Makara Journal of Science

Volume 20
Issue 3 *September*

Article 3

9-20-2016

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Recommended Citation
Suryadi, Yadi; Samudra, I Made; Priyatno, Tri Puji; Susilowati, Dwi Ningsih; Lestari, Puji; Fatimah; and Kadir, Trini Suryani (2016) "Determination of Pathotypes from Indonesian Xanthomonas oryzae Pv. Oryzae Population causing Bacterial Leaf Blight and their Reactions on Differential Rice," *Makara Journal of Science*: Vol. 20 : Iss. 3 , Article 3.
DOI: 10.7454/mss.v20i3.6241
Available at: https://scholarhub.ui.ac.id/science/vol20/iss3/3

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Cover Page Footnote

The author would like to gratefully acknowledge APBN 2012 Project No. 1798.009.001.011 for funding this study and the Microbiology Laboratory technicians for their cooperation in preparing the greenhouse and field research.

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Makara Journal of Science, 20/3 (2016), 109-118
doi: 10.7454/mss.v20i3.6241

Determination of Pathotypes from Indonesian *Xanthomonas oryzae* Pv. *Oryzae* Population causing Bacterial Leaf Blight and their Reactions on Differential Rice

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Received September 9, 2014 | Accepted April 25, 2016

Abstract

The objective of this work was to study the virulence of 15 *Xanthomonas oryzae* pv. *oryzae* (Xoo) isolates collected in three provinces in Indonesia (North Sumatra, South Sumatra, and South Sulawesi) based on five Indonesian differential rice genotypes and 10 near isogenic lines (NILs) that have been known to differ in their resistance to bacterial leaf blight (BLB), in a greenhouse assessment. In addition, this study also aims to monitor the responses of 31 rice genotypes (21 NILs, five differentials, five improved cultivars) to BLB disease in a field experiment. The 15 isolates showed different virulence patterns on the NILs'genotypes with a single resistance gene indicating the pathogen diversity. Eight different pathotypes were present, as demonstrated by a particular virulence pattern of each isolate on the genotypes. Determination of Xoo pathotype revealed that Xoo pathotypes responded differently based on their reaction to NILs and Indonesian differential genotypes. The field assessment demonstrated the incidence and severity of BLB disease on rice genotypes ranging from 25% to 100% and 5.5% to 72.91%, respectively, while the mean disease index ranged from 1.15% to 72.9%. The disease response varied among rice genotypes. IRBB50 *(Xa4+xa5)*, IRBB51 (*Xa4+xa13)*, IRBB52 (*Xa4+Xa21)*, IRBB53 (*Xa4+Xa21)*, IRBB56 (*Xa4+xa5+xa13)*, IRBB57 (*Xa4+xa5+Xa21)*, IRBB59 (*Xa4+xa13+Xa21)*, IRBB64 (*Xa4+xa5+Xa7+Xa21)*, IRBB66 (*Xa4+xa5+Xa7+xa13+Xa21)*, IRBB7(*Xa7)*, Angke (*Xa4+xa5)* and Code *(Xa4+Xa7)* were revealed to be highly resistant to the BLB pathogen. These genotypes have potential as genetic material for the pyramiding of several resistance genes for the development of rice resistance to BLB disease in Indonesia.

Abstrak

Penentuan Patotipe *Xanthomonas oryzae* pv *oryzae* Asal Populasi Indonesia Penyebab Penyakit Hawar Daun Bakteri dan Reaksinya pada Padi Diferensial. Tujuan dari penelitian ini adalah untuk mempelajari virulensi 15 isolat *Xanthomonas oryzae* pv. oryzae (Xoo) yang dikumpulkan dari tiga provinsi di Indonesia (Sumatera Utara, Sumatera Selatan, dan Sulawesi Selatan) berdasarkan reaksinya pada lima genotipe padi diferensial Indonesia dan 10 galur padi isogenik (NIL) yang telah diketahui perbedaan ketahanannya terhadap penyakit hawar daun bakteri (HDB) pada uji di rumah kaca. Selain itu, penelitian ini juga bertujuan untuk memantau tanggap 31 genotipe padi (21 NIL, lima diferensial, lima kultivar unggul) terhadap penyakit HDB pada pengujian di lapangan. Sebanyak 15 isolat menunjukkan pola virulensi yang berbeda pada genotipe NIL dengan gen ketahanan tunggal, yang menunjukkan adanya keragaman patogen. Tercatat delapan patotipe yang berbeda, seperti ditunjukkan oleh pola virulensi tertentu isolat pada setiap genotipe. Hasil penentuan patotipe Xoo mengungkapkan bahwa tanggap reaksi patotipe Xoo berdasarkan reaksinya terhadap NIL dan genotipe diferensial Indonesia berbeda-beda. Hasil penilaian di lapangan menunjukkan kejadian dan keparahan penyakit HDB pada genotipe padi masing masing berkisar dari 25-100% dan 5,5-72,91%, sedangkan indeks penyakit berkisar antara 1,15-72,9%. Tanggap penyakit bervariasi antar genotipe padi. IRBB50 (*Xa4 + xa5*), IRBB51 (*Xa4 + xa13*), IRBB52 (*Xa4 + Xa21*), IRBB53 (*Xa4 + Xa21*), IRBB56 (*Xa4 + xa5 + xa13*), IRBB57 (*Xa4 + xa5 + Xa21*), IRBB59 (*Xa4 + xa13 + Xa21*), IRBB64 (*Xa4 + xa5 + Xa7 + Xa21*), IRBB66 (*Xa4 + xa5 + Xa7 + xa13 + Xa21*), IRBB7 (*Xa7*), Angke (*Xa4 + xa5*) dan Code (*Xa4 + Xa7*) bereaksi sangat tahan terhadap patogen HDB. Genotipe tersebut memiliki potensi sebagai bahan genetik piramida beberapa gen ketahanan untuk mengembangkan padi tahan terhadap penyakit HDB di Indonesia.

Keywords: BLB, pathotype, rice, virulence, Xanthomonas oryzae pv. *oryzae*

Introduction

Among the variety of bacterial diseases found in rice plants, bacterial leaf blight disease (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is one of the most severe and most highly widespread, especially in the irrigated and rainfed lowland ecosystems of Asian tropical countries, as well as in Australia, the United States, and several other rice-growing countries [1]. BLB can significantly decrease rice production by as much as 20-80%. Currently, BLB is reported to not only damage wetland rice but also upland rice in Indonesia [2].

One of the BLB disease control efforts that work well is the use of resistant genotypes. However, pathotype diversity that is varied between seasons and locations can cause the resistance of the rice to cease to be effective. In Indonesia, up to now, at least 12 groups of Xoo pathotypes have been reported [3]. The phenomena of a broad range of Xoo pathotypes, and the fluctuation of the dominant bacterial composition from one region to another, or from one season to another, have been reported in several countries [1,4,5]. As a result, it is apparent that the shifting Xoo population is related to the composition of the host genotype [6]. The choice of a genotype with known resistance genes to certain Xoo pathotypes is important to consider when providing information that can be used in breeding programs to develop effective resistance cultivars that will be planted in certain regions [6]. For example, one study in Vietnam showed that Jasmine 85, OMCS2000, and OM2517 rice cultivars were susceptible to certain Xoo. However, genotypes containing *xa5, Xa7, xa13* and *Xa21* were resistant to Xoo [7]. A similar result in Indonesia reported that Code (*Xa4* + *Xa7*) and Angke (*Xa4* + *xa5*) cultivars could enhance resistance to BLB [8].

Since no effective bactericide or chemical compound is commercially available, growing BLB resistant rice varieties is the most economical means to control this disease. An essential element to resistance breeding programs is the durability of the introduced resistance genes. Resistant rice cultivars mainly based on a single resistance gene have been developed, however, large-scale and long-term cultivation of those varieties and the rapid adaptation of the pathogen result in the eventual breakdown of disease resistance in those cultivars.

More than 30 BLB resistance genes have been identified and some of them have been incorporated into modern rice varieties through conventional breeding. However, the pathogen can easily break down a single resistant gene. The breakdown of resistance in modern and high-yield rice varieties after a few years of cultivation was attributed to rapid-changing pathogens. One useful strategy to prolong the life of major gene resistance is to pyramid several major resistant genes in a resistant cultivar. The gene pyramiding technique creates a broad spectrum resistant cultivar that is an economical and effective method for BLB management [9].

Research on the relationships of different hosts' resistances to the pathogenic isolates of different virulence groups is still very limited. Therefore, regional Xoo pathotype monitoring would be helpful in evaluating the distribution patterns and composition of the dominant pathotypes. The shifting of the pathotype patterns should be evaluated to determine the dominant bacteria groups that can be used in rice breeding programs as a reference to determine suitable rice genotypes for a particular agro-ecosystem. Knowledge of pathogen population structure and genetic resistance mechanism changes in populations of pathogens is very important in determining disease management strategies for long-term BLB disease control [10,11]. Consequently, in this study, five BLB resistance genes, *Xa4, xa5, Xa7, xa13, Xa21* and their mixtures were monitored in a BLB-endemic field for their broad spectrum and durable resistance based on BLB near isogenic lines (NILs) resistance genes. NILs are developed by crossing a resistant individual with universally susceptible plants and backcrossed for six to seven generations with the susceptible recurrent parent to get homozygous resistant lines that only differ in one gene for resistance [12]. These resistance genes were chosen due to their known compatibility reaction against Xoo pathotypes [7,13].

The objective of this study was to determine the interrelationships between 10 differential NILs genotypes with 15 Xoo isolates collected from three provinces in Indonesia under greenhouse assessment. In addition, this study also aimed to compare the relationships between rice (21 NILs, five differential genotypes, and five improved) cultivars and the field bacterial population and monitoring pathotypes of Xoo in BLB endemic areas.

Methods

Preparation of Xoo isolates and plant materials. The experiment was conducted in the ICABIOGRAD greenhouse in Bogor, Indonesia. The 15 Xoo isolates used originated from three provinces in Indonesia *viz*. North Sumatra (six isolates), South Sumatra (four isolates) and South Sulawesi (five isolates) (Table 1) and were obtained from the Biogen CC (culture collection). They were stored in ampoules and tested for their pathogenicity against BLB. Bacterial isolates were prepared as a working culture and maintained in a Wakimoto agar (WA) media (peptone 5g, sucrose 20g, beef extract 3g and agar 15g, $MgSO4.7.H_2O$ 0.05g adjusted to 1 L with dH_2O) [14]. Bacteria that had been propagated in the WA slant media were then grown at 28 °C in a Wakimoto liquid medium for 48 h agitated at100 rpm speed using a shaker incubator (SI 50). The bacterial cells were then suspended in sterile water adjusted to 10^9 CFU/mL.

Table 1. List of Xoo Isolates used in the Study

Isolate code	Pathogen	Isolate origin (rice cultivar host)	Locality/Province
00111	Xoo	Ciliwung	Maros, South Sulawesi
00611	Xoo	Cigeulis	Maros, South Sulawesi
01611	Xoo	Ciherang	Bone, South Sulawesi
03811	Xoo	Cigeulis	Bone, South Sulawesi
06811	Xoo	Inpari13	Sidrap, South Sulawesi
0918	Xoo	Ciherang	Deli serdang, North Sumatra
10811	Xoo	Ciherang	Binjai, North Sumatra
12511	Xoo	Mekongga	Langkat, North Sumatra
17711	Xoo	Bondowoso	Simalungun, North Sumatra
13711	Xoo	Ciherang	Langkat, North Sumatra
20911	Xoo	Ciherang	Batubara, North Sumatra
00212	Xoo	Ciherang	Banyuasin,South Sumatra
00812	Xoo	Ciliwung	OKI, South Sumatra
01612	Xoo	IR42	OKI, South Sumatra
03012	Xoo	Ciherang	OKU Timur, South Sumatra

Xoo inoculation, disease assessment and pathotype grouping in the greenhouse test. NILs and differential rice genotypes used in this study were obtained from the Sukamandi-ICRR collections [3]. The ten rice NILs used were IRBB1 (*xa1*), IRBB3 (*Xa3*), IRBB4 (*Xa4*), IRBB5 (*xa5*), IRBB7 (*Xa7*), IRBB8 (*xa8*) IRBB10 (*xa10*), IRBB11 (*xa11*), IRBB14 (*xa14*) and IRBB21 (*Xa21*). The differential cultivars were Kencana (no R-gene), PB5 (*Xa1+Xakg*), Tetep (*Xa1+xa2*), Kuntulan (*Xaw*) and Java 14 (*Xa1+ xa2+Xakg*). Germinated rice seed was grown in plastic boxes and seedlings were further grown in 30 cm diameter plastic pots containing sterile sandy-loamy soil. The experiment was conducted using a completely randomized design consisting of the treatment of isolates versus rice varieties with three replications. To achieve growth uniformity, rice plant maintenance, such as weeding and fertilizing, was carried out according to the recommended practices. N-P-K fertilizer was applied at a rate of 100-60-40 kg/Ha (10 g mixtures of N-P-K per pot) as the basal dose. Plants were irrigated daily and were top dressed with urea at a rate of 60 kg/Ha (5 g per pot) at 30 days after transplanting (DAT) [9]. Plants were kept in a greenhouse until used for inoculation.

Pure cultures of bacterial Xoo from stock were routinely cultured in a WA medium and incubated at room temperature (approximately 28 °C). The artificial inoculations using 15 Xoo isolates on rice plants were conducted under greenhouse conditions. A total of ten fully developed rice leaves per plant were inoculated with Xoo pathogen bacterial suspensions (10^8 CFU/mL) 45 DAT. The inoculation with Xoo was done by cutting the flag leaf following the method described earlier [14]. The inoculated plants were kept in the greenhouse until they were ready to be assessed. Disease severity was observed based on the lesion length from the cut leaf tips (in centimeters) at 21 days after inoculation.

Monitoring rice cultivars (NILs, differential and improved rice) in the field test. Rice cultivars were tested for their reaction to Xoo at Muara Experiment Station, Bogor, by planting 21 NILs containing a single resistance (R) gene (IRBB1-xa1, IR BB2-*xa2*, IRBB3-*Xa3*, IRBB4-*Xa4*, IRBB5-*xa5*, IRBB7-*Xa7*, IRBB8-*xa8*, IRBB10-*xa10*, IRBB11-*xa11*, IRBB13-*xa13*, IRBB14-*Xa14*, IRBB21-*Xa21*) and a combination of two R-genes (IRRBB50-*Xa4+xa5*, IRBB51-*Xa4+xa13*, IRBB52-*Xa4+Xa21*, IRBB53-*Xa4+Xa21*); three R-genes (IRBB56-*Xa4+xa5+xa13*, IRBB57-*Xa4+xa5+Xa21*, IRBB59-*Xa4+xa13+Xa21*); four R-genes (IRBB64-*Xa4+xa5+Xa7+Xa21*) and five R-genes (IRBB66-*Xa4+xa5+Xa7+xa13+Xa21*). In addition, five differential rice (Kencana-*no R-gene*, PB5-*Xa1+Xakg*, Tetep-Xa1+xa2, Kuntulan-*Xaw*, Java 14-*Xa1+xa2+Xakg*) and five improved rice cultivars containing a single R-gene (IR64-*Xa4*, TN1-*Xa14*, Ciherang-*Xa4*) and two R-genes (Code-*Xa4+Xa7* and Angke-*Xa4+xa5*) were also planted in the field. The site chosen was based on heavy BLB infection throughout planting season (endemic area).

The rice seeds were first sown in a seedbed for 10 days; three seedlings of each line were transferred into a rice plot of 4 x 5 m^2 with 30 x 30 cm plant spacing. Standard crop management practices, such as fertilizer and pesticide applications, watering, and hand weeding were employed following the standard recommended practices. The field plots were irrigated to keep the plants under flooded conditions with about 4 cm standing water above the soil surface. The plots were then fertilized

with urea (N at dose 100 kg/ha) that was applied twice (50% as basal and 50% at 15 DAT). To determine the composition of the dominant pathotypes of Xoo in the trial, five Indonesian differential genotypes (Java 14, Kencana, PB5, Kuntulan and Tetep) were planted around the experimental 2 x 2 m^2 plots. The standard susceptible plant (TN1) was planted prior to the differential planting as the border, and it was allowed to be naturally infected with BLB disease. The BLB infection was observed as characteristic yellow lesions with wavy margins on the leaf blades that may extend to the sheath, followed by the death of the infected tissues that usually starts from the tips of the leaves. The dominant pathotype was determined based on the virulence reaction in differential varieties. The plants were monitored for BLB disease symptoms on fully developed leaves (incidence and severity) at a generative stage based on a standard evaluation system [15]. The disease index was calculated based on Johnsons *et al.* [16] formula as follows: DI=(I x S) x 100, where DI=disease index, I=incidence, S=severity. A disease index of less than 12% was judged as R (resistant), and >12% was S (susceptible).

Results and Discussion

Comparison among isolates and pathotype grouping in the greenhouse test. This study was employed to test the collection of Xoo isolated from three provinces in Indonesia (Table 1). The typical BLB symptoms appeared two to three weeks after inoculation. Some of the lines exhibited a resistant reaction marked by a delayed initiation of the symptoms in which the brown necrotic lesions progressed very slowly. The result of Xoo infection on NILs differential genotypes is presented in Table 2. All of the differential rice NILs genotypes tested showed reactions with varying degrees of resistant reactions (R) and susceptibilities (S) against Xoo.

The mean comparisons among Xoo isolates indicated that two isolates (03012 from OKU Timur, S. Sumatra and 0918 from Deli Serdang, N. Sumatra) were significantly more virulent than the others (Figure 1). Dendrogram analysis showed that the isolates tested were grouped into three categories: i.e., the group of virulent isolates (03012, 0918, 13711), the group of moderate isolates (0612, 20911, 06811), and the group of avirulent isolates (Figure 1). Based on the number of NILs affected, isolates 03012 and 0918 were the most virulent and affected seven NILs and five NILs, respectively. The avirulent isolates were 00611, 01611, 03811, 10811 and 00212 (Figure 2). These results therefore showed the diversity among the isolates studied, although the sample size was limited to 15 isolates. Isolate 03012 exhibited the greatest virulence with a mean lesion length of 15.42 cm, whereas isolates 00212, 00611, 01611, 03811 and 10811 exhibited avirulence to NILs genotypes (Table 3).

IRBB5 and IRBB7, which were inoculated at the generative or flowering stage (45 DAT) with 15 Xoo isolates collected from three provinces in Indonesia, showed that both genotypes were resistant to 14 Xoo isolates. IRBB5 and IRBB7 were susceptible to isolate 03012 from OKU Timur, S. Sumatra. None of the isolates was virulent to IRBB3. Only two isolates, OKU Timur (03012) and OKI, S. Sumatra (01612), could break down the resistance of IRBB21. In a BLB pathotype evaluation based on the Kozaka system [17] using Indonesian differential genotypes, two isolates (03012 and 09811) showed virulence to the genotypes. Lesion lengths ranged from zero to 32.5 cm. Genotype Java 14 containing *Xa1, xa2* and *Xakg* genes showed high resistance to all Xoo isolates (Table 4). Based on the reaction profiles generated from the Indonesian differential genotypes, it was suggested that the isolates belonged to three known pathotypes (VIII, X, XII) and two unknown pathotypes occurred in the regions. However, a comparison to the pathotype evaluation results using NILs and Indonesian differential genotypes showed that these pathotype groups are not related.

Monitoring isogenic lines in the field test. The field test showed that the difference in intensity of BLB disease (incidence and severity) was presumably due to the presence of genetic differences in the host plant. The BLB disease progression in Muara Bogor showed that the tested genotypes have a disease index that ranges from 1.15-72.9% (Table 5). The susceptibility check TN1 (*xa14*) revealead the highest disease index (72.91%), indicated by high disease incidence and severity. Kencana also showed less susceptibility than TN1 with a disease index of 70.14%, while IRBB50 and IRBB66 showed lower BLB incidence and severity. Some genotypes showed high incidence but low disease severity (Table 5). Based on the disease index, 13 NILs genotypes were considered resistant with a disease index of less than 12%. These included Code, IRBB50, IRBB51, IRBB52, IRBB53, IRBB56, IRBB57, IRBB59, IRBB64, and IRBB66, which contained pyramided genes with two to five resistant gene combinations. While the rest (IRBB7, IRBB11, IRBB13, and IRBB21) were single resistance genes (Table 5). The IRBB5 containing *xa5* demonstrated a susceptible reaction with a disease index >12%. However, Angke containing *Xa4+ xa5* remained resistant to the BLB pathogen. Cultivars Ciherang and IR 64 containing *Xa4* are no longer able to overcome Xoo in the field, since both genotypes showed a susceptible reaction to the Xoo field population in Muara Bogor with a disease index of 49.93% and 68.75%, respectively.

Table 2. Relationships between 10 NILs Genotypes and 15 Xoo Isolates

Isolates Xoo	NILs genotypes									
	IRBB1 *xa1*	IRBB3 *xa3*	IRBB4 *Xa4*	IRBB5 *xa5*	IRBB7 *Xa7*	IRBB8 *xa8*	IRBB10 *xa10*	IRBB11 *xa11*	IRBB14 *xa14*	IRBB21 *Xa21*
00111	R	R	R	R	R	R	S	R	R	R
00611	R	R	R	R	R	R	R	R	R	R
01611	R	R	R	R	R	R	R	R	R	R
03811	R	R	R	R	R	R	R	R	R	R
06811	R	R	R	R	R	R	R	R	S	R
0918	S	R	S	R	R	S	S	S	R	R
10811	R	R	R	R	R	R	R	R	R	R
12511	R	R	R	R	R	R	S	R	R	R
17711	R	R	R	R	R	R	S	R	R	R
13711	R	R	S	R	R	R	S	S	R	R
20911	R	R	S	R	R	R	R	R	R	R
00212	R	R	R	R	R	R	R	R	R	R
00812	R	R	R	R	R	R	S	R	R	R
01612	R	R	R	R	R	R	S	R	R	S
03012	S	R	S	S	S	S	S	S	S	S

Note: R = resistant (< 6 cm lesions length), S = susceptible (> 6 cm lesions length)

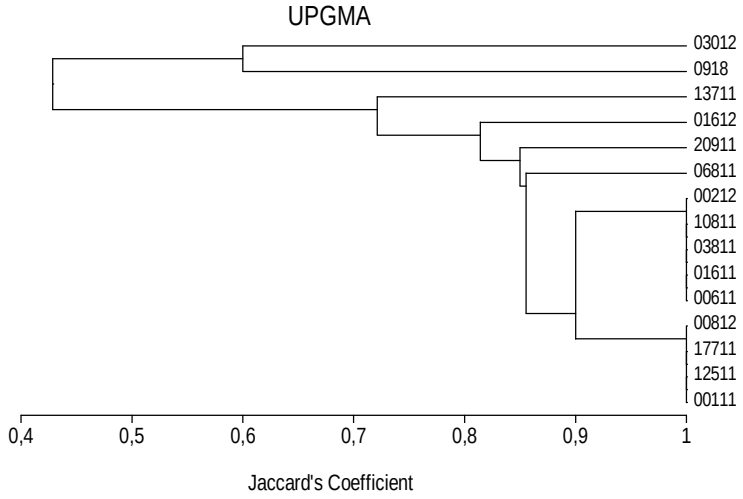

Figure 1. Dendrogram of Similarity among Xoo Isolates based on their Reaction to NILs

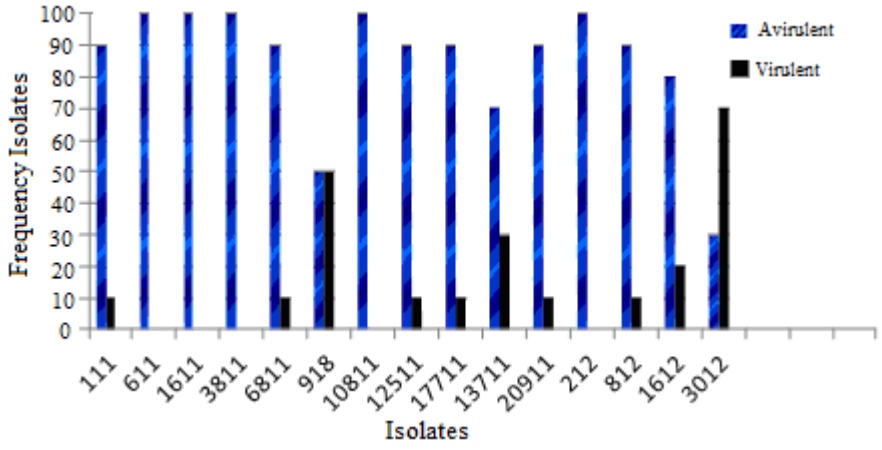

Figure 2. Frequency of Xoo Isolates based on their Virulent Reaction to NILs

Table 3. **Response of 15 Xoo Isolates to Rice NILs and Their Virulence Group Distribution**

NILs	Resistance gene	Xoo virulence group							
		I	II	III	IV	V	VI	VII	VIII
IRBB1	*xa1*	R	R	R	R	R	S	S	S
IRBB3	*Xa3*	R	R	R	R	R	R	R	R
IRBB4	*Xa4*	R	R	R	R	S	S	S	S
IRBB5	*xa 5*	R	R	R	R	R	R	R	S
IRBB7	*Xa7*	R	R	R	R	R	R	R	S
IRBB8	*xa8*	R	R	R	R	R	R	S	S
IRBB10	*xa 10*	R	S	R	S	R	S	S	S
IRBB11	*xa 11*	R	R	R	R	R	S	S	S
IRBB14	*xa14*	R	R	S	R	R	R	R	S
IRBB21	*Xa21*	R	R	R	S	R	R	R	S
Frequency of virulence isolates*		5/15	4/15	1/15	1/15	1/15	1/15	1/15	1/15
Group of isolates		00611;01611; 0318;10811; 00212	00111;1215; 17711;00812	06811	01612	20911	13711	09811	03012

*Frequency was determined based on number of isolates from total 15 isolates

Table 4. **Reaction of 15 Xoo Isolates to Indonesian Rice Differential**

Genotypes	Designated Xoo pathotypes (Kozaka System)				
	?*	?*	VIII	X	XII
Kencana (no R gene)	R	R	S	S	R
PB5 (*Xa1+Xakg)*	R	R	S	R	R
Tetep (*Xa1+xa2)*	R	S	S	S	R
Kuntulan (*Xaw)*	R	R	S	S	S
Java 14 (*Xa1+xa2+Xakg)*	R	R	R	R	R
Frequency of virulence	8/15	2/15	1/15	1/15	3/15
Group of isolates	00611;01611; 10811;17711; 20911;00212; 00812;01612	00111; 12511	03012	09811	0318; 06811; 13711

?*= unknown pathotypes (have not been determined based on present-established pathotypes)

Six NILs genotypes with single resistance genes, namely, IRBB1, IRBB2 and IRBB4, IRBB8, IRBB10, and IRBB14, exhibited susceptible reactions to BLB pathogens (disease index >12%). Java 14 and PB5 are considered resistant genotypes to BLB. None of remaining three genotypes of Indonesian differential lines was resistant to BLB. Based on the typical reactions to Indonesian differential genotypes (Kozaka system) in the field test, it was assumed that the majority of isolates present in Muara, Bogor belong to pathotype X.

In this study, the evaluation of a pathotype's specific resistance and the monitoring of pyramiding genes conferring broad spectrum to BLB were successfully conducted. In Indonesia to date, at least 12 pathotype groups of Xoo have been reported as occurring in the field [3,4]. Consequently, durable broad spectrum resistant genotypes should be developed to protect against diverse pathotypes of Xoo. This evaluation concurs with a previous study that reported that a broad range of virulent Xoo existed in several regions and in among a variety of different rice cultivar groups as hosts [18,19].

In this study, only single resistance genes to Xoo from 10 NILs genotypes were used to characterize the virulence of 15 isolates originating from three provinces in Indonesia. The analysis revealed a diversity of Xoo isolates, even within a limited geographic range in Indonesia. Such diversity among Xoo isolates was similarly reported in the Philippines by Ardales *et al.* [10] who, by sampling areas within field sites and cultivars, pointed out that genetic differences in the population structure of Xoo occur at the agro-ecosystem level.

Table 5. Monitoring of genotype reaction of some NILs and Indonesian differential varieties to BLB infection in the field test (Muara Exp. St), Bogor, West Java

Genotype	Resistance gene	Mean Incidence (%)	Mean Severity (%)	Mean Disease Index (DI) (%)	Reaction*
IRBB1	*xa1*	84.3	39.93	37.78	S
IRBB2	*xa2*	45.8	33.34	23.26	S
IRBB3	*Xa3*	75	36.80	27.95	S
IRBB4	*Xa 4*	65.62	29.51	19.46	S
IRBB5	*xa5*	53.12	25	13.37	S
IRBB7	*Xa7*	37.5	9.72	3.77	R
IRBB8	*xa 8*	51.25	25.34	14.62	S
IRBB10	*xa 10*	51.25	22.91	13.02	S
IRBB11	*xa11*	46.87	20.83	9.01	R
IRBB13	*xa 13*	53.12	19.09	8.31	R
IRBB14	*xa14*	62.5	22.22	13.89	S
IRBB21	*Xa21*	37.5	8.33	3.12	R
Ciherang	*Xa4*	93.75	51.73	49.93	S
IR 64	*Xa4*	96.87	68.75	68.75	S
TN 1	*Xa14*	100	72.91	72.91	S
Angke	*Xa4 +xa5*	43.75	16.66	7.29	R
Code	*Xa4 + Xa7*	40.62	14.23	6.01	R
IRBB50	*Xa4+xa5*	25	7.63	2.17	R
IRBB51	*Xa4+xa13*	28.12	5.90	1.71	R
IRBB52	*Xa4+Xa21*	34.37	7.29	2.53	R
IRBB53	*Xa4+Xa21*	34.37	7.29	2.53	R
IRBB56	*Xa4+xa5+xa13*	21.87	5.20	1.15	R
IRBB57	*Xa4+xa5+Xa21*	46.87	11.49	5.36	R
IRBB59	*Xa4+xa13+Xa21*	28.12	6.59	1.89	R
IRBB64	*Xa4+ xa5+Xa7+ Xa21*	34.37	7.29	2.58	R
IRBB66	*Xa4+xa5+Xa7+xa13 +Xa21*	21.87	5.55	1.21	R
Kencana	(No R gene)	100	70.14	70.14	S
Pb5	*Xa1+Xakg*	46.87	17.01	8.05	R
Tetep	*Xa1+xa2*	78.12	38.54	31.79	S
Kuntulan	*Xaw*	100	65.28	65.28	S
Java 14	*Xa1+ Xa2+Xakg*	25	5.9	1.47	R

S=susceptible, R=resistant

To classify the isolates collected from three different provinces in Indonesia into specific pathotypes, this study showed that the 15 isolates tested on 10 NILs exhibited different phenotypes of virulence, since each isolate induced a particular reaction on the differentials. This suggests that eight different Xoo virulence group/ pathotypes (pathotypes I to VIII) exist in Indonesia; however, there is a need to test more isolates to improve the classification into distinctive Xoo pathogens from the country's different rice growing areas. Using a molecular method, George *et al*. [20] reported that at least 13 unique IS1113 DNA fingerprints and a number of bands per haplotype were identified in 540 isolates collected from four provinces in Java, Indonesia.

Genotype TN1, which was previously reported to be susceptible and hence used as a universal susceptibility check against various Xoo isolates, showed that it was susceptible to all isolates tested in India [21]. However, among the single resistance genes observed in the greenhouse test, none of the NILs was completely broken down by the 15 isolates collected from three provinces in Indonesia. Most of isolates (33%) were incompatible with 10 NILs. Although resistance gene *Xa21* was reported to be effective and stable against multiple Xoo isolates in Asia and Africa [22-24], isolates 01612 and 03012 originating from OKI and OKU Timur, S. Sumatra were virulent to the NILs containing the *Xa21* gene. It was also reported that some

Nepalese, Japanese and Korean Xoo isolates were also capable of overcoming *Xa21* resistance [24].

As an important note, different patterns between Asian and African Xoo isolates occur at the pathological level. African strains possess an avirulent gene that is specifically recognized by the IR24 line. The dissimilarity between African and Asian Xoo races gives further credence to a more local focus on breeding efforts [25-27]. In particular, the single dominant *Xa4* gene that conferred durable resistance in cultivars IR20 and IR64 is widely used in Asian rice breeding programs [28]. The susceptible genotypes harboring only *Xa4* in our study is therefore in agreement with previous reports. The breakdown of *Xa4*-mediated resistance was manifested by significant changes in the qualitative action of *Xa4* and by a quantitative reduction of 50% in the magnitude effect of the *Xa4* gene [27]. Davierwala *et al*. [28] reported that *Xa4* and *Xa21* are now susceptible to BLB in the Philippines, India, Indonesia, and China.

Rice cultivars containing the *xa5* gene provided resistance to BLB in parts of Southeast and Northeast Asia but not in South Asia [29]. The *xa5* gene possesses particularly high resistance and has broad resistance to all Xoo isolates, making it potentially useful in many Asian countries. The result of the field test as indicated in this study showed that resistance genes possessed by IRBB5 (*xa5*) and Angke (*Xa4+ xa5*) differ in their reactions to local Xoo populations in Muara, Bogor.

The resistance gene *Xa7* conveys resistance at the flowering stage [30]. It expressed the most resistant to BLB in Indonesia [3] and also showed lower levels of susceptibility to Korean Xoo races [29]. This resistance gene was also reported to be resistant to Xoo Philippine races 2 and 3 [31]. Code (*Xa4+Xa7*) and Angke (*Xa4+ xa5*) cultivars have genetic backgrounds and traits that are similar to IR64, therefore they are recommended for cultivation in the same areas as IR64. Both of these varieties are more resistant to BLB and demonstrate potential to replace IR64 in areas of BLB endemic disease. In BLB endemic regions, such as Muara (Bogor) and Ciranjang (Cianjur), cultivars Code and Angke are able to produce 10.0% higher yields than IR 64 (*Xa4*) [8].

Various studies have shown that the lines with two, three and four gene combinations exhibit more effective and durable resistance to Xoo isolates than the resistance that is conferred by single genes individually [32]. In a previous study, the reactions of lines IRBB50 (*Xa4* and *xa5),* IRBB52 (*Xa4* and *Xa21)* and IRBB54 (*xa5* and *Xa21)* against most Korean isolates revealed a significantly increased resistance [29]. This was reported to be due to the combined effects of the resistance genes or due to the effect of one resistance gene that masks the susceptibility of the defeated resistance genes. These resistance genes interacted with each other independently and collectively, resulting in a quantitative or qualitative complementation. In genotypes IRBB51 (*Xa4* and *xa13)* and IRBB53 (*xa13* and *Xa21),* particularly, the interactions often resulted in quantitative compensation wherein a susceptible defeated resistance gene has a tendency to reduce the resistance gene effects of others [28,33]. Such a phenomenon was observed in this study, but it differed from that of Jeung *et al*. [29]. The pyramided lines containing resistance (R) genes (*Xa4, xa5, xa13* and *Xa21*) in different combinations, such as IRBB51 and IRBB66, exhibited complex genetic effects with resistance levels that were higher than those of the corresponding monogenic NILs, depending on the nature of the cultivar-isolate interactions. The combination of *Xa4+xa13* (IRBB51) showed an increased resistance level (quantitative complementation) to the BLB pathogen.

In this study, *Xa4* (IR64) showed a susceptibility level against Xoo pathotype X. In addition, the resistance level of *Xa4* on Ciherang was defeated by Xoo in the field test. Quantitative complementation was observed for *Xa4+xa 5* (Angke, IRBB50), *Xa4+xa7* (Code), *Xa4+xa13* (IRBB51), *Xa4+Xa21* (IRBB52, IRBB53), *xa5+Xa21* (IRBB54), *Xa4+xa5+xa13* (IRBB56), *Xa4+xa5+Xa21* (IRBB57), and *Xa4+xa13+Xa21* (IRBB 59). The genetic effects of *Xa21* were quantitatively compensated by the gene *Xa4+xa5+Xa7* (IRBB64) and *Xa4+xa5+Xa7+xa13* (IRBB66). Therefore, the complex R-genes involving *Xa21* and *Xa4, xa5, xa13, Xa7* were much more resistant to BLB in the field. In line with a previous study carried out in Vietnam [34], *Xa4, xa5, Xa21* pyramiding lines in this study expressed high levels of resistance to Xoo pathotypes in Bogor, W. Java.

According to Huang *et al*. [35] the pyramiding of two or more resistance genes should lead to more durable resistance in rice. The pyramiding lines with two, three, and four resistance genes had shown a broader spectrum and a higher level of resistance than lines with only a single gene. The pyramiding lines obtained in our study can be used as genetic resources for BLB to achieve better disease management. Moreover, the success of this study will facilitate future efforts to transfer combinations of BLB resistance genes into other preferred rice cultivars. The pyramiding lines had a higher level of resistance and a broader spectrum of resistance than the parental lines or lines with a single gene. This might be due to interaction and/or complementation between the resistance genes [35]. The information collected in this study has a significant implication for regional gene deployment. Based on this study, the *xa5, Xa7* and *Xa21* genes are relatively stable against the majority of Xoo isolates. The results also support the findings of a previous study conducted by

Fatimah *et al*. [36] using Xoo isolates collected from four provinces in Java. Hence, those resistance genes could be incorporated into rice breeding programs. To improve the BLB resistance of Indonesian cultivars, pyramid lines containing a single gene of *xa5, Xa7* and *Xa21*, or a combination, such as IRBB64 and IRBB66, would be the most valuable and promising as donors for BLB resistance.

From this study, it may be concluded that a larger collection of isolates is recommended to more fully understand the total Xoo population structure in order to identify which potential pathotypes exist in Indonesia. Furthermore, continuously observing the reactions of NILs against Xoo pathogens in endemic areas along with leading cultivars would assist in monitoring changes in the pathogen population followed by deployment of a new resistant genotype. The pyramid lines observed in this study can be used as genetic resources for further improvement of BLB resistance in breeding programs. It is suggested that isolate 03012 from OKU Timur should be used as a virulent Xoo pathotype for screening a germplasm's resistance to BLB.

Conclusion

The population of Xoo isolated from three different rice-producing provinces in Indonesia (North Sumatra, South Sumatra and South Sulawesi) is very diverse. Hence, the effectiveness of BLB resistance genes varies in locations within the region. The monitoring study showed that pyramiding lines containing more than two resistance genes are more resistant to BLB than those with a single gene. However, *xa5, Xa7 and Xa21* single resistance genes are still effective against BLB in the field, hence these R-genes (or combinations thereof) may be evaluated and incorporated in breeding programs to create rice BLB resistance.

Acknowledgement

The author would like to gratefully acknowledge APBN 2012 Project No. 1798.009.001.011 for funding this study and the Microbiology Laboratory technicians for their cooperation in preparing the greenhouse and field research.

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