Screening for disease resistance in rice varieties against bacterial panicle blight disease (*Burkholderia glumae*) in Northern Sumatra of Indonesia

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Abstract. In Indonesia, bacterial panicle blight (BPB) disease affecting rice caused by seedborne pathogen, *Burkholderia glumae* has emerged as a serious problem in rice growing areas in several provinces in Java and Sulawesi since 2014, but not yet in Northern Sumatra. This pathogen is listed as a quarantine pathogen which has to limit the spread. Rice import from China, India and Philippines, where are the disease outbreak locations, is the main cause of the dissemination of BPB disease in Indonesia. As rice is the main staple food in Indonesia, significant crop losses have had huge impact to food security. Four of twenty-three bacterial strains isolated from infected rice fields of six districts in Northern Sumatra were identified as *B. glumae* on the basis of morphological, biochemical and molecular characterizations as well as pathogenicity assay. These four bacterial strains were tested on five rice cultivars in the greenhouse.

1. Introduction

Rice (*Oryza sativa* L.) is an important food plant because it has become the staple food for more than a half of human population in the world. In Indonesia, rice is a main commodity for people’s food material [1]. The main failure that farmers face in rice cultivation is pests which can decrease rice productivity per hectare and further can cause crop failure or phuso. The average of rice yield loss that causes by plant pests is 30% each year [2].

Emerging infectious disease (EID) includes diseases that have rapid incidence, larger geographical distribution and pathogenicity changes from one area to the other areas or the entrance of exotic disease from one country to the other countries, which are mainly from seeds importing activities [3]. The current spread of new plant diseases in Indonesia was triggered by several factors, such as climate, the spread of pathogens cross-region/country, the change of cultivation’s technique and the change of pathogen’s genetic. However, the main factor of emerging disease existence is from seeds importing activities. In recent years, the incidence of *Bacterial Panicle Blight* (BPB) disease as a new emerging infectious disease has been confirmed in several locations in West Java, Central Java, East Java [3] and Southern Sulawesi [4]. Recent report form [5] also detected *B. glumae* in Medan and Deli Serdang districts in Northern Sumatra [5]. In Indonesia, this pathogen is categorized as a quarantine pathogen Group I, Category of A2, which cannot be removed from the carrier through quarantine treatments [6].

Bacterial panicle blight has been reported in several rice producing countries, including China, Korea, Vietnam, Philippines, Japan, India, Sri Lanka, Thailand, Malaysia, Latin America, Africa and
The United States [7], *Bacterial Panicle Blight* is not present in Australia [8]. Yield losses due to this pathogen can reach 75% in several locations in the USA [9].

This research was studied to screen five rice varieties that were resistant to BPB. The use of resistant varieties might reduce the serious problems caused by plant pathogens in the field.

2. Materials and Methods

2.1 Bacterial isolates

Three bacterial isolates, which were confirmed as *B. glumae* from the previous study [5] were re-cultured on King’s B semi selective medium and incubated at 37 °C for 48 hours. The isolates were DSMZ 9512ᵀ as positive control, IRP SID, IRC PRC, TH BJ and ARS.

2.2 Preparation of rice seeds

Five certified rice varieties including Mekongga and Cibogo were obtained from Balai Benih Murni, Tanjung Morawa [Pure Seed Hall, Tanjung Morawa], IR64 and Ciherang were obtained from Sang Hyang Seri, and IP30 was obtained from Unit Pengelolaan Benih Sumber [Source Seed Management Unit], Miring Market, Deli Serdang, North Sumatra.

2.3 Preparation of seedlings and planting

Seeds were planted in seedling containers with soils from rice farming for 10-15 days. The 15 days old seedlings were moved into 5 kg polyethylene bags with soils from rice farming. The planting was conducted in a Glasshouse at Faculty of Agriculture, Universitas Sumatera Utara for 16 weeks.

2.4 Inoculation of *B. glumae* into rice plants

A half millilitre of bacteria suspension (5x10⁸ CFU/ml measured with spectrophotometry at OD 600, ABS 0.5), was injected into leaf sheath on day 60 (booting stage) using 1 ml sterilized needle (BD™ 23G1). Sterile aquadest and bacterial type strain (DSMZ 9517ᵀ) were used as a negative control and a positive control respectively. The humidity (>80%), warm temperature (28-30°C) and disease development was controlled in the glasshouse [10].

2.5 Plant management

Plants management activities included manual pest and disease management, watering and fertilization once after the plants were planted.

2.6 Experimental design

This experiment was designed using Randomized Block Design (RBD) Factorial with 2 factors; Factor 1: I0 (sterile water/ negative control), I1 (DSMZ 9512ᵀ isolate/ positive control), I2 (*B. glumae* isolate IRP SID), I3 (*B. glumae* isolate IRC PRC), I4 (*B. glumae* isolate TH BJ) and I5 (*B. glumae* isolate ARS). Factor 2: V1 (Ciherang variety), V2 (Cibogo variety), V3 (Mekongga variety), V4 (IP 30 variety) and V5 (IR 64 variety).

2.7 Data analysis

Data were analysed using Analyse of Variance (ANOVA) for Randomized Block Design (RBD) with 2 factors and 3 replications and Duncan’s Multiple Range Test (DMRT) with SPSS Software if the treatment significantly affected the parameters.

3. Results and Discussion

3.1 Disease incidence

Visual symptoms on leaf sheaths and panicles were observed from 1 week after the bacterial inoculation until harvesting.
The symptoms were observed as rot on sheath and discoloration of seeds’ hull to light and medium brown (Figure 1a & b). *B. glumae* (formerly *Pseudomonas glumae*) was first described in Japan as causing grain rot, sheath rot and seedling rot, depending on the rice growth stages [11, 12, 13]. A linear lesion extending downwards from the leaf blade collar forms on the flag leaf sheath and may be several inches in length; this lesion had a reddish-brown border and a centre that becomes grey and necrotic grains which fill but are infected later in the season have a light to medium brown discoloration on the lower third to half of the hulls.

![Figure 1](image_url)

**Figure 1.** a. Symptoms appeared as rot on sheath; b. discoloration of seed’s hull

| Table 1. Disease incidence from week 1 to week 3 after inoculation (%) |
|---------------------------------------------------------------|
| **Disease incidence week 1 (%)**                             |
| Treatments (Rice varieties) | Bacterial Isolates | Mean |
|---------------------------|-------------------|------|
| V₁                        | 33.33             | 44.44|
| V₂                        | 66.67             |      |
| V₃                        | 33.33             | 50.00|
| V₄                        | 66.67             | 55.56|
| V₅                        | 66.67             | 50.00|
| Mean                      | 53.33             |      |

| **Disease incidence week 2 (%)** |
|----------------------------------|
| V₁                               | 66.67             | 61.11|
| V₂                               | 100.00            |      |
| V₃                               | 66.67             | 72.22|
| V₄                               | 66.67             |      |
| V₅                               | 66.67             | 72.22|
| Mean                             | 73.33             |      |

| **Disease incidence week 3 (%)** |
|----------------------------------|
| V₁                               | 100.00            | 100.00|
| V₂                               | 100.00            |      |
| V₃                               | 100.00            | 100.00|
| V₄                               | 100.00            |      |
| V₅                               | 100.00            | 100.00|
| Mean                             | 100.00            |      |
Disease incidence occurred at all of the isolates including I0 (negative control) at 1 week after inoculation (Table 1). Isolate IRC PRC had the highest average of disease incidence (66.67%). There are differences of growth speed and spread of the pathogens in the host and the destruction of host’s tissue [14].

Based on observation on disease incidence, either plants that inoculated with B. glumae isolates or plants that inoculated with sterile water were infected (symptom occurred). The symptoms appeared on the uninoculated plants might because the pathogen were already in the seeds (seed borne). Although this study used certified seeds, because B. glumae has not been on the lists of seed health assay, it was not detected.

3.2 Disease severity
The data of disease severity were collected after harvesting and disease severity was calculated using formula of Agrios [15] and categorized by using scale of [16].

Table 2 showed that varieties and isolate were very significantly affected disease severity. Variety Inpari 30 had the highest average of disease severity (79.63%) and the lowest was variety Ciherang (54.94%). ARS isolate caused the highest average of disease severity (82.23%) and the IRP SID caused the lowest disease severity (58.52%). [15] stated that the variation of susceptibility to pathogen that belongs to plants is because there are differences of species and it could be the difference of number of resistance genes in each variety.

The interaction between varieties and isolates significantly affected disease severity. The interaction between variety Inpari 30 and isolate TH BJ had highest average of disease severity (100%) and the lowest average of disease severity was the interaction between variety Mekongga and isolate IRP SID (35.80%). [14] stated that contact between bacterial cells and host cells occur in a compatible interaction. In this process, bacteria induce plant to release nutrient or stimulant which is needed for bacteria growth by destructing host cell.

| Rice Varieties | Bacterial isolates | Mean |
|----------------|--------------------|------|
|                | I₀  | I₁  | I₂  | I₃  | I₄  | I₅  |      |
| V₁             | 40.72z | 51.85u-x | 41.97z | 79.01f-j | 55.56uv | 60.49o-t | 54.94d |
| V₂             | 91.36bcd | 65.43n-q | 70.37k-n | 53.07uvw | 77.78f-k | 97.53ab | 75.93ab |
| V₃             | 50.62v-y | 55.56uv | 35.8z | 75.31h-m | 60.49o-t | 77.78f-l | 59.26d |
| V₄             | 62.97n-r | 59.26o-u | 81.48e-i | 81.48e-h | 100.00a | 92.59bc | 79.63a |
| V₅             | 65.43nop | 66.67no | 62.97n-s | 87.66de | 83.89ef | 82.72efg | 74.89abc |

The numbers followed by the same letter are not significantly different on Duncan’s multiple range test with level of α=5%

3.3 The percentage of empty seeds
Rice varieties affected percentage of empty seeds significantly. Variety Cibogo had the lowest average of empty seeds (24.08%) and the highest average of empty seeds was variety IR 64 (48.59%). Bacteria colonized and multiplied itself in seeds soon after infected by using sugars that needed for seeds development [17].
Table 3. The percentage of empty seeds caused by Bacterial Panicle Blight (%)

| Rice varieties | Bacterial isolates | Mean |
|----------------|--------------------|------|
|                | I₀  | I₁  | I₂  | I₃  | I₄  | I₅  |      |
| V₁ (Ciherang)  | 34.21| 42.13| 34.85| 34.34| 55.75| 41.11| 40.4b |
| V₂ (Cibogo)    | 19.34| 18.43| 20.52| 33.89| 14.01| 38.29| 24.08d|
| V₃              | 23.40| 22.85| 17.58| 32.09| 26.76| 37.61| 26.71d|
| V₄              | 40.01| 34.06| 32.12| 48.99| 25.52| 37.03| 36.29bc|
| V₅              | 45.12| 65.85| 43.66| 26.63| 51.17| 59.11| 48.59a|
| Mean            | 32.41| 36.67| 29.75| 35.19| 34.64| 42.63|      |

Note: The numbers followed by the same letter notations in the same column are not significantly different on Duncan’s Multiple Range Test with level of α=5 %

3.4 Plant Resistance
All of the five rice varieties were very susceptible to Bacterial Panicle Blight (Table 4). A plant is classified resistance if is not infected or only minor destruction that occurs caused by that pathogen infection. The susceptibility of a plant is determinate conversely, which is the number of infection and destructions caused by the pathogens [18]. The resistance of a plant to diseases can be affected by external and internal factors, such as host, pathogen and environment [19].

Table 4. The percentage of empty seeds caused by Bacterial Panicle Blight (%).

| Rice Varieties | Disease Incidence (%) | Resistance   |
|----------------|-----------------------|--------------|
| V₁ (Ciherang)  | 100%                  | Very Susceptible |
| V₂ (Cibogo)    | 100%                  | Very Susceptible |
| V₃ (Mekongga)  | 100%                  | Very Susceptible |
| V₄ (Inpari 30) | 100%                  | Very Susceptible |
| V₅ (IR 64)     | 100%                  | Very Susceptible |

4. Conclusions
It is concluded that Bacterial Panicle Blight pathogen (B. glumae) affected rice plants both in vegetative and generative phases because it causes sheat rot, seed rot and seed emptiness. All varieties tested in this study including Ciherang, Cibogo, Mekongga, Inpari 30 and IR 64 were very susceptible to Bacterial Panicle Blight disease.

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**Acknowledgement**

The authors gratefully acknowledge that this research was supported by Grant of TALENTA 2018, Universitas Sumatera Utara, Indonesia (Contract number 339/UN5.2.3.1/PPM/KP-TALEN TA USU/2018).