite markers. These two primers showed clear polymorphism which were used in genotyping the varieties. RM 122 and RM 390 were used for this. The details of the primers are given in Table 02.

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Table 02. Summary of SSR marker used for the study

Primer name	Molecular Weight	Chromosome locus	Sequence		Anneling Temp (°C)
RM 122	7464	5	Rev.	5'GAAGGAGGTATGCCTTTGTTG GAC	55
	7408		For.	5'GAGTCGATGTAATGTCATCAG TGC	
RM 390	6204	5	Rev.	5'CGTCAATGGGGTAGGTCTTG	55
	6305		For.	5'GGAGGCCAAGGAAGAGGTAG	

Preparation of PCR Cocktail: The following components were used to prepare PCR cocktail (Table 03). The total volume of PCR cocktail for this study was 8.37 µl per sample.

Table 03. Components of PCR cocktail (For 17 reactions)

SL	Component	Quantity (for single reaction)	Total (17 reactions)
1	10X Buffer (Tris with 15 nMMgCI$_2$)	1.0 µl	17.0 µl
2	dNTPs	1.0 µl	17.0 µl
3	Primer forward	1.0 µl	17.0 µl
4	Primer reverse	1.0 µl	17.0 µl
5	Taq DNA polymerase	0.25 µl	4.25 µl
6	Sterile ddH$_2$O	4.12 µl	70.04 µl
Total		8.37µl	142.29µl

PCR Amplification Profile: The PCR tubes were set on the wells of the thermo cycler plate. Then the machine was run according to the following setup: Initial denaturation at 94°C for 3 min, Denaturation at 94°C for 1 min, Annealing at 55°C for 1 min, Polymerization at 72°C for 2 min, Cycle to step 2 for 34 more time and Incubation at 72°C for 7 min. PCR Products Conformation was done by Agarose (1.5%) Gel Electrophoresis. Then, Polyacrylamide (8%) Gel Electrophoresis was done for Microsatellite Analysis.Polyacrylamide Gel Electrophoresis of PCR Products was stained with silver nitrate following (Ethidium Bromide Staining) Pro-mega silver staining protocols with some modification.

Analysis of Microsatellite Data: 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 (Yeh *et al.*,1999) 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 (Liu and Muse, 2005) was used to export the data in binary format (allele presence = 1 and allele absence = 0) for analysis with NTSYS-PC Version 2.2 (Rohlf, 2002). Polymorphisms information content (PIC) value of a marker was calculated according to a simplified version after Andersons *et al.* (1993).

$$PIC_i = 1- \sum X_i^2{}_j$$

Where, Xijis 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 (Liu and Muse, 2005). Expected (H_e) and observed heterozygosity (Ho) were also calculated after (Nei, 1972) using the following formula and with the help of POPGENE (version 1.31) (Yeh *et al.*, 1999) computer package program:

$$h_e = 2n\,(1 — \sum x_i^2/N)/\,(2n — 1)$$

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Where, he ~ is the expected heterozygosity of each locus, n is the number of sampled individuals, x_i is the frequency of i-th allele at each locus and N is the number of loci examined.
Thus, the average heterozygosity (H_e) was calculated as,

$$H_e = \sum h_e / r$$

Where, r is the number of loci examined (Nei and Roychoudhury, 1973).

Nei's (1972) genetic distance value was computed using the formula as described in the POPGENE (Version 1.31) software user manual (Yeh et al., 1999). Genetic distance values (D) (Nei, 1972) were calculated as

$$D = - I_n J_{xy/} \sum J_x J_y$$

Where, $J_x = \sum X_i^2 / r$ in population X, $J_y = \sum Y_i^2 / r$ in population Y, $J_{xy} = \sum X_i Y_i$, X_i and Y_i are the frequency of the i-th allele of a given locus in the two populations compared andr = the number of allele frequencies for all possible pairs of populations.

For the unrooted phylogenic tree, genetic distance was calculated using the 'C.S. Churd 1967' distance (Cavalli-Sforza and Edwards, 1967) 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 (Rohlf, 2002). 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 dendrogram showing relationship among the genotypes. The similarity matrix was also used for principal coordinate analysis (PCoA) with Dcenter, Eigen, Output and MX Plat subprograms in computer program Numerical Taxonomy and Multivariate analysis system (NTSYS-PC). The software GDA (Lewis and Zaykin, 2001) was used for estimating genetic distance. The unweighted pair-group method with arithmetic mean (UPGMA) dendrogram was drawn by using the software TREE VIEW (NTSYS-PC Version 2.2) (Rohlf, 2002).

III. Results and Discussion

Allelic and loci variation within the genotypes

The microsatellite enriched libraries were constructed using the standard procedure with some modifications. In this study 17 varieties of rice were analyzed using two primer pairs (RM122 and RM390). Amplified microsatellite loci were analyzed for polymorphism using Polyacrylamide Gel Electrophoresis (PAGE) (Figure 01-02) and the result revealed that all the primer pairs detected polymorphism among the rice genotypes analyzed. The microsatellite loci were also multi-allelic (seven to thirteen allele per locus with a mean of 10/locus in the present study) and the alleles were co-dominant suggesting their relative superiority in detecting DNA polymorphism over some other markers.

Number of alleles per locus

Using two SSR markers, a total of 20 alleles were detected among the 17 rice varieties. The average number of allele per locus was 10, with a range of 7 (RM122) to as many as 13 (RM390) (Table 04).

Rare alleles

Rare alleles were observed at all of the SSR loci in one or more of the 17 accessions with an average of 7 rare alleles per locus and a total of 14 across all the loci (Table 04). Among 17 rice varieties marker RM390 detected the greatest number of alleles (13), which also detected higher number of

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rare alleles (9) and marker RM122 detected the lower number of alleles (7), which also detected lowest number of rare alleles (5). Rare alleles are highly informative in fingerprinting of the varieties.

Gene diversity

According to Nei's; (1972), the highest level of gene diversity value (0.9135) was observed in loci RM390 and the lowest level of gene diversity value (0.7059) was observed in loci RM122 with a mean diversity of 0.8097 (Table 04). The maximum number of repeats within the SSRs was also positively correlated with the genetic diversity.

Allele size range

The size variation between the smallest and the largest allele at a given SSR locus was correlated with the number of alleles per locus. Thus, RM390 presented the smallest allele size range (76-128bp) and had 13 alleles in a locus, while RM122 had the largest allele size range (229-244 bp) and a total of 7 alleles (Table 04).

PIC values

Asa measure of the in formativeness of microsatellites, the PIC values ranged from a low of 0.6715 (RM122) to a high of 0.9069 (RM390) and averaged of 0.7892 (Table 04). PIC values also showed a significant, positive correlation with the number of alleles and allele size range for microsatellites evaluated in this study. The allele size range and the number of alleles were themselves also highly correlated.

Table 04. The microsatellite markers used the chromosome location of the loci, the number of alleles, Gene Diversity and the diversity index of 17 rice varieties

Primer name	Size range (bp)	No. of Alleles	Rare Allele	Gene Diversity	Diversity index PIC = $1-\sum xi^2$	Average PIC value
RM122	229-244	7	5	0.7059	0.6715	0.7892
RM390	76-128	13	9	0.9135	0.9069	

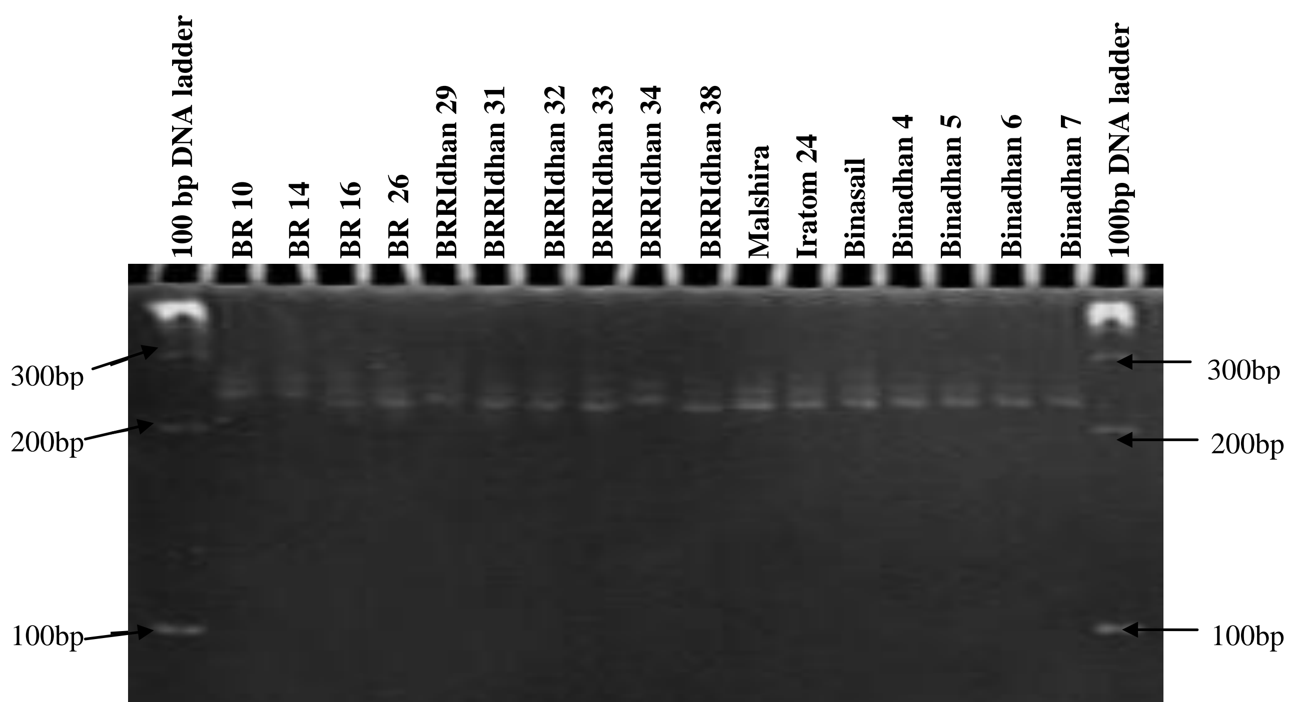

Figure 01. Microsatellite profiles of 17 rice varieties at loci RM122

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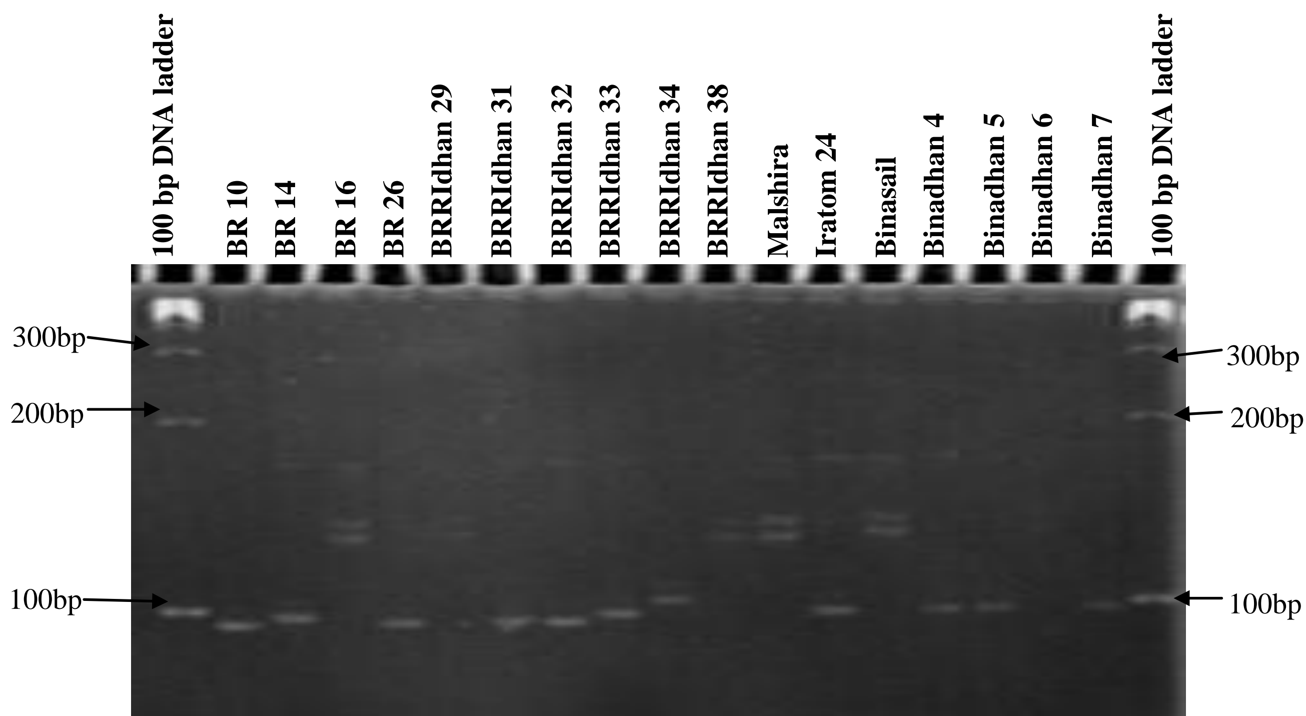

Figure 02. Microsatellite profiles of 17 rice varieties at loci RM390

Table 05. Size, Frequency, Variance and Standard Deviation of alleles at two SSR loci of 17 rice germplasm

SL.No.	Locus	Allele size (bp)	Frequency	Variance	SD
1.	RM122	229	0.0588	0.00325667	0.0571
		231	0.0588	0.00325667	0.0571
		234	0.2353	0.01058416	0.1029
		236	0.4706	0.01465500	0.1211
		239	0.0588	0.00325667	0.0571
		241	0.0588	0.00325667	0.0571
		244	0.0588	0.00325667	0.0571
2.	RM390	76	0.0588	0.00325667	0.0571
		77	0.1176	0.00610625	0.0781
		78	0.1176	0.00610625	0.0781
		82	0.1176	0.00610625	0.0781
		83	0.0588	0.00325667	0.0571
		84	0.0588	0.00325667	0.0571
		86	0.0588	0.00325667	0.0571
		87	0.0588	0.00325667	0.0571
		120	0.0588	0.00325667	0.0571
		122	0.1176	0.00610625	0.0781
		123	0.0588	0.00325667	0.0571
		127	0.0588	0.00325667	0.0571
		128	0.0588	0.00325667	0.0571

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Major allele

Data on sample size, number of observation, major alleles and polymorphism information content (PIC) found among 17 rice genotypes for 2 SSR markers were observed in table 06.

Table 06. Data on sample size, number of observation, major alleles (size and frequencies) and polymorphism information content (PIC) found among 17 rice genotypes for 2 SSR markers

Locus	SampleSize	No. ofObservations	Major allele		PIC
			Size (bp)	Frequency (%)	
RM122	17	17	236	47	0.6715
RM390	17	17	122	11	0.9069
Mean	17	17	179	29	0.7892

Heterozygosity for allmicrosatellite loci of 17 rice varieties*:* The expected heterozygosity was 0.9340 for RM390 and 0.7273 for RM122. The heterozygosity observed was zero for RM122 and RM390 (Table 07).

Table 07. Heterozygosity for allmicrosatellite loci of 17 rice varieties

Microsatellite loci	N	Ho	He	Polymorphism
RM122	7	0.000	0.7273	100
RM390	13	0.000	0.9340	100

(N=Number of alleles, H_o=heterozygosity observed, H_e=heterozygosity expected)

Deviation from Hardy-Weinberg proportion: There are no significant deviations from Hardy-Weinberg Equilibrium (HWE) for all microsatellite loci (Table 08).

Table 08. Deviation from Hardy-Weinberg expectations in 17 rice varieties according to locus

Locus name	Degrees of Freedom	Chi-square (χ^2)	G-square
RM122	21	184.	59.9
RM390	66	324	96.9

Gene diversity and gene flow in 17 rice varieties according to locus:According to Nei's (1987) the highest level of Shannon's information index (2.4255) was observed in loci RM390 and the lowest level of Shannon's information index (1.5285) was observed in loci RM122 (Table 09). The highest effective number of allele (10.7037) was observed in loci RM 390 and lowest effective number of allele (7.0519) was observed in loci RM122 (Table 09). Average gene flow for all microsatellite loci in 17 rice varietiesis 0.00 (Table 10).

Table 09. Genetic variation statistics for all loci

Locus name	na*	ne*	I*
RM122	7	3.4	1.5
RM390	13	10.7	2.4
Mean	10	7.1	1.9
S.D.	3	5.2	0.63

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

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Table 10. F-Statistics and gene flow for all microsatellite loci of 17 rice varieties

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.

Detection of BLB resistance and susceptible rice varieties

Amplification of *xa5* linked DNA fragment was carried out using tightly linked PCR based markers RM 122 (Chen *et al.,* 1997). The bands of *xa5* gene were standardized by the amplified DNAs of IRBB-5 and IR-24 used as control. IRBB-5 showed the amplification DNA fragment having a gene of about 240 bp was considered as resistance line and IR-24 showing the DNA band of about 230 bp was considered as susceptible line (*Naveed et al.,* 2010).The PCR product was measured as polymorphic bands pattern (Table 13).

Genetic distance: The values of pair-wise comparisons of Nei's (1972) genetic distance (GD) between varieties were computed from combined data for the 2 primers, ranged from 0.000 to 0.500 (Table 14). The average genetic distance among the 17 rice varieties was quantified as 0.139 (Figure 03). The means of genetic distances between varieties were used to evaluate the genetic diversity of different rice varieties. From the difference between the highest and the lowest genetic distance value it was revealed that there were wide variability's among 17 rice varieties and genotypes. High genetic variability within varieties and significant difference between varieties indicate rich genetic material of a species. This study indicated that varieties those showed the lowest genetic variation can be used as parental source for breeding line to improve rice varieties.

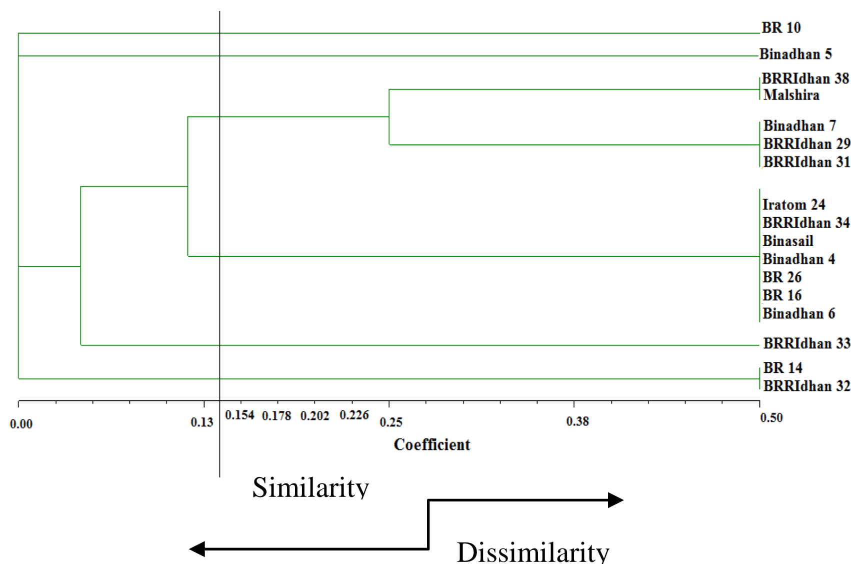

Figure 03. UPGMA dendrogram based on Nei's (1972) genetic distance summarizing on differentiation the data among 17 rice varieties according to SSR analysis

Genetic similarity analysis using UPGMA: A dendrogram was constructed based on the Nei's genetic distance calculated from the 20 SSR alleles generated from the 17 rice genotypes. All 17 rice varieties could be easily distinguished. The Unweighted Pair Group Method with Arithmetic Means (UPGMA) cluster tree analysis led to the grouping of the 17 rice varieties in six major clusters, BR10 and Binadhan 5, which were single variety formed cluster-1 and cluster-2 respectively. BRRIdhan 38, Malshira, Binadhan 7, BRRIdhan 29, BRRIdhan 31 was grouped in cluster-3, in which sub cluster-I includesBRRIdhan 38, Malshira and sub cluster-II includes Binadhan 7, BRRIdhan 29, BRRIdhan31.

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Cluster-4 was the largest cluster comprised of seven rice varieties. These are Iratom 24, BRRIdhan 34, Binasail, Binadhan 4, BR 26, BR 16 and Binadhan 6. Only BRRIdhan 33 was formed cluster-5. BR 14 and BRRIdhan 32 were formed cluster-6. From this study, the dendrogram revealed that the genotypes that derivatives of genetically similar type clustered together.

IV. Conclusion

BR 14 and BR 10 were showed the DNA band of 244bp and 241bp using the same marker RM122. BR 14 and BR 10 are complete resistant or partial resistant to BLB diseases. Binadhan 5 was showed the DNA band of 239bp. Binadhan 5 may be partial resistant to BLB diseases. Malshira, Iratom 24, Binasail, Binadhan 4, Binadhan 6, BR16, BR 26, BRRIdhan 34 were showed the DNA band of 236bp which was considered at the middle between 240bp of IRBB-5 and 230bp of IR-24. Malshira, Iratom 24, Binasail, Binadhan 4, Binadhan 6, BR16, BR 26, BRRIdhan 34 may be partial resistant and partial susceptible to bacterial blight diseases. BRRIdhan 38, Binadhan 7, BRRIdhan29, BRRIdhan 31 were showed the DNA band of 234bp which is partially same with the band of 230bp of IR-24. BRRIdhan 38, Binadhan 7, BRRIdhan29, BRRIdhan 31 may be partial or complete susceptible to BLB diseases. BRRIdhan 32 and BRRIdhan 33 were showed the DNA band of 231bp and 229bp respectively. BRRIdhan 32 and BRRIdhan 33 are complete susceptible to bacterial blight diseases.The results may be utilized as the local source of *xa*5 gene for elite molecular breeding program and used as a baseline for improvement of rice varieties in Bangladesh.

V. References

Anderson, J. A., Churchil, G. A., Autrique, J. E., Tanksley, S. D. & Sorrells, V. (1993). Optimizing parental Selection for genetic linkage maps. *Genome*, 36:81-186.

Bhuiyan. M. A. R. (2005). Efficiency in evaluating salt tolerance in rice using phenotypic and marker assisted selection. M. S. Dissertation, Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh, Bangladesh. 96p.

Bhuiyan, N. I., Paul, D. N. R. & Jabber, M. A. (2002). Feeding the extra millions by 2025-challenges for rice research and extension in Bangladesh. A keynote paper presented on national workshop on rice research and extension, 29-31 January, BARI, Gazipur, p.9

Cavalli-Sforza, L. L. & Edwards, A. W. F. (1967). Phylogenetic analysis models and estimation procedures. *Am. J. Hum. Gent*.19:233-257.

Chakravarthi, B. K. & Naravaneni, R. (2006). SSR marker based DNA fingerprinting and diversity study in rice (*Oryza sativa. L*). *African J. Biotechnol.*, 5 (9): 684-688.

Chen,X., Temnykh, S., Xu, Y., Cho, Y. G. & McCouch, S. R. (1997). Development of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). *Theor. Appl. Genet*. 95: 553-567.

Conway, G. & Toenniessen, G. (2000).Feeding the world in the twenty-first century. Nature (suppl), 402: c55-c58.

Frey, J. E., Frey, B., Sauer, C. & Kellerhals, M. (2004). Efficient low cost DNA extraction and multiplex fluorescent PCR method for marker assisted selection in breeding. *Plant Breed*. 123: 554-557.

Gracia, A. A. F., Benchimol, L. L., Antonica, M. M., Geraldi, I. O. & Deuza, A. P. (2004). Comparison of RAPD, RFLP, AFLP and SSR marker for diversity studies in tropical maize inbred lines. *Euphytica* 108: 53-63.

Islam M. M. (2004). Mapping salinity tolerance genes in rice (*Oryza satiya* L.) at reproductive stage. Ph. D. dissertation.University of the Philippines Los Banos, College, Laguna, Philippines.150 p.

Jayamani, P., Negrao, S., Martins, M., Macas, B. & Oiveira, M. M. (2007). Genetic relatedness of Portuguese rice accessions from diverse origins as assessed by microsatellite markers. *Crop Sci*., 47: 879-889.

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Joshi, S. P., Gupta, V. S., Aggarwal, R. K., Ranjekar, P. K. and Brar, D. S. (2000). Genetic diversity and phylogenetic relationship as revealed by Inter simple sequence repeat polymorphism in the genus *Oryza*. *Theor. Appl. Genet.* 100:1311-1320.

Khush, G. S. (2005). What it will take to feed 5.0 billion rice consumers in 2030. *Plant Molecular Biology,* 59: 1-6.

Lewis, P. O. & Zaykin, D. (2001). Genetic Data Analysis V1. *http://www.eeb.uconn.edu/people/plewis/software.php.*

Liu, K. & Muse, S. V. (2005). Power Marker: Integrated analysis environment for genetic marker data. *Bioinformatics*, 21:2128-2129.

Naveed, S. A., Baba, M., Arif, A., Zafar, Y., Sabar, M., Ali, I., Chragh, M. (2010). Detection of bacterial blight resistant gene xa5 using linked marker approaches. *Afri. J. Biotechnol.*, 9:3549-3554.

Nei, M. (1972).Genetic distance between populations. *Am. Nat.* 106: 283-292.

Nei, M. and Roychoudhury, A. K. (1973). Probability of an over-dominant mutation. *Genetics*, 74:371-324.

Nei, M. (1987). Molecular Evolutionary Genetics. Columbia University Press, New York.

Nino-Lui, D. O., Ronald, P. C. & Bogdanove, A. J. (2006). Pathogen profile *Xanthomonas oryzae* pathovars: model pathogens of a model crop. *Molec.Plant Pathol*. 7(5): 303-324.

Niones, J. M. (2004). Fine mapping of the salinity tolerance gene on chromosome 1 of rice (*Oryza sativa* L.) using near-isogenic lines.M.S. dissertation.University of the Philippines Los Banos, College, Laguna, Philippines.78 p.

Ou, S. H. (1985). Rice disease. 2nd edn. Commonwealth Mycology Institute, England.

Rohlf, F. (2002). NTSYS-PC: Numerical taxonomy and multivariate analysis system, 2.2 edn. Department of Ecology and Evolution, State University of NY, Stony Brook.

Singh, K., Vikal, Y., Mahajan, R., Cheema, K. K., Bhatia, D., Sharma, R., Lore, J. S. &Bharaj, T. S. (2007). Three novel bacterial blight resistance genes identified, mapped and transfer to cultivated rice *O. sativa L.,* In: The 2nd International Conference on Bacterial Blight of Rice. Nanjing, China, 82-84.

Singh, R. K. & Chaudhary, B. D. (1977). Biometrical methods in quantitative genetic analysis. Kalyani Publishers. New Delhi.

Wang,C. T., Wen, G. S., Lin, X. H., Liu, X. Q. & Zhang, D. P. (2009). Identification and fine mapping of the new bacterial blight resistance gene, *Xa31(t)*, in rice, *Eur. J. Plant Pathol.*, 123: 235-240.

Xia, C., Chen, H. & Zhu, X. (2012). Identification, Mapping, Isolation of the Genes Resisting to Bacterial Blight and Breeding Application in Rice. *Molecular Plant Breeding*., 3(12): 120-130.

Yeh, F. C., Yang, R. C., Boyle, T. B. J., Ye, Z. H. & Mao, J. X. (1999). POPGENE, the user-friendly software for population genetic analysis. *Mol. Biol. and Biotech.Cent.*, University of Alberta, Canada.

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Table 11. Allele frequency of two SSR markers in all 17 rice varieties

Marker	Allele	BR 10	BRRI dhan38	Malshira	Iratom 24	Binasail	Binadhan 4	Binadhan 5	Bina dhan6	Bina dhan7	BR 14	BR 16	BR 26	BRRI dhan 29	BRRI dhan31	BRRI dhan32	BRRI dhan33	BRRI dhan 34
RM122	229	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	0
RM122	231	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	0	0
RM122	234	0	**1**	0	0	0	0	0	0	**1**	0	0	0	**1**	**1**	0	0	0
RM122	236	0	0	**1**	**1**	**1**	**1**	0	**1**	0	0	**1**	**1**	0	0	0	0	**1**
RM122	239	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0
RM122	241	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RM122	244	0	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0
RM390	120	0	0	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0
RM390	122	0	**1**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RM390	123	0	0	0	0	0	0	0	0	0	0	0	0	**1**	0	0	0	0
RM390	127	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0
RM390	128	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0
RM390	76	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RM390	77	0	0	0	0	0	0	0	0	0	0	0	**1**	0	**1**	0	0	0
RM390	78	0	0	0	0	0	0	0	0	0	**1**	0	0	0	0	**1**	0	0
RM390	82	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	**1**	0
RM390	83	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0
RM390	84	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0
RM390	86	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0
RM390	87	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**

Table 12. Identification of BLB resistance and susceptible rice varieties using the allele frequency of SSR Marker RM122

Allele	BR 10	BRRI dhan38	Malshira	Iratom 24	Binasail	Binadhan 4	Binadhan 5	Bina dhan6	Bina dhan7	BR 14	BR 16	BR 26	BRRI dhan 29	BRRI dhan 31	BRRI dhan32	BRRI dhan33	BRRI dhan34
229	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
231	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
234	0	1	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0
236	0	0	1	1	1	1	0	1	0	0	1	1	0	0	0	0	1
239	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
241	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
244	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0

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Table 13. Detection of BLB resistance and susceptible rice varieties using the allele (bp) DNA band of SSR Marker RM122

Rice Variety	Allele (bp) DNA Band	BLB complete resistant genotype	BLB partial resistant genotype	BLB partial susceptible genotype	BLB complete susceptible genotype
BR 10	241	+	+	-	-
BRRIdhan38	234	-	-	+	+
Malshira	236	-	+	+	-
Iratom 24	236	-	+	+	-
Binasail	236	-	+	+	-
Binadhan 4	236	-	+	+	-
Binadhan 5	239	-	+	-	-
Binadhan6	236	-	+	-	-
Binadhan7	234	-	-	+	+
BR 14	244	+	+	-	-
BR 16	236	-	+	+	-
BR 26	236	-	+	+	-
BRRIdhan 29	234	-	-	+	+
BRRdhan 31	234	-	-	+	+
BRRIdhan32	231	-	-	-	+
BRRIdhan33	229	-	-	-	+
BRRIdhan34	236	-	+	-	-

(+) 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.

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Table 14. Summary of Nei's (1972) genetic distance (below diagonal) values for different cultivar pairs of rice

Genotypes	BR10	BRRI dhan 38	Malshira	Iratom 24	Binasail	Bina dhan 4	Bina dhan 5	Bina dhan 6	Bina dhan 7	BR 14	BR 16	BR 26	BRRI dhan 29	BRRI dhan 31	BRRI dhan 32	BRRI dhan 33	BRRI dhan 34
BR-10	****																
BRRIdhan 38	0.000	****															
Malshira	0.000	0.500	****														
Iratom 24	0.000	0.000	0.500	****													
Binasail	0.000	0.000	0.500	0.500	****												
Binadhan 4	0.000	0.000	0.500	0.500	0.500	****											
Binadhan 5	0.000	0.000	0.000	0.000	0.000	0.000	****										
Binadhan 6	0.000	0.000	0.500	0.500	0.500	0.500	0.000	****									
Binadhan 7	0.000	0.500	0.000	0.000	0.000	0.000	0.000	0.000	****								
BR 14	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	****							
BR 16	0.000	0.000	0.500	0.500	0.500	0.500	0.000	0.500	0.000	0.000	****						
BR 26	0.000	0.000	0.500	0.500	0.500	0.500	0.000	0.500	0.000	0.000	0.500	****					
BRRIdhan 29	0.000	0.500	0.000	0.000	0.000	0.000	0.000	0.000	0.500	0.000	0.000	0.000	****				
BRRIdhan 31	0.000	0.500	0.000	0.000	0.000	0.000	0.000	0.000	0.500	0.000	0.000	0.500	0.500	****			
BRRIdhan 32	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.500	0.000	0.000	0.000	0.000	****		
BRRIdhan 33	0.000	0.000	0.000	0.500	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	****	
BRRIdhan 34	0.000	0.000	0.500	0.500	0.500	0.500	0.000	0.500	0.000	0.000	0.500	0.500	0.000	0.000	0.000	0.000	****

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