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Research Article

The Resistance of Local Pigmented Rice Varieties against Bacterial Leaf Blight

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ABSTRACT

Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is an important disease in rice plants worldwide, including in Indonesia. The use of resistant variety against BLB is the best effort in disease control because it is considered as the most effective and environmentally friendly. This study aimed to evaluate the resistance of local varieties, red rice (cv. Sembada Merah) and black rice (cv. Sembada Hitam), against Xoo compared to white rice variety (Ciherang), commonly cultivated by the farmers. The study was conducted in a greenhouse and arranged in Factorial of Completely Randomized Design (CRD) with 5 replications and 2 treatment factors: variety (Sembada Hitam, Sembada Merah, and Ciherang) and inoculation (with and without inoculated by Xoo bacterial suspension). Xoo inoculation was performed by clipping the leaves that close to the reproductive phase when the heading begins to be produced. The disease intensity and Area Under Disease Progress Curve (AUDPC) over two weeks were used to determine the level of resistance of the tested plant varieties. The results showed that black rice and red rice had better levels of resistance against BLB compared to white rice. At two weeks after inoculation, Sembada Hitam had the lowest AUDPC value followed by Sembada Merah, while the Ciherang had the highest AUDPC value and was categorized as a susceptible variety to Xoo.

Keywords: area under disease progress curve, black rice, red rice, resistance response, *Xanthomonas oryzae* pv. *oryzae*

INTRODUCTION

Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is an important diseases of rice caused by pathogenic bacteria (Nino-Liu *et al*., 2006) aside from bacterial grain rot caused by *Burkholderia glumae* (Joko, 2017; Handiyanti *et al*., 2018). In Indonesia, yield losses caused by Xoo can reach 21–36% in the rainy season and 18–28% in the dry season (Suparyono *et al*., 2004). Furthermore, in India yield losses reached 6–60% and in Japan reached 20–50% (Adhikari *et al*., 1995). In addition, Xoo can also reduce the quality of rice. Xoo can infect the vegetative to generative phases of the plants by entering the plant tissues through natural openings, i.e. hydatodes and contaminated seeds (Adhikari *et al*., 1999). Like other hemibiotrophic pathogens, Xoo produces extracellular enzymes such as cellulases and proteases as one of the virulence factors (Yun *et al*., 1992; Xu & Gonzalez, 1989; Joko *et al*., 2000; Trianom *et al*., 2019). These extracellular enzymes are needed to soften plant tissues so hence the pathogen can penetrate the intercellular spaces between plant cells. Yun *et al*. (1993) reported that cellulase enzymes were also required for survival when Xoo lived as saprophytes in dead plant tissue.

BLB is difficult to control because the causal pathogen has many pathotypes and its population in rice plantations is very diverse and dynamic (Akhtar *et al*., 2008). The variability of the Xoo pathotype is increasingly diverse along with the formation of new pathotypes that break plant resistance in the field (Sudir *et al*., 2012). In Indonesia, there are 12 pathotypes of Xoo with different levels of virulence and being a major threat to national rice production (Wahyudi *et al*., 2011). Several pathotypes are found in a certain region with one or several dominant pathotypes. The population structure of Xoo can be influenced by environmental changes such as seasonal differences and the presence of resistance genes in rice plants (Dardic *et al*., 2003). The continues planting of one type of variety on a large scale may cause a change in the pathotype hence Xoo becomes more virulent (Suparyono *et al*., 2004). Sudir *et al.* (2012) reported that Xoo pathotype in the central production of rice in Java consisted of pathotype III (12.8%), IV (18.6%), and VIII (68.6%), the highest level of virulence is pathotype IV. The use of resistant varieties against dominant pathotype in certain areas is one of the best strategies in controlling Xoo. Besides to be effective, the control technique using the resistant varieties is economical way and easy to do as long as resistant varieties are available. In Indonesia, breeding of resistant varieties through selection has been done for a long time and has successfully obtained several resistant varieties to Xoo (Herlina & Silitonga, 2011). Resistant varieties are recommended to be used in efforts to control Xoo because it is more environmentally friendly than the use of bactericides that leave residues and triger pathogens resistance.

Indonesia has various local rice cultivars that potential to be developed as functional food, one of those is pigmented rice which is rich in fiber, i.e. red rice and black rice (Kristamtini & Purwaningsih, 2009). Those rice are also high in vitamin, mineral and anthocyanin content (Kristamtini *et al*., 2012). Apart from being the functional food; red and black rice also be developed as resistant local cultivars to disease. Mau *et al.* (2018) reported the resistance of local red and black rice cultivars to blast disease caused by *Pyricularia grisea*. According to Gianinetti *et al.* (2018), the accumulation of pigment in the caryopsis layer in red and black rice is a barrier to protect the plants from pathogen infections. Therefore, this study aimed to determine the resistance response to BLB of local varieties of red and black rice originated from Sleman Regency, the Special Region of Yogyakarta.

MATERIALS AND METHODS

Test Plants and Bacterial Isolates

The rice varieties used for the resistance test were black rice (Sembada Hitam), red rice (Sembada Merah), and white rice as a control (Ciherang). The rice was grown until close to the reproductive phase (when the headings started to be produced). This research was arranged in Factorial of Completely Randomized Design (CRD) where each pot contained 3 rice leaves as a sample, with 5 replications and 2 treatment factors: variety (V1, Sembada Hitam; V2, Sembada Merah, and V3, Ciherang) and inoculation (P1, Xoo inoculation; P2, without Xoo inoculation) (Wibowo *et al*., 2010; Widyaningsih *et al*., 2017). Therefore, this study has 6 treatment combinations:

V1P1: Sembada Hitam with Xoo inoculation
V1P2: Sembada Hitam without Xoo inoculation
V2P1: Sembada Merah with Xoo inoculation
V2P2: Sembada Merah without Xoo inoculation
V3P1: Ciherang with Xoo inoculation
V3P2: Ciherang without Xoo inoculation

The bacterial pathogen (Xoo) was isolated from Sleman Regency, the Special Region of Yogyakarta. Xoo isolates were grown on Peptone Sucrose Agar (PSA) medium. Preparation of PSA medium was employed by mixing 5 g of sucrose, 1.25 g of peptone, 0.125 g of K_2PO_4, 0.0625 g of $MgSO_4.7H_2O$, and 5 g of agar into 250 ml of distilled water in Erlenmeyer. The pH of 7.2–7.4 was measured before agar was put into Erlenmeyer using a universal pH indicator paper (Nurjanah *et al*., 2017). Xoo culture was incubated for 48 hours at room temperature, then a suspension was made using sterile distilled water, and cell density were measured using a spectrophotometer to obtain a density of 10^8 CFU/ml.

Inoculation of **Xanthomonas oryzae** *pv.* **oryzae**

Xoo inoculation to rice plants was conducted using the clipping method by soaking the sterile scissors in Xoo suspension or sterile distilled water for a few seconds, then the scissors were used to cut rice leaves with a distance of 2 cm from the leaf tip (Djatmiko *et al*., 2011). Then the plants were covered overnight with plastic to maintain the moisture.

The Resistance of Rice Against Bacterial Leaf Blight

The resistance of rice against BLB was tested by observing the development of disease symptoms, the disease intensity, and conducting an AUDPC (Area Under Disease Progress Curve) analysis. In this study, the observation of the symptoms of BLB was employed 2 times over 14 days after inoculation: the first and the second week. The damage level (Table 1) used to calculate the disease intensity on each leaf inoculated by Xoo was calculated according to Suparyono *et al.* (2004):

$$\text{Damage level} = \frac{\text{symptom length}}{\text{leaf length}} \times 100\%$$

Table 1. Score of damage level (Solekha *et al.*, 2019)

Score	Damage level (%)
0	asymptomatic
1	1−6
3	> 6−12
5	>12−25
7	>25−50
9	>50−100

The disease intensity was calculated based on the score from the damage level using the following formula (Ismiyatuningsih *et al.*, 2016; Widyaningsih *et al.*, 2019):

$$DI = [\Sigma\ (ni \times vi) / (N \times V)] \times 100\%$$

Remarks:
DI: disease intensity
ni: number of the plant with a score of i
vi: disease score of i
V: the highest score that can be achieved
N: number of plants observed

The development of BLB during observation in this study could be analyzed using the Area Under Disease Progress Curve (AUDPC) obtained from the disease intensity. AUDPC values were calculated according to Sutrisno *et al.* (2018) as follows:

$$AUDPC = \frac{\sum_{i=1}^{n}\left(\frac{x_{i+1}+x_i}{2}\right)x[t_{i+1}-t_i]}{N-1}$$

Remarks:
Xi: the disease intensity at the time of observation
Q: time after the infection appears in the field (days)
n: number of observations

Statistical Analysis

AUDPC were statistically analyzed with ANOVA by IBM SPSS version 23 and further analyzed using LSD (P = 0.05).

RESULTS AND DISCUSSION

Xoo infects plants through hydathodes and wounds on the leaves. In this study, the entry of infection was an artificial wound made by cutting the leaf tip using scissors dipped in the Xoo suspension. In the vascular system, bacteria reproduce and move in two ways, i.e. vertically through the main vein and laterally through the connecting vein (Sharma *et al.*, 2017). In addition, extracellular production of polysaccharides, extracellular enzymes, and toxins are important factors for the virulence of pathogenic bacteria (Suvendra *et al.*, 2000; Joko *et al.*, 2018). These factors will produce a leaf blight symptom in the form of a lesion that extends through the leaf vein (Figure 1).

The results showed that all varieties produce symptoms of BLB but they have different intensity levels according to the defense mechanism of each variety. The development of BLB in each variety was determined based on the disease intensity in rice leaves after Xoo infection. In the first week, the disease intensity in Ciherang has reached 46.7%, while in Sembada Merah and Sembada Hitam were 24.4% and 28.9%, respectively. In the second week, the disease intensity in Ciherang increased to 86.7%, while in Sembada Merah and Sembada Hitam were 73.3% and 46.9%, respectively (Table 2). Thereby, the highest disease intensity of disease is in Ciherang. Pigmented rice, i.e. Sembada Merah and Sembada Hitam have better resistance, based on the value of disease intensity and lower AUDPC compared to Ciherang which is commonly cultivated by farmers, but the variety is susceptible to BLB. Djatmiko *et al.* (2011) reported that the widespread planting of susceptible variety (Ciherang) triggered the development of a new patotype group of Xoo in Banyumas Residency.

The disease intensity of BLB increased every week in all varieties. That finding showed that Xoo strain has a high virulence resulting in an increase in disease intensity. The different plant responses cause the intensity levels of each variety also different. The differences in disease intensity of the three varieties began to appear in the first week, disease intensity in Sembada Merah and Sembada Hitam was lower than Ciherang until the second week might cause by the differences in the flavonoid content of the three varieties, especially the anthocyanin content (Hidayah, 2017). Kangatharalingam *et al.* (2002) reported that in cotton leaves, anthocyanin production in the leaf epidermis is an indicator of resistance to leaf blight (*X. campestris* pv. *malvacearum*). Therefore, the pigmented rice which has a higher anthocyanin content might have better resistance than Ciherang. Furthermore, pigment accumulation in pigmented rice caryopsis layer also acts as a protective barrier against pathogenic attack (Gianinetti *et al.*, 2018).

Disease development in a certain time of period can be evaluated by comparing disease progress among varieties to determine the resistance level of

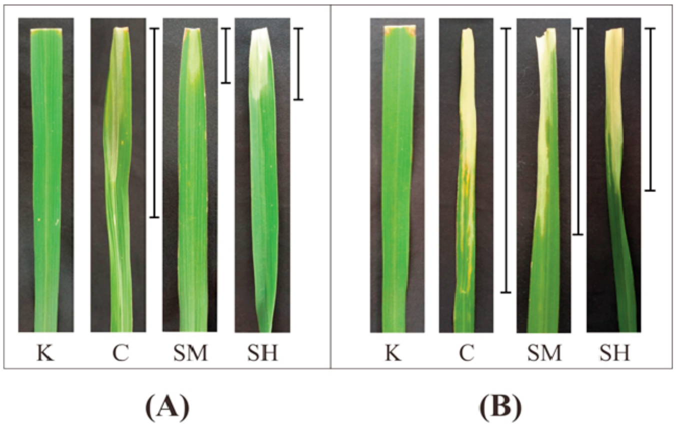

Figure 1. Symptoms of bacterial leaf blight during 2 times observations, in the first week (A) and the second week (B); inoculation of *Xanthomonas oryzae* pv. *oryzae* was performed on white rice cultivar (Ciherang, C), red rice cultivar (Sembada Merah, SM), and black rice cultivar (Sembada Hitam, SH); as a control (K), the rice leaves were inoculated with mock treatment: suspension of *X. oryzae* pv. *oryzae* replaced with sterile aquadest

Table 2. The disease intensity of bacterial leaf blight and AUDPC in three rice varieties over 2 weeks after inoculated by *Xanthomonas oryzae* pv. *oryzae*

No.	Variety	Disease intensity (%) week of		AUDPC
		1	2	
1	Ciherang	46.7	86.7	466.90a
2	Sembada Merah	24.4	73.3	341.95b
3	Sembada Hitam	28.9	46.9	265.30b

Remarks: values followed by the same letters were not significant difference according to LSD (P = 0.05).

plants to the disease. In this study, the resistance of rice varieties to BLB caused by Xoo was evaluated by observing the development of the disease in three rice varieties using AUDPC to assess disease resistance quantitatively. The higher the disease progression, the greater the AUDPC value. The resistance test to three varieties of rice against Xoo infection showed that pigmented rice had a lower AUDPC value and was significantly different compared to that of white rice (Table 2). This indicated that pigmented rice has a better level of resistance. Table 2 also revealed that the development of BLB in Ciherang variety was higher than pigmented rice. The highest AUDPC value was in Ciherang, a susceptible variety to bacterial leaf blight as control, while lower AUDPC values were in pigmented rice varieties. The resistance of pigmented rice varieties, namely Sembada Merah and Sembada Hitam, against Xoo was similar. This showed that Sembada Merah and Sembada Hitam are resistant to BLB, whereas the Ciherang is susceptible to BLB.

Specific defense mechanisms and systems in each plant are the cause of differences in plant responses to BLB. The disease development in susceptible varieties is faster than medium resistant or resistant varieties. Resistance to BLB was showed by Sembada Merah and Sembada Hitam might be caused by the ability of recognition by plant cells to pathogens, Xoo. This recognition induces a defense response by resistant plants to prevent Xoo colonization and disease development. The defense response involves a resistance gene (*R*–gene). Some *R*–gene products act as receptors and initiate defense responses. The rice resistance gene against Xoo is named with the prefix *Xa* followed by a specific number determined based on the time of discovery of the *Xa* gene. Recently, 40 *Xa* resistance genes have been identified in a series from *Xa1* to *Xa40* (Kim *et al.,* 2015). The first *R*-gene will activate another *R*–gene. This ongoing gene expression could strengthen and multiply defense signals and produce responses (antimicrobial metabolites, proteins related to pathogenesis, enzymes that protect against oxidative stress, stress-related hormones, cell wall lignification, fortification, and hypersensitive responses), which leads to cell death to block the spread of pathogens. In addition, race-specific pathogenic resistance in rice against Xoo is determined by the major disease resistance (MR). Thirty–seven MR genes are identified as encoding resistance against Xoo (Zhang & Wang, 2013). Pigmented rice is most likely has an MR gene expressed better than white rice in response to resistance against Xoo.

CONCLUSION

The local Pigmented rice varieties, which is originated from Sleman Regency, the Special Region of Yogyakarta, i.e. Sembada Merah and Sembada Hitam, have better resistance against BLB compared to Ciherang which is widely cultivated by the farmers.

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