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DIFFERENTIAL DISEASE REACTION OF RICE PATHOGEN *XANTHOMONAS ORYZAE* PV. *ORYZAE* PREVAILING IN INDIA ON RICE CULTIVARS

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ABSTRACT

In the present study, the virulence of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) causing bacterial blight disease in rice was analyzed using 200 isolates of *Xoo* on near isogenic lines (NILs) and rice differentials having single or different gene combinations at field condition. These isolates were assembled from major rice growing areas of Punjab, Haryana, Uttar Pradesh, Bihar, West Bengal, Odisha, Andhra Pradesh, Tamilnadu and Kerala state. From these isolates, 20 pathotypes (races) on NILs and 12 pathotypes on rice differentials were identified based on disease reaction. In this analysis, more number of pathotypes were identified in Bihar state (10 pathotypes) followed by Uttar Pradesh (7 pathotypes), Haryana, West Bengal, Andhra Pradesh, Kerala (6 pathotypes), Odisha (4 pathotypes), Punjab (3 pathotypes) and Tamilnadu (2 pathotypes). Among these, pathotype I was virulent to all rice cultivar harbouring single resistant gene (*Xa*4, *xa*5, *Xa*7, *Xa*10 and *xa*13) and it was found to be prevalent throughout India. Significantly, isolates of pathotype III prevailing in Haryana state were noted that they breakdown the resistance of IRBB21 (*Xa*21) and IRBB52 (*Xa*4 + *Xa*21). Thus, in this analysis, data clearly is revealed that almost all the isolates were able to overcome only those NILs which harbored a single resistant gene (*i.e.* IRRB4) or two gene (*i.e.* IRBB52/DV85) for BB resistance and not those NILs (*i.e.* IRBB57-IRBB60) which harbored either a 3-gene/4-gene combinations.

INTRODUCTION

Bacterial blight (BB) caused by *X. oryzae*. pv. *oryzae* is one of the oldest and serious diseases of rice. BB was first noticed by farmers in Japan in 1884 and now, it occurs globally from Asia to Africa to the Americas with varying degrees of virulence and endemic in many parts of Asia. It is a widespread and destructive disease in irrigated and rainfed environments and it causes 30 to 50% yield loss (Ou 1985). In India, bacterial leaf blight emerged as a serious disease of rice after the introduction of semidwarf, high yield and susceptible rice cultivars (Shrivastava *et al.*, 1967) and it occurs in many states of India with yield loss up to 60-80% in severe infections. To manage this disease, a most effective way *i.e.* host plant resistance is being practiced due to ineffective and inconsistent of chemical and biological control method, respectively. So far, more than thirty five different BB resistant (R) genes (called *Xa* genes) that confer resistance against various races and pathotypes of *Xoo* have been identified (Suh *et al.*, 2013). These resistance genes display specificity with regard to their effectiveness against different pathogen races. Therefore, the use of resistant crop varieties is an inexpensive and environmentally friendly approach to crop protection. However, over time, virulent strains of the pathogen breakdown the resistance of rice cultivars. For instance, breaking down of resistance of IRBB21 rice line which harbouring dominant gene *Xa*-21 by some of *Xoo* isolates from Japan, Nepal, Korea and India have been reported in earlier studies (Lin *et al.*, 1996; Venkatesan and Gnanamanickam, 1999). This is due to resistance specificity of R-genes to particular pathogen sub-populations. This circumstance necessitates understanding the structure of pathogen populations to determine an appropriate R-gene for deployment of resistance in rice cultiva. In this study, virulence analysis was done using 200 isolates of *X. oryzae*. pv. *oryzae* assembled from major rice growing areas in India on near isogenic lines (NILs) and rice differentials for selecting appropriate R-genes/gene-combinations in rice breeding programme based on information of virulence of *Xoo* and its distribution.

MATERIALS AND METHODS

Rice seeds

Near isogenic rice lines (NILs) and rice differentials used for pathotyping experiment were imported from the International Rice Research Institute (IRRI), Philippines, with import permits

supplied by the National Bureau of Plant Genetic Resources (NBPGR), New Delhi. Seeds were multiplied by scientists at Hybrid Rice International Ltd, Hyderabad.

Collection of infected rice leaf samples

To study the existing pathogen population, bacterial blight infected rice leaf samples were collected from major rice cultivating areas in Haryana, Punjab, Uttar Pradesh, Bihar, West Bengal, Odisha, Andhra Pradesh, Tamilnadu and Kerala state of India. Infected samples were collected from each and every corner of the field and collected samples were maintained into deep freezer until *Xoo* isolation complete (Table 1).

Isolation of X. oryzae pv. oryzae

An infected leaf tissue with progressing lesion was cut into smaller bits of two to three centimeter long. They were surface sterilized with 1% sodium hypochlorite solution for 5 minutes. Later, the leaf bits were washed in 60% ethanol for 1 minute. Washed leaf bits were rinsed twice in sterile distilled water and dried on a sterile blotting sheet. Moisture-free bits of infected samples were ground in 1-2 mL of sterile distilled water with the help of a pestle and mortar. A loopful of the extract was streaked onto a peptone sucrose agar (PSA) medium and incubated at 28ºC for 72h. Isolated, single golden yellow colonies of the pathogen were picked and sub cultured on a fresh PSA plate. The strains were cultured on peptone sucrose agar and preserved in 20% glycerol at-80ºC (Yashitola *et al.*, 1997).

Pathotyping of X. o. pv. oryzae

Virulence analysis was done for 200 isolates of *Xoo* obtained from different states as mentioned in the Table 1. For this analysis, 13 near isogenic rice lines (NILs) and 4 rice differentials carrying single/two/three/four-gene combination, IR24 (susceptible check) and local rice cultivar (Jyothi) were used (Table 2). This study was carried out at Regional Agricultural Research Station (RARS), Pattambi, Kerala. For this analysis, rice seedlings were raised in a nursery and they were transplanted at 21-day old seedling stage in plots with 3x1m size. In a plot, each line was transplanted with ten plants at 10 cm distance from each other and was separated by a space of 15cm between rows. Necessary fertilizers were applied one week after transplanting. *Xoo* isolates grown for 72 h at 28ºC in peptone sucrose agar (PSA) medium were suspended in distilled water and the concentration of the suspension was adjusted to 0.1 OD at 600nm which is equivalent to 10^6cfu/ml. When the rice plants were 45 days old they were inoculated with *Xoo* by the clip-inoculation method. About 2-3 fully expanded young leaves per plant were inoculated with the help of sterile scissors dipped in the bacterial cell suspension (Kauffman *et al.*, 1973). All ten plants in a line were thus inoculated. Disease reactions were scored by BB lesion lengths measured 14 days after inoculation. Leaves showing lesion length below 5 cm were considered as BB resistance and those with lesion lengths longer than 5 cm as BB susceptible (Gnanamanickam *et al.*, 1994). Based on their virulence spectrum to different genes and gene combinations carried by the rice NILs and rice differentials, they were grouped into pathotypes (races).

RESULTS AND DISCUSSION

Sample collection and Isolation of X. oryzae pv. oryzae

In the sample collection, 647 isolates were isolated from 1600 infected samples. From each state, minimum 20 isolates (Haryana) and maximum 140 isolates (Kerala) were obtained (Table 1). From each state, *Xoo* isolates were taken in the

Table 1: Details of collection sites of infected rice samples, number of *Xoo* isolates obtained and used for pathotyping.

Region	State	Collection Site collected	Number of samples	Host cultivar obtained	Number of isolates	Number of isolates used for pathotyping
North West	Haryana	Hansi	50	Unknown	20	5
	Punjab	Kupkala, Ludhiana	40	Pusa, Pusa Basmati	30	5
North	Uttar Pradesh(UP)	Ranichouri, Raniket, Pantnagar, Faizabad	200	DX16, Bhona	90	25
	Bihar(B)	Samastipur, Pusa, Musaferpur	200	Unknown	80	30
East	West Bengal (WB)	Howrah	200	Unknown	46	30
	Odisha(O)	Cuttack	100	Unknown	35	25
South	Andhra Pradesh(AP)	Cutappah, Tada, Nandiyal	250	Unknown	96	30
	Tamil Nadu(TN)	Madurai, Theni, Trichy, Tanjur	350	ADT36, 46, Ponni, IR20,CO43	110	25
	Kerala(K)	Phalgat, Tirur, Kannur, Velacherry	260	Tiruvani, ADT36, Jyothi, ADT39, Badra, Shoranam	140	25
		Total	1,600		647	200

range of 5-30 isolates for the virulence analysis.

Disease reaction on NILs

In this analysis, 20 distinct pathotypes (races) were revealed based on disease reaction of NILs harbouring single/two/three/four gene combinations. Among them, more number of pathotypes were identified in Bihar state (10 pathotypes) followed by Uttar Pradesh (7 pathotypes), Haryana, West Bengal, Andhra Pradesh, Kerala (6 pathotypes), Odisha (4 pathotypes), Punjab (3 pathotypes) and Tamilnadu (2 pathotypes) on NILs (Table 3a; Fig.1a). In previous studies, the presence of different pathotypes in India has been reported such as 9 pathotypes in Odisha, Madhya Pradesh and Uttar Pradesh states (Shanti *et al.*, 2001), 4 pathotypes in Kerala state (Brindha *et al.*, 2002) and 17 pathotypes in Punjab state (Singh *et al.*, 2003). Here, isolates of pathotype I were found to be highly virulent to all rice cultivars which are having single R-gene (*Xa*4, *xa*5, *Xa*7, *Xa*10, and *xa*13), except IRBB21. This pathotype was detected to be widely distributed in all states (Tamilnadu, Kerala, Odisha, West Bengal, Bihar, Uttar Pradesh, Punjab and Haryana) except Andhra Pradesh with highest frequency rate (63%) of total isolates (Fig. 2a). Generally, pathogen populations within a region are similar and in some cases, genetically similar strains are detected in different regions due to consequence of germ plasm exchange or slow migration of pathogen population (George *et al.*, 1997). The remaining pathotypes were found in less than 5% frequency. Even though, some of them like pathotype IV present in Haryana state is identified as highly virulent to rice cultivar, IRBB52 having two-gene combination (*Xa*4 + *Xa*21) and also IRBB21 (*Xa*21). And, pathotype III has broken down the resistance of IRBB21 rice cultivar. Similarly, in earlier studies also, overcoming the resistance of IRBB21 (*Xa*-21 gene) have been reported in Japan, Korea, Nepal and India (Lin *et al.*, 1996; Venkatesan and Gnanamanickam, 1999; Lee *et al.*, 1999) but not on rice cultivar which are having more than two R-genes. Contrastingly, in this analysis, we found that some isolates belonging to pathotype XX in Uttar Pradesh state are not acute and they were virulent only to receive gene, *xa*5.

In this study, we noted the gene-specificity among pathotypes *i.e.* isolates of pathotype VII in West Bengal and pathotype X in Andhra Pradesh, Bihar and Uttar Pradesh states caused disease reaction only on IRBB10 (*Xa*10gene) and IRBB7 (*Xa*7gene), respectively, but not on others. At the same time, some of the pathotypes like pathotype V has showed compatible reaction to many R-genes (*xa*5, *Xa*7, *Xa*10 and *xa*13). In Haryana, isolates belonging to pathotype IV have showed compatibility to dominant gene, *Xa*21 but

Table 2: Details of near isogenic rice lines (NILs) and differential cultivars of rice used for pathotyping of *X. o. pv. oryzae* isolates

Sl.No.	Rice line / cultivar	R – gene(s) present
1.	IRBB4	*Xa4*
2.	IRBB5	*Xa5*
3.	IRBB7	*Xa7*
4.	IRBB10	*Xa10*
5.	IRBB13	*Xa13*
6.	IRBB21	*Xa21*
7.	IRBB52	*Xa4/Xa21*
8.	IRBB54	*Xa5/Xa21*
9.	IRBB55	*Xa13/Xa21*
10.	IRBB57	*Xa4/xa5/Xa21*
11.	IRBB58	*Xa4/xa13/Xa21*
12.	IRBB59	*Xa5/xa13/Xa21*
13.	IRBB60	*Xa4/xa5/xa13/Xa21*
14.	IR20	*Xa4*
15.	DV85	*Xa5/Xa7*
16.	Cas209	*Xa*10
17.	IR8	None
18.	IR24	None
19.	Jyothi	None

Table 3a: Data of BB disease reaction on near isogenic lines (NILs), number and distribution of pathotypes.

Near isogenic lines (NILs)														Pathotype	States in which pathotypes distributed
IRBB4	IRBB5	IRRB7	IRBB10	IRBB13	IRBB21	IRBB52	IRBB54	IRBB55	IRBB57	IRBB58	IRBB59	IRBB60	IR24		
S	S	S	S	S	R	R	R	R	R	R	R	R	S	I	TN, WB, O, B,UP, K, P, H
S	S	S	S	R	R	R	R	R	R	R	R	R	S	II	TN, AP, K, UP
S	S	S	S	S	S	R	R	R	R	R	R	R	S	III	H
R	S	S	S	S	S	S	R	R	R	R	R	R	S	IV	H
R	S	S	S	S	R	R	R	R	R	R	R	R	S	V	WB,O,B, UP,AP,P
R	R	R	R	R	R	R	R	R	R	R	R	R	S	VI	O, B, UP, K
R	R	R	S	R	R	R	R	R	R	R	R	R	S	VII	WB
R	R	S	S	R	R	R	R	R	R	R	R	R	S	VIII	WB, O, B, AP
R	R	S	S	S	R	R	R	R	R	R	R	R	S	IX	AP, UP, B,WB,P
R	R	S	R	R	R	R	R	R	R	R	R	R	S	X	B, UP, AP
S	S	S	R	R	R	R	R	R	R	R	R	R	S	XI	AP, B, O
S	S	R	R	R	R	R	R	R	R	R	R	R	S	XII	K
R	S	S	R	R	R	R	R	R	R	R	R	R	S	XIII	AP, K
S	S	S	R	S	R	R	R	R	R	R	R	R	S	XIV	B, AP
R	S	S	S	R	R	R	R	R	R	R	R	R	S	XV	AP, UP
R	S	S	R	S	R	R	R	R	R	R	R	R	S	XVI	O
S	R	S	S	S	R	R	R	R	R	R	R	R	S	XVII	K, UP
S	R	S	R	R	R	R	R	R	R	R	R	R	S	XVIII	B
S	R	S	R	S	R	R	R	R	R	R	R	R	S	XIX	B
R	S	R	R	R	R	R	R	R	R	R	R	R	S	XX	UP

Table 3b: Data of BB disease reaction on rice differentials, number and distribution of pathotypes.

Rice differentials IR20	IR8	DV85	Cas209	Jyothi	No. of Pathotype	Pathotype prevailing state
S	S	S	S	S	I	TN, AP, K, O, WB, P, H
R	S	R	S	S	II	TN, AP, K, UP, B, O, WB, H
S	S	R	S	S	III	TN, AP, K, WB, P, H
R	S	S	S	S	IV	AP, K, UP, O, WB, P
R	R	R	R	S	V	AP, B, O
R	R	S	S	S	VI	UP, O
R	R	R	S	S	VII	AP, K, UP, B
R	R	R	S	R	VIII	UP, B
R	R	R	R	R	IX	UP
R	R	S	R	S	X	K
S	R	S	S	S	XI	AP
S	R	R	S	S	XII	O, UP

R-refers to a resistant disease reaction observed as < 5cm lesion length; S-refers to a susceptible disease reaction observed as > 5cm lesion length; Each observation is an average of 6 measurements.

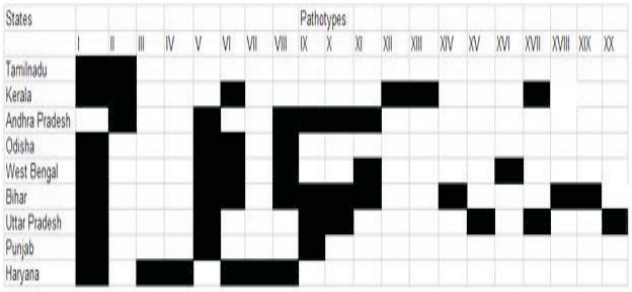

Figure 1a: shows the distribution of pathotypes based on NILs in states of India

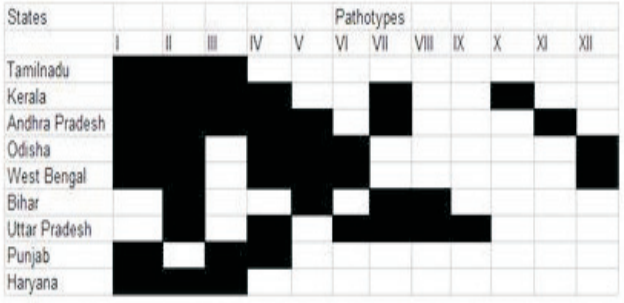

Figure 1b: shows the distribution of pathotypes based on rice differentials in states of India

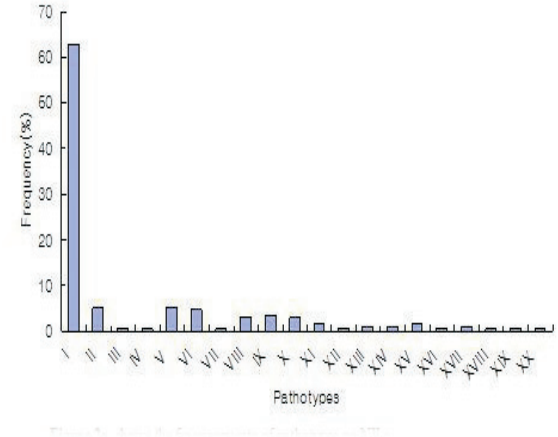

Figure 2a: shows the frequency rate of pathotypes on NILs

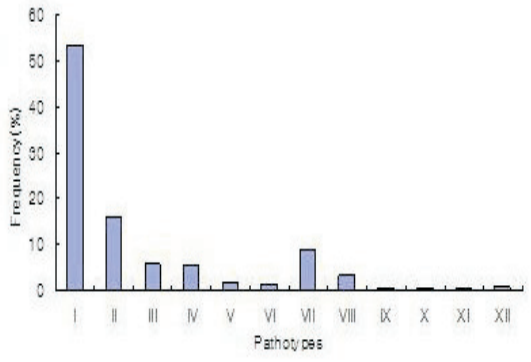

Figure 2b. shows the frequency rate of pathotypes on rice differentials

incompatibility to *Xa*4 gene. Similarly, pathotype XVII in Kerala and Uttar Pradesh, pathotype XVIII and XIX in Bihar were identified to be virulent to dominant R-gene, *Xa*4, but not to recessive gene, *xa*5. Because *X. o.* pv. *oryzae* strains play an important role in host or cultivar specificity and it determines the spectrum of bacterial virulence based on the compositional differences of exopolysaccharide(EPS) (Singh *et al.*, 2006). Interestingly, there were *Xoo* isolates with less virulence (pathotype VI) also were identified in Odisha, Bihar, Uttar Pradesh and Kerala states and they were avirulent to all NILs with R-genes except IR24 (susceptible check) in this analysis. Thus, based on virulence analysis on NILs, more number of pathotypes (16 pathotypes) are found to be compatible to *Xa*7 gene followed by 12 pathotypes to *xa*5 gene, 9 pathotypes to *Xa*4 and *Xa*10 gene, 8 pathotypes to *xa*13 gene, 2 pathotypes to *Xa*21 gene and 1 pathotype to gene-combination (*Xa*4 + *Xa*21) (Fig. 3a). In a screening for R-gene source in aromatic rice (basmati landraces), the *Xa*7 gene is found to be the most prevalent among cultivars and landraces. Unfortunately, in cultivated germplasm of the Indian

Figure 3a: shows the level of compatible/incompatible reaction of pathotypes on NILs harboring R-genes

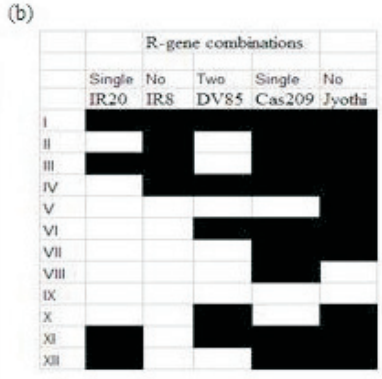

Figure 3b: shows the level of compatible/incompatible reaction of pathotypes on rice differentials harboring R-genes.

subcontinent, genetic diversity is low which necessitates screening of diverse resources (Ullah *et al.*, 2013; Zainab *et al.*, 2013).

Disease reaction on rice differentials

Based on disease reaction on four rice differentials, only 12 distinct pathotypes were revealed among 200 isolates of *Xoo*. In this study, more number of pathotypes were identified in Andhra Pradesh, Odisha, West Bengal, Uttar Pradesh (6 pathotypes) followed by Bihar (4 pathotypes), Punjab, Haryana, Tamilnadu and Kerala (3 pathotypes) (Table 3b; Fig.1b). Among them, isolates of pathotype I were presented in many states (Tamilnadu, Kerala, Andhra Pradesh, Odisha, West Bengal, Punjab and Haryana) with high frequency rate (53.5%). And, they were highly virulent to all rice differentials harbouring single/two genes and local cultivar, Jyothi (Fig. 2b). Pathotype V present in Andhra Pradesh, Odisha and Bihar states showed virulence reaction only on local cultivar (Jyothi) with 2% frequency rate. Similarly, no disease incidence was found on all rice differentials including local cultivar, except IR24 with isolates of pathotype IX in Uttar Pradesh state. Here, most of the pathotypes obtained on rice differentials (8/12 pathotypes) exhibited incompatible reaction to IR20 (*Xa*4) as well as IR8 (no R-gene) compared to DV85 having two-gene combination (*xa*5/*Xa*7) (7/12 pathotypes). But, Cas209 having *Xa*10 gene was more susceptible to most of the pathotypes (10/12 pathotypes) (Fig. 3b). So far, there are many studies have reported that those NIL lines carrying R-genes *Xa*1, *Xa*3, *Xa*4, *Xa*7, *Xa*10, *Xa*11 and *Xa*14 individually have showed distinctly susceptible to BB disease and all the gene combinations were resistant (Sridar, 2000). But, here, rice line harbouring two-gene combination (*Xa*4 + *Xa*21) also has showed susceptible reaction to *Xoo* isolates prevailing in Haryana state. Therefore, selection of donor line with more than two-gene combination will be highly resistant due to gene interaction or quantitative complementation between R-genes. In this way, many studies to select effective R-gene combination against all prevailing pathotypes in particular region or throughout India have been carried out in different places and identified better lines with different gene combination (Shanti *et al.*, 2001; Brindha *et al.*, 2002; Singh *et al.*, 2001). Very recently also, Suh *et al.* (2013) have improved rice variety using three-gene combination NIL, IRBB57 (*Xa*4, *xa*5 and *Xa*21) since this line showed highly resistant against all used BB isolates compared to lines with single R genes *Xa*4, *xa*5 and *Xa*21 individually.

In conclusion, this study is very comprehensive in its nature as it takes into account the pathogen population of *X. o.* pv. *oryzae* that prevails in India, analyzes for its pathotypic and distribution and links the information on pathogen diversity to two approaches of BB management in India. In spite of the fact that bacterial blight caused by strains of *X. o.* pv. *oryzae* continues to devastate rice production every year in several parts of India, BB management in the rice fields has been mostly through the use of disease resistant cultivars. In our traditional agricultural system, very little has been done to tackle the problem with careful linking of the information on pathogen diversity to molecular approaches. Data of pathotype clearly reveal also that almost all the strains were able to overcome only those NILs which harbored a single R-gene for BB resistance and not those NILs (IRBB54 to IRBB60) which harboured either a 2-gene/3-gene/4-gene combinations. The utility of the international rice differentials in identifying pathotypes/races of *X. o.* pv. *oryzae* is less as compared to the rice NILs. Only 12 pathotypes could be identified from among the 200 pathogen strains. The valuable information that emerged from the pathotyping is this: rice cultivars that carry single R-genes for BB resistance are likely to break down in the field and a pyramid of R-genes for BB resistance is likely to be durable. The results highlighted in this study show that this strategy has been followed as strategy for the management of rice bacterial blight in India. In the days to come, they will be very useful for the plant pathologists and rice breeders in their joint effort of rice disease management.

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