



UNIVERSITI PUTRA MALAYSIA

***DEVELOPMENT OF BLAST RESISTANT RICE VARIETY DERIVED FROM
CROSSING BETWEEN MR219 AND PONGSU SERIBU 2 THROUGH
MARKER-ASSISTED BACKCROSS BREEDING***

TANWEER FATAH

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By

TANWEER FATAH

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

November 2015

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DEDICATION

I dedicate this thesis to my beloved parents, wife, son, brother, his family and sister 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

DEVELOPMENT OF BLAST RESISTANT RICE VARIETY DERIVED FROM CROSSING BETWEEN MR219 AND PONGSU SERIBU 2 THROUGH MARKER-ASSISTED BACKCROSS BREEDING

By

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November 2015

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Faculty : Agriculture

Rice blast caused by fungus *Magnaporthe oryzae* is a major rice disease due to significant yield losses worldwide as well as in Malaysia. Cultivating blast-resistant rice varieties is the most effective, economical and practical approach to prevent blast disease. Marker-assisted backcross breeding contribute an effective and vital role in incorporating blast resistant genes into blast susceptible high yielding rice varieties.

In this study two blast resistant genes (putative *Pi-b* and *Pi-kh*) were identified from the Malaysian rice variety Pongsu Seribu 2 and revealed that it contain Nucleotide Binding Site-Leucine Rich Repeat (NBS-LRR) domain. The blast resistant genes (*Pi-b* and *Pi-kh*) were introgressed from Pongsu Seribu 2 variety using marker-assisted backcross breeding strategy into a high-yielding blast susceptible rice variety MR219. Therefore crosses were made between the MR219 used as recurrent parent and Pongsu Seribu 2 as donor parent to transfer blast resistant genes into MR219 without losing their actual quality and yield sustainability. Eleven SSR molecular markers linked to rice blast resistant genes were found polymorphic between the two parental varieties and used to find the potential relation with blast resistance in the present developed backcross population. The polymorphic markers were used in the further subsequent generation for confirmation of blast resistant genes. Out of the 11 markers, only 2 markers RM208 (located on chromosome 2 linked to *Pi-b* gene) and RM206 (located on chromosome 11 linked to *Pi-kh* gene) conferring blast resistance were confirmed and used in F₁, BC₁F₁, BC₂F₁ and BC₂F₂ generations providing resistance against most virulent Malaysian rice blast fungus *M. oryzae* pathotype P7.2.

300-SSR markers were screened, out of them 72 markers were found to be polymorphic between the parental lines and used for background recovery of the recurrent parent (MR219) in each backcross population. The inheritance patterns and identification of microsatellite markers linked to the rice blast resistance were observed in BC₂F₁ and BC₂F₂ generations. The recurrent parent MR219 showed susceptibility with lesion 5 to 7 score, and donor parent PS2 showed resistivity with lesion 0 to 2 while challenging to pathotype P7.2 fungus inoculum under control conditions. In

BC₂F₁ generation, 320 plants were inoculated with pathotype P7.2 and 154 plants showed the resistance mechanism while another 166 plants showed susceptible reaction to blast. Chi-square test (χ^2 for the single-gene model was applied for testing goodness of fit of observed frequencies. The two linked markers RM208 ($\chi^2 = 1.5130$; $p = 0.2188$) and RM206 ($\chi^2 = 0.6130$; $p = 0.4338$) for blast resistance to pathotype P7.2 showed good fit with expected test cross ratio (1:1) for single-gene model analysis. The markers RM208 and RM206 found suitable for marker-assisted selection of *Pi-b* and *Pi-kh* blast resistance genes conferring resistance against the blast pathotype P7.2. Phenotypically BC₂F₂ population segregated into 3:1 ratio. The genotypic segregation of the BC₂F₂ population segregated into 1:2:1 ratio. The background selection analysis for the recovery of MR219 variety among the best improved lines ranged from 73 to 94% in BC₁F₁, 79.4 to 96.1 in the BC₂F₁ and 94 to 97.5% in BC₂F₂ generations. The average proportions of the recurrent parent genome in the selected 15 improved lines of BC₂F₂ were 96.17%, explaining that very close phenotypic resemblance to the recurrent parent MR219. The 15 homozygous lines carrying blast resistant genes with similar background to MR219 were selected for the development of improved blast resistant rice variety. The agro-morphological traits of the improved lines and recurrent parent showed no significant difference between those lines. In conclusion, from the present rice breeding program, 15 homozygous advanced blast resistant rice lines were developed with a high potential to be released as a new variety for commercial cultivation.

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

**PEMBANGUNAN VARIETI PADI RINTANG KARAH MENERUSI
KACUKAN ANTARA MR219 DAN PONGSU SERIBU 2 MELALUI
PEMBIAKBAKAN KACUKBALIK BANTUAN PENANDA**

Oleh

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Karah padi adalah disebabkan oleh fungi *Magnaporthe oryzae*, merupakan satu penyakit utama padi yang mengakibatkan penurunan hasil yang ketara di seluruh dunia serta di Malaysia. Penanaman padi rintang karah merupakan pendekatan yang paling berkesan, ekonomik dan praktikal untuk mencegah penyakit karah ini. Pembiakbakaan kacukbalik bantuan penanda menunjukkan impak yang berkesan dan penting dalam memindahkan gen kerintangan karah ke varieti padi berhasil tinggi yang rentan penyakit.

Dalam kajian ini dua gen kerintangan karah (putatif *Pi-b* dan *Pi-kh*) telah dikenalpasti dari varieti padi Malaysia, Pongsu Seribu 2 dan didapati ianya mengandungi domain *Nucleotide Binding Site-Leucine Rich Repeat* (NBS-LRR). Gen kerintangan (*Pi-b* dan *Pi-kh*) telah diintrogresikan dari varieti padi Pongsu Seribu 2, dengan menggunakan strategi pembiakbakaan kacukbalik bantuan penanda, ke dalam satu varieti padi berhasil tinggi yang rentan karah, MR219. Kacukkan telah dilakukan antara MR219 sebagai induk penerima dan Pongsu Seribu 2 sebagai induk penderma untuk memindahkan gen kerintangan karah dari Pongsu Seribu 2 ke MR219 tanpa kehilangan kualiti dan prestasi hasil induk penerima. Sebelas penanda molekul SSR yang berkait rapat dengan gen kerintangan karah padi memberikan polimorfik diantara kedua induk tersebut dan telah digunakan untuk menentukan potensinya sebagai penanda untuk gen kerintangan karah dalam populasi kacukbalik yang sedang dibangunkan ini. Dari 11 penanda polimorfik tersebut, hanya dua SSR penanda iaitu RM208 (terletak pada kromosom 2 dikaitkan dengan gen *Pi-b*) dan RM206 (terletak pada kromosom 11 dikaitkan dengan gen *Pi-kh*) menunjukkan kerintangan karah telah dikenalpasti dan telah digunakan dalam generasi F_1 , BC_1F_1 , BC_2F_1 dan BC_2F_2 terhadap patotip fungi karah padi Malaysia *M. oryzae* yang virulen, P7.2.

Daripada 300-penanda SSR yang telah diuji, didapati 72 penanda polimorfik antara kedua-dua induk, dan penanda tersebut telah digunakan untuk pemulihan genom induk penerima (MR219) dalam setiap populasi kacukbalik. Pewarisan gen kerintangan dicerap dalam generasi BC_2F_1 dan BC_2F_2 . Induk penerima, MR219 menunjukkan

kerentanan dengan skor lesion 5 hingga 7, dan induk penderma, PS2 menunjukkan kerintangan dengan lesion 0 hingga 2 terhadap fungsi patotip P7.2 yang diinokulum di bawah keadaan terkawal. Dalam generasi BC₂F₁, 320 pokok telah diinokulasi dengan patotip P7.2, dan 154 pokok telah menunjukkan mekanisme kerintangan, manakala 166 pokok lagi menunjukkan reaksi rentan terhadap karah. Ujian Chi-NXDVDGXD\$QWXN model gen tunggal telah dijalankan ujian padanan yang baik dengan frekuensi yang GLFHUDES 'XD SHQDQGDWHUVHEXW50\$\$ GDQ50\$ (0,6130; p = 0.4338) untuk kerintangan karah bagi patotip P7.2 menunjukkan padanan yang tepat dengan nisbah dijangka (1:1) untuk analisis model gen tunggal. Kedua-dua penanda tersebut didapati sesuai untuk pemilihan bantuan penanda kerintangan gen karah, *Pi-b* dan *Pi-*kh** yang memberikan rintangan terhadap patotip P7.2. Secara fenotipik populasi BC₂F₂ memberikan kadar segregasi 3:1 terhadap karah patotip P7.2. Segregasi genotip populasi BC₂F₂ adalah dengan nisbah 1:2:1. Analisis pemulihan genom bagi varieti MR219 dikalangan titisan maju terbaik adalah dari 73 hingga 94% dalam generasi BC₁F₁, 79.4 hingga 96.1% dalam BC₂F₁ dan 94 hingga 97.5% dalam BC₂F₂. Purata peratusan genom induk penerima dalam 15 titisan maju terpilih adalah 96.17%, yang menunjukkan persamaan fenotip sangat rapat dengan induk penerima, MR219. Lima belas titisan homozaigus yang mempunyai gen kerintangan serta dengan kesamaan genom MR219 yang tinggi telah dipilih untuk pembangunan varieti padi yang rintang penyakit karah. Ciri agro-morfologi diantara titisan maju tersebut dengan induk penerima MR219 menunjukkan tidak terdapat perbezaan yang bererti. Kesimpulannya, dari program pembiakbakaan padi ini, 15 titisan maju homozaigus rintang karah telah dihasilkan dan ianya sangat berpotensi untuk menghasilkan varieti padi baru bagi penanaman secara komersial.

I certify that a Thesis Examination Committee has met on 17 November 2015 to conduct the final examination of Tanweer Fatah on his thesis entitled "Development of Blast Resistant Rice Variety Derived from Crossing between MR219 and Pongsu Seribu 2 Through Marker-Assisted Backcross Breeding" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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LIST OF ABBREVIATIONS

QTL	Quantitative trait loci
DNA	Deoxyribonucleic acid
MAS	Marker-assisted selection
MABB	Marker-assisted backcross breeding
MABC	Marker-assisted backcrossing
SSR	Simple sequence repeat
MT	Million tons
Mb	Mega base
NBS-LRR	Nucleotide binding site- Leucine rich repeat
PCR	Polymerase chain reaction
BAC	Bacterial artificial chromosome
YAC	Yeast artificial chromosome
AFLP	Amplified fragment length polymorphism
STS	Sequence tag site
RAPD	Randomly Amplified Polymorphic DNA
SNP	Single nucleotide polymorphism
RIL	Recombinant inbred line
CTAB	Cetyltrimethylammonium bromide
EDTA	Ethylenediamine Tetraacetic Acid
dNTP	Deoxynucleotide triphosphate
UV	Ultra violet
LB	Lysogeny broth
SOB	Super Optimal Broth
rpm	Revolutions per minute
Tris	tris(hydroxymethyl)aminomethane
DTT	Dithiothreitol
ATP	adenosine-triphosphate
PEG	polyethylene glycol
Taq	Thermus aquaticus
RGA	Resistance gene analogue
EST	Expressed sequence tag
RPG	Recurrent parent genome
RIL	Recombinant inbred line
RH	Relative humidity
SMA	Single marker analysis
SES	Standard evaluation score
TE	Tris/EDTA
V	volt
v/v	Volume/volume
w/v	Weight/volume
- ME	-mercaptoethanol
ddH ₂ O	Double distilled water
BLD	Blast lesion degree
BLT	Blast lesion type
%DLA	Percent diseased leaf area

CHAPTER 1

INTRODUCTION

1.1 General introduction

Rice (*Oryza sativa* L.) is the most widely consumed staple dietary food and largest cereal crops throughout the world, especially in Asia (Khush, 2005). Rice is not only used for human consumption, but it is also used for animal feeding and provides the major source of income for rural people (Datta, 2004). Rice provides nutrition for six billion people worldwide (Maclean *et al.*, 2002). Considering its nutritional value, along with carbohydrates rice also provides other essential nutrients such as protein, iron, calcium, thiamine, riboflavin, niacin and vitamin E, to the human body (Akinbile *et al.*, 2011).

In Malaysia, rice is mostly grown in eight granary areas of Peninsular Malaysia, Sabah and Sarawak, mainly under flooded conditions (Akinbile *et al.*, 2011). Malaysia is ranked 24th in terms of rice production in the world with annual production of 2.750 million tons (FAO, 2014). Total area under rice cultivation in Malaysia in 2014 was 692,340 ha, out of which 43,353 ha are situated in Sabah and 121,921 in Sarawak (DOA, 2014). Presently, Muda Agricultural Development Authority (MADA) and Kemudu Agriculture Development Authority (KADA) are two main rice growing areas in Malaysia. At present Malaysia in term of rice production is not self-sufficient. The main factor in limiting the rice production includes various biotic and abiotic stresses.

Among biotic stresses, diseases are one of the main factor that reduces rice production. The only best way to minimize the yield losses is the production of high-yielding resistant rice varieties. Blast caused by the fungal pathogen *Magnaporthe oryzae* is one of the most serious diseases because the yield loss caused by blast range from 1 to 50 % worldwide including Malaysia. On the other hand Malaysia has targeted to achieve 80% rice-sufficiency by 2020 (FAO, 2014). There is an urgent need to expand research in rice in order to assure rice sufficiency by controlling disease and increasing yield potential. Recently, several techniques have been adopted to control the blast disease, including chemical control, disease forecasting, cultivation practice, but these control measures are not very effective (Srinivasachary *et al.*, 2002). The recent advent of DNA marker technology, gene cloning, development of numerous markers, molecular breeding approaches helped plant breeders to produce rice varieties with blast resistance and higher yield potential. The cloning and characterization of blast resistant genes facilitated the exploration of mechanism of host-pathogen interaction. DNA markers also acted as a valuable resource to localize the genes/QTL controlling important traits such as plant disease resistance (Yano and Sasaki, 1997; Paran and Zamir, 2003). Currently SSR markers are widely used to screen the rice population for blast resistance. Several SSR markers tightly linked to blast resistant genes have been identified; therefore tagging of blast resistant genes until several generations are possible (Miah *et al.*, 2013a). Furthermore the application of marker-assisted selection

(MAS) and marker-assisted backcross breeding (MABB) could greatly improve the potential of modern rice varieties through introgression of novel resistant genes.

1.2 Significance of the study

Achieving stable resistance to blast is perhaps the most important goal in managing blast disease. Plant breeding has been very successful in improving blast susceptible varieties using marker-assisted selection (Zhao *et al.*, 2010). In Malaysia several upland and traditional rice varieties are available conferring strong resistance against the blast disease. Among them Pongsu Seribu 2 is one of blast resistant rice variety conferring broad spectrum resistance against the different blast pathotypes. Another Malaysia rice variety MR219 remained very popular among farmers for a long time because of high yield, long grain and good eating quality (Alias, 2002). At present MR219 is highly susceptible to blast disease. MR219 rice cultivar can be improved by the introduction of blast resistant genes from Pongsu Seribu 2 variety. Considering the above discussed advancement of marker-assisted selection the aim of this research was to identify the blast resistant genes from Pongsu Seribu 2 and tightly linked SSR marker associated with blast resistance against the most virulent pathotype P7.2 of *M. oryzae*. In the present study marker-assisted backcross breeding approach was also used to incorporate blast resistant genes into MR219 rice variety. The introgression of blast resistant genes in MR219 will retain its high yield and will be widely adopted again by local rice farmers of Malaysia.

1.3 Problem statement

In Malaysia, rice blast has become noticeable disease due to occurrence in main and off-season across the rice growing regions. Several outbreaks occur in different areas of peninsular Malaysia and about 3000 farmers lost RM5 million (DOA, 2014). The major outbreak was in the year of 2006 at KADA, Pasir Puteh, and Kelantan where 60% of 4,000 ha land cultivated with rice was affected due to blast disease and caused heavy economic loss (Ashkani *et al.*, 2011). In the recent years the frequency of blast occurrence has increased with invasion into new areas. Moreover, there is not cultivated blast resistant rice variety in Malaysia. Research for developing highly blast resistant rice varieties are urgently needed to reduce the yield losses and supporting the poor farmers by combating blast disease.

1.4 Research objectives

The main objective of the study was to develop blast resistant, high yielding rice variety suitable to local environments through marker-assisted backcrossing. Accordingly, the specific objectives were:

1. To clone and sequence the blast resistant genes from resistant rice variety Pongsu Seribu 2.

2. To identify suitable segregating SSR markers for blast resistance using inheritance and disease reaction analysis in BC₂F₁ population.
3. To estimate the recovery of recurrent parent genome (MR219) contribution in marker-assisted backcross populations for introgression of blast resistant genes.
4. To evaluate, verify and select the advanced blast resistant lines from BC₂F₂ population.



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