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SHORT COMMUNICATION

Rice Blast Field Assessment in Three Regencies Underlies the Importance of Fungicide Resistance Studies in West Java, Indonesia

Asesmen Lapangan Penyakit Blas Padi Mendasari Pentingnya Kajian Resistensi Fungisida di Jawa Barat, Indonesia

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ABSTRACT

In recent decades, research about rice blast disease in Indonesia has not been focused on the dynamics of the fungus (*Pyricularia oryzae*) and fungicide use, which resulted in undiscovered pathogen mutations. Observations in Bogor, Cianjur, and Sukabumi Regencies in West Java reveal a high disease incidence (53%–100%), with severity ranging from 8% to 67%. The highest disease severity was recorded in Cikembar District, which is located at the foothill of Mount Gede Pangrango, Sukabumi Regency. Despite a prolonged drought caused by *El Niño* in 2023, Cikembar still experienced a relatively high disease severity (36%), confirming that this area remains an endemic blast area. The high disease severity, although fungicides were widely used in Cikembar, raises concerns that *P. oryzae* resistance to fungicides has developed, mainly to isoprothiolane which has been yearly deployed in this area. Farmers are already confronting extra challenges such as unfavorable acidic soil and differences in fungicide use practices decision-making which complicates their control efforts. Laboratory investigations are needed to validate evidence of the emergence of *P. oryzae* mutations against isoprothiolane in order to provide long-term recommendations for the most effective fungicide use.

Keywords: Cikembar, fungicide use practice, mutation, *Pyricularia oryzae,* rice cultivar

ABSTRAK

Dalam dekade terakhir, penelitian penyakit blas padi di Indonesia belum membahas dinamika antara cendawan *Pyricularia oryzae* dan penggunaan fungisida sehingga belum ada laporan terkait mutasi patogen ini. Pengamatan di Kabupaten Bogor, Cianjur, dan Sukabumi, Jawa Barat menunjukkan insidensi penyakit yang tinggi (53%–100%) dengan keparahan berkisar 8%–67%. Keparahan penyakit tertinggi terjadi di Kecamatan Cikembar, daerah di kaki Gunung Gede Pangrango, Kabupaten Sukabumi. Pengamatan lebih lanjut menunjukkan bahwa meski dilanda kemarau panjang akibat fenomena *El Niño* pada sepanjang tahun 2023, Cikembar masih mengalami keparahan penyakit yang relatif tinggi (36%) mengonfirmasi daerah ini masih merupakan daerah endemik blas. Tingginya keparahan penyakit meski fungisida diaplikasikan secara intensif di Cikembar memperkuat dugaan telah munculnya kasus resistensi

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P. oryzae terutama terhadap fungisida isoprotiolan yang telah digunakan bertahun-tahun. Kelompok tani Cikembar juga menghadapi tantangan lain, yaitu tanah masam dan perbedaan taktik antarpetani dalam penggunaan fungisida yang memperumit upaya pengendalian penyakit secara terpadu. Bukti kemunculan mutasi *P. oryzae* terhadap fungisida isoprotiolan perlu dikonfirmasi melalui studi laboratorium sehingga diperoleh rekomendasi penggunaan fungisida yang lebih baik dalam jangka panjang.

Kata kunci: Cikembar, kultivar padi, mutasi, praktik penggunaan fungisida, *Pyricularia oryzae*

The Asia continent, as the primary rice producer, is an essential site for the global rice supply and a source of accessible disease inoculum. With yearly crop losses ranging from 10% to 30%, the rice blast fungus (*Pyricularia oryzae*; teleomorph *Magnaporthe oryzae*) is the most destructive pathogen to rice productivity and food security worldwide (Dean *et al*. 2012; Nayak *et al.* 2021). The symptoms include gray-brown neck rot and diamond-shaped lesions on rice leaves. Additionally, brown blotches on the grain may indicate this disease, particularly if the panicles, leaves, and neck have previously been affected. During the off-season, rice blast disease can infect rice seedlings' debris and is primarily transmitted by raindrops and wind. The degree of yield loss is largely determined by the compatibility of host susceptibility and pathogen virulence, as well as the presence of favorable environmental conditions that support a perpetual cycle of pathogenesis (Devanna *et al*. 2022).

To directly target the fungi, synthetic chemical fungicide is still considered more practical for rice blast control. Fungicides damage fungal cell membranes, interfere with fungal energy production, and work either before or after infection and symptom development (Caffi and Rossi 2018). However, the continuous use of a specific type of fungicide may lead to reduced sensitivity of the fungal strain due to genetic pressure when the fungus adapts to severe environmental stressors. Over the previous decade, rice blast research in Indonesia has focused on epidemiology, yield loss estimation, breeding line evaluation, and biological control. There has been limited research into fungicide resistance. However, the occurrence of mutation leading to fungicide resistance in Indonesia raises a high concern since abundance reports published in other countries (Ishii 2006).

Regular observation is the first step in recognizing potential incidences of fungicide resistance, particularly in endemic areas with extensive fungicide use over many years. Therefore, establishing the disease severity status in the field is a crucial issue to consider when deciding if fungicide resistance research is worthwhile. Along with climatic change, shifts over time, and changes in farmer cultivation methods, disease fluctuations occur, resulting in diverse dynamics based on their agroecological origin (Asibi *et al.* 2019). We conducted a study on blast disease control utilizing synthetic chemical fungicides and their relationship with disease incidence and severity in three regencies in West Java, the second-largest rice-producing province in Indonesia. In addition, fungicide use is closely related to farmers' social and economic position. As a result, the difficulties farmers face in controlling blast disease were also recorded in this study. The expected outcome of this research is a better understanding of the situation with blast disease, its relationship to agro-climatic and socioeconomic factors, and the identification of crucial spots for early detection of fungicide resistance emergence.

This study was undertaken in December 2021 and November 2023. Communication with local agricultural officers from the Ministry of Agriculture provided information on blast-affected areas in three regencies i.e. Bogor, Cianjur, and Sukabumi. Purposive sampling was conducted to measure the disease incidence and severity in 2 plots per regency with a total of 30 plants per plot. At the time of observation, temperature and humidity were recorded and confirmed by climatic information from the BMKG (2023). Farmers provided information on rice cultivars and fungicides employed. Cooke's (1998) formula was used for disease measurement, along with IRRI's (2014) scoring scale, and analysed by Tukey test α 0.05 following the one-way ANOVA.

$$\text{DI (\%)} = \frac{n}{N} \times 100\%\text{, with}$$

DI, disease incidence; n, number of infected plants; and N, total number of observed plants.

$$\text{DS (\%)} = \frac{\sum_{i=1}^{9} (n_i \times v_i)}{N \times Z} \times 100\%\text{, with}$$

DS, disease severity; n_i, number of plants infected in a particular scale; v_i, scale score; N, total number of observed plants; and Z, maximal disease scale.

Disease severity was re-examined in five plots in the district with the highest infection rates under varied agroclimatic conditions (dry season in 2023). The five rice plots were chosen based on differences in rice cultivars and fungicides used. At the time of observation, the temperature and humidity were recorded. Additionally, two observation points were taken from each plot to test soil acidity. A total of 11 farmers were interviewed to gather information about cultivation techniques related to blast development. Questions included cultivation practices, production cost, and yearly and dramatic yield loss records.

The blast disease survey area in this study is located around Mount Gede Pangrango, West Java Province, with two rice fields each in Bogor (Situgede and Ciherang), Cianjur (Bojongpicung and Gekbrong), and Sukabumi (Kadudampit and Cikembar). These six fields are at an altitude of 180–900 masl (Figure 1). On average, each rice plot covers an area of 600–700 m^2. Both vegetative and generative stages were found in the same large area, allowing the disease cycle to continuously occur alongside *P. oryzae* infection at different stages including rice seedlings (Figure 2a).

Three monitored regencies were experiencing the rainy season in 2021, with the number of rainy days reaching 20–25 days per month. The average temperature fluctuates between 19–31 °C, with humidity reaching 98%, which is ideal for the development and blast disease progress in the field (Table 1). According to Kirtphaiboon *et al.* (2021), rice blast disease spreads quickly at temperatures ranging from 25 to 29 °C, humidity levels ranging from 60% to 95%, and soil temperatures ranging from 28 to 31 °C. Aside from that, rising atmospheric CO_2 levels create climatic circumstances favorable to the growth of *P. oryzae* (Luck *et al.* 2011).

Symptoms of blast disease observed include chlorotic and necrotic areas in the form of brown spots on the leaves, which then

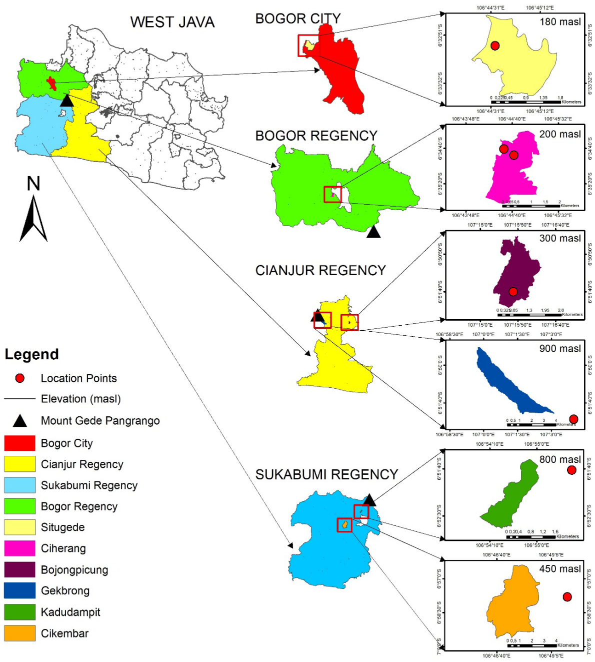

Figure 1 Maps of rice blast disease survey area.

develop into a diamond shape with brown edges and gray center (Figure 2a–b). On symptomatic leaves that are still infectious, aggregates of conidia are visible, indicated by white powder attached to the center of the symptoms, often found in multiple spots on a leaf (Figure 2c). Meanwhile, the symptoms of neck blast disease in brown spots circling the neck (Figure 2d). Plants infected with neck blast often experience empty grains.

Farmers generally plant Ciherang and Inpari cultivars three times a year and apply various fungicides to control blast disease five to six times during each planting season. Despite the continuous and widespread use of these rice cultivars and intensive application of fungicides, as well as the favorable environmental conditions mentioned above, the disease intensity remained varied. The incidence of blast disease ranged from 53% to 100%, with severity ranging from 8% to 67%. Compared to Bogor and Cianjur, Sukabumi Regency had the highest incidence of blast diseases which is heavily influenced by the high rainfall rate (545 mm) and high temperature (27 ^{o}C) (Table 1). In addition, all plots observed in Sukabumi have both leaf and neck blast. Cikembar District in Sukabumi Regency had the highest disease incidence and severity, with values of 100% and 67%, respectively, and clear symptoms could have been noticed in 2-week-old seedlings (Table 2; Figure 2a). Disease dynamics in Cikembar are interesting to be investigated more thoroughly.

The persistent availability of *P. oryzae* fungal inoculum both during and outside of the growing season is one of several variables that contribute to extremely high levels of infection in this location. This is also supported by the geomorphology of Sukabumi, which appears as a terraced valley, providing for continual trapping of high humidity (Hardini *et al.* 2019; Jamil *et al.* 2022). Associated with fungicide usage, isoprothiolane (IPT) is the current commonly used fungicide by farmers in Cikembar District to control blast disease, as it remains the most effective fungicide among several alternative fungicide options (Table 2). Farmers, on the other hand, report that the efficacy of isoprothiolane is declining.

Farmers who were usually able to harvest 6 tonnes ha^{-1} have only harvested 5 tonnes ha^{-1} after using IPT continuously in the past three

a b c d

Figure 2 Symptoms of blast disease observed. a, occurrence of leaf blast in 2-weeks old seedlings in Cikembar District; b, light leaf infection; c, severe leaf infection; and d, neck blast.

Table 1 Average temperature, relative humidity, rainfall days, and rainfall rate in Bogor, Cianjur, and Sukabumi Regency in December 2021

Climatic factors	Bogor	Cianjur	Sukabumi
Temperature (^{o}C)	20–33 (av.[a] 26)	19–31 (av. 24)	19–28 (av. 27)
Relative humidity (%)	54%–98%	76%–97%	67%–98%
Rainfall days (days per month)	20	23	25
Rainfall rate (mm)	279	353	545

[a]Average

years. This 16% decline caused uncertainty, and farmers believe that the effectiveness of IPT has been lowered, prompting them to begin looking for substitutes. However, genetic alterations in *P. oryzae* that allow it to become less sensitive to IPT may be the source of decreasing control performance, rather than the fungicide no longer being effective. This concern requires additional confirmation by laboratory tests of isolates from field collections that have been extensively exposed to IPT.

Given that farmers have reported that their predominant fungicide has reduced performance, as demonstrated by the high disease severity, it is thought that the *P. oryzae* population in this area was subjected to a mutation. Mutation from fungicide resistance can result from alterations to genes that encode fungicide targets or from a wide range of mechanisms triggered by sub-lethal fungicide exposures (Deising *et al.* 2008). Isoprothiolane is a systemic fungicide reported to stimulate resistance in *P. oryzae* lab-generated mutants, among other fungicides reported overseas, thus comparable examples of resistance reports have probably evolved in Indonesia requiring further research (Table 3).

Aside from the indispensable of *P. oryzae* genetic variability reflected in disease dynamics

Table 2 Disease incidence and severity of rice blast in six fields in Bogor, Cianjur, and Sukabumi with different cultivars and fungicides used (2021)

District	Rice cultivars	Type of blast		Fungicide	Incidence (%)[a]	Severity (%)[a]
		Leaf	Panicle			
Situgede	Ciherang	√	×	Azoxystrobin	70.0 a	20.7 a
Ciherang	Inpari 32	√	√	Propiconazole	53.3 a	8.1 a
Bojongpicung	Inpari 32	√	×	Difenoconazole	73.3 a	16.3 a
Gekbrong	Inpari 33	√	√	Thiophanate-methyl	86.7 a	31.1 ab
Kadudampit	Inpari 32	√	√	Tricyclazole	76.7 a	21.8 a
Cikembar	Ciherang	√	√	Isoprothiolane	100.0 a	67.4 b

[a]Means followed by the same letter are not significantly different by Tukey test α 0.05

Table 3 Published reports on the fungicide resistance of *Pyricularia* species

Active ingredients	Species	Host	Country of occurrence	References
Azoxystrobin	*P. oryzae*	Rice	Brazil, Japan, Italia	D'Avila *et al.* (2021); Ishii (2006); Tenni *et al.* (2021)
Isoprothiolane	*P. oryzae*	Rice	China	Wang *et al.* (2018) Meng *et al.* (2023)
Triazole	*P. grisea, P. graminis-tritici, P. pennisetigena*, *P. urashimae*	Wheat	Brazil	Dorigan *et al.* (2019)
Tricyclazole	*P. oryzae*	Rice	Brazil	Bezerra *et al.* (2021); D'Avila *et al.* (2021)

Table 4 Disease incidence and severity in five plots in Cikembar, West Java with different cultivars and fungicides used (2023)

Plot	Rice cultivars	Fungicide	Incidence (%)	Severity (%)
1	Mixture	Isoprothiolane	90 ± 8.2	28.5 ± 6.8
2	Ciherang	Carbendazim	100 ± 0.0	33.3 ± 10.1
3	IR64	Tricyclazole + Azoxystrobin	97 ± 4.7	20.4 ± 2.6
4	Ciherang	Isoprothiolane	90 ± 8.2	30.7 ± 8.6
5	Local	Tricyclazole	100 ± 0.0	35.6 ± 6.3

and geographical conditions supporting the disease development, farmers face some other challenges when managing blast disease. Acidic soil (pH 4.1 ± 0.2) is a current challenge that farmers also encounter in Cikembar District (Figure 3). This acidic soil is favorable for pathogen development but is less favorable for plant growth in nutrient absorption, particularly Na, Ca, Mg, and Cl (Thakur *et al.* 2021). All interviewed farmers had experienced 10%–15% production loss per year owing to blast, with the worst condition ever being 55%, thus they designed several strategies to mitigate this threat in the future. Farmers continue to prefer the Ciherang cultivar due to its high yield potential and social acceptability but also plant the IR64 cultivar considering lower production loss when the rice blast outbreaks due to IR64's higher performance of resistance.

Other challenges in integrated rice blast management are seedlings and fungicide costs which reach 24% of total production expenditures. To cut production expenses, some farmers discontinue using fungicides or limit their frequency of use, especially if the yield that can be saved is predicted not to cover the whole production cost. The differences in decision-making are driven by the quantity of land possessed and the land tenure system, which classifies certain farmers as cultivators rather than landowners (Kihoro *et al.* 2013). However, these varied methods and economic volatility increase the rice blast progress, availability and dispersion of inoculum sources are more problematic.

The recurrence of blast disease in Cikembar during the dry season has raised concern about whether the infection cycle could be highly suppressed. At the time of observation, the air temperature ranged between 25 and 32 ^{o}C, with a humidity of 75%. The number of rainy days is only about 7–11 days per month, with rainfall of less than 100 mm per day. This is heavily influenced by the length of the dry season in 2023. A moderate *El Niño* has been present in West Java Province since mid-2023 (BKMG 2023; Ludher and Teng 2023). This climate anomaly causes a long dry season (more than 60 days) on Java Island and should be not ideal for blast development in the field. Not only affect fungal development, but drought stress also causes less sensitivity to abscisic acid (ABA) and salicylic acid (SA) activities and several downregulations of defense-related transcripts making the plants more susceptible to new infection (Roussin-Léveillée *et al.* 2024).

Global climate changes have been shown to have an impact on both plant health and the activity of pathogenic fungi performing a novel state of disease triangle including the rice–*P. oryzae* pathosystem. Elevated temperatures decrease SA production while increasing jasmonic acid (JA) resulting in an SA/JA antagonism and imbalance of defense-related phytohormone. High relative humidity decreases the ACC deaminase enzyme which acts as an ethylene suppressor and alters the responsiveness of stomatal guard cells leading to a decrease in the response for PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI). In other words, climate change impacts both plants and pathogens, but this fungus can immediately adapt to become more resilient and virulent (Roussin-Léveillée *et al.* 2024).

The higher adaptability of *P. oryzae* in facing climate change is noted by the disease incidence in Ciherang, IR64, local, and mixed cultivars was above 90% in the dry season (Table 4). Although the daily humidity is lower, the presence of dew in the morning provides adequate water for conidial germination (Everts 1990). Meanwhile, the

Figure 3 Device used by farmers to measure the soil pH showing acidic soil (pH 4.2).

severity of the disease produced was moderate (20%–36%). Given that the plots were in the same location and had similar sources and amounts of inoculum, the IR64 cultivar had the lowest disease severity (20%) and undeveloped symptoms confirmed by *halo* encircling most of the small spots (Table 4; Figure 4c). According to Santoso *et al.* (2021), different rice cultivars performed a diverse host response against 200 Indonesian strains of *P. oryzae* and its main races. IR64 is known to include 11 blast resistance genes that are effective in blast control tactics, particularly in tropical countries (Fukuta *et al.* 2022).

The use of isoprothiolane on mixed cultivars yielded intriguing results. The plant's response to this practice resembles that of IR64 with undeveloped multiple spots (Figure 4a). This is thought to be the result of varying resistance degrees provided by the multiline. Jamaloddin *et al.* (2021) explained resistance rice cultivars are capable of exhibiting multiple pathogen races (vertical resistance) or numerous diseases simultaneously (horizontal resistance). In addition, isoprothiolane activity conveyed fungal cell membrane disorganization in the choline biosynthesis pathway (Uesugi 2001). Suganda *et al.* (2016) indicated that isoprothiolane fungicide could reduce potential yield losses by 28% when compared to untreated plots in the Ciherang variety. This suggests that the use of isoprothiolane is still considerable, with the precautions taken against fungicide resistance. Amid the complexity of blast disease control, farmers rely on resistant cultivars that exhibit varying levels of resistance, as well as fungicides, to mitigate yield losses caused by rice blast.

Uncertain climatic conditions necessitate a more constructive strategy for rice blast disease control, including the monitoring of mutation occurrence. Sukarta *et al.* (2018) model that increased rainfall in West Java predicts an outbreak of blast diseases between 2030 and 2036. Other global climate changes e.g. elevated temperature also increase the fungus mutation rate (Habig 2021). Despite intensive regular application and reports of declining performance by farmers as well as recent updates of fungicide resistance overseas, resistance of *P. oryzae* to isoprothiolane probably has emerged in Indonesia. It will be of interest to understand the novel dynamics of rice-*P.oryzae*-fungicide due to pathogen mutation and how this information translates to possible efforts to have better strategies in long-term rice blast control.

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Figure 4 Common leaf blast appearance observed in the combination of cultivars and fungicide usage in Cikembar District. a, Mixture – isoprothiolane; b, Ciherang – carbendazim; c, IR64 – tricyclazole + azoxystrobin; d, Ciherang – isoprothiolane; and e, Local – tricyclazole.

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