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Pathotype characterization of *Xanthomonas oryzae* pv *oryzae* isolates of West Bengal and evaluation of resistance genes of bacterial blight of rice (*Oryza sativa* L.)

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ABSTRACT

Bacterial blight is the second most important disease of rice. Hence characterization of the pathotype that causes bacterial blight and identification of resistance genes were attempted as a preliminary step towards identifying some resistant sources. In order to do that, six isolates collected from different parts of West Bengal were screened against near isogenic lines to identify variability in virulence. All the NILs were found to possess varying degree of susceptibility to resistance against all the isolates with significant differences in disease progress. The pyramided lines showed broad spectra of resistance against all the Xoo isolates and most durable resistant monogenes were xa5, xa13 and Xa21.

Keywords: Bacterial Blight, Near-Isogenic lines (NILs), Xanthomonas oryzae pv. oryzae (Xoo)

Rice is more than a staple food as well as a model cereal all over the world. The disease incidence of *X. oryzae* in rice, limits the production of this staple food, of more than half the world's population. Bacterial blight of rice, caused by *Xanthomonas oryzae* pv. *oryzae* is known to occur in epidemic form in many parts of the world, causing losses to the extent of 6-60% or even upto 81% in some cultivars (Shehzad *et al.,* 2012). Breeding for resistance is thus the best option to encounter the disease. The variability of the pathogen is also a problem to achieve this goal. High rate of mutation of the pathogen makes the approach further difficult. However, a continual search for new source of resistance is necessary. The availability of several genes responsible for resistance may help the breeders to go for various breeding strategies like gene rotation, gene deployment and gene pyramiding. Hence, it is essential to have a standard race against which the disease reaction may be tested. Pathogenic variability and evaluation of resistance genes are therefore, imperative to the plant breeders. Hence, near isogenic lines (NILs) carrying different resistant genes are employed to evaluate pathogenicity of *Xoo* isolates (Gnanarmanickam *et al.,* 1999) as well as to evaluate different resistant genes*viz*. *xa5, xa13* and *Xa21*, which were noticed to be the durable mono genes, that expressed under this terai agro-climatic region of West Bengal, to combat the disease. All the pyramided lines showed high and broad spectra of resistance against all the *Xoo* isolates (Debnath *et al.,* 2012).

MATERIALS AND METHODS

For identification of the most virulent pathotypes, six *Xoo* isolates were collected from the diseased leaf samples of rice from six districts of West Bengal *viz*. Burdwan (*Xoo1*), East Midnapore (*Xoo2*), North Dinajpur (*Xoo3*), Malda (*Xoo4*), Cooch Behar (*Xoo5*) and North 24 Parganas (*Xoo6*). The isolates were characterized by employing NILs. Out of those isolates, the isolate 5 collected from Cooch Behar, was the most virulent.

Bacterium containing solution was streaked on the Yeast Glucose Chalk Agar (yeast extract-10g, dextrose-10g, calcium carbonate-20g, agar-20g, water-1000ml) medium. Bacterial pure culture was suspended in sterile distilled water for preparation of inoculum. Cell density was adjusted to 10^9cfuml^{-1}.

For field screening inoculation was done by dipping sterile scissors into bacterial solution following clip-inoculation method (Kauffman *et al.,* 1973). Full grown leaves were clipped at maximum tillering stage from the upper 3-4 cm of the leaf. Near isogenic lines (NILs) comprising of different resistance gene(s) were evaluated to characterize different pathotypes. The NILs and the genotypes were classified as resistant and susceptible following Ogawa's method of identification of resistance genes to bacterial leaf blight (Ogawa.1993). Twelve near-isogenic lines were screened with the six isolates of *X. oryzae* pv *oryzae* collected from different parts of West Bengal. Genotypes of rice were screened in a randomized completely block design with three replications to test their level of resistance against bacterial blight. All the field tests were carried out at the experimental field of the Department of Genetics and Plant Breeding, Uttar Banga Krishi Viswavidyalaya, Coochbehar.

RESULTS AND DISCUSSION

Screening of near isogenic lines (NILs)

Twelve near-isogenic lines were screened with six isolates of *X. oryzae* pv *oryzae* collected from different parts of West Bengal. The NILs were developed in the background of IR 24 at the International Rice Research Institute, Philippines. Among the NILs, three were pyramided lines containing multiple genes for resistance. IRBB 55

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having two genes, *xa* 13 and *Xa* 21 while IRBB 59 contains three genes for resistance to bacterial blight, *viz*. *xa* 5, *xa* 13 and *Xa* 21. The pyramided genotype IRBB 60 carries 4 genes, *viz*. *Xa* 4, *xa5*, *xa* 13 and *Xa21*. All the NILs were considered for analysis of variance (Table 2). Screening of twelve NILs with the first isolate (*Xoo1*) categorized the genotypes into resistant and susceptible types (Table 1**)**.

Table1: Length of lesions of six bacterial isolates of *X. oryzae* pv *oryzae* on different NILs after inoculation

NILs	Resistant genes	*Xoo*1 Lesion length (cm)	*Xoo*2 Lesion length (cm)	*Xoo*3 Lesion length (cm)	*Xoo*4 Lesion length (cm)	*Xoo*5 Lesion length (cm)	*Xoo*6 Lesion length (cm)
IRBB3	*Xa 3*	7.26 (MR)	6.83(MR)	18.41(S)	5.10(R)	20.40(S)	19.73(S)
IRBB4	*Xa 4*	11.16(M)	6.05(MR)	19.04(S)	5.90(MR)	20.60(S)	19.43(S)
IRBB5	*xa 5*	0.52(R)	0.50(R)	1.02(R)	1.00(R)	0.61(R)	0.75(R)
IRBB7	*Xa 7*	3.61(R)	9.18(MR)	5.45(MR)	9.00(MR)	17.13(S)	20.26(S)
IRBB8	*xa8*	7.66(MS)	8.31(MR)	23.10(S)	6.66(MR)	22.00(S)	26.28(S)
IRBB10	*Xa 10*	4.80(MR)	5.86(MR)	16.72(S)	7.26(MR)	17.66(S)	21.73(S)
IRBB13	*xa 13*	2.70(R)	1.25(R)	2.85(R)	2.26(R)	9.83(R)	2.26(R)
IRBB14	*Xa 14*	3.78(R)	5.83(MR)	13.40(S)	6.36(MR)	17.51(MR)	15.48(S)
IRBB21	*Xa 21*	0.43(R)	0.41(R)	0.70(R)	0.56(R)	1.68(R)	1.00(R)
IRBB55	*xa 13, Xa 21*	0.52(R)	0.56(R)	0.43(R)	0.91(R)	0.56(R)	0.90(R)
IRBB59	*xa 5, xa 13, Xa 21*	0.43(R)	1.00(R)	0.73(R)	0.83(R)	1.01(R)	0.56(R)
IRBB60	*Xa 4, xa 5, xa 13, Xa 21*	0.36(R)	0.23(R)	0.46(R)	0.30(R)	0.48(R)	0.36(R)

Note: S=Susceptible, R=Resistant, MS=Moderately susceptible, MR=Moderately resistant
Scale: < 5cm-Resistant, 5 to10cm-Moderately Resistant, 10-15cm-Moderately Susceptible, > 15cm-Susceptible

Table 2: ANOVA for lesion length of twelve NILs screened with six isolates

Isolates	df	MS		LSD (0.05)
Xoo1	11	37.89	P<0.01	0.33
Xoo2	11	35.98	P<0.01	0.64
Xoo3	11	235.09	P<0.01	1.45
Xoo4	11	29.98	P<0.01	0.61
Xoo5	11	280.76	P<0.01	0.59
Xoo6	11	337.02	P<0.01	0.65

Table 3: Joint ANOVA for NIL X isolate interaction

Sources of variation	df	MS		LSD (0.05)
Replication	2	1.09	-	-
NIL	11	711.15	P<0.01	0.36
Isolate	5	408.34	P<0.01	0.26
NIL× Isolate	55	47.52	P<0.01	

Among the lines containing single gene for resistance, IRBB 21 showed the lowest lesion length of 0.43 cm while the highest value of 11.16 cm was observed in IRBB 4. IRBB 7, IRBB 10, IRBB 14 showed moderate resistance with lesion length of 7.26 cm. Among the lines having single gene for resistance, IRBB 5 and IRBB 21 showed high degree of resistance. The lines with multiple genes for resistance *i.e.* IRBB 55, IRBB 59 and IRBB 60 were highly resistant against *Xoo1*. Mean lesion length over all genotypes was recorded as 3.6 cm. Differences among the NILs were found to be significant with a critical difference of 0.33. Differences among all the resistant lines were found to be non-significant. IRBB 3 and IRBB 8 showed non-significant differences. Similarly IRBB 4 and IRBB 14 also showed non-significant difference between them. All the NILs screened with the second isolate (*Xoo2*) resulted six lines *viz*., IRBB 13, IRBB 21, IRBB 55, IRBB 59 and IRBB 60 to be resistant. Lowest disease progress was recorded as 0.23 cm for IRBB 60. Five genotypes *viz*., IRBB 3, IRBB 4, IRBB 8, IRBB 10 and IRBB 14 were found to be moderately resistant. Highest disease progress of 9.18 cm was recorded in IRBB 7. Mean lesion length of 3.8 cm was found for all the NILs. These genotypes were grouped based on least significant difference showed that IRBB7 and IRBB 8 were the most susceptible. Moderately susceptible IRBB 3, IRBB 4, IRBB 8, IRBB 10 and IRBB 14 were significantly different from the group of resistant genotypes. Mean lesion length of 8.5 cm was recorded for all the NILs screened with the third isolate (*Xoo3*). IRBB 8 exhibited highest disease progress (23.1 cm). Five genotypes were found to be highly susceptible. IRBB 7 was noticed to be moderately resistant with a lesion length of 5.45 cm. IRBB 8 exhibited significantly less disease development than all other genotypes. No significant difference was found among the susceptible genotypes, *viz*. IRBB3, IRBB4 and IRBB 10**.** All the genotypes showed resistant to moderately resistant reaction with the bacterial isolate 4 (*Xoo4*). An average lesion length of 3.8 cm was noted for this isolate. Lowest lesion length was noted for this isolate. Lowest lesion length was observed in the pyramided line IRBB 60 of 0.3 cm. IRBB 7 showed highest disease progress with a lesion length of 9 cm. Significant differences in lesion length among the NILs was revealed from F-test. IRBB 7 exhibited significant differences from rest of the genotypes. The fifth isolate (*Xoo5*) produced an average lesion length of 10.05 cm over all NILs. Among the susceptible NILs, IRBB 5 exhibited

lowest lesion length of 0.61 cm. All the pyramided lines showed high level of resistance. No moderately susceptible reaction was found. IRBB 8 produced the highest lesion length of 22 cm against *Xoo5*. This genotype was significantly different from all other genotypes. Six genotypes, viz. IRBB 3, IRBB 4, IRBB 8, IRBB 10 and IRBB 14 showed high degree of susceptibility with lesion length of 19.73cm, 21.7cm, 26.28 cm, 21.73cm and 15.48 cm respectively when all the NILs were screened against the sixth isolate (*Xoo6*). Average lesion length of 10.85 cm was observed in this case. Significant differences were found between the NILs as evidenced from F-test. Highest lesion length was found to be of 26.28 cm for IRBB 8. It is the highest lesion length among all the NILs screened with six bacterial isolates. IRBB 8 differed significantly from the rest of the genotypes. A grand mean of 6.76 cm of lesion length was observed over all isolates and NILs.

The joint analysis of variance was done to partition the total variability. Variation due to isolates, NILs and interaction showed highly significant difference (Table 3). *Xoo6*, *Xoo5* and *Xoo3* showed significant differences from the *Xoo4*, *Xoo2* and *Xoo1*. No significant differences were found among *Xoo4*, *Xoo2* and *Xoo1* over all genotypes. IRBB 8 showed highest mean value over isolates. IRBB 60 revealed the lowest average lesion length over all the isolates. Cluster analysis was performed using squared Euclidean distance and average linkage analysis by taking all near-isogenic lines to observe relationship among the six isolates. A dendogram was constructed from the cluster values. Highest squared Euclidean distance was found between *Xoo1* and *Xoo6* (Fig.1). The closest isolates were *Xoo2* and *Xoo4*. Three pathotypic groups were visible from this experiment. Pathotype I included *Xoo2*, *Xoo4* and *Xoo1* whereas, Pathotype II included *Xoo5* and *Xoo6* and the third pathotype group included *Xoo3*.

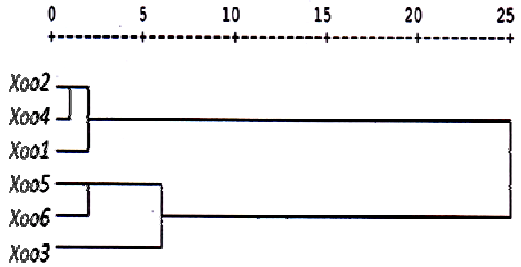

Fig.1: Dendogram using average linkage of isolates

Three NILs, having multiple genes for resistance, pyramided in them were included in our study to visualize the effects of multiple resistance genes on the isolates. IRBB 5, IRBB 13 and IRBB 21 were found to be resistant to all the isolates (Li *et al.*, 2001). Hence it was concluded that, *xa5*, *xa13* and *Xa21* were the durable mono genes that expressed under this agro-climatic region, to combat the disease. All the pyramided lines showed high and broad spectra of resistance against all the *Xoo* isolates (Le *et al.*, 2006). A quantitative complementation among the genes might also have taken place. Therefore, these genes should be induced for bacterial blight resistance breeding programme against all the pathotypes. Adhikari *et al*. (1999) showed that, pyramided lines have displayed higher levels and or wider spectra of resistance to bacterial blight than parental NILs with single resistance genes, suggesting synergism and complementation among resistant genes. Our results also confirmed with the findings of Adhikari *et al*. (1999). The rest NILs showed susceptible to moderately susceptible reaction to all the isolates, therefore these genes were unable to prevent the attack of different pathotypes in the present case.

NILs were the best material to study pathogenecity and value of resistance genes. Pyramided lines were found to be most effective to combat bacterial blight here. So, for breeders' perspective, gene pyramiding was proved to be novel way to achieve a solution against the occurrence of the disease. Besides, some single gene resistance was also found to be effective for terai agro-climatic region of West Bengal.

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