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RESEARCH PAPER

The characterization, pathotype distribution and genetic diversity of *Xanthomonas oryzae* pv. *oryzae* in rice production centers of the Special Region of Yogyakarta, Indonesia

Dea Musytari Intan Irpawa[1], Triwidodo Arwiyanto[2], & Sri Sulandari[2]

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ABSTRACT

Xanthomonas oryzae pv. *oryzae* (Xoo) is a pathogenic bacterium that induces bacterial leaf blight in rice. This disease is challenging to treat due to the presence of multiple pathotypes that can harm the plants. Characterizing and determining the distribution of pathotypes and genetic diversity of Xoo in the Special Region of Yogyakarta is essential for evaluating the appropriate approach to managing rice leaf blight in different districts of Yogyakarta. This study aimed to ascertain the characterization, distribution of pathotypes, and genetic diversity of Xoo in the Special Region of Yogyakarta. This study involved the isolation and characterization of Xoo from various rice production centers in Yogyakarta. The distribution of Xoo pathotypes was determined using five differential varieties (*Tetep* (4251), *PB 5* (4827), *Java 14* (11022), *Kencana Bali* (4477), and *Kuntulan* (1529)). Pathogenicity testing was conducted on six common varieties used by Yogyakarta farmers (*IR64, Ciherang, C4, Mekongga, Menthik Wangi,* and *Inpari 42*). Additionally, molecular characterization of Xoo was performed. The bacterial leaf blight that affects rice plants in the Yogyakarta region, caused by Xoo, is identified by yellow circular colonies. It exhibits a negative Gram staining response, positive catalase activity, negative oxidase activity, and does not hydrolyse starch. The Xoo pathotypes identified in Yogyakarta are IV, VIII, and XI. All six prevalent cultivars utilized by farmers in Yogyakarta are susceptible to Xoo. Out of each pathotype, four isolates were chosen, and they were divided into two distinct groups based on the DNA banding pattern they formed. Among these isolates, three had the lowest base sequence at 200 bp, while one isolate had a different DNA banding pattern with the lowest base sequence between 250–300 bp.

Key words: bacterial leaf blight, genetic diversity, pathotype, rice, *Xanthomonas oryzae* pv. *oryzae*

INTRODUCTION

Rice (*Oryza sativa* L.) is a highly significant food crop, widely used as a staple food in almost every part of the world, particularly in Asia. According to the Wahyudi (2021), Indonesia's potential rice field area in 2021 was 10.52 million hectares, with an expected total production of 55.27 million tonnes. This data highlights that rice is a significant contributor to the agricultural sector and a major source of livelihood in Indonesia.

Various constraints, including the incidence of plant diseases, hinder the optimization of rice production in Indonesia. *Xanthomonas oryzae* pv. *oryzae* (Xoo) is a significant pathogen responsible for bacterial leaf blight. The symptoms induced by Xoo consist of gray to brown lesions along the leaf vasculature of rice plants, which subsequently lead to progressive blight symptoms. In areas with highly prevalent epidemic circumstances, this disease has the potential to cause a significant decrease in crop output, reaching up to 60%. This impact is particularly pronounced in irrigated rice fields (Tian et al., 2014).

Several control measures have been documented, including the use of bactericides dissolved in water to sterilize seeds. However, this approach can result in pesticide residues, which may contaminate water in rice fields (Ranjani et al., 2018). Hot water treatment is another control method that has been found to impact seed germination rates (Müller et al., 2008). Adopting resistant plant cultivars is the primary method for controlling bacterial leaf blight (HDB). However, this approach becomes less effective as the pathogen develops pathotypes that can overcome resistance traits. The pathogen responsible for HDB exhibits

Corresponding author:
Triwidodo Arwiyanto (triwid@ugm.ac.id)

[1]Postgraduate Program Phytopathology, Department of Plant Protection, Faculty of Agriculture, Gadjah Mada University, Jl. Flora, Bulaksumur, Sleman, Yogyakarta, Indonesia 55281
[2]Department of Plant Protection, Faculty of Agriculture, Gadjah Mada University, Jl. Flora, Bulaksumur, Sleman, Yogyakarta, Indonesia 55281

multiple pathotypes that can inflict harm on rice plants at different growth stages, making the treatment of this disease challenging. The Xoo pathotypes can be classified into 11 types, but the pathotypes typically observed in rice fields in Indonesia are pathotypes III, IV, and VIII (Sudir et al., 2009). In a study conducted by Sudir & Yuliani (2016), it was found that pathotype III was the most prevalent in three provinces: Yogyakarta, South Sulawesi, and South Sumatra. Pathotype IV was dominant in North Sumatra, Lampung, and West Nusa Tenggara. Pathotype VIII, on the other hand, was the most common in four provinces: West Java, Banten, Central Java, and East Java.

Understanding the prevalence of different strains of this disease in a particular area will enhance the efficacy of utilizing resistant plant varieties as a means of disease management. The determination of pathotypes and genetic diversity of Xoo in four districts in Yogyakarta has not been previously conducted. This work focuses on characterising Xoo isolates from four Yogyakarta districts associated with rice plants. The characterization includes analyzing their physiology-biochemistry, hypersensitivity reactions, starch hydrolysis, and pathotype distribution. Xoo exhibits a significant level of genetic diversity. The wide range of genetic variations in rice types used by farmers in Yogyakarta leads to several vulnerabilities. This study aimed to ascertain the characterisation, distribution of pathotypes, and genetic diversity of Xoo in the Special Region of Yogyakarta.

MATERIALS AND METHODS

Research Site. The research was conducted from July 2022 to January 2023, in the Laboratory and Greenhouse of Plant Disease, Department of Plant Protection, Faculty of Agriculture, Gadjah Mada University.

Collection of Samples. Leaf samples of rice plants affected by bacterial leaf blight were gathered from multiple rice-producing centres in Yogyakarta. The sampling procedure employed the Simple Random Sampling technique, where 2–3 symptomatic leaves were selected from each clump. Ten infected leaf samples were gathered from the field. The sampling process involved placing the symptomatic rice leaves in a 60 × 100 cm plastic bag. The bag was stored in a 24-L cooler box and promptly transported to the laboratory for further examination.

Isolation and Characterization of *Xanthomonas oryzae* pv. *oryzae.* The Xoo isolation was conducted by cultivating the bacteria on potato sucrose agar (PSA) media. The leaves of rice plants exhibiting bacterial leaf blight were sterilized using 70% alcohol. They were then sliced into sections approximately 1 cm in length at the boundary between healthy and diseased areas. These sections were placed in a test tube containing 5 mL of sterile distilled water and were then spread over PSA media. The samples were incubated at room temperature for 72 to 120 hours, during which a solitary yellow colony developed. To prove their pathogenicity, the isolated bacteria were tested for hypersensitivity reaction on tobacco. Physical and biochemical tests were also performed to confirm the presence of the Xoo bacterial leaf blight pathogen. These tests included the Gram staining test, catalase enzyme production, oxidase test, growth in anaerobic conditions, and starch hydrolysis (Ghasemie et al., 2008).

Macroscopic and Microscopic Morphology of *Xanthomonas oryzae* pv. *oryzae.* The selected Xoo isolates were examined for macroscopic morphological characteristics, including surface texture, edges, shape, and colour. Their microscopic morphology was also observed using a Scanning Electron Microscope (SEM) SNE4500M equipped with EDAX Elements at the Integrated Testing and Research Laboratory of Gadjah Mada University (LPPT UGM).

Pathotype Determination of *Xanthomonas oryzae* pv. *oryzae.* The Xoo isolates were inoculated into five distinct rice varieties, namely *Tetep* (4251), *PB 5* (4827), *Java 14* (11022), *Kencana Bali* (4477), and *Kuntulan* (1529). These varieties were selected from the Rice Plant Research Centre and were specifically bred to possess diverse genetic origins. The five rice varieties are commonly used as standard test varieties to identify and classify pathotypes of Xoo isolates (Lee et al., 2003). The density of Xoo inoculum used to inoculate rice plants was 10^9 CFU/mL (Yashitola et al., 1997). The Xoo isolates were inoculated by cutting the tip of rice leaves, measuring approximately 2-3 cm, using scissors dipped in Xoo suspension. This was done 40 days after planting, when the leaves were fully mature (10 leaves). The inoculated leaves were then covered with plastic for 24 hours and placed in an incubator at a temperature of 30 ºC (EPPO, 2007). Observations were conducted 14 days after inoculation to assess the response of each test variety to all Xoo bacterial isolates.

The formula proposed by Suparyono et al.

(2004) was used to calculate the disease intensity:

$$IP = \frac{a}{b} \times 100\%$$

IP = Disease intensity;
a = Length of bacterial blight symptoms (cm);
b = Overall leaf length (cm).

Subsequently, the results are displayed in Table 1.

Pathogenicity of *Xanthomonas oryzae* pv. *oryzae* on Several Varieties of Rice Seeds. The Xoo isolates were subsequently inoculated into six other rice varieties: *IR64*, *Ciherang, C4, Mekongga, Menthik Wangi,* and *Inpari 42*, which are the original host plants of the isolates. The density of Xoo inoculum applied to rice seedlings was 10^9 CFU/mL (Yashitola et al., 1997). The Xoo isolates were inoculated by cutting the tips of rice leaves, measuring around 2–3 cm, with scissors dipped in Xoo suspension. This was done 40 days after planting when the leaves were fully matured (10 leaves). The inoculated leaves were subsequently covered with plastic and incubated at a temperature of 30 °C for 24 hours (EPPO, 2007). Observations were conducted 14 days after inoculation to assess each test variety's response to the 10 Xoo isolates.

The calculation of disease intensity used the formula as described by Suparyono et al. (2004), based on the previous formula. The tested varieties were declared resistant if the disease intensity was ≤ 11% and susceptible if the severity disease was ≥ 12% (Sudir et al., 2009).

Molecular Characterization of *Xanthomonas oryzae* pv. *oryzae* DNA Extraction. The process of extracting DNA was carried out using the CTAB (Cetyl Trimethyl Ammonium Bromide) method (Ausubel et al., 2003). Xoo isolates were placed in a 1.5 mL tube with 540 µL of TE buffer. A volume of 30 µL of 10% sodium dodecyl sulphate (SDS) was introduced into the tube, followed by incubation at 37 ºC for 1 hour. Subsequently, 100 µL of 5 M NaCl and 80 µL of CTAB/NaCl were added to the tube, followed by incubation at 65 ºC for 10 minutes. Then, 750 µL of CIAA (chloroform: isoamyl alcohol, 24:1) were added, and the solution was mixed. The tube was centrifugated at 9000 g for 10 minutes. Subsequently, the liquid portion above the sediment, known as the supernatant, was carefully transferred to a new tube. After adding 100 µL of 5 M NaCl and 80 µL of CTAB/NaCl, the tube was incubated at 65 ºC for 10 minutes. Following the addition of 750 µL of CIAA (24:1), the solution was mixed and centrifuged at 9000 g for 10 minutes. The supernatant was transferred to a fresh tube and extracted by adding 600 µL of PCIAA (phenol: chloroform: isoamyl alcohol, 25:24:1). The sample was centrifuged at 9000 g for an additional 10 minutes. A volume of approximately 500 µL of the supernatant was withdrawn, and DNA was precipitated by adding 0.6× isopropanol (approximately 300 µL) or chilled 95% ethanol (approximately 1 mL), followed by incubation at -20 ºC for one hour. The precipitate was washed with 70% ethanol and then centrifuged at 9000 g for 10 minutes. After the supernatant was discarded, the pellet was dried in a laminar airflow hood (LAF) before being resuspended in 20-40 µL of TE buffer and stored at -20 ºC.

Molecular Detection of *Xanthomonas oryzae* pv. *oryzae*. A total of 20 Xoo isolates were identified using the Polymerase Chain Reaction (PCR) method. The primers used were Xoo3350-F (5'GCAAGCTGATCGGTATCCTC-3') and Xoo3350- R (5'-GCGAGACCTTGAACTGGAAC-3'), targeting a 300 base pair region (Lang et al., 2010). The amplification process was conducted in a 25 µL reaction mixture containing 2 µL of DNA template, 2 µL of each primer, 12.5 µL of MyTaq Red Mix (Bioline), and 6.5 µL of ddH2O. The reaction commenced with pre-denaturation at 95 ºC for 5 minutes, followed by denaturation at the same temperature for 30 seconds. Subsequently, annealing occurred at 58 ºC for 30 seconds, followed by extension at 72 ºC for 30 seconds. The reaction concluded with a final exten-

Table 1. Patterns of plant reactions

Variety	Pathotype											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Kencana Bali	S	S	S	S	S	S	S	S	S	S	S	R
PB5	R	S	S	S	R	R	S	S	S	R	S	R
Tetep	R	R	S	S	R	S	S	R	R	S	R	R
Kuntulan	R	R	R	S	S	R	S	S	S	S	S	S
Java 14	R	R	R	S	R	R	R	R	R	R	S	R

R = Resistance, disease intensity ≤ 11%; S = Susceptible, disease intensity ≥ 12% (Sudir et al., 2009).

sion at 72 ºC for 5 minutes, and the resulting mixture was stored at 14 ºC. The PCR was conducted for 35 cycles. PCR products were visualized by electrophoresis on a 1% agarose gel stained with ethidium bromide. A total of 5 µL of the PCR products were used for this purpose. The DNA bands were observed using a UV transilluminator.

Four Xoo samples were chosen based on the findings of prior pathotype determination. These samples include SlMo_3 (pathotype 4), SlPk_1 (pathotype 11), KpSg_4 (pathotype 8), and BaK_2, which exhibited the highest level of virulence. The four samples that produced PCR products measuring 300 base pairs were selected for sequencing using the 1st BASE Sanger sequencing service. Additionally, the sequences were subjected to phylogenetic analysis using Mega XI software and compared with sequences from the Xoo species complex obtained from GenBank (http://www.ncbi.nlm.nih.gov).

Analysis of Genetic Diversity of 4 Xoo Isolates. The four selected isolates were subsequently examined using a PCR machine with the assistance of two primers, JEL1 (5'-CTCAGGTCAGGTCGCC-3') and JEL2 (5'-GCTCTACAATCGTCCGC-3') (Islam et al., 2016). The amplification process was carried out in a 25 µL reaction mixture containing 2 µL of DNA template, 2 µL of each primer, 12.5 µL of MyTaq Red Mix (Bioline), and 6.5 µL of ddH2O. The reaction commenced with pre-denaturation at 95 ºC for 5 minutes, followed by denaturation at the same temperature for 30 seconds. Annealing occurred at 57.9 ºC for 30 seconds, followed by extension at 72 ºC for 30 seconds and a final extension at the same temperature for 5 minutes. The reaction was then stored at 14 ºC. The PCR process was conducted for a total of 35 cycles. Electrophoresis was used to visualize 5 µL of PCR products on a 1.5% agarose gel stained with ethidium bromide. The DNA bands were observed using a UV transilluminator.

The pattern of each DNA band was recorded in binary form using a numerical system, where 1 represented the presence of a band, and 0 represented its absence. This analysis was conducted using AlphaImager software. The dendrogram was created using the unweighted pair group method with arithmetic mean (UPGMA) in the NTSYS program (Islam et al., 2016).

RESULTS AND DISCUSSION

Collection of Samples. Twenty Xoo isolates were successfully obtained from rice production centers in several subdistricts in Yogyakarta, each located at different altitudes. The sampling locations are listed in Table 2 and depicted in Figure 1.

Isolation and Characterization of *Xanthomonas oryzae* pv. *oryzae*. The leaf samples exhibiting bacterial leaf blight were isolated and cultivated on PSA media, as described by Adachi et al. (2012). The results of the physiological and biochemical characterisation tests are presented in Table 3. The findings (Table 3) indicated that all isolates are gram-negative, have positive reactions in hypersensitivity, catalase, and oxidative growth tests, and display negative oxidase activity with an inability to hydrolyse starch. These characteristics are similar to those of Xoo isolates collected by Ghasemie et al. (2008) in Iran.

After 24 hours of inoculation on tobacco leaves, all Xoo isolates exhibited tissue death or necrosis. Xoo can elicit hypersensitivity and pathogenicity due to its reliance on the hrp gene, a common feature of gram-negative plant pathogenic bacteria, including *Xanthomonas* sp. (Zhu et al., 2000). Most bacteria belonging to the genus *Xanthomonas* exhibit catalase activity, require oxygen for growth, and do not produce spores (Niño-Liu et al., 2006). Bacterial catalase is involved in breaking down H_20_2 into H_2O and O_2, converting the harmful hydrogen peroxide into harmless molecules (Cappucino & Sherman, 2001).

Macroscopic and Microscopic Morphology of *Xanthomonas oryzae* pv. *oryzae*. All Xoo isolates cultivated on PSA media exhibit a colony morphology characterized by a spherical, convex shape, slimy texture, and yellow color. This is due to the production of the Xanthomonas pigment, a distinctive feature of the *Xanthomonas* genus (Figure 2A). The colonies have an oval-shaped morphology with rounded edges. The individual cells within the colonies vary in size, ranging from approximately 0.7 µm to 2.0 µm in length and 0.4 µm to 0.7 µm in width (Niño-Liu et al., 2006). The morphology of Xoo closely resembles the description provided by Liu et al. (2006).

Pathotype Determination of *Xanthomonas oryzae* pv. *oryzae*. Determining pathotype distribution in different rice-growing regions is conducted as a preventive measure to enhance the efficacy of resistant cultivars for disease control.

The virulence results of all Xoo isolates on the different varieties—*Kencana Bali, PB 5, Tetep, Kuntulan,* and *Java 14*—exhibited a wide range of

Table 2. Xoo isolates from 4 districts in Yogyakarta

No	Isolate code	Origin	Coordinate
1	GkPo_1 (209 masl)	Ponjong, Gunung Kidul	7º57’59.3”S 110º42’17.2”E
2	BaK_1 (64 masl)	Kasihan, Bantul	7º50’31.4”S 110º19’43.7”E
3	BaK_2 (64 masl)	Kasihan, Bantul	7º50’31.4”S 110º19’43.7”E
4	BaK_3 (64 masl)	Kasihan, Bantul	7º50’31.4”S 110º19’43.7”E
5	BaK_4 (64 masl)	Kasihan, Bantul	7º50’31.4”S 110º19’43.7”E
6	BaK_5 (63 masl)	Kasihan, Bantul	7º50’43.4”S 110º19’09.5”E
7	BaJ_1 (119 masl)	Jetis, Bantul	7°55’58.0”S 110°21’36.8”E
8	BaJ_2 (119 masl)	Jetis, Bantul	7°56’26.9”S 110°21’41.5”E
9	BaP_2 (55 masl)	Pleret, Bantul	7º52’48.4”S 110º25’08.0”E
10	BaS_1 (56 masl)	Sewon, Bantul	7º52’14.2”S 110º20’20.7”E
11	BaS_2 (51 masl)	Sewon, Bantul	7º52’36.3”S 110º20’38.1”E
12	KpSg_4 (137 masl)	Samigaluh, Kulon Progo	7º42’24.5”S 110º12’04.6”E
13	KpNg_1 (137 masl)	Nanggulan, Kulon Progo	7º45’00.8”S 110º11’34.2”E
14	SlPk_1 (430 masl)	Pakem, Sleman	7º39’47.5”S 110º25’12.9”E
15	SlPk_3 (553 masl)	Pakem, Sleman	7º38’25.9”S 110º25’26.4”E
16	SlMo_3 (99 masl)	Moyudan, Sleman	7º45’22.9”S 110º13’52.3”E
17	SlMi_1 (118 masl)	Minggir, Sleman	7º44’12.4”S 110º14’46.3”E
18	SlB_1 (104 masl)	Berbah, Sleman	7º47’54.5”S 110º26’36.3”E
19	SlB_2 (108 masl)	Berbah, Sleman	7º47’39.5”S 110º26’31.6”E
20	SlMl_2 (172 masl)	Mlati, Sleman	7º44’11.1”S 110º21’27.0”E

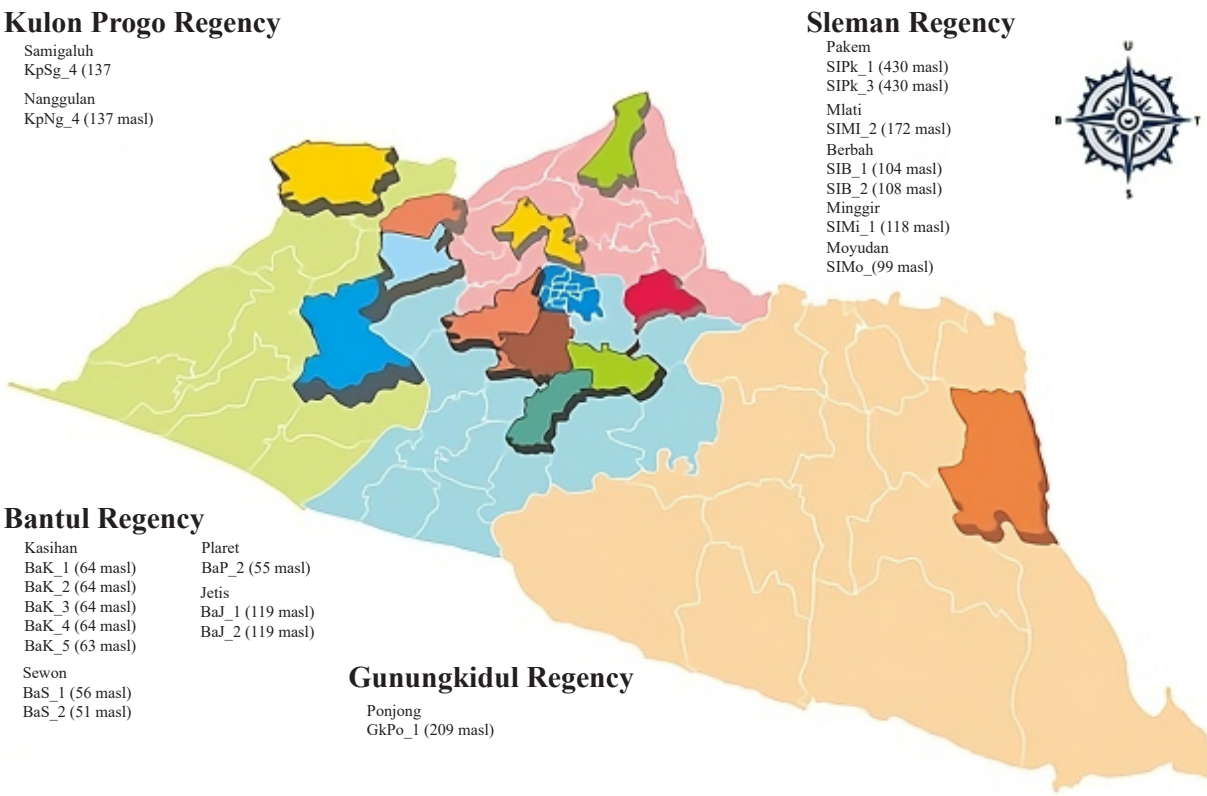

Figure 1. Sampling map of several sub-districts from Sleman, Kulon Progo, Bantul, and Gunungkidul districts.

Table 3. Characterization *Xanthomonas oryzae* pv. *oryzae*

No	Isolate	Hypersensitive Reaction	Gram	Catalase	Oxidase	Oxidative	Fermentative	Starch Hydrolysis
1	GkPo_1	+	-	+	-	+	-	-
2	BaK_1	+	-	+	-	+	-	-
3	BaK_2	+	-	+	-	+	-	-
4	BaK_3	+	-	+	-	+	-	-
5	BaK_4	+	-	+	-	+	-	-
6	BaK_5	+	-	+	-	+	-	-
7	BaJ_1	+	-	+	-	+	-	-
8	BaJ_2	+	-	+	-	+	-	-
9	BaP_2	+	-	+	-	+	-	-
10	BaS_1	+	-	+	-	+	-	-
11	BaS_2	+	-	+	-	+	-	-
12	KpSg_4	+	-	+	-	+	-	-
13	KpNg_1	+	-	+	-	+	-	-
14	SlPk_1	+	-	+	-	+	-	-
15	SlPk_3	+	-	+	-	+	-	-
16	SlMo_3	+	-	+	-	+	-	-
17	SlMi_1	+	-	+	-	+	-	-
18	SlB_1	+	-	+	-	+	-	-
19	SlB_2	+	-	+	-	+	-	-
20	SlMl_2	+	-	+	-	+	-	-

+ = Positive reaction, - = Negative reaction.

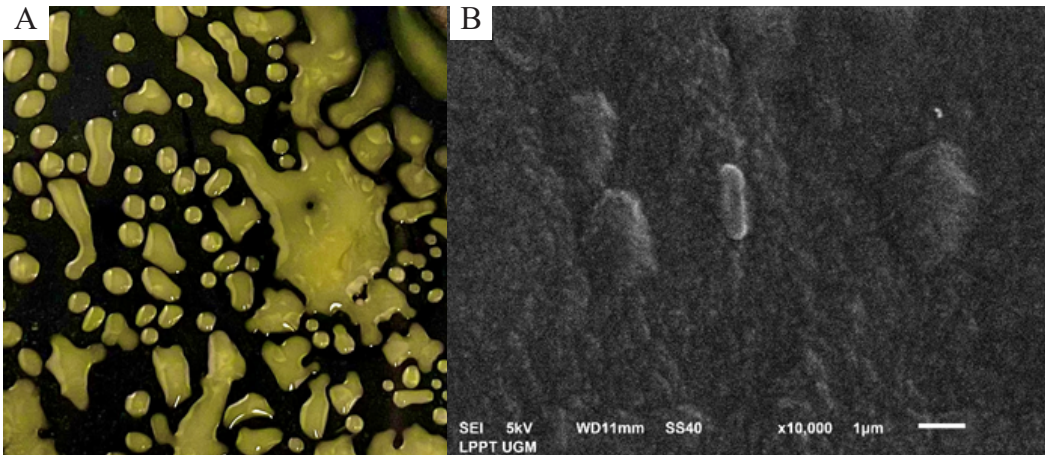

Figure 2. *Xanthomonas oryzae* pv. *oryzae*. A. Morphology of 72-hour-old Xoo colonies on PSA medium; B. Scanning electron microscopy of Xoo.

outcomes. The variation in virulence levels results from the interaction between the virulent genes of each Xoo bacterial isolate and the resistance genes in each differential variety (Sudir et al., 2013). The test results indicated that the bacterial isolates found in Yogyakarta Province can be categorised as pathotypes IV, VIII, and XI. The Xoo strain categorized as pathotype IV exhibits a high level of pathogenicity against all differential varieties. Xoo of pathotype VIII exhibits high virulence against *Kencana Bali, PB 5, Tetep,* and *Kuntulan* varieties but low virulence against *Java 14*. Conversely, Xoo of pathotype XI displays high virulence against *Kencana Bali, PB 5, Kuntulan*, and *Java 14* varieties but low virulence against *Tetep*.

Table 4. The results of inoculation of all Xoo isolates against differential varieties

No	Isolate	*Kencana Bali*		*PB 5*		*Tetep*		*Kuntulan*		*Java 14*		Pathotype
		DI (%)	Rating	DI (%)	Rating	DI (%)	Rating	DI (%)	Rating	DI (%)	Rating	
1	GkPo_1	54.90	S	87.13	S	34.13	S	61.31	S	49.54	S	IV
2	BaK_1	61.69	S	77.70	S	74.10	S	75.92	S	51.20	S	IV
3	BaK_2	99.30	S	96.72	S	84.62	S	97.09	S	98.72	S	IV
4	BaK_3	83.46	S	90.84	S	75.29	S	87.39	S	64.21	S	IV
5	BaK_4	68.95	S	92.22	S	83.27	S	34.17	S	96.71	S	IV
6	BaK_5	91.37	S	90.63	S	72.07	S	86.87	S	40.08	S	IV
7	BaJ_1	88.78	S	91.00	S	66.34	S	60.88	S	44.74	S	IV
8	BaJ_2	87.00	S	80.93	S	81.32	S	62.36	S	49.17	S	IV
9	BaP_2	86.30	S	93.34	S	83.24	S	85.10	S	30.66	S	IV
10	BaS_1	88.20	S	63.58	S	84.08	S	97.30	S	64.34	S	IV
11	BaS_2	88.65	S	91.93	S	78.44	S	85.38	S	32.03	S	IV
12	KpSg_4	82.37	S	97.24	S	92.99	S	63.29	S	7.85	R	VIII
13	KpNg_1	55.72	S	75.09	S	73.25	S	70.50	S	37.21	S	IV
14	SlPk_1	80.59	S	74.18	S	0.98	R	42.59	S	37.98	S	XI
15	SlPk_3	46.93	S	49.07	S	5.90	R	49.99	S	12.75	S	XI
16	SlMo_3	79.44	S	96.13	S	73.71	S	90.46	S	35.06	S	IV
17	SlMi_1	90.10	S	75.76	S	66.38	S	76.62	S	30.17	S	IV
18	SlB_1	76.42	S	77.20	S	81.02	S	75.52	S	77.93	S	IV
19	SlB_2	88.60	S	84.94	S	68.94	S	87.95	S	48.02	S	IV
20	SlMl_2	76.05	S	80.39	S	54.84	S	32.95	S	21.87	S	IV

DI = Disease intensity, R = Resistant, S = Susceptible.

Pathotype IV exhibits greater virulence than pathotypes VIII and XI, as indicated by its virulence reactivity to differential varieties. Table 4 displays the identification of Xoo pathotypes from different rice-producing centres in Yogyakarta using five distinct varieties.

The results of inoculation and the diversity of reactions to different varieties from 20 Xoo bacteria originating from various rice production centers in Yogyakarta showed that 17 isolates (85%) belonged to the pathotype IV group, one isolate (5%) belonged to the pathotype VIII group, and two isolates (10%) belonged to pathotype XI group. Pathotype IV is the most prevalent in the Bantul and Sleman districts. Pathotype VIII is found exclusively in the Kulon Progo district. The two pathotype XI isolates are primarily found in the Sleman district, particularly in the Pakem sub-district. This supports the finding of the Sudir & Yuliani (2016) study, which reported that the Xoo pathotypes prevalent in rice production centers in Yogyakarta were primarily pathotypes III, IV, and VIII.

The diversity of pathotypes in a given area is influenced by variations in plant physiological characteristics and altitude, which in turn affect temperature and humidity levels (Djatmiko et al., 2011). Isolates, mainly consisting of pathotypes IV and VIII, are found at similar elevations (as shown in Table 1), ranging from low to moderate heights below 200 metres above sea level (msal). The two isolates classified in pathotype XI share a similar elevation, specifically above 400 masl, which is considered relatively high. Suparyono et al. (2004) found that pathotype VIII, prevalent in Java, thrives in regions with low and medium altitudes, while pathotypes III and IV are limited to lower altitudes. Additional factors contributing to this phenomenon include genetic changes, inherent heterogeneity within the pathogen population, and the development of adult-plant resistance, a feature that manifests in plants at a specific stage of maturity (Sudir & Yuliani, 2016).

Pathogenicity of *Xanthomonas oryzae* pv. *oryzae* Against Several Varieties of Rice Seeds. The virulence results of all Xoo isolates were consistent when inoculated on six rice varieties, which included both the host plants of the isolate origin and common varieties used by farmers in Yogyakarta. These results are summarised in Table 5. Of all the isolates, 60% exhibited a high level of virulence against all varieties;

Table 5. The results of inoculation of all Xoo isolates against several varieties of rice seeds in Yogyakarta

No	Isolate	*IR64*		*C4*		*Ciherang*		*Mekongga*		*Inpari 42*		*Menthik Wangi*	
		DI (%)	Rating	DI (%)	Rating	DI (%)	Rating	DI (%)	Rating	DI (%)	Rating	DI (%)	Rating
1	GkPo_1	60.14	S	50.47	S	45.00	S	62.82	S	67.86	S	1.97	R
2	BaK_1	55.17	S	86.65	S	96.51	S	86.06	S	87.19	S	6.67	R
3	BaK_2	65.56	S	88.48	S	96.16	S	90.36	S	64.82	S	10.62	R
4	BaK_3	63.09	S	66.53	S	89.58	S	95.21	S	98.37	S	17.84	S
5	BaK_4	52.53	S	72.35	S	87.19	S	68.31	S	88.05	S	59.81	S
6	BaK_5	76.51	S	91.26	S	79.08	S	96.16	S	82.14	S	36.57	S
7	BaJ_1	57.76	S	84.83	S	82.01	S	96.69	S	99.42	S	54.97	S
8	BaJ_2	78.30	S	77.05	S	94.02	S	91.28	S	89.95	S	61.21	S
9	BaP_2	63.27	S	54.93	S	80.96	S	65.38	S	80.77	S	8.42	R
10	BaS_1	60.65	S	71.17	S	80.12	S	74.11	S	70.97	S	42.37	S
11	BaS_2	80.12	S	80.37	S	95.03	S	87.16	S	84.15	S	0.95	R
12	KpSg_4	83.31	S	71.76	S	99.66	S	96.64	S	71.56	S	79.67	S
13	KpNg_1	77.04	S	53.93	S	77.97	S	70.37	S	63.64	S	49.51	S
14	SlPk_1	19.93	S	50.81	S	62.18	S	51.89	S	52.98	S	7.65	R
15	SlPk_3	52.89	S	20.81	S	54.22	S	11.95	S	23.02	S	1.12	R
16	SlMo_3	67.38	S	86.33	S	97.03	S	92.24	S	78.21	S	21.48	S
17	SlMi_1	78.51	S	85.63	S	79.77	S	92.71	S	76.65	S	38.96	S
18	SlB_1	65.88	S	61.87	S	87.79	S	80.63	S	75.63	S	9.69	R
19	SlB_2	68.48	S	46.02	S	57.53	S	61.64	S	83.43	S	20.33	S
20	SlMl_2	68.48	S	46.02	S	57.53	S	61.64	S	83.43	S	20.33	S

DI = Disease intensity, R = Resistant, S = Susceptible.

however, 40% showed low virulence, specifically against the *Menthik Wangi* variety. This demonstrates that all regularly utilized varieties in Yogyakarta are susceptible to Xoo.

The prevalence of pathotype IV in the Yogyakarta area contributes to Xoo's high virulence against the *Ciherang* variety. According to Suprihatno et al. (2010), the *Ciherang* variety is resistant to Pathotype III but is sensitive to pathotypes IV and VIII.

Molecular Characterization of *Xanthomonas oryzae* pv. *oryzae*. The visualisation of the oryza PCR product revealed the detection of 20 isolates using Xoo-specific primers. All isolates produced DNA bands of 300 base pairs (bp) in size, as shown in Figure 3. This suggests that all the obtained isolates are Xoo bacteria. According to the BLAST program's comparison results, the four selected isolates exhibited 100% similarity with the Xoo species from GenBank (Figure 4).

Four representative isolates from each pathotype were chosen for DNA sequencing at LPPT UGM. The findings revealed the presence of four distinct clusters. Notably, isolate KpSg_4 exhibited a close relationship with Xoo strain IX-280 from Tanuku, India, and clustered with Xoo strain ScYc-b from Sichuan, China, Xoo strain K3 from Iksan-si, South Korea, and isolate BaK_2. Isolate SlMo_3 is genetically similar to Xoo XM9 from Pingtung, Taiwan, and is grouped with Xoo strain ScYc-b from Sichuan, China and Xoo strain AUST2013 from Australia. SlPk_1 is genetically similar to Xoo strain NX0260 from Nepal and Xoo strain PXO61 from the Philippines. The four isolates are not genetically related to Xoo strain CIAT from Colombia, suggesting they belong to a distinct bacterial species. In case of a discrepancy between the BLAST and phylogenetic tree analysis results, it is advisable to determine identification using a phylogenetic tree generated through global alignment. Since BLAST analysis is a form of local alignment, phylogenetic analysis tis more reliable for accurately assessing identity (Clarridge, 2004).

Figure 5 displays the DNA banding pattern of the four chosen Xoo isolates analysis. It is evident that three samples—samples 3, 16, and 12—exhibit comparable DNA banding patterns. The largest base size among these samples is 1000 base pairs, while the smallest

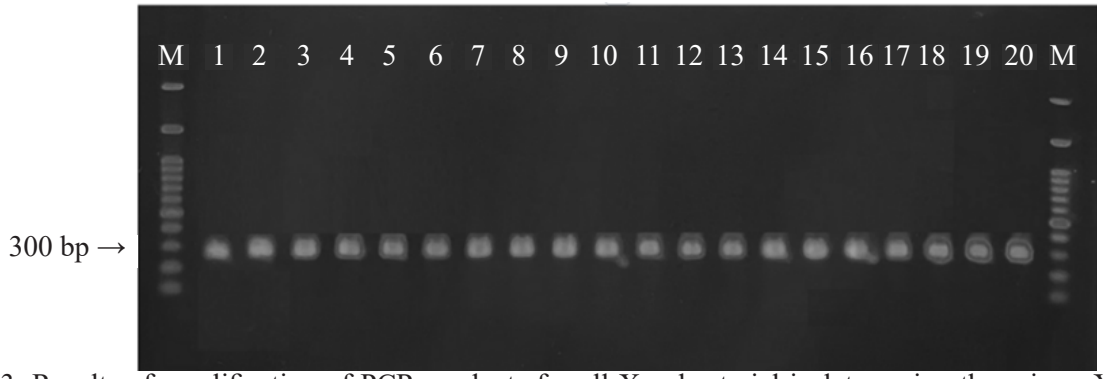

Figure 3. Results of amplification of PCR products for all Xoo bacterial isolates using the primer Xoo3350-F/ Xoo3350-R. The number above indicates the isolate code number as listed in Table 1; M = marker.

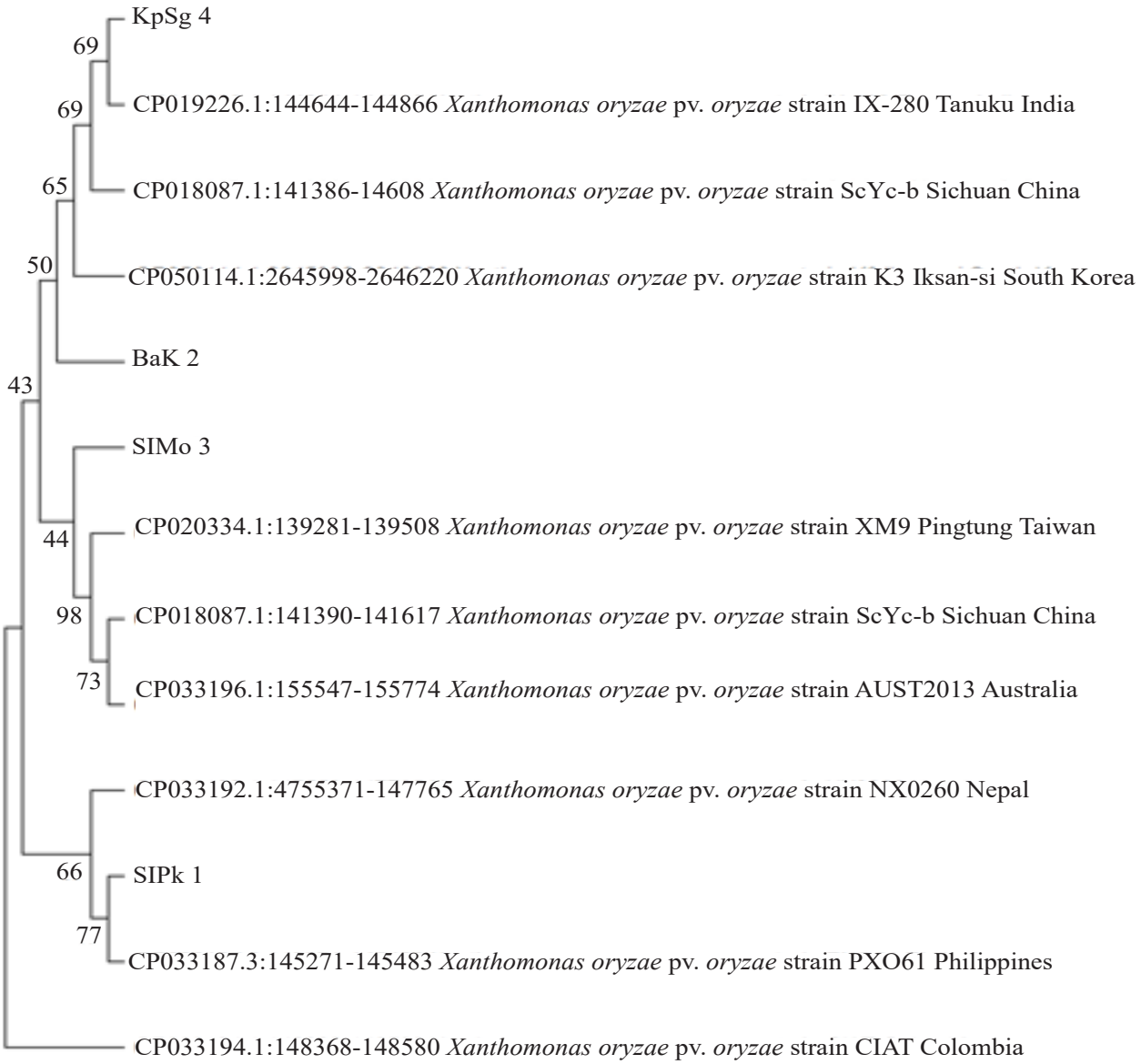

Figure 4. Phylogeny tree of Xoo detected from samples of rice leaf from Yogyakarta, constructed using Neighbor-Joining method with 1000 bootstraps (used Mega 11 program).

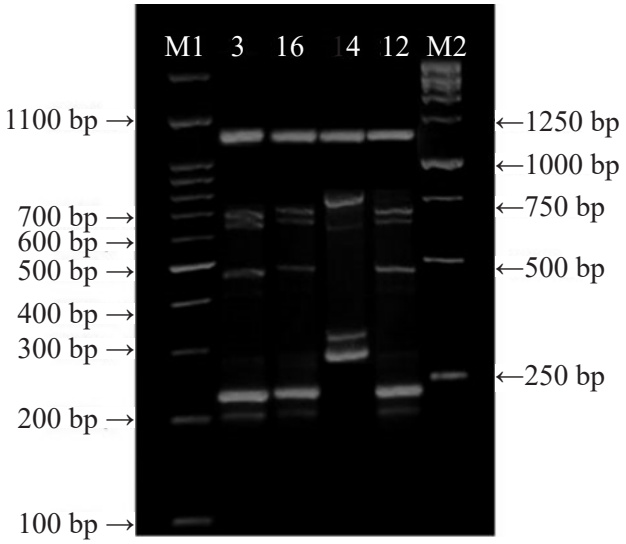

Figure 5. DNA banding patterns of the four selected Xoo isolates were generated using JEL1/JEL2 primers by PCR.

base size is 200 base pairs. Sample 14 has a distinct DNA banding pattern compared to the others, with the largest base size exceeding 1000 bp and the smallest within the range of 250–300 bp. The differences in the size and quantity of DNA bands indicate genetic variations among isolates. This suggests the presence of two distinct groups among the four isolates. Figure 4 illustrates these two distinct groups of Xoo isolates: BaK_2, SlMo_3, and KpSg_4 in one group and SlPk_1 in another. The coefficient indicates the percentage similarity between these isolates.

The yellow circular colonies distinguish the bacterial leaf blight caused by Xoo, which affects rice plants in the Yogyakarta region. It exhibits a negative Gram response, positive catalase activity, negative oxidase activity and does not hydrolyze starch. The Xoo pathotypes identified in Yogyakarta include IV, VIII, and XI. All six prevalent cultivars planted by farmers in Yogyakarta are susceptible to Xoo. Four isolates chosen from each pathotype exhibited two distinct groups based on the pattern of DNA bands produced.

CONCLUSION

Bacterial leaf blight that infects rice plants in the Yogyakarta region is caused by Xoo, which is characterized by yellow round colonies, a Gram-negative reaction, positive catalase activity, negative oxidase activity, and negative starch hydrolysis. The Xoo pathotypes found in Yogyakarta are pathotypes IV, VIII, and XI. The six common varieties used by farmers in Yogyakarta are susceptible to Xoo. Analysis of the four selected isolates from each pathotype revealed two distinct groups based on the pattern of DNA bands formed.

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AUTHORS' CONTRIBUTIONS

DMII is the first author, contributing to the research and writing of this manuscript. TA, as the corresponding author, considered the experimental design, act as a proofreader, revised the manuscript, and led and supervised the project. SS revised the methods and data analysis. The authors provided responses

and comments on the research flow, data analysis, interpretation, and the shape of the manuscript. All the authors have read and approved the final manuscript.

COMPETING INTEREST

The authors declared that there is no potential conflict of interest.

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