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# 学 位 論 文 内 容 の 要 旨

博士の専攻分野の名称： 博 士（食資源学）

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## 学 位 論 文 題 名

Isoprothiolane resistance in the rice blast fungus in Indonesia: Field survey of its impact and molecular analysis of mutations

（インドネシアにおけるイソプロチオラン耐性イネいもち病菌：その影響のフィールド調査および突然変異の分子解析）

### 1. General Overview

Rice productivity in Indonesia is challenged by fluctuating production, harvest deterioration, climate instability, land conversion to urban centers, and disease outbreaks. Rice blast disease is caused by an ascomycete fungus, *Pyricularia oryzae*, which continues to become a major threat to rice production and global food security. Infection of leaf blast and neck blast both often lead to empty grains plant death resulting in annual 10% - 30% yield loss. Rice blast fungus (*P. oryzae*) exhibits a disease cycle both in vegetative and generative stages and during the off-season, this fungus survives in seeds and seedling debris and reentry the infection cycle by dissemination to healthy plants primarily by wind and raindrops. Efforts to control this disease are the use of resistant cultivars, biological control, habitat management unfavorable to the fungus development, as well as fungicide application. However, *P. oryzae* has high genetic variability and can adapt rapidly to various stressor stimuli, including mutation in sub-lethal doses of fungicide by altering the genes encoding fungicide targets, enabling this fungus to perform the continuous infection cycle and change the race dominance within a couple of growing seasons complicates the efforts to safeguard rice production. Fungicide resistance is a global issue and its discovery in Indonesia as an important rice-producing country is critical to assess.

### 2. Assessment of Blast Diseases in Three Regencies in West Java and Monitoring in an Endemic Area

*P. oryzae* will continuously perform the pathogenesis while compatibility presence of favorable environmental conditions, rice susceptibility, and virulence determined the degree of yield loss. A field survey in Bogor, Cianjur, Sukabumi Regencies in West Java, the second-largest rice-producing province in Indonesia was undertaken to investigate the pathological and socioeconomic aspects of blast disease endemic, dynamics of rice-fungus-fungicide, strategies, and challenges employed by farmers in controlling rice blast disease. The average temperature of 27 °C, humidity >90%, and rainfall days reaching 25 days per month were recorded during the survey and were favorable for disease development. Cikembar District in Sukabumi Regency is an endemic area where farmers rely on isoprothiolane fungicide but reported that the efficacy has declined. In Cikembar, 67% of disease severity was developed while 35% of severity was maintained during dry season. *P. oryzae* isolates were collected and tested by a series of laboratory works to validate this anecdotal evidence. Acidic soil, seedlings, and fungicide cost, the diverse controlling strategy applied by farmers are among the current challenges making the integrated disease management effort of the farmers' group more complicated.

### 3. Sensitivity Assays of *Pyricularia oryzae* Isolates Collected from Three Regencies in West Java

A total of 14 Indonesian *P. oryzae* field isolates have white to gray colonies, slow-growing on potato dextrose agar (PDA) medium, and have the closest genetic relationship with isolates from India and Thailand. These isolates were tested for a sensitivity test in isoprothiolane (IPT)-amended PDA both commercial and reagent-grade IPT. Isoprothiolane is a systemic fungicide that targets Kennedy's pathway in *P. oryzae* choline biosynthesis. Ina86-137, Japanese isolate was used as Control. Six isolates were

indicated as mutants with the ability to grow at 20 mg L<sup>-1</sup> IPT while maintaining radial growth and EC<sub>50</sub> at 4-6 mg L<sup>-1</sup>, 10-fold higher than the recommended concentration. At 0 mg L<sup>-1</sup>, mutant colonies grow slower than the sensitive isolates. Colonies from the IPT-exposed PDA were subcultured to a fresh PDA medium and most of the isolates can regrow as their original colonies, while a few are not reversed expressed by irradiated growth and sector development, indicating a fitness cost. Mutants (isolates 1-6) were continued to mutation analysis on *Magnaporthe-oryzae* isoprothiolane resistance-related (*MoIRR*) gene.

#### **4. Mutation Analysis of Isoprothiolane Resistance from Indonesian *Pyricularia oryzae* Field Isolates**

Resistant *P. oryzae* mutants are reported to possess varying responses to an active ingredient and cross-resistance to different active ingredients compared to that of lab-generated mutants. Identification of the *MoIRR* gene revealed that bases shift (G→A changed Alanine and Threonine at codon 396 and T→A changed Asparagine to Lysine at codon 555) occurred in isolate 1 and 6, respectively, while isolate indicated an insertion of G base at codon 288 changed the subsequent Isoleucine to Asparagine followed by a stop codon. Isolates 2, 3, 5, and a sector that emerged from Ina-86 137 did not have a mutation in *MoIRR*. Identification of the *MoVelB* gene demonstrated in isolate 2, insertion of A at codon 437 changed Aspartic Acid to Arginine and repeated 2 times before a stop codon appeared, while still no mutation was identified in isolate 3, 5, and SectorIna. Mutation may occur in other unknown genes. *P. oryzae* easily mutates as confirmed by SectorIna with lesser sensitivity to IPT with EC<sub>50</sub> 2 times lower than Ina86-137. Sector Ina also has a fitness penalty while its growth is inhibited in the absence of IPT but higher growth, conidial formation, and viability in challenged growing resources.

#### **5. General Discussion**

Since its first appearance in dryland rice in 1985, rice blast (*P. oryzae*) has been the most important disease in irrigated rice in Indonesia with a hundred races where the dominance of its main races alternates over the decades. Its novel state of virulence has also emerged while tackling rice resistance, dramatic climate changes, and fungicides, like what has been observed during the field survey in West Java. Fungicide resistance has occurred in Indonesia with multiple mutation mechanisms in the *MoIRR* and *MoVelB* gene. A combination of IPT and IR64 or a mixture of rice cultivars is detected as promising for short-term disease control implying the variant sources of pressure to minimize a higher possibility of *P. oryzae* mutations. It is highly recommended to temporarily stop the use of IPT or substitute it with other active ingredients to delay the development of emerging *P. oryzae* mutants in the field.