

Genetic Diversity of Bacterial Leaf Blight Disease in a Set of Rice Landraces of West Bengal

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Abstract

Bacterial Leaf Blight (BLB) is a major disease of rice in tropical monsoon and sub tropical rice growing regions of the world. BLB substantially reduces the yield of the crop affecting food security. Rice is the second most important food crop, after wheat, in the world; hence the disease must be effectively controlled.

As chemical control is impractical due to unavailability of specific bactericides, the focus is on the use of resistant genotypes. Landraces are repositories of resistance to biotic and abiotic stresses. During the process of domestication and cultivation of crop plants, a wealth of genetic diversity has been created, utilized and partly preserved with valuable allelic variations of traits of economic significance. These variations can be discovered and effectively used to meet the existing and emerging challenges that threaten world food security.

In this study we have used a set of 30 rice landraces from West Bengal to assess the genetic diversity

1. At the disease phenotype level through artificial inoculation and measurement of lesion length.
2. In the variation pattern of bacterial infection of the xylem vessel in the resistant, partially resistant and susceptible accessions using scanning electron microscopy, and
3. At the DNA level using six markers linked to the resistant genes xa-5, Xa 21, xa13, Xa 10, xa 8, and xa 24.

Substantial diversity was observed and the results on these three areas are presented.

In the future, this set of landraces will be assessed for diversity using primer pairs designed from the conserved regions of cloned and characterized BLB resistance genes, and sequencing the amplified products. Attempts will be made to correlate the band length and sequence variations with the disease phenotype.

Keywords- *Oryza sativa*; Landrace; Xoo;BLB;Genetic Diversity; Scanning Electron Microscopy; Linked Markers.

1. Introduction

Rice, a member of the grass family (Poaceae), is largely grown in monsoon tropical and sub tropical regions of the world (Ezuka and kaku, 2000). This unique grain helps sustain two-thirds of the world's population. Rice cultivation is the principal activity and source of income for billion of households in Asia and Africa. Over 90 percent of the world's rice is produced and consumed in Asia. Rice is a valuable crop and considerable research has to be done for its betterment, so that it can meet the future food demand which is increasing with time.

Bacterial Leaf Blight Disease (BLB), one of the major diseases of rice, known to occur in epidemic proportion in many part of the world, can reduce production by more than 50% (Dai et.al, 2007). Caused by the bacteria *Xanthomonas oryzae* pv. *oryzae* (Xoo), it is one of the oldest known diseases and was first noticed by the farmers of Japan in 1884 (Tagami and Mizukami 1962). Hydathodes at the leaf tips and margin are the portal of entry of the organism. The Xoo multiplies inside the xylem vessel clogging it and causing wilting of the plant. Filling of grain is inhibited and chaffy grains are produced (Khush et.al, 1990). As chemical control is impractical due to unavailability of specific bactericides; the focus is on the use of resistant genotypes (Gnanamanickam S. S et.al, 1999).

Genetic diversity substantially decreases a crop's vulnerability to diseases and traditional landraces are ingrained with enormous diversity. During the process of domestication and cultivation of crop plants, a wealth of genetic diversity has been utilized and partly preserved. But thousands of valuable allelic variations of traits of economic significance remain unutilized. These can be discovered and effectively used to meet the existing and emerging challenges that threaten world food security. Hence there is an urgent need to conserve and characterize rice landraces to identify useful alleles for disease resistance and other agronomic traits.

Till date 34 different BB resistance genes have been identified of which Xa1, Xa2, Xa3, Xa4, Xa7, Xa10, Xa11, Xa12, Xa14, Xa16, Xa17, Xa18, Xa21, Xa22 are dominant and xa 5, xa 19, xa 28, xa 8, xa 20, xa 32t, xa 13, xa 24, xa 34t, xa 15, xa 26b are recessive (Khush and Kinoshita, 1991; Lin etal., 1996; Chun et al., 2007, S. Chen,2008).

Molecular markers have the potential to detect genetic diversity and to aid the management of plant genetic resources (Ford-Lloyd et al. 1997; Virk et al. 2000; Song et al. 2003). In contrast to morphological traits, DNA markers can reveal differences among genotypes at the sequence level, providing a more direct, reliable

and efficient tool for germplasm characterization, conservation and management. Information on the genetic diversity within and among closely related crop varieties is essential for a rational use of genetic resources and is of fundamental interest to plant breeders (Chakravarthi & Naravaneni 2006).

In this project we have used a set of 30 rice landraces from West Bengal to assess the genetic diversity

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3. At the DNA level using six markers linked to the resistant genes xa-5, Xa 21, xa13, Xa 10, xa 8, xa 24.

2. Material and Methods

2.1 Plant materials

A total of 30 rice landraces were collected from different state of West Bengal. The name, source and category of each rice accession are given in Table 1.

Table 1. Names, source and category of genotypes included in this study

<i>Genotypes</i>	<i>Source</i>	<i>Category</i>
Badshabhog	RRS, Chinsurah	Aromatic
Bangalakshmi	ATC, Fulia	Non aromatic
Bangladeshipatnai	ATC, Fulia	Non aromatic
Danaguri	RRS, Chinsurah	Aromatic
Gandhamalati	SARF, Kashipur	Aromatic
Gobindobhog	RRS, Chinsurah	Aromatic
Jarava	SARF, Kashipur	Non aromatic
Joha	RRS, Chinsurah	Aromatic
Kabirajsail	RRS, Chinsurah	Non aromatic
Kalindi	RRS, Chinsurah	Non aromatic
Kalobhog	RRS, Chinsurah	Aromatic
Kalosethe	RRS, Chinsurah	Non aromatic
Kaminibhog	SARF, Kashipur	Aromatic
Kataribhog	RRS, Chinsurah	Aromatic
Kerelasundori	ATC, Fulia	Non aromatic
Lalbinni	ATC, Fulia	Non aromatic
Lathisail	ATC, Fulia	Non aromatic
Lilabati	RRS, Chinsurah	Non aromatic
Lunishree	SARF, Kashipur	Non aromatic
Mohanbhog	RRS, Chinsurah	Aromatic
Medi	RRS, Chinsurah	Non aromatic

Motibhog	RRS, Sekhampur	Aromatic
Nagra	RRS, Sekhampur	Non aromatic
Narayanpurna	ATC, Fulia	Non aromatic
Radhunipagol	RRS, Chinsurah	Aromatic
Radhatilak	RRS, Chinsurah	Aromatic
Sitabhog	SARF, Kashipur	Aromatic
Talmari	RRS, Chinsurah	Non aromatic
Tulsibhog	RRS, Sekhampur	Aromatic
Lakshmansail	RRS, Chinsurah	Non aromatic
Ajaya	RRS, Chinsurah	Resistant checks
IR24	RRS, Chinsurah	Susceptible checks

Abbreviations: RRS-Rice Research Station, SARF-State Agricultural Research Farm, ATC-Agriculture Training Centre

2.2 Leaf Inoculation

Thirty rice genotype were planted in pots and proper management practices were done. The plants were allowed to grow up to tillering stage and inoculated with 36 hr old culture of *X. oryzae* following leaf clipping method. Lesion length was measured 14 days after infection.

Table2. Scoring System of lesion

Lesion length (Cm)	Description
0 – 5	Resistant
> 5 - 10	Moderately resistant
>10 - 15	Moderately susceptible
>15	susceptible

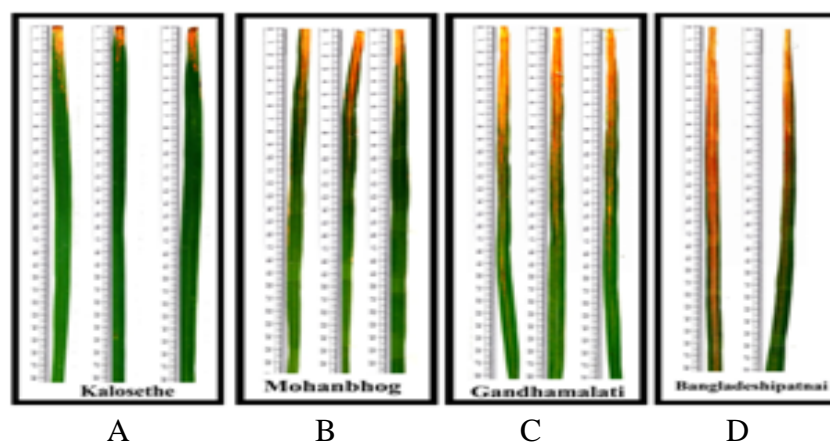


Figure1. Representative image of leaf infection. A-Resistant, B-moderately resistant, C-Moderately susceptible, D-Susceptible.

2.3 Scanning Electron Microscopy

Rice genotypes were grown in boxes with proper inlet and outlet in order to create a warm and damp environment suitable for infection. Equal amount of soil was mixed with soilrite and sand. The box was filled with two inch thick soil layer. Sufficient amount of water was added to prepare seed bed. Seeds were sown in a row with one inch distance between two varieties. After 21 days seedlings were inoculated with *Xanthomonas* culture by leaf clipping method. Samples were collected 1 day and 5 days after infection.

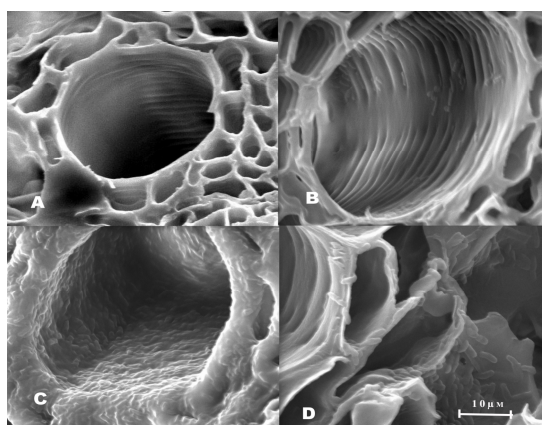


Figure 2. SEM. A control, B resistant variety, C partially resistant variety, D susceptible variety

2.4 Isolation of genomic DNA and PCR amplification

Three day-old rice seedlings germinated from 10 well developed grains from a single plant of each genotype were used for genomic DNA isolation according to the method of Walbot (1988). PCR amplification of this DNA was done with 6 pairs of linked SSR markers. The name, chromosomal location, annealing temperature of the markers, expected product size and corresponding references are given in Table 2. DNA amplification was carried out in 25 μ l volumes in a MJR thermal cycler (USA). Each reaction mixture contained 1 μ l of genomic DNA (100 ng), 0.5 μ l of each primers (at a concentration of 10 pmole/ μ l), 2.5 μ l of 10 \times PCR buffer, 0.75 μ l of 50 mM MgCl₂, 0.25 μ l of 2.5 mM dNTP mixture, 0.2 μ l (1 unit) of 5 unit/ μ l Taq DNA polymerase and 19.3 μ l of PCR-grade water. The temperature profile of the first PCR cycle was 97 $^{\circ}$ C for 5 mins, 55-60 $^{\circ}$ C (as necessary in accordance to Table 2) for 2 min; followed by 35 cycles of 1 min at 95 $^{\circ}$ C, 1 min at 55-60 $^{\circ}$ C and 2 min at 72 $^{\circ}$ C. The final extension was at 72 $^{\circ}$ C for 10 min.

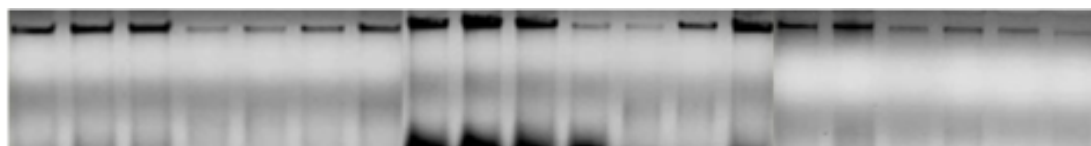


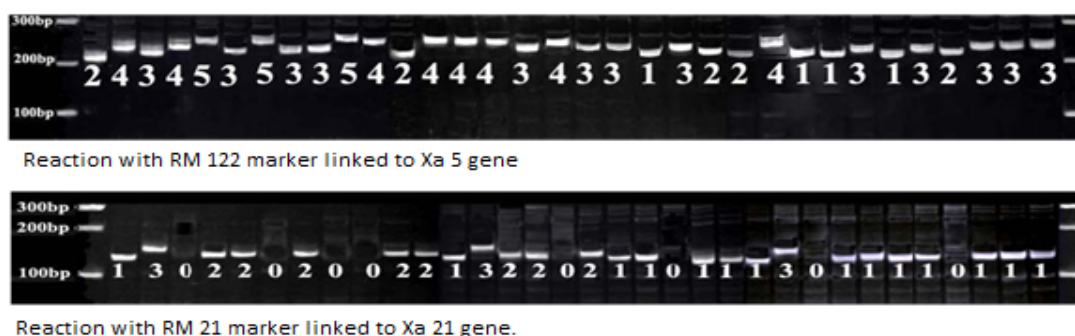
Figure 3. Image for genomic DNA extraction.

Table 2. Name, chromosomal location, annealing temperature, expected product size and corresponding references of markers used for this study

Name of primer	Chromosomal location	Annealing temp.	Product size	reference
RM 122	xa 5	60° C	227	Wu and Tanksley, 1993
RM 21	Xa 21	55° C	157	Chen <i>et al.</i> , 1997
RM230	xa 13	55° C	257	Chen <i>et al.</i> , 1997
RM 206	Xa 10	55° C	147	Panaud <i>et al.</i> , 1996
RM 263	xa 8	55° C	199	Chen <i>et al.</i> , 1997
RM 138	xa 24	55° C	233	Wu and Tanksley, 1993

2.5 Polyacrylamide Gel electrophoresis

The PCR products were resolved in native polyacrylamide gel electrophoresis (PAGE) according to Sambrook *et al.* in 6% gel in vertical electrophoresis tank (gel size of 16 cm × 14 cm, Biotech, India) with Tris-Acetate-EDTA buffer at 150 V. The gel was stained with ethidium bromide (5 µg of EtBr in 200 ml of Tris-Borate-EDTA buffer) washed twice with distilled water and analyzed in Gel Documentation System (Biorad, USA).

**Figure 4.** Representative Image for Polyacrylamide gel electrophoresis.

3. Results

3.1 Leaf Inoculation: Disease Response

Lesion length was measured in the leaves after BLB infection. The lesion length varied considerably among the genotypes. Lesion length even longer than IR24, considered as the susceptible check, was found in the Bangladeshipatnai (figure1) and lunishree. Lesion comparable to IR24 was found in Kabirajsail, Danaguri, Talmari, motibhog, Kaminibhog, Radhunipagol and Gobindobhog. Kalosethe (figure. 1) showed lesions smaller than Ajaya, the resistant check, while kalindi developed lesions comparable to Ajaya. Medi, Keralasundori, Bangalaksmi, Lathisail and Mohanbhog showed lesions longer than the resistant phenotype but smaller than the moderately susceptible ones.

3.2 Analysis of the Variation of the Disease Response through Scanning Electron Microscopy

SEM showed significant differences in the Xylem vessel in resistant, partially resistant and susceptible genotypes. Clean vessel was observed in control variety (Figure2A). The resistant genotype showed bacterial infestation limited to the vessel wall (Figure2B). The bacterial titer was observed to be very low. But for the partially resistant varieties heavy bacterial titer was found to infest the vessel wall with no clogging of the vessel (Figure2C). In case of the susceptible varieties, the bacteria causes clogging of the vessel which is clearly visible in the figure 2D and causes wilting of the plant.

3.3 Genetic Diversity Analysis using the Six BLB-linked SSR Markers

The following are the observations on the genetic diversity obtained through the 6 SSR markers in the set of the 30 rice landraces. No attempt has been made to correlate the alleles obtained and the disease phenotype.

The Six Linked markers used generated 35 alleles. A total of five alleles were produced by the SSR marker RM 122 (Figure 4). Allele 1, 2 and 3 were found in susceptible and moderately susceptible genotypes while allele 4 and 5 were present in resistant and moderately resistant genotypes. Similarly for RM21 (Figure 4), three allele could be distinguished. 8 genotypes contain null allele, which includes 3 most susceptible genotypes Bangladeshipatnai, Nagra and Lunishree. The rest are moderately susceptible. The resistant genotypes possess allele number 2 and 3. Allele number 1 is possessed by moderately susceptible genotypes. Only Mohanbhog which is moderately resistant contains allele number 1. Three alleles were identified for RM 230. Two most resistant genotypes contain allele number 2. The two most susceptible genotypes contain allele number 1. Allele number 3 was found in the most moderately susceptible genotypes. For RM206 and RM 263, 9 alleles could be identified while RM 138 could amplify 6 alleles.

4. Conclusions

The disease response of a set of 30 rice landraces of West Bengal to BLB infection has been assessed at the phenotype and at the genotypic level. Two landraces, Kalindi and Kalosethe were found to be resistant while five landraces Lunishree, Kalobhog, Gandhamalati, Bangladeshipatnai and Nagra were found to be highly susceptible. Mohanbhog, an aromatic rice like Gobindobhog, was found to be moderately resistant while the other aromatic rice landraces were either moderately susceptible or susceptible. Analyzing the diversity of disease response at the phenotypic and genetic level will enable us to develop rice varieties with durable resistance to diseases and hence higher yield potential.

References

- [1] Dai, L.Y., X.L. Liu, Y.H. Xiao and G.L. Wang. Recent advances in cloning and characterization of disease resistance genes in rice. *J. Integr. Plant Biol*, 2007, 49: 112-119
- [2] Ezuka A, Kaku H. A Historical Review of Bacterial Blight of rice. National Institute of Agrobiological Resources Bulletin 2000, Japan. p. 207.
- [3] Ford-Lloyd BV, Jackson MT, Newbury HJ, 1997. Molecular markers and the management of genetic re-sources in seed genebanks: a case study of rice. In: Callow JA, Ford-Lloyd BV, Newbury HJ, eds. *Biotechnology and plant genetic resources: conser-vation and use*. Wallingford, CAB International.
- [4] Gnanamanickam S. S., Brindha Priyadarisini V , Narayanan N. N., Vasudevan Preeti and Kavitha S. An overview of bacterial blight disease of rice and strategies for its management. *Current science* 1999, 77: NO. 11.
- [5] Khush, G. S., Bacalangco, E. and Ogawa, T., *Rice Genet. Newsl.* 1990, 7, 121–122.
- [6] Sambrook J, Fritsch EF, Maniatis T: *Molecular cloning: A laboratory manual*. 2nd edition. New York: Cold Spring Harbour Laboratory Press; 1989.
- [7] Song ZP, Xu X, Wang B, Chen JK, et al. (2003). Genetic diversity in the northernmost *Oryza rufipogon* populations estimated by SSR markers. *Theor. Appl. Genet.* 107: 1492-1499.
- [8] Tagami, Y. and T. Mizukami 1962. Historical review of the researches on bacterial leaf blight of rice caused by *Xanthomonas oryzae* (Uyeda et Ishiyama) Dowson. Special report of the plant diseases and insect pests forecasting service No. 10. Plant protection Division, Ministry of Agriculture and Forestry, Tokyo, Japan. 112 pp.
- [9] Virk PS, Ford-Lloyd BV, Jackson MT and Newbury HJ (1995). Use of RAPD for the study of diversity within plant germplasm collections. *Heredity* 74 (Pt 2): 170-179.
- [10] Walbot V: Preparation of DNA from single rice seedling. *Rice Genet Newsl* 1988, 5:149–151.