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Pathotype Determination of *Xanthomonas oryzae* pv. *oryzae (Xoo)*, the causal agent of Bacterial Leaf Blight and Resistance Reactions of several Rice Varieties in South Sulawesi

Nurmujahidin*, Baharuddin Patandjengi, Tutik Kuswinanti, & Andi Alfian Darmawan

Department of Plant Pests and Diseases, Faculty of Agriculture, Hasanuddin University, Makassar, South Sulawesi, Indonesia, 90245

*Corresponding Author: nurmujahidin@unhas.ac.id

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ABSTRACT

Bacterial leaf blight (BLB), caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo), is a major disease of rice. This study aimed to determine the Xoo pathotypes present in rice-growing areas of South Sulawesi and to evaluate the resistance of selected rice varieties. The research was conducted at the Agricultural Biotechnology Research and Development Center and the Plant Pests and Diseases Laboratory, Hasanuddin University, Makassar. Xoo isolates were cultured on Wakimoto medium and inoculated using the clipping method. Five differential varieties (Kinmaze, Wase Aikoku, Tetep, Java 14, and Kogyoku) were used to classify the pathotypes, while five commercial rice varieties (Inpari 6, Ciliwung, Ciherang, IR 64, and Pioneer) were tested for resistance. Six Xoo isolates were classified into pathotypes VI, VII, VIII, and XI, with pathotype VIII being the most dominant (33.3%). Inpari 6 and Ciliwung showed resistance to all tested pathotypes. Ciherang was resistant to most pathotypes but moderately susceptible to pathotype VIII. IR 64 and Pioneer displayed varying degrees of resistance and moderate resistance. These findings highlight the presence of diverse Xoo pathotypes and identify rice varieties with broad-spectrum resistance, providing valuable information for the development of BLB management strategies suited to specific regional conditions.

Keywords: diversity, management, resistance, screening

Penentuan Patotipe *Xanthomonas oryzae pv. oryzae* (Xoo), penyebab Hawar Daun Bakteri, dan Reaksi Ketahanan beberapa Varietas Padi di Sulawesi Selatan

ABSTRAK

Hawar daun bakteri (HDB), yang disebabkan oleh *Xanthomonas oryzae* pv. *oryzae (Xoo)*, merupakan salah satu penyakit utama pada tanaman padi. Penelitian ini bertujuan untuk menentukan patotipe *Xoo* yang terdapat di daerah sentra produksi padi di Sulawesi Selatan serta mengevaluasi ketahanan beberapa varietas padi terpilih. Penelitian dilaksanakan di Pusat Penelitian dan Pengembangan Bioteknologi Pertanian serta Laboratorium Hama dan Penyakit Tumbuhan, Universitas Hasanuddin, Makassar. Isolat *Xoo* dikulturkan pada media Wakimoto dan diinokulasikan menggunakan metode pemotongan daun (*clipping method*). Lima varietas diferensial (Kinmaze, Wase Aikoku, Tetep, Java 14, dan Kogyoku) digunakan untuk mengklasifikasi patotipe, sedangkan lima varietas padi komersial (Inpari 6, Ciliwung, Ciherang, IR 64, dan Pioneer) diuji untuk mengetahui reaksi ketahanannya. Enam isolat *Xoo* berhasil diklasifikasikan ke dalam patotipe VI, VII, VIII, dan XI, dengan patotipe VIII sebagai yang paling dominan (33,3%). Varietas Inpari 6 dan Ciliwung menunjukkan ketahanan terhadap seluruh patotipe yang diuji. Ciherang tahan terhadap sebagian besar patotipe, namun cukup rentan terhadap patotipe VIII. IR 64 dan Pioneer menunjukkan variasi tingkat ketahanan dari tahan hingga cukup tahan. Temuan ini menunjukkan keberadaan patotipe *Xoo* yang beragam serta mengidentifikasi varietas padi dengan ketahanan luas, yang dapat menjadi dasar penting dalam pengembangan strategi pengelolaan HDB yang sesuai dengan kondisi spesifik wilayah setempat.

Kata Kunci: keberagaman, ketahanan, pengelolaan, skrining

INTRODUCTION

Bacterial leaf blight (BLB), caused by *Xanthomonas oryzae* pv. *oryzae (Xoo)*, is a serious disease affecting rice cultivation in Asian countries, including Indonesia (Suparyono *et al.,* 2004; Hoang *et al*., 2008; Nayak *et al.,* 2008). In Indonesia, this disease is generally found in various types of rice fields in the lowland, midland, and even upperland areas (Suparyono *et al.,* 2003, Kadir *et al.,* 2009). In rice plants at the early growth stage, *Xoo* induces wilting symptoms known as kresek whereas at the tillering, flowering, and grain maturation stages, it manifests as blight symptoms (Suparyono *et al.,* 2004). Kresek and blight symptoms typically initiate at the leaf margins,

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progressing to a grayish discoloration and eventually leading to leaf desiccation. In susceptible rice cultivars, the symptoms of bacterial leaf blight may become systemic, resembling leaf scorch (Sudir *et al.,* 2012). This disease has the potential to cause a significant decrease rice production by as much as 15-80% depending on crop stage when the disease arises (Reddy & Shang-zi 1989; Shanti *et al.,* 2010).

Infections by *Xoo* cause significant yield losses, highlighting the need for effective control strategies. The use of resistance rice varieties remains a cost-effective and practical approach, especially for economically constrained farmers. However, continuous deployment of resistance varieties poses a challenge, as *Xoo* rapidly evolves, producing more virulent pathotypes that can overcome plant resistance. This high variability in virulence often leads to the breakdown of resistance and emerge new strains in the field (Suryadi & Kadir, 2004).

Pathotypes are identified based on their virulence or interactions with a selected set of differential rice varieties. *Xoo* is classified into 12 distinct pathotypes according to its virulence against the differential rice varieties Kinmaze, Kogyoku, Tetep, Wase Aikoku, and Java 14, each of which carries unique resistance genes (Suparyono *et al.,* 2003; Kadir *et al.,* 2004).

According to Ogawa (1993), effective management of diseases caused by pathogens with the ability to form distinct pathotypes, such as bacterial leaf blight, requires the strategic rotation of resistance rice varieties. the strategic rotation of resistance rice varieties must be carefully planned. This approach helps anticipate variations in *Xoo* strains, ensuring that varietal resistance remains effective and sustainable over time. Nayak *et al.,* (2008) & Hoang *et al.,* (2008) also stated that this strategy requires comprehensive data support, particularly concerning the mapping of existing pathotypes within a given ecosystem and the resistance background of the rice varieties intended for cultivation.

South Sulawesi is a major rice-producing region in Indonesia. According to Statistics Indonesia (BPS, 2024), rice production in the region declined from 5.36 million tons in 2022 to 4.82 million tons in 2024. One of the predominant factors contributing to this disease is the outbreak of *Xoo,* the causal agent of bacterial leaf blight. Therefore, as a fundamental strategy for managing bacterial leaf blight through the application of resistance rice varieties in South Sulawesi, research is required to evaluate the resistance responses of various rice varieties and to determine the distribution of *Xoo* pathotypes that are dominant in South Sulawesi.

MATERIALS AND METHODS

This research was conducted at the Agricultural Biotechnology Research Center Activity and the Plant Pests and Diseases Laboratory, Hasanuddin University, Makassar. The research activities were divided into two parts: Pathotype determination of *X. oryzae* pv. *oryzae* on differential varieties and evaluation of resistance reactions in several commercial varieties in South Sulawesi to *X. oryzae* pv. *oryzae.*

Research Methods

Rejuvenation of *X. oryzae* pv. *oryzae* Isolates

The *Xoo* isolates used in this research were obtained from a previous research performed by Andi Herwati (2014) and consisted of six distinct isolates (Table 1). Cultures of *Xoo* were streaked on Wakimoto Agar (WA) media with IRRI (1996) modification (300 gr potato in infusion form, 17 g sucrose, 7 g peptone, 0.5 g $Ca(NO_3)_2{\cdot}4H_2O$, 1g $Na_2HPO_4{\cdot}12H_2O$, 17 g bacto agar, with the volume adjusted to 1 L using distilled water (dH_2O). The isolates were incubated for 2-3 days and stored at room temperature. The presence of bacterial colonies on the medium is indicated by the appearance of yellowish-white colonies (Semangun, 2000).

Pathotype Determination of *X. oryzae* pv. *oryzae* Using Differential Rice Varieties

Rice seeds of Tetep, Kinmaze, Kogyoku, Wase Aikoku, and Java 14 were used in this research. These five varieties are widely recognized as differential hosts for the identification and classification of *Xoo* pathotypes (Lee *et al.,* 2003). Seeds were initially germinated in a substrate composed of soil, dung, and husk at 2:1:1 ratio. Each variety was cultivated in three pots, with each pot serving as a replicate, and one seedling was grown per pot. After 10 days, the seedlings were transplanted into pots filled with medium composed of topsoil and dung in 3:1 ratio and cultivated for 30 days before further treatment.

Table 1. Origin of *X. oryzae pv. oryzae* bacterial isolates

No.	Isolate Code	Rice Variety	Place of Origin	Hypersensitive reaction on Tobacco Leaves
1.	MG 02	Mekongga	Gowa	+
2.	IT 01	IR 64	Bantaeng	+
3.	CB 01	Cisantana	Barru	+
4.	MS 01	Mekongga	Soppeng	+
5.	CT 03	Cisantana	Bantaeng	+
6.	*Xoo* III	*Xoo* III	Bogor	+

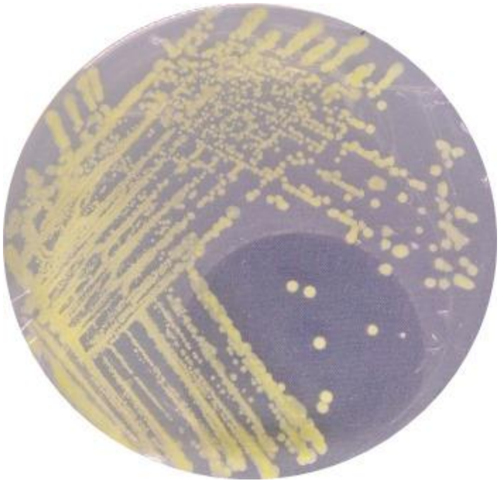

Figure 1. *X. oryzae* pv. *oryzae* colonies on Wakimoto Agar

Inoculum was prepared by transferring a loopful of *Xoo* colony into 10 mL of liquid Wakimoto medium, and incubating it on a rotary shaker for 48 hours at room temperature (Tasliah *et al.,* 2013). The bacterial inoculum suspention was adjusted to a density of 10^9 CFU mL^{-1} (Yashitola *et al.,* 2007), six characterized *Xoo* isolates were inoculated onto the differential varieties using clipping method by wounding 2–3 cm of leaf tips with scissors dipped in a bacterial suspension. Inoculated plants were covered with plastic for 24 hours and incubated. Pathotype classification was based on the interaction between differential varieties and isolate virulence (Table 2). Treatments were replicated three times.

The determination of *Xoo* pathotypes was based on their virulence levels on differential rice varieties, which possess distinct resistance genes to bacterial leaf blight, as defined by Suparyono *et al.,* 2003.

Table 2. Pathotype grouping of *X. oryzae* pv. *oryzae* with differential varieties

Differential variety	Resistance genes	Resistance response to *Xoo*											
Kinmaze	None	S	S	S	S	S	R	S	S	S	S	S	R
Kogyoku	Xa-1, Xa-12	R	S	S	S	R	R	S	S	S	R	S	R
Tetep	Xa-1, Xa-2	R	R	S	S	R	S	S	S	R	S	R	R
Wase Aikoku	Xa-3 (Xa-12)	R	R	R	S	S	R	R	S	S	S	S	S
Java 14	Xa-1, Xa-2, and Xa-12	R	R	R	S	R	R	S	R	R	R	S	R
Xoo pathotype grouping		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII

Remarks: R = Resistance, disease intensity <11%; S = Susceptible, disease intensity >11%

Evaluation Of Resistance Reactions in Several Commercial Varieties to *X. oryzae* pv. *oryzae*

Resistance reactions was performed on five commercial rice varieties including Ciherang, Inpari 6, Ciliwung, IR 64, and Pioneer with three replications (each pot serving as a replicate). Plants at the peak tillering stage (30-40 days old) were inoculated following the same method as for differential varieties. Resistance to *Xoo* was assessed based on symptoms development 15 days after inoculation.

The intensity of the disease was measured following the method proposed by Suparyono *et al.,* 2004, as shown on the following formula:

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

DI = Disease Intensity
a = Length of bacterial blight symptoms (cm)
b = Overall leaf length (cm)

The resistance reaction of each varieties were evaluated using the Standard Evaluation System (SES) published by the International Rice Research Institute (IRRI, 1996) as shown on Table 3 below.

Table 3. Scale for bacterial leaf blight disease

Disease scale	Disease Intensity (%)	Disease reaction
0	0%	Highly resistance (HR)
1	1-5%	Resistance (R)
3	6-12%	Moderately resistance (MR)
5	13-25%	Moderately susceptible (MS)
7	26-50%	Susceptible (S)
9	51-100%	Highly susceptible (HS)

RESULT AND DISCUSSIONS

Pathotype Determination of *X. oryzae* pv. *oryzae* on Differential Varieties

A total of six *Xoo* isolates exhibited diverse pathotypes, as determined by their interactions with five differential rice varieties. This was determined by the range of disease intensity observed on the inoculated differential varieties, with symptoms varying from mild (below 10%) to highly severe (more than 60%). Table 4 below shows that the composition and distribution of these *Xoo* pathotypes varied across the five major rice-producing regencies in South Sulawesi.

Table 4. Pathotype grouping of *Xoo* based on resistance reactions on differential varieties

Isolate code	Kinmaze			Kogyoku			Tetep			Wase Aikoku			Java 14			Pathotype Group
	DI (%)		Rt	DI (%)		Rt	DI (%)		Rt	DI (%)		Rt	DI (%)		Rt	
CB 01	6.79	± 0.56	R	4.84	± 0.43	R	33.2	± 1.37	S	4.79	± 0.16	R	9.21	± 0.31	R	VI
IT 01	14.6	± 2.01	S	13.2	± 2.19	S	6.25	± 2.17	R	36.7	± 0.93	S	17.3	± 0.10	S	XI
MG 02	13.4	± 0.30	S	29.1	± 2.53	S	31.3	± 1.96	S	7.55	± 0.42	R	31.4	± 3.05	S	VII
CT 03	23.7	± 1.43	S	27.0	± 1.08	S	22.3	± 0.99	S	15.0	± 0.95	S	9.40	± 1.11	R	VIII
MS 01	43.4	± 2.25	S	14.5	± 1.24	S	42.5	± 0.96	S	67.8	± 0.53	S	7.83	± 1.94	R	VIII
Xoo III	25.5	± 3.86	S	37.0	± 1.38	S	12.9	± 0.82	S	9.90	± 0.58	R	10.7	± 0.24	R	III

Remarks: DI = Disease Intensity; Rt = Rating
R = Resistance, disease intensity <11%; S = Susceptible, disease intensity >11% (Suparyono *et al.,* 2003).

Based on their interaction with five differential varieties, these isolates were determined as four pathotype groups, namely VI, VII, VIII, and XI. Among these, pathotype VIII was the most dominant, representing 33.3% of the total, while pathotypes VI, XI, and VII were each constituted 16.67%. Kinmaze is a differential rice variety that does not contain any resistance genes, while Kogyoku carries two dominant resistance genes, Xa-1 and Xa-12. Tetep also possesses two dominant genes, Xa-1 and Xa-2. Wase Aikoku includes Xa-3 and Xa-12, and Java 14 contains three resistance genes: Xa-1, Xa-2, and Xa-12 (Suparyono *et al.,* 2003; Nayak *et al.,* 2008). Xoo isolates tested in this study were highly virulent to Kinmaze, Kogyoku, and Tetep, while their virulence was relatively low when tested against Wase Aikoku and Java 14.

Isolate CB 01, grouped as *Xoo* pathotype VI, exhibits high virulence on the differential variety Tetep, but shows lower virulence on Wase Aikoku, Kinmaze, Java 14, and Kogyoku. Isolate IT 01, grouped as pathotype XI, exhibits high virulence across all differential varieties except Tetep. Meanwhile, isolate MG 02 is grouped into pathotype VII and displays strong virulence against Kinmaze, Kogyoku, Tetep, and Java 14, but low virulence on Wase Aikoku. Isolates CT 03 and MS 01, identified as pathotype VIII, shows high virulence on Kinmaze, Kogyoku, Tetep, and Wase Aikoku, while exhibiting low virulence on Java 14.

Variation in disease intensity among rice varieties is primarily influenced by the interaction between host resistance genes and the virulence genes of *Xoo* isolates. Factors such as adult-plant resistance, environmental conditions, genetic mutations, and the natural heterogeneity of pathogen populations contribute to this variation. Adult-plant resistance, which appears at specific growth stages, significantly affects disease intensity and yield loss (Suparyono *et al.,* 2003). Khaeruni (2001) reported that the spread and development of pathogens are also influenced by environmental conditions specific to a given location, which may vary considerably. High humidity and dense planting spacing are key factors that facilitate the transmission of diseases among plants. According to Ou (1985), *Xoo* Bacteria grows optimally under conditions of humidity exceeding 90% and temperature between 25-30ºC. Environmental factors such as wet and dry seasons also significantly influence the pathotype diversity of *Xoo*. Suparyono *et al.,* (2003) reported that in the 2001 dry season, pathotypes III and VIII were equally dominant (42.7% and 42% respectively) in several Java provinces. However, during the 2001/2002 rainy season, pathotype VIII became dominant (63%), followed by IV (29%) and III (9%), reflecting a seasonal shift in pathotype composition. Natural genetic diversity within microbial populations also plays a role (Sudir *et al.,* 2013). Suparyono *et al.,* (2003) noted that genetic variation among rice varieties influences the diversity of *Xoo* pathotypes. Pathotype classification is based on specific virulence genes in the pathogen that interact with resistance genes in the host, following the gene-for-gene relationship model (Agrios, 2005). Sudir & Suprihanto (2008) found that rice growth stages influence *Xoo* pathotype distribution, with pathotype VIII dominant during tillering and flowering, and pathotype III appearing at ripening.

Evaluation of Resistance Reactions in Several Commercial Varieties to *X. oryzae* pv. *oryzae*

A total of five commercial rice varieties that inoculated with *Xoo* isolates developed bacterial leaf blight symptoms, with leaf tips initially turning pale green or grayish, followed by yellowing. Symptoms first appeared at 7 days after inoculation and progressed along the leaf margins, originating from one or both edges and extending across the entire leaf blade. By 15 days after inoculation, lesion lengths had reached an average of 1 cm.

All rice varieties inoculated with *Xoo* showed typical bacterial leaf blight symptoms. These symptoms were characterized by elongated lesions with wavy edges starting at the leaf tip and spreading along the margins, with discoloration progressing from pale green or grayish to yellow blight (fig. 2). Symptoms became visible approximately two weeks after inoculation. These findings align with the descriptions provided by Liu *et al.,* (2006) and Kadir *et al.,* (2009). Infected leaf showed lesions that first emerged at the cut site of the leaf tip. Bacterial leaf blight can infect rice plants through mechanical wounds or natural openings such as stomata and hydathode, subsequently spreading into the xylem tissue (Agrios, 2005; Wahyudi *et al.,* 2011).

The *Xoo* bacterial isolates after being tested for virulence on differential rice varieties were inoculated into several commercial rice varieties. The results are shown in table 5.

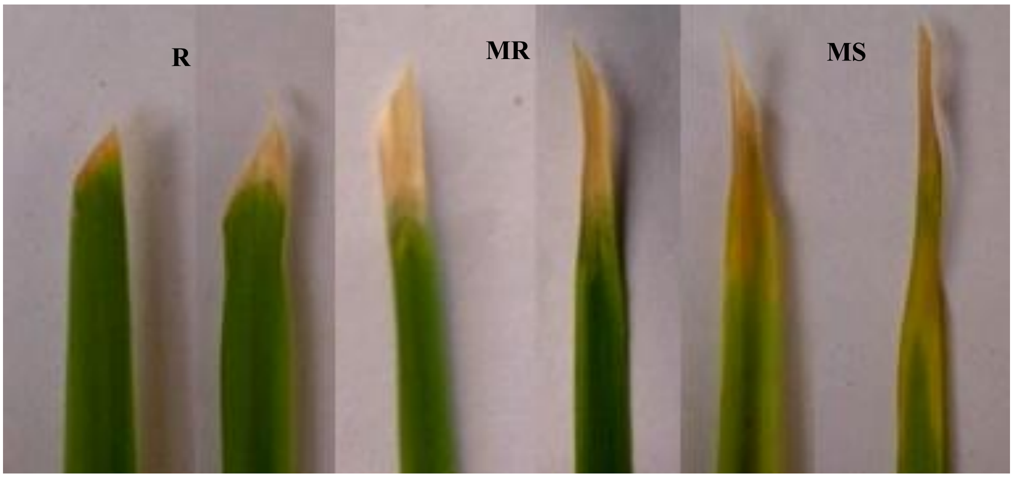

Figure 2. Responses of several rice varieties to inoculation with *Xoo* R = Resistance; MR = Moredately Resistance; MS = Moderately Susceptible

Table 5. Resistance reactions of five commercial rice varieties to *X. oryzae* pv. *oryzae*

Variety	CB 01 (VI)		IT 01 (XI)		MG 02 (VII)		CT 03 (VIII)		MS 01 (VIII)		*Xoo* III	
	DI (%)	Rt	DI (%)	Rt	DI (%)	Rt	DI (%)	Rt	DI (%)	Rt	DI (%)	Rt
Pioneer	2.30 ± 0.45	R	2.10 ± 0.33	R	3.56 ± 0.61	R	7.05 ± 1.21	MR	2.69 ± 0.63	R	3.16 ±0.88	R
Inpari 6	3.19 ± 1.02	R	3.71 ± 0.52	R	3.09 ± 0.74	R	4.51 ± 2.01	R	3.63 ± 1.07	R	3.41 ±0.26	R
IR 64	2.14 ± 0.52	R	2.18 ± 0.29	R	8.41 ± 0.81	MR	4.06 ± 0.74	R	3.38 ± 0.52	R	9.35 ±0.95	MR
Ciliwung	3.31 ± 0.42	R	3.32 ± 1.03	R	2.28 ± 0.31	R	3.95 ± 1.27	R	3.61 ± 1.07	R	4.22 ±0.48	R
Ciherang	2.58 ± 0.52	R	2.31 ± 0.25	R	5.21 ± 2.08	R	4.41 ± 2.21	R	14.81± 0.83	MS	3.62 ±0.26	R

Remarks: DI = Disease Intensity; Rt = Rating
R = Resistance (1-6%); MR = Moderately Resistance (6-12%); MS = Moderately Susceptible (13-25%)

Observations conducted 15 days after inoculation showed that Inpari 6 and Ciliwung varieties resistance to *Xoo* pathotypes III, VI, XI, VII, and VIII with low disease intensity (2.28–4.22%). These varieties showed the highest level of resistance compared to other tested varieties (Pioneer, Ciherang, and IR 64). This indicated that the presence of multiple *Xa* resistance genes in both varieties contributes to their broad-spectrum resistance against various pathotypes. According to Nafisah *et al.,* (2007), varieties with more *Xa* genes tend to show stronger resistance, supporting the high resistance observed in these two varieties.

The Pioneer variety showed strong resistance to pathotypes III, VI, XI, and VII, particularly to pathotype XI (2.10% disease intensity). It presented moderate resistance to pathotype VIII from Bantaeng (7.05%) but higher resistance to the same pathotype from Soppeng (2.69%). This variation likely results from interactions between plant resistance genes and the virulence of local *Xoo* strains. Suparyono *et al.,* (2003) stated that resistance durability is influenced by factors such as pathotype composition, mutation rate, planting frequency, and genetic diversity of rice varieties planted in specific time and location. Mew (1989) stated that pathogen species exhibit genetic variability, leading to distinct races that, while morphologically similar, vary in their ability to infect specific host varieties. This explains why a resistance variety in one region or time may become susceptible elsewhere or later, due to shifts in the infecting pathogen race.

The IR 64 variety showed moderate resistance to pathotypes VII and III, and strong resistance to pathotypes VI, XI, and VIII. Ciherang showed resistance to pathotypes III, VI, XI, and VII, however, it was moderately susceptible to pathotype VIII, with a disease intensity of 14.81%, the highest among all tested varieties inoculated with *Xoo*. This may be attributed to Ciherang's specific resistance only to pathotypes III and IV (DPTP, 2011).

The findings of this research are expected to serve as a reference for varietal zoning strategies in major rice-producing regencies of South Sulawesi based on the dominant *X. oryzae* pv. *oryzae* pathotypes present in specific areas. For instance, in regions where pathotype VIII is dominant, the use of varieties resistance to this pathotype is advised. Similar recommendations apply to areas where pathotypes VI and XI are predominant. Therefore, selecting rice varieties based on their resistance to the dominant pathotypes present in a given area can effectively reduce the incidence of bacterial leaf blight disease.

CONCLUSION

Determination of rice plant pathotypes in several regencies in South Sulawesi grouped into four types of pathotype, namely VI, VII, VIII, and XI pathotypes, with pathotype VIII was dominant, representing 33.3% of the total, followed by pathotypes VI, XI, and VII were each observed at frequency of 16.67%. This information showed that their distribution dominance varied across regencies.

The Inpari 6 and Ciliwung varieties were resistance to pathotypes III, VI, VII, VIII, and XI. Ciherang was resistance to pathotypes III, VI, VII, and XI, but moderately susceptible to pathotype VIII. IR 64 showed resistance to pathotypes VI, VIII, and XI, and moderate resistance to pathotypes III and VII. Pioneer showed resistance to pathotypes III, VI, VII, and XI, with moderate resistance to pathotype VIII.

Based on the results of this research, Inpari 6 and Ciliwung are recommended for wide-scale cultivation across all four examined regencies (Gowa, Bantaeng, Barru, and Soppeng). The Ciherang variety is suitable for planting in Gowa and Barru but is less recommended for Bantaeng and Soppeng. IR 64 demonstrates high suitability in Barru, Bantaeng, and Soppeng, and moderate suitability in Gowa. Lastly, the Pioneer variety is strongly recommended for Gowa and Barru and is also considered appropriate for Bantaeng and Soppeng. Cultivating resistant rice varieties that are specifically to the dominant *Xoo* pathotypes in a certain regencies can significantly contribute to reducing the incidence and intensity of bacterial leaf blight disease.

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