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Screening rice lines with various resistant genes and combinations against *Xanthomonas oryzae* pv *oryzae* strains from Malaysian rice granaries

Kogeethavani, R.[1*], Fatin, N. A.[1], Nurshamiza, M. Y.[1] and Muhammad Rusydi, S.[1]

[1]*Paddy and Rice Research Centre, MARDI Seberang Perai, Jalan Paya Keladi/Pinang Tunggal, 13200 Kepala Batas, Pulau Pinang, Malaysia*

Abstract

Bacterial blight caused by *Xanthomonas oryzae* pv *oryzae* is the most destructive disease of rice in Malaysia due to its high epidemic potential. Therefore, the most effective solution for disease management, considering both environmental safety and production costs, is the utilisation of resistant rice varieties. This study evaluated the performance of 10 near-isogenic lines and 16 pyramided lines carrying known bacterial blight resistance genes against 12 different *Xanthomonas oryzae pv oryzae* pathotypes collected from Malaysian rice granaries. Lesion length percentage was measured 21 days after inoculation and converted into various disease reaction categories. The study revealed that single resistance genes, *xa*5 and *Xa*7, found in IRBB5 and IRBB7, respectively, were effective against Malaysian strains of *Xanthomonas oryzae pv oryzae*. Furthermore, the combination of these genes with *Xa*21 (IRBB21) in pyramided lines IRBB54, IRBB60, IRBB64, IRBB65 and IRBB66 exhibited strong resistance against Malaysian *Xanthomonas oryzae pv oryzae* strains. This information supports the use of resistant cultivars in Malaysia as part of a gene deployment strategy to manage the disease.

Keywords: bacterial blight, resistance gene, near-isogenic lines, rice, *Xanthomonas oryzae* pv *oryzae*

Introduction

Xanthomonas oryzae pv. *oryzae* (*Xoo*) is responsible for the bacterial blight (BB) of rice, which is considered one of the most severe bacterial diseases of rice (Swings et al. 1990). In areas where epidemic occur and in cases of severe infections, the disease can cause yield losses of up to 80% (Mishra et al. 2013; Bharathkumar et al. 2014), posing a significant threat to food security in rice-producing countries worldwide (Busungu et al. 2018; Chien et al. 2019).

In Malaysia, susceptible rice varieties can experience yield losses of up to 50% (Saad 1995). From 1982 to 1994, a severe outbreak of BB on the local susceptible variety MR 84 was projected to incur a cost of RM50 million (Saad and Habibuddin 2010). In February 2014, a BB outbreak occurred in Padang Besar, Perlis, affecting approximately 60,000 MT of rice (Jonit et al. 2016). More recently, in 2016, another disease outbreak affected Kuala Selangor and Sabak Bernam, with approximately 56% of paddy cultivation areas in Selangor being affected and resulting in yield losses of up to 60% (Berita Pas 2017). Varieties such as MR 284, MR 263 and MR 220 CL2 were severely impacted. MR 220 CL2 is the most extensively cultivated rice variety in Peninsular Malaysia, accounting for around 54% of total paddy production in rice granaries, followed by MR 219 and MR 263, each at 17% (DOA 2016). MR 220 CL2 and MR 219 continue to be the most popular varieties planted in Peninsular Malaysia (MAFI 2019). According to in-house screening conducted at MARDI Seberang Perai, both varieties are susceptible and prolonged usage could lead to another bacterial blight outbreak (Unpublished data).

Conventional remedies for managing BB disease, including chemicals, antibiotics, biological control agents, and cultural practices, have been recommended. However, their effectiveness is limited, particularly when the disease is widespread (Sundaram et al. 2008; Gnanamanickam, 2009). Resistant varieties offer the most effective solution for managing the disease while considering environmental safety and production costs. Unfortunately, no natural resistance to BB has been identified in local rice varieties

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Authors' full names: Kogeethavani Ramachandran, Fatin Nurliyana Ahmad, Nurshamiza Mat Yusoff and Muhammad Rusydi Sarifhudin
Corresponding author: kogeetha@mardi.gov.my

thus far (Chukwu et al. 2019). Therefore, incorporating resistant genes into local rice varieties could prove to be the most effective approach to enhance BB disease resistance in Malaysia.

Near-isogenic lines (NILs) carrying known resistant genes can serve as suitable donors for introducing targeted gene(s), into desired recurrent parent(s) through individual incorporation or pyramiding. To date, approximately 43 BB resistant (R) genes have been identified and designated as *Xa*1 to *Xa*43 ((Busungu et al. 2016; Kim and Reinke 2019). These genes have been discovered in cultivated, mutant, and wild rice varieties. They provide complete and race specific resistance against *Xoo*, with the durability of resistance depending on the prevalence of different pathogen races over time and across different geographical locations (Lore et al. 2011; Pandey et al. 2013; Aafreen et al. 2019)

The objective of this study is to assess the resistance levels of NILs and pyramided lines carrying single and multiple resistance genes against *Xoo* strains found in Malaysia. The evaluation of these genes' resistance levels provides valuable information for the management of rice BB. It aids in the selection of effective resistant genes for future breeding programs, enabling the incorporation of durable resistance into promising high-yielding rice varieties.

Materials and methods

Plant materials and Xoo strains

The seeds of International Rice Bacterial Blight (IRBB) lines, consisting of 26 NILs and pyramids, were obtained from the International Rice Research Institute (IRRI), Philippines. These lines were developed in the genetic background of IR 24. Among them, there were 10 lines carrying single resistance genes and 16 pyramided lines containing two to five gene combinations (*Table 1*). For comparison, the local MR 84 variety, known to be susceptible to BB, was used. The virulence of the IRBB lines was assessed against 12 *Xoo* strains isolated from major rice granaries, representing the prevailing *Xoo* pathotypes in Malaysia (*Table 1*). The classification of pathotypes was based on the virulence of *Xoo* isolates using six different varieties: IR 8, Cempo Selak, Zenith, IR 20, IR 1545-339 and DV 85 as reported by Saad and Habibuddin, 2010.

Field establishment

The experiment was conducted in the MARDI Seberang Perai research field during the wet season of 2017 using a Randomised Complete Block Design (RCBD) split-plot design. The trial consisted of three replications. Individual line seedlings were transplanted into the field after 21 days of sowing. The main plots were dedicated to planting the IRBB lines, while the *Xoo* pathotypes were inoculated in subplots. The spacing between plants and rows was maintained at 20 cm × 20 cm. Rice cultivation and field management practices followed the recommendations outlined by Saad and Habibudin (2010).

Inoculum preparation and inoculation

The 12 *Xoo* strains, which were stored in 20% glycerol at -80°C, were revived by culturing on peptone sucrose agar (PSA) medium at a temperature of 28°C for a duration of 72 hr. Inoculums were prepared from these three-day-old cultures. For each strain, the bacterial cells were suspended in sterile distilled water, and the concentration was adjusted to reach 10^8 colony-forming units (CFU) per mL. Inoculation was performed by dipping sterilised scissors into the bacterial suspension and clipping off the leaves approximately 2 to 3 cm from the leaf tip. This procedure was carried out from the panicle initiation stage to the booting stage of the plants (Kauffman et al. 1973; Habarurema et al. 2013). To assess the virulence of each isolate, a total of 20 fully extended leaves (5 leaves × 4 replicates) for each NIL were inoculated. As a negative control, NILs were inoculated with distilled water.

Data assessment

The percentage of lesion length was determined by measuring the length of lesions from the leaf tips at 21 days after inoculation (DAI) (*Figure 1*). This measurement was conducted for each *Xoo* pathotype subplot. The calculation of lesion length percentage was done using the following formula: Lesion length (%) = (Lesion length / Total leaf length) × 100 (Gnanamanickam et al. 1999). The reactions of the NILs were then classified based on the mean lesion length percentage using scales from the Standard Evaluation System of Rice (SES) developed by the IRRI (Anon 1996; Samiullah et al. 2015). Refer to *Table 2* and *Figure 1* for the classification and representation of the results.

Statistical analysis

The mean percentage of lesion length was calculated using Microsoft Excel, and the data were subjected to statistical analysis using the SAS statistical package (version 9.4, SAS Institute, Cary, NC, USA). Analysis of variance (ANOVA) was performed to assess the statistical significance of the results. For multiple mean comparisons, Duncan's Multiple Range Test (DMRT) was utilised at a significance level of 5%.

Table 1. The rice genotypes included 10 near-isogenic lines (NILs), 16 pyramided lines harbouring *Xa* genes, and a local susceptible check MR 84 to evaluate for resistance to bacterial blight disease. The *Xanthomonas oryzae* pv *oryzae* strains comprise 12 different pathotypes collected from Malaysian rice ecosystem

Differential rice lines					*Xoo* strains	
Rice genotypes	*Xa*-gene(s)	Source	Parentage	Category	Pathotypes	Strain no/ID
IRBB1	*Xa*1	IRRI	IR24*5/KOGYOKU	NIL	P0.0	MXO1058
IRBB3	*Xa*3	IRRI	IR24*5/CHUGOKU45	NIL	P1.0	MXO1031
IRBB4	*Xa*4	IRRI	IR24*5/IR20	NIL	P1.2	MXO1063
IRBB5	*xa*5	IRRI	IR24*5/IR1545-339	NIL	P1.3	MXO1043
IRBB7	*Xa*7	IRRI	IR24*5/DV85	NIL	P3.0	MXO1050
IRBB8	*Xa*8	IRRI	IR24*5/P1231129	NIL	P3.1	MXO1016
IRBB10	*Xa*10	IRRI	IR24*5/CAS209	NIL	P3.3	MXO1037
IRBB13	*xa*13	IRRI	BJ1/5*IR24	NIL	P5.1	MXO1124
IRBB14	*Xa*14	IRRI	TAICHUNG NATIVE 1/5*1R24	NIL	P5.3	MXO1079
IRBB21	*Xa*21	IRRI	IR 24*8/O BARTHII	NIL	P7.1	MXO1122
IRBB51	*Xa*4 + *xa*13	IRRI	IRBB4/IR66699-9-1-1-5-2	Pyramid	P7.3	MXO1072
IRBB52	*Xa*4 + *Xa*21	IRRI	IRBB4/IR66700-3-3-3-4-2	Pyramid		
IRBB53	*xa*5 + *xa*13	IRRI	IRBB4/IR66699-9-1-1-5-2	Pyramid		
IRBB54	*xa*5 + *Xa*21	IRRI	IRBB4/IR66700-3-3-3-4-2	Pyramid		
IRBB55	*xa*13 + *Xa*21	IRRI	IR66699-9-1-1-5-2/IR66700-3-3-3-4-2	Pyramid		
IRBB56	*Xa*4 + *xa*5 + *xa*13	IRRI	AY4 + 5/IR68311-13-3-42	Pyramid		
IRBB57	*Xa*4 + *xa*5 + *Xa*21	IRRI	AY4 + 5/IR66700-4-2-9-5-2	Pyramid		
IRBB58	*Xa*4 + *xa*13 + *Xa*21	IRRI	NH11-35/NH9-53	Pyramid		
IRBB59	*xa*5 + *xa*13 + *Xa*21	IRRI	NH11-35/NH9-53	Pyramid		
IRBB60	*Xa*4 + *xa*5 + *xa*13 + *Xa*21	IRRI	NH11-35/NH9-53	Pyramid		
IRBB61	*Xa*4 + *xa*5 + *Xa*7	IRRI	IRBB7/IRBB60	Pyramid		
IRBB62	*Xa*4 + *Xa*7 + *Xa*21	IRRI	IRBB7/IRBB60	Pyramid		
IRBB63	*xa*5 + *Xa*7 + *xa*13	IRRI	IRBB7/IRBB60	Pyramid		
IRBB64	*Xa*4 + *xa*5 + *Xa*7 + *Xa*21	IRRI	IRBB7/IRBB60	Pyramid		
IRBB65	*Xa*4 + *Xa*7 + *xa*13 + *Xa*21	IRRI	IRBB7/IRBB60	Pyramid		
IRBB66	*Xa*4 + *xa*5 + *Xa*7 + *xa*13 + *Xa*21	IRRI	IRBB7/IRBB60	Pyramid		
MR 84	Unknown	MARDI	CR261-7039-236/MR50	Susceptible check		

Table 2. Bacterial blight disease scale and reaction under the Standard Evaluation System of Rice (SES) (Anon 1996; Samiullah et al. 2015). The assessment was based on the mean percentage lesion length observed on the rice leaf.

Scale	Lesion length (%)	Disease reaction
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)

Figure 1. Bacterial blight lesion development and their reaction at 21 days after inoculation. The reaction was categorized based on percentage of lesion length calculated by dividing lesion length and leaf length as shown. The reaction C=Control Negative (0%), R=Resistant (1-5%), MR=Moderate Resistant (6-12%), MS=Moderate Susceptible (13-25%), S=Susceptible (26-50%) and HS=Highly Susceptible (>50%).

Results

Interaction between NILs and Xoo pathotypes

Table 3 presents the percentage of lesion length caused by BB for 10 NILs against 12 *Xoo* pathotypes. The results show significant differences ($p<0.001$) between NILs and the interaction between NILs and *Xoo* pathotypes. The lesion length varied among the NILs, ranging from 5.58% in IRBB21 (P1.0) to 51.56% in IRBB1 (P1.3). Most *Xoo* pathotypes exhibited significantly higher lesion lengths, ranging from 10.75 – 51.56%, when inoculated on IRBB1, which carries the *Xa*1 gene. IRBB13 (*xa*13) and IRBB14 (*Xa*14) also had relatively high lesion lengths, except for pathotype P1.2, which showed a significantly lower lesion length of 10.75% on IRBB14. Comparatively, the lesion length (%) of the local susceptible check, MR 84, was slightly lower than that of the NILs, ranging from 11.02 – 44.54%. Among the NILs, IRBB7 (*Xa*7) displayed the lowest lesion length, ranging from 5.58 – 24.26%. This was followed by IRBB21 (*Xa*21), IRBB5 (*xa*5) and IRBB3 (*Xa*3). However, pathotype P7.3 exhibited a higher lesion length of 17.52% on IRBB5, while pathotype P5.1 showed a slightly lower lesion length on IRBB3.

Table 4 displays the disease reactions of 10 NILs towards 12 *Xoo* pathotypes. Except for pathotype P1.3, which exhibited highly susceptible (HS) reaction to IRBB1, most pathotypes showed moderate susceptibility (MS) to susceptibility (S) reactions on the majority of NILs. However, several NILs demonstrated moderate resistance (MR) against specific pathotypes. These included IRBB5 and IRBB7 showing MR reactions to P0.0, P1.0, P1.1, P1.2 and P1.3; IRBB21 exhibiting MR reactions to P0.0 and P1.0; IRBB3 demonstrating MR reactions to P1.0 and P5.1; and IRBB14 displaying MR reactions to P1.2. Among the pathotypes used in the study, P1.0 was reported as the most prevalent pathotype in Malaysian rice granaries (Saad and Habibudin, 2010). The remaining pathotypes were considered minor, with P7.3 being one of the most virulent. Pathotype P1.0 showed MR reactions only on four NILs, namely IRBB5, IRBB7, IRBB21 and IRBB3, while pathotype P7.3 displayed MS reactions on all NILs. None of the NILs carrying a single *Xa* gene exhibited complete resistance to the tested *Xoo* pathotypes.

In general, the NILs carrying the *xa*5 and *Xa*7 genes demonstrated good resistance *against* Malaysian *Xoo* populations, while *Xa*21 did not provide high resistance as a single gene. Previous reports from various rice-growing countries have also highlighted the effectiveness of the recessive *xa*5 gene and the dominant *Xa*7 and *Xa*21 genes against *Xoo* populations (Chukwu et al. 2019). These genes have been successfully incorporated into breeding programs to confer durable resistance to several popular rice varieties (Perumalsamy et al. 2010; Arshad et al. 2016; Nguyen et al. 2018; Chukwu et al. 2019).

Table 3. The mean percentage of lesion length on 10 near-isogenic lines (NILs) consisted of single *Xa* genes and susceptible check MR 84 against 12 different *Xanthomonas oryzae* pv *oryzae* pathotypes. Mean differences were indicated with the different superscripted letters (a-d)

IRBB lines	*Xoo* Strains (pathotypes)										
	P0.0	P1.0	P1.1	P1.2	P1.3	P3.0	P3.1	P3.3	P5.1	P5.3	P7.1	P7.3
IRBB1	38.61^{a}	15.01^{a}	45.51^{a}	30.58^{a}	51.56^{a}	46.57^{a}	25.10^{a}	38.49^{a}	24.21^{abc}	19.17^{a}	36.98^{ab}	15.07^{ab}
IRBB3	16.47^{c}	6.10^{b}	20.34^{c}	24.26^{ab}	18.71^{c}	21.47^{cd}	12.60^{b}	16.89^{c}	10.06^{e}	18.12^{a}	18.69^{c}	19.38^{a}
IRBB4	26.72^{b}	13.59^{a}	35.40^{b}	15.17^{bc}	32.81^{b}	38.45^{ab}	17.93^{ab}	18.95^{c}	21.93^{bcd}	14.57^{a}	30.22^{abc}	16.08^{ab}
IRBB5	10.35^{c}	5.93^{b}	12.17^{de}	10.45^{c}	11.86^{cd}	24.86^{cd}	16.20^{ab}	17.62^{c}	17.78^{b-e}	19.95^{a}	28.81^{abc}	17.52^{a}
IRBB7	8.83^{c}	7.87^{b}	6.86^{d}	11.30^{c}	8.46^{d}	13.10^{d}	16.42^{ab}	17.29^{c}	15.19^{de}	16.19^{a}	17.11^{c}	14.29^{ab}
IRBB8	30.01^{ab}	16.23^{a}	38.47^{ab}	18.67^{abc}	35.62^{b}	30.54^{bc}	17.17^{ab}	17.47^{c}	20.60^{bcd}	18.27^{a}	22.61^{bc}	14.41^{ab}
IRBB10	36.88^{a}	16.66^{a}	44.17^{ab}	20.39^{abc}	49.07^{a}	39.70^{ab}	14.25^{ab}	23.10^{bc}	19.21^{bcd}	16.62^{a}	23.23^{bc}	14.04^{ab}
IRBB13	37.12^{a}	15.53^{a}	46.58^{a}	21.69^{abc}	49.91^{a}	43.48^{ab}	17.01^{ab}	34.08^{a}	31.55^{a}	20.88^{a}	37.56^{a}	13.78^{ab}
IRBB14	33.99^{ab}	15.39^{a}	46.98^{a}	10.75^{c}	44.95^{a}	39.88^{ab}	15.94^{ab}	22.67^{bc}	24.55^{ab}	20.44^{a}	38.28^{a}	17.90^{a}
IRBB21	12.46^{c}	5.58^{b}	14.44^{de}	14.19^{bc}	13.16^{cd}	15.91^{d}	14.23^{ab}	18.41^{c}	16.55^{cde}	21.36^{a}	17.31^{c}	14.53^{ab}
MR 84	34.64^{ab}	16.57^{a}	44.54^{ab}	19.11^{abc}	36.56^{b}	32.90^{bc}	18.15^{ab}	31.32^{ab}	16.24^{cde}	22.89^{a}	29.74^{abc}	11.02^{b}

Note: Means with the same letter along the column are not significantly different at $p<0.05$ according to DMRT

Table 4. Bacterial blight disease reaction of 10 near-isogenic lines (NILs) consisted of single *Xa* genes and susceptible check MR 84 against 12 different *Xanthomonas oryzae* pv *oryzae* pathotypes

IRBB Lines	*Xoo* Strains (pathotypes)											
	P0.0	P1.0	P1.1	P1.2	P1.3	P3.0	P3.1	P3.3	P5.1	P5.3	P7.1	P7.3
IRBB1	S	MS	S	S	HS	S	MS	S	MS	MS	S	MS
IRBB3	MS	MR	MS	MS	MS	MS	MS	MS	MR	MS	MS	MS
IRBB4	S	MS	S	MS	S	S	MS	MS	MS	MS	S	MS
IRBB5	MR	MR	MR	MR	MR	MS	MS	MS	MS	MS	S	MS
IRBB7	MR	MR	MR	MR	MR	MS	MS	MS	MS	MS	MS	MS
IRBB8	S	MS	S	MS	S	S	MS	MS	MS	MS	MS	MS
IRBB10	S	MS	S	MS	S	S	MS	MS	MS	MS	MS	MS
IRBB13	S	MS	S	MS	S	S	MS	S	S	MS	S	MS
IRBB14	S	MS	S	MR	S	S	MS	MS	MS	MS	S	MS
IRBB21	MR	MR	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS
MR 84	S	MS	S	MS	S	S	MS	S	MS	MS	S	MS

Note: R= Resistant, MR= Moderate resistant, MS= Moderate susceptible, S= Susceptible and HS=Highly susceptible

In this study, *Xoo* strains exhibited varying levels of virulence, resulting in a range of compatible and incompatible reactions with the NILs. Some strains showed high virulence, some were less virulent, and others demonstrated intermittent. Similar observations have been reported by other researchers (Priyadarsini et al. 1999; Thimmegowda et al. 2011). The interaction between Avirulence (*Avr*) genes in *Xoo* and resistance (R) genes in the host plant follows a gene-for-gene interaction, as proposed by Flor in 1955. *Avr* genes in *Xoo* encode pathogenic proteins that are recognized by R genes (such as *Xa* genes). This recognition leads to the activation of plant defence responses, triggering incompatible interactions between the pathogen and the host plant. The function of R genes requires the presence of a recognizable *Avr* ligand in the pathogen. Moreover, there is evidence suggesting that some *Avr* genes are involved in disease symptom expression and pathogenic fitness, both of which are important components of the pathogen's ability to cause disease. The dual function of these genes (avirulence and fitness) suggests that R genes corresponding to these *Avr* genes may exhibit greater durability in the field (Aafreen et al. 2019). Several *Avr* genes have been extensively studied in *Xoo*, including *avrXa*2, *avrXa*3, *avrXa*4, *avrXa*7, *avrXa*10, and *avrXa*21 (Song et al. 1995; Iyer and McCouch 2004; Tian et al. 2014; Sekhwal et al. 2015). These genes play a crucial role in the interaction between *Xoo* and rice plants and have been the focus of research aiming to develop durable resistance against BB.

Interaction between pyramided lines and Xoo pathotypes

Table 5 displays the percentage lesion length of pyramided lines against the 12 *Xoo* pathotypes, while *Table 6* presents their corresponding reactions. Significant differences ($p < 0.001$) were observed among the pyramided lines and in the interaction between the pyramided lines and *Xoo* strains. The lesion length caused by *Xoo* inoculation on the pyramided lines varied, ranging from 4.09% in IRBB57 (P1.0) to 42.46% in IRBB51 (P1.1). In comparison, the local susceptible check MR 84 generally exhibited significantly higher lesion lengths for most *Xoo* pathotypes. Among the pyramided lines, IRBB51 (*Xa*4 + *xa*13), followed by IRBB52 (*Xa*4 + *Xa*21), showed significantly higher lesion lengths ranging from 6.21 – 44.54%, except for pathotype P7.3, which had a significantly lower lesion length (11.88%) for IRBB52. With the exception of pathotype P5.1, which showed significantly higher lesion length on IRBB 56 (*Xa*4 + *xa*5 + *xa*13), and pathotypes P3.3 and P7.3, which exhibited significantly higher lesion lengths on IRBB57 (*Xa*4 + *xa*5 + *Xa*21), the other pyramided lines generally displayed relatively lower lesion lengths. IRBB60 (*Xa*4 + *xa*5 + *xa*13 + *Xa*21) had the lowest lesion length, followed by IRBB65 (*Xa*4 + *Xa*7 + *xa*13 + *Xa*21) and IRBB64 (*Xa*4 + *xa*5 + *Xa*7 + *Xa*21), with lesion lengths ranging from 3.80 – 22.95%. These results indicate the varying levels of resistance conferred by different pyramided lines against the *Xoo* pathotypes tested.

Most pyramided lines exhibited resistant (R) to moderately resistant (MR) reactions against pathotypes P0.0, P1.0, P1.1, P1.2, P1.3 and P3.0. However, they showed moderately susceptible (MS) reactions to pathotypes P3.1, P3.3, P5.1, P5.3, P7.1 and P7.3. The prevalent pathotype P1.0 displayed R to MR reactions in all pyramided lines except for IRBB51, which showed MS. On the other hand, the virulent pathotype P7.3 exhibited R to MR reactions in all lines except for IRBB52 and IRBB54, which showed MS. The presence of the IRBB51 gene resulted in MS reactions to all *Xoo* pathotypes. However, the combination of *xa*5 and *Xa*7 genes in the pyramided lines significantly enhanced resistance, even in the presence of susceptible genes like *Xa*4 and *xa*13. The *Xa*4 gene is widely used in many Asian rice breeding programs and has provided long-term resistance in numerous commercial rice cultivars (Mew et al. 1992).

Table 5. The mean percentage of lesion length on 16 pyramided lines consisted of multiple *Xa* genes and a susceptible check MR 84 against 12 different *Xanthomonas oryzae* pv *oryzae* pathotypes. Mean differences were indicated with the different superscripted letters (a-d).

Rice Lines	*Xoo* Strains (pathotypes)											
	P0.0	P1.0	P1.1	P1.2	P1.3	P3.0	P3.1	P3.3	P5.1	P5.3	P7.1	P7.3
IRBB51	31.08^{ab*}	13.28^{b}	42.46^{a}	13.89^{ab}	34.71^{a}	28.08^{a}	19.00^{a}	22.21^{b}	17.20^{ab}	26.35^{a}	33.81^{a}	14.39^{bcd}
IRBB52	21.56^{bc}	10.68^{b}	16.86^{b}	19.09^{a}	17.70^{b}	16.44^{b}	15.72^{a}	21.65^{b}	19.45^{ab}	18.71^{a}	29.77^{ab}	11.88^{d}
IRBB53	12.02^{dc}	6.21^{c}	11.32^{bc}	10.23^{bc}	12.13^{bc}	11.70^{bc}	14.23^{a}	19.03^{bc}	18.71^{ab}	20.16^{a}	18.36^{bc}	14.02^{bcd}
IRBB54	6.22^{d}	5.55^{c}	5.90^{c}	7.94^{bc}	5.83^{c}	6.27^{bc}	17.43^{a}	19.71^{bc}	15.22^{b}	15.16^{a}	24.88^{abc}	11.50^{d}
IRBB55	11.63^{dc}	5.41^{c}	11.42^{bc}	11.71^{abc}	11.13^{bc}	14.04^{bc}	12.00^{a}	16.36^{bc}	13.71^{b}	17.14^{a}	22.30^{bcd}	15.80^{bcd}
IRBB56	13.48^{dc}	5.35^{c}	8.13^{c}	10.35^{bc}	10.89^{bc}	10.92^{bc}	13.77^{a}	18.33^{bc}	27.25^{a}	16.06^{a}	18.04^{cd}	14.78^{bcd}
IRBB57	5.56^{d}	4.09^{c}	5.65^{c}	4.44^{c}	8.44^{bc}	7.34^{bc}	14.97^{a}	20.47^{b}	22.35^{ab}	17.93^{a}	19.25^{cd}	22.69^{a}
IRBB58	10.34^{dc}	5.76^{c}	8.28^{c}	7.91^{bc}	11.49^{bc}	10.47^{bc}	14.41^{a}	15.30^{bc}	18.06^{ab}	17.02^{a}	18.62^{cd}	15.87^{bcd}
IRBB59	9.74^{dc}	4.97^{c}	5.27^{c}	5.78^{c}	5.50^{c}	7.43^{bc}	14.94^{a}	15.00^{bc}	21.61^{ab}	18.07^{a}	14.92^{cd}	14.32^{bcd}
IRBB60	7.70^{d}	4.45^{c}	6.71^{c}	5.41^{c}	4.94^{c}	6.98^{bc}	12.85^{a}	15.00^{bc}	14.40^{b}	15.33^{a}	17.32^{cd}	19.76^{ab}
IRBB61	7.62^{d}	5.09^{c}	6.01^{c}	6.53^{bc}	6.56^{c}	7.42^{bc}	14.59^{a}	16.40^{bc}	16.33^{ab}	17.08^{a}	13.72^{d}	18.93^{abc}
IRBB62	13.96^{dc}	6.15^{c}	16.48^{b}	4.42^{c}	13.59^{bc}	14.54^{bc}	12.93^{a}	17.25^{bc}	17.87^{ab}	16.47^{a}	17.62^{cd}	14.57^{bcd}
IRBB63	8.14^{d}	6.31^{c}	11.62^{bc}	5.16^{c}	7.94^{bc}	6.47^{bc}	14.35^{a}	15.99^{bc}	14.53^{b}	17.38^{a}	17.54^{cd}	15.69^{bcd}
IRBB64	6.46^{d}	3.80^{c}	5.23^{c}	6.19^{bc}	3.95^{c}	8.36^{bc}	14.48^{a}	11.96^{c}	21.29^{ab}	16.62^{a}	22.86^{bcd}	14.78^{bcd}
IRBB65	9.03^{dc}	4.36^{c}	6.53^{c}	4.69^{c}	7.84^{bc}	3.70^{c}	13.20^{a}	16.20^{bc}	22.95^{ab}	15.17^{a}	15.22^{cd}	16.40^{bcd}
IRBB66	14.91^{dc}	4.19^{c}	4.58^{c}	6.43^{bc}	4.19^{c}	4.35^{c}	14.70^{a}	16.57^{bc}	18.86^{ab}	16.98^{a}	22.65^{bcd}	12.89^{cd}
MR 84	34.64^{a}	16.57^{a}	44.54^{a}	19.11^{a}	36.56^{a}	32.97^{a}	18.15^{a}	31.32^{a}	16.24^{ab}	22.89^{a}	29.74^{ab}	11.02^{d}

Note: Means with the same letter along the column are not significantly different at $p < 0.05$ according to DMRT

Table 6. Bacterial blight disease reaction of 16 pyramided lines consisted of multiple *Xa* genes and a susceptible check MR 84 against 12 different *Xanthomona*s *oryzae* pv *oryzae* pathotypes.

Rice Lines	*Xoo* Strains (pathotypes)											
	P0.0	P1.0	P1.1	P1.2	P1.3	P3.0	P3.1	P3.3	P5.1	P5.3	P7.1	P7.3
IRBB51	S	MS	S	MS	S	S	MS	MS	MS	S	S	MS
IRBB52	S	MR	MS	MS	MS	MS	MS	MS	MS	MS	S	MR
IRBB53	MR	MR	MR	MR	MR	MR	MS	MS	MS	MS	MS	MS
IRBB54	MR	MR	MR	MR	MR	MR	MS	MS	MS	MS	MS	MR
IRBB55	MR	R	MR	MR	MR	MS	MR	MS	MS	MS	MS	MS
IRBB56	MS	R	MR	MR	MR	MR	MS	MS	S	MS	MS	MS
IRBB57	MR	R	MR	R	MR	MR	MS	MS	MS	MS	MS	MS
IRBB58	MR	MR	MR	MR	MR	MR	MS	MS	MS	MS	MS	MS
IRBB59	MR	R	R	MR	MR	MR	MS	MS	MS	MS	MS	MS
IRBB60	MR	R	MR	R	R	MR	MS	MS	MS	MS	MS	MS
IRBB61	MR	R	MR	MR	MR	MR	MS	MS	MS	MS	MS	MS
IRBB62	MS	MR	MS	R	MS	MS	MS	MS	MS	MS	MS	MS
IRBB63	MR	MR	MR	R	MR	MR	MS	MS	MS	MS	MS	MS
IRBB64	MR	R	R	MR	R	MR	MS	MR	MS	MS	MS	MS
IRBB65	MR	R	MR	R	MR	R	MS	MS	MS	MS	MS	MS
IRBB66	MS	R	R	MR	R	R	MS	MS	MS	MS	MS	MS
MR 84	S	MS	S	MS	S	S	MS	S	MS	MS	S	MR

Note: R= Resistant, MR= Moderately Resistant, MS= Moderately Susceptible, S= Susceptible and HS=Highly Susceptible

The pyramided lines containing *Xa*4 and other resistance genes demonstrated a broader spectrum of resistance and higher resistance levels compared to single-gene lines (Huang et al. 1997). These findings corroborated our results, which have shown that *Xoo* populations may be susceptible to the *Xa*4 gene but exhibit strong resistance when combined with other genes in pyramided lines.

Performance of NILs and pyramided lines against Xoo population

The resistance frequency was calculated by determining the ratio of *Xoo* pathotypes that induced incompatible or resistant reactions (MR and R) to the total number of *Xoo* pathotypes (12) tested on each NILs, pyramided lines and susceptible check (Li et al. 2009). Among the 10 NILs carrying a single *Xa* gene (*Figure 2*), IRBB5 (*xa*5) and IRBB7 (*Xa*7) exhibited the highest resistance frequency, at 42%. These two NILs demonstrated incompatible reactions to five different pathotypes. IRBB21 (*Xa*21) and IRBB3 (*Xa*3) had a resistance frequency of 17%, as they showed incompatible reactions to two different pathotypes. The remaining NILs displayed compatible reactions (MS and S) to all 12 pathotypes. Based on these findings, it can be concluded that IRBB5 (*xa*5) and IRBB7 (*Xa*7) are potential single resistant genes against the *Xoo* population in Malaysian rice granaries, as they showed the highest resistance frequencies among the tested NILs.

Among the pyramided lines (*Figure 3*), IRBB54 (*xa*5 + *Xa*21) exhibited the highest resistance frequency of 58%. It demonstrated incompatibility with seven pathotypes. Additionally, IRBB60 (*Xa*4 + *xa*5 + *xa*13 + *Xa*21), IRBB65 (*Xa*4 + *Xa*7 + *xa*13 + *Xa*21), and IRBB64 (*Xa*4 + *xa*5 + *Xa*7 + *Xa*21) showed a resistance

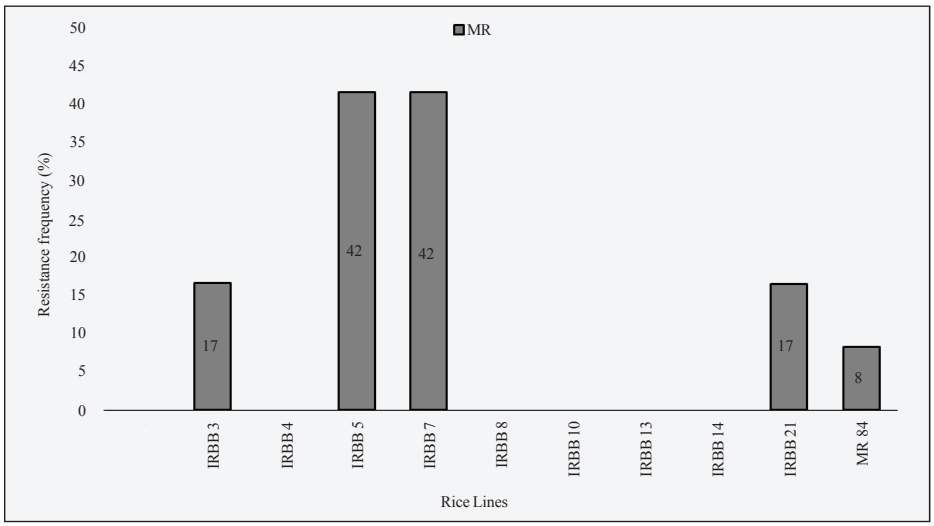

Figure 2. Resistance frequencies of 10 NILs consisted of single Xa genes, and a susceptible check (MR 84) based on the percentage of incompatible reactions which include R=Resistant (1-5%), MR=Moderate Resistant (6 – 12%) reaction to Xanthomonas oryzae pv oryzae population (12 pathotypes)

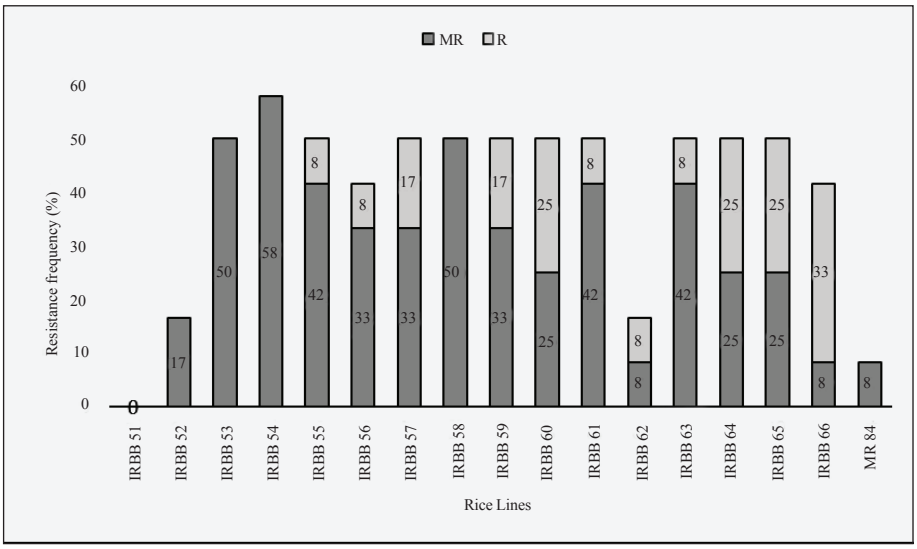

Figure 3. Resistance frequencies of 16 pyramided lines consisted of different combinations of Xa genes and a susceptible check (MR 84) based on the percentage of incompatible reactions which include R=Resistant (1 – 5%), MR=Moderate Resistant (6 – 12%) to Xanthomonas oryzae pv oryzae population (12 pathotypes)

frequency of 50% as they displayed incompatibility with six pathotypes. Furthermore, IRBB66 (*Xa*4 + *xa*5 + *Xa*7 + *xa*13 + *Xa*21) had a resistance frequency of 41%, with 33% of the pathotypes showing high incompatibility to this line. Therefore, it can be concluded that IRBB54, IRBB60, IRBB64, IRBB65 and IRBB66 lines are effective and expected to provide optimal resistance against the Malaysian *Xoo* population. These pyramided lines have also shown effectiveness against *Xoo* populations in certain regions of Indonesia, Bangladesh, and Pakistan (Suryadi et al. 2016; Mubassir et al. 2016; Qudsia et al. 2019). The combination of multiple resistance genes against *Xoo* strains resulted in a higher resistance level (Bharathkumar et al. 2008; Khan et al. 2012; Rizwan et al. 2017). Gene pyramiding, which involves combining multiple resistance genes, offers a better chance of achieving long-term resistance to both biotic and abiotic stresses in plants. Developing resistant varieties through marker-assisted backcrossing, as seen in the development of these pyramided lines, is a cost-effective and ethical approach for achieving durable resistance (Mamadou et al. 2015; Kumar et al. 2016; Ramalingam et al. 2017; Hsu et al. 2020; Ramalingam et al. 2020).

The study conducted on several elite rice varieties in Malaysia, such as MR 284, MR 84, MR 219, MARDI Siraj 297, MR 303, and MR 307, revealed that these varieties lack the presence of important resistance genes, namely *Xa*4, *xa*5, *xa*13, and *Xa*21 (Hasan et al. 2020). This finding suggests that the low resistance observed in these local varieties against BB may be attributed to the absence of these resistant genes. To effectively control BB disease, it is recommended to initiate a breeding program aimed at transferring and pyramiding the potential resistance genes into high-quality Malaysian rice varieties. By incorporating these resistance genes, breeders can enhance the resistance level of local varieties and develop new varieties that are better equipped to withstand BB infections. This breeding approach is crucial for improving the overall resistance of rice varieties and ensuring sustainable and effective management of BB in Malaysian rice production.

Conclusion

The NILs IRBB5 and IRBB7 carrying the single genes *xa*5 and *Xa*7 have demonstrated effectiveness in providing resistance against Malaysian *Xoo* strains. These genes can be utilized to deploy resistant varieties that offer protection against BB disease in farmers' fields, helping to prevent outbreaks and minimize yield losses. Moreover, the pyramided lines IRBB54, IRBB60, IRBB64, IRBB65, and IRBB66, which possess a combination of *xa*5, *Xa*7 and *Xa*21 genes, exhibit strong resistance to BB disease. Utilising effective genes and employing gene pyramiding strategies in breeding programs can significantly enhance the resistance of rice varieties to BB disease. This approach ensures a more durable and sustainable solution for managing BB and contributes to the development of improved rice cultivars that can withstand the challenges posed by the pathogen.

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