UNIVERSITI PUTRA MALAYSIA

VALIDATION OF GENE AND QUANTITATIVE TRAIT LOCI ASSOCIATED WITH Nilaparvata lugens Stål RESISTANCE IN Oryza sativa L. CULTIVAR RATHU HEENATI

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By

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Chair : Noor Azmi Bin Shaharuddin, PhD
Faculty : Biotechnolgy and Biomolecular Sciences

The use of resistant varieties has been recognized as more economical and environmental friendly approach to manage brown planthopper *Nilaparvata lugens* Stål (BPH) populations in the rice fields. Rathu Heenati has a broad spectrum resistance against BPH. At least, four genes and QTLs were reported in Rathu Heenati, namely *Bph3* located on chromosome 6, *Qbph3* (Chr 3), *Qbph4* (Chr 4), and *Qbph10* (Chr 10). Microsatellite markers, flanking these gene/QTLs has been identified and made available in the literatures. This study was aimed to validate the presence of those gene/QTLs in Rathu Heenati by using the F_2 population of a cross between Rathu Heenati/MR219 with the final aim to introgress them into MR219 through marker-assisted selection (MAS). Resistance assessment were based on plant damage score (a measure of tolerance) and amount of honeydew excretion (a measure of antibiosis) while the presence of the gene/QTLs in the individual F_2 plants was based on the polymorphism of their respective markers on F_2 plants, *Qbph3* (RM7 and RM1256), *Qbph4* (RM8213 and RM5473), *Bph3* (RM8072 and RM588) and *Qbph10* (RM5352, RM228 and RM5471). Levels of resistance of individual plants were estimated. Cluster analysis manages to divide the plants into four clusters at 0.06 semi partial R-square value. These clusters represent groups of resistant plants (IV), moderately resistant (III), moderately susceptible (II), and susceptible (I). Correlation analysis showed significant correlation between *Bph3* presence and the amount of honeydew excretion (r = -0.200*), while *Qbph10* presence is correlated with the plant damage score (r = -0.196*). There was no correlation observed between *Qbph3* or *Qbph4* presence to any of the two phenotypic parameters measured. This study indicated that the presence of *Bph3* and *Qbph4* from Rathu Heenati contributed to the BPH resistance among the progenies of Rathu Heenati/MR219 cross. Their flanking markers were successfully utilised in the marker-assisted selection to monitor the gene introgression among the progenies of the cross. There were 59 F_2 plants in the group IV which are resistant to BPH which could be promoted for further evaluation at the F_3 generation.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Sarjana Sains

VALIDASI KE ATAS GEN DAN LOKUS TRAIT KUANTITATIF YANG BERSANGKUTAN DENGAN KERINTANGAN TERHADAP *Nilaparvata lugens* Stål DI DALAM KULTIVAR PADI *Oryza sativa* L. RATHU HEENATI

Oleh

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Penggunaan varieti padi rintang telah dikenalpasti sebagai satu kaedah pengawalan populasi serangga bena perang (BPH), *Nilaparvata lugens* Stål yang berkesan, murah lagi mesra alam di sawah-sawah padi. Varieti padi Rathu Heenati mempunyai spektrum kerintangan yang tinggi dan meluas terhadap serangga ini. Sekurang-kurangnya empat gen telah dilaporkan wujud dan berperanan mengatur kerintangan Rathu Heenati terhadap bena perang iaitu *Bph3* yang dilaporkan terletak pada kromosom 6, manakala lokus-lokus trait kuantitatif *Qbph3,Qbph4,* dan *Qbph10* pula adalah terletak pada kromosom-kromosom 3, 4 dan 10 setiap satunya. Penanda-penanda mikrosatelit yang mengapit setiap gen atau QTL ini telah pun dikenalpasti dan dilaporkan di dalam pelbagai literatur sebelum ini. Kajian yang dijalankan ini pula adalah bertujuan untuk mengesahkan kehadiran gen atau QTL ini di dalam varieti padi Rathu Heenati. Kajian dijalankan dengan menggunakan populasi F_2 hasil dari kacukan Rathu Heenati dengan MR219. Matlamat akhir kajian ini adalah bagi membolehkan pengintrograsian gen/QTL ini ke dalam varieti padi MR219 dengan menggunakan kaedah pemilihan berbantukan penanda molekul (MAS). Tahap kerintangan pokok adalah berdasarkan kepada tahap skoran kerosakan pokok (i.e. suatu bentuk pengukuran tahap toleransi) dan jumlah rembesan serangga (satu bentuk pengukuran tahap antibiosis). Kehadiran gen/QTL dalam setiap pokok F_2 pula adalah berasaskan kepada bentuk polimorfisma penanda-penanda molekul berkenaan, di mana *Qbph3* adalah berdasarkan polimorfisma penanda molekul RM7 serta RM1256, *Qbph4* (RM8213 serta RM5473), *Bph3* (RM8072 serta RM588) dan *Qbph10* (RM5352, RM228 serta RM5471). Tahap kerintangan yang ditonjolkan oleh setiap gen secara sendirian atau kombinasi sesama gen juga telah dianggarkan. Analisa kluster telah membahagikan pokok-pokok F_2 ke dalam empat kluster pada tahap 0.06 bagi nilai semi partial R-square. Kluster ini mewakili pokok-pokok F_2 dari kumpulan rintang (IV), sederhana rintang (III), sederhana rentan (II) dan kumpulan rentan (I). Analisa menunjukkan kehadiran korelasi yang bermakna antara kehadiran gen *Bph3* dengan jumlah rembesan serangga (r = -0.200*), sementara kehadiran *Qbph10* pula berkorelasi dengan skoran kerosakan pokok (r = -0.196*). Tidak wujud korelasi yang

bermakna di antara kehadiran *Qbph3* atau *Qbph4* dengan kedua-dua parameter fenotip yang diukur. Kajian ini telah menunjukkan bahawa kehadiran *Bph3* dan *Qbph10* dari Rathu Heenati telah menyumbang kepada kerintangan terhadap BPH dalam kalangan progeni-progeni kacukan Rathu Heenati/MR219. Penanda-penanda molekul yang mengapit kedua-dua gen/QTL ini telah berjaya digunakan dalam pemilihan berbantukan penanda molekul (MAS) bagi mengesan kewujudan intrograsi gen berkenaan dalam kalangan progeni kacukan. Sejumlah 59 pokok F_2 dari kluster IV telah menunjukkan tahap kerintangan tinggi terhadap BPH, yang boleh dimajukan ke generasi F_3 bagi penilaian selanjutnya.

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Declaration by graduate student

I hereby confirm that:

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LIST OF ABBREVIATIONS

AFLP	Ampilified Fragment Length Polymorphism
ANOVA	Analysis of Variance
BLB	Bacterial Leaf Blight Disease
bp	Base Pair
BPH	Brown Planthopper
Chr	Chromosom
CV	Coefficients of Variation
ddh²O	Deionized Water
D	Dark
DNA	Deoxyribonucleic Acid
FPLI	Funtional Plant Loss Index
GS	Honeydew excretion
H	Height
Hb	Heritability
hr	Hour
ICIIM	Inclusive Composite Internal
IRMI	International Rice Microsatellite Initiative
IRRI	International Rice Research Institute
K	Potassium
Kg	Kilogram
L	Light
MAB	Marker-Assisted Backcrossing
MARDI	Malaysian Agricultural Research and Development
MAS	Marker-Assisted Selection
mg	Milligram
ml	Millilitre
mm	Millimetre
N	Nitrogen
nm	Nanometre
O_2	Oxygen
P	Phosphorus
PCR	Polymerase Chain Reaction
PS	Plant damage Score
QTL	Quantitative Trait Loci
R	Radius
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RH	Rathu Heenati
rpm	Revolutions per minute
SAS	Statistical Analysis System
SE	Standard Error
SNP	Single Nucleotide Polymorphism
SSL	Self-sufficiency
SSR	Simple Sequence Repeat
SSST	Standard Seedbox Screening Test
TN1	Taichung Native 1
%	Percentage
°C	Degree Celsius

µg	Microgram
µl	Microliter
µm	Micrometer

CHAPTER 1

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important crops in the world. It is the staple food for more than 3 billion people in Asia and several other countries. A further increment of more than 60% is needed to fulfill the demand of the growing world population by 2025 (Norimah *et al*., 2008; Ghaffar, 2012; Mamaduo *et al*., 2015). However, biotic and abiotic factors numerously impact rice productivity in many tropical regions that may affect the expected production increment. Amongst major biotic agents which may cause significant yield reduction or losses are diseases and insect pests. Globally, 52% of rice production is lost annually due damages caused by biotic factors, of which 21% is attributed to the infestation by various species of insect pests (Yarasi *et al*., 2008).

There are more than 100 species of insects are found in the rice crop. About 20 species were identified as insect pests that have potential to cause significant damage affecting productivity of the crop. These included many species of stem borers, leafhoppers and planthoppers (Pathak and Khan, 1994). Rice brown planthopper (BPH) *Nilaparvata lugens* (Stål) is identified as one of the important insect pests in the world. This insect caused significant yield reduction to rice crops in many countries (Huang *et al*., 1997; Sogawa *et al*., 2003; Sun *et al*., 2005; Shabanimofrad *et al*., 2017). Invading BPH population congregates at the base of paddy plants and sucks the sap from the stem and leaf through phloem ingestion. Excessive feeding caused plant dehydration and loss of nutrition. The symptoms of damage can be identified by the leaves turn yellowing initially, followed by complete wilting and drying of rice plants. Under severe cases, paddy field looks a 'burnt-like' appearance in circles known as "hopperburn". The insect is also capable of transmitting two viral diseases, the ragged stunt virus and grassy stunt virus (Bhogadhi *et al*., 2015).

The rice crops in several South and South East Asian countries have been damaged by BPH in a large scale since 1970s (Dyck and Thomas, 1979; Latif, 2000). Annually, about 1 million tons of rice losses have been recorded in China due to brown planthopper infestation. A loss of about 2.7 million tons was recorded in year 2005 and 2008 (Brar *et al*., 2009; Hu *et al*., 2016), while in 2006 and 2007, a bigger damage was observed resulting with 9.4 million and 8.7 million ton loss (Catindig *et al*., 2009). Japan and Korea also affected by the numerous BPH outbreaks in 2005 (Otuka, 2013). In 2007, due to the high losses in rice production by BPH in Vietnam, rice exportation has been suspended by several producing countries (IRRI, 2011). Over tens of thousands hectares of rice fields were also reportedly affected in Indonesia since 2008. BPH infestations and its associated virus diseases (ragged and grassy stunt diseases) were also reported in 2009 in Central Thailand, southern provinces of China, northern Vietnam, and Indonesia. The BPH infestation in Thailand affected more than 3 million hectares of rice growing areas, causing losses in excess of 1.1 million tons, with an export potential of US$275 million (IRRI, 2014). In 1967, a brown planthopper outbreak was occurred in Terengganu affecting more than 6, 000 ha of rice. Another outbreak was also reported in Tanjong Karang in 1977 affecting a large area of rice

fields (Lim and Heong, 1977). Several other outbreaks were also reported in the subsequent years (Habibuddin, 2012) and thus BPH was identified as one of the important insect pests in Malaysia.

Traditionally, BPH population in Malaysia is controlled by using insecticides. However, application of insecticides increases the cost of rice production. An indiscriminate use of them may also affecting environment and the health of farmers. As such, utilization of resistant varieties to lower the potential loss and preventing BPH outbreaks was later adopted and promoted (Habibuddin, 2012). Many resistant genes to BPH were identified and utilized in the breeding program in Malaysia to develop BPH resistant varieties. Among them are *Bph1* from the donor variety Mudgo, *bph2* gene from ASD7, *Bph3* gene from Rathu Heenati and *bph4* gene from rice variety Babawee. So far, the *indica* rice cultivar Rathu Heenati was found to show high resistance and found to be resistant to all the four BPH biotypes, biotype 1, biotype 2, biotype 3 and biotype 4 worldwide (Li *et al*., 2017; Jairin *et al*., 2007a), including to BPH populations in Malaysia (Ito *et al*., 1994).

Conventional BPH resistant rice breeding protocol based on symptoms or phenotypic characterization was useful but it had certain limitations. Among the limitation of conventional resistant breeding program is the long duration period taken in the breeding processes to identify resistant breeding lines. It also has difficulty in identifying resistant genes playing their roles in those resistant lines, especially when the breeding processes were involving multiple crosses involving multiple donor parents. However, these limitations might be overcome by utilizing molecular DNA markers in the marker-assisted selection (MAS) breeding program. The presence of the desired targeted genes, of which in this case the BPH resistant gene(s), will be confirmed through the identification of their close-linked flanking DNA markers.

The *Bph3* gene in Rathu Heenati was mapped on the short arm of chromosome 6, flanking by the linked markers RM589 and RM588 (Jairin *et al*., 2007a). There were also reported findings that resistance of Rathu Heenati to BPH is also contributed by other quantitative trait loci (QTLs), reportedly located on different chromosomes (Sun *et al*., 2005; Jairin *et al*., 2007a; Kumari *et al*., 2010; Hu *et al*., 2016). Three QTLs were assigned to chromosome 3, 4, and 10, respectively (Sun *et al*., 2005). The loci in chromosome 3 which was designated as *Qbph3*, was found located between markers RM313 and RM7. The second QTL, *Qbph4*, was found located between markers RM8213 and RM5953 on the short arm of chromosome 4, with a map distance of 3.6 cM and 3.2 cM, respectively. On the other hand, the *Qbph10* on the chromosome 10 was flanked between markers RM484 and RM496 (Sun *et al*., 2005). The other major BPH resistance gene in Rathu Heenati was designated as *Bph17,* which was mapped between markers RM8213 and RM5953 on the chromosome 4 (Sun *et al*., 2005). However, there were also reports of contradictory findings on the identification and loocations of these genes in the variety.

In Malaysia, MR219 has been grown as a popular variety for more than 10 years, covering more than 90% of the planted area. The variety which is carrying *Bph1* gene was resistant to BPH when released, but was succumbed to BPH infestation in recent years. There is a need to incorporate other resistant genes from Rathu Heenati into

MR219 so that improved MR219 varieties with higher resistance levels to local BPH population could be introduced, and durabilities of these varieties could be prolonged in the fields. Hence, the present studies were conducted with the following objectives;

1.1. To validate several resistance genes and quantitative trait loci (QTLs) reportedly controlling BPH resistance in Rathu Heenati.

1.2. To assess and validate the flanking markers of the identified gene and QTLs for their possible application in a marker assisted selection program.

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