MARKER-ASSISTED SELECTION FOR BACTERIAL LEAF BLIGHT RESISTANCE IN MR219 × IRBB60 RICE (Oryza Sativa L.) VARIETY

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By

ZAKIAH BINTI MOHD ZUKI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

December 2018

DEDICATION

THIS THESIS IS SPECIALLY DEDICATED

TO

MY LATE GRANDMA,

MY PARENTS,

AND

BELOVED FAMILY

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

MARKER-ASSISTED SELECTION FOR BACTERIAL LEAF BLIGHT RESISTANCE IN MR219 × IRBB60 RICE (*Oryza Sativa* L.) VARIETY

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December 2018

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

Rice is one of the most important food sources for more than half the world population, which its yield constantly affected by more than 70 diseases. Bacterial leaf blight (BLB) is a major problem in rice production across the world including Malaysia. One of the main reasons was lack of BLB resistant varieties were planted for commercial cultivation in Malaysia. Marker-assisted selection (MAS) has been proven to be useful in breeding and development of variety resistance in rice. Therefore, the main objective of this study was to develop BLB resistance rice lines using simple sequence repeat (SSR) markers-assisted selection between resistant BLB rice line (IRBB60) and high yielding but susceptible variety (MR219). The specific objectives were to determine highly BLB resistant near isogenic lines for crossing program, to identify SSR markers associated with BLB resistance R-gene, to quantify phenotypic and genotypic inheritance resistance pattern of the R-gene in F_2 rice population and to select BLB resistant lines in the F_2 population. From nine near isogenic BLB resistant lines, IRBB60 was the most resistance to BLB disease in local environment. This line was used to cross with high yielding local variety, MR219 for development of BLB resistant variety. For genotyping segregation analyses, a total of 129 SSR markers were tested on parental lines MR219 × IRBB60 for BLB resistance, and only 42 (32%) SSR primers show distinct polymorphism. Out of the 42 polymorphic markers, the best 18 including R-gene based markers were used to screen on 345 F_2 progenies for resistance to bacterial leaf blight. The patterns of all the 15 markers were similar with segregation ratio is 1:2:1 except the others 3 markers (RM400, RM264 and RM281). The chi-square analysis (χ^2) confirmed that the genotypic ratio shows a good fit to expected Mendelian segregation ratio (1:2:1) for 15 SSR markers, RM13 *(xa5)*, RM21 *(Xa21)*, RM122 *(xa5)*, RM153 *(xa5)*, RM164 *(Xa13)*, RM206 *(Xa10)*, RM5509, RM20B, RM25, RM163, RM169, RM218, RM267, RM276, and RM334, for a single gene model (d.f. =2.0, $p \leq 0.05$). For phenotypic ratio, the F_2 population segregated in a 3:1 (R:S) for resistant and susceptible plants, respectively. This indicated that resistance to bacterial leaf blight caused by pathotype *Xoo* $P_{7.0}$ in the MR219 × IRBB60 F_2 population is controlled by single dominant genes. Seventy-five highly resistant lines derived from MR219 × IRBB60 consist of four BLB R-genes (*xa5*, *Xa10*, *Xa13 and Xa21*) in the homozygous form were identified for further evaluation.

The result indicated that a significant finding of this rice breeding program using MAS for development of high yield with durable bacterial leaf blight resistance in Malaysia.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

PEMILIHAN BANTUAN PENANDA MOLEKUL TERHADAP KERINTANGAN PENYAKIT HAWAR DAUN BAKTERIA BAGI VARIETI PADI (*Oryza sativa* L.) MR219 × IRBB60

Oleh

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Padi merupakan salah satu sumber makanan yang paling penting bagi lebih separuh daripada penduduk dunia, dimana hasilnya sentiasa dipengaruhi oleh lebih daripada 70 jenis penyakit. Penyakit hawar daun bakteria (BLB) masih merupakan masalah utama dalam pengeluaran padi di seluruh dunia termasuk Malaysia. Salah satu sebab utama adalah kekurangan varieti yang rintang terhadap penyakit BLB yang ditanam bagi penanaman secara komersial di Malaysia. Kaedah pemilihan bantuan penanda molekul (MAS) telah terbukti berguna dalam pembiakbakaan dan pembangunan pokok padi yang rintang terhadap pelbagai penyakit padi. Oleh yang demikian, objektif utama kajian ini adalah untuk menghasilkan titisan padi yang rintang terhadap penyakit BLB dengan menggunakan pemilihan bantuan penanda jujukan mudah berulang (SSR) di antara titisan padi yang rintang BLB (IRBB60) dengan varieti yang mempunyai hasil yang tinggi tetapi rentan penyakit (MR219). Objektif spesifik adalah untuk menentukan titisan isogenik yang mempunyai kerintangan yang tinggi terhadap BLB untuk program kacukan, untuk mengenalpasti penanda SSR yang berhubungkait dengan rintangan R-gen BLB, untuk menganggarkan corak fenotip dan genotip perwarisan rintangan daripada R-gen dalam populasi padi F_2, dan untuk memilih titisan yang rintang terhadap BLB dalam populasi F_2. Daripada sembilan titisan isogenik yang rintang terhadap BLB, IRBB60 adalah yang paling rintang terhadap penyakit BLB dalam persekitaran setempat. Titisan ini telah digunakan untuk kacukan dengan varieti tempatan MR219 yang mempunyai hasil yang tinggi bagi menghasilkan varieti yang rintang penyakit BLB. Bagi analisa segregasi genotip, sejumlah 129 penanda SSR telah diuji ke atas titisan-titisan induk MR219 × IRBB60 terhadap kerintangan BLB, dan hanya 42 (32%) penanda SSR menunjukkan perbezaan polimorfism yang ketara. Daripada 42 penanda polimorfik tersebut, 18 penanda yang terbaik termasuk penanda berasaskan R-gen telah digunakan untuk memilih 345 progeni F_2 yang rintang terhadap penyakit hawar daun bakteria. Corak bagi kesemua 15 penanda adalah sama iaitu menunjukkan nisbah segregasi 1:2:1 kecuali bagi 3 penanda yang lain (RM400, RM264 dan RM281). Analisa khi-kuasa dua (χ^2) ke atas nisbah genotip menunjukkan padanan yang sangat baik mengikut nisbah

segregasi Mendel dijangka (1:2:1) bagi model gen tunggal (df=2.0, $p \leq 0.05$) untuk semua 15 penanda SSR, iaitu RM13 (*xa5*), RM21 (*Xa21*), RM122 (*xa5*), RM153 (*xa5*), RM164 (*Xa13*), RM206 (*Xa10*), RM5509, RM20B, RM25, RM163, RM169, RM218, RM267, RM276, dan RM334. Bagi nisbah fenotip pula, populasi F_2 tersebut telah bersegregasi dengan nisbah 3:1 (R:S) masing-masing ke atas pokok yang rintang dan rentan. Ini membuktikan bahawa kerintangan di dalam MR219 × IRBB60 populasi F_2 terhadap penyakit hawar daun bakteria yang diakibatkan oleh patogen *Xoo* $P_{7.0}$ adalah dikawal oleh gen dominan tunggal. Tujuh puluh lima titisan sangat rintang yang dihasilkan dari kacukan MR219 × IRBB60 yang mempunyai empat R-gen BLB (*xa5, Xa10, Xa13* dan *Xa21*) homozigot telah dikenalpasti untuk penilaian selanjutnya. Keputusan kajian ini memberikan penemuan yang sangat signifikan bagi program pembiakbakaan padi ini yang menggunakan MAS bagi pembangunan varieti yang berhasil tinggi serta rintang terhadap penyakit hawar daun bakteria di Malaysia.

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This thesis was submitted to the senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows;

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	Percentage
<	Less than
>	More than
±	Plus minus
µl	Micro liter
^{O}C	Degree celcious
σ^2e	Variance error
σ^2g	Variance genetic
σ^2p	Variance phenotypic
χ^2	Chi-square analyses
ALFP	Amplified Fragment Length Polymorphism
ANOVA	Analysis of variance
BLB	Bacterial leaf blight
BLS	Barat Laut Selangor Integrated Agricultural Development Area
bp	Base pair
Chr	Chromosome
CL	Clearfield
cm	Centimeter
CTAB	Cetyltrimethylammonium bromide
CV	Coefficient of variation
DAN	Dasar Agromakanan Negara
DLA	Disease leaf area
DMT	Day to maturity
DNA	Deoxyribonucleic acid
dNTP	2'- Deoxynucleoside 5'- triphosphate
DAS	Days after sowing
DOA	Department of Agriculture
DOS	Department of Statistics
EDTA	Ethylenediamine tetra-acetic acid
F_1	First generation
F_2	Second generation
F_3	Third generation
FG	Filled grain per panicle
FM	Functional marker
g	Gram
$_g$	Number of progeny
GA	Genetic advance
GCV	Genotypic coefficient of variation
GFSI	Global Food Security Index
gwt	Grain weight per panicle
H	Segregant / heterozygous

H^2_B	Broadsense heritability
ha	Hectare
HD	50% heading days
HS	Highly susceptible
IADA	Integrated Agricultural Development Area
IRRI	International Rice Research Institute
ISSR	Inter Simple sequence repeat
KADA	Kemubu Agricultural Development Authority
KERIAN	Kerian Integrated Agricultural Development Area
KETARA	Northern Terengganu Integrated Agricultural Development Area
kg	Kilogram
L	Ladder
LL	Lesion length
LSD	Least significant different
m	Meter
M	Molar
MADA	Muda Agricultural Development Authority
MARDI	Malaysian Agriculture Research and Development Institute
MAS	Marker assisted selection
mg	Miligram
MgCl	Magnesium chloride
min	Minute
ml	Mililiter
mM	Milimolar
MOA	Ministry of Agriculture and Agro-based Industry
MR	Moderate resistance
MS	Moderate susceptible
MSE	Mean square error
MSG	Mean square progeny
NaCl	Sodium chloride
ng	Nanogram
NIL	Near isogenic line
no	Number
O	*Oryza*
p	Phenotypic
PCR	Polymerase chain reaction
PCV	Phenotypic coefficient of variation
PH	Panicle per hill
PHT	Plant height
pH	Potential of hydrogen
PL	Panicle length
PROC CORR	Phenotypic correlation coefficients
QTL	Quantitative trait loci
R	Resistant

RAPD	Random Amplification Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RR	Homozygous resistant plants
Rr	Heterozygous plants
rr	Homozygous susceptible plants
r	Number of replications
RCBD	Randomized complete block design
REP	Repetitive extragenic palindromic
RM	Rice microsatelite
RNAse	Ribonuclease
rpm	Round per minutes
S	Susceptible
s	Second
SAS	Statistical analysis system
SD	Standard deviation
SE	Standard error
SES	Standard evaluation system
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeat
SSLP	Simple Sequence Length Polymorphism
STR	Short Tandem Repeat
TBE	Tris-borate-EDTA
TE	Tris-EDTA
Tris-HCL	Tris (trisaminomethane) hydrochloride
TSP	Total spikelet per panicle
USDA	United States Department of Agriculture
UV	Ultra violet
V	Voltant
×	Crossing
Xoo	*Xanthomonas oryzae* pv. *oryzae*
YH	Yield per hill

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

Rice is a staple food feeding almost one third of the world's population including Malaysia. Rice is considered as one of the source food crops and plays a substantial role in the nutrition (Khan *et al.,* 2015). It shows the second position after wheat in term of consumer and producer in Asia (Rajamoorthy *et al.,* 2015).

Malaysia's expected total rice planted area is 730,016 ha and eleven granary areas in Peninsular Malaysia that covering an area about 558,074 ha released by Department of Agriculture, Malaysia for planted areas in 2015 (GAIN, 2017). Rice production has increased in 2014 and 2015 to 2.849 million metric tonnes and 3.322 million metric tonnes, respectively compared with 2.604 million metric tonnes in 2013. Similar trend also observed for milled rice production, 1.677 million metric tonnes in 2013 to 1.835 and 2.141 million metric tonnes in 2014 and 2015, respectively (DOS, 2016). This increase will help to achieve self-sufficiency level to 86% to meet the country's food security policy by 2020 (MARDI, 2016). Malaysia was ranked 35th out of 113 countries worldwide in terms of food security (GFSI, 2016). Despite this, Malaysia still imports rice from neighboring countries (Thailand, Vietnam and Cambodia) to meet up domestic rice demand (Agrofood Statistics, 2015).

Rice production is constantly affected by more than 70 pests and diseases (Ou, 1985). Among them, bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae (Xoo)* is a serious vascular disease accounting for yield loss of 20 – 30% (Ou, 1985), more seriously at 80% (Singh *et al.,* 1997) and almost 100% (Zhai and Zhu, 1999). According to Khush and Virmani (1985), Mew (1987), and Mew *et al.* (1993), bacterial leaf blight occurs as vascular wilt at the early stages of crop development (seedling to tillering stage) and at later stages as leaf blight (panicle initiation to flowering stage).

Research on bacterial blight of rice begins in Japan in early ninety centuries, and the efforts were focused mainly on ecological studies and chemical control (Ou, 1985). Since then, significant gains have been made in understanding BLB through analysis of the interactions between *Xoo* and rice at different growth stages. This study has been carried out on the epidemiology, population biology, physiology, cell biology, biochemistry, and molecular genetics of the host pathogen interaction.

The recent outbreak in Feb 2014 was reported from Padang Besar, Perlis about 60,000 metric tonnes were affected (Utusan, 2014) and become more serious 50 - 70% of yield losses in Sabak Bernam (The Star, 2016). Various strategies were conducted over the years worldwide including Malaysia to control this disease. Controlling BLB pathogen is very difficult because chemical application is not very effective. Therefore, the development of resistant varieties is the economical, effective and eco-friendly strategy, for controlling this endemic pathogen to minimize disease incidence and yield losses (Jagjeet *et al.,* 2011; Ogawa, 1993).

Marker-assisted selection (MAS) is increasingly popular as an efficient method in rice breeding program. Different types of molecular markers are used in MAS for detecting the rice diseases like BLB. More than 32 bacterial blight resistance genes in a series from *Xa1* to *Xa34* has been discovered, identified and designated as reported by Ram *et al.* (2010). According to Debabrata *et al*. (2008), pathotype analysis and studies of DNA fingerprinting has indicated a significant diversity in the *Xoo* populations in other rice-growing countries.

MAS using simple sequence repeat (SSR) marker was selected as molecular markers for the present study, due to co-dominant nature of SSR which can detect both hetero- and homozygous alleles. Other than that, SSR markers show high level of polymorphism (Ishii *et al.*, 2001; He *et al.*, 2003). According to Rahman *et al.* (2009), SSR markers can be greatly and efficiently applied for developing unique DNA profiles of rice genotypes because having high level of polymorphism and greater information.

Bacterial leaf blight is a major problem in rice production in Malaysia, and no resistant variety is available for commercial cultivation. Therefore, the main objective of this study was to develop BLB resistant rice lines through marker-assisted selection for future rice breeding programs.

The specific objectives were:

1. To identify highly BLB resistant near isogenic lines (NILs) for crossing program.
2. To identify SSR markers associated with BLB resistance R-gene from crosses between resistant parental line and susceptible MR219 rice variety.
3. To quantify phenotypic and genotypic inheritance resistance of *Xoo* in F_2 rice population.
4. To identify BLB resistant lines conferring resistance to *Xoo* in the F_2 population.

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