ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2018; 4(8): 65-68
www.allresearchjournal.com
Received: 04-06-2018
Accepted: 06-07-2018

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Identification of pathotype (s) of *Xanthomonas oryzae* pv. *oryzae* (Xoo) causing bacterial leaf blight (BB) and the gene(s) responsible for resistance in rice

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Abstract
A series of bacterial leaf blight disease samples of rice were collected from different locations of Nagaon district of Assam to identify pathotype (s) of *Xanthomonas oryzae* pv. *oryzae* (Xoo) and gene(s) responsible for resistance in rice against bacterial leaf blight disease. Altogether, 6 (six) isolates were isolated through serial dilution techniques in nutrient agar medium. Isolates were then individually inoculated to set of 8 (eight) differential rice cultivars and another set of 23 near isogenic lines of rice by clipping method. Following pathogenicity pattern of the bacterial isolates on 8 (eight) differential rice cultivars, pathotype of *Xanthomonas oryzae* pv. *oryzae* has been identified as 'pathotype Ia'. Further, based on pathogenicity pattern on 23 near isogenic lines of rice, single gene Xa4, Xa7, xa13 and Xa21 showed resistant further, the gene pyramiding Xa4 + xa5, Xa4 + xa13, Xa4 + Xa21, xa5 + xa13, xa5 + Xa21, Xa4 + xa5 + xa13, Xa4 + xa5 + Xa21, xa5 + xa13 + Xa21 and Xa4 + xa5 + xa13 + Xa21 will also confer a stable resistant to 'pathotype Ia'. Therefore, rice variety (ies)/cultivar(s) with any one of the above mentioned gene combinations or alone will provide stable resistant against *Xanthomonas oryzae* pv. *oryzae* (pathotype Ia). The present study has identified the pathotype of *Xanthomonas oryzae* pv. *oryzae* as 'pathotype Ia' for first time from Assam.

Keywords: Rice, pathotype, gene, *Xanthomonas oryzae* pv. *oryzae*, bacterial leaf blight (BB)

Introduction
The bacterial blight of rice caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) [Ishiyama (1922) [8] and Swings *et al*. (1990) [22]] is an economically important disease (Mew, 1987) [14] and is first time reported from Maharashtra, India by Srinivasan *et al*. (1959) [20]. This is one of most destructive disease of rice in both irrigated and rainfed environments in Asia (Mew, 1987) [14]. It leads to loss from 20 to 30 per cent in Japan (Mizukami and Wakimoto, 1969) [16], West Africa from 2.7 to 41 per cent (Awoderu *et al*., 1991) [2] and in India, yield loss was recorded to be varied from 6 to 70 per cent (Srivastava *et al*., 1982; Joshi, 1977 & Raina *et al*., 1981) [21, 9, 18]. The bacterial leaf blight of rice occurs every year in endemic to epidemic form in southern part of Gujarat (Chauhan, 1973) [3]. In Assam, this disease is very much common and invariably encountered in rice every year during growing seasons. However, information regarding pathotype of bacterial leaf blight disease caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) and gene(s) responsible for resistance in rice against the pathogen occurred in this region is very limited. That is why, an attempt has been made to identify pathotype of *Xanthomonas oryzae* pv. *oryzae* prevailing of the region and resistance gene(s) that confer resistant to the pathogen.

Methodology
Bacterial leaf blight infected leaves were collected from nearby rice field of Regional Agricultural Research Station, Shillongani, Nagaon within a radius of 2 km during *Boro* season. For isolation of causal organism *Xanthomonas oryzae* pv. *oryzae* the infected leaves were cut into small pieces and followed by surface sterilization. After that leaves were dip in sterile water to allow maximum particles of pathogen to be come out of pieces of infected leaves. Through serial dilution technique, pure cultures of the pathogen were isolated separately in nutrient agar medium. Eight differential rice cultivars and 23 near isogenic lines of rice along with Ajay a and TN1 as local resistant and susceptible checks respectively, were

grown on nursery bed and then 30 day-old-seedlings were transplanted under natural epiphytotic condition which was initially fertilized with 120:30:0 NPK kg/ha in *summer* season 2017 at Regional Agricultural Research Station, Shillongani, Nagaon. Each line (cultivar) was grown in two lines of 2.5 m length with 20 x 20 cm spacing plant to plant and row to row in two lines (Anonymous, 1997) [1]. Isolates were then multiplied in nutrient broth medium at 28± 2 ^{0}C for 48 hours to get 10^9 cells/ml concentration of bacteria. The experimental field was kept free from weeds by adopting manual weeding and need based plant protection measures were undertaken to prevent pests damage during crop growth.

Inoculation of different sets of differential rice cultivars and near isogenic lines of rice were done at maximum tillering stage by clipping method (Kauffman *et al.,* 1973) [10] at 15:00 hours (evening time) with already prepared inoculums. An initial record was done 7 (seven) days after inoculation and final record was done when susceptible check express high susceptible reaction. Disease scoring was done following 0-9 scale (IRRI, 1996).

0 = Immune
1 = Resistant (1 – 5% area affected)
3 = Moderately resistant (6 – 12% area affected)
5 = Moderately susceptible (13 – 25% area affected)
7 = Susceptible (26 – 50% area affected)
9 = Highly susceptible (51 – 100% area affected)

The per cent disease index (PDI) was worked out following formula

$$\text{Per cent disease index (PDI)} = \frac{\text{Sum of numerical ratings}}{\text{Number of leaves observed x Max. scale rating}} \text{ x } 100$$

The pathotypes (s) of *Xanthomonas oryzae* pv. *oryzae* was grouped according to the methods given by Reddy *et al*. (1989) [19].

Results and Discussion

It has been observed in table 1 that all the 6 (six) isolates showed same reactions on 8 (eight) differential cultivars where BJ1, DV 85 and IR 20 showed resistant reactions carrying resistance genes Xa4, Xa7 and xa13 (Table 1) and rest were susceptible. It reflects that the isolates is carrying avirulence genes in respect of those resistance genes in host. Further, it has also observed that the pathogen is carrying virulence genes against Xa1, Xa3, xa11 and Xa14 genes showed susceptible reaction in differential cultivars, supports the earlier observation made by Dissanayake *et al.* (1992) and Reddy *et al.* (1989) [19]. Reddy *et al.* (1989) [19] reported prevalence of 3 pathotypes of *Xoo* namely, patholtype Ia, Ib and II in India. From this study, it revealed that considering the disease reactions on differential cultivars against *Xoo*, the prevailing pathotype of *Xanthomonas oryzae* pv. *oryzae* according to Reddy *et al.* (1989) [19] was grouped as 'pathotype Ia'. Hence, the prevailing pathotype of *Xanthomonas oryzae* pv. *oryzae* causing bacterial blight disease of rice in this region is 'pathotype Ia'.

It has been revealed from table 2 that most of the cultivars containing single gene for resistance were found susceptible except Xa4, Xa7, xa13 and Xa21 genes against the 'pathotype Ia' that means there was a corresponding gene for avirulence in the pathogen. This supports gene-for-gene hypothesis suggested by Flor (1942) [5] where inter-relationship between the host and pathogen existed. These genes for resistance will provide vertical resistance to the cultivar/host. This type of resistance is effective only against one or a few specific pathotype of pathogen until and unless a new strain has developed (Vanderplank, 1963) [23]. However, cultivar containing a single major resistance gene proved susceptible due to pathogen mutation (Rajpurahit *et al.,* 2010) [17]. The Xa4 gene alone was not effective against many of the *Xanthomonas oryzae* pv. *oryzae* pathotypes evaluated from Punjab (Lore *et al.,* 2011) [11]. Therefore, chances of development of new pathotype in nature can not be ignored, which leads to break down of resistant character governed by single gene in a cultivar. To avoid such chances, gene pyramiding / multigene for resistance will provide a long term solution to prevent frequent break down of resistant character in cultivar/variety by the prevailing pathogen and able to deliver durable resistance (Rajpurahit *et al.,* 2010) [17]. The pyramided with Xa4 and other resistance genes showed a wider spectrum and a higher level of resistance than the cultivar with a single resistance gene (Huang *et al.,* 1997) [6]. The Xa4 gene is one of the most exploited resistance genes in many Asian rice breeding programmes and it conferred durable resistance in many commercial rice cultivars (Mew *et al.,* 1992) [15]. It has also been observed that all different combinations of genes or gene pyramiding were recorded to confer resistant against the 'pathotype Ia'. However, long term / stable / horizontal resistance could be expected from multiple resistance genes *viz.,* Xa4 + xa5, Xa4 + xa13, Xa4 + Xa21, xa5 + xa13, xa5 + Xa21, Xa4 + xa5 + xa13, Xa4 + xa5 + Xa21, xa5 + xa13 + Xa21 and Xa4 + xa5 + xa13 + Xa21 (Table 2).

The reactions in host plants against different pathogenic races are controlled by many genes for resistance (Mehrotra, 1988) [13]. Therefore, gene pyramiding with multiple resistance genes in a cultivar will show horizontal resistance character until and unless pathogen breaks it down. To break down the resistance genes of a cultivar, the pathogen has to develop all virulence genes on same time against those resistance genes in the host. Therefore, in nature chances of development different virulence genes on same time in a pathogen against a host is very remote.

It may be concluded through this investigation that the prevailing pathotype of *Xanthomonas oryzae* pv. *oryzae* isolate causing bacterial blight disease of rice has been identified as 'pathotype Ia' for the first time from Assam, carrying avirulence genes for Xa4, xa13 and Xa21 genes. Further, it can also reveal that the gene pyramiding (Xa4 + xa5, Xa4 + xa13, Xa4 + Xa21, xa5 + xa13, xa5 + Xa21, Xa4 + xa5 + xa13, Xa4 + xa5 + Xa21, xa5 + xa13 + Xa21 and Xa4 + xa5 + xa13 + Xa21) will provide horizontal / durable resistance to the cultivar. This information may be utilized by the rice breeder for developing resistant variety against rice bacterial blight disease.

Table 1: Pathogenicity pattern of *Xanthomonas oryzae* pv. *oryzae* on differential rice cultivars

S. No.	Differential rice cultivars	Resistance genes	Reaction –BB (Score 0-9 scale)	Disease reaction	Pathotype group
1.	BJ1	xa13	1	R	
2.	DV85	Xa7	3	MR	
3.	Java14	Xa1	7	S	
4.	Cempo Selak	Xa3	7	S	Pathotype Ia
5.	IR8	xa11	7	S	
6.	IR20	Xa4	3	MR	
7.	Ajaya	-	3	MR	
8.	TN1	Xa14	7	S	

Table 2: Reactions of different gene combinations against *Xanthomonas oryzae* pv. *oryzae*

S. No.	Near Isogenic lies of rice	Gene combination (s)	Reaction-Blb (Score 0-9 scale)	Reactions
1.	IRBB-1	Xa1	7	S
2.	IRBB-3	Xa3	7	S
3.	IRBB-4	Xa**4**	3	**MR**
4.	IRBB-5	xa5	5	MS
5.	IRBB-7	Xa7	3	MR
6.	IRBB-8	xa8	5	MS
7.	IRBB-9	Xa9	7	S
8.	IRBB-10	Xa10	7	S
9.	IRBB-11	xa11	7	S
10.	IRBB-13	xa13	1	R
11.	IRBB-14	Xa14	7	S
12.	IRBB-21	Xa21	1	R
13.	IRBB-50	Xa4 + xa5	3	MR
14.	IRBB-51	Xa4 + xa13	1	R
15.	IRBB-52	Xa4+ xa21	3	MR
16.	IRBB-53	xa5 + xa13	3	MR
17.	IRBB-54	xa5 + Xa21	3	MR
18.	IRBB-55	xa13 + Xa21	1	R
19.	IRBB-56	Xa4 + xa5 + xa13	1	R
20.	IRBB-57	Xa4+ xa5 + Xa21	1	R
21.	IRBB-58	Xa4 + xa13 +Xa21	1	R
22.	IRBB-59	xa5 + xa13 +Xa21	1	R
23.	IRBB-59	Xa4+ xa5 + xa13 +Xa21	1	R
24.	Ajaya	Resistant check	3	MR
25.	T(N)1	Susceptible check	9	HS

Acknowledgement
The author is pleased to acknowledge the office of the Chief Scientist, Regional Agricultural Research Station, Shillongani, Nagaon-782002, Assam, India for necessary assistance and time to time advice during the course of investigation.

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