Screening of Indonesian rice cultivars against bacterial leaf blight disease under acidic soil condition

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Abstract. Marginal lands, which often have poor soil, are largely found in North Sumatra, Indonesia. Acidic soil, one of marginal lands, become an obstacle for agriculture including rice growing. Additionally, bacterial leaf blight disease which is caused by Xanthomonas oryzae pv oryzae, is also the main problem for rice production in Indonesia and worldwide. This study was aimed to screen rice cultivars that were tolerant to acidic soils and showed resistance to bacterial leaf blight (X. oryzae pv. oryzae). This research was conducted at the Screenhouse in Faculty of Agriculture, Universitas Sumatera Utara from July to November 2020. This experiment was designed as factorial Randomized Block Design (RBD) by 3 factors, i.e. Factor 1: rice cultivars (Inpari 30, Inpara 5, Inpago 9 and Inpago Unsoed 1); Factor 2: soil pH (control (pH 6.8) and pH 4.0); Factor 3: isolate of X. oryzae pv oryzae (Xoo 1) with three replications. The results showed that all cultivars grown in acidic soil were susceptible to bacterial leaf blight diseases, while all cultivars grown in control soil pH were moderately susceptible. Disease incidence for all cultivars were 100% starting from the fifth week after planting. Plant growth in acidic soil was affected more severely than that of on neutral soil, while the highest disease severity was 75% (Inpari 30).

1. Introduction
Marginal lands, which are unproductive due to poor soil quality, are widespread in Indonesia (approximately 90 million hectares) including in North Sumatra (approximately 8 million hectares) [1]. As a part of Indonesian agricultural extensification strategies, managing marginal lands can be used for optimising land productivity which can increase agricultural production.

Rice is one of the main food commodities that become Indonesian government’s priority because it is the staple food of majority Indonesian people. The high consumption of rice causes high demand for domestic rice and sometimes unbalanced with the available supply. Consequently, Indonesian government has imported rice from neighbouring countries including China, Thailand, Vietnam, Pakistan, India and the Phillipines. Main problems in rice production include pest and diseases and also the ineffective use of marginal lands. Bacterial leaf blight disease causes by Xanthomonas oryzae pv oryzae (Xoo) is one of the important rice diseases in Indonesia [2] and other rice-growing countries such as Japan, India, China, and Phillipines [3]. The disease is responsible for a yield loss of more than 70% under favorable conditions on susceptible varieties during rainy season [4, 5]. Several studies showed the great variability of strains of Xoo pathotypes in several rice-growing countries [6, 7, 8]. The dominant Xoo pathotypes found in rice fields in Indonesia were pathotypes III, IV and V, however pathotypes VI and VIII were also dominant [9]. Additionally, Noer et al. [10] revealed that
A pathotype IV isolate of Xoo was found in large number in North Sumatra. Environmental factors including climate changes, rice varieties and high gene mutability resulted in high variability of bacterial pathotypes which affect the plant resistance in the field [11].

One alternative method is by agricultural extensification to use marginal lands [12], particularly acidic soil, to grow rice and select the cultivars which manage the obstacles occur by growing rice in acidic soil including plant diseases. This method can therefore increase the rice production in specific environment in Indonesia. Plant breeding program for biotic and abiotic stress is more prioritized on breeding of tolerant cultivars of the growing environment than managing the growing environment because it is quite costly. Consequently, the first step to the purpose of breeding of tolerant cultivars is direct selection on the biotic and abiotic stress environment. Thus, this research was aimed to screen rice cultivars that were tolerant to acidic soil and showed resistance to pathotype IV isolate of Xoo.

2. Materials and Methods

2.1. Preparation of acidic soil
The acidic soil with pH 4.0 was obtained from Selotong, Langkat district, North Sumatra, Indonesia. The neutral soil with pH 6.8 was top soil, which was used as control.

2.2. Bacterial isolate
Isolate of Xoo 1 was a collection in Laboratory of Plant Pathology, Faculty of Agriculture, Universitas Sumatera Utara, which was originated from Labuhan Batu district, North Sumatra. This isolate was identified as X. oryzae pv oryzae using morphological, physiological, biochemical, and molecular approaches and grouped as pathotype IV according to pathotyping system from the previous study (unpublished). There are at least 12 pathotypes of X. oryzae pv. Oryzae, which show different pathogenicity, have been reported in Indonesia and pathotype IV is found in North Sumatra [10]. Bacterial isolate was re-cultured in King’s B media and incubated at 28°C for 48 hours.

2.3. Preparation seedlings and planting
The four selected rice cultivars including Inpari 30, Inpara 5, Inpago 9 and Inpago Unsoed 1 were tolerant cultivars to acidic soils on the basis of the previous study which tested several rice cultivars on different acidic solution in nutrient culture assay (unpublished). According to Indonesian rice research department [18], Inpago 9 is moderately tolerant to aluminium toxicity at 60 ppm, Inpago Unsoed 1 is moderately tolerant until tolerant to ferrum toxicity and no information regarding tolerance to acidic soil for Inpari 30 and Inpara 5. The seeds were planted in seedling containers with top soils for 15 days. After 15 days, the seedlings were moved into 5 kg polyethylene bags with soils according to the treatments. The planting was conducted in a Screenhouse at Faculty of Agriculture, Universitas Sumatera Utara for 14 weeks.

2.4. Bacterial inoculation into the rice plant
Xoo 1 isolate were grown on King’s B media at 28°C for 48 hours. The four week-old plants were artificially inoculated by the 10^6 cfu/ml bacterial suspension according to the clip method by Kauffman et al. [13] with modification. A sterilized surgical scissor was dipped in bacterial suspension. Three leaves in a polyethylene bag were grasped in one hand and top 1-3 inches of the leaves were clipped off simultaneously. Sterile water was used as control treatment. Inoculation was carried out on three individual plants and repeated three times. Inoculated plants were covered by polyethylene bags for 24 hours. The symptoms were observed after 48 hours until 14 days.

2.5. Determination of disease incubation period, plant resistance category, disease incidence and disease severity during vegetative and generative phase
Disease incubation period is the time period to cause the first visual symptoms of leaf blight disease after inoculated by Xoo. Plant resistance was recorded according to the category of IRRI [14], and the
infection percentage to determine the plant response was calculated per one leaf after 14 days of inoculation (Table 1).

**Table 1. Plant resistance criteria to bacterial leaf blight of rice.**

| Infection % | Host response         |
|-------------|-----------------------|
| 0           | Highly resistant (HR) |
| 0 - 10      | Resistant (R)         |
| 11 – 30     | Moderately resistant (MR) |
| 31 – 50     | Moderately susceptible (MS) |
| 51 – 75     | Susceptible (S)       |
| 76 – 100    | Highly susceptible (HS) |

Disease incidence was observed on the basis of visual symptoms on leaf sheaths from 1 week until 14 weeks after the bacterial inoculation, with the following formula [15]:

\[
DI = \frac{a}{b} \times 100\%
\]

where,

- \( DI \): Incidence of bacterial leaf blight disease
- \( a \): Number of plants infected by bacterial leaf blight disease
- \( b \): Number of observed plants

Disease severity was scored by measuring the ratio between the length of leaves with symptom and the length of overall leaves, stated in percentage (%), and measured from 1 week until 14 weeks after inoculation with the following formula [16]:

\[
DS = \frac{\sum (nsv)}{Nz} \times 100\%
\]

where,

- \( DS \): disease severity
- \( N \): number of plant each score
- \( v \): disease scale value each individual plant
- \( Z \): the highest disease scale
- \( n \): number of observed plants

2.6 **Experimental design**

This experiment was designed using Randomized Block Design (RBD) Factorial with 3 factors; Factor 1: rice cultivars, including Inpari 30, Inpara 5, Inpago 9, and Inpago Unsoed 1. Factor 2: acidic soils including pH 6.8 as control and pH 4.0. Factor 3: isolate of Xoo 1.

3. **Results and Discussion**

3.1. **Disease incubation period**

The fastest disease incubation period was occurred in cultivar Inpari 30 and Inpara 5 infected by Xoo isolate both in control treatment (pH 6.8) and acidic soil, which were 5 hours after inoculation respectively (Table 2). While incubation period of cutivarInpago 9 and Inpago Unsoed 1 isolated by Xoo isolate both in control and acidic soil was 10 hours respectively, Xoo 1 which grouped as pathotype IV strain is very virulent because plant showed the disease symptoms within 5 and 10 hours after inoculation for the tested cultivars. According to Khaeruni et al. [17], the fastest incubation period of IR 64 cutivar inoculated by Xoo isolate was 4.25 days after inoculation.

3.2. **Plant resistance**

After infected by Xoo, all cultivars reacted as moderately susceptible on control soil pH and susceptible on acidic soil (Table 3). According to the cultivar description [18], the selected rice cultivars were resistant to bacterial leaf blight pathogen pathotype IV (Inpara 5), susceptible to
bacterial leaf blight pathogen pathotype IV (Inpari 30), and moderately resistant to bacterial leaf blight pathogen pathotype III (Inpago 9) [18].

The results of this study were different from the cultivar description [18], because \textit{Xoo} 1 isolate pathotype IV is a very virulent isolate because it showed the first visual symptoms on leaves 5 and 10 hours after inoculation (Table 2). When the plants were under more stress due to salinity, the plants were more susceptible to the pathogen compared to the the plants that were grown on neutral soil pH.

**Table 2.** Disease incubation period of four rice cultivars isolated by \textit{Xanthomonas oryzae pv oryzae} on acidic soil.

| Treatments          | Incubation period (hours) |
|---------------------|---------------------------|
| **Bacterial isolate** | **Cultivar** | **pH** |                      |
| \textit{Xoo} 1      | Inpari 30                | 6.8    | 5                    |
| \textit{Xoo} 1      | Inpari 30                | 4.0    | 5                    |
| \textit{Xoo} 1      | Inpara 5                 | 6.8    | 5                    |
| \textit{Xoo} 1      | Inpara 5                 | 4.0    | 5                    |
| \textit{Xoo} 1      | Inpago 9                 | 6.8    | 10                   |
| \textit{Xoo} 1      | Inpago 9                 | 4.0    | 10                   |
| \textit{Xoo} 1      | Inpago Unsoed I          | 6.8    | 10                   |
| \textit{Xoo} 1      | Inpago Unsoed I          | 4.0    | 10                   |

**Table 3.** Plant resistance of four cultivars inoculated by \textit{Xanthomonas oryzae pv oryzae} on acidic soil.

| Treatments          | Disease percentage (%) | Host response |
|---------------------|------------------------|---------------|
| **Bacterial isolate** | **Cultivar** | **Soil pH** |                      |
| \textit{Xoo} 1      | Inpari 30              | 6.8           | 41.4                  | MS                     |
| \textit{Xoo} 1      | Inpari 30              | 4.0           | 58.0                  | S                      |
| \textit{Xoo} 1      | Inpara 5               | 6.8           | 42.5                  | MS                     |
| \textit{Xoo} 1      | Inpara 5               | 4.0           | 56.6                  | S                      |
| \textit{Xoo} 1      | Inpago 9               | 6.8           | 44.4                  | MS                     |
| \textit{Xoo} 1      | Inpago 9               | 4.0           | 57.4                  | S                      |
| \textit{Xoo} 1      | Inpago Unsoed I        | 6.8           | 43.1                  | MS                     |
| \textit{Xoo} 1      | Inpago Unsoed I        | 4.0           | 61.3                  | S                      |

**Note:** MS: moderately susceptible; S: susceptible

#### 3.3. Disease incidence

The results showed that all cultivars had 100% disease incidence both in control and acidic soil, started from the fifth week after planting (one week after inoculation) (Table 4).

#### 3.4. Disease severity

The results showed that after one week of bacterial inoculation (week 5 after planting) all cultivars that were grown on control soil pH had a range of 35 - 40.3% of disease severity (Table 5). While all cultivars that were grown on acidic soil had a range of 54.6 - 57.6% of disease severity. Inpari 30 cultivar had the lowest disease severity (54.6%) when they were grown on acidic soil, but the disease developed steadily until it reached 75% of disease severity at harvesting period (14 weeks after planting). The disease severity value of Inpari 30 cultivar was higher than the disease severity of control soil pH, which was only 59.6% at 14 weeks after planting (10 weeks after inoculation). All cultivars that were grown on control soil had the disease severity less than 60.6%, while all cultivars
Table 4. Disease incidence of four rice cultivars isolated by *Xanthomonas oryzae* pv *oryzae* on acidic soil (14 weeks after planting).

| Bacterial isolate | Cultivars     | Soil pH | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 |
|-------------------|---------------|---------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| *Xoo* 1           | Inpari 30     | 6.8     | 0  | 0  | 0  | 0  | 100| 100| 100| 100| 100| 100| 100| 100| 100| 100|
| *Xoo* 1           | Inpari 30     | 4.0     | 0  | 0  | 0  | 0  | 100| 100| 100| 100| 100| 100| 100| 100| 100| 100|
| *Xoo* 1           | Inpara 5      | 6.8     | 0  | 0  | 0  | 0  | 100| 100| 100| 100| 100| 100| 100| 100| 100| 100|
| *Xoo* 1           | Inpara 5      | 4.0     | 0  | 0  | 0  | 0  | 100| 100| 100| 100| 100| 100| 100| 100| 100| 100|
| *Xoo* 1           | Inpago 9      | 6.8     | 0  | 0  | 0  | 0  | 100| 100| 100| 100| 100| 100| 100| 100| 100| 100|
| *Xoo* 1           | Inpago 9      | 4.0     | 0  | 0  | 0  | 0  | 100| 100| 100| 100| 100| 100| 100| 100| 100| 100|
| *Xoo* 1           | Inpago Unsoed 1 | 6.8   | 0  | 0  | 0  | 0  | 100| 100| 100| 100| 100| 100| 100| 100| 100| 100|
| *Xoo* 1           | Inpago Unsoed 1 | 4.0   | 0  | 0  | 0  | 0  | 100| 100| 100| 100| 100| 100| 100| 100| 100| 100|

Table 5. Disease severity of four rice varieties cultivars isolated by *Xanthomonas oryzae* pv *oryzae* on acidic soil (14 weeks after planting).

| Bacterial isolate | Cultivars     | Soil pH | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 |
|-------------------|---------------|---------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| *Xoo* 1           | Inpari 30     | 6.8     | 0  | 0  | 0  | 0  | 35.0| 45.3| 48.0| 49.3| 51.0| 52.6| 55.6| 57.0| 59.0| 59.3|
| *Xoo* 1           | Inpari 30     | 4.0     | 0  | 0  | 0  | 0  | 54.6| 65.0| 66.3| 68.0| 69.3| 70.6| 71.6| 72.6| 74.0| 75.0|
| *Xoo* 1           | Inpara 5      | 6.8     | 0  | 0  | 0  | 0  | 40.1| 44.8| 46.0| 47.3| 50.3| 51.3| 52.3| 55.6| 56.6| 57.6|
| *Xoo* 1           | Inpara 5      | 4.0     | 0  | 0  | 0  | 0  | 55.6| 66.0| 67.0| 68.0| 69.0| 70.6| 73.6| 75.0| 76.0| 70.3|
| *Xoo* 1           | Inpago 9      | 6.8     | 0  | 0  | 0  | 0  | 40.3| 46.0| 47.0| 48.3| 49.6| 52.0| 54.0| 57.0| 58.0| 60.6|
| *Xoo* 1           | Inpago 9      | 4.0     | 0  | 0  | 0  | 0  | 56.0| 66.0| 67.3| 68.3| 64.6| 68.0| 69.6| 71.3| 72.3| 72.3|
| *Xoo* 1           | Inpago Unsoed 1 | 6.8   | 0  | 0  | 0  | 0  | 39.6| 46.0| 48.0| 49.0| 50.3| 52.6| 54.6| 55.6| 56.6| 58.0|
| *Xoo* 1           | Inpago Unsoed 1 | 4.0   | 0  | 0  | 0  | 0  | 57.6| 64.0| 64.6| 66.3| 67.6| 68.6| 69.6| 71.6| 73.0| 74.3|
that were grown on acidic soil had disease severity more than 70.3%. Symptoms of infected plants showed leaf stripes and the leaves became yellowing, wilt and roll up and turned to grayish green (Figure 1). The plant symptoms that were grown on acidic soil was more severe than the plants grown on neutral soil pH.

According to cultivar description by Indonesian rice research department [18], the selected rice cultivars were resistant to bacterial leaf blight pathogen pathotype IV (Inpara 5), susceptible to bacterial leaf blight pathogen pathotype IV (Inpari 30), moderately resistant to bacterial leaf blight pathogen pathotype III and moderately tolerant to Aluminium toxicity at 60 ppm (Inpago 9), and tolerant to iron toxicity (InpagoUnsoed I). This study showed different results with the exception of Inpari 30. Starting from the 5-week old plant showed the lowest disease severity of 35% on the neutral soil pH and 54.6% on the acidic soil (Inpari 30). The plant ages affect the disease development of bacterial leaf blight, which the younger the plant, the faster the disease development [17]. Rice is a highly salt-sensitive crop but there is considerable variation in tolerance [19, 20]. Rice is usually tolerant of salt stress during germination, tillering, and until maturity phase and is sensitive during early seedling and reproductive stages [21]. However, the plants in this study had both abiotic and biotic stress in the same time during the growth. With the effect both very virulent bacterial pathogen that infected the plants and salinity, all cultivars could not manage the stress obstacles.

4. Conclusion
The results showed that all cultivars that were grown in acidic soil were susceptible to bacterial leaf blight disease, while all cultivars that were grown in control soil pH were moderately susceptible.

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