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# Resistance Test Of Several Rice (*Oryza sativa* L.) Varieties Against Bacterial Leaf Blight Disease (*Xanthomonas oryzae* pv. *oryzae*)

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**Abstract**

Rice (*Oryza sativa* L.) is a great staple crop in Asia, particularly in Indonesia, where it serves as a primary source of carbohydrates. One of the major challenges to rice cultivation is bacterial leaf blight, caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo). To address this issue, effective and environmentally sustainable control strategies, such as the development and use of resistant varieties are essential. This study aimed to evaluate the resistance of several rice varieties to bacterial leaf blight. Research was conducted at the Phytopathology Laboratory and Greenhouse, Department of Plant Pests and Diseases, Plant Protection Study Program, Faculty of Agriculture, Lambung Mangkurat University, Banjarbaru. A Completely Randomized Design (CRD) was used, including five rice varieties (Mayang, Pandak, Karang Dukuh, Siam Madu Murakarta, and Siam Marli) and control. Each treatment was replicated four times, and each control was replicated twice, resulting in a total of 30 experimental buckets. The variables assessed included disease incubation period and disease intensity. Karang Dukuh variety exhibited the longest incubation period (9.48 days), while Siam Marli had the shortest (7.10 days). After three weeks of observation, the final disease intensity values are as follows: Mayang 1.51%, Pandak 1.55%, Karang Dukuh 1.05%, Siam Madu Murakarta 1.09%, and Siam Marli 1.77%. This result indicate that all tested varieties fall within the resistant category.

*Keywords* : *Bacterial Leaf Blight; Xanthomonas oryzae* pv. *oryzae; Rice; Resistant Varieties*

## Introduction

Rice (*Oryza sativa* L.) is a cultivated plant that is very important for every resident in the world, especially in Indonesia, because rice as processed is the main source of food and carbohydrates (Utama, 2015). Based on data from the Central Statistics Agency (BPS, 2023), national rice production experienced a decline in 2023. In 2022, rice production reached 54.75 million tons with a land area of 10,452,672 Ha, but fell to 53.63 million tons with a land area of 10.196,887 Ha. In contrast, rice paddy production in South Kalimantan increased from 819.42 thousand tons to 835.28 thousand tons, although the land area decreased from 214,909 Ha to 211,765 Ha.

One important factor that can affect the productivity of rice plants is the attack of plant pest organisms, especially bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae*. This disease has become a serious problem in various rice-producing countries, including in Indonesia since the 1980s (Wening *et al*., 2016). According to Herlina & Tiur (2011) bacterial leaf can infect rice plants in the vegetative phase to the generative phase and can reduce the quantity of rice yield by 30-40% and reduce the quality of rice produced.

One of the efforts that can be made to overcome the problem of bacterial leaf

blight is the development and use of resistant rice varieties, which is an effective and environmentally sustainable control strategy. According to Suryaningsih *et al*., (2023), the selection of rice varieties resistant to bacterial leaf blight can help reduce the risk of infection and yield losses. This study aims to determine the level of resistance of five rice varieties (Mayang, Pandak. Karang Dukuh, Siam Madu Murakarta and Siam Marli) to bacterial leaf blight caused by the pathogen *Xanthomonas oryzae* pv. *oryzae*,

## Research Method

This study used a completely randomized design (CRD) which included five rice varieties (Mayang, Pandak, Karang Dukuh, Siam Madu Murakarta, and Siam Marli) and a control. Each treatment was repeated four times, and each control per treatment was repeated twice, so the total experiment was 30 buckets.

### Research Preparation

### Tool Sterilization

Tools that will be used for research must be sterilized first to avoid contamination from unwanted live microorganisms. Sterilization is carried out using an oven for 1 hour at 170°C.

### Preparation of YDC (*Yeast Dextrose Calcium Carbonate*) Media

YDC media (*Yest Dextrose Calcium Carbonate*) is a selective media that can be used to grow Xanthomonas oryzae bacteria, YDC media is made from 1000 ml of distilled water, 10 g yeast extract, 20 g dextrose, 20 g Calcium carbonate ($CaCO_3$) and 15 g agar. All ingredients are mixed one by one and heated, then put into a glass bottle and closed using aluminum foil and cling wrap, after that sterilize the media using an autoclave with a pressure of 15 psi at a temperature of 121°C for 30 minutes.

### Pathogen Isolate Preparation

Bacterial leaf blight-symptomatic rice plants obtained in the field (Sungai Ulin Banjarbaru area, South Kalimantan) had symptoms of gray-brown leaves and drying. Rice plant samples were taken and put into clear plastic for isolation. Pathogen isolation is carried out by cutting the leaves of symptomatic rice plants, then put in 70% alcohol and rinsed with sterile water, do so until the third sterile water and dried on sterile tissue. After that, the leaves of symptomatic plants are crushed using a mortar and pestle until smooth, then added with 1 ml of sterile water, and gently smooth. From this extract, 1 ml of suspension is put into a test tube that has been filled with 9 ml of sterile water to dilute, then vortexed so that the suspension is homogeneous. In the same way, dilutions continue to be carried out successively up to 4 times ($10^4$dilution) (Herawati *et al*., 2017).

The dilution results were then grown on YDC media and incubated for 2-3 days, then bacterial colonies suspected of *Xanthomonas oryzae* pv. *oryzae* were grown on new YDC media until a single colony was obtained. According to Herawati *et al*. (2017), bacterial colonies suspected of *Xanthomonas oryzae* pv. *oryzae* are yellow, have a round colony shape, show a gram-negative reaction and are pathogenic to plants.

**Observation of Pathogenic Isolates**

Pathogenic isolates were tested for gram reaction using 3% KOH solution. Bacterial colonies are taken from the culture using an ose needle, then placed on a preparate glass and dripped with 3% KOH solution, the mixture is then stirred evenly with an ose needle while gently lifting it as high as 0.5-1 cm. If the colony looks slimy and attached, it shows a positive reaction indicating that the bacteria are classified as Gram Negative (G-), and vice versa if it is not slimy and easily removable, then the results show a negative reaction indicating that the bacteria are classified as Gram Positive (G+). *Xanthomonas oryzae* pv. o*ryzae* bacteria showed a positive reaction, namely slimy and attached which included Gram Negative (G-) (Herawati *et al*., 2017).

**Soil Strelization**

The planting medium used to grow rice consists of a mixture of soil and manure. Before use, the planting media must be sterilized first using a soil sterilizer.

**Seeding**

The seeds to be sown are selected first by soaking the rice seeds, for water adjusted to the number of seeds, soaking is done for 24 hours. The sunken seeds are then dried and matured for 24 hours on paper towels. After that, the seeds are arranged in trays with moist and watery soil conditions, and stored in a dark place until the seeds germinate, maintained for 14 days.

**Planting**

Rice seedlings that were 14 days old (2 weeks) after sowing were transferred to buckets that contained planting media in the form of a mixture of soil and sterile manure as many as 10 seedlings/bucket.

**Maintenance**

Maintenance is done by watering and weeding. Watering is done every day in the morning and evening to keep the media moist and wet, while weeding is done every week by pulling weeds around the test plants.

**Research Implementation**

The inoculation process of *Xanthomonas oryzae* pv. *oryzae* was carried out once when the rice plants were 42 days after transplanting (HST) or in the vegetative phase. Inoculation is done with the scissors method for leaf opening as an entry point for bacterial infection, by cutting leaves transversely 5 cm long using sterile scissors and which have been dipped in *Xanthomonas oryzae* suspension pv. *oryzae*. After cutting, 2 cm long leaves are dipped in *Xanthomonas oryzae* pv. *oryzae* suspension with a density of $10^8$ (Herlina & Tiur, 2018). Inoculation should be done in the afternoon at 15.00 - 17.00 WIB to avoid the heat of the sun and high evaporation (Hadianto *et al*., 2015). After inoculation, the plants are covered using plastic to maintain the moisture of the plants.

**Observation Variable**

The variables observed were incubation period and disease intensity. The incubation period is the time required for a pathogen to infect a plant. The incubation

period was observed every day after inoculation, until disease symptoms appeared on the test plants. While disease intensity is the percentage of tissue area in plants attacked by pathogens from the total area carried out in the observation, disease intensity is observed every week after inoculation (MSI) for 3 observations.

Calculation of disease intensity, bacterial leaf blight according to Khaeruni *et al*., 2016 as follows:

IP = $\frac{n}{N}$ $x$ 100%

Description :
IP : Disease Intensity (%)
n : *Xoo* Symptom Length (cm)
N : Overall Leaf Length (cm)

Resistance of rice plants to bacterial leaf blight was measured based on disease intensity and then entered into the *Standart Evaluation System for Rice* (IRRI, 1996).

Table 1. Criteria for resistance of rice varieties to HDB disease (IRRI, 1996).

| Scale | Symptom Area in Leaf Area (%) | Resilience Level |
|---|---|---|
| 0 | No attack | Very Resistant (ST) |
| 1 | Leaf area with blight symptoms 1-5% | Resistant (R) |
| 3 | Leaf area with blight symptoms 6-12% | Somewhat Resistant (AT) |
| 5 | Leaf area with blight symptoms 13-25% | Somewhat Vulnerable (AR) |
| 7 | Leaf area with blight symptoms 26-50% | Vulnerable (R) |
| 9 | Leaf area with blight symptoms 51-100% | Highly Vulnerable (SR) |

## Results and Discussion

### Incubation Period

The incubation period is the time required for a pathogen to cause disease symptoms on plants after inoculation, observations of the incubation period were made every day. Based on observations, the onset of bacterial leaf blight symptoms in each rice variety tasted showed different incubation periods, ranging from 7.10 – 9.48 days (Table 2).

Table 2. Incubation period of bacterial leaf blight in rice varieties inoculated with *Xanthomonas oryzae* pv. *oryzae*

| Varieties | Incubation Period (HSI) |
|---|---|
| Mayang | 8,55 |
| Pandak | 8,48 |
| Karang Dukuh | 9,48 |
| Siam Madu Murakarta | 9,10 |
| Siam Marli | 7,10 |

Description: HSI = Days After Inoculation

The Marli Siamese variety has the fastest incubation period, at 7.10 days. This is followed by the Pandak variety with 8.48 days and the Mayang variety with 8.55 days. Meanwhile, the Madu Murakarta and Karang Dukuh Siamese varieties have the longest incubation period, 9.10 days and 9.48 days respectively. According to research by

Hadianto *et al*., (2015), the incubation period of each rice variety against bacterial leaf blight ranged from 4 to 10.67 days after inoculation. Differences in the incubation period of each variety are not only influenced by plant genetic factors, but can also be influenced by environmental factors. According to Tasliah (2012), the development of bacterial leaf blight is influenced by warm temperatures ranging from 25-30$^{o}$C, high humidity and rainfall, and non-optimal water management.

The initial symptoms of bacterial leaf blight are characterized by the presence of gray-brown spots on the tips of the leaves after inoculation (Figure 1A) and slowly spread towards the base of the leaves which can eventually cause the leaves to wilt and dry out (Figure 1B). This is in accordance with the opinion of Yuliani *et al*., 2017) which states that symptoms arise from the edges of brown to grayish leaves and over time will roll up and dry out, also in line with Rismawati *et al*., 2024 that blight symptoms appear from the tips and edges of the leaves of inoculated plants, the leaves become brown to grayish and dry out.

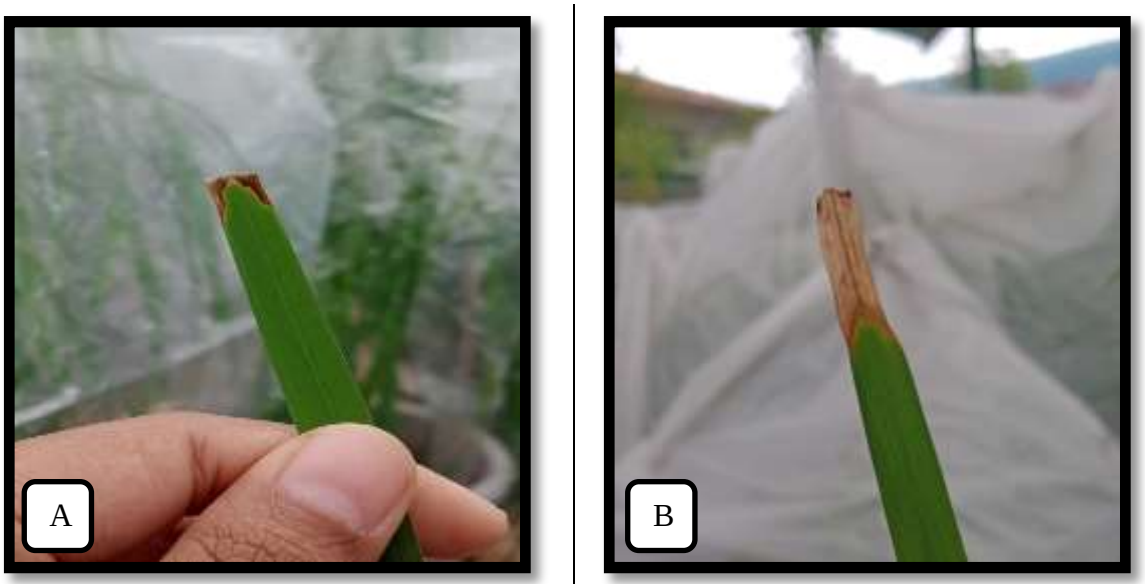


Figure 1. Symptoms of bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*) on inoculated leaves; A. early symptoms, B. late symptoms

**Disease Intensity**

Observation of disease intensity were made in week 1 (7 HSI), week 2 (14 HSI), and week 3 (21 HSI). The observation results can be seen in table 3.

Table 3. Disease intensity and resistance of rice varieties to bacterial leaf blight (Xanthomonas oryzae pv. oryzael based on the Rice Standard Evaluation System (IRRI, 1996).

| Varieies | 7 HSI | | 14 HSI | | 21 HSI | |
|---|---|---|---|---|---|---|
| | IP (%) | KK | IP (%) | KK | IP(%) | KK |
| Mayang | 0,18 | ST | 0,67 | ST | 1,51 | T |
| Pandak | 0,25 | ST | 0,99 | ST | 1,55 | T |
| Karang | 0,19 | ST | 0,48 | ST | 1,05 | T |
| Dukuh | | | | | | |
| Siam | 0,15 | ST | 0,56 | ST | 1,09 | T |
| Madu | | | | | | |
| Murakarta | | | | | | |
| Siam | 0,20 | ST | 0,67 | ST | 1,77 | T |
| Marli | | | | | | |

Notes: HSI = Days After Inoculation, IP = Disease Intensity. KK = Resistance Category. ST = Very Resistant, T = Resistant

Based on the observation of disease intensity, all rice varieties tested showed a high resistance response to pathogen attack. In week 1 (7 HSI) and week 2 (14 HSI) observations, all rice varieties were categorized as highly resistant with disease intensity <1%. Meanwhile, in the 3rd week observation (21 HSI) although there was an increase in disease intensity, all varieties were still included in the resistant category, with a range between 1.05% (Karang Dukuh) - 1.77% (Siam Marli). The criteria for assessing resistance refers to the Standard Evaluation for Rice by IRRI (1996) (Table 1), which states that varieties with disease intensity <5% are generally categorized as resistant or very resistant to bacterial leaf blight.

In general, resistance is the ability of a plant that is used to resist or avoid pest or disease attacks. Each plant has different plant resistance to pathogens, plant resistance is divided into 2 mechanisms, namely active resistance and passive resistance. Active resistance is a reaction caused by a plant after being infected by a pathogen. In this mechanism the plant will release certain chemical compounds such as phenolic compounds that are antimicrobial. While passive resistance is a form of defense that a plant already has naturally before pathogen infection enters because the plant has a thick cuticle on the surface of the leaves and a waxy layer (Adikarsih & Ruly, 2015).

According to Carsono *et al*., (2021), plants with high resistance or resistance to disease will not provide opportunities for the proliferation of bacteria or pathogens, while in plants with low resistance or susceptible defense mechanisms are not able to inhibit the development of pathogens so as to provide opportunities for bacteria or pathogens to proliferate quickly. The difference in resistance of each rice variety to *Xanthomonas oryzae* pv. *oryzae* is also influenced by genetic factors, especially resistance genes controlled by the R. major gene (Liu *et al*., 2006, in Djatmiko & Fatichin, 2009). According to Rahim *et al*., (2012), varieties with a longer incubation period may have better resistance to a pathogen.

In addition, inoculation in this study was carried out at the age of 60 days after planting (HST), later than the planned time of 42 HST. This delay was caused by technical constraints in the field, one of which was the difficulty of obtaining pathogen isolates within the specified time. The time difference is expected to affect the results of

observationsespecially in the response of plant resistance to disease. Plants inoculated at an older age have generally entered the generative phase, while the age of 42 HST is still in the vegetative phase. Djatmiko & Fatichin. (2009) stated that the vegetative phase is a phase that is more vulnerable to pathogen infection than the generative phase, because in the generative phase the plant tissue is more mature and able to show a more effective defense response.

## Conclusion

The conclusion of this research is that the tested rice varieties have different incubation periods, ranging from 7.10 - 9.48 days and all varieties are included in the category of resistance to bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*), with a disease intensity value of less than 2%.

Suggestions for further research, it is necessary to test bacterial leaf blight resistance on other types of rice plant varieties, in order to obtain more resistant varieties and when inoculation can be done on time.

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