Test of resistance in some of rice varieties to pathogenic bacteria *Burkholderia Glumae*

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**Abstract.** *Burkholderia glumae* is a bacterium that causes grain rot disease in rice plants. In Indonesia, the disease inflicts yield losses to farmers but there have been no clear reports of the level of the attacks. This study was aimed to determine the development of the grain rot symptoms and the resistance of several rice varieties to grain rot disease. Eleven rice varieties that had been widely used by the farmers in South Sulawesi Province tested in this experiment. The *B. glumae* isolate used came from previous studies in our laboratory. *B. glumae* inoculation was performed in the generative phases of the rice plants by spraying the flowering panicles. Observations in the form of the impact of *B. glumae* inoculation on symptom incubation, and resistance of the varieties against the rice grains disease. In the generative phase, typical symptoms of grain rot disease developed on the inoculated plants. The varieties reaction against the disease varied. Varieties of Inpari 30, Inpari 4, Cigeulis, Ciliwung, Ciherang, and Inpari were highly resistant; Mekongga, Inpari 32 and Sidenuk varieties were medium to strong, then Inpari 33 was medium. Then the Cisantana variety was low to moderate. Of the eleven varieties, Cisantana variety has the highest severity of 61.02% and variety resistance is weak to medium. Further testing in the laboratory is needed to determine the presence of *B. glumae* which has been inoculated into plant tissue by sampling symptomatic and asymptomatic rice plants.

1. Introduction

Increase rice production that the Indonesian government continues to do does not necessarily run smoothly and is free from various obstacles. One problem is plant disease by *Burkholderia glumae*. *B. glumae* is a bacterium that causes grain rot disease in rice plants. In 2015, the regulation Number 51/Minister of Agriculture Regulation/KR.010/9/2015 stated that *B. glumae* is one of the types of Quarantine Plant Pest Organisms (QPPO) A2 Group 1 whose existence cannot be released from the carrier media through quarantine treatment [1]. In 2018, in Minister of Agriculture Regulation No. 31/Minister of Agriculture Regulation/KR.010/7/2018 concerning the type of QPPO stated *B. glumae* has been released from QPPO which means it has become a common pest in Indonesia [2].

In Indonesia, the existence of this disease has existed since 1987 as stated in a press release from Ministry of Agriculture, but after that severe damage due to this disease has not been reported until the existence of this disease has been reported again since 2015 in several regions in Indonesia. Losses caused by panicle blight in Indonesia have never been reported before. But in terms of ecology, climate conditions in Indonesia are very suitable for the development of panicle blight. This certainly will have an effect on increasing the intensity and disease severity [3]. Therefore, this research aimed
to test the resistance of rice varieties against the severity and development of diseases that have been inoculated with *B. glumae* into plant tissues.

2. Materials and Methods

2.1 Sample Preparation

Eleven varieties with seed sources were available in Table 1. Samples of rice were used as samples of isolates of *B. glumae* pathogenic bacteria obtained from previous studies. Isolate *B. glumae* was rejuvenated on Nutrient Agar (NA) media and then incubated for 3 x 24 hours, then made a suspension with a concentration of $10^8$ cfu/ml dissolved sterile distilled water and sterile distilled water as a control treatment.

**Table 1. Samples of Rice Varieties**

| No | Varieties | Seed Source | Plant Age |
|----|-----------|-------------|-----------|
| 1  | Cigeulis  | ICDF/COE in collaboration with FAPERTA UNHAS | 115-125   |
| 2  | Sidenuk   | ICDF/COE in collaboration with FAPERTA UNHAS | ±103      |
| 3  | Inpari 32 | ICDF/COE in collaboration with FAPERTA UNHAS | 120       |
| 4  | Inpari 33 | ICDF/COE in collaboration with FAPERTA UNHAS | ±100      |
| 5  | Inpari 30 | Maros Protection Center                   | 111       |
| 6  | Inpari 7  | Maros Protection Center                   | 110-115   |
| 7  | Inpari 4  | Maros Protection Center                   | 115       |
| 8  | Mekongga  | Maros Protection Center                   | 116-125   |
| 9  | Ciherang  | Maros Protection Center                   | 116-125   |
| 10 | Ciliwung  | Maros Protection Center                   | 117-125   |
| 11 | Cisantana | Maros Protection Center                   | 118       |

2.2 Rice Planting

Planting is done inside the Greenhouse. A 10-liter bucket is prepared and filled with soil as a medium for growing rice. The seeds are soaked in water for 24 hours, then sown on soil-filled media until the age of the plant is 19 days, then transplanted into a media bucket. In each variety there were 2 replications of control and 3 replications of *B. glumae*, 3 replicates were planted for 3 planting holes.

2.3 Inoculation

The method of *B. glumae* inoculation modified from Mizobuchi [4] by spraying on rice panicles on the third day after flowering, this period is the most vulnerable condition with the highest infection [5].

2.4 Observation

The resistance of rice varieties to *B. glumae* disease was observed through disease severity each affected variety. The severity of the disease is calculated using the following formula:

$$IP = \sum_{l=0}^{n} \left( \frac{n_l \times v_i}{Z \times N} \right) \times 100\%$$

*IP* is the intensity of disease severity (%), this is the number of plants or parts of plant samples with the scale of damage, *vi* is the value of scale damage to example-1, *N* is the number of plants sampled, and *Z* is the highest damage scale value. Each variety that was inoculated with *B. glumae* was based on observation score of grain rot severity. The disease severity score from *B. glumae* followed Karki [6] using a scale of 0 - 9 (Table 2).
Table 2. Scoring of disease severity in rice panicles

| Scale | Disease Incidence (%)                      |
|-------|--------------------------------------------|
| 0     | no symptoms                                |
| 1     | 10% are symptomatic                        |
| 2     | 11 – 20% are symptomatic                   |
| 3     | 21 – 30 % are symptomatic                  |
| 4     | 31 – 40 % are symptomatic                  |
| 5     | 41 - 50 % are symptomatic                  |
| 6     | 51 – 60 % are symptomatic                  |
| 7     | 61 – 70 % are symptomatic                  |
| 8     | 71 – 80 % are symptomatic                  |
| 9     | > 80 % are symptomatic                     |

Standard for variety selection resistance from grain rot according to Mizobuchi [4] was chosen according to level of resistance and relative maturity using a scale of 1-5 (Table 3).

Table 3. Scoring Resistance varieties based on disease severity score

| Scale | Level of Resistance                  |
|-------|-------------------------------------|
| 1     | < 3 (Strong)                        |
| 2     | ≥ 3 dan < 4 (Medium to Strong)      |
| 3     | ≥ 4 dan < 6 (Medium)                |
| 4     | ≥ 6 dan < 7,5 (Weak to Medium)      |
| 5     | ≥ 7,5 (Weak)                        |

2.5 Data analysis
Each planting of rice varieties inoculated with rotten disease and control as a comparison. This study was analyzed in a qualitative test using group design with descriptive statistics.

3. Results and Discussion

Table 4. Level of varieties resistance and severity of grain rot at 2 WAI (Week After Inoculation)

| No. | Varieties | Disease Incidence (%) | Level of varieties Resistance |
|-----|-----------|-----------------------|-------------------------------|
| 1   | Cigeulis  | 8,55                  | Strong                        |
| 2   | Sidenuk   | 31,39                 | Medium to strong              |
| 3   | Inpari 32 | 24,12                 | Medium to strong              |
| 4   | Inpari 33 | 50,73                 | Medium                        |
| 5   | Inpari 30 | 6,71                  | Strong                        |
| 6   | Inpari 7  | 18,08                 | Strong                        |
| 7   | Inpari 4  | 7,58                  | Strong                        |
| 8   | Mekongga  | 21,99                 | Medium to strong              |
| 9   | Ciherang  | 14,82                 | Strong                        |
| 10  | Ciliwung  | 9,34                  | Strong                        |
| 11  | Cisantana | 61,02                 | Weak to medium                |

Source: Primary data after processing, 2019

Level of disease severity of grain rot in rice plants varies by variety (Table 4). Based on the level of resistance to disease severity in rice panicles, there were four groups. In the first group with a scale of 1 (varieties resistance: strong) obtained six varieties, namely varieties Cigeulis, Inpari 30, Inpari 7, Inpari 4, Ciherang, and Ciliwung. In the second group with a scale of 2 (varieties resistance: medium
to strong) three varieties were obtained, namely Sidenuk, Inpari 32 and Mekongga. In the third group with a scale of 3 (varieties resistance: medium), namely Inpari 33 varieties, and in the fourth group with a scale of 4 (varieties resistance: weak to medium), namely the Cisantana variety.

At Greenhouse, it was recorded that daytime temperatures ranged from 25 - 41°C and were at most 42°C, and humidity ranged from 31 - 95%. Symptoms begin to appear in the generative phase (78-93 day after planting) but with disease severity varies by variety. Observation of severity was carried out at 2 weeks after inoculation, in table 5 it was shown that the lowest severity level was in Inpari 30 varieties of 6.71% until the highest severity level was in Cisantana variety at 61.02%.

Symptoms of this disease arise when plants enter the flowering phase and bacteria multiply rapidly on the panicle surface [7]. Zhou-qi [8] suggested that brown spots on grains and lesions on flag leaves were an effect caused by toxoflavin and fervenulin produced by B. glumae.

The resistance of varieties from bacterial grain rot has not been developed. The urgent need for the development of bacterial grain rot-resistant cultivars includes the background as follows: the absence of highly effective pesticides for bacterial rot, sudden outbreaks due to environmental changes, and the occurrence of panicles and nurseries [4]. Because bacterial grain rot tends to be strongly influenced by environmental conditions such as humidity and temperature, it is difficult to evaluate the resistance of different canopy date varieties using field inoculation [9].

Fory [10] reported that of the 14 asymptomatic seed samples, there were 3 samples were positively infected with B. glumae. This situation arises because if pathogenic carrier seeds have survived the post-germination phase, most of the vegetative phase of plant shows no symptoms until grain formation, where symptoms of the disease appear again [11,12].

4. Conclusion

The resistance of rice varieties to disease severity has varied results based on variety. Of the eleven varieties tested there were six varieties whose disease severity was low and included a strong level of variety resistance. Of the eleven varieties, Cisantana variety has the highest severity of 61.02% and variety resistance is weak to medium. Further research is needed to the identification of B. glumae presence in plant tissue sampling both asymptomatic and symptomatic seeds of each variety.

References

[1] Appendix of Decree of the Minister of Agriculture Indonesia, Number 51 / Minister of Agriculture Regulation / KR010 / 9 / 2015, concerning Types of Quarantine Plant Pest Organisms (QPPO) Categories A1 and A2

[2] Appendix of Decree of the Minister of Agriculture Indonesia, Number 31 / Minister of Agriculture Regulation / KR.010 / 7 / 2018, concerning Types of Quarantine Plant Pest Organisms (QPPO) Categories A1 and A2

[3] Joko T. 2017. *Burkholderia glumae as Emerging Pathogens: Status, Potential Damage, and Control Strategies*. National Phytopathology Symposium "Emergence of New Diseases and Import of Seeds" p. 27-35, Bogor

[4] Mizobuchi R, Fukuoka S, Tsuiki C, Tsushima S, and Sato H. 2018. *Evaluation of major japanese rice cultivars for resistance to bacterial grain rot caused by Burkholderia glumae and identification of standard cultivars for resistance*. Research paper. Breeding Science Preview. doi:10.1270/jsbbs.18018

[5] Shahjahan, A. K. M., M. C. Rush, C. E. Clark, and D. E. Groth. 1998. *Bacterial sheath rot and panicle blight of rice in Louisiana*. Proc. 27th RTWG. 27: 31-32

[6] Karki H S, Shrestha B K, Han J W, Groth D E, Barphagha I K, Rush M C, Melanson R A, Kim B S, and Han J H. 2012. *Diversities in virulence, antifungal activity, pigmentation and DNA fingerprint among strains of Burkholderia glumae* PLoS One, 7:e45376

[7] Miller S A, Beed F D, and Harmon C L. 2009. *Plant disease diagnostic capabilities and networks*. Annu. Rev. Phytopathol. 47:15-38

[8] Zhou-qi [8] suggested that brown spots on grains and lesions on flag leaves were an effect caused by toxoflavin and fervenulin produced by B. glumae.
[8] Zhou-qi, C., Z. Bo, X. Guan-lin, L. Bin and H. Shi-wen. 2016. Research Status and Prospect of Burkholderia glumae, the Pathogen Causing Bacterial Panicle Blight. Rice Science 23:111–118

[9] Tsushima S. 1996. Epidemiology of bacterial grain rots Of rice caused by Pseudomonas glumae. JARQ 30 (2): 85-89

[10] Fory PA, Triplett L, Ballen C, Abello JF, Duitama J, Aricapa MG, Prado GA, Correa F, Hamilton J, and Leach JE. 2014. Comparative analysis of two emerging rice seed bacterial pathogens. Phytopathology. 104:436–444

[11] Hikichi, Y., Okuno, T. and Furusawa, I. 1993. Immunofluorescent antibody technique for detecting Pseudomonas glumae on rice plants. Japanese J. Phytopathol. 59:477-480

[12] Li, L., Wang, L., Liu, L., Hou, Y., Li, Q. and Huang, S. 2016. Infection process of Burkholderia glumae before booting stage of rice. J. Phytopathol. 164:825-832