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Pathotypic diversity of *Xanthomonas oryzae* pv. *oryzae*, and stringent evaluation of resistance lines of Rice in Bangladesh

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Abstract *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) poses a major risk to worldwide rice cultivation due to its ability to cause bacterial blight (BB). Identifying the *Xoo* race patterns, and using resistant genes specific to a particular race is a promising strategy to develop varieties with durable resistance. In the present research, 300 *Xoo* isolates were confirmed and purified from 40 rice-producing areas of Bangladesh to determine the existing races/pathotypes of *Xoo*. The sensitive rice varieties IR24, BRRI dhan49, and Purbachi showed susceptible reactions against the tested isolates. Fourteen monogenic differentials and 18 pyramid lines were challenged against 300 isolates of *Xoo*. Bacterial blight resistance genes *Xa1*, *Xa2*, *Xa3*, *Xa4*, *xa5*, *Xa7*, *xa8*, *Xa10*, *Xa11*, *xa13*, *Xa14*, *Xa21*, *Xa23*, and *Xa27* were found in each monogenic differential. By analyzing patterns of the reaction of 300 *Xoo* isolates on monogenic differentials, 13 pathotypes/races were determined. The effectiveness of the host plant *R* genes *Xa4*, *xa5*, *Xa7*, *xa8*, *Xa10*, *xa13*, *Xa14*, *Xa21*, and *Xa27* against bacterial blight has been determined by analyzing frequency resistances and the responses of near isogenic and pyramid lines. Races 1, 3, and 6 were dominantly widespread across the country and were regarded as important races since they had the greatest number of isolates (25%, 23.33%, and 9.67% respectively). Race 2 was the most ubiquitous among the pathotypes, whereas Race 3 was the most virulent, having circumvented every evaluated resistance gene. The bacterial-blight resistant *R* genes *Xa21* and *Xa27* have shown resistance against eight and ten races out of thirteen different races, respectively (i.e., 54.7 and 44.3% of the isolates tested). In the evaluation of 50 pyramid lines against the 5 most virulent races, the combinations

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of *Xa4, Xa7, xa13,* and *Xa21* or the combinations of *Xa4, xa5, Xa7, xa13,* and *Xa21* genes were effective. At present, the suitable and effective *R* genes i.e., *xa5*, *Xa7*, *xa8*, *xa13*, *Xa21*, *Xa23,* and *Xa27* could be utilized for the development of a durable BB-resistant variety in Bangladesh.

Keywords Bacterial blight · *Xanthomonas oryzae* pv. *oryzae* · Pathotypic diversity · Monogenic differential · Pyramid lines · Races

Introduction

The pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is responsible for bacterial blight (BB), a significant leaf disease that severely affects rice. Rice acts as an essential dietary component for more than 50% of the worldwide population (Khush, 2005; Phillips et al., 2024), the food security of Asian nations is contingent upon rice production. A significant annual reduction of 10–20% even up to 80% in rice production is due to the infection induced by bacterial blight disease (Xia et al., 2012). Typically, *Xoo* penetrates the rice leaf by the margin's hydathodes and exists in the intercellular spaces of the surrounding epithelial tissues, and then migrates to the xylem arteries to create an infection caused by chronic bacterial blight (Niño-Liu et al., 2006). Distinguishing races of *Xoo* is possible according to their capacity to cause infection in various rice varieties. Currently, there are more than thirty recognized pathotypes of Xoo identified globally (Mishra et al., 2018; Reddy et al., 1979; Tekete et al., 2020). In favorable conditions, *Xoo* is capable of causing a maximum yield reduction of around 70% (Reddy et al., 1979). The degree of yield reduction depends on the stage of the crop, the level of sensitivity, and environmental conditions, even though BB impacts all stages of rice development (Mew, 1987; Ou, 1984).

The most economical and environmentally sustainable method of managing rice diseases is the use of resistant cultivars (Pinta et al., 2013). For the development of rice cultivars that are incompatible with a broad spectrum of *Xoo* races, experimenting with *Xoo* races is essential. The mutations of the pathogens are responsible for the breaking down of the host resistance resulting the severe emergence of bacterial blight disease. It has been observed that major rice-growing nations in Asia, including Bangladesh, India, Indonesia, China, Korea, Sri Lanka, Malaysia, Nepal, Japan, and the Philippines, exhibited a considerable degree of genetic variation in *Xoo* strains (Alam et al., 2016; Mishra et al., 2013; Nayak et al., 2008; Noer & Suryanto, 2018; Tekete et al., 2020). According to NIÑO-LIU et al. (2006), the pathogenic diversity of *Xoo* strains, which originated in the Chinese province of Yunnan, revealed that the strains are virulent and polymorphic along 12 Monogenic differentials. An evaluation of molecular and pathogenic variability of *Xoo* in India demonstrated that all isolates could overcome resistance genes except for *xa5, Xa10, xa13,* and *Xa21*. These four resistance genes were still effective in controlling the disease, suggesting their potential for developing durable bacterial blight resistant varieties (Rashid et al., 2021; L. J. Reddy et al., 2009).

In Bangladesh, 32 rice diseases have been documented (Haq et al., 2011; Khatun et al., 2021). Among those, BB is one of the most devastating diseases affecting rice production all over the world (Mew, 1987; Mew et al., 1993), including Bangladesh (Khan et al., 2009). Every year, the disease reappears in Bangladesh varying in severity (Jalaluddin & Kashem, 2013) and causes yield loss ranging from 5.8 to 30.4% may take place depending on the growth phases of crops and environmental factors (Ansari et al., 2019). The different pathotypes of *Xoo* that were found in Bangladesh's main rice-growing areas and identified through investigating how those affected the Monogenic differentials of rice (Alam et al., 2016; Islam et al., 2016; Khan et al., 2009; Rashid et al., 2021). So far, a comprehensive study on analyzing the field evaluation of the *Xa27* gene against different isolates of *Xoo*, has not yet been reported for the assessing of its resistancy and pathotypic variation in Bangladesh.

At present, a total of 48 resistant *R* genes for BB have been identified on various chromosomes, and several of these genes have been characterized and identified in native wild rice. (Chen et al., 2020; Gu et al., 2004a; Kumar et al., 2012; Sinha et al., 2023; W.-Y. Song et al., 1995; Tan et al., 2004; Zhang et al., 2001) However, rapid alterations in *Xoo*'s pathogenicity and the emergence of novel *Xoo* races may decline the resistance acquired by *R* genes (Khan et al., 2014; Mew, 1987). Before trying to fix the issue of *Xoo* resistance breaking down,

more researches need to be done on pathotypic variation, along with the effective resistant genes selection, and their different combinations to introduce broad-spectrum of resistance. It is critical to develop a thorough understanding of both the resistance mechanisms of hosts and the racial makeup of pathogen populations to develop an effective strategy for implementing resistance genotypes.

The monogenic differential having the *Xa27* resistance gene was introduced for the first time in Bangladesh to identify the BB pathotypes. Present studies were undertaken to determine the pathotypic variation of *Xoo* in Bangladesh using 14 monogenic and 18 pyramid lines. The current investigation was also aimed to identify effective pyramid lines with different combinations of BB resistance genes for developing durable resistant rice cultivars in Bangladesh.

Materials and Methods

Bacterial blight-affected leaf collection

A total of 920 BB-infected leaf samples were collected from 40 widely rice-growing locations in Bangladesh (Fig. 1). Infected leaf samples were obtained from the following rice cultivars: BR3, BR11, BR22, BR23, BRRI dhan34, BRRI dhan46, and BRRI dhan49, Samba mashuri, Swarna, Kalizira, Paizom, and hybrid rice. Prior to *Xoo* isolation, the BB infected leaf samples were stored in air-dried paper envelopes at 4 °C.

Identification, purification, and isolation of *Xoo* isolates

A successful isolation of 350 *Xoo* isolates was obtained on peptone sucrose agar (PSA) medium by the methodology outlined by Rashid et al. (2021). Purified and identified by the methodology outlined by Jalaluddin and Kashem (2013), the isolated bacteria were thereafter briefly stored in a refrigerator set at 4 °C. The steps are shown in Fig. 2. During culture, few isolates were contaminated, or even fewer were not revived. Hence, for the final inoculation, 300 isolates out of 350 were effectively preserved in cryovials at −80 °C for further use.

Xoo isolates are confirmed via pathogenicity testing

The maintained bacterial isolates were subjected to three susceptible checks: IR24, BRRI dhan49, and Purbachi for *Xoo* confirmation by pathogenicity test. For *Xoo* inoculation, thirty-day-old seedlings were transferred from a net house to an earthen pot. PSA medium was used to culture the bacterial isolates to ensure optimal bacterial growth for two to three days at a temperature of 28 °C. A spectrophotometer was used to determine that the resuspended culture in distilled water had an optical density of OD600 = 1, which corresponds to a bacterial cell number of 3.3×10^8 CFU/ml method was followed to determine pathogenicity, as described by (Kauffman et al., 1973). Virulent isolates were identified 14 days after inoculation if they displayed bacterial blight lesions greater than 3 cm in diameter. The virulent isolates were kept at -80 °C in an NBY liquid medium containing 40% glycerol (Rashid et al., 2021). They were designated as BXoN (Bangladeshi *Xanthomonas oryzae*, where N denotes the isolate number).

Pathotypic variation of *Xoo* isolates determination

Materials for the plants and experimental site

The races were determined by analyzing the disease response of several *Xoo* isolates to monogenic differentials under artificial inoculation conditions. This research used a set of fourteen monogenic differentials i.e., IRBB1, IRBB2, IRBB3, IRBB4, IRBB5, IRBB7, IRBB8, IRBB10, IRBB11, IRBB13, IRBB14, IRBB21, IRBB23 and IRBB27; and 18 BB resistant pyramid lines IRBB50, IRBB51, IRBB52, IRBB53, IRBB54, IRBB55, IRBB56, IRBB57, IRBB58, IRBB59, IRBB60, IRBB61, IRBB62, IRBB63, IRBB64, IRBB65, IRBB66 and IRBB67 that were collected from the International Rice Research Institute (IRRI), Philippines. The identification of the race or races were accomplished by utilizing disease reactions to differential variations following the gene theory. At 14 days after inoculation, based on the cut leaf tip, the percentage of the 20 damaged leaves areas was measured. The percentage of the leaf regions impacted by the disease has been used to determine disease reactions. The classification of disease reactions was based on lesion length; lesions less than 3 cm were considered resistant (R),

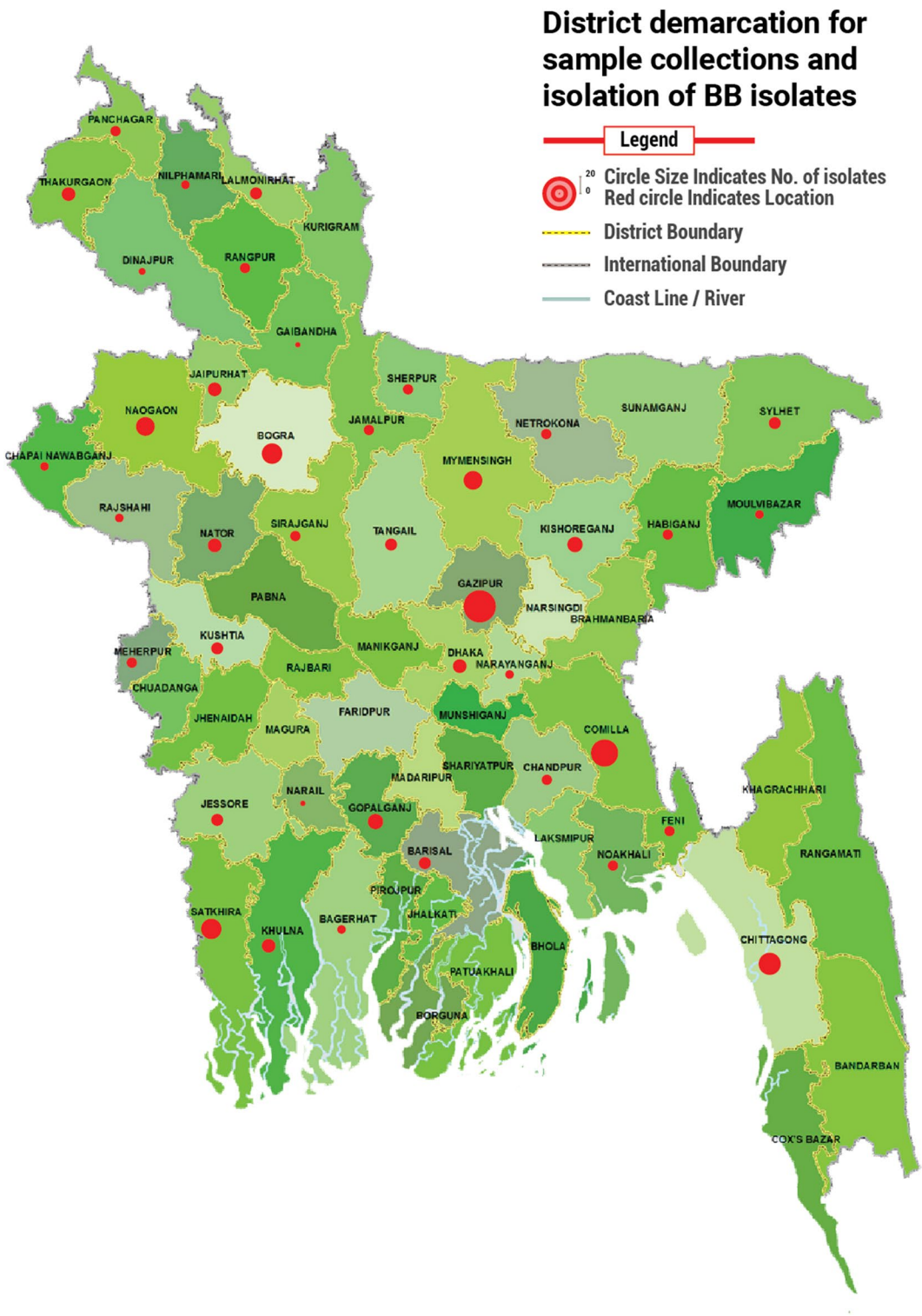

Fig. 1 District demarcation for sample collections and isolation of BB isolates

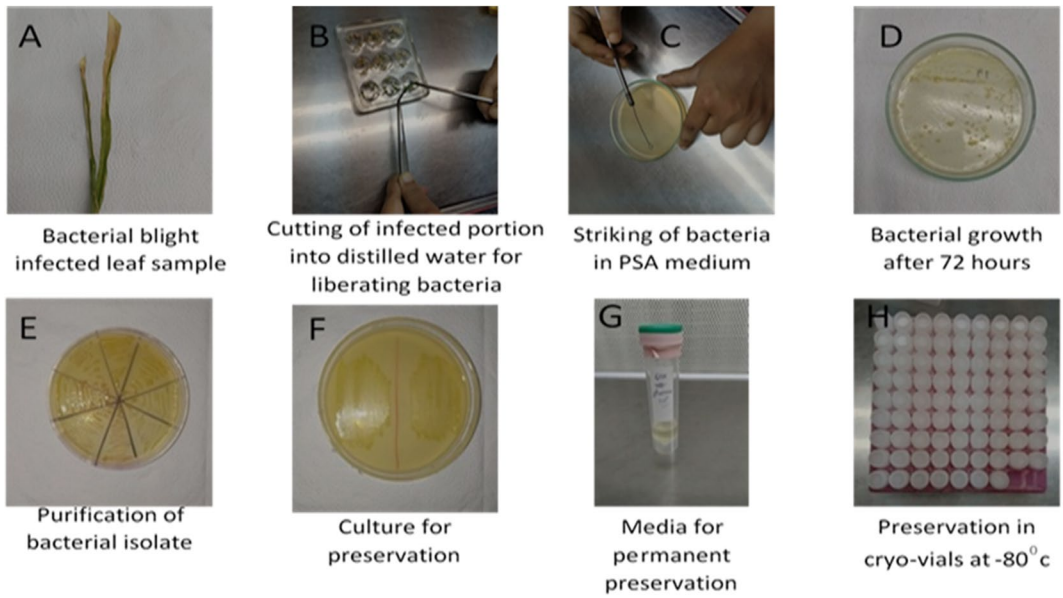

Fig. 2 The process of isolation, purification and preservation of bacterial blight isolates (**A**-**H**)

whereas lesions more than 3 cm were considered sensitive (S) (Li et al., 2009).

The use of pyramid lines and monogenic differentials to ascertain the pathotypes of Xoo isolates

In this investigation, fourteen monogenic differentials with known single resistance genes, i.e., IRBB1, IRBB2, IRBB3, IRBB4, IRBB5, IRBB7, IRBB8, IRBB10, IRBB11, IRBB13, IRBB14, IRBB21, IRBB23, and IRBB27 were utilized to determine the reaction pattern of 300 *Xoo* isolates collected from Bangladesh, while 18 pyramid lines containing multiple resistance genes were also assessed against same *Xoo* isolates. To differentiate among the *Xoo* pathotypes, pathogenicity assessment was done by inoculating on each group of genotypes during the maximum tillering stage.

Stringent evaluation of advanced materials against Xoo isolates

A total of 50 experimental materials, that were collected from the International Rice Research Institute (IRRI), Philippines, along with 2 resistant checks (IRBB60 and IRBB65) and 3 susceptible checks (BRRI dhan49, IR24, and purbachi) were used in both T. Aman and T. Aus season to assess the resistance against bacterial blight pathogen. The research was conducted in the Bangladesh Rice Research Institute's (BRRI) experimental field, Plant Pathology Division, located in Gazipur, Bangladesh. Thirty-day-old seedlings were transplanted in the field maintaining 20 cm × 20 cm spacing. The experiment was conducted under field conditions by artificial inoculation. Over 48–72 hours at a temperature of 28 °C, a Petri dish containing PSA medium was utilized to cultivate 300 BB isolates. To prepare the inoculum for each isolate, the bacterial culture was diluted with distilled water. An adjustment was made to the inoculum concentration to roughly OD600 = 1 (3.3×10^8 CFU/ml). Plants were inoculated with 5 virulent isolates (BXo6, BXo13, BXo9, BXo22, BXo31) of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) during the Aus and T. Aman seasons at the highest tillering period by using the leaf clipping technique (Kauffman et al., 1973). Lesion length data from about 20 infected leaves were collected for disease grading fourteen days after inoculation. Click or tap here to enter text. Categorization of the entries was done by measuring the lesion length on the inoculated leaves as Highly Resistant (HR) <1 cm (Score 0), Resistant (R) 1–3 cm (Score 1), Moderately Resistant (MR) 3–5 cm (Score 3), Moderately Susceptible (MS) 5–10 cm (Score 5),

Susceptible (S) 10–15 cm (Score 7), and Highly Susceptible (HS) >15 cm (Score 9) (Kim et al., 2015; Lore et al., 2011; Neelam et al., 2020). A scale was used to measure the length of the lesion, including the entire leaf-infected area of 20 leaves of each monogenic line (Table S1 and S2).

Results

Pathotype determination of *Xoo* isolates through the utilization of monogenic differentials and effective resistance genes

The 300 *Xoo* isolates exhibited characteristic symptoms of bacterial blight disease on 14 monogenic lines. Based on the response patterns exhibited by the *Xoo* isolates to Monogenic differentials, thirteen pathotypes/races were determined (Table 1). The most virulent race was 1 followed by 2, 6, and 12, while the least virulent race was 8 followed by 13, 9, 4, 7, etc. There were no resistance reactions observed in the genes *Xa1, Xa2, xa3*, and *Xa11* against 300 *Xoo* isolates. In Table 1, *Xa27, Xa23, Xa21, xa13*, *Xa7*, *Xa8*, and *xa5* genes were effective against BB races in Bangladesh. Here, the *Xa21* gene exhibited the maximum frequency of resistance, i.e. the most effective (54.7%), to the greatest number of isolates, whereas the resistant frequencies of the remaining genes *xa27, Xa13, xa7, Xa23, Xa8, xa13, and Xa5 were as follows: 44.3, 29.0, 15.00, 6.00, 5.33, and 5.0%,* respectively (Table 1 and Fig. 3).

Isolates frequency and their location-wise distribution

The location-wise race distributions of 300 isolates originating from various regions in Bangladesh. The location-wise race distributions of 300 isolates show significant variation among the rice-growing regions (Table 3). Race 1(23.3%) and 2(7.33%) from Gazipur were found as the highest no of isolates, 8 and 4, respectively. This location also showed the maximum frequency of isolate distribution (6.33%). Race 3 (25%) was the predominant and most widely dispersed race, followed by Race 1 (23.33%), Race 6 (9.67%), and Race 7 (8.67%) also being identified as major races of the bacterial blight diseases in Bangladesh (Figs. 4 and 5).

The effect of *Xoo* isolates on resistant pyramid lines

In addition, the resistance and susceptibility of 18 pyramid lines to 300 tested BB isolates were evaluated to determine the prevalence of resistance for various gene combinations (Table 2). All sensitive checks demonstrated a compatible reaction to the tested isolates, confirming that *Xoo* isolates exhibited the virulence strain. In the current investigation, pyramid lines containing the combinations of *Xa4, Xa7, xa13,* and *Xa21* or the combinations of *Xa4, xa5, Xa7, xa13,* and *Xa21* genes displayed the greatest frequency of resistance (60%). Furthermore, pyramid line IRBB64 containing *Xa4*, *Xa7, xa13*, and *Xa21* genes exhibited the second-highest frequency of resistance (59%), while IRBB60 (*Xa4*, *xa5, xa13*, and *Xa21*) and IRBB63 (*xa5, Xa7*, and *xa13*) genes exhibited 52% frequency of resistance. The frequency of resistance was greatest (52–60%) when the *Xa21* gene was combined with the *xa5, Xa7* or *xa13* gene (Table 2 and Fig. 4).

The effect of *Xoo* isolates on advanced lines

Out of 50 advanced lines, these 3 lines IR 129336:11–37 (*Xa4-xa5-xa13*(H)-*Xa21*(H)-*Xa23*), IR 127164:11–26 (Xa4-xa5(H)-Xa7(H)-xa13-Xa21(H)) and IR 129337:37–79 (Xa4-xa5-xa13(H)--Xa23) showed highly resistant to the 5 most virulent races, while 31 lines showed resistant reaction for both T. Aman and T. Aus seasons. Moreover, 14 lines showed moderately resistant reaction. In contrast, 3 lines showed the moderately susceptible reaction and 2 lines showed the susceptible reaction, while Swarna-Sub1 was highly susceptible (Table S1 and S2).

Discussion

Almost every rice-producing location on Earth has extensive documentation of the variations of virulence in *Xoo* isolates (Kaur et al., 2023; Song et al., 2023). The variability of the virulence of *Xoo* in Bangladesh is also the subject of considerable research. Thirteen pathotypes or races were identified from 300 *Xoo* isolates using 14 monogenic lines by their reaction patterns. Based on the reaction pattern of monogenic lines against BB isolates, previous studies also identified different pathotypes of *Xoo*

Table 1 Pathogenic diversity and resistance frequency of 300 *Xoo* isolates based on 14 Monogenic differentials reactions

Races	No. of isolates	% of isolates	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)*	IRBB27 *(Xa27)*
1	71	23.67	S	S	S	S	S	S	S	S	S	S	S	S	S	S
2	78	1.33	S	S	S	S	S	S	S	S	S	S	S	R	S	S
3	13	24.67	S	S	S	S	S	S	S	S	S	S	S	R	S	R
4	21	4.33	S	S	S	S	S	S	S	S	S	R	S	R	S	R
5	29	7.00	S	S	S	S	S	S	S	S	S	R	S	S	S	R
6	18	9.67	S	S	S	S	S	S	S	S	S	R	S	S	S	S
7	26	6.00	S	S	S	S	S	R	S	S	S	S	S	R	S	R
8	5	8.67	S	S	S	R	R	R	R	R	S	R	R	R	R	R
9	10	1.67	S	S	S	S	R	R	S	S	S	R	S	R	S	R
10	7	3.33	S	S	S	S	S	S	R	S	S	S	S	R	S	R
11	13	2.33	S	S	S	S	S	S	S	S	S	S	S	S	R	R
12	5	4.33	S	S	S	S	S	S	S	R	S	S	S	S	S	R
13	4	1.67	S	S	S	S	S	R	R	S	S	R	S	R	S	R
Total	**300**	**Resistance freq. (%)**	**1.33**	**0**	**0**	**1.67**	**5**	**15**	**5.33**	**3.33**	**0**	**29**	**1.67**	**54.7**	**6**	**44.3**

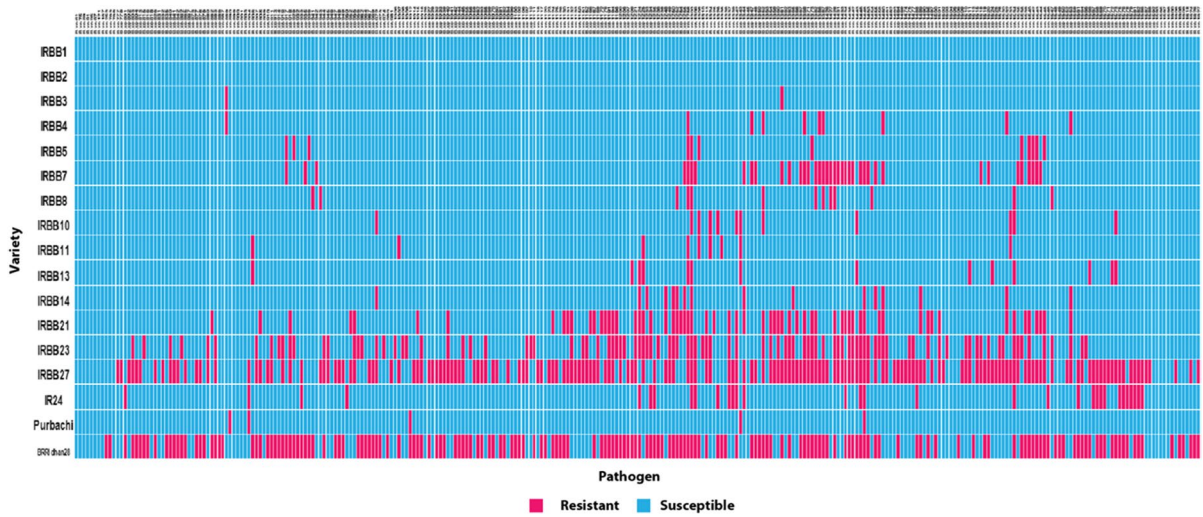

Fig. 3 Reaction of BB isolates against 14 monogenic lines

Fig. 4 Reaction of BB isolates against 18 resistant pyramid lines. Note: Reaction showing lesion length < 3 cm was considered as resistant (R) and > 3 cm were considered as susceptible (S)

in Bangladesh, India, Pakistan, and China (Gautam et al., 2015; Kaur et al., 2023; Rashid et al., 2021; Song et al., 2023). A monogenic line exhibited vertical resistance to a single isolate, while the same isolate exhibited diverse interactions with different Monogenic differentials; our finding corroborated with the previous studies as well (Rashid et al., 2021; C. Wang et al., 2005).

The distribution of significant pathotypic diversity was observed in 300 isolates collected from 40 different locations of Bangladesh. Gazipur and Cumilla had been noted to have the highest number of pathotypes, making those the most vulnerable locations to bacterial blight (Khan et al., 2009; Rashid et al., 2021). Additionally, the utilization of a greater number of isolates from these specific regions might contribute to maximum variation of *Xoo* races. *Xoo*, consisting of various pathotypes, exhibits diverse pathogenic characteristics in rice cultivars that possess varied resistance genes. Thus, it is imperative

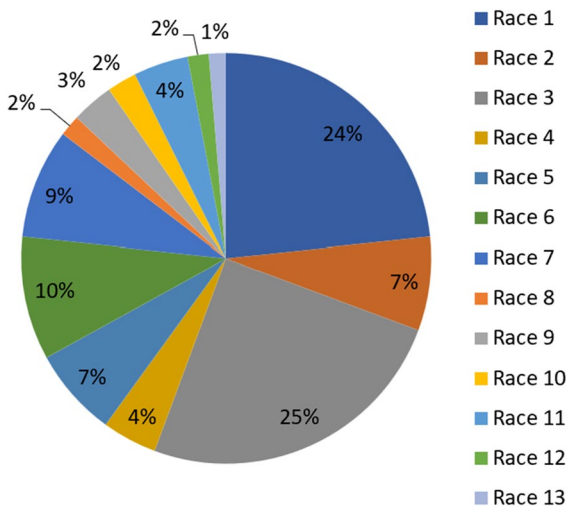

Fig. 5 *Xoo* isolates frequency of obtained races among the total isolates tested

to comprehend the diversity of the *Xoo* population in order to effectively incorporate race-specific resistance genes in the program of variety development.

In our study, the most effective *R*-genes was *Xa21* (54.7%), followed by *Xa27* (44.3%), *xa13* (29%), *Xa7*(15%), *Xa23* (6%), and *xa5*(5%). Our study corroborated with the findings of previous studies, such as Khan et al. (2009) reported that the most commonly employed resistance genes for rice breeding to enhance resistance to bacterial blight is *Xa21* across the world including Bangladesh. In addition, another study had also recorded a significant resistance of *Xa23* to bacterial blight, which encodes an executor R protein to confer broad-spectrum of resistance to bacterial blight (Rashid et al., 2021; C. Wang et al., 2015). *Xa27*'s ectopic expression evaded the prerequisite for *AvrXa27* and provided resistance to suitable strains, revealing that resistance is a result of *Xa27* expression at the post-transcriptional level, with specificity being determined by the *Xa27* promoter (Gu et al., 2005). Although the resistance spectrum displayed by *Xa27* significantly overlapped with that

Table 2 Resistance genes in pyramid lines and their frequency to Bangladeshi *Xoo* isolates

#	Pyramid line	Resistant gene	Resistance frequency (%)
1	IRBB50	*Xa4*, *xa5*	17
2	IRBB51	*Xa4*, *xa13*	26
3	IRBB52	*Xa4, Xa21*	40
4	IRBB53	*xa5, xa13*	28
5	IRBB54	*xa5, Xa21*	48
6	IRBB55	*xa13, Xa21*	49
7	IRBB56	*Xa4, xa5, xa13*	48
8	IRBB57	*Xa4, xa5, Xa21*	41
9	IRBB58	*Xa4, xa13, Xa21*	41
10	IRBB59	*xa5, xa13, Xa21*	43
11	IRBB60	*Xa4, xa5, xa13, Xa21*	52
12	IRBB61	*Xa4, xa5, Xa7*	35
13	IRBB62	*Xa4, Xa7, Xa21*	40
14	IRBB63	*xa5, Xa7, xa13*	52
15	IRBB64	*Xa4, xa5, Xa7, Xa21*	59
16	IRBB65	*Xa4, Xa7, xa13, Xa21*	60
17	IRBB66	*Xa4, xa5, Xa7, xa13, Xa21*	60
18	IRBB67	*Xa4, Xa7*	10
15	IR24 (Sus. Ck.)	*Xa18*	0
16	Purbachi (Sus. Ck.)	–	0
17	BRRI dhan49 (Sus. Ck.)	=	0

Sus., susceptible, Ck., check

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

Location	Race-wise Number of isolates (*Xoo*)													% of isolate Location-wise
	1	2	3	4	5	6	7	8	9	10	11	12	13	
Bagerhat			2		1	2								1.67
Barisal	1		2			3					1			2.33
Bogura	3	1	3			1	1	2	1					4.00
Chandpur				1			1	1	1	1		1		2.00
Chapai Nawabganj	1		1		1							2		1.67
Chattagram		1	4	3	2	1		1			1			4.33
Comilla	4	1	2			3	4			1	1			5.33
Dhaka			1	1		3	2				1			2.67
Dinazpur	2		2											1.33
Feni			3		3									2.00
Gaibandha	1		1			1								1.00
Gazipur	8	4	2				1		2				2	6.33
Gopalganj		1	1				4			1	2			3.00
Habiganj	1	2	2	1										2.00
Jaipurhat	3		2	1		1					1			2.67
Jamalpur	1		3			1	1							2.00
Jessore		1			1		1					2	2	2.33
Khulna	1	1	1		1		3		1					2.67
Kishoreganj	2	1	3	1	1						1			3.00
Kustia	2	1	1			1				2				2.33
Lalmonirhat	2		4			1								2.33
Meherpur	1	1	3			1								2.00
Moulavibazar	1		2	2										1.67
Mymensingh	4	1	2		2	1				1				3.67
Naogoan	6	1			1	2			1					3.67
Narail	1		1			1								1.00
Narayanganj	1						3				1			1.67
Natore	4		1						3					2.67
Netrokona			1		3						2			2.00
Nilphamari	3		2											1.67
Noakhali	2		4											2.00
Panchagar	1		4			1								2.00
Rajshahi	2	1				1					1			1.67
Rangpur			4		1	1								2.00
Shatkhira	3	1	1		1		3	1	1	1				4.00
Sherpur	1		1			1	2				1			2.00
Shylet	2	1	1	1	2									2.33
Sirajganj	1	1	2	1	1									2.00
Tangail	2	1	3	1										2.33
Thakurgaon	3		3			2								2.67
% of isolate Race-wise	23.33	7.33	25.00	4.33	7.00	9.67	8.67	1.67	3.33	2.33	4.33	1.67	1.33	

of *Xa21*, the IRBB27 cultivars' BB lesion length was less than the IRBB21's ones, indicating that *Xa27* provided greater resistance to the *Xoo* strains when evaluated compared to *Xa21*. (Gu et al., 2004b). So far, *Xa27* has not been reported yet in Bangladesh. The present study reported first to differentiate the Bangladeshi *Xoo* isolates (BXoN) by utilizing the *Xa27* gene. From our findings, it is Comprehensible that the effective single R genes, *Xa21, Xa27, xa13, Xa7, Xa23,* and *xa5,* in different combination could have great possibilities to develop a durable BB-resistant variety in Bangladesh.

Pathogenicity testing on monogenic differentials and pyramid lines, as well as the distribution of *Xoo* isolates and the number of isolates, were utilized to determine the extent of resistance among genotypes. The results of this study indicate that isolates from the same region had separate pathotypes, but isolates from other locations were found to have identical pathotypes. This discovery provided evidence that the 300 *Xoo* isolates that were e*xa*mined are exceptionally dynamic, with distinct population structures observed in different localities. For instance, our previous study revealed the 10 and 08 races out of 12 from Comilla and Gazipur respectively (Rashid et al., 2021), whereas our present investigation found the 07 and 06 out of 13 races from the same locations. Region specific resistant variety could be developed with the conformity of result. Alam et al. (2016) documented variations in the virulence characteristics of *Xoo* isolates originating from the same regions. In addition, Ardales et al. (1996) concluded, an examination of various agro-ecosystems and cultivars in the Philippines revealed that the degree of variation among the hosts had a little impact on the diversity of pathogens.

Implementing host resistance is the most sustainable, environment friendly, and cost-effective method of combating the bacterial blight disease (Gautam et al., 2015). Additionally, every resistant *Xa* genes associated with bacterial blight disease has already been catalogued along with its country of origin and source (Khan et al., 2014). In this experiment, the *Xa7*, *xa13*, *Xa21*, and *Xa27* genes were shown to be resistant to the majority of the bacterial isolates, while the *xa5*, *xa8* and Xa23 genes exhibited only modest resistance to the isolates. Although, the majority of the isolates of our study exhibited resistance to the *Xa21* gene, which corroborates other findings, while a considerable proportion of those from Bangladesh, Korea, Sri Lanka, Pakistan, and Nepal demonstrated virulence towards *Xa21* (Alam et al., 2016; Khan et al., 2012; Mazzola et al., 1994). Alam et al. (2016) determined, in accordance with the virulence profiles of 96 *Xoo* isolates, that Monogenic differentials carrying *R*-genes *xa5* exhibited the highest resistance performance (66.67%) in Bangladesh, followed by *Xa2* and *Xa21* (65.63%). Furthermore, Adhikari et al. (1995) and Yang et al. (2013) reported identical findings, namely that the *xa5* gene provided resistance to the majority of *Xoo* isolates. While the *Xa21* gene has been identified as the most efficacious resistant gene against bacterial blight disease, additional *Xa* genes are essential for enhancing the action of the *Xa21* gene and attaining long-lasting resistance (Jeung et al., 2006). In Pakistan, sixteen pyramid lines having two to five R genes were employed in an experiment against sixteen *Xoo* isolates; the results indicated that the *xa13* and *xa21* genes provided resistance to the majority of the isolates (Khan et al., 2014). In Bangladesh, based on the reaction pattern of 300 *Xoo* isolates on 18 pyramid lines, among those lines, IRBB65 *(Xa4, Xa7, xa13,* and *Xa21),* IRBB66 (*Xa4, xa5, Xa7, xa13,* and *Xa21),* IRBB64 (*Xa4*, *Xa7, xa13*, and *Xa21),* IRBB60 (*Xa4*, *xa5, xa13*, and *Xa21*) and IRBB63 (*xa5, Xa7*, and *xa13*) may be the best sustainable donor parent for the development of bacterial blight-resistant varieties. Furthermore, from screening assessment of 50 advanced lines during T. Aman (July to November) and T. Aus (mid-March to August) seasons, the efficient single R genes' combinations, i.e., *(Xa4, xa5, Xa7, xa13, Xa21*), (*Xa4, xa5, xa13, xa21, Xa23*), and (*Xa4, xa5, xa13, Xa23*) offered highly resistant against the 5 most virulent races of *Xoo*. In agreement with the resuts of the present study, several authors showed similar findings (Sukhwinder-Singh et al., 2003; Sundaram et al., 2008; Wang et al., 2020). The different findings of monogenic, pyramid and advanced lines showed integrity in terms of R gene resistance and suggested that the combination of *Xa4* or/and *xa5* or/and *Xa7* or/and *xa13* or/and *Xa21* or/and *Xa23* or/and *Xa27* could make the great pave to develop the most durable BB-resistant variety. Planning and designing are necessary to execute comprehensive research on population structures and their genetic compositions with regard to host-plant resistance in every rice-growing country to develop BB-resistant varieties. However,

pathotype research with differential cultivars can fail to reveal the true extent of genetic variability present within a given pathogen population (Yashitola et al., 1997).

The bacterial blight-resistant variety can be developed for a country or within a country or in a particular location by a resistance breeding program utilizing the effective *Xa* genes identified in this work. Furthermore, Plant Pathologists and Plant Breeders will find the results of this research beneficial in gaining a deeper comprehension of the attributes of bacterial blight races in Bangladesh. This understanding will facilitate the development of BB-resistant varieties and the formulation of an effective and sustainable management approach to control the bacterial blight disease.

Conclusion

Our investigation on the pathogenicity test of 300 *Xoo* isolates collected from 40 districts in Bangladesh revealed 13 distinct pathotypes. A proportion of *Xoo* bacterial isolates comprising 48.33% belonged to pathotype/race 1 and 3. The resistant genes *xa5*, *Xa7*, *xa8*, *xa13*, *Xa21*, and *Xa27* were effective against the 300 *Xoo* isolates. The gene combinations of advanced lines including IR 129336:11–37 (*Xa4-xa5-xa13*(H)-*Xa21*(H)-*Xa23*), IR 127164:11–26 (Xa4-xa5(H)-Xa7(H)-xa13-Xa21(H)), and IR 129337:37–79 (Xa4-xa5-xa13(H)--Xa23) were also highly resistant to BB disease in rice growing zones of Bangladesh. These genes may therefore be useful for the development of a sustainable bacterial blight resistant cultivars. Additionally, gene pyramiding could be an efficient approach for developing durable resistant varieties to bacterial blight disease in Bangladesh.

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Data availability The datasets created and/or analyzed during the current investigation are available upon reasonable request from the respective authors.

Declarations

Conflict of interest The authors reported no possible conflicts of interest.

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