Response of a local rice variety Pandanwangi to infection of bacterial leaf blight \textit{Xanthomonas oryzae} pv. \textit{oryzae} strain III, IV, and VIII

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Abstract. Bacterial leaf blight (BLB) caused by \textit{Xanthomonas oryzae} pv. \textit{oryzae} (\textit{Xoo}) is one of the limiting factors in rice production. A local cultivar, rice "Pandanwangi" is a superior variety much preferred and cultivated by the farmers in Cianjur, West Java, Indonesia. The information about the response of "Pandanwangi" to \textit{Xoo} is still poorly understood. This paper reports the results of the evaluation of "Pandanwangi" response against BLB. This research was conducted in a greenhouse with artificial inoculation using \textit{Xoo} strains III, IV, and VIII with bacterial suspension at $10^8$ cfu.mL$^{-1}$. The results showed that the response of cv Pandanwangi to \textit{Xoo} infection was different from the strain of \textit{Xoo}. "Pandanwangi" cultivar was susceptible to \textit{Xoo} strain III and VIII and very susceptible to strain IV.

1. Introduction

Food needs always increase along with the increase in population. The Indonesian population continues to grow every year. If it is not accompanied by increasing food production, it will have the opportunity to face problems in food needs in the future [1]. Rice is a staple food for most Indonesian population [2]. A rice cv Pandanwangi is superior rice originated from Cianjur, West Java, Indonesia. It has high economic value due to by very specific flavor taste [3].

Increasing rice production is an effort to fulfill the growing food demand. Efforts to increase rice production always get disturbances from pests and diseases [4]. Bacterial leaf blight (BLB) is a limiting factor in rice production [5,6]. BLB is an important disease in lowland rice in all world regions, including in Indonesia [7]. \textit{Xanthomonas oryzae} pv. \textit{oryzae} (Ishiyama) Swings et al. (\textit{Xoo}) is the causal agent of BLB [8]. \textit{Xoo} can attack in all stages of rice [9]. The pathogen attacking earlier will cause higher yield losses [10].

BLB can reduce rice production by up to 90% [11]. Use tolerant varieties is one of the control alternatives of BLB. Tolerant varieties are considered more effective, inexpensive, and safe to use because they do not pollute the environment [12]. Commonly tolerance character is owned by local variety. One of the popular local varieties of rice in Indonesia is "Pandanwangi". The cultivar has high economic value due to the specific flavor. The response of plants against disease infection will be...
determined by the strain of the pathogen [10]. Therefore, it is necessary to do a research on the \textit{Xoo} bacterial strain that attacks the rice production of "Pandanwangi".

2. Materials and methods

The research was conducted in the greenhouse at the Faculty of Agriculture, Universitas Sebelas Maret. The research is geographically located at 7°33'41" S.L. and 110°51'32" E.L. The study was conducted from October 2018 to February 2019, with daily temperatures ranging from 30–43°C.

The research used a nested design. The rice varieties used are Pandanwangi, IR64 as susceptible control, and Cilamayamuncul as a resistant control. I.R. 64 rice variety is susceptible to BLB disease [13]. Cilamayamuncul variety is rice that is resistant to BLB disease [14]. The \textit{Xoo} isolates used were strains III, IV, and VIII, the most abundant in Indonesia [15].

For each variety of rice, 3 seedlings of rice were planted in one polybag, then inoculated with one \textit{Xoo} bacterial strain with $10^8$ cfu by cutting method on 45 days after planting (DAP) for IR64, and 61 DAP for Pandanwangi and Cilamayamuncul variety. The \textit{Xoo} cell density with a value of $10^8$ cfu was determined using a spectrophotometer with O.D. = 0.2 at a wavelength of 600 nm [16]. Cutting is done on the ten youngest leaves. The variables observed included disease severity, incubation time, infection rate, the area under the disease progress curves (AUDPC), yield, yield losses, and intolerance value. Observation of disease severity is carried out every three days after inoculation (DAI) to harvest with the calculation of the formula:

$$DS = \frac{\sum (n \cdot v)}{N \cdot Z} \cdot 100\%$$

where \(D.S\). is disease severity, \(n\) is observed sample, \(v\) is the disease score, \(N\) is the number of samples observed, and \(Z\) is the highest disease score (Table 1).

| Scoring value | Disease area of blight (%) | Figure (Tip – Base) |
|---------------|---------------------------|----------------------|
| 1             | 1–5                       | Figure 1             |
| 3             | 5–12                      | Figure 2             |
| 5             | 12–25                     | Figure 3             |
| 7             | 25–50                     | Figure 4             |
| 9             | 50–100                    | Figure 5             |

Incubation time was calculated from the appearance of the first symptom after the test plant was inoculated with pathogenic bacteria. Observation of infection rates was calculated based on the formula [18]:

$$r = \frac{2.3}{t} \left( \frac{1}{1-Xt} - \frac{1}{1-Xo} \right)$$

with \(r\) = the infection rate, \(Xo\) = proportion of initial disease, \(Xt\) = proportion of disease at time \(t\), and \(t\) = time of observation.
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AUDPC observation is calculated using the formula:  
\[
AUDPC = \sum_{i}^{n-1} \left( \frac{X_i + X_{i+1}}{2} \right) t_{i+1} - t_i
\]
with \(X_i\) = the first observation data, \(X_{i+1}\) = i + 1 observation data, \(t_i\) = 1\textsuperscript{st} observation time, \(t_{i+1}\) = i - 1 observation time + 1 [19]. After harvest, observation of harvested dry grain weight is carried out by weighing the harvested dry grain for each crop. Tolerance values are measured by regression between disease severity and yield losses. Plants that are tolerant of BLB have significant differences in regression coefficients compared to intolerant plants [20]. Data were analyzed using the \(F\) test with a confidence level of 95%, then continued with the DMRT test.

3. Results and discussion

Symptoms of BLB disease are found in all experimental units. The development of BLB could be seen in Figure 1. Symptoms start from the edges and tips of yellowish leaves, then gray or dry. BLB symptoms start from the edges and tips of gray then die [21,22]. The overall severity of the disease increases with increasing age. The severity value that appears determines the resistance category [17,23].

**Figure 1.** Progress of BLB disease severity in several rice varieties against several Xoo strains.

Different strains of Xoo gave different respond to plants. Strain III of Xoo bacteria caused the lowest damage in all varieties, while strain IV caused the most severe damage. Strain III has the lowest virulence compared to strains IV and VIII [24], while strain IV has the highest virulence [25]. IR64 has the most severe damage among other varieties because it is susceptible to strain III, IV, and VIII of Xoo bacteria [26]. Pandanwangi showed a susceptible response to strains III and VIII, whereas strain IV showed very susceptible. Pandanwangi’s response was statistically significantly different from the resistant and susceptible comparators used (Table 2).

Different responses are caused by interactions between virulent genes in each isolate and resistant genes in each variety [27]. Strain III has a high virulence against Kinmaze (plants without resistant
gene), Kogyoku (dominant genes Xa-1 and Xa-kg), and Tetep (two dominant genes, Xa-1 and Xa-2). Strain III is not virulent against Wase Aikoku (Xa-3) and Java 14 (dominant genes Xa-1, Xa-2, and Xa-kg). Strain IV has a high virulence in all varieties. Strain VIII has high virulence towards Kinmaze, Kogyoku, Tetep, and Wase Aikoku. Strain VIII is less virulent towards Java 14 [27,28].

The incubation time shows the resistance of plants to a pathogen. Plants with long incubation times indicate their resistance to a pathogen. Plant resistance to pathogens occurs because plants produce phytoalexin, which inhibits bacterial growth due to host-pathogen interactions [18]. The incubation time was divided into three, namely long (> 10 DAI), medium (8–10 DAI), and fast (<8 DAI) [13]. Pandanwangi against Xoo strain III is relatively fast compared to strains IV and VIII, classified as medium (Table 2).

Infection rate is the rapid development of disease as measured by disease severity differences at initial observation with at the end of disease observation per unit in time [29]. The infection rate is also one of the reactions of plant resistance to pathogens. Based on observations, Pandanwangi against Xoo strain III has the lowest infection rate after strain IV and strain VIII. This value is statistically significantly different compared to the control varieties. Pathogens with high virulence and susceptible plants cause higher infection rates than low virulence pathogens and resistant plants [18]. This shows that the fastest development of Xoo in the Pandanwangi varieties is in strains IV, VIII, and III, respectively (Table 2).

AUDPC is used to measure disease resistance quantitatively [30]. AUDPC value is used for measuring disease development [31]. AUDPC is sharper to be used as a rapid development of plants cause higher infection rates [32]. High AUDPC values describe rapid disease progression [16]. Pandanwangi AUDPC value of Xoo strain III was smaller and statistically significantly different from strains IV and VIII inoculated. In contrast, the Pandanwangi AUDPC against Xoo IV strain was not different from VIII strain. This finding shows that the infection progress of Xoo strain III is slower than those IV and VIII strains on cv Pandanwangi (Table 2).

### Table 2. Effect of Xoo strain on the development of BLB disease in some rice varieties

| Varieties     | Xoo strain | Incubation period (DAI) | Infection Rate (Unit.day⁻¹) | AUDPC | Disease Severity (%) | Response Category* |
|---------------|------------|-------------------------|-----------------------------|-------|----------------------|-------------------|
| IR 64         | Strain III | 4.92a                   | 0.0258                      | 2777.50e | 80.35f               | VS                |
|               | Strain IV  | 7.15b                   | 0.0326                      | 3112.60f | 87.17g               | VS                |
|               | Strain VIII| 7.48b                   | 0.0290                      | 3275.90f | 83.93g               | VS                |
| Pandanwangi   | Strain III | 7.23b                   | 0.0053                      | 772.09b  | 28.42b               | S                 |
|               | Strain IV  | 9.16c                   | 0.0126                      | 1309.50d | 54.74e               | VS                |
|               | Strain VIII| 8.95c                   | 0.0106                      | 1340.90d | 48.63d               | S                 |
| Cilamayamuncul| Strain III | 11.83d                  | 0.0024                      | 460.55a  | 14.45a               | M                 |
|               | Strain IV  | 11.33d                  | 0.0062                      | 974.53c  | 32.68c               | S                 |
|               | Strain VIII| 11.60d                  | 0.0049                      | 668.75b  | 26.61b               | S                 |

Note: the average followed by different letters in the same column is significantly different (P <0.05).

*VS = Very Susceptible, S = Susceptible, M = Moderate

Xoo bacteria can significantly reduce rice production both in quality and quantity [33]. Based on observations, Xoo inoculation decreases rice production and causes the number of empty rice grains. Yield losses were determined by variety and bacteria strain. Xoo can reduce yield by more than 50% on cv Pandanwangi and up to 56.99% on cv I.R. 64. Xoo strain IV caused the highest yield losses (Table 3). Xoo bacteria can disrupt the grain’s filling process so that the unoccupied grain is even empty [34].

The response of cv Pandanwangi as a local rice variety depends on pathogenic Xoo strains, which is shown in the observation of the progress of BLB on the test varieties (Table 2) and plant growth (Table 3). Losses of yield and disease severity can be regressed to find intolerance values.
Table 3. Effect of *Xoo* strains on growth plants in several rice varieties

| Varieties       | *Xoo* strain | Yield (ton. ha⁻¹) | Yield Losses (%) |
|-----------------|--------------|-------------------|------------------|
| I.R. 64         | No Inoculation | 4.28<sup>bc</sup> | -                |
|                 | Strain III    | 2.77<sup>ab</sup> | 35.20<sup>bc</sup> |
|                 | Strain IV     | 1.77<sup>a</sup>  | 56.99<sup>c</sup>  |
|                 | Strain VIII   | 1.86<sup>c</sup>  | 56.13<sup>c</sup>  |
| Pandanwangi     | No Inoculation | 6.45<sup>def</sup>| -                |
|                 | Strain III    | 4.52<sup>bcd</sup>| 24.00<sup>ab</sup> |
|                 | Strain IV     | 3.23<sup>ab</sup> | 50.73<sup>c</sup>  |
|                 | Strain VIII   | 4.71<sup>bde</sup>| 33.74<sup>bc</sup> |
| Cilamayamuncul  | No Inoculation | 8.83<sup>g</sup>  | -                |
|                 | Strain III    | 8.17<sup>g</sup>  | 4.15<sup>a</sup>  |
|                 | Strain IV     | 5.88<sup>de</sup> | 25.16<sup>ab</sup> |
|                 | Strain VIII   | 6.72<sup>ef</sup> | 17.24<sup>ab</sup> |

Note: the average followed by different letters in the same column is significantly different (*P* <0.05).

Plants tolerant of pathogens quantitatively can be measured from a regression analysis between loss of yield and disease severity. Rice varieties tolerant to BLB have significant differences in the regression coefficient compared to intolerant plants [20]. The regression model that corresponds to the quantitative relationship between disease severity and yield losses in this study is *Y* = *a* + *bX*, where *Y* represents yield losses and *X* represents disease severity. The tolerance value used is a reverse value of the coefficient of *b*, which means that a smaller *b* value is higher tolerance to disease infection. Tolerant varieties are plants that cannot inhibit pathogenic infections but can minimize the impact of the infection [35]. Based on the measurement, the results showed no significant difference in the regression coefficient indicates that Pandanwangi is intolerant of *Xoo* strains III, IV, and VIII (Table 4).

Table 4. Intolerance value of plants to BLB disease in some rice varieties

| Varieties       | *Xoo* strain | Intolerance Value |
|-----------------|--------------|-------------------|
| I.R. 64         | Strain III   | 0.989<sup>ab</sup> |
|                 | Strain IV    | 0.554<sup>a</sup>  |
|                 | Strain VIII  | 1.096<sup>ab</sup> |
| Pandanwangi     | Strain III   | 1.732<sup>c</sup>  |
|                 | Strain IV    | 1.501<sup>c</sup>  |
|                 | Strain VIII  | 0.789<sup>a</sup>  |
| Cilamayamuncul  | Strain III   | 0.740<sup>a</sup>  |
|                 | Strain IV    | 0.955<sup>ab</sup> |
|                 | Strain VIII  | 0.924<sup>ab</sup> |

Note: Numbers followed by different letters in the same column are significantly different (*P* <0.05).

4. Conclusions
A rice local variety Pandanwangi is a non-tolerant variety to BLB strains III, IV, and VIII. The cv Pandanwangi is moderately susceptible to infection of *Xoo* strains III and VIII, and highly susceptible to *Xoo* strain IV.

5. Suggestion
A local rice variety "Pandanwangi" should not be planted on an endemic area of BLB dominated by *Xoo* strain III, IV, and VIII because it is susceptible and very susceptible to those strains.
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