Pathotype profile of *Xanthomonas oryzae* pv. *oryzae* isolates from North Sumatera

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Abstract. The Bacterial blight disease caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is one of the most important diseases and has caused crop failure in rice crops. This pathogen infects the leaves in all plant growth phases. The purpose of this study is to investigation 10 Xoo isolates pathotype obtained from North Sumatra based on their interactions with 10 near-isogenic rice lines (NIL) of IRRI. The results showed that there are 6 pathotypes of virulence in North Sumatra, they are; pathotype I with incompatible interaction to all Xa genes, pathotype II with compatible interaction to *Xa1* and *Xa3* genes, while it has incompatible interaction to other genes, pathotype III with compatible interaction to *Xa1*, *Xa5*, *Xa7*, *Xa8*, *Xa10* and *Xa11* genes, but it has incompatible interaction to other genes, pathotype IV with compatible interaction to all Xa genes, pathotype V with compatible interaction to *Xa1* gene and incompatible interaction to other genes, and pathotype VI with compatible interaction to *Xa3* gene and incompatible interaction to other genes. Based on the resistant genes in each individual *Xa2*, *Xa4*, and *Xa21* genes are the combination of Xa genes which are most suitable for use in the development of rice cultivars in North Sumatra.

1. Introduction
Rice is a staple food and is a worldwide model cereal [1]. Bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is one of the most important diseases in rice-producing countries in Asia, including in Indonesia [2][3]. In Indonesia, this particular disease is found in various rice cultivation ecosystems and spread in the lowlands [4]. Bacterial blight is endemic and has been known to cause rice crop failures in irrigated and lowland areas across Asia [5]. Bacterial blight caused by Xoo appears in the form of epidemics in many parts of the world, resulting in 6-81% loss in some rice varieties. [6].

Bacterial blight generally infects leaves, but its distribution depends highly on its environmental conditions [3]. Infection usually starts from the tip of the leaf and spreads all the way to the base. Symptoms that occur in rice plants during its vegetative phase are called kresek, and in its generative phase they’re called blight [7]. Pathogenic infections cause a disruption of photosynthetic functions, leading to infected plants producing much more empty grains compared to healthy plants [8]. If pathogen attacks the plant during the seed phase, its leaves will wither, curl, and become grayish-green in color. If the attack occurs on mature plants, the leaves will turn pale yellow [9].
The spread of Xoo-resistant rice varieties is a more sustainable solution for disease control than the use of chemicals [10]. The resistance of a rice variety to Xoo largely depends on the availability of resistance genes and information on virulence estimates in pathogen populations [11]. Information on the virulence of pathogenic populations is essential for efficient cultivation programs [12]. The population structure was determined through a series of tests using varieties with confirmed resistance genes, followed by classification of Xoo isolates into different pathotypes. Pathotypes are unique to one another because the researchers used various differential genotypes, both from Japan and the Philippines, as well as the newly selected differential genotypes in their research [13].

More than thirty bacterial leaf blight-resistance genes (called Xa genes) have been identified to provide resistance to a wide variety of race and pathotypes of Xoo [14]. Pathogens can easily damage a single-resistance gene [15]. One strategy that can be used to prolong the life of major gene resistance is the pyramid of several major resistant genes in resistant varieties [16]. This resistance gene shows specificity in terms of effectiveness against different pathogen races. As such, knowledge of diversity of pathotypes in target pathogenic population is essential to select resistant genes to be used in cultivation programs [10].

Rotation of resistant varieties to control bacterial leaf blight disease must be carefully designed, as they can last longer in the field. This strategy requires the support of various data, especially regarding the profile of Xoo pathotype in a population and the resistance history of the varieties to be planted. The purpose of this research is to identify the profile of 10 Xoo isolates obtained from the province of North Sumatra, Indonesia, using 10 differential genotypes of near-isogenic lines (NILs) from IRRI.

2. Material and Methods

2.1. Pathogen collection and artificial inoculation

Ten pathogenic strains representing the North Sumatra region were collected in 2016 and 2017. Xoo isolates were grown on YDC (yeast extract-dextrose-CaCO3) or SPA (sucrose pepton agar) medium. The collected isolates were cultured and treated at the Pest and Disease Laboratory of the University of North Sumatra and the Laboratory of Indonesian Biotechnology and Genetic Resources Research and Development Center. Leaves with symptoms of bacterial blight underwent surface sterilization using 70% alcohol, then rinsed with sterile water three consecutives times, dried and cut to 5x5 mm pieces and eventually soaked in sterile water for 5 minutes in the test tube. The suspension was scratched on the medium, incubated for 48-72 hours until a single yellow colony is obtained and then stored in SPA medium at 4°C. The test plant was inoculated with Xoo at age 35 days after transplanting with scissors method[4]. The tip of rice leaves in three different plant varieties for every genotype was cut to a 10 cm piece using inoculation shear with 48 hours-old bacteria inoculum with a concentration of 108 cfu. Inoculation was carried out on three individual plants in each strain and repeated three times.

2.2. IRBB lines and their evaluation

To identify the type of reaction of all isolates collected from North Sumatra, 10 different rice varieties with known resistant gene differences were employed. There were 10 rice genotypes consisting of IRBB-1, IRBB-2, IRBB-3, IRBB-4, IRBB-5, IRBB-7, IRBB-8, IRBB-10, IRBB-11, and IRBB-21 from IRRI (Table 1).

2.3. Disease scoring, virulence analysis and morphological characterization

Scoring is done by measuring the length of symptoms 15-30 days after inoculation. The severity of the disease was measured on three inoculated plant species by examining the five longest symptomatic leaves in each clump. The severity of the disease is determined by the ratio between the length of the symptoms and the length of the leaves. The resistance of varieties was grouped according to disease severity upon observation 30 days after the inoculation. The final grouping is based on the Standard Evaluation System (IRRI, 1996). The data were then used to calculate the disease intensity of each tested genotype. The crop endurance category was determined using terms based on lesion length (cm) with resistance (R; ≤ 10 mm) and susceptibility (S; > 10 mm)
3. Result and Discussion

3.1. Result

A total of 10 Xoo isolates were collected from 10 different regions in North Sumatra, Indonesia, from March 2016 to March 2017. All isolates were tested for pathotype using 10 NILs with Xoo-resistant genes of Xa1, Xa2, Xa3, Xa4, Xa5, Xa7, Xa8, Xa10, Xa11, and Xa21. The results showed that there are 6 different pathotypes in this research (Table 1). Pathotype IV has the highest-frequency and accounts for 50% of all isolates. The Xoo strain included in this pathotype is widespread across North Sumatra. Compared with this pathotype, the other five are more rarely present, each accounting for 10% of the total batch (Figure 1).

Table 1. Pathotyping of North Sumatera, Indonesia of X. oryzae pv. oryzae

| Host differential  | X. oryzae pv. oryzae Pathotype |
|--------------------|--------------------------------|
|                    | I    | II   | III  | IV   | V    | VI   |
| IRBB-1 (Xa1)       | R    | S    | S    | S    | S    | R    |
| IRBB-2 (Xa2)       | R    | R    | R    | S    | R    | R    |
| IRBB-3 (Xa3)       | R    | S    | R    | S    | R    | S    |
| IRBB-4 (Xa4)       | R    | R    | R    | S    | R    | R    |
| IRBB-5 (Xa5)       | R    | R    | S    | S    | R    | R    |
| IRBB-7 (Xa7)       | R    | R    | S    | S    | R    | R    |
| IRBB-8 (Xa8)       | R    | R    | S    | S    | R    | R    |
| IRBB-10 (Xa10)     | R    | R    | S    | S    | R    | R    |
| IRBB-11 (Xa11)     | R    | R    | S    | S    | R    | R    |
| IRBB-21 (Xa21)     | R    | R    | S    | S    | R    | R    |

R: Resistance; S: Susceptible

Figure 1. Frequency of X. oryzae pv oryzae pathotypes in North Sumatera, Indonesia. Six different pathotypes, designated as I to VI, were identified in this study. The x-axis indicates pathotype and the y-axis indicates frequency among the 10 isolates that were collected and analyzed.

Pathotype I shows incompatibility with all differential plants, which indicates that this pathotype consists of a less virulent strain. This pathotype is present in 10% of the total sample of Xoo isolates. The possibility that pathotype I is a grouping of strains from another pathotype for unknown reasons has lost its virulence. Loss of virulence has been reported in the aging culture of Xoo [17]. The results of Xoo infection on NIL genotype are presented in Table 2. All the NIL genotypes of the differential rice varieties tested in this research show various reactions of resistance levels and susceptibilities to Xoo.

Average comparison of Xoo isolates shows that 5 isolates (Xo16-011, Xo16-222, Xo16-230, Xo16-254, and Xo17-624) are significantly more virulent than other isolates. The dendogram analysis shows that the tested isolates are grouped into three main categories: resistant isolates (40%), moderately resistant isolates (10%), and susceptible isolates (50%) (Figure 2).

Table 2. Relationship between 10 NILs Genotypes and 10 Xoo isolates
| Isolate     | IRBB-1 | IRBB-2 | IRBB-3 | IRBB-4 | IRBB-5 | IRBB-7 | IRBB-8 | IRBB-10 | IRBB-11 | IRBB-21 |
|------------|--------|--------|--------|--------|--------|--------|--------|---------|---------|---------|
| Xo16-011   | S      | S      | S      | S      | S      | S      | S      | S       | S       | S       |
| Xo16-222   | S      | S      | S      | S      | S      | S      | S      | S       | S       | S       |
| Xo16-230   | S      | S      | S      | S      | S      | S      | S      | S       | S       | S       |
| Xo16-254   | S      | S      | S      | S      | S      | S      | S      | S       | S       | S       |
| Xo16-080   | R      | R      | R      | R      | R      | R      | R      | R       | R       | R       |
| Xo16-090   | S      | R      | S      | R      | R      | R      | R      | R       | R       | R       |
| Xo16-089   | S      | R      | R      | S      | S      | S      | S      | S       | R       | R       |
| Xo16-100   | S      | R      | R      | R      | R      | R      | R      | R       | R       | R       |
| Xo16-003   | R      | R      | S      | R      | R      | R      | R      | R       | R       | R       |
| Xoo-7624   | S      | S      | S      | S      | S      | S      | S      | S       | S       | S       |

R: Resistance; S: Susceptible

**Figure 2.** Dendogram of similarity among Xoo isolate based on their reaction to NILs

Based on the resistant genes in each individual, the genes \( Xa2, Xa4, \) and \( Xa21 \) are the most effective genes that are resistant to 50% isolates, followed by \( Xa5, Xa7, Xa8, Xa10 \) and \( Xa11 (40\%), Xa3 (30\%), \) and \( Xa1 (20\%). Xa2, Xa4, \) and \( Xa21 \) genes are a combination of \( Xa \) genes that are most suitable for cultivating rice in North Sumatra (Figure 3).
3.2. Discussion

Virulence of bacterial isolates in host genotype can be determined by the presence or absence of substantial differential interactions between the two [2]. Significant differential interactions between differences and Xoo isolates in the current research suggest that pathogen isolates have different in virulence responses, whereas host genotypes show vertical resistance [18]. Xoo isolates which are unique pathogens provide molecular mechanisms of plant pathogenic interactions. The diversity of Xoo isolates has been demonstrated in previous studies [13][19][20].

The reaction of different isolates on different rice varieties [21]. The grouping of isolates into different pathotypes follows a scheme provided by IRRI with slight modification [22]. Pathotype is numbered according to the pathotyping scheme designed by the Directorate of Rice Research. Numbering is based on virulence strength, from the least to the most virulent. The results show that isolates from different regions are classified as the same pathotype and at the same time, different isolates from the same region are grouped into different pathotypes. This shows that these pathogens are highly dynamic and their population in a particular region varies with different pathotypes [21]. Variations of virulence profiles between Xoo isolates from the same area have been reported previously [23] resulting in a strong selection pressure. The Xoo population produces variations of the virulence profile.

Bacterial blight is a major rice disease in the tropical and subtropical rice-growing countries. Cultivation for host resistance is the most effective, simple, economical, and environmentally-friendly method of controlling this disease [24]. To date, all Xa genes that provide resistance to these bacteria have been listed together with their source and country of origin [25]. In this study, the effective genes for all bacterial isolates were the genes Xa2, Xa4 and Xa21, while the weakest against isolates were the genes Xa1 and Xa3 (Figure 3). Although the Xa21 gene is resistant to most isolates, several isolates from Sri Lanka, Korea, Nepal, Bangladesh, and Pakistan have demonstrated virulence to Xa21 [26]. Therefore, other Xa genes are indispensable to complement the Xa21 gene in achieving longer lasting effects [27]. [28] conducted a screening study on 16 pyramid genes which carry the 2-5 resistance gene tested against 16 isolates and concluded that the genes Xa13 and Xa21 provide resistance to most of the Xoo isolates in Pakistan.

A better understanding of the population and its genetic structure is essential for planning and implementing better cultivation programs on the host plant’s resistance. However, a study of the varieties themselves fails to show the true level of genetic diversity in the pathogenic population [29]. This study can be further developed using plants with Xa gene combinations in a pyramid, using
molecular markers and transfer of plant resistance to bacterial blight through binary quarantine with local varieties and resistant varieties. The use of resistance genes found to be effective in this research can be carried out through dispersion under tropical climate conditions. This finding is also useful for pathologists to better understand the characteristics of bacterial isolates blight in North Sumatra in order to develop an efficient and long-lasting management strategy.

4. Conclusion
Analysis of rice plant patotypes in North Sumatra grouped into six types of patotype. A total of 50% of Xoo bacteria isolates belong to the type of patotype IV. Dendogram analysis showed that the isolates grouped into three categories: resistant isolates (40%), moderately resistant isolates (10%), and susceptible isolates (50%). Gene Xa2, Xa4, and Xa21 are the most effective genes against isolate resistance (50%). Research can be continued by testing isolates using differential plants with pyramid Xa gene combinations to obtain which combinations of genes are most effective against Xoo bacteria.

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