Faculty of Resource Science and Technology

QUALITATIVE AND QUANTITATIVE OF RESISTANCE OF SARAWAK RAIN-FED LOWLAND RICE TO *PYRICULARIA ORYZAE*

LAI KIM YEN (47236)

Bachelor of Science with Honours
(Plant Resource Science and Management)
2017

UNIVERSITI MALAYSIA SARAWAK

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Final Year Project Report ☑

Masters ☐

PhD ☐

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Programme Coordinator
Department of Plant Science & Environmental Ecology
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Qualitative and Quantitative of Resistance of Sarawak Rain-fed Lowland Rice to *Pyricularia oryzae*

Lai Kim Yen

This project is submitted in partial fulfilment of the requirement for the degree of Bachelor of Science with Honours
(Plant Resource Science and Management)

Supervisor: Dr Freddy Yeo Kuok San

Plant Resource Science and Management
Department of Plant Science and Environment Ecology

Faculty of Resource Science and Technology
Universiti Malaysia Sarawak

2017

Approval Sheet

Name of candidate: Lai Kim Yen

Matric number: 47236

Title of thesis: Qualitative and Quantitative of Resistance of Sarawak Rain-fed Lowland Rice to *Pyricularia oryzae*

Approved by,

____

(Dr. Freddy Yeo Kuok San)

Supervisor

Plant Resource Science and Management

Faculty of Resource Science and Technology

Date:

____

(Dr. Freddy Yeo Kuok San)

Coordinator

Plant Resource Science and Management

Faculty of Resource Science and Management

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(Lai Kim Yen)

Plant Resource Science and Management

Department of Environmental Science and Ecology

Faculty of Resource Science and Technology Universiti Malaysia

Acknowledgements

Great thanks to my supervisor, Dr Freddy Yeo Kuok San for his valuable guidance, suggestion and very constructive criticism which allowed me to improve myself either in lab work or in thesis writing. Not to forget, the two lovely master students under Dr. Freddy, Ain and Karimah who had guided and assisted me along my project in lab and also in field trips. Lab will be dull without lab mates, special thanks to Hakim, Aliah, Raffar, Adrian and Izzatty. We always supported and motivated each other and never forget to have fun when doing overtime lab work at night.

I would like to thanks Agriculture Research Centre for providing different local rice varieties and local rice blast isolates for this research as well as their research knowledge on rice blast isolation. Protocol was given by them is very helpful in my project.

Other than that, I would also like to express my deepest appreciation to my partner, Ten Hui Yin who had similar title as mine. Lot of helps you had given to me and we always troubleshoot problems together until it is resolved. Beside, when I was feeling exhausted and hopeless, you always be there to help and motivate me.

I also would like to thank all my friends who support me direct or indirectly throughout this project, imcluding Mei Chee, Andy, Mugu, Carlson, Leonard, Aimi, Dijah, and many mores. Lastly, I would like to thank my family for their moral and financial support throughout my project.

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List of Abbreviations

AVR	Avirulent
R gene	Resistance gene
PRR	Pattern recognition receptor
MAMP	Microbial associated molecular patterns
PAMP	Pathogen associated molecular patterns
LRR	Leucine-rich repeat
NB	Nucleotide-binding
FLS 2	Flagellin sensitive 2
PTI	PAMP-Triggered Immunity
ETI	Effector-triggered immunity
HR	Hypersensitive response
MABC	Marker-Assisted Backcrossing
QTLs	Quantitative trait loci
OMA	Oatmeal agar
ARC	Agriculture Research Centre
WA	Water Agar

OMA	Oatmeal Agar
FOMA	Filtered Oatmeal Agar
PDA	Potatoes Dextrose Agar

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Qualitative and Quantitative of Resistance of Sarawak Rain-fed Lowland Rice to

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Lai Kim Yen

Plant Resource Science and Management
Faculty of Resource Science and Technology
University Malaysia Sarawak

Abstract

Local rice production is threatened by rice blast disease, caused by the fungus, *Pyricularia oryzae*. Yield can be greatly reduced by this disease as it can attack all the aerial parts of rice. Use of resistant cultivar can control the disease effectively and can be produced from resistant breeding. Knowledge on fungus population in Sarawak and resistant local rice varieties are important for resistant breeding. Disease samples were collected from selected paddy fields in Sarawak. B2PG and 7^6 isolates have been collected and their morphology variations have been documented. B2PG is used for resistant screening of six selected Sarawak lowland rain-fed rice varieties, including Padi Bubuk, Padi Semanggang, Padi Entangor, Padi Perintah, Padi Hitam and Padi Rendah. Only Padi Hitam and Padi Entangor are found to be susceptible towards B2PG isolate of *P. oryzae*. Resistant varieties might carry one or more R genes which can be utilised in breeding strategies such as gene pyrimading.

Keyword: Local rice production**,** rice blast, *Pyricularia oryzae*, resistant breeding, Sarawak lowland rain-fed rice

ABSTRAK

Pengeluaran beras tempatan diancam oleh penyakit karah beras, yang disebabkan oleh kulat, Pyricularia oryzae. Hasil boleh dikurangkan dengan penyakit ini kerana ia boleh menyerang semua bahagian padi tanah. Penggunaan kultivar yang tahan penyakit karah dapat mengawal penyakit ini dengan berkesan dan boleh dihasilkan daripada pembiakan. Pengetahuan mengenai populasi kulat and ketahanan penyakit dalam varieti tempatan sangat penting untuk pembiakan tahan. Sampel daun yang berpenyakit dikumpulkan dari beberapa sawah padi di Sarawak. B2PG dan 7^6 *isolat telah dikumpulkan dan variasi morfologi mereka telah didokumenkan. B2PG digunakan untuk ujian ketahanan penyakit daripada enam varieti padi sawah Sarawak yang terpilih, termasuk Padi Bubuk, Padi Semanggang, Padi Entangor, Padi Perintah, Padi Hitam dan Padi Rendah. Hanya Padi Hitam dan Padi Entangor didapati tidak terdedah terhadap isolat B2PG. Ketahanan penyakit karat dalam varieti mungkin terdapat satu atau lebih gen R yang boleh digunakan dalam strategi pembiakan seperti gen pyrimading.*

Kata kunci: *Beras tempatan, penyakit karah, Pyricularia oryzae, pembiakan tahan, varieti Padi sawah Sarawak*

CHAPTER 1: INTRODUCTION

1.1 Research Background

Rice (*Oryza sativa*) is the third most important crops in Malaysia after oil palm and rubber with areas of cultivation accumulated up to 688, 207 ha and acts as the main staple food in the country (Harun, 2015). Due to economic, political and social importance of rice, there are more than 100, 000 farmers who rely on rice production for their subsistence and many of them are working in rice-related industry (Siwar *et al.*, 2014). However, rice production is threatened by various factors such as pests, diseases, weeds, drought and others in every growth stage and may cause heavy losses in rice yield.

Rice blast is one of the most destructive diseases which caused by an ascomycete fungus named *Pyricularia oryzae* Cavara. Rice blast was reported to destroy rice production which could be enough to feed an estimated of 60 million people in each year (Roy-Barman & Chattoo, 2005). This is due to the virulence of *P. oryzae* which able to strike any aerial part of rice plant (Wilson & Talbot, 2009). The leaves and panicles are the most commonly infected organs. Infected leaves greatly reduce photosynthetic areas of the plant which can lead to plant death due to insufficient food generated. Panicle infection is recorded as the most significant infection with highest economic losses compared with leaf (Castejón-Muñoz *et al.*, 2007).

To prevent heavy yield loss of rice blast might cause, chemical control is largely adopted by farmers for an immediate inhibition of rice blast compared to other strategies such as biological, cultural control and host resistance. Application of systemic fungicide such as triazoles and strobilurins can greatly control the disease and reduce the yield lost especially spraying is done at heading stage (IRRI, n.d.). However, fungicides application are only for treating the disease temporarily by inhibiting the growth of fungi and the use of chemical in large scale farm is neither practical nor environmental friendly (Chaudhary *et al.*, 2005). Therefore, utilization of host resistance by planting resistance variety is more effective and recommended (IRRI, n.d., Chen *et al.*, 2001; Ghazanfar *et al.*, 2009).

Nevertheless, *P. oryzae* had been reported to have high pathogenic variation due to the ability for the fungus to evolve or adapt with dynamic environment (Ou, 1980; McDonald & Linde, 2002). High pathogenicity variation in fungus increases challenges in producing resistant cultivar due to interaction specificity of isolate with cultivar. Once there is emergence of a new or more virulent pathogen race, resistance conferred by major gene will be broken down results the cultivar becomes susceptible (*Vanaraj et al.*, 2013). To perform effective and durable resistant breeding, one must understand the pathogenic variation of *P. oryzae* and also enrich the genetic resources for host resistance (rice) are crucial.

1.2 Problem Statement

High pathogenic variability of *Pyricularia oryzae* is due to constant evolution or new recombination of different *P. oryzae* races to create new variance. This allows *P. oryzae* easily overcome resistant rice cultivars available to farmers. To understand their pathogenic variation, one must collect blast isolates from geographical different areas. Diverse isolate collection can provide better understanding of fungus population present in paddy fields of Sarawak.

Some local rice varieties in Malaysia might carry blast resistance or defense genes in their genome without being discovered. Those unknown resistance or defense genes can be utilized in breeding to improve blast resistance of rice variety. One of the known examples is Pongsu Seribu 2 originated from Peninsular. It is the most resistant and had been used extensively in resistant breeding in Malaysia (Rahim *et al.*, 2013; Hasan *et al.*, 2015). However, there is no proper documentation and characterization of resistance against *P. oryzae* in rain-fed lowland rice varieties in Sarawak. More resistant varieties should be discovered and documented.

1.3 Objectives

- To isolate *Pyricularia oryzae* from selected paddy fields in Sarawak.
- To identify the rain-fed lowland rice variety with resistance towards *Pyricularia oryzae* in Sarawak.
- To characterize the resistance observed in the rain-fed lowland rice variety.

CHAPTER 2: LITERATURE REVIEW

2.1 Rice Blast Fungi (*Pyricularia oryzae*)

2.1.1 Background

Pyricularia oryzae is a type of filamentous ascomycete fungus which can cause blast disease in rice (*Oryzae sativa*) with its synonym name, *Pyricularia. grisea* (Rossman *et al*., 1990). *P. oryzae* is a member under Ascomycota, having *Magnaporthe oryzae* as teleomorph (sexual) stage. The taxonomy classification of *M. oryzae* is shown in Table 1. Meanwhile, *M. grisea* is also used to describe teleomorph of *P. oryzae* (Scheuermann *et al*., 2012; Hosseyni-Moghaddam & Soltani, 2013). However, a new phylogenetic analysis shown there is new clades segregating within *M. grisea* resulted in two distinct clades after analyzing actin, beta-tubulin, and calmodulin compounds from these two fungi. One clade is for the original species, *M. grisea* which is associated with grass genus *Digitaria.* While another new sister clade formed is *M. oryzae* which is associated with *Oryzae sativa*. The result concluded *M. oryzae* is a new species distinct from *M. grisea* which is the correct name for isolates affecting the rice (Brett & Linda, 2002).

Table 1: Taxonomy classification of *Magnaporthe oryzae* (Mycobank, n.d.)

Taxonomic Rank	**Classification**
Kingdom	Fungi
Phylum	Ascomycota
Class	Sordariomycetes
Order	Sordariomycetidae
Family	Magnaporthaceae
Genus	*Magnaporthe*
Species	*Magnaporthe oryzae* Couch

Typical blast lesion in elliptical shape with dark borders and whitish grey area in mild could be observed on leaves where it is the most common diagnostic symptoms for rice blast. Besides on the leaves, symptoms can also be observed on other aerial parts of rice such as node, neck, and collar (Figure 1) (Wilson & Talbot, 2009). Infection on nodes causes the culm to break, leading to the plant death (Figure 1a). Infected neck usually observed at the base of panicles where the rotting could destroy the entire panicle. If the neck rot occurs before milky stage of panicles (Figure 1b), no grains would be produced and results in white panicles. In contrast, poor quality of grains could be produced when the infection occurs after milky stage. For collar infection, lesions are formed at the junction of leaf blade and leaf sheath which cause the premature leaf to fall (Figure 1c).

Figure 1: Aerial parts of rice infected by blast, (a) Node blast, (b) Neck blast, (c) Collar blast (Wilson & Talbot, 2009).

2.1.2 Morphology Characteristic

M. oryzae has black and globose perithecia, a prominent beak and two layers of perithecial wall. Ascus is clavate with a refractive apical ring. Conidiophore of teleomorph *M. oryzae* is single or clustered, septated and slightly brown in colour. It has simple or rarely branched structure which exhibits sympodial growth (Scheuermann *et al.*, 2012). At the tip of conidiophore, conidia formed at point that ascends sympodially and have tapering structure towards the tips (Figure 2). In anamorph *P. oryzae*, pyriform shaped of conidia with one or two septa with rounded base can be found (Figure 2). Those conidia are slightly darkened and hyaline (Scheuermann *et al.*, 2012).

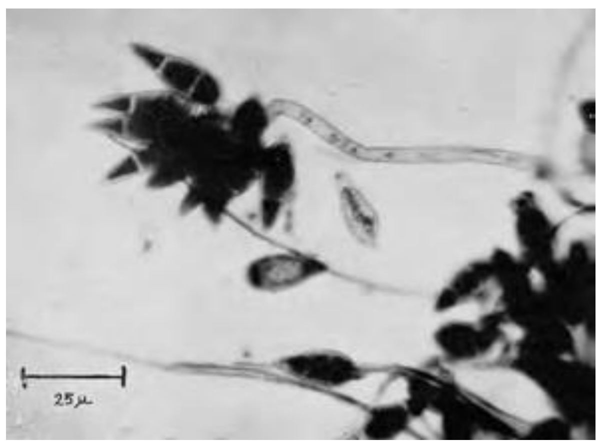

Figure 2: Conidia and conidiophore of blast (International Seed Testing Association, 2008)

2.1.3 Life Cycle of Blast

Life cycle of *P. oryzae* can be studied from crossing compatible and suitable isolates to produce teleomorph *M. oryzae* in which cannot find in nature. Blast is air-borne disease where the life cycle is initiated after an asexual conidium landed on a leaf surface (Figure 3). Rapid germination of *M. oryzae* is induced within two hours of landing on leaf while a polarized germ tube will be formed. Meanwhile, mucilage is produced to assists conidia to stick tightly on the leaf surface (Talbot, 2003).

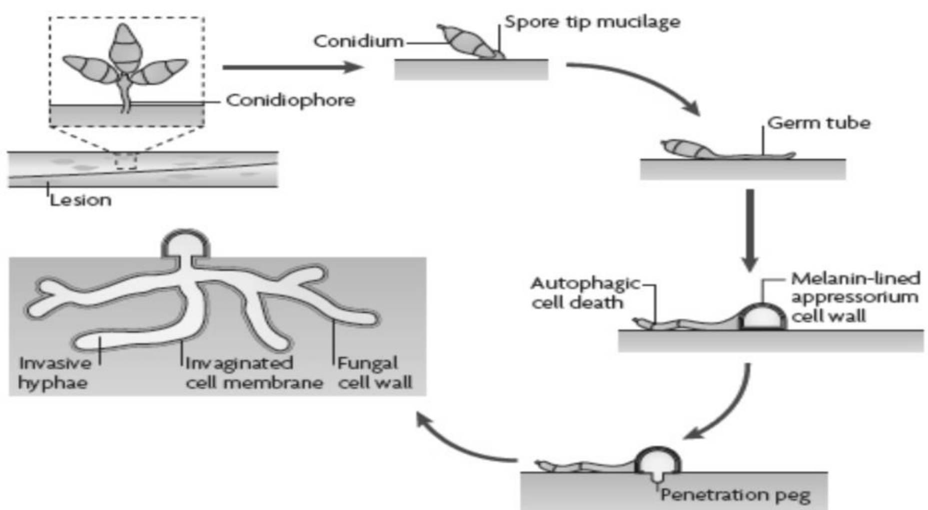

Figure 3: Life cycle of *Magnaporthe oryzae.* (Wilson & Talbot, 2009)

Then 'hooking' process occurs to precede development of the appressorium. The germ tube emerges from tapering end of conidium and grows across the leaf surface with extended and swollen tips. Development of appressorium requires a hard and hydrophobic surface. Wax monomers such as 1,16-hexadecanediol induces appressorium formation even on non-inductive surface (Gilbert *et al.*, 1996). Once the appressorium is formed, a daughter nucleus enters and the whole three-celled conidium is collapsed and degraded (Scheuermann *et al.*, 2012).

The dome shaped appressorium has a specialized cell wall structure, chitin rich and contain a layer of melanin on the inner cell wall. Melanin together with glycerol accumulation creates high turgor pressure which allows the peg of appressorium to penetrate plant epidemic cell forcefully. The nucleus inside the appressorium migrates into the peg and continues mitotic events to generate a series of bulbous and branched invasive hyphae (Scheuermann *et al.*, 2012).

In infected cell, *M. oryzae* continuos to invade other neighboring cells without damaging cell wall by the means of plasmodesmata (Wilson & Talbot, 2009). This shown biotrophic phase in early rice blast infection by using those invasive hyphae. When the fungi proceed to infect neiboring cells, the previous infected cells are dead and result in the apprearance of large elliptical or spindle-shaped lesion with dark borders and whitish to grey in mild on leaves (Figure 4). At this point of infection, *M. oryzae* shown necrotrophic properties when consistent lesions appeared on leaf surface, approximately 74 to 96 hours after the condia land on leaf surface. Then, the fungus is able to sporulate profusely from present lesions under conditions of high relative humidity (Wilson & Talbot, 2009). However, *M. oryzae* is neither oblique-biotroph nor oblique-necrotroph, it is classfied as hemibiotroph (Scheuermann *et al.*, 2012).