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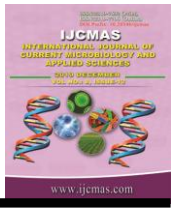

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Evaluating Pathogenic Diversity of Seven Isolates of *Xanthomonas oryzae* pv *oryzae* Derived from Different Hotspot of Chhattisgarh on near Isogenic lines (NILs)

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A B S T R A C T

The Bacterial blight disease caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is one of the most important disease and has caused crop failure in rice crops. This pathogen infects the leaves in all plant growth phases. The purpose of this study is to work out pathogenic diversity of seven Xoo isolates obtained from Chhattisgarh based on their interactions with 24 near-isogenic rice lines (NILs). The results showed, all the isolates were varying in their interaction with NILs. The most virulent isolate was XO-BLRP which was virulent on all the 11 lines having single BB resistance genes and it is also able to infect lines having two (*xa13 + Xa21*), three (*Xa4 + xa5 + xa13*), four (*Xa4 + Xa7 + xa13 + Xa21*) and five (*Xa4+ xa5 + Xa7 + xa13+ Xa21)* gene combinations. The next most virulent isolates were XO-BTP, XO-RPR and XO-SRP. The best gene combinations, so far identified are *Xa4 + xa5, Xa4 + Xa21, xa5+ xa13+ Xa21 Xa4 + xa5+ Xa7, Xa4 + Xa7 + Xa21* and *Xa4 + xa5 + xa13 + Xa21*. None of the isolate was able to infect the plants with these genes combinations. These gene combinations can be used in further resistance breeding programs for development of varieties with durable resistance to bacterial blight along with higher yield potential.

Introduction

Bacterial blight (BB) of rice, caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the oldest known and most destructive disease prevalent in major rice growing countries of Asia (Wang *et al.*, 2006), was first noticed by the farmers of Japan in 1884 (Tagami and Mizukami, 1962). In India, BB is a serious problem under irrigated and high fertilizer input conditions, which are conducive for disease development. In some

areas of Asia, it can reduce crop yield by up to 50% (Khush *et al.,* 1989) or even up to 80% (Singh *et al.,* 1977). Crop loss assessment studies have revealed that this disease reduces grain yield to varying levels, depending on the stage of the crop, degree of cultivar susceptibility and to a great extent, the conduciveness of the environment in which it occurs.

Host plant resistance has been used extensively for disease control in many crop species. However, resistance is not long lasting as a result of rapid changes in pathogen populations. Durability of resistance (*R*) genes is thus central to sustainable disease management (Shanti *et al.,* 2010). Information on the virulence of pathogenic populations is essential for deploying durable resistance (Arshad *et al.,* 2017). About 43 BB resistance (R) genes, designated from *Xa1* to *Xa43* (Cheema *et al.,* 2008, Kim *et al.,* 2015. Busungu *et al.,* 2016, Kim and Reinke, 2019) conferring resistance against various strains of *Xoo*, have been identified from cultivated, mutant population, and wild rice species. These genes have been mapped on 6 of the 12 rice chromosomes. Most of these genes provide complete and race-specific resistance to *Xoo* and have been used singly or in combination in rice breeding for BB resistance (Kinoshita, 1995; Zhang *et al.,* 1998; Gao *et al.,* 2001; Sundaram *et al.,* 2008; Perumalsamy *et al.,* 2010; Pandey *et al.,* 2013). This resistance gene shows specificity in terms of effectiveness against different pathogen races. As such, knowledge of diversity of pathotypes in target pathogenic population is essential to select resistant source to be used in resistance breeding programs (Mishra *et al.,* 2013).

Though a lot of research has been done on the R genes and pyramiding into different cultivars of interest, there is not much study on the pathogen population especially in India, where there is a diverse geographical variation within the country. Knowledge of the pathogen structure and changes in the races of the contemporary pathogen are very vital areas of research.

The present study was undertaken to know the population structure and virulence pattern of BB pathogen in Chhattisgarh region of India and to apply knowledge of pathogen population structure to the deployment of resistance genes. It has been undertaken to identify genes and gene combinations conferring resistance to the contemporary BB pathogen and for deploying of such genes in future breeding programs so as to incorporate durable levels of resistance in promising high yielding varieties.

Materials and Methods

Bacterial culture

Infected leaf samples collected from seven different regions of Chhattisgarh: Dhamtari (XO-DMT), Balrampur (XO-BLRP), Durg (XO-DUR), Raipur (XO-RPR), Mahasamud (XO-MHS), Surajpur (XO-SRP) and Bahatapara (XO-BTP). Bacteria were isolated using the procedure described by Kotasthane (2003). Isolates were purified and maintained in Wakimoto's medium. These seven isolates were used to work out pathogenic diversity of Xoo in Chhattisgarh region.

Plant population: Differential rice lines

Set of 24 NIL's and Pyramids (11 lines with single resistance genes and 13 pyramided lines with two, three, four and five gene combinations) obtained from IRRI, Philippines were used for pathotyping of *Xoo* isolates.

(NIL's:- IRBB1, IRBB2, IRBB3, IRBB4, IRBB5, IRBB7, IRBB8, IRBB10, IRBB11,

IRBB13, RBB14, IRBB21. Pyramids: - IRBB50, IRBB51, IRBB52, IRBB54, IRBB55, IRBB56, RBB57, IRBB60, IRBB62, IRBB65).

Phenotyping of plant population

During the wet season 2017, the experimental plant material were grown in field and inoculated at maximum tillering stage with bacterial culture following the clip inoculation technique (Kauffman *et al.*, 1973). After 21 days of inoculation, Phenotyping of plant population was done by scoring following Standard Evaluation System of Rice developed by IRRI, Philippines. The lesion length and total leaf length were observed and percent disease leaf area was calculated. Inoculated plants were rated as Highly Susceptible, Susceptible, Resistant and Highly Resistant. In this study, plants with score 1 and 3 were considered as resistant and plants with score 5 and above were considered as susceptible. Plants were categorized into three categories highly resistant (score 0), resistant (score1 and 3) and susceptible (score 5, 7 and 9).

Results and Discussion

Bacterial blight (BB) caused by the pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most serious diseases of rice. The pathogen varies in its virulence to different rice varieties. Six races of the bacterium have been identified in Philippines. Each race has specific virulence to varieties with different resistance genes, showing a gene-for-gene relationship in the host-pathogen interaction (Mew, 1987; Vera Cruz and Mew, 1989). Several qualitative traits including bacterial leaf blight (BLB) resistance have been mapped and candidate genes were identified by physical mapping and map-based cloning approaches (Kameswara Rao *et al.*, 2002, Nino-Liu *et al.*, 2006). To facilitate characterization of *Xa* genes and *X. oryzae* pv. *oryzae* isolates, IRRI developed a set of near isogenic lines (NILs) in the 1980s. Each NIL contained a single *Xa* gene in the background of the susceptible cultivar IR-24 (Ogawa *et al.*, 1988). In this study 24 NILs were used to work pathogenic diversity of seven Xoo isolates (Table 2). Knowledge of pathogenic diversity of pathogen population is essential for selection of disease resistance sources for a regional crop breeding program (Leung *et al.*, 1993). The present study was undertaken to provide information about the population structure of *Xanthomonas oryzae* pv. oryzae, the bacterial blight pathogen of rice in Chhattisgarh.

Each of seven isolates was inoculated on 24 Near Isogenic Lines (NILs). 21 days after inoculation disease reaction were recorded as Highly Resistance (HR), Resistance (R) and Susceptible (S) (Table 2).

Pathogenic diversity of seven *Xoo* isolates

More the virulence of pathogen more it is difficult to breed for resistance and selection of resistance sources. Despite having resistance genes in each of 24 lines all of the isolates were able to infect many of the NILs. This is due to absence of matching avirulence genes in the pathogen. Xoo has capacity of rapid evaluation. So there is more chance of breaking down of resistance in cultivars with R genes. It is necessary to know its virulence nature and pathogenic diversity for incorporating long lasting resistance in rice cultivars.

It can be understand with the help of graph 2 that no isolate were able to show complete virulence in all inoculated lines. All the isolates showed varying degrees of resistance and susceptibility. The most virulent isolate was XO-BLRP which was able to infect all the 11 lines having single BB resistance genes and it is also able to infect lines having two (*xa13 + Xa21*), three (*Xa4 + xa5 + xa13*), four (*Xa4*

+ Xa7 + xa13 + Xa21) and five (*Xa4+ xa5 + Xa7 + xa13+ Xa21)* gene combinations. The next most virulent isolates were XO-BTP, XO-RPR and XO-SRP showing virulence to eleven lines. These isolates were able to knockout 10 single genes out of eleven BB genes used in this study. The least virulent isolates among seven was XO-DUR followed by XO-DMT and XO-MHS, which showed virulence against 5, 6 and 9 lines respectively.

Resistant spectrum of selected Near Isogenic Lines (NILs)

The single BB resistance genes and their combinations were chosen for this study. The results based on the mean disease reaction observed among the 24 Near Isogenic Lines (NILs) developed by incorporating BB resistance gene in the background of IR-24. Eleven among 24 lines were having single gene and thirteen lines had genes in many combinations. Disease scoring results suggested that no single gene confers complete resistance to all the isolates. Lines with individual genes showed susceptibility as compared to lines with gene combinations (Graph 2). Among all single genes used in the study to evaluate their disease spectrum, the dominant gene *Xa21, Xa7* and *Xa8* showed a higher degree of resistance whereas *Xa4, xa5, Xa10* and *xa13* showed the lowest degree of resistance being susceptible to all seven isolates.

The two, three and four gene combinations performed better than single genes. Interestingly, *Xa4+Xa21* combination performed well (resistant to all 7 isolates). This is in complementation with the earlier studies showing that *Xa4* though referred to as a defeated gene, it was found that this gene when in combination with *Xa21* showed higher level of resistance (Jeung *et al.,* 2006). Similarly *Xa4 + xa5* combinations were found to be effective against all seven isolates. The best gene combinations are *Xa4 + xa5, Xa4 + Xa21, xa5+ xa13+ Xa21 Xa4 + xa5+ Xa7, Xa4 + Xa7 + Xa21* and *Xa4 + xa5 + xa13 + Xa21*, all the seven isolates were avirulent against these gene combinations. Next best gene combinations are X*a4 + xa13, xa5 + Xa21, Xa4 + xa5 + xa13, Xa4 + xa5 + Xa21, Xa4 + Xa7 + xa13 + Xa21,* resistant against six isolates (graph2). Unexpectedly, five gene combination *Xa4+ xa5 + Xa7 + xa13+ Xa21* performed least in among all gene combinations. This might be due to negative epitasis of genes. It is difficult for pathogen to infect plants with multiple genes in the back ground. This advantage is being used in resistant breeding programs. Till date many varieties has been released by incorporating multiple gene combination in the background as Pusa Basmati-1 and Sambha Mashuri. But pathogen is dynamic enemy and continuously evolving. So the durability of resistance is always questionable. This is why, it is necessary to know best gene combination to be deployed in the given region.

Avirulence pattern of *Xoo* isolates

The *R* genes used in plant disease management direct the recognition of pathogenic protein encoded by avirulence (*avr*) genes; this relationship is referred to as a gene-for-gene interaction (H. H. Flor, 1955).

The majority *R* genes encode leucine-rich repeat domains, which mediate specific protein–protein interactions. The R proteins are predicted to act as receptors to bind specifically to a pathogen-produced ligand, which is produced directly or indirectly by the *avr* gene. This direct interaction of the R protein and Avr ligand results in activation of the plant defense response, which often involves a hypersensitive response (HR) leads to incompatible interaction between pathogen and host.

Table.1 Standard Evaluation System (IRRI 1980), developed for assessing diseased leaf area.

The mean percentage of diseased leaf area (%DLA) on the upper 3 leaves of plants was assessed

Scale	Disease leaf area	Description
0	0-1%	**Highly Resistant (HR)**
1	1-5%	**Resistant (R)**
3	6-12	**Moderate resistance (MR)**
5	13-25 %	**Moderate susceptible (MS)**
7	26-50 %	**Susceptible (S)**
9	**>50 %**	**Highly Susceptible (HS)**

Table.2 Disease reactions of seven *Xoo* isolates on 24 NILs

NILs	Gene/ gene combination	NILs /Isolates	XO-BTP	XO-DMT	XO-DUR	XO-BLRP	XO-MHS	XO-RPR	XO-SRP
IRBB1	*Xa1*	NIL1	S	HR	R	S	R	S	R
IRBB2	*Xa2*	NIL2	S	R	R	S	S	S	S
IRBB3	*Xa3*	NIL3	S	S	R	S	S	S	S
IRBB4	*Xa4*	NIL4	S	S	S	S	S	S	S
IRBB5	*xa5*	NIL5	S	S	S	S	R	S	S
IRBB7	*Xa7*	NIL6	S	R	R	S	S	R	S
IRBB8	*xa8*	NIL7	R	R	R	S	S	S	S
IRBB10	*Xa10*	NIL8	S	S	S	S	S	S	S
IRBB13	*xa13*	NIL9	S	S	S	S	S	S	S
IRBB14	*Xa14*	NIL10	S	R	R	S	S	S	S
IRBB21	*Xa21*	NIL11	S	R	HR	S	R	S	S
IRBB50	*Xa4 + xa5*	NIL12	R	R	R	R	R	R	R
IRBB51	*Xa4 + xa13*	NIL13	S	R	R	R	R	R	R
IRBB52	*Xa4 + Xa21*	NIL14	R	HR	HR	R	R	HR	R
IRBB54	*xa5 + Xa21*	NIL15	R	HR	HR	HR	HR	R	S
IRBB55	*xa13 + Xa21*	NIL16	HR	R	HR	S	HR	S	R
IRBB56	*Xa4 + xa5 + xa13*	NIL17	R	HR	HR	S	R	R	R
IRBB59	*xa5+ xa13+ Xa21*	NIL18	R	R	HR	R	R	HR	R
IRBB57	*Xa4 + xa5 + Xa21*	NIL19	R	S	HR	R	HR	R	HR
IRBB60	*Xa4 + xa5 + xa13 + Xa21*	NIL 20	R	HR	HR	R	HR	R	R
IRBB61	*Xa4 + xa5+ Xa7*	NIL 21	R	R	R	R	HR	R	R
IRBB62	*Xa4 + Xa7 + Xa21*	NIL 22	R	R	R	R	R	HR	HR
IRBB65	*Xa4 + Xa7 + xa13 + Xa21*	NIL 23	HR	HR	R	MR	HR	HR	HR
IRBB66	*Xa4+ xa5 + Xa7 + xa13+ Xa21*	NIL 24	R	R	S	S	S	R	R

Table.3 Speculation of avirulence gene present in isolates under study based on interaction with NILs containing respective resistance genes

Sr. no.	NILs	Speculation of avirulence gene(s)	Frequency
1	XO-BTP	*avrxa8*	1
2	XO-DMT	*avrXa2, avrXa7, avrxa8, avrXa10, avrXa21*	5
3	XO-DUR	*avrXa2, avrXa3, avrXa7, avrxa8, avrXa10, avrXa21*	6
4	XO-BLRP		0
5	XO-MHS	*avrXa5, avrXa21*	1
6	XO-RPR	*avrXa7*	1
7	XO-SRP		0

Graph.1 Virulence spectrum of seven *Xoo* isolates on 24 NIL: graph showing number of susceptible Near Isogenic Lines for each of seven *Xoo* isolates

Graph.2 Frequency distribution of 24 Near Isogenic Lines inoculated with seven *Xoo* isolates

Therefore, the function of the *R* gene depends on the presence of a recognizable Avr ligand in the pathogen. Many Avr gene has been well characterized in Xoo i.e. avrXa2, *avrXa3, avXa4, avrXa7, avrXa10 and avrXa21* (Song *et al.*,1995, Iyer and McCouch, 2004, Tian *et al.,* 2014, Sekhwal *et al.,* 2015,). The *Xoo* In addition to their role in avirulence, several *avr* genes possess a function in aggressiveness (amount of disease) or disease symptom expression, both of which are components of pathogenic fitness. The dual functions (avirulence and fitness) associated with these genes has led to speculation that the *R* genes corresponding to these *avr* genes may be more durable in the field.

During the present investigation on inoculation of two different isolates on NILs and Pyramids, which carried different known R genes and gene combinations, it was observed that the isolates had specific interactions with some of these lines and as a consequence incompatible interaction were observed (Table 2). The incompatible interactions, which is accompanied by hypersensitive response predicts that it is a consequence of recognition of protein encoded by avirulence gene in the pathogen by corresponding R gene in the host. Based on the specific interactions observed during the present investigation on NILs it was speculated for the presence of corresponding *avr* gene(s) in the pathogen.

Based on incompatible interaction between NILs and Xoo isolates, avirulence genes can be speculate in the pathogen according to corresponding R gene present in respective NIL. Isolate XO-RPR has *avrXa7* because it was incompatible on IRBB7 containing Xa7 R gene. Similarly Isolate XO-DMT has *avrXa2, avrXa7, avrxa8, avrXa10, avrXa21,* Isolate X-DURG has *avrXa2, avrXa3, avrXa7, avrxa8, avrXa10, avrXa21*avirulence gene, isolate XO-MHS has *avrXa5* and avrXa7, XO-BTP has *avrxa8* (Table 3).

Bacterial blight has shown high race-cultivar specificity. Information regarding population structures of pathogen is necessary to deploy resistance genes into cultivars for incorporating long lasting resistance, which can not be broken by pathogen. Seven isolates of pathogen *Xanthomonas oryzae* pv *oryzae* were collected from Chhattisgarh region. These isolates were inoculated on 24 Near Isogenic lines to work out their pathogenic diversity. Field screening results suggested that one of the isolate XO-BLRP was highly virulent and able to breakdown all the single gene as well as multiple gene combinations, followed by two isolates XO-RPR and XO-SRJ. Best combinations of resistance genes identified in this study were *Xa4 + xa5, Xa4 + Xa21, xa5+ xa13+ Xa2, Xa4 + xa5+ Xa7, Xa4 + Xa7 + Xa21, Xa4 + Xa7 +xa13+ Xa21* and *Xa4 + xa5 + xa13 + Xa21.* These combinations can be used for planning regional breeding program against Bacterial Blight of rice and deploying durable resistance in region.

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