



UNIVERSITI PUTRA MALAYSIA

***DEVELOPMENT OF BLAST-RESISTANT RICE VARIETY DERIVED
FROM CROSSING BETWEEN MR219 AND PONGSU SERIBU 1
THROUGH MARKER-ASSISTED SELECTION***

GOUS MIAH

ITA 2015 13



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By
GOUS MIAH

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfillment of the Requirement for the Degree of Doctor of Philosophy**

January 2015

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DEDICATIONS

I dedicate this thesis to my beloved parents, wife, son and daughter, brother and sisters for their kind and loving support.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Doctor of Philosophy

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January 2015

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Marker-assisted selection (MAS) is very effective and powerful method for efficient selection of the target gene. MAS can potentially accelerate breeding programs for varietal resistance. Marker-assisted backcrossing (MABC) is the most-effective way of transferring specific gene(s) to an agronomically superior variety. Rice blast disease, caused by the fungus *Magnaporthe oryza*, is one of the most devastating and destructive disease causing significant yield loss worldwide as well as Malaysia. The most-effective approach to preventing this disease is the genetic improvement using resistant varieties. In this study, blast resistance gene (putative *Piz*) was transferred from an *indica* rice donor Pongsu Seribu 1, using MABC method, into a blast susceptible elite *indica* rice variety, MR219. Therefore, a cross between rice variety MR219 and Pongsu Seribu 1 was performed to incorporate blast resistance gene(s) into MR219 variety without losing their quality. Sixteen microsatellite markers tightly linked to the blast resistant genes were selected and used in this study to determine potential association with blast resistance gene. These polymorphic foreground markers were used for confirmation of blast resistant genes in F₁ population. After that only two microsatellite markers, RM6836 and RM8225, that conferred blast resistance gene (putative *Piz*) located on chromosome 6 were used to BC₁F₁, BC₂F₁ and BC₂F₂ populations related to resistance against the most-virulent Malaysian rice blast pathotype P7.2 of *Magnaporthe oryzae*. Out of 375-microsatellite markers, 70 markers were found to have polymorphism between the parental lines and were used for background analysis in each generation. The inheritance of the resistance gene against the pathotype P7.2 was investigated on BC₂F₁ and BC₂F₂ populations. The parent MR219 showed susceptible reaction with lesion score 5 to 7, whereas Pongsu Seribu 1 was found as resistant producing lesion score 0 to 2 after exposing to P7.2 inoculum in the glasshouse. In BC₂F₁ population, 333 plants were challenged with pathotype P7.2. Among them, 159 plants showed

resistant reaction and another 174 plants showed susceptible reaction to blast. The observed frequencies were tested for goodness of fit with chi-square (χ^2) test for single dominant gene model. The goodness of fit ($p=0.4463$) to the expected test cross ratio (1:1) showed that the resistance is controlled by a single nuclear gene. The plants resistant to blast pathotype P7.2 from BC₂F₁ population showed good fit with two markers, RM6836 ($\chi^2=0.20$; $p=0.6547$) and RM8225 ($\chi^2=1.20$; $p=0.2733$) with expected test cross ratio (1:1) for single gene model. These two markers were found suitable for marker-assisted selection conferring blast resistance gene against the pathotype P7.2. The phenotypic reaction on the blast incidence of BC₂F₂ population segregated in 3:1 ratio (resistance to susceptible). The genotypic segregation of BC₂F₂ population using RM6836 and RM8225 markers showed 1:2:1 ratio. Results confirmed that single dominant gene governs blast resistance in Pongsu Seribu 1 variety. The background analysis of the improved lines indicated recurrent parent genome recovery ranging from 75.40 % to 91.30 % in BC₁F₁, 80.40% to 96.70% in BC₂F₁ and 92.7% to 97.7% in BC₂F₂ generation. The average proportion of the recurrent parent in BC₂F₂ selected improved lines was 95.98%, explaining the very close similarity at phenotypic level with the parental variety, MR219. Thirteen homozygous plants consist of blast resistant gene which is phenotypically similar with MR219 backgrounds were selected as an improved blast resistant breeding lines. The agronomic characters showed no significant difference between MR219-parent and blast resistant MR219 improved lines. The improved lines possessing blast resistant gene (putative *Piz*) with desirable agronomic traits that can be used as a valuable source for further blast resistance rice breeding programs. In conclusion, from this research rice blast resistance in Pongsu Seribu 1 is governed by a single dominant gene located on chromosome 6, which is linked to RM6836 and RM8225 markers. This information could be used in MAS for blast resistance in other rice crosses involving Pongsu Seribu 1.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PEMBANGUNAN VARIETI PADI RINTANG KARAH DARI KACUKAN
ANTARA MR219 DAN PONGSU SERIBU 1 MELALUI PEMILIHAN
BANTUAN PENANDA**

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Pemilihan bantuan penanda (MAS) adalah kaedah yang sangat berkesan dan berkeupayaan tinggi dalam memilih gen yang dikehendaki. MAS juga berpotensi untuk mempercepatkan program pemilihan varieti yang rintang penyakit. Kacuk balik bantuan penanda (MABC), adalah cara paling berkesan untuk memindahkan gen spesifik ke varieti yang mempunyai ciri agronomi yang unggul. Penyakit karah padi, yang disebabkan oleh kulat *Magnaporthe oryza*, adalah salah satu penyakit yang paling utama dan merosakkan tanaman, yang mengakibatkan penurunan hasil yang ketara dalam pengeluaran padi di seluruh dunia dan juga Malaysia. Pendekatan yang paling berkesan untuk mencegah penyakit ini adalah dengan memperbaiki kandungan genetik menggunakan varieti yang rintang penyakit. Dalam kajian ini, gen rintang karah (putatif *Piz*) telah dipindahkan dari penderma padi *indica* (Pongsu Seribu 1), menggunakan kaedah MABC, ke satu varieti padi *indica* unggul yang rentan karah, MR219. Oleh itu, kacukan antara varieti padi MR219 dan Pongsu Seribu 1 telah dijalankan untuk menggabungkan gen rintang karah ke atas varieti penerima, padi MR219 tanpa kehilangan kualiti asal MR219. Enam belas penanda mikrosatelit yang berkait rapat dengan gen kerintangan karah (*Pi*-gen) telah dipilih dan digunakan dalam kajian ini bagi menentukan potensi pengabungannya dengan gen kerintangan karah. Penanda polimorfik utama ini telah digunakan bagi mengenal pasti gen kerintangan karah pada populasi F_1 . Selepas itu hanya dua penanda mikrosatelit, RM6836 dan RM8225, yang menentukan gen kerintangan karah (putatif *Piz*) yang terletak di atas kromosom 6 telah digunakan pada populasi BC_1F_1 , BC_2F_1 dan BC_2F_2 yang menunjukkan kerintangan terhadap patotip P7.2 *Magnaporthe oryzae*, iaitu, patogen karah yang paling virulen di Malaysia. Daripada 375 penanda mikrosatelit, 70 penanda didapati polimorfik di antara kedua induk dan telah digunakan untuk analisis pemulihan dalam setiap generasi. Pewarisan gen kerintangan karah padi terhadap patotip P7.2 telah dinilai ke atas populasi BC_2F_1 dan BC_2F_2 . Induk MR219 menunjukkan reaksi paling rentan dengan

skor *lesion* 5 hingga 7, manakala induk Pongsu Seribu 1 yang mempunyai kerintangan tinggi dengan skor *lesion* dari 0 hingga 2 apabila dirawat dengan inokulum P7.2 di rumah kaca. Pada populasi BC₂F₁, 333 pokok telah diinokulasi dengan patotip P7.2. Dari jumlah tersebut, didapati 159 pokok menunjukkan kerintangan dan 174 pokok lagi didapati reaksi rentan karah. Frekuensi yang dicerap telah diuji untuk keserasian menggunakan ujian khi-kuasa dua (χ^2) bagi model gen tunggal. Keserasian ($p=0.4463$) kepada ujian nisbah kacuk yang dijangka (1:1) menunjukkan kerintangan tersebut dikawal oleh gen tunggal. Pokok rintang karah terhadap patotip P7.2 dari populasi BC₂F₁ menunjukkan padanan yang baik dengan dua penanda RM6836 ($\chi^2=0.20$; $p=0.6547$) dan RM8225 ($\chi^2=1.20$; $p=0.2733$) bagi nisbah dijangka (1:1) untuk model gen dominan tunggal. Kedua-dua penanda ini didapati sesuai digunakan untuk pemilihan bantuan penanda bagi gen kerintangan karah patotip P7.2. Reaksi fenotip jangkitan karah pada populasi BC₂F₂ bersegregasi pada nisbah 3:1 (nisbah rintang ke rentan). Segregasi genotip dalam populasi BC₂F₂ dengan menggunakan penanda RM6836 dan RM8225 memberikan nisbah 1:2:1. Keputusan mengesahkan bahawa gen dominan tunggal yang mengawal kerintangan terhadap karah pada varieti Pongsu Seribu 1. Analisa pemulihan pada titisan maju menunjukkan pemulihan genom induk penerima dari 75.40% kepada 91.30% pada BC₁F₁, 80.40% kepada 96.70% pada BC₂F₁ dan 89.7% kepada 97.7% pada generasi BC₂F₂. Purata pemulihan genom titisan induk penerima pada BC₂F₂ terpilih adalah 95.98%, yang menunjukkan persamaan yang rapat pada fenotip dengan varieti induk, MR219. Tiga belas pokok homozigot mempunyai gen rintang karah dengan fenotip yang sama dengan MR219 telah dipilih bagi membangunkan varieti yang rintang karah. Ciri agronomi menunjukkan tiada perbezaan yang bererti di antara induk MR219 dan waris MR219 maju rintang karah. Titisan maju yang telah diperbaiki memiliki putatif gen *Piz* mempunyai ciri agronomi yang dikehendaki dan boleh digunakan secara langsung sebagai sumber untuk program seterusnya bagi pembiakbakaan padi kerintangan karah. Kesimpulannya, hasil dari penyelidikan kerintangan terhadap karah padi Pongsu Seribu 1, ianya dikawal oleh gen dominan tunggal yang terletak pada kromosom 6, yang berkait dengan penanda RM6836 dan RM8225. Maklumat ini boleh digunakan dalam MAS untuk kerintangan karah pada kacukan padi lain yang melibatkan varieti Pongsu Seribu 1.

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LIST OF ABBREVIATIONS

bp	Base pair
cM	Centi Morgan
cm	Centimeter
CTAB	Cetyltrimethylammonium bromide
χ^2	Chi-square
°C	Degree celsius
df	Degree of freedom
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EST	Expressed sequence tag
g	Gram
MABC	Marker-assisted backcrossing
MAS	Marker-assisted selection
Mbp	Mega base pair
m	Metre
μ	Micro
ml	Milliliter
mm	Millimeter
min	Minute
M	Molar
n	Nano
PCR	Polymerase chain reaction
QTL	Quantitative trait loci
%	Percentage
RPG	Recurrent parent genome
SSR	Simple sequence repeat
RAPD	Randomly amplified polymorphic DNA
RIL	Recombinant inbred line

RFLP	Restriction fragment length polymorphism
RH	Relative humidity
RNA	Ribonucleic acid
rpm	Rounds per minute
s	Second
SMA	Single-marker analysis
SNP	Single-nucleotide polymorphism
SES	Standard evaluation score
TE	Tris/EDTA
UV	Ultra violet
V	Volt
v/v	Volume/volume
w/v	Weight/volume
β-ME	β-mercaptoethanol
ddH ₂ O	Double distilled water
BLD	Blast lesion degree
BLT	Blast lesion type
%DLA	Percent diseased leaf area
GDP	Gross domestic product

CHAPTER 1

INTRODUCTION

1.1 General introduction

Rice (*Oryza sativa* L.) is one of the most vital food sources of humans and the second major global calories contributor (FAO, 2008). Rice is also the staple food of Malaysia, which is the significant source of employment and earnings to the rural farmers. Rice is prone to a range of man-made environmental or natural stresses. All these stresses cause modifications in an extensive range of physiological, biochemical and molecular processes in plants which ultimately lead to poor growth and low crop yield (Ashraf and Harris, 2013; Ashraf, 2014). The rice consumer is increasing, and demand for rice is also moving up due to better living standards. Various studies have shown that to meet the increasing demand for rice, production has to be increased more than 40 % by 2030 (Khush, 2005). This challenge has to be overcome by the development of high-yielding rice varieties with resistance or tolerance to biotic and abiotic stresses (Selvaraj *et al.*, 2011). Among the biotic stresses, blast disease is the most harmful threat to high productivity of rice (Fahad *et al.*, 2014). The rice yield loss should be minimized in order to help the marginal and poor farmers of developing countries (Latif *et al.*, 2011a). However, the potential yield of rice in Malaysia is around 10 tons ha⁻¹ (Abdullah *et al.*, 2010). Unfortunately, until now the rice production in Malaysia has not met the national demand (Ghee-Thean *et al.*, 2012). Consequently, research is needed to expand and to increase rice yields by preventing diseases in order to supply sufficient food for a rising population. Malaysia is targeting 80% rice-sufficiency by 2020 that is why the government has increased four new granary areas under rice cultivation around 2.5% in year 2013 (FAO, 2013).

At least 85% of the Malaysian rice farmers reported that pests and diseases are the major problems in rice production (Amir *et al.*, 2012). The evolution of new biotypes of pests and diseases, and the pressures of climate change, pose thoughtful challenges to rice breeders, who would like to increase rice production by introducing resistance to numerous biotic and abiotic stresses (Miah *et al.*, 2013). Blast disease can be managed by the use of genetic resistance rice varieties, with cultural practices and the use of fungicides (Fahad *et al.*, 2014). In modern years, many techniques have been developed to control the rice blast disease. The chemical and biological methods, cultivation practices and disease forecasting methods are applied widely to control the spread of this disease (Jeger *et al.*, 2007). Unfortunately, these methods are not very effective. The use of fungicides is expensive as well as neither practical nor environmental-friendly. The use of resistant rice varieties is a powerful tool to reduce the use of environmentally destructive fungicides.

The molecular role of some blast resistance genes has been defined and many quantitative trait loci (QTLs) for blast resistance have been mapped (Dai *et al.*, 2007). New information and knowledge gained from molecular biology research on disease

resistance gene-mediated defense responses which is undoubtedly offer new perceptions into the nature of rice disease resistance. So, understanding and application of molecular biology is a prerequisite for accelerating the development of the blast resistance. Many rice researchers and breeders (Zhao *et al.*, 2010; Wang *et al.*, 2011; Swamy and Sarla, 2011) have developed new improved cultivated rice for resistance against the blast through molecular breeding approach involving DNA markers, marker-assisted selection (MAS) and genetic transformation. The PCR-based allele-specific markers provide an efficient marker system for MAS for blast resistance breeding (Latif *et al.*, 2011b). Conventional rice breeding is a slow process, typically requires 10 to 15 years from initiation to varietal release and the release of improved varieties cannot be assured (Werner *et al.*, 2005; Zhang *et al.*, 2006). Moreover, MAS offer better selection tactics in rice breeding with a short period. Marker-assisted selection is more efficient, effective and reliable than phenotypic selection. Advent of DNA marker technology, development of numerous types of molecular markers and molecular breeding approaches offered possibilities to plant breeders and geneticists to overcome several problems faced during conventional breeding. In specific, microsatellite-based methods offer a fascinating high-throughput and non-labor-intensive technique to tag blast resistance genes in rice breeding programs.

Oryza sativa ssp. *indica* cv. MR219 a Malaysian elite rice variety which have the characteristics of high-grain yield, short maturation period and good eating quality (Alias, 2002). MR219 is the premier variety released by Malaysian Agricultural Research and Development Institute (MARDI) in 2001 and widely planted in Malaysia. The highest potential yield recorded is 10.75 tons ha⁻¹ and with long panicles at 23.2 cm (Alias, 2002). MR219 is grown more than 90% of rice fields in Malaysia due to its high-production potentiality (Fasahat *et al.*, 2012). When MR219 is released by MARDI, it was moderately resistant against blast fungal. But after a longtime cultivation and regeneration of seeds and due to climate change new blast pathotypes have been developed and recently MR219 broken down blast resistant ability and affected severely with blast pathogens. These informations highlighted the opportunity to develop additional high-yielding varieties that are adapted to other regions or environments. Here, the objective of this research was to convert widely cultivated variety MR219 to blast resistant variety with MR219 genetic backgrounds through backcross breeding and MAS. MR219 is not a blast resistant variety, and farmers are facing either very low yield or did not get any yield in the affected area. More supportable and everlasting solutions are needed to overcome this problem. One of the most encouraging solutions is to develop high-yielding varieties that are blast disease resistant and that are more likely to be rapidly adopted by the farmers in the target regions.

Pongsu Seribu 1 is Malaysian local rice variety which is resistant against blast fungal *Magnaporthe oryzae*. The choice of Pongsu Seribu 1 as a donor parent for the current breeding program was based on the fact that this variety is blast resistant, has a great percentage of the existing genetic difference for *Oryza* genus even though it's phenotype is agronomically unattractive. Information on genetic control of blast resistance in Pongsu Seribu 1 is very limited.

Recent advances in rice genomics have now made it possible to identify and map a number of genes through linkage to existing DNA markers. There are more noteworthy examples of molecular markers that tightly linked with the genes in rice confer resistance to blast. Therefore, in combination with conventional breeding methods, MAS can be used in breeding populations to monitor the presence or lack of these genes. For instance, MABB has been used to incorporate important genes with meaningfully biological effects into a number of regularly grown rice varieties. The use of finely mapped microsatellite markers and MAS strategies provide opportunities for breeders to develop high-yield, blast resistance rice varieties. Therefore, there is an urgent need to develop novel strategies to breed new rice varieties that confer high and stable resistance to this important rice disease in order to ensure food security in this country. Taking consideration above all these things in mind, blast resistant gene was introgressed into the rice genotype, MR219 which will help much to reduce yield losses of rice, as well as improvement livelihood to the people of Malaysia. In this study, microsatellite markers were successfully used to introgress blast resistance gene into a popular Malaysian rice variety MR219 having high-yield potential through marker-assisted backcrossing (MABC).

1.2 Significance of the study

In agriculture, one of the main objectives of plant breeder is to improve the existing varieties, which are deficient in one or more traits by crossing such varieties with lines that possess the desired traits. Conventional breeding program is laborious and time consuming; involving several crosses, several generations and careful phenotypic selection, and the linkage drag may make it further difficult to achieve the desired objectives. Molecular breeding approach involving MAS for resistance against blast has been used in developing new improved rice varieties by many rice researchers and breeders (Zhao *et al.*, 2010; Wang *et al.*, 2011; Swamy and Sarla, 2011). Marker-assisted selection is a recent tool in breeding to improve resistance to rice blast (Chowdhury *et al.*, 2012). Marker-assisted selection provides the potential solution to some of the problems that conventional breeding cannot resolve. In recent years, blast resistant genes have introgressed into Luhui 17, G46B, Zhenshan 97B, Jin 23B, CO39, IR50, Pusa1602 and Pusa1603 lines through MAS. Introduction of exotic genes for resistance induced the occurrence of new races of blast fungus; therefore, breeding work should be concentrated in local resistance genes (Miah *et al.*, 2013b). In Malaysia, several upland and traditional rice varieties are known to be resistant to blast disease. Among them, Pongsu Seribu 1 is one of the Malaysian local rice variety which is resistant against blast fungal. A better understanding of resistant genes is needed to enhance the development of blast-resistant varieties especially those with long-lasting, durable resistance (Tyng *et al.*, 2010). Information on genetic control of blast resistance in Pongsu Seribu 1 is very limited. Therefore, Pongsu Seribu 1 can be used in order to develop new rice variety with blast resistant in the current Malaysian rice breeding program. In the present study molecular marker technology and MABC were used to

introgress blast resistant gene in MR219 rice variety and to identify tightly linked markers conferring blast resistance against the most virulent rice blast fungus *Magnaporthe oryzae* pathotype P7.2. The improved blast resistant MR219-rice variety will reduce the yield loss and helpful to achieve rice production at self-sufficiency level in Malaysia.

1.3 Problem statement

Rice blast disease, caused by the fungus pathogen *Magnaporthe oryzae*, is one of the most serious diseases of rice. More than 85 countries around the world have reported the blast occurrence (Wang *et al.*, 2014), leading to the yield loss 10 million tons of rice every year (Wen and Gao, 2011). Therefore, rice blast is a significant economic and humanitarian problem. In Malaysia, rice blast has become a considerable disease of rice. Outbreaks often occur in seasons and off-seasons across the rice growing areas. The most popular rice variety MR219 is susceptible to blast disease. In Malaysia, a severe outbreak of blast disease occurred in 2006 at Kemubu Agriculture Development Authority (KADA) and Kelantan and yield losses were more than 60% from 4,000 hectares of rice cultivated area (Ashkani, 2011). In the recent years, in Malaysia, frequency of blast occurrence has increased with invasion into new areas. Moreover, there is no cultivated blast resistant rice variety in Malaysia. These results highlighted the opportunity to develop additional blast resistant high-yielding MR219 rice variety that will be adapted to Malaysia.

1.4 Objectives of the research

1. To analyse SSR markers linked with blast resistant gene in BC₂F₁ population derived from crosses between MR219 and Pongsu Seribu 1.
2. To analyse the recurrent parent genome recovery contribution in marker-assisted backcross breeding programs for the introgression of blast resistant gene.
3. To introgress blast resistant gene in MR219-rice variety through marker-assisted selection.

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