



**UNIVERSITI PUTRA MALAYSIA**

***DEVELOPMENT OF BLAST RESISTANT RICE VARIETY THROUGH  
MARKER-ASSISTED BACKCROSSING BETWEEN VARIETIES MR263  
AND PONGSU SERIBU 1***

***MUHAMMAD MAHMUDUL HASAN***

**FP 2015 40**



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AND PONGSU SERIBU 1**

**By**

**MUHAMMAD MAHMUDUL HASAN**

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

**August 2015**

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## DEDICATION

*Dedicated to my beloved parents*



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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MARKER-ASSISTED BACKCROSSING BETWEEN VARIETIES MR263 AND  
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By

**MUHAMMAD MAHMUDUL HASAN**

**August 2015**

**Chairman: Professor Mohd Rafii Yusop, PhD**  
**Faculty: Agriculture**

The rice blast caused by *Magnaporthe oryzae* is the most important and potentially damaging rice disease globally. Its frequent appearance during all stages of plant growth greatly decreases yield and grain quality. Marker-assisted backcrossing (MABC) selection can play a vital role in developing disease resistant, high-yield or quality rice varieties by incorporating a gene of interest into an elite variety that is already cultivated by farmers. The main objective of this study was to develop a blast resistant rice variety from cross between MR263 and Pongsu Seribu 1 (PS1). The specific objectives were to introgress blast resistant genes derived from a resistant variety, PS1 into the popular but blast susceptible Malaysian rice variety MR263 through MABC, to identify polymorphic SSR markers associated with blast resistant genes for foreground selection, to identify polymorphic SSR markers between the two parents, and to evaluate agronomic performance of advanced blast resistant rice lines of BC<sub>2</sub>F<sub>3</sub> generation. A total of 450 SSR markers were used among which 65 polymorphic markers were identified including *Pi* gene based markers and background markers to identify the segregation ratio in 300 individuals of BC<sub>2</sub>F<sub>1</sub> rice population derived from MR263 × PS1. For the phenotypic study, the most virulent blast (*Magnaporthe oryzae*) pathotype P7.2 was used to screen BC<sub>2</sub>F<sub>1</sub> individuals to determine the inheritance of blast resistance as well as linkage association with the SSR markers. Among the 65 polymorphic markers, 16 markers showed a heterozygous band in the BC<sub>2</sub>F<sub>1</sub> population. From the 16 polymorphic markers, only eight markers (RM5961, RM263, RM163, RM224, RM262, RM168, RM229 and RM169) showed a good fit to the expected segregation genotypic ratio (1:1) for the single dominance gene model (df = 1.0,  $P < 0.05$ ) using chi-square ( $\chi^2$ ) analysis. Phenotypic data analysis of the BC<sub>2</sub>F<sub>1</sub> population also showed a good fit to the expected phenotypic ratio (1:1; R:S) for resistant and susceptible plants. Resistance to blast Pathotype P7.2 in PS1 is most likely controlled by a single dominant gene whereby the eight markers are linked to rice blast resistance. These linked-markers could be used in marker-assisted selection programme to develop a durable blast resistant rice variety. From BC<sub>2</sub>F<sub>2</sub> generation, four blast resistant lines (MR263-BR-3-2, MR263-BR-4-3, MR263-BR-13-1 and MR263-BR-26-4) were selected for rice blast resistant varietal development. These lines were identified to carry the *Pi-7(t)*, *Pi-d(t)1*, and *Pir2-3(t)* genes and the *qLN2* QTL as determined by markers RM5961 and RM263 (linked-marker to the blast

resistant genes and QTL). Background selection analysis using 65 polymorphic SSR markers revealed that high recurrent parent genome recovery was 96.0% in MR263-BR-4-3, 94.1% in MR263-BR-3-2, 89.5% in MR263-BR-26-4 and 89.1% in MR263-BR-13-1, and these four resistant BC<sub>2</sub>F<sub>2</sub> lines were selfed to develop BC<sub>2</sub>F<sub>3</sub> population. Finally, thirty advanced blast resistant BC<sub>2</sub>F<sub>3</sub> lines were selected for agro-morphological evaluation. Out of 30 blast resistant lines, 12 lines (Lines 12, 6, 7, 5, 21, 22, 26, 11, 8, 10, 13 and 15) produced comparable grain yield per hill, number of tillers per hill, panicles per hill and percentage of filled grain to variety MR263. The estimates broad-sense heritability values in the selected rice population were high (70 to 95%) for all the traits indicating that these characteristics are highly heritable to the next generation. From cluster analysis based on the agro-morphological traits, 20 advanced lines were grouped into similar cluster with MR263 variety. This result was expected due to a high genome recovery (mean 96%) of MR263 variety into the advanced lines. The 12 advanced rice lines are recommended for multi-location trial to select blast resistant high yielding lines for development of highly potential rice variety in Malaysia.

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

**PEMBANGUNAN VARIETI PADI RINTANG KARAH MELALUI BANTUAN  
PENANDA KACUKBALIK DI ANTARA VARIETI MR263 DAN PONGSU  
SERIBU 1**

Oleh

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Karah padi disebabkan oleh *Magnaporthe oryzae* adalah merupakan penyakit padi utama yang penting dan mengakibatkan kerosakkan padi seluruh dunia, termasuk di Malaysia. Serangannya berlaku disemua peringkat pertumbuhan pokok padi yang mengakibatkan penurunan ketara hasil dan kualiti bijian. Pemilihan kacukbalik bantuan-penanda (MABC) dapat memainkan peranan penting dalam pembangunan varieti padi yang rintang penyakit serta berhasil tinggi atau beras berkualiti dengan menggabungkan gen yang dikehendaki ke dalam varieti elit yang ditanam oleh petani. Objektif utama kajian ini adalah untuk membangunkan satu varieti padi yang rintang karah daripada kacukan antara MR263 dan Pongsu Seribu 1 (PS1). Objektif khusus adalah untuk intrograsi gen rintang karah dari varieti yang rintang, PS1 ke dalam varieti popular di Malaysia tetapi rentan penyakit karah, MR263 melalui MABC, untuk mengenal pasti penanda SSR polimorfik yang berkait rapat dengan gen kerintangan karah untuk digunakan sebagai penanda yang pemilihan gen karah, untuk mengenal pasti penanda SSR polimorfik antara dua induk tersebut, dan untuk menilai prestasi agronomi waris maju padi rintang karah dari generasi  $BC_2F_3$ . Daripada 450 penanda SSR, 65 penanda polimorfik termasuk penanda berdasarkan gen *Pi* dan penanda pemulihan genom telah digunakan untuk mengenalpasti nisbah segregasi dalam 300 individu populasi padi  $BC_2F_1$  dari kacukan  $MR263 \times PS1$ . Kajian fenotip, patotip karah (*Magnaporthe oryzae*) yang paling virulen, Patotip P7.2 telah digunakan untuk pemilihan pokok  $BC_2F_1$  bagi menentukan pewarisan kerintangan karah serta perkaitannya dengan penanda SSR. Dari 65 penanda polimorfik, 16 petanda memberikan *band* yang heterozigot dalam populasi  $BC_2F_1$  tersebut. Dari 16 penanda polimorfik tersebut, hanya lapan penanda (RM5961, RM263, RM163, RM224, RM262, RM168, RM229 dan RM169) menunjukkan padanan yang baik bagi nisbah genotip dijangka (1:1) untuk model gen dominan tunggal ( $df = 1.0$ ,  $P < 0.05$ ) berdasarkan analisa khi-kuasa dua ( $\chi^2$ ). Analisis data fenotip populasi  $BC_2F_1$  juga menunjukkan padanan yang baik kepada nisbah fenotip yang dijangka (1:1; R:S) di antara pokok padi rentan dan rintang. Oleh itu, kerintangan karah Patotip P7.2 pada PS1 adalah kemungkinan besar dikawal oleh gen rintang dominan tunggal. Ini membuktikan bahawa lapan penanda tersebut adalah berkait rapat dengan gen kerintangan karah padi. Penanda tersebut boleh digunakan dalam program pemilihan bantuan-penanda untuk membangunkan varieti rintang karah dengan ketahanan yang stabil. Dari generasi

BC<sub>2</sub>F<sub>2</sub>, empat waris rintang karah (MR263-BR-3-2, MR263-BR-4-3, MR263-BR-13-1 dan MR263-BR-26-4) telah terpilih untuk pembangunan varieti rintang karah seterusnya. Waris tersebut telah dikenal pasti mengandungi gen *Pi-7 (t)*, *Pi-d (t) I*, dan *Pir2-3 (t)* dan QTL *qLN2* yang telah ditentukan oleh penanda RM5961 dan RM263 (penanda berkait-rapat kepada gen rintang karah dan QTL ). Analisa pemilihan pemuliharaan genom menggunakan 65 penanda SSR polimorfik menunjukkan pemuliharaan genom induk penerima adalah tinggi iaitu 96.0% dalam MR263-BR-4-3, 94.1% dalam MR263-BR-3-2, 89.5% dalam MR263-BR-26-4 dan 89.1% dalam MR263-BR-13-1, dan keempat-empat waris tersebut BC<sub>2</sub>F<sub>2</sub> rintang karah tersebut diswa-kacuk untuk menghasikan populasi BC<sub>2</sub>F<sub>3</sub>. Akhir sekali, 30 waris maju rintang karah dari populasi BC<sub>2</sub>F<sub>3</sub> telah dipilih untuk penilaian agro-morfologi. Daripada 30 waris rintang karah tersebut, 12 waris (Waris 12, 6, 7, 5, 21, 22, 5, 26, 11, 8, 10, 13 dan 15) telah memberikan hasil bijirin per perdu, bilangan anak padi per perdu, bilangan tangkai per perdu dan peratusan bijirin-berisi adalah setanding dengan varieti MR263. Anggaran nilai heritabiliti luas bagi populasi padi terpilih tersebut adalah tinggi (70 hingga 95%) untuk kesemua ciri, menunjukkan bahawa ciri tersebut kebolehwarisan yang tinggi kepada generasi seterusnya. Dari analisa kluster berdasarkan ciri agro-morfologi, 20 waris maju telah terkumpulkan kedalam kelompok yang sama dengan varieti MR263. Keputusan ini telah dijangkakan berdasarkan pemuliharaan genom yang tinggi (purata 96%) daripada varieti MR263 ke dalam waris maju tersebut. Dua belas waris maju padi tersebut adalah disyorkan untuk dinilai di pelbagai lokasi bagi memilih waris rintang karah serta berhasil tinggi bagi pembangunan varieti padi yang berpotensi tinggi di Malaysia.



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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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## LIST OF ABBREVIATIONS

BLD	Blast Lesion Degree
BLT	Blast Lesion Type
bp	Base pair
CTAB	Cetyltrimethylammonium bromide
$\chi^2$	Chi-square
df	Degree of Freedom
DNA	Deoxyribo Nucleic Acid
EDTA	Ethylene Diamine Tetra Acetate Acid
GA	Expected Genetic Advance
GCV	Genotypic Coefficient of Variance
MABC	Marker Assisted Backcrossing
MAS	Marker Assisted Selection
MS	Mean Square
MSE	Mean Square of Error
MSG	Mean Square of Genotypes
PCA	Principal component analysis
PCR	Polymerase Chain Reaction
PCV	Phenotypic coefficient of variance
PDA	Potato Dextrose Agar
PVP	Polyvinyl Pyrrolidone
QTL	Quantitative Trait Loci
SSR	Simple Sequence Repeat
RAPD	Randomly Polymorphic DNA
RCBD	Randomized Complete Block Design
RH	Relative Humidity
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic Acid
rmp	Rounds per Minute
TE	Tris/EDTA
UV	Ultra Violet



# CHAPTER 1

## INTRODUCTION

### 1.1 General Introduction

Rice (*Oryza sativa* L.) is one of the world's highest value crops and the second leading cereal crop after wheat. Approximately 480 million metric tons of milled rice is produced annually (Muthayya *et al.*, 2014). It is the major food source for more than half of the world's population (IRRI, 2010). Over 95% of the world's rice crop is used for human food. It is one of the principal food crops and plays a significant role in the diet of more than three billion people around the globe (Khush, 2005). It does not only provide carbohydrates but also supplies essential food elements including protein, iron, calcium, thiamine, riboflavin, niacin and vitamin E to the human body (Akinbile *et al.*, 2011). The global need for rice has been forecasted to increase by 25% from 2001 to 2025 to cope with the increasing population (Maclean *et al.*, 2002). Therefore, it is an important challenge of great importance to meet the increasing rice demand with diminishing natural resources.

Rice is also the staple food of Malaysia and the principal source of carbohydrates. Malaysia is the 25<sup>th</sup> highest volume rice producer in the world with fairly constant land area cultivated for rice (Akmbile *et al.*, 2011). The yield of rice in Malaysia is around 10 tons ha<sup>-1</sup> (Abdullah *et al.*, 2010). Still now the rice production in Malaysia has not met the national demand (Ghee-Thean *et al.*, 2012). Malaysia produces 70% of the rice it consumes. Hence, it needs to import the balance of 30% from other countries. There is therefore a vital need to increase rice production in Malaysia. The government is currently encouraging local production to obtain 100% self-sufficiency by 2020 (Rafii *et al.*, 2014). Considering above matter, research is needed to expand and to increase rice yields by preventing diseases in order to supply sufficient food for a rising population.

The rapidly increasing world population, reduction in land cultivable for rice, decreased water table, emergence of new diseases and pests in the environment, and climate change are major challenges in the future. However, increasing rice production is becoming more difficult due to biotic and abiotic stresses.

Diseases are among the most important limiting biotic factors that affect rice production. The potentially devastating economic impact resulting from disease infection has impelled worldwide efforts for the development of new rice varieties. More than 70 diseases including those resulting from bacteria, fungi, viruses and nematodes have been reported in rice (Ou, 1985). Disease management can be accomplished through chemical protection, host plant resistance, and biological control. Chemical controls are not always effective, and some are injurious to the rice plants (Mizukami and Wakimoto, 1969). Moreover, chemicals are not eco-friendly. The use of bio control agents to control blast remains to be explored in detail. Therefore, an efficacious, cost-effective

and eco-friendly blast management strategy is crucial for sustainable rice production not only for Malaysia but also for the whole world.

Among the biotic stresses, blast is the most harmful threat to high productivity of rice (Fahad *et al.*, 2014). The rice blast caused by *Magnaporthe oryzae* is the most important and potentially damaging rice disease worldwide (Ou, 1985; Latif *et al.*, 2011; Lee *et al.*, 2011). It is responsible for yield losses ranging from 35 to 50% throughout the world (Padmavathi *et al.*, 2005). The humid tropical environments in Asia are highly conducive to blast disease (Mew *et al.*, 2004). In recent years, the frequency of blast occurrence in Malaysia has increased with invasion into new areas. 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). In Malaysia, several 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. There is no recognized blast resistant rice variety with high yield in Malaysia.

MR263 is a popular rice variety in Malaysia. MR263 matures approximately 97 to 104 days after seeding, approximately seven days in advance of MR219. MR263 plants produce lower heights than MR219, approximately 59 to 71 cm compared to 71 to 84 for MR219. Low MR263 trees are usually more resistant to lodging. Even so, the nurse leaves are quite long but straight and bend slightly if they become too long. MR263 also has better head rice recovery after milling, likely due to its rice grain length, which is shorter and broader than that of MR219. Its seeds are only slightly lower in number than those of MR219. MR263 seeds can be classified as oval but not as oval as those of MR219 (3:39 length ratio from 4.12 to MR 219). This is because the seeds are shorter and wider than those of MR219. The weight of MR263 seeds was also lower than that of MR219 seeds.

MR263 is a moderately blast resistant rice variety. However, its resistance ability has gradually been reduced due to climatic changes. As a result, new pathotypes are entering the environment. This variety is now severely affected by new blast pathotypes that cause resistance loss. A severe outbreak of blast disease occurred in 2006 in Kedah and Kelantan. Yield losses were more than 60% (Malaysian rice news, TV-3) in 2006. Chemical control is practiced in mainly at the field level to control disease. Other options, particularly water management, are difficult to practice. Chemical use is discouraged to save the environment. Therefore, emphasis has been given to host plant resistance, which is an economically viable and environmentally friendly technique for disease management (Latif *et al.*, 2011).

It was suggested by Adhikari *et al.*, (1995) that using disease resistant varieties can reduce the cost of production and be an environmentally friendly approach for crop protection. Many new varieties can be developed from those rice genotypes that are the sources of various types of resistance. The use of resistant cultivars is a potential tool to decrease harmful pesticides (Wu-ming *et al.*, 2009). In Malaysia, some upland and traditional rice varieties (Anak Ikan China, Pongsu Seribu II and Biji Terong) are known to be blast resistant (Abdullah *et al.*, 1991). For the development of a blast resistant rice

variety, incorporation of resistance genes into a blast susceptible elite variety is appropriate for long-lasting resistance (Tyng *et al.*, 2010). Pongsu Seribu 1, a local Malaysian variety obtained from the Malaysian Rice Research Centre, Malaysian Agricultural Research Development Institute (MARDI), has been used as a source for blast resistance genes in a broad spectrum as well as a donor parent in marker-assisted backcrossing programmes. Pongsu Seribu 1 has been selected as a donor parent on the basis of a large portion of genetic variation for the *Oryza* genus, despite having undesirable agronomic characteristics (Xiao *et al.*, 1998; Sabu *et al.*, 2006; McCouch *et al.*, 2007).

Marker-assisted backcrossing (MABC) is one of the most promising approaches to developing blast resistant rice varieties, using molecular markers to identify and select for genes controlling the traits of interest. MABC can play a vital role in the development of resistant, high-yield or quality rice varieties by incorporating genes of interest into the elite variety MR263 that is already well adapted to farming. Recently, MABC has been widely used in plant breeding programs to develop new varieties of rice.

MABC is superior to conventional backcrossing in precision and efficiency. Background selection can greatly accelerate a backcrossing program compared to conventional backcrossing (Frisch *et al.*, 1999a). This approach has been widely used, and is likely to continue being a successful approach due to the prevalence of several rice “mega varieties” (IR64, BR11, Mashuri, Swarna, Samba Mashuri, KDML105) (Machill *et al.*, 2005).

Using marker-assisted backcrossing (MABC), it is possible to develop new varieties within a short period of time. This technique shortens the total breeding cycles. It offers great potential and a novel strategy for rice variety development and is expanding daily. It is strongly believed that this approach will be very informative and helpful for rice researchers, especially plant breeders who want to develop new varieties. Marker-assisted backcrossing provides a potential method for developing a new blast resistant rice variety. A molecular breeding approach involving MABC is a recent tool in breeding for improving blast resistance in rice (Chowdhury *et al.*, 2012). In view of the aforesaid discussion, to achieve the goal, the following objectives were pursued:

## 1.2 Research objectives

### Main objective:

To develop a high yielding blast resistant rice variety from a cross between MR263 and Pongsu Seribu 1.

### Specific objectives:

1. To introgress blast resistant genes derived from Pongsu Seribu 1 into rice variety MR263 through marker-assisted backcrossing.
2. To analyze SSR markers associated with blast in the BC<sub>2</sub>F<sub>1</sub> population.
3. To evaluate agronomic performance of advanced blast resistant rice lines in BC<sub>2</sub>F<sub>3</sub> population.

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