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SHORT COMMUNICATION

Detection of the presence of bacteria causing grain rot disease (*Burkholderia glumae*) in some rice seed producers in South Sulawesi, Indonesia

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ABSTRACT

One of the constraints in rice production is grain rot disease caused by *Burkholderia glumae*, which can be carried by seeds grain. An observation to determine the presence of *B. glumae* in different grain yield classes of seeds was conducted by taking samples derived from several seed producers in South Sulawesi. This research was carried out by first taking seed samples at the South Sulawesi Agricultural Technology Study Center (BPTP), Tungro Research Workshop, South Sulawesi Main Seed Center (Balai Benih Induk), PT. Sang Hyang Seri, PT. Pertani and PT. Harmoni were then tested at the Agricultural Quarantine Laboratory of Makassar. Based on the observations, it was concluded that all samples in the sowing seed class tested positive for *B. glumae*, supported by an average percentage of disease incidence of 25.13%, namely foundation seeds from the Balai Benih Induk, as well as foundation seeds and stock seeds from AIAT.

Key words: *B. glumae*, extension seed, foundation seed, stock seed

INTRODUCTION

The high demand for rice must be supported by increased rice production. According to data from the Central Statistics Agency of Indonesia (CSA), the productivity of the national rice yield in 2023 reached 52,59 quintals per hectare. One of the factors affecting rice productivity is the quality of seeds and the use of disease-free seeds. Increasing rice productivity does not always run smoothly and tends to encounter problems. One of these problems is plant diseases. Several new diseases have been discovered in Indonesia, including bacterial grain rot caused by *Burkholderia glumae* (Wahidah et al., 2019; Sahlan et al., 2023). The presence of *B. glumae* can also result in various diseases, including sheath rot and seedling rot, commonly referred to as panicle blight (Nandakumar et al., 2009; Ham et al., 2011).

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Grain rot disease in rice was first reported in Japan in 1950 and has since become one of the important diseases affecting rice plants worldwide (Zhou-qi et al., 2016). *B. glumae* is one of the most damaging seed-borne pathogens in many rice-producing regions. The most severe infections have been linked to yield losses ranging from 15% to 80% (Fang et al., 2009). According to Regulation No. 51 of 2015 from the Minister of Agriculture of the Republic of Indonesia, *B. glumae* has been detected in rice plantations located on the Java, Sumatra, and Kalimantan islands. This disease is more prevalent in rice grains, earning it the name "grain rot" in several Asian countries (Wamishe et al., 2014).

The spread of *B. glumae* must be monitored because this pathogen affects both the quality and quantity of rice. *B. glumae* infestation in rice plantations in Indonesia has not yet reached the severity of bacterial leaf blight caused by *Xanthomonas oryzae* and blast disease caused by *Pyricularia oryzae* (Handiyanti et al., 2018). In terms of ecological conditions, Indonesia is an ideal location for the spread of bacterial grain rot, given its hot and dry climate and high precipitation levels. The presence of high nighttime temperatures and frequent precipitation are environmental factors that tend to exacerbate this disease (Karki et al., 2012).

The presence of bacterial grain rot disease has existed since 1987, but since then, there have been no reports indicating that this disease has caused severe damage (Indonesian Ministry of Agriculture, 2015). The

presence of *B. glumae* has reportedly spread to several areas in Indonesia such as Sulawesi, Java, North Sumatra (Aflaha et al., 2020; Widarti et al., 2020; Hasibuan et al., 2018). Since 2015, however, the existence of this disease has begun to be reported again in several regions of Indonesia. Due to global climate change, *B. glumae* has been identified as an emerging pathogen in several countries. Importing seeds from countries where rice grain rot has recently been prevalent can serve as a source of new inoculum (Handiyanti et al., 2018).

Molecular detection was performed to identify the presence of *B. glumae* in multiple seed samples. Molecular biology-based identification is considered more accurate than morphological identification. Using molecular identification techniques, the identities of various plant diseases, including those whose pathogens could not be isolated on artificial media, were revealed. The Polymerase Chain Reaction (PCR) technique is one of the molecular detection methods. PCR, a highly sensitive technique, allows the detection of low-abundant, slow-growing, or non-culturable cells (Schaad et al., 2003). This method has a high level of sensitivity and can be completed in a shorter amount of time than other methods commonly used to detect pathogens (Venbrux et al., 2023).

In this article, we provide an overview of grain rot diseases and the incidence of the disease in the fields of six South Sulawesi-based rice seed producers. Additionally, we determined that grain rot disease caused by *B. glumae* was detected using the PCR technique with specific primers in some rice seed producers in South Sulawesi.

MATERIALS AND METHODS

Research Site. The research was conducted at the Agricultural Quarantine Laboratory of Makassar, Indonesia.

Seed Sampling. The rice seed samples weighed 100 g per seed class in six seed sources, including three seed-producing government agencies (BP= The South Sulawesi Agricultural Technology Study Center (BPTP); LT= Lolit Tungro (Tungro Research Workshop); BB= Main Seed Center (Balai Benih Induk)) and three private producing companies (SS= PT. Sang Hyang Seri; PT= PT. Pertani; HR= PT. Harmoni). Seed classes were further categorized as 1= Foundation seed, 2= Stock seed, and 3= Extension Seeds. The combination of the following symbols:

SS1 = Foundation seed samples from PT. Sang Hyang Seri;
SS2 = Stock seed samples from PT. Sang Hyang Seri;
SS3 = Extension seed samples from PT. Sang Hyang Seri;
PT3 = Extension seed samples from PT. Pertani;
HR3 = Extension seed samples from PT. Harmoni;
LT1 = Foundation seed samples from Lolit Tungro;
LT3 = Extension seed samples from Lolit Tungro;
BP1 = Foundation seed samples from BPTP;
BP2 = Stock seed samples from BPTP;
BP3 = Extension seed samples from BPTP;
BB1 = Foundation seed samples from Main Seed Center;
BB2 = Stock seed samples from Main Seed Center;
BB3 = Extension seed samples from Main Seed Center.

Disease Incidence of Symptomatic Rice Grains. The observation of seed morphology involved selecting seeds exhibiting symptoms of stripes or brown spots on the surface of rice grains from each seed class. From a 1 kg sample for each seed class from every seed producer, 400 seeds were randomly selected to calculate the percentage of symptom occurrence by distinguishing between symptomatic and asymptomatic seeds. The percentage of symptomatic seeds was calculated using the formula:

$$DI = \frac{a}{b} \times 100\%$$

DI = Disease Incidence;
a = The number of symptomatic seeds;
b = The total number of seeds observed.

Morphology and Molecular Identification of *B. glumae*. The observation of seed morphology involved selecting seeds that exhibited symptoms of stripes or brown spots on the surface of rice grains from each type of seed source.

Extraction of each sample was performed to obtain total DNA, utilizing the DNeasy Plant Mini Kit (QIAGEN) protocol. DNA amplification was carried out using PCR reagents, including pure Taq bead ready to go (GE Health), a pair of specific primers (1418S-1418A), and nuclease-free water. The specific primers used to amplify the DNA of each bacterial isolate were 1418S: 5'-GCG ATA TGG CAA GAC GCA AA-3 and Primer 1418A: 5'-AGT CAT ACC CTT TGT CAG CGT-3'

(Aflaha et al., 2020).

The DNA amplification process was conducted using a PCR Thermal Cycler machine (Applied Bio System). The amplification reaction consisted of denaturation at 94 ºC for 30 s, annealing at 58 ºC for 20 s, and extension at 72 ºC for 30 s. This cycle was repeated 29 times, with the final extension at 72 ºC for 5 min.

The amplified DNA bands were visualized through electrophoresis using 1.5% agarose at 90 volts for 45 min. DNA band visualization was carried out with Sybr green staining. The DNA marker employed was a 100 bp ladder (Thermo), mixed with 5 µL, 2 µL of loading buffer 5×, and 2 µL Sybr green. Detection was performed using UV light on the gel documentation system (UVP UPLAND CA). The presence of *B. glumae* was confirmed by observing DNA bands with a size of 571 bp (Aflaha et al., 2020).

RESULTS AND DISCUSSION

Infestation and Morphology of Rice Seeds Infected with *B. glumae*. Observation of seed morphology was conducted by selecting seeds exhibiting symptoms of stripes or brown spots on the surface of the rice grains from each type of seed source (Figure 1).

Table 1 showed that the intensity of attack symptoms on the extension seeds was higher than on the stock and foundation seeds. Extension seeds from PT. Sang Hyang Seri had the highest percentage, reaching 32.25%, followed by 22% for stock seeds, and 16.25% for foundation seeds from Lolit Tungro.

***B. glumae* DNA Amplification by PCR.** The presence of *B. glumae* was successfully detected by PCR. The presence of ±575 bp DNA bands confirmed positive amplification reactions in six seed samples (Figure 2).

Figure 2 showed the samples from Balai Benih Induk tested positive for the stock seed and extension seed samples. Samples from AIAT and PT. Sang Hyang Seri tested positive only for the extension seeds, while samples from Lolit Tungro (foundation and stock seeds), PT. Harmoni (extension seeds), and PT. Pertani (extension seeds) also tested positive. However, the foundation seed samples from Balai Benih Induk, as well as the foundation seeds and stock seeds from AIAT, tested negative using the PCR detection test for *B. glumae* bacteria that causes seed rot disease.

The disease that causes grain rot by *B. glumae* is one of the bacteria that causes significant crop loss worldwide. A study conducted by Mulaw et al. (2018) reported that 45 out of 175 samples of bacterial panicle

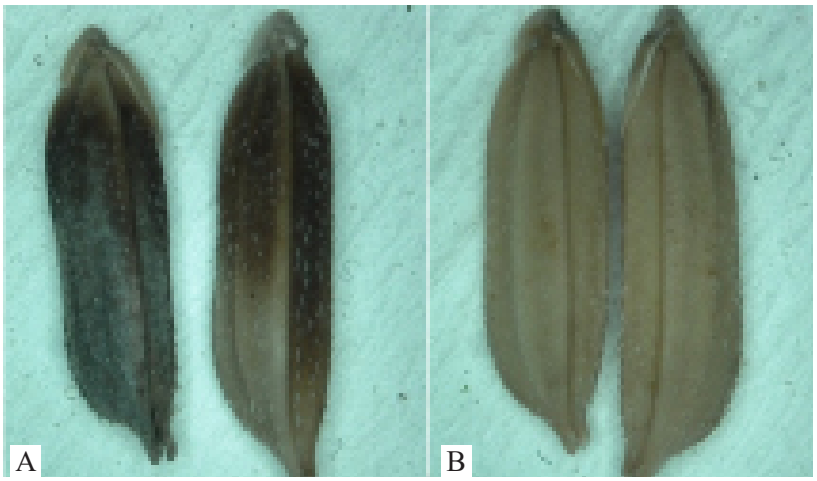

Figure 1. Differences in samples of rice seeds. A. Symptoms of grain rot; B. Healthy seeds.

Table 1. Percentage of symptomatic seeds

Seed sources	The percentage of symptoms (%)		
	Foundation seed	Stock seed	Extension seed
PT Sang Hyang Seri (SS)	11.75	22.00	32.25
PT Pertani (PT)	-	-	25.75
PT Harmoni (HR)	-	-	26.50
Lolit Tungro (LT)	16.25	-	27.50
BPTP (BP)	6.00	17.50	18.25
Balai Benih Induk (BB)	8.25	14.25	20.50

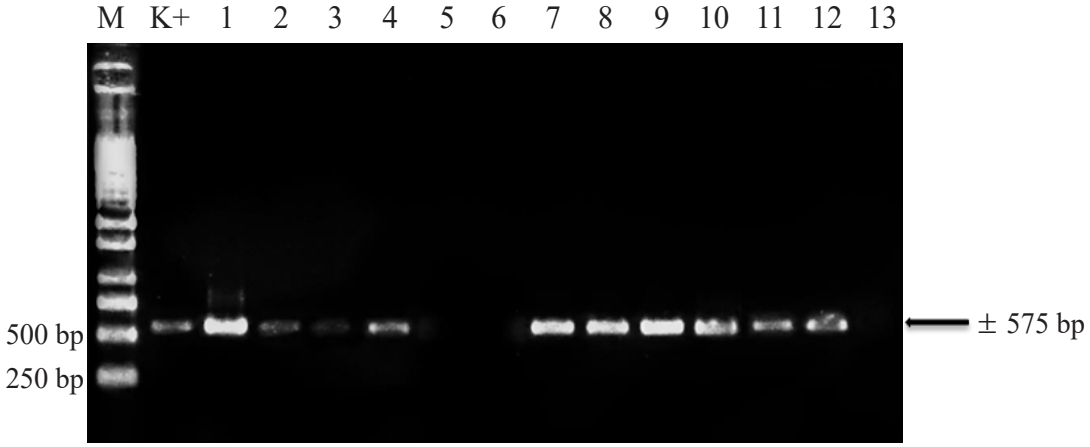

Figure 2. Results of *B. glumae* DNA amplification from rice seeds. M, DNA Marker 1 Kb (Thermo Scientific USA); Rice seed samples: 1. PT3; 2. HR3; 3. LT1; 4. LT3; 5. BP1; 6. BP2; 7. BP3; 8. SS1; 9. SS2; 10. SS3. 11. BP3; 12. BP2; 13. BP1.

blight in Arkansas were caused by *B. glumae,* and no disease was found to be caused by *B. gladioli. B. glumae* infects rice grains, invades the plumule through stomata and wounds, and spreads in the spaces between parenchyma cells during the germination process. Bacterial proliferation in the plumule supports bacteria to produce toxic compounds such as toxoflavin, which can cause the grain to rot (Zhou-qi et al., 2016). One of the infected grains is sterile or causes the grains to turn brown at the base (Goto et al., 1988).

The results of molecular detection showed that not all seed classes were positively infected by *B. glumae*. Samples from the seed class all tested positive for *B. glumae* bacteria. Therefore, it becomes a concern for seed producers in producing extension seeds. This information will assist seed producers in selecting the seeds to be produced. *B. glumae,* which was previously only a minor pathogen, has increased its status to become a major pathogen in rice. This is related to favorable weather changes, namely warm night conditions and high humidity and rainfall during the growing season (Zhou-qi et al., 2016; Suharti et al., 2017). Geographically, countries with semi-tropical and tropical climates are relatively vulnerable to *B. glumae* attacks because the optimum temperature for the growth of this bacterium is relatively high, ranging from 30 to 35 ºC (Ham et al., 2011).

According to Pedraza et al. (2018), the primary means of transmission for *B. glumae* is via seeds that have been contaminated. The presence of the pathogen in the soil or on infected plants poses a risk of seed contamination during different stages, including harvesting, processing, and storage (Kouzai & Akimoto-Tomiyama, 2022). The aforementioned scenario may lead to the extensive dissemination of the pathogen among seed-producing enterprises. Furthermore, it has been observed that *B. glumae* exhibits the ability to endure and maintain its population during both the vegetative and reproductive stages of rice plants (Pedraza et al., 2018). Consequently, in situations where the initial contamination stems from the seeds, the pathogen possesses the ability to endure, reproduce, and spread throughout the complete life cycle of the plant, encompassing the pivotal stage of seed production.

The introduction and sustained presence of the pathogen within seed-producing companies are influenced by its existence in the environment, including the soil and other plant species. *B. glumae* exhibits a broad spectrum of plant invasion capabilities, encompassing both monocotyledonous and dicotyledonous plant species (Compant et al., 2008). This implies that the bacteria exhibit the ability to persist in the soil through the process of infecting and establishing colonies within various host plants. Therefore, rice is widely regarded as the plant species most vulnerable to invasion by *B. glumae*.

Apart from environmental factors, the pathogenicity of *B. glumae* is significantly influenced by the presence of a type 3 secretion system (T3SS) (Wallner et al., 2021). The pathogenicity of *B. glumae* is contingent upon the utilization of quorum sensing (QS) mechanisms to facilitate the production and secretion of toxoflavin, a compound that is primarily responsible for the extensive harm inflicted upon rice crops (Ham et al., 2011). This phenomenon entails the ability of bacteria to engage in intercellular communication and synchronize their assault on the host plant, thereby potentially facilitating its dissemination.

Rice bacterial diseases are relatively difficult to control because the pathogen and its genetic

characteristics are easily mutated, especially its virulence against rice varieties. In the United States, it was reported that there were more than 400 strains isolated from rice plantations in various states (Nandakumar et al., 2009). There are variations in virulence from highly virulent to low virulence. *B. glumae* is known to produce a toxin that is suspected as a virulence factor (Jeong et al., 2003). Currently, there is no standard technique for forecasting bacterial diseases, especially for the tropics, so the exact timing of control cannot be determined.

The main control of rice diseases caused by bacteria is by using resistant varieties. The resistance of rice plants to *B. glumae* is highly dependent on the type of variety used (Amirullah et al., 2020). Furthermore, the effective implementation of environmental management strategies is also of paramount importance in mitigating the spread of *B. glumae*. These measures include agricultural techniques such as crop rotation, weed control, and soil management, which collectively strive to reduce the prevalence of the pathogen in the surrounding ecosystem (Akimoto-Tomiyama, 2021).

The use of resistant varieties is the main option for controlling rice grain rot, but until now, there has been no report on resistant varieties. The utilisation of pathogen-free seeds is the primary preference due to the inherent characteristics of the pathogen carried by the seeds. However, it should be remembered that even seeds that do not show disease symptoms are not necessarily free from *B. glumae.* Therefore, the health factor of the seeds must also be one of the parameters of seed testing, in addition to agronomic factors.

The implementation of comprehensive seed testing protocols is a crucial measure in protecting the agriculture sector against inadvertent transmission of *B. glumae*-infected seeds. Seed-producing companies can enhance their biosecurity measures and guarantee the provision of uncontaminated seeds to farmers by adopting sophisticated detection technologies, conducting routine inspections, engaging in collaboration with relevant stakeholders, and making investments in employee training.

CONCLUSION

The symptom of stripes or brown spots on the grain surface of rice was found in all six different seed-producing companies and all classes. According to the results of molecular detection with specific primers, some of the samples were infected by *B. glumae*. The highest proportion of symptomatic seeds in every seed resource was shown by the extension seed class. To figure out the taxonomy of the bacteria at the level of individual strains, sequencing reads need to be matched to the bacteria.

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AUTHORS' CONTRIBUTIONS

BP and RJ considered and planned the experiment. AR and AY carried out monitoring rice fields in six different locations and taking seeds samples. AR performed molecular work and analysis. AY collecting data on the plant damage area caused by *B. glumae*. AKFB perform analysis and interpreting the plant damage and prepared the manuscript. The authors provided responses and comments on the research flow, data analysis and interpretation as well as shape of the manuscript. All the authors have read and approved the final manuscript.

COMPETING INTEREST

We declare there are no relevant financial or nonfinancial competing interests to report.

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