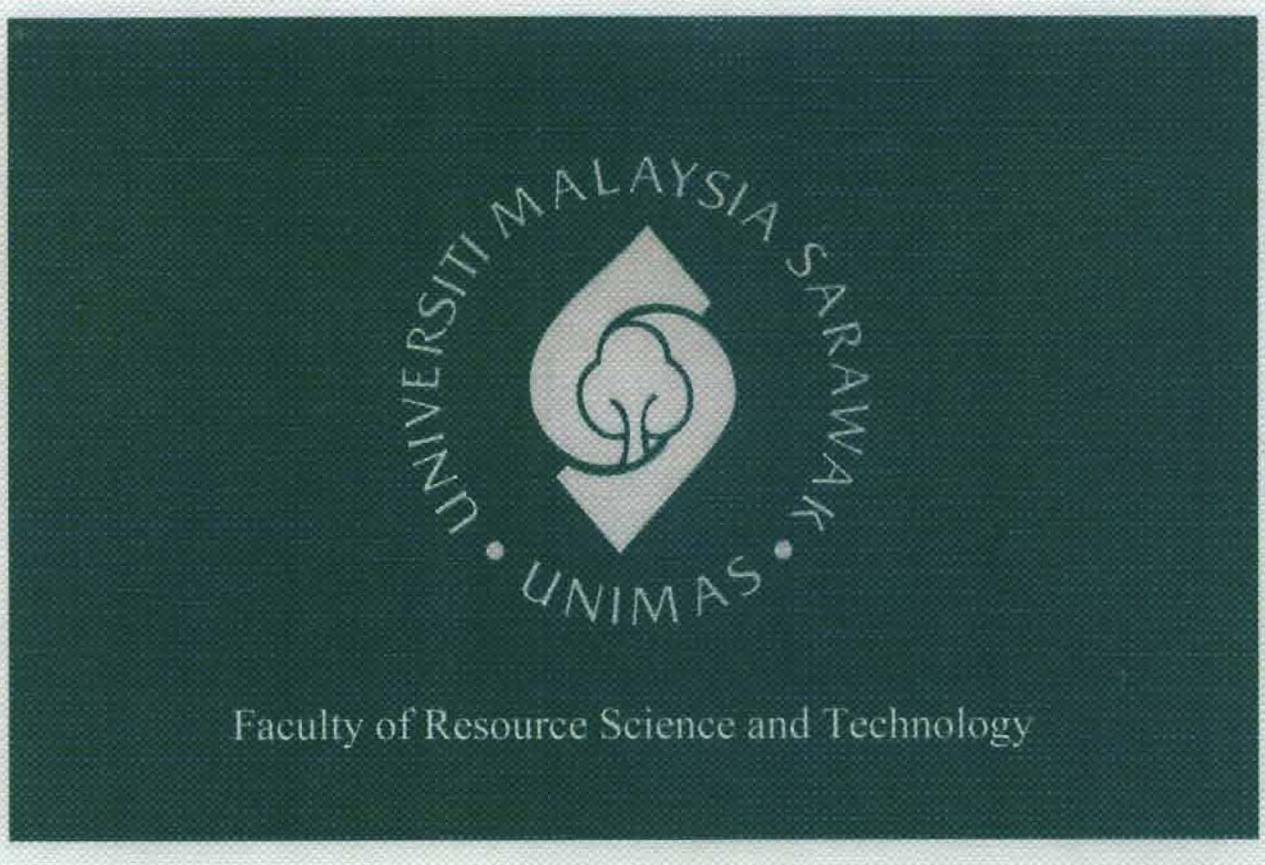

RICE BLAST DISEASE SCREENING AND PHYTOCHEMICAL ANALYSIS OF LOCAL RICE VARIETIES

Chia Yi Zhen

Bachelor of Science with Honours
(Plant Resource Science and Management)
2015

UNIVERSITI MALAYSIA SARAWAK

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Final Year Project Report ☑

Masters ☐

PhD ☐

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Rice Blast Disease Screening and Phytochemical Analysis of Local Rice

Chia Yi Zhen

(35723)

This project is submitted in partial fulfilment of the requirement for degree of Bachelor of Science with Honours

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

Faculty of Resource Science and Technology
University Malaysia Sarawak

Approval Sheet

Name of candidate: Chia Yi Zhen

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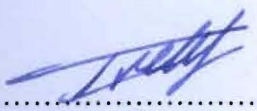

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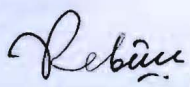

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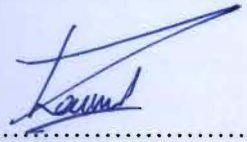

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List of Abbreviations

SM	Secondary Metabolite
hai	Hours after inoculation
OMA	Oatmeal agar
PDA	Potato dexterous agar
O.sativa	*Oryza sativa*
ARC	Agriculture Research Centre
QTLs	Quantitative trait loci
R gene	Resistant gene
MR	Malaysian Rice
bp	Base pair
AVR	Avirulent

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Rice Blast Disease Screening and Phytochemical Analysis of Local Rice

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Abstract

Rice (*Oryza sativa*) is a major food crop throughout the world. In Sarawak, there are at least 10 types of rice varieties available; however rice blast disease caused by *Magnaporthe oryzae* is still threatening to local rice production. Disease screening had found out that the seedling of Bario Halus is susceptible, while Biris and MR219 were resistance to local rice blast varieties *Magnaporthe oryzae* 2(1)'. The flavonoid profiling for eight local rice varieties had been done, intending to associate with the resistance of local rice varieties to rice blast. Unfortunately, establishing relation of local rice variety flavonoid profile to rice blast disease resistance was not as facile as expected due to low conidiation of local rice blast isolates that hampered the disease inoculation. A test of fungal growth rate was then conducted and the result suggests local rice blast pathogen in pH 5 and pH 7 OMA at 28°C grown best. The primers were also designed for four Avr genes while degenerate primers were design to two Avr genes.

Keyword: local rice, rice blast (*Magnaporthe oryzae* 2(1)'), resistance screening, fungal growth rate, flavonoids, primer

ABSTRAK

Beras (oryza sativa) adalah tanaman makanan utama di seluruh dunia. Di Sarawak, terdapat sekurang-kurangnya 10 jenis varieti padi; walaubagaimanapun penyakit kulat disebabkan oleh *Magnaporthe oryzae* masih mengancam pengeluaran beras tempatan. Ujian ketahanan penyakit telah mendapati bahawa anak benih Bario Halus mudah dijangkiti, manakala ketahanan penyakit Biris dan MR219 terhadap penyakit kulat beras tempatan *Magnaporthe oryzae* 2(1) adalah tinggi. Profil flavonoid terhadap lapan jenis beras tempatan telah dilakukan, dengan tujuan untuk mengaitkan ketahanan penyakit varieti beras tempatan terhadap penyakit kulat beras. Akan tetapi, untuk mewujudkan hubung kait antara beras profil flavonoid tempatan dengan ketahanan penyakit kulat beras tidak semudah yang diharapkan. Ini disebabkan kadar rendah penghasilan konidia kulat beras tempatan telah menjejaskan inokulasi penyakit ini. Ujian kadar pertumbuhan kulat kemudiannya dijalankan dan hasilnya menunjukkan kondusi terbaik untuk pertumbuhan kulat beras tempatan adalah pH 5 dan pH 7 OMA pada 28°C. Primer juga direka untuk empat gen AVR manakala primers merosot direka untuk dua gen AVR.

Kata kunci: beras tempatan, kulat rice (*Magnaporthe oryzae* 2 (1)'), ujian ketahanan penyakit, kadar pertumbuhan kulat, flavonoid, primer

Chapter 1: Research Background

1.1 Introduction

Rice (*Oryza sativa*) is one of the important food crops and is attacked by large number of diseases including nematodes, fungi, bacteria and viruses. Rice blast, caused by *Magnaporthe. oryzae*, is the most serious disease that leads to major lost in rice harvest. Case study on rice blast fungus state that rice blast epidemics in China (2001-2005) had caused destruction of 5.7 million hectares of rice ("Rice Blast Fungus", n.d.).

M. oryzae is a necrotroph, causing dead spots on grains, panicles, nodes and leaves of rice – a host. *M. oryzae* obtain nutrient from the dead host cells. (Long, Lee, & TeBeest, 2000; van Kan, 2006). The point of infection remains greyish, whitish or bluish, small and water-soaked before fully developed. The point of infection spreads quickly under a humid condition and will form into a lesion. A lesion will develop into a brown necrotic spot. In shady condition, the infected point will develop into a necrotic spot with very little brown margin, sometimes with yellow halo around the spot (Ou, 1985).

Researchers have been continuously screening for resistance in rice against rice blast (Wisser, Sun, Hulbert, Kresovich, & Nelson, 2005). There are two types of resistance viz. qualitative and quantitative resistance. Qualitative resistance is the resistance governed by a R gene which gives complete resistance. The resistance confirmed by R genes is race specific, functions on a gene-for-gene basis (Flor 1971). The R genes from plant will interact with a corresponding avirulence gene (Avr) from pathogen. One of the cloned R genes is *Pita* gene. After introducing *Pita* gene into a susceptible rice cultivar, the cultivar became

resistant against rice blast (Bryan et al., 2000). Although resistance based on R genes gives complete resistance against a pathogen, the resistance effect of R genes is not durable.

Quantitative or partial resistance is governed by resistance quantitative trait loci (QTL) i.e. polygenic. Quantitative resistance gives an incomplete resistance against rice blast but they slow down the fungal development (Agrios, 2005). Hypothetically, quantitative resistance has a longer durability (Niks and Marcel, 2009). Breeding resistant rice cultivar will help the farmers to counter the yield threat from rice blast. Before breeding is possible, one must identify the source of resistance against rice blast. One of the sources of resistance is from the local varieties (land races). Sarawak has interesting local rice varieties such as Bajong, Bario, Biris and Wai. The resistance of Sarawak local rice varieties against rice blast is not documented. We propose to screen a selection of Sarawak local rice varieties for quantitative and qualitative resistance to rice blast.

Screening for disease resistance against rice blast is not simple as the different rice varieties may have differed in degree of resistance across their stages of development (Hao, Wang, Li, Huang, & Tao, 2014). For instant, the panicle from the main culm might have different degree of resistance when compared with the first primary tiller in the same variety. Stage dependent resistance is depending on the expression of genes across the different stages of development. Breeding strategy for resistance to rice blast will be influenced by the information available on the stage dependency of the resistance. We propose to screen a selection of Sarawak local rice varieties aged one month and two month.

There are different methods used for screening rice blast resistance. Each method has its advantages and disadvantages (Berruyer, Poussier, Kankanala, Mosquera, & Valent, 2006).

The method of selection is depending on the purpose of study. For example, screening for resistance from different varieties or plant selection in a breeding program will prefer simple method and producing relatively reliable result. We propose to test different methods for screening rice blast resistance on a selection of Sarawak local rice varieties. The result will be valuable for deciding which method to be used for future large scale screening and breeding program.

1.2 Problem Statements

1. No documentation of local rice and MR varieties resistance to local rice blast isolates.
2. Flavonoid of Sarawak local rice varieties isolate is not documented.
3. Local rice blast isolates are still not well studied and no documentation of Avr genes in local rice blast isolates.
4. Slow growth rate and low sporulation of local *M. oryzae* cause lack of spore during inoculation and waste of too much resource for local *M. oryzae* culture for inoculation.

1.3 Objectives

1. Resistance screening of Sarawak local rice varieties and MR varieties against local *M. oryzae* isolate.
2. Determine best agar media, pH and temperature condition for *M. oryzae* growth.
3. Profile local rice varieties and MR varieties flavonoid
4. Design primers for Avr genes cloning.

Chapter 2: Literature Review

2.1 Rice (*Oryza sativa*)

Rice – *O. sativa indica* – is one of the global main food sources. Closely related edible rice species includes *O. sativa japonica* and *O. rufipagon*. These species produce sterile offsprings when cross bred, indicating they are distinct species (Linares, 2002).

However, these varieties of edible rice are commonly susceptible to a lot of diseases. The most commonly reported rice disease is rice blast disease, caused by the fungus *Magnaporthe grisea* (IRRI, n.d.)

2.1.1 Rice Blast Epidemic Documentation

Rice blast has always threatened the food supply of nations producing rice even since ancient era. One of the earliest reports of rice blast epidemic was documented during Ming Dynasty in China whereby paddy fields was destroyed by this fungus, leading to severe famine (Ou, 1985).

Today, in spite of the efforts put in to prevent this epidemic, the fungus is still a major threat to rice production industries. Resent report shows that there are still 85 countries throughout the world facing this challenge, among which, upland region in tropical Africa, Latin America and Asia and lowland region in subtropical and temperate Asia are more prompt to *M. oryzae* attack. (Dillon, Overton, Grayer & Harborne, 1997; IRRI, n.d.).

2.2 *Magnaporthe oryzae*

M. oryzae – rice blast or neck rot – is also known as *Pyricularia oryzae*. The common names were given as the disease blast like lesion symptoms on rice. This fungus is one of the causes of blast in rice, other cereals and grass species such as barley, wheat, pearl millet and turf grasses (Anderson, Cunningham, Patel, Morales, Epstein, & Daszak, 2004).

2.2.1 Taxonomic Nomenclature

The taxonomic nomenclature of rice blast (*M. oryzae*) at the order level is still remain undistinctive due to unclear identification and too closely related characteristics between families related to the Magnaporthaceae family. This results in uncertainties on which order to place Magnapothaceae familly within the class of Sordariomycetidae (Thongkantha, Jeewon, Vijaykrishna, Lumyong, McKenzie, & Hyde, 2009).

Different generic names and species names are given to rice blast. In 1880, the generic names of this fungus were used to be known as *Magnaporthe* and *Dactylaria* and the spelling of *Magnaporthe* was changed to *Piricularia* by Hughes in the year of 1958 (Ou, 1985). However, International Rules of Botanical Nomenclature enforce to as *Magnaporthe* instead of *Piricularia* (Ou, 1985). Rice blast can be known as *M.oryzae* and *M. grisea* which created confusions. *M. grisea* that is should be used according the regulation of nomenclature as the first existing binomial name. However, *P. oryzae* is already established and is commonly used (Ou, 1985).

2.2.2 Pathology and Life Cycle

M. oryzae is not a host specific pathogen; it attacks a wide range of host ranging from cereals to grasses. The hosts include rice, barley, and sugarcane (Ou, 1985). In spite of the wide range of host plant, this fungus uses a similar mechanism to penetrate the host plant tissue.

Infection begins when viable conidia (asexual spore) of *M. oryzae* landed on leaf surface of host plant under moist condition which allows the spore tip mucilage to be hydrated and adhere to the surface in a non-host specific manner. The spore tip mucilage is a droplet like substance forming at the spore apical (Talbot, 2003).

After spore attachment, the spore will germinate producing a germ tube which arises from mitosis of a single nucleus in the conidial cell; one daughter cell remains in the conidium while the other daughter cell travels along with the germ tube and forms appressorium upon reaching leaf region with suitable substrates for penetration (Howard & Valent, 1996). For complete appressorium formation, a cyclic AMP is needed at an optimum quantity at the germ tube tip. The MPG1 hydrophobic gene is also highly express during this stage to ensure appressorium attached onto the hydrophobic region of leaf (Talbot, 2003). When the germ tube reaches the hydrophobic region, the tube apex bends and swells and flattens against plant surface, forming a hook-like structure (Howard & Valent, 1996; Talbot, 2003), then flatten part then rises into a high differentiated dorm-shap appressorium, the appressorium morphogenesis requires combination of signals (Talbot, 2003).

In the process of penetrating the plant surface, the fungus cell wall which is rich in chitin first attached to the leaf surface follow by layer of melanin production inside the cell wall of

appressorium to accumulate sufficient turgor by preventing solute efflux. The turgor pressure is sufficient to open the plant cuticle surface enable formation of penetration peg (Talbot, 2003). During turgor pressure increases within the appressorium, the fungal plasmalemma enclosed and direct contact to the plant surface. The bilayer appressorium pore covering forms, the peg then emerges into the substratum bounded by a single cell wall layer. The penetrated hypha differentiates into bulbous brunched infectious hyphae and starts to infest adjacent cells. The hyphae produce after filling the initial epidermal cell regains the cylindrical hyphae and start infest the adjacent cell (Talbot, 2003).

2.3 Plant Resistance

Plant resistance can also be classified into nonhost resistance and host resistance. Nonhost resistance occur when the host specific pathogen could not interact or infect with other nonhost plant other than the host plant (Niks & Marcel, 2009). For example, *Blumeria graminis* infect barley could not cause disease symptoms in wheat. This kind of resistance can be the result of absence of essential factor for pathogen infection, plant structural defence or existing chemical defence in plant (Agrios, 2005).

The term 'host' in host resistance is used to describe where by a pathogen can recognise and is compatible to individuals within a species when access is granted. In this case, the plant species become a host to that pathogen (Niks & Marcel, 2009). Unlike nonhost resistance which lack of certain essential factors that cause incompatibility, host plant species that are susceptible does not have these factors. However within the host plant, some individuals have pre-existing resistance against the pathogen or resistance are developed, this resistance type is known as the host resistance.

The host resistance in plant can be grouped into vertical and horizontal resistance. Vertical resistance is conferred by monogenic resistant (R) gene. The presence or absence of R gene will result in complete resistance or susceptible, respectively towards pathogen infection. Horizontal resistance in rice is conferred by quantitative gene loci (QTL)s. Horizontal resistance is also known as partial, polygeneic or quantitative resistance. Effect due to the presence of quantitative resistance in host plant can either slow down pathogen infection rate or cripple pathogen reproduction ability (Agrios, 2005).

2.4 Rice Blast Pathosystem

Qualitative resistance in rice against blast is a complete resistance due to presence of a single resistance gene (Agrios, 2005). The presence and expression of the gene enable the rice to produce specific protein known as R protein, to recognise the gene of the avirulent protein produced by *M. oryzae*. The recognition of *M. oryzae* by R protein will cause a series of resistant reaction to occur. The resistance of rice against *M. oryzae* is easily identified either with a complete resistance or complete susceptible host plant as shown in Figure 1 (Bryan et al., 2000). Detail researches have revealed several classes of R gene associated protein. In general, these proteins contain similar structures of nucleotide binding site (NBS) and leucine rich repeat (LRR) domains (Jones & Dangl, 2006).

Agrios (2005) presented R gene proteins classification into five different classes according to the protein structures. The first class R gene encodes for serine-theonine kinase (STK) signal transduction protein, the second class R gene encodes for proteins with cytoplasmic STK and extracellular leucine repeats transmembrane receptor protein. The third R gene encodes for cytoplasmic protein containing LRR, NBS and a Toll/interleukin 1 receptor

(TIR). The third class proteins serve as receptor to activate a series of chain reaction which lead to hypersensitive response. The forth class R gene protein is also a cytoplasmic protein, consisting of a similar structures as the third class R gene but the protein do not has a TIR domain But a putative leucine zipper domain. The last class of the R gene protein locate outside the cell with a transmembrane region attaching to the plant cell. This class of protein contain mainly rich leucine repeats.

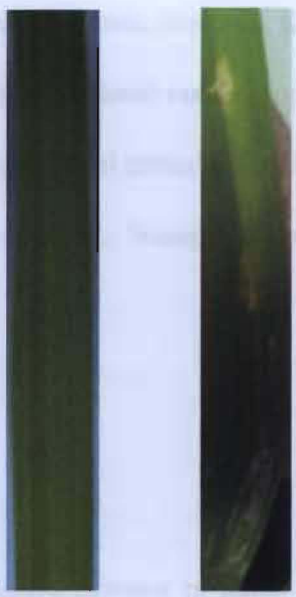

Figure 1: Left: leaf shows total resistance against pathogen; Right: leaf shows early susceptible symptom against pathogen.

The quantitative resistance is a partial resistance against pathogen. Unlike the qualitative resistance, quantitative resistance involve a few loci or QTLs in the gene to acquire a partial resistance against the pathogen. Most mode of action of the quantitative resistance QTLs involve in a structural and biochemical defence when encounter pathogen invasion (Agrios, 2005). Presence of quantitative resistance can be indicated by the degree of resistance

reaction ranging within complete resistance and complete susceptible (Jia, Valent, & Lee, 2003).

Quantitative resistance is influenced by the surrounding environment factors, gene combination effect of host and pathogen, plant growth stage and nature of infected tissue (Wang et al., 2010). In rice, presences of quantitative resistance lengthen latent period (take longer time to show susceptible symptoms), reduce infection efficiency, lesion size, and lesion expansion and reduce disease epidemic (Seebold, Kucharek, Datnoff, Correa-Victoria & Marchetti, 2001). Barley – a closely related cereal crop with the presence of quantitative resistance also prove to have a longer latent period at similar condition when inoculated with leaf rust (Neervoort & Parlevliet, 1978; L. Wang, Y. Wang, Z. Wang, Marcel, Niks & Qi, 2010).

2.5 Flavonoid

Flavonoid is a secondary metabolites existing in plant. Early science and discovery had labelled these SM as waste product of plant in the "waste product" hypothesis (Seigler, 1998). This hypothesis labelled SM as an error during primary plant compound production and is only related to plant taxonomic studies.

However the increasing studies on flavonoids in the late 50 years have led to blooming of synthesis of active compounds used in pharmaceuticals and plant biotechnologies research (Bourgaud, Gravot, Milesi & Gontier, 2001). Studies also allow understanding benefits of these compounds in curative product and human health-care product.