

Pathotype profiling, distribution and virulence analysis of *Xanthomonas oryzae* pv. *oryzae* causing bacterial blight disease of rice in Bangladesh

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Funding information

Bangladesh academy of sciences

Abstract

Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is a serious threat to rice production in the world. Identification of *Xoo* races pattern, distribution and deployment of race-specific resistant genes is a sustainable strategy to develop bacterial blight resistant varieties. In this study, a total of 118 isolates of *Xoo* were collected from 8 rice-growing regions of Bangladesh to identify the existing pathotypes of *Xoo*. All tested isolates were virulent on the susceptible rice varieties viz. IR24, Purbachi and BR11. The tested isolates were evaluated on 13 near-isogenic lines of rice and each line consisted of a single bacterial blight resistant gene viz *Xa1*, *Xa2*, *Xa3*, *Xa4*, *xa5*, *Xa7*, *xa8*, *Xa10*, *Xa11*, *xa13*, *Xa14*, *Xa21* and *Xa23*. Based on reaction patterns of 118 isolates of *Xoo* on near-isogenic lines, 12 pathotypes/races were first time identified in Bangladesh. Race 1, Race 2 and Race 3 were predominantly distributed in the most of the areas of Bangladesh and considered as major races containing the maximum number of isolates (48%, 14% and 11% respectively). Among these, Race 1 was the most prevalent and widely distributed while Race 5 was the most virulent circumventing all of the resistance genes tested. Race 1 was recorded from six rice-growing regions of Bangladesh. Host plant *R*-genes *xa5*, *xa8*, *xa13*, *Xa21* and *Xa23* have been found as effective against bacterial blight based upon resistance frequencies and the reactions of near-isogenic lines and pyramid lines. The bacterial blight resistant gene *Xa21* showed a resistant reaction against 11 of 12 races (i.e. 94.91% of the isolates tested). Effective genes and information of races generated from this study could be deployed for the development of race-specific bacterial blight resistant varieties in Bangladesh.

KEYWORDS

bacterial blight, near-isogenic lines, pyramid lines, races, *Xanthomonas oryzae* pv. *oryzae*

1 | INTRODUCTION

Bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) causes one of the major foliar diseases of rice called bacterial blight (BB). Rice (*Oryza sativa* L.) is the staple food for over half of the world's population (Khush, 2005) and Asian country's food security depends on rice security. Every year a significant amount of rice yield loss occurs due to infection of bacterial blight disease. *Xoo* can cause bacterial blight disease in major rice sub-species, *O. sativa* subsp. *japonica* and *O. sativa* subsp. *indica*. *Xoo* enters rice leaf typically through the hydathodes of leaf margin, multiplies in the intercellular spaces of the underlying epithelial tissues and shifts to the xylem vessels to cause systemic bacterial blight infection (Niño-Liu et al., 2006). *Xoo* can be further classified into races based on the ability to infect different rice cultivars. To date, over 30 races of *Xoo* have been discovered all over the world (Mishra et al., 2013; Reddy, 1979; Tekete et al., 2020) and *Xoo* can cause up to 70% yield loss under favourable condition (Reddy, 1979). All growth stages of rice are susceptible to BB albeit the amount of yield loss depends on crop stage, degree of susceptibility and environmental conditions (Mew, 1993; Ou, 1984).

Deployment of varietal resistance is the most suitable environment-friendly and economic strategy for controlling rice diseases (Pinta et al., 2013). The study of *Xoo* races is essential to developing rice cultivars resistant to a wide range of *Xoo* races. Genetic mutation of the pathogens is responsible for breaks down the host resistance and causing the devastating outbreak of bacterial blight disease. A high degree of genetic variation of *Xoo* strains was reported from major rice-growing countries of Asia such as Bangladesh, China, India, Indonesia, Korea, Malaysia, Nepal, Sri Lanka, Japan and Philippines (Alam et al., 2016; Mishra et al., 2013; Nayak et al., 2008; Noer et al., 2018; Tekete et al., 2020). Pathogenic diversity of *Xoo* strains from Yunnan province of China revealed that the *Xoo* strains were polymorphic and virulence to 12 near-isogenic lines (NILs) (Niño-Liu et al., 2006). The assessment of genetic and pathogenic diversity of *Xoo* in India showed that *Xoo* isolates were compatible with all resistance genes except *xa5*, *Xa10*, *xa13* and *Xa21*, indicating that these genes are effective for the deployment of bacterial blight resistance (Reddy et al., 2009).

Thirty-two rice diseases are reported in Bangladesh (Haq et al., 2010). Among the major rice diseases, bacterial blight is one of the most destructive diseases of rice throughout the world (Mew, 1987, 1993) including Bangladesh (Khan et al., 2009). The disease appears every year with different degrees of severity in Bangladesh (Jalaluddin & Kashem, 1999), and it can cause around 5.8%–30.4% yield loss though it depends on the crop stages and environmental condition (Ansari et al., 2019). Based on the pathogenic variation on NILs of rice, several pathotypes of *Xoo* were identified from the major rice-growing areas of Bangladesh (Alam et al., 2016; Islam et al., 2016; Khan et al., 2009). Intriguingly, a comprehensive report on the field-based assessment of BB and the pathotypic variation of BB pathogen from different parts of Bangladesh and a new BB resistant gene *Xa23* has not been reported yet.

To date, at least 46 race-specific bacterial blight resistance (*R*) genes were identified from cultivated rice, wild rice and artificial mutants (Chen et al., 2020). However, the resistance provided by *R*-genes could break due to the emergence of new *Xoo* races and rapid changes in the pathogenicity of *Xoo* (Khan et al., 2014; Mew, 1987). To solve the problem of *Xoo* resistance breakdown, pathotypic variation as well as the selection of effective resistant genes and their combination for broad-spectrum resistance needs to be investigated first. Understanding of both pathogen population structures (races) as well as host resistance is the prerequisite in designing an effective strategy for deployment of resistance.

In consideration of the above facts, the present study has been undertaken to know the pathotypic variation, virulence patterns and distribution of *Xoo* in Bangladesh by using 13 near-isogenic lines and 14 pyramid lines. Moreover, a new *Xa23* resistant gene has been deployed for the first time to differentiate pathotypic variation among the collected isolates.

2 | MATERIALS AND METHODS

2.1 | Collection of bacterial blight diseased leaf samples

A total of 300 bacterial blight infected leaf samples were collected from eight major agro-ecological rice-growing regions, that is Gazipur, Cumilla, Rangpur, Habiganj, Barishal, Patuakhali, Satkhira and Rajshahi districts of Bangladesh. Infected leaf samples were collected from rice cultivars viz. BR3, BR11, BR22, BR23, BRRI dhan34, BRRI dhan46, BRRI dhan49, local cultivars like Swarna, Samba mashuri, Paizom and Kalizira. The infected samples were air-dried in paper envelopes and stored at 4°C until isolation of *Xoo*.

2.2 | Isolation, identification and purification of *Xoo* isolates

A total of 165 isolates of *Xoo* were successfully isolated on peptone sucrose agar (PSA) medium by following the method described by Islam et al., 2016. Isolated bacteria were purified and identified according to the description of Jalaluddin & Kashem, 1999 and stored temporarily in a 4°C refrigerator. During maintenance few isolates were contaminated and few were not revived and so out of 165 isolates, 118 isolates were successfully maintained for final inoculation.

2.3 | Confirmation of *Xoo* isolates by pathogenicity test

Three susceptible checks, BR11, IR24 and Purbachi were used against the preserved bacterial isolates for *Xoo* confirmation by pathogenicity test. Thirty-day-old seedlings were transplanted into an earthen pot in the net house for *Xoo* inoculation. The bacterial isolates were

cultured in a PSA medium and incubated 2–3 days at 28°C for proper growth of bacteria. The culture was resuspended in distilled water at an optical density of $OD_{600} = 1$ (equivalent to bacterial cell number 3.3×10^8 CFU/ml) measured by a spectrophotometer. Pathogenicity test was done by leaf clipping method of Kauffman et al., 1973. After 14 days of inoculation, isolate exhibiting more than 3 cm bacterial blight lesions were confirmed as virulent isolates. These virulent isolates were permanently preserved in NBY 40% glycerol liquid medium at -80°C and named as BxoN (Bangladeshi *Xanthomonas oryzae* and N indicates isolate number).

2.4 | Determination of pathotypic variation of *Xoo* isolates

2.4.1 | Plant materials and experimental location

Seeds of 13 NILs as well as 14 pyramid lines were obtained from International Rice Research Institute (IRRI), Philippines. The experiment was conducted at the experimental field of Plant Pathology Division, Bangladesh Rice Research Institute (BRRI), Gazipur,

Bangladesh during T. Aman, 2017 season. Thirty-day-old seedlings were transplanted in the field maintaining 20×20 cm spacing. A total of 13 NILs and 14 pyramid lines along with susceptible checks Purbachi, IR24 and BR11 were transplanted for virulence analysis of 118 isolates of *Xoo*.

2.4.2 | NILs and pyramid lines for the determination of pathotypes of *Xoo* isolates

To identify the reaction pattern of collected 118 *Xoo* isolates of Bangladesh (Figure 1), thirteen NILs, that is IRBB1, IRBB2, IRBB3, IRBB4, IRBB5, IRBB7, IRBB8, IRBB10, IRBB11, IRBB13, IRBB14, IRBB21 and IRBB23 having known single resistant gene were used in this study. Moreover, fourteen pyramid lines IRBB50, IRBB51, IRBB52, IRBB53, IRBB54, IRBB55, IRBB57, IRBB58, IRBB59, IRBB60, IRBB61, IRBB63, IRBB64, IRBB65 consisting of multiple resistant genes were also evaluated in this study. Three susceptible checks IR24, Purbachi and BR11 were maintained with these tested lines. Inoculation was done at the maximum tillering stage of each set of genotypes to differentiate the *Xoo* pathotypes based on the pathogenicity test.

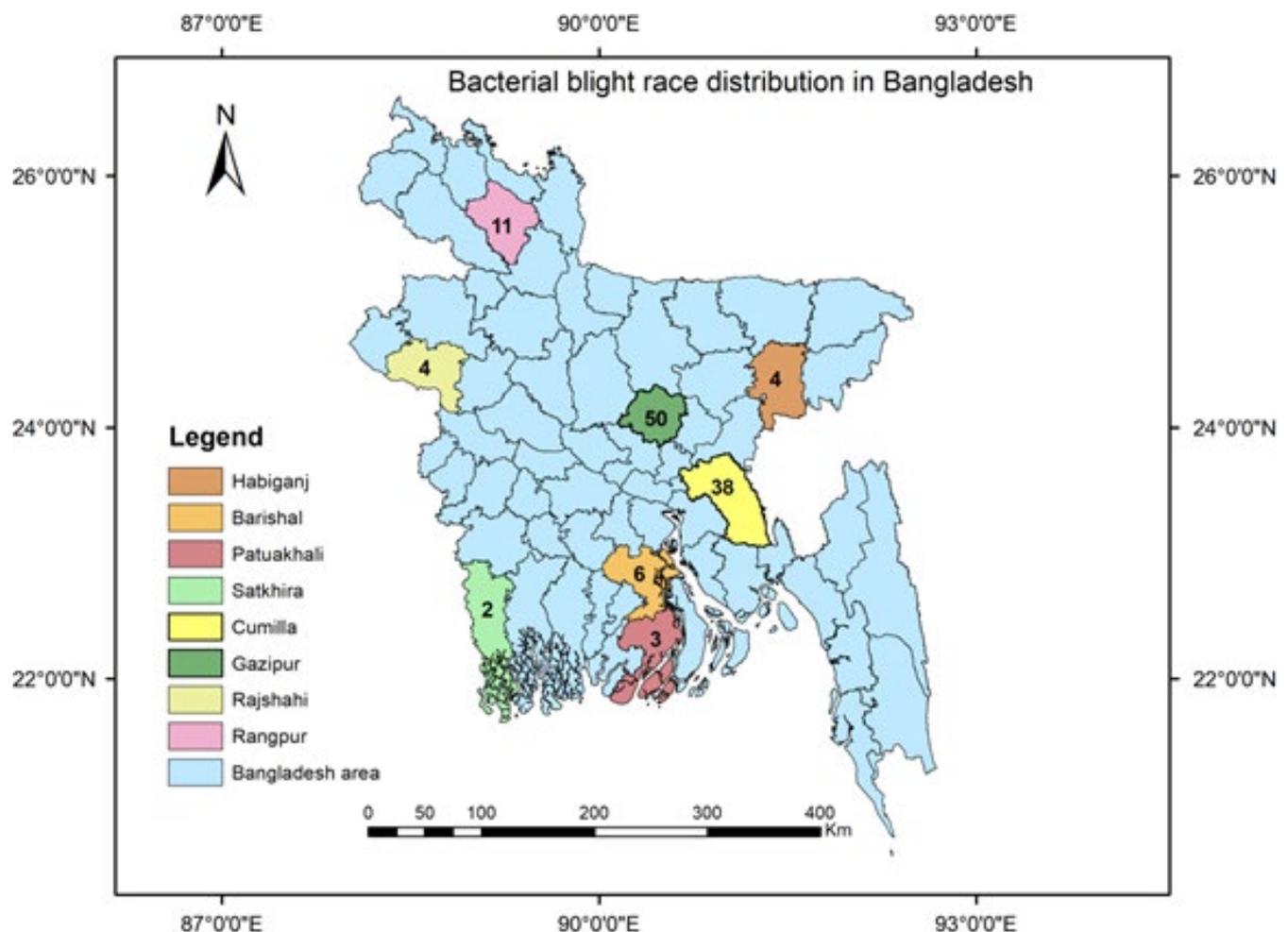


FIGURE 1 Location-wise (circles) BB isolates (number in the circles) collection areas of Bangladesh

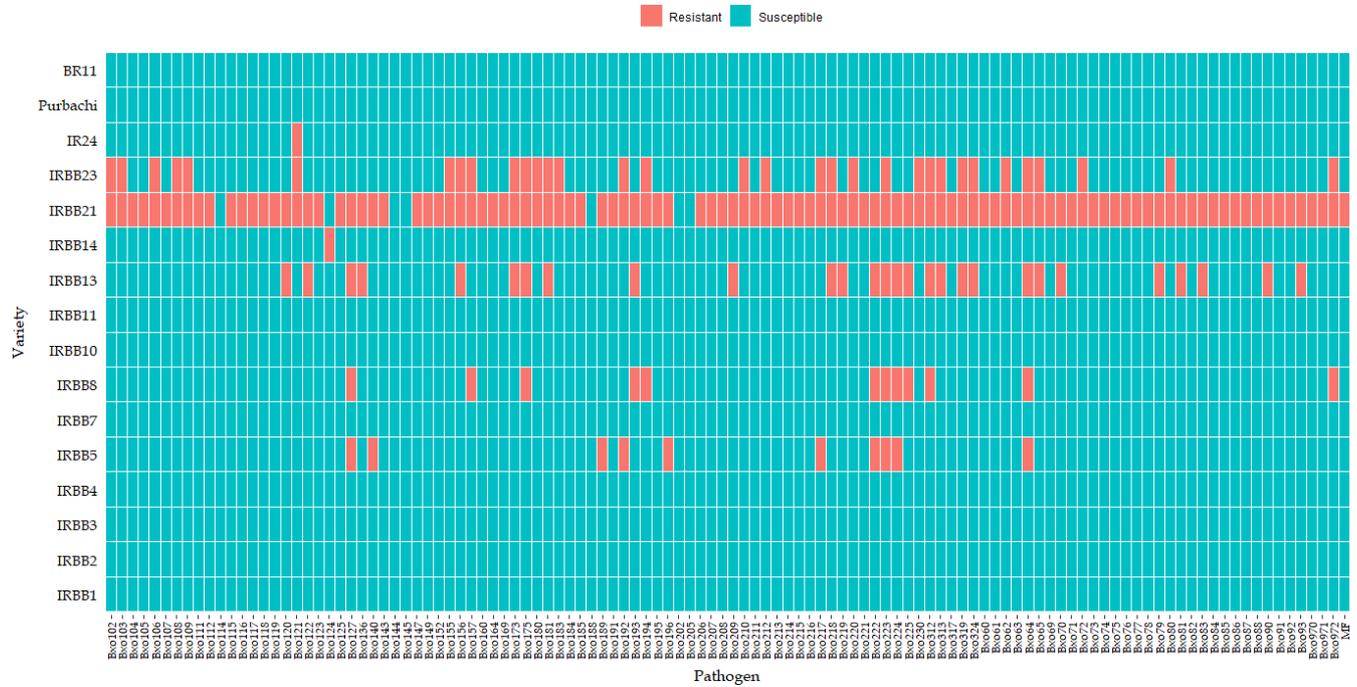


FIGURE 2 Reaction of BB isolates against 13 near-isogenic lines

2.4.3 | Inoculum preparation, inoculation and disease assessment

A total of 118 BB isolates were grown on Petri dish containing PSA medium for 48–72 hr at 28°C temperature. The inoculum of each isolate was prepared by mixing the bacterial culture with distilled water. The concentration of the inoculum was adjusted approximately to $OD_{600} = 1$ (3.3×10^8 CFU/ml). At the maximum tillering stage, 3 hills of each line (about 30 leaves) were cut by following the leaf clipping method for *Xoo* isolates inoculation. For disease scoring, lesion length data of 20 inoculated leaves were recorded 14 days after inoculation. Disease reactions were categorized based on lesion length, where <3 cm was considered as resistant (R) and >3 cm was rated as susceptible (S) (Li et al., 2009).

3 | RESULTS

3.1 | Determination of pathotypes of *Xoo* isolates using NILs and effective resistant genes

The studied 118 *Xoo* isolates produced typical bacterial blight disease symptoms on susceptible check varieties IR24, Purbachi and BR11. Based on reaction patterns of *Xoo* isolates on NILs, a total of 12 pathotypes/races were identified (Table 1 and Figure 2). However, Race 5 had a compatible reaction (susceptible reaction) to all 13 *Xa* resistant genes. Race 1 had an incompatible reaction (resistant reaction) to only the *Xa21* gene, while it had a compatible reaction to the other 12 genes. Race 2 showed an incompatible reaction to *Xa21* and *Xa23* genes, but it had a compatible reaction to other genes. Race 3 showed an incompatible reaction to *xa13* and

Xa21 genes. Race 4 had an incompatible reaction to *xa13*, *Xa21* and *Xa23* genes and a compatible reaction to other genes. Race 6 had an incompatible reaction to *xa8*, *xa13*, *Xa21* and *Xa23* genes and a compatible reaction to the rest of the genes. Race 7 had an incompatible reaction to *xa8*, *Xa21* and *Xa23* genes while it showed a compatible reaction to other genes. Race 8 had an incompatible reaction to *xa5*, *xa8*, *xa13* and *Xa21* genes and a compatible reaction to others. Race 9 had an incompatible reaction to *xa5* and *Xa21* genes and a compatible reaction to others. Race 10 had an incompatible reaction to *xa8*, *xa13* and *Xa21* genes and a compatible reaction to other genes. Race 11 showed an incompatible reaction to *xa5*, *xa8*, *xa13*, *Xa21* and *Xa23* genes and a compatible reaction to other genes. Race 12 had an incompatible reaction to *xa5*, *Xa21* and *Xa23* genes and a compatible reaction to other genes.

3.2 | Resistant frequency of *Xa* or *xa* genes in Bangladesh

The genes *Xa1*, *Xa2*, *xa3*, *Xa4*, *Xa7*, *Xa10*, *Xa11* and *Xa14* didn't show any resistant reactions against 118 *Xoo* isolates. Only the *Xa21* gene showed the highest resistant frequency (94.91%) to maximum isolates while the other genes such as *xa5*, *xa8*, *xa13* and *Xa23* were showed 8.47, 11.02, 25.42 and 28.81% resistant frequency, respectively (Table 2).

3.3 | Distribution of pathotypes/races of *Xoo*

The race-wise distributions of 118 isolates of different areas are shown in Table 3. Most of the isolates of Gazipur (29 isolates) were

TABLE 1 Pathogenic diversity of the 118 *Xoo* isolates based on the reaction against 13 NILs

Race/Pathotype	No. of isolates	Near-isogenic lines (NILs) and known resistance genes												
		IRBB1 (Xa1)	IRBB2 (Xa2)	IRBB3 (Xa3)	IRBB4 (Xa4)	IRBB5 (xa5)	IRBB7 (Xa7)	IRBB8 (xa8)	IRBB10 (Xa10)	IRBB11 (Xa11)	IRBB13 (xa13)	IRBB14 (Xa14)	IRBB21 (Xa21)	IRBB23 (Xa23)
1	57	S	S	S	S	S	S	S	S	S	S	S	S	S
2	17	S	S	S	S	S	S	S	S	S	S	S	R	R
3	13	S	S	S	S	S	S	S	S	S	R	S	R	S
4	7	S	S	S	S	S	S	S	S	S	R	S	R	R
5	6	S	S	S	S	S	S	S	S	S	S	S	S	S
6	3	S	S	S	S	S	S	R	S	S	R	S	R	R
7	3	S	S	S	S	S	S	R	S	S	S	S	R	R
8	3	S	S	S	S	S	R	R	S	R	S	S	R	S
9	3	S	S	S	S	S	R	S	S	S	S	S	R	S
10	2	S	S	S	S	S	S	R	S	R	S	S	R	S
11	2	S	S	S	S	S	S	R	S	R	S	S	R	R
12	2	S	S	S	S	S	R	S	S	S	S	S	R	R

under Race 1 followed by Cumilla (18 isolates). The isolates of Gazipur and Cumilla were highly diverged and comprised most of the *Xoo* races (Table 3). The major and widely distributed race was Race 1 (48%) followed by Race 2 (14%) and Race 3 (11%) and they were found as major races of bacterial blight disease of Bangladesh. Race 1 of BB pathogen was widely distributed in Gazipur (51%) and Cumilla (32%) compared to other rice-growing areas. Race 5 was obtained from the isolates of Cumilla and Patuakhali and was found as a virulent *Xoo* strain against all NILs (Table 1). Host-pathogen interaction revealed that most of the *Xoo* isolates (57) were under Race 1 and showed the second most virulent reaction after Race 5 (Table 1). Isolates of Cumilla were divided into 10 races based upon host-pathogen interaction and race variation was found higher in Cumilla compared to other regions (Table 3). Moreover, isolates of Gazipur were grouped into 8 races and positioned as the region of second highest race diversity.

3.4 | Efficacy of BB resistant genes against *Xoo* races

In view of the performance of individual *R*-gene against the *Xoo* races, *R*-genes, *xa13*, *Xa21* and *Xa23* were found effective conferring 50%, 91.66%, 50% resistance respectively compared to the other two genes, *xa5* (33.33%) and *xa8* (41.66%) (Figure 3).

3.5 | Reaction of *Xoo* isolates on resistant pyramid lines

The reaction of 14 pyramid lines viz. IRBB50, IRBB51, IRBB52, IRBB53, IRBB54, IRBB55, IRBB57, IRBB58, IRBB59, IRBB60, IRBB61,

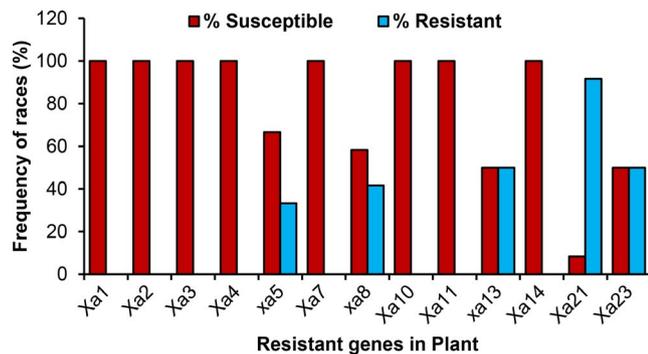
TABLE 2 Resistant genes in rice NILs and their resistance frequency to 118 *Xoo* isolates of Bangladesh

NILs	Resistant gene	Resistance frequency (%)
IRBB1	<i>Xa1</i>	0
IRBB2	<i>Xa2</i>	0
IRBB3	<i>Xa3</i>	0
IRBB4	<i>Xa4</i>	0
IRBB5	<i>xa5</i>	8.47
IRBB7	<i>Xa7</i>	0
IRBB8	<i>xa8</i>	11.02
IRBB10	<i>Xa10</i>	0
IRBB11	<i>Xa11</i>	0
IRBB13	<i>xa13</i>	25.42
IRBB14	<i>Xa14</i>	0
IRBB21	<i>Xa21</i>	94.91
IRBB23	<i>Xa23</i>	28.81

Abbreviation: NILs; Near-isogenic lines.

TABLE 3 Distribution of the major races of *Xoo* in different rice-growing districts of Bangladesh

Location	Race-wise isolates of <i>Xoo</i>												Location-wise isolate %
	1	2	3	4	5	6	7	8	9	10	11	12	
Gazipur	29	5	7	4		1	2				1	1	42
Cumilla	18	4	1	3	5	2	1		2	1		1	32
Rangpur	4	2	3					1	1				9
Habiganj	2	1	1										3
Barishal	3	3											5
Patuakhali	1	1			1								3
Satkhira		1	1										2
Rajshahi								2		1	1		3
% of total isolates	48	14	11	6	5	3	3	3	3	2	2	2	

**FIGURE 3** Efficacy of plant *Xa* genes against *Xoo* races. Note: Reaction showing lesion length <3 cm was considered as resistant (R) and >3 cm were considered as susceptible (S)

IRBB63, IRBB64, IRBB65 and 3 susceptible checks against 118 BB isolates were also evaluated to know the resistant frequency of multiple gene combinations (Figure 4). All the susceptible checks were exhibited a susceptible reaction against the tested isolates which confirmed that the isolates belonged to *Xoo*. The pyramid lines having the combination of *xa5* & *xa13* or *xa13* & *Xa21* genes exhibited the highest amount of resistant frequency (100%) and the pyramid lines containing *Xa4*, *xa13*, *Xa21* genes combination showed the second highest resistant frequency (99%) in the present study. The resistant frequency was the highest (97%–100%) when the gene, *xa5* or *xa13* was combined with the *Xa21* gene. Moreover, *Xa4* and *xa5* were influential genes of *xa13* and *Xa21* gene function, because the presence of *Xa4* and *xa5* genes influenced the resistant frequency of pyramid lines containing *xa13* or *Xa21* or both (Table 4).

4 | DISCUSSION

Variability of pathogen needs to be unveiled to find the effective resistant gene (s) and to develop race-specific resistant variety. A total of 12 pathotypes or races were identified based upon the reaction

pattern of *Xoo* isolates on IRBB lines. Virulence of bacterial blight isolates on near-isogenic lines can be determined by the presence or absence of substantial differential interactions between the host and pathogen (Nayak et al., 2008). A near-isogenic line showed vertical resistant reaction to the single isolate but the same isolate showed divergence interaction with different NILs and this finding corroborated with the study of Wang et al., 2005. The highest number of pathotypes were identified from Gazipur and Cumilla, which are the most BB prone areas of Bangladesh (Khan et al., 2009). Moreover, a higher number of isolates was used from these two regions which might be another cause of the highest variation of *Xoo* races. *Xoo* subsisted in different pathotypes with pathogenic diversity on rice cultivars carrying distinct resistance genes. Therefore, it is necessary to understand *Xoo* population diversity for the deployment of race-specific resistant genes in the variety development programme.

The variability of *Xoo* isolates has been reported from many countries including Bangladesh (Alam et al., 2016; Islam et al., 2016; Noer et al., 2018; Tekete et al., 2020). But no one yet uses the *Xa23* gene for the identification of pathotypic variations in *Xoo*. Identification of 12 *Xoo* pathotypes/races of Bangladesh by using *Xa23* and other resistant genes are unique from the previous studies.

Pathotype numbering was done based on the distribution of *Xoo* isolates, number of isolates and pathogenicity test on NILs and Pyramid lines. The result of the present study exhibited that isolates from different regions were clustered into the same pathotype and at the same time, different isolates from the same region were grouped into different pathotypes. This finding proved that the tested 118 *Xoo* isolates are highly dynamic and their population structures in a particular region differ from other regions. Variations in virulence profiles of *Xoo* isolates from the same areas have been reported previously (Alam et al., 2016). Moreover, based on a comparison of different agro-ecosystems and cultivars in the Philippines, Ardales et al., 1996 suggested that variation on the host did not affect the pathogen diversity.

In this study, 13 R-genes were tested and it was found that *Xa21* (94.91%) provided the highest broader resistance followed by *Xa23*

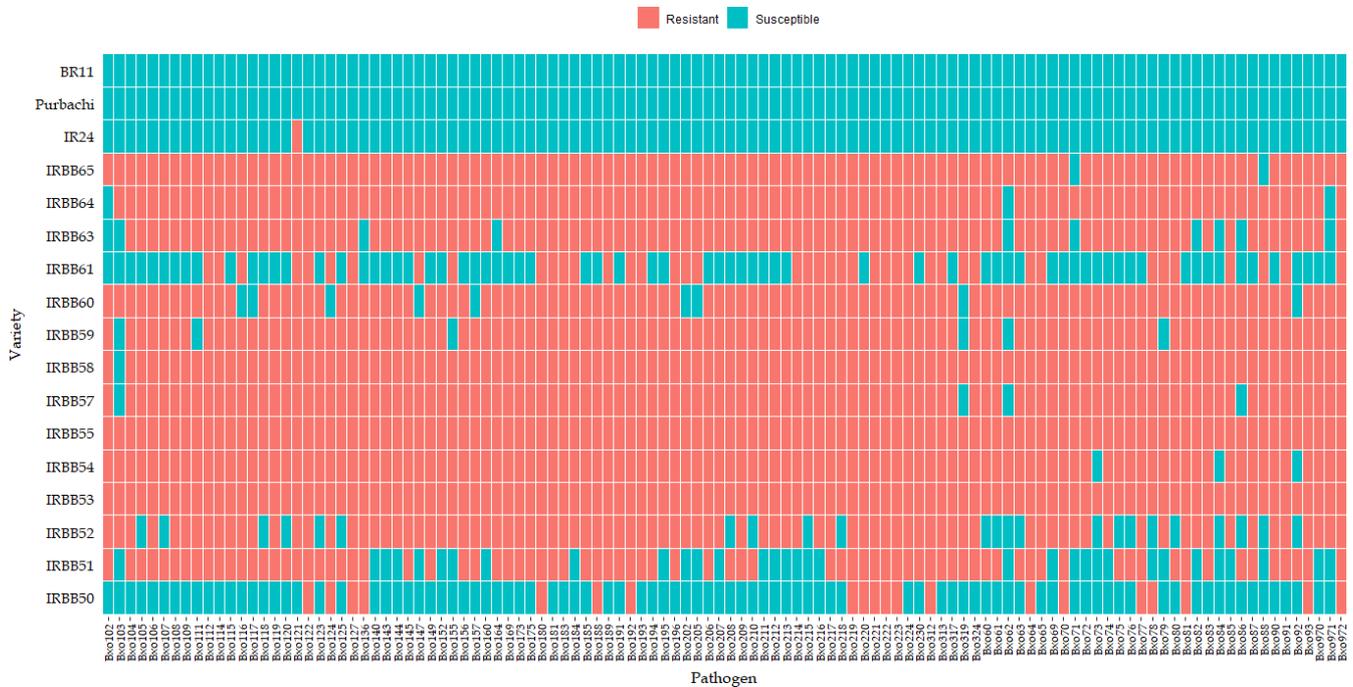


FIGURE 4 Reaction of BB isolates against 14 resistant pyramid lines

TABLE 4 Resistant genes in rice pyramid line and their resistance frequency to 118 *Xoo* isolates of Bangladesh

#	Pyramid line	Resistant gene	Resistance frequency (%)
1	IRBB50	<i>Xa4, xa5</i>	18
2	IRBB51	<i>Xa4, xa13</i>	71
3	IRBB52	<i>Xa4, Xa21</i>	81
4	IRBB53	<i>xa5, xa13</i>	100
5	IRBB54	<i>xa5, Xa21</i>	97
6	IRBB55	<i>xa13, Xa21</i>	100
7	IRBB57	<i>Xa4, xa5, Xa21</i>	97
8	IRBB58	<i>Xa4, xa13, Xa21</i>	99
9	IRBB59	<i>xa5, xa13, Xa21</i>	95
10	IRBB60	<i>Xa4, xa5, xa13, Xa21</i>	92
11	IRBB61	<i>Xa4, xa5, Xa7</i>	38
12	IRBB63	<i>xa5, Xa7, xa13</i>	92
13	IRBB64	<i>Xa4, xa5, Xa7, Xa21</i>	97
14	IRBB65	<i>Xa4, Xa7, xa13, Xa21</i>	98
15	IR24 (Sus. Ck.)	<i>Xa18</i>	0
16	Purbachi (Sus. Ck.)	-	0
17	BR11 (Sus. Ck.)	-	0

Abbreviations: Ck., check; Sus., susceptible.

(28.81%). Including Bangladesh, *Xa21* provides broad-spectrum resistance against the BB pathogen in different countries of the world (Khan et al., 2009). *Xa23* gene encoded executor protein to confer resistance against *Xoo* (Wang et al., 2015) and the present study

reported first to differentiate the *Xoo* isolates of Bangladesh by using the *Xa23* gene.

Deployment of host resistance is the most effective, simple, economical and environment-friendly sustainable approach to combat the bacterial blight disease (Gautam et al., 2015). To date, all resistant *Xa* genes that provide resistance to this disease have been listed together with their source and country of origin (Khan et al., 2014). In this study, *xa13*, *Xa21* and *Xa23* genes have been found as resistant against most of the bacterial isolates where *xa5* and *xa8* showed moderately resistant reactions against the studied isolates. Though the *Xa21* gene was resistant to most of the isolates of the present study, a good number of isolates from Bangladesh, Korea, Sri Lanka, Pakistan and Nepal were virulent to *Xa21* (Alam et al., 2016; Khan et al., 2012; Mazzola, 1994). Based on the virulence patterns of 96 *Xoo* isolates, Alam et al., 2016 found that NILs containing *R*-genes *xa5* performed better resistance (66.67%) followed by *Xa2* and *Xa21* (65.63%) in Bangladesh. Additionally, Yang et al., 2013 and Adhikari et al., 1995 found the same result where the *xa5* gene conferred resistance against most of the *Xoo* isolates. Though the *Xa21* gene is found as the most effective resistant gene, other *Xa* genes are also indispensable to enhance the *Xa21* gene function for achieving durable disease resistance against bacterial blight disease (Jeung et al., 2006). An experiment was conducted in Pakistan where 16 pyramid lines carrying 2–5 *R*-genes were used against 16 *Xoo* isolates and the results revealed that *xa13* and *Xa21* genes conferred the resistance to most of the isolates (Khan et al., 2014). However, our study suggests that the combination of *xa5* or *xa8* or *xa13* with *Xa21* and *Xa23* can be the most sustainable combination for bacterial blight resistant variety development in Bangladesh. On host plant resistance, a better understanding of the population and its genetic structure is essential for planning and implementing improved cultivation programmes. Nevertheless, pathotype studies based on

differential cultivars may not reveal the exact level of genetic variation within a set of pathogen population (Yashitola et al., 1997).

Effective *Xa* genes of this study can be used in a resistant breeding programme to develop bacterial blight resistant variety. Moreover, the findings of this study will be useful to Plant Pathologists or Plant Breeders for a better understanding of the characteristics of bacterial blight isolates in Bangladesh to develop BB resistant variety as well as to develop an efficient and sustainable management strategy to control bacterial blight disease.

5 | CONCLUSION

Pathogenicity analysis of 118 *Xoo* isolates of Bangladesh grouped into 12 types of pathotypes. A total of 48% of *Xoo* bacterial isolates belonged to pathotype/Race 1. From this study, *xa5*, *xa8*, *xa13*, *Xa21* and *Xa23* resistant genes and their combinations are found as effective against bacterial blight pathogen and these genes could be suitable to develop sustainable bacterial blight resistant rice variety in Bangladesh. Moreover, Gene pyramiding of effective resistant genes could be the best option to develop durable bacterial blight resistant varieties in Bangladesh.

ACKNOWLEDGEMENTS

The authors are grateful to BAS-USDA PALS BRRRI CR-03 project and Plant Pathology Division, BRRRI Gazipur for providing financial and research support, respectively. The authors would like to acknowledge International Rice Research Institute (IRRI) for providing the near-isogenic lines and pyramid lines for race identification.

CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/jph.13000>.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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REFERENCES

- Adhikari, T. B., Vera Cruz, C. M., Zhang, Q., Nelson, R. J., Skinner, D. Z., Mew, T. W., & Leach, J. E. (1995). Genetic diversity of *Xanthomonas oryzae* pv. *oryzae* in Asia. *Applied and Environmental Microbiology*, 61(3), 966–971.
- Alam, M. S., Islam, M. R., Hossain, I., Bhuiyan, M. R., & Khan, M. A. I. (2016). Pathotypic variation of *Xanthomonas oryzae* pv. *oryzae* in Bangladesh. *Archives of Phytopathology and Plant Protection*, 49(1–4), 31–42. <https://doi.org/10.1080/03235408.2016.1150633>
- Ansari, T. H., Ahmed, M., Akter, S., Mian, M. S., Latif, M. A., & Tomita, M. (2019). Estimation of rice yield loss using a simple linear regression model for bacterial blight disease. *Bangladesh Rice Journal*, 23(1), 73–79. <https://doi.org/10.3329/brj.v23i1.46083>
- Ardales, E. Y., Leung, H., Vera Cruz, C. M., Mew, T. W., Leach, J. E., & Nelson, R. J. (1996). Hierarchical analysis of spatial variation of the rice bacterial blight pathogen across diverse agroecosystems in the Philippines. *Phytopathology*, 86(3), 241.
- Chen, S., Wang, C., Yang, J., Chen, B., Wang, W., Su, J., Feng, A., Zeng, L., & Zhu, X. (2020). Identification of the novel bacterial blight resistance gene *Xa46(t)* by mapping and expression analysis of the rice mutant H120. *Scientific Reports*, 10(1), 12642.
- Gautam, R. K., Singh, P. K., Sakthivel, K., Srikumar, M., Kumar, N., Kumar, K., Singh, A. K., & Dam Roy, S. (2015). Analysis of pathogenic diversity of the rice bacterial blight pathogen (*Xanthomonas oryzae* pv. *oryzae*) in the Andaman islands and identification of effective resistance genes. *Journal of Phytopathology*, 163, 423–432.
- Haq, M., Mia, M. A. T., Rabbi, M. F., & Ali, M. A. (2010). Incidence and severity of rice diseases and insect pests in relation to climate change. In R. Lal, M. V. K. Sivakumar, M. A. Faiz, A. H. M. Mustafizur Rahman, & K. R. Islam (Eds.), *Climate change and food security in South Asia* (pp. 445–457). Springer Netherlands.
- Islam, M. R., Alam, M. S., Khan, A. I., Hossain, I., Adam, L. R., & Daayf, F. (2016). Analyses of genetic diversity of bacterial blight pathogen, *Xanthomonas oryzae* pv. *oryzae* using IS1112 in Bangladesh. *Comptes Rendus Biologies*, 339(9–10), 399–407.
- Jalaluddin, M., & Kashem, M. (1999). Pathogenic variability in *Xanthomonas oryzae* pv. *oryzae* in Bangladesh. *Indian Journal of Agricultural Sciences*, 69, 25–27.
- Jeung, J. U., Heu, S. G., Shin, M. S., Vera Cruz, C. M., & Jena, K. K. (2006). Dynamics of *Xanthomonas oryzae* pv. *oryzae* populations in Korea and their relationship to known bacterial blight resistance genes. *Phytopathology*, 96, 867–875.
- Kauffman, H. E., Reddy, A. P. K., Hsieh, S. P. Y., & Merca, S. D. (1973). An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae*. *Plant Disease*, 57, 537–541.
- Khan, J. A., Arshad, H. M. I., Saleem, K., Sandhu, A. F., Hasnain, S., & Babar, M. M. (2012). Evaluation of resistance genes in rice against local isolates of *Xanthomonas oryzae* pv. *oryzae* in Punjab province of Pakistan. *Archives of Phytopathology and Plant Protection*, 45, 1826–1839.
- Khan, M. A. I., Kabir, M. S., Monsur, M. A., Ali, M. A., & Mia, M. A. T. (2009). Pathogenic diversity of *Xanthomonas oryzae* pv. *oryzae* in Bangladesh. *Bangladesh Journal of Plant Pathology*, 25(2), 1–6.
- Khan, M. A., Naeem, M., & Iqbal, M. (2014). Breeding approaches for bacterial leaf blight resistance in rice (*Oryza sativa* L.), current status and future directions. *European Journal of Plant Pathology*, 139(1), 27–37.
- Khush, G. S. (2005). What it will take to feed 5.0 billion rice consumers in 2030. *Plant Molecular Biology*, 59(1), 1–6.
- Li, G., Song, C. F., Pang, X. M., Yang, Y., & Wang, J. S. (2009). Analysis of pathotypic and genotypic diversity of *Xanthomonas oryzae* pv. *oryzae* in China. *Journal of Phytopathology*, 157, 208–218.
- Mazzola, M. (1994). Analysis of the interaction between *Xanthomonas oryzae* pv. *oryzae* and the rice cultivars IR24 and IRBB21. *Phytopathology*, 84(4), 392. <https://doi.org/10.1094/Phyto-84-392>
- Mew, T. W. (1987). Current status and future prospects of research on bacterial blight of rice. *Annual Review of Phytopathology*, 25(1), 359–382.
- Mew, T. W. (1993). Focus on bacterial blight of rice. *Plant Disease*, 77(1), 5.

- Mishra, D., Vishnupriya, M. R., Anil, M. G., Konda, K., Raj, Y., & Sonti, R. V. (2013). Pathotype and genetic diversity amongst Indian isolates of *Xanthomonas oryzae* pv. *oryzae*. *PLoS One*, 8(11), e81996.
- Nayak, D., Bose, L. K., Reddy, P. R., & Nayak, P. (2008). Host-Pathogen interaction in rice-bacterial blight pathosystem. *Journal of Plant Protection Research*, 48, 371–384.
- Niño-Liu, D. O., Ronald, P. C., & Bogdanove, A. J. (2006). *Xanthomonas oryzae* pathovars: Model pathogens of a model crop. *Molecular Plant Pathology*, 7(5), 303–324.
- Noer, Z., Hasanuddin, L., & Suryanto, D. (2018). Pathotype profile of *Xanthomonas oryzae* pv. *oryzae* isolates from North Sumatera. In IOP conference series: Earth and environmental science.
- Ou, S. H. (1984). Exploring tropical rice diseases: A reminiscence. *Annual Review of Phytopathology*, 22(1), 1–11.
- Pinta, W., Toojinda, T., Thummabenjapone, P., & Sanitchon, J. (2013). Pyramiding of blast and bacterial leaf blight resistance genes into rice cultivar RD6 using marker assisted selection. *African Journal of Biotechnology*, 12(28), 4432–4438. <https://doi.org/10.5897/AJB12.2028>
- Reddy, J. L., Sirisha, C., & Sambasiva Rao, K. R. S. (2009). Assessment of genetic and pathogenic diversity of *Xanthomonas oryzae* pv. *oryzae* on high yielding local variety, Tella Hamsa, from farmer fields in Gagillapur and Kompally, Andhra Pradesh. Taiwan.
- Reddy, K. A. P. (1979). Relationship of bacterial leaf blight severity to grain yield of rice. *Phytopathology*, 69(9), 967.
- Tekete, C., Cunnac, S., Doucouré, H., Dembele, M., Keita, I., Sarra, S., Dagno, K., Koita, O., & Verdier, V. (2020). Characterization of new races of *Xanthomonas oryzae* pv. *oryzae* in Mali informs resistance gene deployment. *Phytopathology*, 110, 267–277.
- Wang, C., Su, C., Zhai, H., & Wan, J. (2005). Identification of QTLs underlying resistance to a virulent strain of *Xanthomonas oryzae* pv. *oryzae* in rice cultivar DV85. *Field Crops Research*, 91(2-3), 337–343.
- Wang, C., Zhang, X., Fan, Y., Gao, Y., Zhu, Q., Zheng, C., Qin, T., Li, Y., Che, J., Zhang, M., Yang, B., Liu, Y., & Zhao, K. (2015). XA23 Is an executor r protein and confers broad-spectrum disease resistance in rice. *Molecular Plant*, 8(2), 290–302.
- Yang, S.-Q., Liu, S.-Y., Zhao, S., Yu, Y.-H., Li, R.-B., Duan, C.-J., Tang, J.-L., & Feng, J.-X. (2013). Molecular and pathogenic characterization of new *Xanthomonas oryzae* pv. *oryzae* strains from the coastline region of Fangchenggang city in China. *World Journal of Microbiology and Biotechnology*, 29(4), 713–720.
- Yashitola, J., Krishnaveni, D., Reddy, A. P. K., & Sonti, R. V. (1997). Genetic diversity within the population of *Xanthomonas oryzae* pv. *oryzae* in India. *Phytopathology*, 87, 760–765.

How to cite this article: Rashid MM, Nihad SAI, Khan MAI, et al. Pathotype profiling, distribution and virulence analysis of *Xanthomonas oryzae* pv. *oryzae* causing bacterial blight disease of rice in Bangladesh. *J Phytopathol*. 2021;00:1–9. <https://doi.org/10.1111/jph.13000>