CHARACTERIZATION OF *Pyricularia oryzae* CAUSING RICE BLAST FROM RICE GRANARY IN PENINSULAR MALAYSIA

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LIST OF SYMBOLS

%	Percentage
°C	Degree celcius
™	Trade mark
±	Plus minus
≤	Less than or equal to

LIST OF ABBREVIATIONS

µL	Microliter
µm	Micrometer
bp	Base pair
cm	Centimeter
d	Day
DNA	Dinucleotide
DOA	Department of Agriculture
g	Gram
h	Hour
ha	Hectar
IRRI	International Rice Research Institute
kb	Kilo base
L	Litre
M	Molar
min	Minute
ml	Mililitre
mm	Milimetre
$MgCl_2$	Magnesium chloride
OMA	Oat Meal Agar
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
RAPD	Random Amplified Polymorphism DNA
Rep-PCR	Repetitive element-based PCR
RFLP	Restriction Fragmen Length Polymorphism
rRNA	Ribosomal Ribonucleic Acid
sp	Species
TBE	Tris-Borate-EDTA
WA	Water Agar

PENCIRIAN PATOGEN PENYAKIT KARAH PADI *Pyricularia oryzae* DARI JELAPANG PADI DI SEMENANJUNG MALAYSIA

ABSTRAK

Penyakit karah yang disebabkan oleh kulat *Pyricularia oryzae*, adalah antara penyakit utama tanaman padi di Malaysia dan boleh menyebabkan kehilangan hasil sehingga 70% sekiranya berlaku wabak. Penanaman varieti padi yang rintang merupakan cara yang berkesan untuk mengawal penyakit ini. Walau bagaimanapun, penggunaan monovarieti dalam liputan kawasan yang luas dan dalam jangkamasa yang lama telah menyebabkan penurunan tahap kerintangan akibat daripada perubahan populasi atau kemunculan patotip baru di jelapang padi. Objektif kajian ini adalah untuk mencirikan pencilan-pencilan *P. oryzae* berdasarkan ciri-ciri morfologi, mengenalpasti patotip *P. oryzae* berdasarkan tindak balas penyakit karah terhadap varieti pembeza Malaysia (MDVs), menentukan kepelbagaian genetik pencilan-pencilan *P. oryzae* dan mengenalpasti corak kevirulenan pencilan-pencilan (patotip) *P. oryzae* terhadap titisan-titisan monogenik karah IRRI (IRBLs). Sejumlah 65 pencilan dipencilkan daripada sampel penyakit padi (daun, panikel dan kolar) yang diperolehi dari pelbagai jelapang padi di Semenanjung Malaysia. Berdasarkan pencirian morfologi, sejumlah 51 pencilan telah dicirikan sebagai *P. oryzae* berdasarkan ciri konidianya yang berbentuk pirifom, mempunyai tiga sel, dua septa dan konidia berukuran 20.1–34.5 µm X 7.8–11.8 µm. Morfologi koloni dari segi warna didapati berwarna kelabu kehijauan, kuning pucat, kuning pucat ke kelabu kehijauan, kuning cair ke kelabu kehijauan, kelabu, kelabu cerah, kelabu cerah ke coklat dan coklat kehijauan. Pengenalpastian patotip dilakukan melalui inokulasi 65 pencilan *P. oryzae* terhadap varieti pembeza Malaysia iaitu Mahsuri, Setanjung

(MR1), Bahagia, Engkatek, Seribu Gantang, Tadukan, Pankhari 203 dan Pongsu Seribu 2. Penilaian penyakit dilakukan mengikut ‘Standard Evaluation System of Rice IRRI’ dan seterusnya diklasifikasikan mengikut sistem penamaan patotip. Pencilan-pencilan berkenaan telah diklasifikasikan kepada tujuh patotip iaitu P0.0 (13 pencilan), P0.2 (lima pencilan), P1.0 (10 pencilan), P2.0 (satu pencilan), P3.0 (tiga pencilan), P7.0 (23 pencilan), dan P9.0 (10 pencilan). Patotip P7.0 adalah patotip dominan dengan frekuensi pencilan 35.4% di mana kebanyakannya dipencilkan dari varieti padi MR219 dan MR220CL2 memandangkan liputan penanaman varieti popular ini telah melebihi 50% daripada kawasan penanaman padi di Semenanjung Malaysia semasa persampelan dijalankan. Kepelbagaian genetik pencilan *P. oryzae* dicirikan dengan menggunakan tindak balas rantai polimerase berasaskan elemen berulang (rep-PCR) dengan primer komplimentari elemen *Pot2*-TIR, iaitu elemen ‘transposable’ yang terdapat pada *P. oryzae*. Berdasarkan pencapjarian DNA ini, jalur DNA bersaiz antara 0.6kb hingga 5.0kb dan bilangan jalur DNA sebanyak dua hingga lapan jalur DNA bagi setiap pencilan telah diamplifikasi. Analisis kluster pencapjarian DNA telah mengklusterkan 51 pencilan *P. oryzae* kepada enam kluster iaitu I-A, I-B, I-C, I-D, I-E and II-F, di mana ianya bertepatan dengan pencilan-pencilan klasifikasi patotip masing-masing iaitu P0.2, P7.0, P9.0, P0.0, P1.0 dan P3.0. Selain itu, corak kevirulenan enam patotip telah dinilai berdasarkan tindak balasnya kepada 22 titisan monogenik (IRBLs) yang setiap satunya mempunyai 22 gen rintang karah iaitu *Pish, Pib, Pit, Pia, Pii, Pi3, Pik-s, Pik-m, Pi1, Pik-h, Pik, Pik-p, Pi5(t), Pi7 (t), Pi9, Piz, Piz-5, Piz-t, Pita-2, Pita, Pi19 (t),* dan *Pi20 (t)*. Analisa kevirulenan mendapati bahawa *Pii, Pik, Pik-h, Piz, Pish*, *Pi7(t)* dan *Pi9* adalah calon gen-gen rintang karah yang sesuai untuk dijadikan gen penderma dalam proses pembaikbakaan varieti padi baharu yang rintang terhadap penyakit karah. Kesimpulannya, pencilan-pencilan *P. oryzae* telah berjaya diperolehi dan tujuh patotip telah dikenalpasti. Walaupun pencapjarian DNA menggunakan rep-PCR mendapati pencilan-pencilan *P. oryzae* mempunyai kepelbagaian yang tinggi, namun analisa ini berupaya mengklusterkan pencilan-pencilan tersebut mengikut kumpulan patotip. Analisa corak kevirulenan pencilan *P. oryzae* mendapati tujuh gen rintang (*Pii*, *Pik*, *Pik-h, Piz, Pish*, *Pi7(t)* dan *Pi9)* adalah calon gen kerintangan yang sesuai bagi pembaikbakaan varieti padi baharu rintang penyakit karah. Informasi dari hasil kajian ini adalah penting untuk pemantauan dan mengenalpasti populasi patotip di lapangan serta ianya perlu bagi merancang strategi dalam pembangunan varieti padi baharu rintang penyakit karah.

CHARACTERIZATION OF *Pyricularia oryzae* CAUSING RICE BLAST FROM RICE GRANARY IN PENINSULAR MALAYSIA

ABSTRACT

Rice blast disease caused by the fungus *Pyricularia oryzae*, is the most destructive diseases of rice in Malaysia which is responsible for yield loss in the epidemically favourable areas and crop seasons. Planting resistant rice varieties normally is the effective way to manage the disease. However, the extensive use of monovariety has led to the breakdown of resistance after a few seasons of its released due to the emergence of new virulent or changing in the dominant pathotype in the rice fields. The objectives of this study were to characterize *P. oryzae* isolates based on their morphological characteristics, identify *P. oryzae* pathotypes based on disease reaction to Malaysian Differential Varieties (MDVs), determine the genetic diversity of *P. oryzae* isolates based on molecular characterization and identify the virulence pattern of identified selected *P. oryzae* isolates (pathotypes) against specific gene for resistance harbored in the IRRI bred blast monogenic lines (IRBLs). A total of 65 isolates were obtained from rice disease samples (leaf, panicle and collar) collected from various rice granaries in Peninsular Malaysia. Based on morphological characteristics, 51 isolates were identified as *P. oryzae* by its conidial characters of pyriform shape, 3-celled and 2-septate with the size ranged 20.1–34.5 µm X 7.8–11.8 µm. Colony morphology varied from olivaceous grey, buff, buff to olivaceous grey, beige to olivaceous grey, grey, light grey, light grey to brown, and olivaceous brown. Pathotype identification was carried out via inoculation of 65 isolates to Malaysian own differential varieties, namely Mahsuri, Setanjung (MR1), Bahagia, Engkatek, Seribu Gantang, Tadukan, Pankhari 203 and Pongsu Seribu 2.

Disease assessment was done according to IRRI's Standard Evaluation System for Rice for further pathotype designation. Seven pathotypes were designated as pathotype P0.0 (13 isolates), P0.2 (five isolates), P1.0 (10 isolates), P2.0 (one isolate), P3.0 (three isolates), P7.0 (23 isolates), and P9.0 (10 isolates). Pathotype P7.0 was a dominant pathotype with isolates frequency of 35.4% and mostly isolated from MR219 and MR220CL2 considering cultivation of these popular rice varieties has dominating more than 50% of the rice planted area in Peninsular Malaysia during the disease sampling. Genetic diversity of *P. oryzae* isolates were further characterized using repetitive element-based polymerase chain reaction (rep-PCR) with primer complementary to sequence of *Pot2,* the transposable element found in *P. oryzae*. Based on DNA fingerprinting, bands pattern ranging 0.6kb to 5.0kb in size and in number from two to eight bands per isolate were amplified. Cluster analysis of DNA fingerprinting clustered 51 *P. oryzae* isolates into six clusters namely, I-A, I-B, I-C, I-D, I-E and II-F which corresponded to the pathotype group of pathotypes P0.2, P7.0, P9.0, P0.0, P1.0 and P3.0, respectively. Furthermore, virulence pattern of six pathotypes was evaluated based on the reactions to 22 monogenic lines IRBLs harboring 22 resistance genes namely *Pish, Pib, Pit, Pia, Pii, Pi3, Pik-s, Pik-m, Pi1, Pik-h, Pik, Pik-p, Pi5(t), Pi7(t), Pi9, Piz, Piz-5, Piz-t, Pita-2, Pita, Pi19(t),* and *Pi20(t)*. The virulence analysis has determined that *Pii, Pik, Pik-h, Piz, Pish, Pi7(t)* and *Pi9* as suitable candidate of resistance genes to be incorporated for developing future blast resistant variety for combating the existing pathotypes in the fields. In conclusion, isolates of *P. oryzae* were successfully identified from diseased plants and seven pathotypes were determined. Although rep-PCR fingerprinting demonstrated the isolates were variable, the analysis was able to distinguish the *P. oryzae* isolates according to the pathotypes. Virulence patterns analysis indicated seven resistance genes (*Pii, Pik, Pik-h, Piz, Pish, Pi7(t)* and *Pi9*) as suitable candidate genes for development of rice variety resistant to blast disease. The information from the present study is important for monitoring and identification of *P. oryzae* pathotypes population in the field as well as to strategize in developing new blast resistant rice variety.

CHAPTER 1

INTRODUCTION

Rice blast caused by a highly variable fungal pathogen, *Pyricularia oryzae* is one of the most destructive diseases of rice. It is responsible for yield loss in many of the epidemically favourable areas and crop seasons and hence posing a major threat to world's food supply (Telebanco-Yanoria et al., 2008).

Previously, either *Magnaporthe oryzae*/*Magnaporthe grisea* or *Pyricularia oryzae*/*Pyricularia grisea* were used to refer to the fungal pathogen responsible for rice blast disease. *Magnaporthe* is the teleomorph stage or sexual stage of *Pyricularia*. Based on 'one fungus, one name concept', Zhang et al. (2011) and Luo & Zhang (2013) proposed that the name *Pyricularia oryzae* should be adopted for rice blast fungus based on phylogenetic analysis using multiple markers that revealed that *Magnaporthe* from rice blast forms distinct clade or phylogenetic group from other species of Magnaporthaceae including *Magnaporthe* spp. and *Gaeumannomyce*s spp. Therefore, in the present study *P. oryzae* is used when refer to the rice blast pathogen. However, other species name of blast pathogen is still being used in this literature which followed accordingly the species name stated in the cited literature.

Rice blast can infect different parts of rice plants; leaf, collar, stem, node and panicle; and at different stages of rice cultivation from seedling to ripening stage. Typically, the yield losses caused by blast disease is 10–30% (Talbot & Wilson, 2009; Dean, 2012). In Malaysia, the yield loss due to this disease is estimated up to 50% (Elixon et al., 2017). However, infection of the panicle at grain filling to ripening stage can lead to complete yield loss due to empty grain (Dean, 2012).

There were series of rice blast disease incidence been occurred in Malaysia. Recently, about 3,400 farmers in Sabak Bernam, Selangor have been affected by the outbreak of panicle blast disease. Yield obtained from the field infected by this disease was about 4 tonne/ha instead of the average yield usually achieved by the farmers in Selangor is about 6 tonne/ha. Yield loss was estimated about 30% (Sinar Harian, 2020). In 2015, about 8,512 ha of rice production area in Malaysia was reported to be affected by rice diseases. From this, an area of 3,400 ha was infected by leaf blast disease and another 4,752 ha was infected by panicle blast or neck rot disease of which the estimated yield loss were RM13 million and RM19 million, respectively (Harian Metro, 2015). Although the affected area was less than 5% of the total rice production area in the country the yield loss caused by blast on the affected fields is as high as 50-70%. Considering the average farm size of Malaysian farmers is small, the affected field certainly affected the income of those small farmers (Habibuddin, 2012).

Rapid resistance breakdown issue could possibly be managed if several issues on host-pathogen interaction are fully understood. On the pathogen side, the variability of blast pathogen should be estimated, including on the composition of variable pathotypes, both avirulent and virulent as well as their dominant existence. On the host plant side, the available resistant genes should be classified as a narrow spectrum resistance gene of which resistant only to a particular pathotype, or broad spectrum which are resistant to groups of pathotypes. Rice varieties often remain resistant for only a few years after released before the emergence of the new virulent or dominant pathogenic races that lead to rapid resistance breakdowns (Saleh et al., 2014; Li et al., 2017; Zhou et al., 2020).

The rapid breakdown of the introduced resistant varieties is partly due to the lack of information on pathogen population and composition at the areas of which those resistant varieties were introduced resulted with improper gene deployment strategy (Chen et al., 2001). The information required including occurrence of rice blast pathotypes as blast pathogen can evolved rapidly. Due to its pathogenic variability and genetic instability, new pathotype may emerged in the population (Bryan et al., 2000; Jiang et al., 2012). To identify blast pathogen pathotype, suitable differential set of rice cultivars or varieties are used.

Blast pathotypes (historically called races) is referred to group of blast isolates that infect rice classified into infection types (based on pathogenicity testing) which is based on infection assays involving a set of differential rice cultivars (Latterell et al., 1965; Ling & Ou, 1969; Kim et al., 2017). After 1980, the differential set of rice varieties generally consisted of seven or more cultivars that differ from one another by one or more resistance genes to analyze the race of rice blast (Ou, 1985; Kim et al., 2017).

In MARDI, eight Malaysian differential varieties namely Mahsuri, MR1, Bahagia, Engkatek, Seribu Gantang, Tadukan, Pankhari 203 and Pongsu Seribu 2 with different degree of resistance to blast infection have been used to identify the pathotype of blast isolates. Differential varieties could also be used for monitoring of blast population structure and to predict the emerging of new pathotypes (Telebanco-Yanoria et al., 2010).

Blast isolates are generally consisted of several pathotypes which were identified and characterized based on their reaction to a set of differential rice varieties. Molecular characterization through DNA fingerprinting can provide genetic definition and genetically resolved the issues of pathotype variations observed in pathogenicity assays (Levy at al., 1991).

During 1990s, molecular characterization using restriction fragment-length polymorphism (RFLP) analysis is known to be a valuable tool to study genetic variation among populations. Repetitive elements in the *M. grisea* genome such as *Magnaporthe grisea* repeat element (MGR) specifically MGR586 element was used as probe in RFLP to characterize population variation among strains of different host origin which enabled blast strains to be grouped into distinct lineages (Hamer et al., 1989; Dobinson et al., 1993; Shull & Hamer, 1996). The limitation is that the method is laborious and expensive. Later, George et al. (1998) used repetitive element-based polymerase chain reaction (rep-PCR) to fingerprinting blast isolates which later used to form groups or clusters of isolates based on the banding pattern similarities.

Suzuki et al. (2006) investigated the use of *Pot2*-TIR single primer for the analysis of population structure that complement the transposable element of rice blast pathogens, *Pot2*. Rep-PCR using *Pot2*-TIR primer produced distinct fingerprint patterns among rice-infecting *P. grisea* isolates collected in Japan with the band size from 0.6 to 6 kb and generated nine haplotypes. Other molecular methods used to characterize blast pathogen includes using internal transcribed spacer (ITS) (Abed-Ashtiani et al., 2016; Mior et al., 2017), Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeat (ISSR) (Abed-Ashtiani et al., 2016). Molecular characyerization using these methods revealed that all isolates obtained were homolog to *M. oryzae* There was correlation between the isolates and their geographical region based on clustering of RAPD and ISSR analyses. In the present study, rep-PCR using *Pot2*-TIR primer was applied to determine genetic diversity and possible population structure of *P. oryzae* isolates collected from rice granary area in Peninsular Malaysia.

In Malaysia, although a number of blast resistant rice varieties have been released such as MR219, MR232, MR253 and MR263 but their resistance is not durable (Habibuddin, 2012). Many reasons had been attributed to blast resistance breakdown. Cultivating of a monovariety for a long duration with wide varietal coverage is one of them. This is particularly true if resistance in the varieties is controlled by narrow spectrum resistance genes that fasten the development of virulent strains or pathotypes (Habibuddin, 2012; Hassan, 2015). The durability of a resistance variety could possibly be prolonged if resistance is controlled by a broad-spectrum resistant gene. This would make utilization of resistant varieties more effective and economical method of disease control (Fukuta et al., 2007).

After blast pathotypes have been identified, the virulence patterns and the resistance genes correspond to the blast pathotypes are determine in order to obtain information on the host resistance and fungal virulence as well as to postulate the resistant genes. To carry out this study, a set of IRRI bred blast resistant lines (IBRLs) containing 22 resistant genes was used in pathogenicity assay against the identified blast pathotypes (Tsunematsu et al., 2000; Telebanco-Yanoria et al., 2008).

Information on the morphological variation, pathotype diversity as well as the genetic variation and virulence patterns of the blast causal pathogen in the country is required as blast disease outbreak keep on occurring in rice producing area in Malaysia. Furthermore, the information on relationship of the isolates obtained (pathotype) and the rice cultivars (resistant gene) was not well studied. Therefore, assessing pathotype diversity of blast populations in a locality or region is needed in order to strategize in developing a successful, long lasting resistant varieties against blast disease.

1.1 Objectives

The objectives of the present study are:

(i) To characterize *P. oryzae* isolates based on their morphological characteristics.

(ii) To identify *P. oryzae* pathotypes based on disease reaction to Malaysian Differential Varieties (MDVs).

(iii) To determine genetic diversity of *P. oryzae* isolates based on molecular characterization.

(iv) To identify the virulence pattern of identified selected *P. oryzae* isolates against specific gene for resistance harbored in the IRRI bred blast monogenic lines (IRBLs).

CHAPTER 2

LITERATURE REVIEW

2.1 Rice (*Oryzae* spp.)

2.1.1 Taxanomy, biology and domestication

Rice is a unique crop, rich in genetic diversity and has an extensive range of geographical and ecological adaptation (Chang, 2003). It is a monocotyledon plant, a cereal food crop belongs to the grass family Poaceae of the plant kingdom and under the genus *Oryza* L. The genus *Oryza* contains 22 species which is widely distributed throughout the tropics. Among the *Oryza* species, eight species have been classified as *Oryza sativa* complex, namely *O. sativa*, *O. rufipogon* and *O. nivara,* which are native in Asia; *O. longistaminatta*, *O. barthii* and *O. glaberrima*, domesticated in West Africa; *O. meridionalis,* is found in Australia; and *O. glumaepatula* only found in Latin America (Lu et al., 2000).

Two cultivated rice spesies, *O. sativa* L. and *O. glaberrima* Steud., which are native to tropical and subtropical southern Asia, and southeastern Africa, respectively are well accepted. *Oryzae sativa* is widely cultivated worldwide (Callaway, 2014).

2.1.2 Rice cultivation and its economic importance

As a cereal grain, rice is the most widely consumed staple food for half of the world's human population, especially in Asia. Based on FAOSTAT (2018) data, rice is the third-highest cereal crop produced in the world (769.7 million tonne), after maize (1.1 billion tonne) and wheat (771.7 million tonne) (http://www.fao.org/faostat/en/#data/QC).

Historically, rice was domesticated more than l2,000 years ago in the river valleys of South and Southeast Asia and China. The history of mankind in Asia is strongly associated with rice cultivation, domestication, as well as seeds diversification, since Asian is known as rice-eating people (Chang, 2003; Gnanamanickam, 2009). More importantly, about 90% of rice is produced and consumed in Asia. Hence, rice production in Asia plays a key role in global food security. Although Asia is the main place of rice cultivation, but it was also produced in other regions including in Latin America, Europe, some parts of Africa and United State of America (Gnanamanickam, 2009; Bandumala, 2018).

Food security issue of most Asian countries including Malaysia is very much related to their self-sufficiency levels (SSL) in domestic rice production (Arshad et al., 2011). Malaysia, for example, has determined to achieve 70% SSL in her rice production. To achieve this target Malaysian government had devised many approaches as spelled out in the National Agriculture Policy 4 (NAP4). Based on Agrifood Statistics 2018 (**Table 2.1**), the policy had received positives impacts in rice production at the first 4 years of its implementation (Rozhan, 2018; Agrifood Statistics, 2018). Rice production was increased from 2.6 million metric tons in 2011 to 2.8 million metric tons in 2014 with the achievable SSL at 72.3% in 2015. Unfortunately, the rice production was decreased during the period of 2016 to 2018.

Table 2.1: Rice production, average yield and popular rice varieties grown, 2011-2018.

Year	Rice production (‘000 metric tonnes)	Average yield (kg/ha)	Popular rice varieties		
			1	2	3
2011	2, 579	3, 748	MR219	MR220	MR84
2012	2, 599	3, 797	MR219	MR220	MR220CL1
2013	2, 604	3, 876	MR219	MR220CL2	MR220
2014	2, 849	4, 194	MR220CL2	MR219	MR263
2015	2, 741	4, 022	MR220CL2	MR263	MR219
2016	2, 740	3, 978	MR220CL2	MR263	MR269
2017	2, 571	3, 750	MR220CL2	MR284	MR263
2018	2, 640	3, 770	MR220CL2	MR297	MR219

Source: Agrifood Statistics (2018).

Currently, more than 95% of rice fields in major granary areas of Malaysia are planted with rice varieties developed and released for commercialization by Malaysian Agricultural Research and Development Institute (MARDI) (Elixon et al., 2017). The rice varieties released by MARDI are well adapted and accepted by farmers which helped them to increase their yield and income. To date, about 50 rice varieties have been released by MARDI since 1964 (Othman et al., 1986; Alias, 2010; Zainudin et al., 2012). The acceptance by farmers varies. Several varieties such as MR84, MR167, MR219 and MR220 used to be widely planted, covered more than 50% of the planting areas in Peninsular Malaysia, while several others faded away due to one or other reasons.

Rice variety MR219 was released in 2001 (Alias et al., 2002) and MR220 in 2003 (Alias et al., 2005). The two varieties dominated rice growing areas of Peninsular Malaysia for more than 10 years. The two varieties remained popular and highly demanded due to their high yield potential. Probably, the two varieties have contributed to the higher rice production during the 2011-2014 period where the yield of more than 10 t/ha had been recorded. Rice varieties MR220CL2 (Azmi et al., 2010) and MR263 (Sariam et al., 2012) were released in 2010. These two remained popular due to their respective advantages, even though their yield was not as high as MR219.

Unfortunately, continuously planting of a variety in large scale for a long period may resulted in the shifted in strains of insect biotypes and disease pathotype capable of attacking the previously resistant varieties, especially if their resistance is not highly resistant or broadly resistant to multiple pathotypes. Susceptibility of high yielding varieties to insects and diseases is the most important limiting factors that affect the overall rice production and farmers income. Series of pests and disease outbreaks had been reported during the period 2011-2019 in major granary areas such as IADA Barat Laut Selangor and MADA that causing yield reduction of the crop (Informasi Pertanian, 2011; Zakaria & Misman, 2018). The infection was detected on the rice variety MR219, which was originally classified as resistant when it was released in 2001 (Alias et al., 2002). The infection resulted in rotten panicles or broken grains or empty hulls, which in turn led to high yield losses.

Life-span of a rice variety is usually lasted for only a few years, and this is due the breakdown of the resistance. The resistance breakdown is not likely due to changing in the varietal genome, but rather due to changing in the prevalence of the biotypes of insects or pathotypes of the diseases in the field (Song & Goodman, 2001). This phenomenon of biotype changes is due to the presence of high variability in the pathogen and insect populations in the fields. Several pest and disease outbreaks may also occur due to intensive agronomic practices including the high fertilizer rate used, application of broad-spectrum pesticides causing resurgence, double cropping system, high density planting and planting susceptible varieties (Saad et al., 2004; Habibuddin, 2012; Elixon et al., 2017).

In Malaysia, there are many insect pests and disease- causing pathogen in the rice fields. However, only a few of them will cause dramatic yield and economic loss. These may include insect pests such as brown plant hopper, stem borers, leaf feeders and grain suckers or pathogen causing diseases such as tungro virus complex, bacterial leaf blight, sheath blight and panicle blast diseases (Saad et al., 2004; Habibuddin, 2012).

2.2 Rice blast disease

Rice blast disease caused by *P. oryzae* is one of the most destructive diseases of rice in all of the rice growing countries in the world (Kato, 2001). The disease occurs in more than 85 countries on all continents where rice is grown, in both rice fields and upland rice cultivation. The first known occurrence of the disease is in China where it was called rice fever disease (Wang and Valent, 2009). Potential yield loss caused by blast disease infection is estimated to be about 10-30% (Talbot, 2003). The potential damage caused by it makes it one of the most economically important and well-studied diseases of rice. It is also been used as a model experimental organism studied for the understanding of plant pathogenic fungi infection processes (Martin-Urdiroz et al., 2016).

In Malaysia, blast disease was first thought to be associated with the rice varieties brought in from Taiwan during the Second World War in 1945. However, the disease was also observed among local varieties grown in widely separated areas all over the country. Therefore, it was then considered that the disease has long been presence in the country, but changes in cultural practices where farmers planting modern bred varieties, with increasing use of fertilizers, particularly nitrogen in rice cultivation had encourage the disease emergence. Historically, series of blast disease outbreaks have been reported in Kelantan (1960 and 1961), in Perlis and Tanjung Karang (1963) and in Province of Wellesley (1966) (Habibuddin, 2012).

In the recent years, outbreaks of rice blast disease in Malaysia had been reported in 2005, affecting about 4033 ha (Department of Agriculture, unpublished data). Although the affected rice area was less than 5% of the total paddy planted area in the country, the yield loss caused by panicle blast incidence was as high as 50-70%. When considering the fact that the average farm size in Malaysia is small, the farmers are greatly affected.

In Main season 2010/2011, incidence of panicle blast was reported to occur in Kuala Muda, Yan and Kota Setar, Kedah. The blast outbreak infected high yielding variety, MR219 which was resistant to blast disease during its released in 2001. Farmers’ income was affected due to the yield loss caused by the panicle blast which the panicle resulted empty grains (Informasi Pertanian, 2011).

Another blast incidence in 2015, had affected about 8, 512 ha of rice production area in Malaysia. An area of 3,400 ha was infected by leaf blast disease and another 4,752 ha was infected by panicle blast or neck rot disease of which the estimated yield loss were RM13 million and RM19 million, respectively (Harian Metro, 2015).

Recently, outbreak of panicle blast disease has affected about 3, 400 farmers in Sabak Bernam, Selangor who suffered yield loss about 30%. Yield obtained from the field infected by this disease was about 4 tonne/ha instead of the average yield usually achieved by the farmers in Selangor is about 6 tonne/ha (Sinar Harian, 2020).

2.2.1 Nomenclature, systematics and morphology of blast causing pathogen

The causal pathogen of rice blast disease is *P. oryzae*, cav., which is a member of the family Pyriculariaceae, in the order of Magnaporthales and in the fungal class of Sordariomycetes (Ascomycota) (Mycobank, 2016).

The Order *Magnaporthales* has about 200 species which includes nearly 50% of important plant pathogens of *Poaceae* or *Gramineae.* Order Magnaporthales has three families, *Pyriculariaceae*, *Magnaporthaceae* and *Ophioceraceae*, which are phylogenetically, morphologically and ecologically distinct (Klaubauf et al., 2014; Zhang et al., 2016).

In particular, the families comprises a few notable important pathogens of cereals and grasses in this order, namely *Pyricularia oryzae (*syn *Magnaporthe oryzae)* (rice blast disease), *Gaeumannomyces graminis* (cereals take-all disease), *Nakataea oryzae (Magnaporthe salvinii)* (rice stem rot disease) and *Magnaporthiopsis poae* (turf grass summer patch disease) (Cannon, 1994, Thongkantha et al., 2009). Another pathogen of special interest is *P. grisea*, a pathogen of crabgrass *(Digitaria sunguinalis* L.) which in earlier studies had made many arguments on its similarity or dissimilarity with *P. oryzae*.

However, advancement in phylogenetic and phylogeneomic studies revealed that *Magnaporthe* which are morphologically indistinguishable (Bussaban et al., 2005; Tosa & Chuma, 2014) or demonstrated cross-infectivity (Choi et al., 2013) was polyphyletic (Couch & Kohn, 2002), meaning they might be derived from the same ancestor, but yet not suitable to be placed in the same taxon. *Magnaporthe* was separated into two distinct clades, namely *M. grisea* - the one that is associated with *Digitaria* (crabgrass)-infecting isolates and *M. oryzae* – the one that is associated with isolates capable of infecting rice, millets and other grasses from the species of *Oryza* spp, *Setaria* spp, *Lolium* spp, *Eragrostis* spp and *Eleusine* spp (Couch & Kohn, 2002).

Phylogenetic analysis studies by Zhang et al. (2011) based on six genes, namely *RPB1*, *TEF1*, *MCM7*, *ITS*, *SSU*, and *LSU*, showed that blast fungus *P. oryzae* or *M. oryzae* forms a separate and distinct clade. Another phylogenetic analysis by Luo and Zhang (2013) on plant pathogenic taxa in Magnaporthaceae which analysis was based on multiple genes including *SSU*, *ITS*, *LSU*, *MCM7*, *RPB1*, and *TEF1*, showed that *P. oryzae* and *P. grisea* also formed distinct clades, and the study showed that the rice blast was not congeneric with *M. salvinii*, the type species of genus *Magnaporthe.* Hence, they suggested that the blast fungus should be designated as *Pyricularia*.

Subsequently, Murata et al. (2014) revealed that phylognetic analsis study based on rDNA-ITS and *RPB1* sequences resulted in two clusters, which grouped *Pyricularia* spp. separately from species of *Magnaporthe* and *Gaeumannomyces*. The study also concluded that blast fungi are a distinct phylogenetic group and was not congeneric with the type species of *Magnaporthe,* i.e. *M. salvinii* and hence suggested that rice blast fungus should be designated as *Pyricularia*. The results were in accordance with the reports by Zhang et al. (2011) and Luo and Zhang (2013).

Consequently, *Pyricularia* which previously was identified as congeneric, meaning of the same genus with *Magnaporthe* when based on the similarity of ascospore morphology as observed in the laboratory crossing experiment (Couch & Kohn, 2002) is revealed not to belong to the type spesies of the *Magnaporthe* namely *M. salvinii* (Zhang et al., 2011; Luo & Zhang, 2013; Luo et al., 2014; Murata et al., 2014; Luo et al., 2015).

The teleomorph stage, *Magnaporthe,* is rarely encountered in nature and therefore, the fungus is generally referred by its anamorph stage name, *Pyricularia*. Thus, the name for the rice blast fungus was changed to *Pyricularia oryzae,* while the name for the rice stem rot is *Nakataea oryzae*, because these are the older names. However the synonym *Magnaporthe oryzae* should be written in publications as "*Pyricularia oryzae* (syn. *Magnaporthe oryzae*) (Zhang et al., 2016). The updates on the taxonomic, biogeography and molecular data on Magnaporthales can be accessed through e-monograph by researchers and the communities worldwide (Luo & Zhang, 2020; https://magnaporthales.sebs.rutgers.edu).

*Pyricularia oryzae (*syn. *Magnaporthe oryzae)* is a heterothallic, haploid ascomycete fungus produces three-celled air-borne conidia as the primary inoculums which may be present all year in the atmosphere to initiate and disseminate blast disease infection in the fields. Mycelia are usually thin, effuse, black coloured in centre with whitish margin. Conidiophores are simple to rarely branched, carrying clusters of conidia at their tips, moderately long, septate, light brown, slightly thickened at the base with denticles at the apex. Conidia are typically obclavate to pyriform, almost hyaline, pale olive or greyish, truncated with a short tooth at the base, 2-septate, rarely 1-3 septate, usually with a pointed acute apex, 20–25 µm × 9–12 µm (**Figure 2.1**) (Ou, 1985; Mew and Misra, 1994; Mew & Gonzales, 2002; Rice & Barr, 2014).

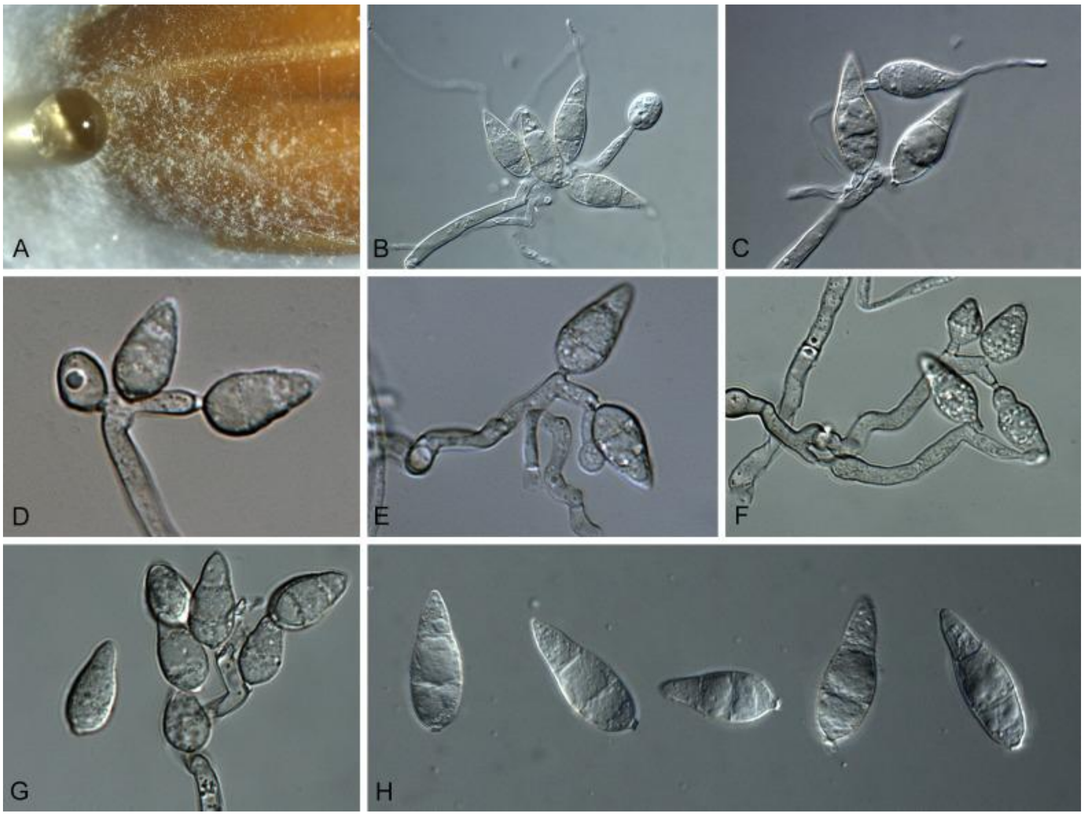

Figure 2.1. *Pyricularia oryzae* (BF0028). A. Sporulation on sterile barley seed on SNA. B–G. Conidiophores and conidia. H. Conidia. Scale bars = 10 µm. (Source: Klaubauf et al., 2014).

2.2.2 Symptoms of rice blast

Generally, *P. oryzae* infects rice plants and produces spots or lesions at different plant parts, leaves, nodes, collar/leaf sheath, different parts of the panicles, grains; and at any growth stage – seedling to tillering, flowering to ripening (Sella et al., 2021). For leaf blast, lesions appeared on the leaves as small whitish or greyish spots that eventually enlarge and become spindle-shaped (diamond-shaped) necrotic spots with brown to reddish brown margins (**Figure 2.2A, B**) (Ou, 1985; Mew & Mistra, 1994; Mew & Gonzales, 2002). Normally, the size of fully developed lesions is 1.0-1.5 cm in length and 0.3-0.5 cm in breadth. Lesions that enlarge and assemble may kill the entire leaves (Mew and Misra, 1994).

Any part of the panicle such as rachis, panicle branches and panicle base (neck) may be infected. Infected panicle breaks easily at the neck, turns black, rotten and leads to unfilled grains. Early infection may cause empty grains which appear as whitehead symptoms similar to stem borer infestation (Bonman, 1992; Kato, 2001; Shahriar et al., 2020). In the case of collar rot, the lesion appears at the junction of the leaf blade and leaf sheath which may kill the leaf (**Figure 2.2D**) (Ou, 1985; Kato, 2001; Shahriar et al., 2020). Meanwhile, symptom on glumes is observed mostly on sterile lemmas of the seed consisting of short, delicate conidiophores carrying clusters of conidia at their tips. The growth rarely covers the whole seed (Rice & Barr, 2014).

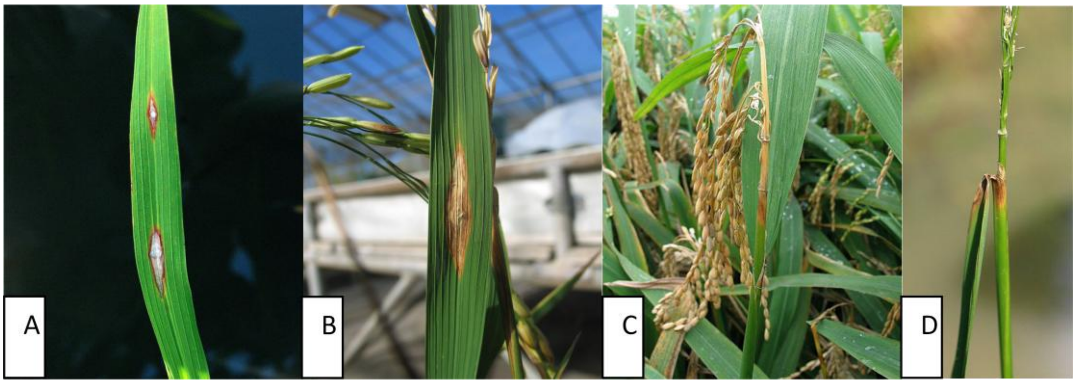

Figure 2.2: Symptoms of rice blast disease on (A & B) Leaf, (C) Panicle (D) Collar. (Source:http://www.knowledgebank.irri.org/training/fact-sheets/pest-management/diseases/item/blast-leaf-collar).

2.2.3 Disease cycle and infection strategies

Infection cycle of the rice blast fungus starts when a three-celled conidium lands on the hydrophobic rice leaf surface (**Figure 2.3**) (Wilson and Talbot, 2009). Morphology of conidia plays a critical role for spore dispersal and virulence of blast pathogen in the field (Bhadauria, et al. 2010). The conidium is tightly attached on the leaf surface by means mucilaginous substances which produced at the tip of the germ tube (Koga & Nakayachi, 2004). Appresorium, a highly melanized structure which produces penetration peg then develops substantial turgor pressure (up to 8.0 MPa) to rupture the cuticle and cell wall (Talbot, 2003; Park et al., 2009; Choi et al., 2011; Galhano & Talbot, 2011).

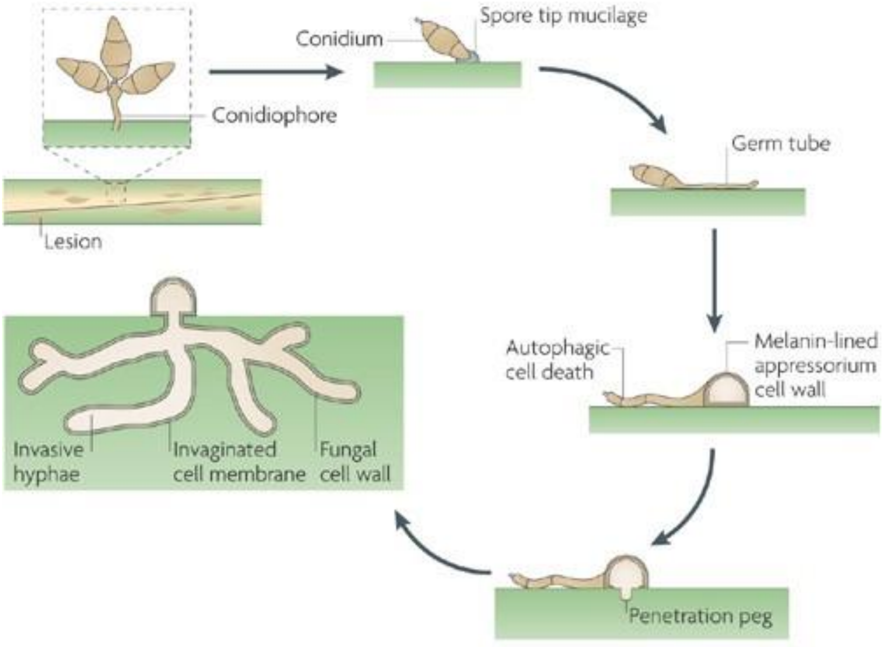

Figure 2.3: Life cycle of the rice blast fungus *Magnaporthe oryzae*. (Source: Wilson & Talbot, 2009).

After penetration invasive fungal hyphae invade biotrophically the host cells at early stage of infection. In late stages of infection, infectious growth of the pathogen switches to a necrotrophic-like phase, resulted in plant cell death and appearance of disease lesions within 72 to 96 hours after the infection. Under favourable conditions such as high humidity, sporulation occurs from the disease lesions and the conidia disperse by wind and dewdrop splash or rain splash. Hence, re-initiate the infection cycle to adjacent rice plants in the field (Ou, 1985; Talbot, 2003; Ribot et al., 2008; Zhang et al., 2014).

Subsequently, the germ tube flattens and hooks at its tip before differentiating into a dome-shaped appressorium. The single-celled appressorium matures and the three-celled conidium collapses and dies in an autophagic cell death programme (Wilson and Talbot, 2009).

2.3 Management of rice blast disease in Malaysia

There are many approaches available to manage rice blast disease in the fields. These include the use of cultural practices, biological control, cultural control, chemical control and utilization of resistant varieties.

2.3.1 Chemical control using fungicides

The most common practice in managing rice blast is fungicides application. However, this method is harmful to the environment and possible to induce fungicide resistance among pathogen populations (Todorova and Kozhuharova, 2010; Suzuki et al., 2010). Ideally, farmers are advice to combine or alternate the fungicides application with different modes of action. This is to decrease the risk of fungicide resistance and preserve full efficacy of the fungicide (Skamnioti and Gurr, 2009).

Since 1960s, several fungicides have been developed and registered to control blast such as O, O-diisopropyl-S-benzyl thiophosphate (IBP) which acts through the inhibition or spore germination and mycelia growth, edifenphos (inhibit spore germination and mycelial growth), probenazole (inhibit penetration and mycelial growth), isoprothiolane (inhibit or penetration and mycelial growth), fthalide (inhibition of penetration), tricyclazole (inhibition of melanin biosynthesis) and benomyl (Amoghavarsha et al., 2021).

Along with azoxystrobin, kresoxim-methyl and trifloxystrobin are quinone outside inhibitors (QoIs) fungicides that represent a group of mitochondrial respiration inhibitors derived from naturally occurring strobilurins (Jin et al., 2009; Kunova et al., 2013). Specifically, these fungicide disrupt the energy cycle of the fungus in the mitochondria (Bartlett et al., 2002) and leads to penetration inhibition of the pathogen into the plant (Brent and Hollomon, 2007).

In Malaysia, tricyclazole, benomyl, isoprothiolane, azoxystrobin and trifloxystrobin are among the fungicides listed in the Rice Subsidy Programme. Applications of the fungicide are recommended when the symptoms appears at seedling to maximum tillering stage (leaf blast) and at milky stage (panicle or neck rot) (Maisarah et al., 2014). Moreover, seed treatment for preventing the blast disease is done by using benomyl slurry at 0.3% by seed weight, or by hot water at 52-57 °C for 15 minutes as recommended by Misra et al. (1994).

2.3.2 Rice blast forecasting system

Forecasting systems of rice blast have been developed in several countries and found to be effective in forecasting the incidence of the disease. Models developed for forecasting rice blast were based on incorporation of many variables or factors that favour rice blast development including meteorological variables such as leaf wetness, air temperature, soil temperature, relative humidity, rainfall, sunlight, near UV light and carbon dioxide (Katsantonis et al., 2017) as well as planting date and number of air-borne spore trapped (Kim et al., 2015).

There are a few rice blast forecasting models developed by researchers worldwide. For instance, rice growth simulation model, CERES-Rice was integrated with blast epidemic simulation model, BLASTSIM (Luo et al., 1998). A generic epidemiological model, EPIRICE, was developed to assess changes in disease probabilities under climate change scenarios and incidences of rice diseases including rice blast (Kim et al., 2015). A simulation model, SiRBInt, for forecasting blast appearance in the field could be utilised for planning proper fungicide application (Biloni et al., 2006). EPIBLAST, a leaf blast simulation model was used for quantitative forecasting of leaf blast incidence, which also predict the peak time of leaf blast epidemic (Kim and Kim, 1993).

Future forecasting system may include parameters such as the diversity of blast pathotypes in the fields, both the virulence and avirulence compositions. Monitoring the changes in pathotype composition in the field may avoid the resistance breakdown in the newly released varieties (Mgonja et al., 2017). Breakdown of resistance are generally caused by the capacity of the fungus to evolve rapidly, emergence of new virulent strains and spread among and between populations (Saleh et al., 2014).

2.3.3 Cultivation of resistant varieties

Amongst many approaches to control blast disease in the fields, utilization of resistant varieties is the best approach due to its environment friendly input and the lowest cost for its adoption. It does not incur the farmers with any additional cost except for the cost of seeds. Most of MARDI released varieties have been ensured to have some degree of resistance during their evaluation prior to release for commercialization (Habibuddin, 2012).

2.3.3(a) History of blast resistant varieties in Malaysia

Development of Malaysian rice varieties started since 1965 and the first few varieties were developed by the Department of Agriculture (DOA), Malaysia. High incidence of neck rot blast disease on local rice varieties Malinja and Mahsuri in 1964 and 1965 has brought the attention of researchers on the importance of breeding for blast disease resistance in the national rice improvement programme (NurulNahar et al., 2020). It was started with the incorporation of systematic blast screening protocol for used in the selection of resistant lines against the disease. Since then, breeding of new rice varieties in MARDI has indicate resistance to blast disease as an important criteria to be taken into consideration for a potential variety to be commercially released (Habibuddin, 2012).

High yielding rice variety, MR219 and MR220 were released in 2001 and 2003, respectively (Alias et al., 2002, 2005). Both varieties were screened and identified as resistant to foliar blast under blast disease evaluation prior to their released (Hassan, 2015). However, five years after they were released, rice blast disease outbreaks were occurred during planting seasons of 2004 and 2005 affecting 4033 hectares at Projek Barat Laut Selangor, MADA, Pulau Pinang, KADA, KETARA, Chuichak and FELCRA Seberang Perak (Department of Agriculture, Unpublished data).

During the planting season of Main Season 2008/2009, the varietal coverage of MR219 was about 90% throughout the Peninsular Malaysia, and this has contributed to the high incidence of panicle blast infection recorded, which caused for the official withdrawal from the fields. However, until now, these two varieties still being planted by a significant number of farmers due to their high yielding potential, capable of producing yield of 10 t/ha (Elixon et al., 2017).

In 2006, MR232 which has a better blast resistance than MR211, MR219 and MR220 was officially released. Besides blast resistant, it also has a good eating quality. Its resistance to blast disease might be inherited from Pankhari 203 as the donor parent (Badrulhadza et al., 2013). Several new rice varieties were introduced by MARDI following the failure of MR219 to withstand blast infection. This included the released of rice varieties MR253, MR263 (Zainudin et al., 2012) and MR269 (Blog Rasmi MARDI, 2012) to reduce the potential major epidemic of blast disease in the country, besides increasing yield potential. The rice variety MR284 which was introduced in 2015 was able to reduce the problems caused by leaves blast, panicle blast, as well as to the brown planthopper infestation (The Borneo Post, 2015**)**. The variety MARDI SIRAJ 297 is another rice variety introduced in 2016 which is also resistant to leaves and panicle blast disease (Harian Metro, 2016).

Recently, Ab Razak et al. (2019) has screened 24 Malaysia released varieties to determine the potential existence of four major blast resistant genes namely *Pi-5, Piz-t, Pi-b* and *Pi-k* in the varieties. Screening of the rice varieties through allele-specific PCR markers revealed the presence of all four resistance genes, *Pi-5, Piz-t, Pi-b* and *Pi-k* in three of Malaysia rice varieties namely Sekembang, Kadaria and MR81. In addition to that, there were another 15 varieties were harboring three genes, one variety harboring two genes and three other varieties are harboring at least one blast resistant gene, respectively. Meanwhile there were only two varieties which did not harbor any of those four resistant genes. This finding gave new information on what are the blast resistance genes harboring in Malaysian rice varieties and could possibly strategize new breeding for blast resistant variety.

However, planting of a mono-variety for a long term in a large area has resulted with the emergence of new pathotypes capable of attacking the previously resistant varieties. Therefore, a suggestion by Ali et al. (1995) that the variety's coverage in a certain area of not be more than 50% might be taken into consideration.

2.4 Diversity of blast causing pathogen

Control management of the disease by using resistant varieties or fungicide application could be affected by the presence of genetic variation within the plant pathogen population (Milgroom and Peever, 2003). For instance, resistance breakdown of the originally introduced resistant rice varieties is associated to the presence of high genetic variability in the pathogen population in the field (Séré et al., 2007). A segment of virulent pathotype within the population will adapt to the pressure exerted by resistant gene in the host, resulted in changing in the prevalence of pathotype in the field, capable of causing resistance breakdown of the variety.

2.4.1 Race classification of *Pyricularia oryzae* isolates

Several studies have been conducted to develop differential systems for the classification and monitoring of the distribution of blast races in the fields. In differential system, each differential variety represents each dominant resistance gene. Therefore, the differential system has the ability to differentiate blast races (Hayashi et al., 2009). Later, studies on race identification were established in several countries by using differential varieties. However the differential system used by different countries were not identical (Sridhar & Singh, 2001: Hayashi et al., 2009).

In Malaysia, Malaysian Agriculture and Research Development Institute (MARDI) use eight differential varieties, namely, Mahsuri, MR1, Bahagia, Engkatek, Seribu Gantang, Tadukan, Pankhari 203, and Pongsu Seribu with varying