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MORPHO-PHYSIOLOGICAL AND MOLECULAR CHARACTERIZATION OF UPLAND RICE GENOTYPES FOR BLAST DISEASE RESISTANCE

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By

TUHINA KHATUN

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

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DEDICATION

In the name of Allah STW, the most gracious, the most merciful

To the departed soul of my beloved father, who always encouraged and wished for my higher education

To my loving mother, who makes sacrifices and does a lot to make me happy

To my younger brothers Tarek and Muaz, and my younger sister Tania who always make me delighted and cheerful

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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Chairman : Professor Mohamed Hanafi Musa, PhD
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Upland rice is a valuable base population for protecting against genetic erosion and broadening the gene pool of rice germplasm. Blast caused by the fungus *Magnaporthe oryzae* is the most impactful rice disease, and it can be severe in upland/dry land cultures. This study aimed to identify and characterize upland rice germplasm for blast disease resistance. Initially, 50 upland rice genotypes, including one resistant check, Pongsu Seribu-1, and one susceptible check, MR219, were evaluated to identify new sources of resistance and assess diversity based on reactions to *M. oryzae*. Resistant reactions were observed in the genotypes Biaw Bood Pae, Blau Noc, Chirikata 2, IPPA, IR 5533-50-1-10, IR 5533-55-1- 11, Ja Hau, Ja No Naq, BR26, BRRI dhan42 and BRRI dhan43. The 50 genotypes were grouped into five clusters based on the greater similarities of their reactions to the blast fungus. The morphological, physiological and yield trait characteristics of 27 globally diverse blast-resistant genotypes were further evaluated. Additionally, the genotypes were analyzed to determine the correlations between and diversity among these characteristics. The BRRI dhan43, C, Choke Tang and Chirikata 2 were identified as early-maturing genotypes that provided the best yields. The largest heritability coupled with a high genetic advancement was recorded for a number of filled grains/panicle and yield/plant, demonstrating that these traits can be successfully transferred to offspring if selection of these characteristics is performed *via* a hybridization program. The 27 genotypes were grouped into six clusters based on the contributing morphological, physiological and yield characteristics. The blast-resistant, early-maturing, high-yield genotype BRRI dhan43 was then finally characterized to determine the molecular mechanism underlying the plant-microbe interaction using next generation sequencing (NGS). The reference-based alignment produced total reads of 66.9 and 66.2 million in the uninoculated and inoculated plant samples, respectively. This study showed a large number of SNPs with the value of more than 95 and 88 thousand for healthy and diseased samples, respectively. A total of 2,733 differentially expressed genes (DEGs) were identified from *Oryza sativa* cv BRRI dhan43 and *M. oryzae* race P7.2 interactions, of which 43% up-regulated and 57% down-regulated, About 9.9% of genes involved in incompatible interaction between BRRI dhan43 and fungus race P7.2 pathosystem. The resistant cultivar, BRRI dhan43

responded upon colonization of virulent fungus P7.2, inducing both pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) and effector-triggered immunity (ETI). Although the functions yet to be known, based on the results of transcriptome analysis, two novel race-specific elicitors AVR4 and AVR9 in virulent race P7.2, and three resistant proteins MLA10, L6 and RPS in rice cultivar BRRI dhan43 were reported for the first time during host-pathogen interaction.

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

PENCIRIAN MORFO-FISIOLOGI DAN MOLEKULAR GENOTIP PADI HUMA UNTUK RESISTEN PENYAKIT KARAH

Oleh

TUHINA KHATUN

Ogos 2016

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Padi huma ialah satu populasi asas berharga untuk dipelihara daripada kepupusan genetik dan memperluaskan kumpulan gen germplasms padi. Penyakit karah disebabkan oleh kulat *Magnaporthe oryzae* adalah penyakit padi yang paling ketara, dan ia boleh menjadi teruk dalam keadaan tanah kering/tanah tinggi. Kajian ini bertujuan untuk mengenal pasti dan mencirikan germplasms padi huma bagi resisten kepada penyakit karah. Pada mulanya, 50 genotip padi huma, termasuk satu cek resisten, ‘Pongsu Seribu-1’ dan satu cek mudah peka, ‘MR219’, telah dinilai untuk mengenal pasti sumber-sumber baharu yang resisten dan menilai kepelbagaian berdasarkan reaksi kepada *M. oryzae*. Reaksi resisten diperhatikan dalam genotip ‘Biaw Bood Pae’, ‘Blau Noc’, ‘Chirikata 2’, ‘IPPA’, ‘IR 5533-50-1-10’, ‘IR 5533-55-1- 11’, ‘Ja Hau’, ‘Ja No Na’, ‘BR 26’, ‘BRRI dhan42’ dan ‘BRRI Dhan43’. Lima puluh genotip telah dikumpulkan ke dalam lima kelompok berdasarkan persamaan daripada tindak balas mereka kepada kulat karah. Morfologi, ciri-ciri sifat fisiologi dan hasil 27 genotip rentan karah pelbagai peringkat global telah dinilai. Selain itu, genotip dianalisis untuk menentukan hubungan dan kepelbagaian di kalangan ciri-ciri ini. ‘BRRI dhan43’, ‘C’, ‘Choke Tang’ dan ‘Chirikata 2’ telah dikenal pasti sebagai genotip awal-matang yang memberikan hasil yang terbaik. Kombinasi warisan dan kemajuan genetik yang tinggi dicatatkan bagi ciri-ciri bijirin berisi/panikel dan hasil/pokok, menunjukkan bahawa sifat-sifat ini boleh berjaya dipindahkan kepada anak-anak jika pemilihan ciri-ciri ini dilakukan melalui program penghibridan. Sebanyak 27 genotip telah dikumpulkan kepada enam kelompok berdasarkan ciri-ciri morfologi, fisiologi dan hasil. Rentan karah, awal-matang, penghasilan tinggi genotip ‘BRRI dhan43’ kemudiannya dicirikan untuk menentukan mekanisme molekul yang menentukan interaksi tumbuhan mikrob dengan menggunakan penjujukan generasi akan datang (NGS). Hasil jajaran mencatatkan jumlah bacaan sebanyak masing-masing 66.9 dan 66.2 juta untuk kawalan dan rawatan tumbuh-tumbuhan. Kajian ini menunjukkan bilangan SNPs yang besar, dengan nilai masng-masing 95 dan 88 ribu bagi kawalan dan sampel dirawat. Sebanyak 2,733 gen terzahir (DEGs) telah dikenal pasti daripada interaksi ‘BRRI dhan43’-patogen, yang mana 43% gen kawal selia huluan dan 57% gen ke bawah. Sebanyak 9.9% gen terlibat dalam interaksi yang tidak serasi antara BRRI dhan43 dan bangsa kulat P7.2 pathosystem. Hasil penjajaran getir interaksi kulat P7.2 dengan kultivar resisten, BRRI dhan43 memberikan kedua-dua corak molekul patogen-berkaitan (PAMP) kekebalan -triggered (PTI) dan imuniti effector-dicetuskan (ETI). Walaupun fungsi belum diketahui, berdasarkan keputusan analisis transcriptome, dua elisitors novel perlumbaan khusus *AVR4* dan *AVR9* dalam getir perlumbaan P7.2, dan tiga proteins tahan MLA10, L6 dan RPS dalam kultivar padi BRRI dhan43 dilaporkan buat kali pertama semasa interaksi host-pathogen.

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I certify that a Thesis Examination Committee has met on 10 August 2016 to conduct the final examination of Tuhina Khatun on her thesis entitled "Morpho-Physiological and Molecular Characterization of Upland Rice Genotypes for Blast Disease Resistance" 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

ANOVA	Analysis of variance
AS	Alternative splicing
BGI	Beijing genomics institute
BLAST	Basic local alignment search tool
BLT	Blast lesion type
bp	Base pair
BRRI	Bangladesh Rice Research Institute
CALM	Calmodulin
CDPK/CPK	Calcium-dependent protein kinase
cDNA	Complementary DNA
CC67	Chlorophyll content at 67 days after transplanting
CC97	Chlorophyll content at 97 days after transplanting
CPC	Coding potential calculator
DAI	Days after inoculation
DAT	Days after transplantation
DEGs	Differentially expressed genes
DF	Days to flowering
Df	Degrees of freedom
DGE	Digital gene expression profiling
DLA	Diseased leaf area
DM	Days to maturity
DNA	Deoxyribonucleic acid
DOA	Department of Agriculture
ds	Double stranded

EM	Expectation-maximization
ESTs	Expressed sequence tags
ET	Number of effective tillers/plant
ETI	Effector-triggered immunity
EtOH	Ethyl alcohol
FG	Number of filled grains/panicle
FL	Flag leaf length
GA	Genetic advance
GATK	Genome analysis toolkit
GB	Grain breadth
GCV	Genotypic coefficient of variance
GL	Grain length
GO	Gene ontology
h^2_B	Heritability in broad sense
HCl	Hydrochloric acid
HI	Harvest index
HR	Hypersensitive response
HYV	High yielding variety
Indel	Small insertions and deletions
IRGSP	International rice genome sequencing project
IRRI	International Rice Research Institute
JA	Jasmonic acid
LAI	Leaf area index
L/B	Grain length/breadth ratio
LSD	Least significant difference

KADA	Kemubu Agricultural and Development Authority
Kb	Kilobase
KEGG	Kyoto encyclopedia of genes and genomes
MAPK	Mitogen-activated protein kinase
MARDI	Malaysian Agricultural Research and Development Institute
Mb	Megabase
MR	Moderately resistant
MS	Moderately susceptible
mRNA	Messenger RNA
MSE	Mean square of error
MSG	Mean square of accessions
NCBI	National center for biotechnology information
ng	Nanogram
NGS	Next generation sequencing
PAMP	Pathogen-associated molecular pattern
PCA	Principal component analysis
PCR	Polymerase chain reaction
PCV	Phenotypic coefficient of variance
PDA	Potato dextrose agar
PE	Paired-end
PH	Plant height
PL	Panicle length
PR	Pathogenesis related
PRRs	Pathogen-recognition receptors
PS-1	Pongsu seribu-1

PTI	PAMP-triggered immunity
QC	Quality control
RBOH	Respiratory burst oxidase
RCBD	Randomized complete block design
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RSEM	RNA seq by expectation maximization
SA-PMPs	Streptavidin magnesphere® paramagnetic particles
SES	Standard evaluation system
SNARE	Soluble N-ethylmaleimide-sensitive factor attachment protein receptor
SNP	Single nucleotide polymorphism
TMRI	Torrey Mesa Research Institute
TT	Total number of tillers/plant
UFG	Number of unfilled grains/panicle
UPGMA	Unpaired group method of arithmetic mean
UPM	Universiti Putra Malaysia
Y	Yield/plant
℃	Degree centigrade
%DLA	Percentage of diseased leaf area
σ^2e	Mean squares of error
σ^2g	Genotypic variance
σ^2p	Phenotypic variance
100 GW	100-grain weight

CHAPTER 1

INTRODUCTION

1.1 Background

Rice (*Oryza sativa* L.) is the most widely consumed grain in the world, playing a vital role in combating global hunger, aiding food security, alleviating poverty and promoting socioeconomic development in most Asian countries (Pranab, 2008). Global rice consumption remains strong, as it is driven by both population and economic growth, especially in Asian and African countries (Ricepedia, 2015). In most of the developing world, rice availability is associated with food security and political stability (Ricepedia, 2015). Rice is the third most significant crop in Malaysia in terms of production, following oil palm and rubber (Karim et al., 2004). Malaysian rice production currently accounts for 72% of domestic consumption, with a goal of reaching 90% over the next 10 years (Isiaka et al., 2015). Rice in Malaysia is generally cultivated as wet paddy (Peninsular Malaysia, 503,184 ha) or upland rice (Sabah and Sarawak, 165,888 ha) per the DOA (2005).

Global agricultural production increased dramatically as a result of the "Green Revolution", which was introduced in Mexico by Norman E. Borlaug between 1950 and 1970 due to technological innovations. These advancements include producing high-yielding crop varieties, as well as using chemical fertilizers, synthetic pesticides and herbicides to control weeds, kill insect pests and prevent diseases. The Green Revolution involved a concerted effort to address the lack of food self-sufficiency (David, 1997). However, the Green Revolution exaggerated both agricultural biodiversity and wild biodiversity (Kilusang, 2007), which extensively affected the environment. In addition, the movement involved the breakdown of both pathogen and insect resistant genes, which cannot be controlled by artificial pesticides. Valuable genetic resources were also permanently lost. The utilization of underground water for irrigating HYV's resulted in water shortages and droughts. In these cases, upland rice provides the best alternative for cultivation because it relies on rain-fed conditions without the use of irrigation water or chemical inputs.

The world will be faced with global rice production challenges in the coming years. Upland rice cultivation offers a promising production technique with minimal negative environmental effects and highly predictable yields (Dzido et al., 2004). Upland rice refers to rice cultures that are grown on undulated land areas with well-drained soils. The rice completely depends on rainfall for its water requirements (Fageria et al., 2011). Approximately 100 million people now depend on upland rice as their daily staple food. Upland rice comprises 11% of the total global rice production and is cultivated on approximately 14 million ha (Sohrabi et al., 2012). In Malaysia, 98,000 ha are used for upland rice cultivation, accounting for approximately 12% of the total domestic rice cultivation area (Ferdous, 2012). In underprivileged areas, the utilization of upland rice varieties will undoubtedly help farmers to combat food security (Dzido et al., 2004).

Upland rice is subject to more pressure from bio-aggressors than is rain-fed lowland rice (Sester et al., 2008). These bio-aggressors include weed infestations and diseases specially blast disease, which is caused by the fungus *Magnaporthe oryzae* (Sester et al., 2008). Blast is one of the most devastating rice diseases, posing a significant threat to global food security (Galhano and Talbot, 2011). The blast fungus causes global rice yield losses of 60-100% annually (Kihoro, 2013), leading to severe epidemics in rice growing regions around the world and increasing rice cultivation costs (Galhano and Talbot, 2011). In such a context, new strategies must be created and evaluated to control blast epidemics and ensure sustainable upland-rice production (Sester et al., 2008).

Next generation sequencing (NGS) is the latest biotechnological approach, providing a complete picture of genomes and genes. Advancements in gene sequencing and subsequent DNA sequence analyses have led to the identification of many novel genes and improved the understanding of various qualitative and quantitative genetic traits in crop plants (Ganapathy et al., 2013). These tremendous advances have created new opportunities for identifying the molecular mechanisms behind host-microbe interactions in plant species. The interaction between the blast fungus *M. oryzae* and rice has been the focus of extensive rice disease resistance and fungal infection mechanism studies (Ribot et al., 2008). A better understanding of the mechanisms associated with *M. oryzae* infection and rice plant damage may lead to new invasive disease control methods (Ribot et al., 2008).

The screening or evaluation of upland rice germplasms is essential for determining new sources of resistance and developing durable blast resistant varieties *via* hybridization programs in blast endemic upland rice areas. Further morphological characterization of blast resistance genotypes is crucial for identifying high-yielding cultivars, which are of significant value to upland farmers. The identification of resistant genes from blast resistant upland rice varieties is essential for rice breeding programme. These genes can then be used to develop durable blast resistant upland rice cultivars in future.

1.2 Problem statement

The global upland rice yield is relatively low (Fageria et al., 2013), and rice blast disease represents the major constraint for a higher yield (Hanafi et al., 2009). Few studies have focused on improving upland rice yields and disease management strategies at the national and international scales. Up to now study on molecular dissection of blast resistant high yielding varieties through NGS are scarce in rice data bases. Currently, almost no high-yielding blast resistant varieties exist for cultivation in upland rice areas. Such varieties would help to meet the future food demands of subsistence farmers.

1.3 Objectives

The general objective of this project was to morphologically characterize upland rice germplasm to identify blast resistant genotypes with high yield potentials and to understand the precise molecular infection mechanisms of host-pathogen interaction that can be used to facilitate the design of novel control strategies. The research was initiated with the following specific objectives:

i) to assess the genetic diversity among upland rice genotypes against virulent race of blast fungus P7.2;
ii) to characterize the blast resistant upland rice genotypes based on morpho-physiological and yield attributes;
iii) to perform transcriptome analysis of blast resistant cultivar BRRI dhan43 through next generation sequencing;
iv) to analyze differentially expressed genes (DEG) in incompatible interaction between BRRI dhan43 and blast fungus P7.2.

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