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GENETIC ANALYSIS AND QTL MAPPING OF BROWN PLANTHOPPER (Nilaparvata lugens Stål.) BIOTYPES 2 AND 3 RESISTANCE IN RICE

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By

MAHMOODREZA SHABANIMOFRAD

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

To my beloved wife

Thanks for her support, understanding, love and encouragement.

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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The use of molecular markers in many aspects of rice (*Oryza sativa* L.) studies such as genetic analysis of insect and diseases resistance genes is on the increase. Molecular markers have played an important role in rice breeding worldwide. Brown planthopper (BPH), *Nilaparvata lugens* is one of the most destructive insect pests in rice growing areas of the world. Several strategies such as QTL mapping are being deployed in breeding for resistance genes into rice varieties have been proposed for combating the BPH insect pest. This study used molecular marker approach in order to analyse molecular genetics of resistance in segregating populations and to identify QTL conferring resistance against two different biotypes of brown planthopper, namely, Biotype 2 and 3, in F_3 generation families derived from the cross between Rathu Heenati (BPH resistant) and MR276 (BPH susceptible) cultivars. One hundred and ten SSR primer pairs related to BPH resistance gene (*Bph* genes) distributed over 12 chromosomes of the rice genome were chosen and used to amplify SSR markers, and to analyse their potential association with *Bph* resistance. Fifty seven of polymorphic markers were used to identify BPH resistant segregation ratios in 176 individuals of F_2 population. Thirty five markers showed a good fit to the expected segregation ratio (1:2:1) for single gene model (df = 1.0, $p \leq 0.05$). The rest of the markers did not fit the expected segregating Mendelian ratios. The F_3 generation families were grown in a greenhouse and infested with two BPH biotypes, Biotype 2 and 3. Chi-square analysis showed a good fit to the phenotypic ratio of 3:1 for the segregation of resistance and susceptibility for the Biotypes 2 and 3 of BPH. Six SSR markers, RM401, RM5953, RM217, RM210, RM242 and RM1103 were found significantly associated with resistance to Biotype 2 and 3 of BPH in rice. These markers showed high selection accuracy for resistant plants with confirmation of resistance effect of about 17 and 20% respectively for phenotypic variation, and can be used in MAS for the resistant gene. The resistance gene markers reported here provide rice breeders and geneticists a valuable tool for marker-assisted selection of the BPH insect resistance gene. A total of 150 F_3 generation families derived from the cross between Rathu Heenati and MR276 were used in this experiment to identify QTLs for resistance to BPH Biotypes 2 and 3. A trait distribution analysis showed continuous variation with normal distribution. Twenty independent QTLs were detected to be associated with BPH resistance on nine chromosomes. Five putative QTL (qBph-1-1, qBph-3-1, qBph-6-1, qBph-7-1 and qBph-3-1) with Logarithmic of Odds (LOD) > 3.0 and five suggestive QTLs (qBph-5-1, qBph-11-1, qBph-6-1, qBph-9-1 and qBph-12-1, LOD < 3.0) were detected for Biotype 2. Meanwhile, two putative QTLs (qBph-3-1 and qBph-6-1) with LOD > 3.0 and eight suggestive QTLs (qBph-1-1, qBph-7-1, qBph-6-1, qBph-9-1, qBph-3-1, qBph-6-1, qBph-10-1 and qBph-12-1, LOD < 3.0) were detected for Biotype 3. The individual locus found in the F_3 population for traits studied, explained 7 to 24% of the

total phenotypic variance in resistance against BPH biotypes. In conclusion, from this research, the QTL identified could help breeders in their programme for marker-assisted selection for rice varietal development with BPH resistance.

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

ANALISIS GENETIK DAN PEMETAAN QTL KERINTANGAN BENA PERANG (*Nilaparvata lugens* Stål) BIOTIPS 2 DAN 3 TERHADAP PADI

Oleh

MAHMOODREZA SHABANIMOFRAD

Jun 2015

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Penggunaan penanda molekul dalam banyak aspek kajian padi (*Oryza sativa* L.) seperti analisis genetik terhadap gen kerintangan serangga dan penyakit semakin meningkat. Penanda molekul telah memainkan peranan penting dalam pembiakbakaan padi di seluruh dunia. Bena perang (BPH), *Nilaparvata lugens* adalah salah satu serangga perosak yang mengakibatkan kerosakkan yang teruk di kawasan penanaman padi di dunia. Beberapa strategi seperti pemetaan QTL sedang diperluaskan dalam pembiakbakaan untuk gen rintang ke dalam varieti padi telah dicadangkan untuk mengatasi serangga perosak BPH. Kajian ini menggunakan pendekatan penanda molekul untuk menganalisis kerintangan genetik molekul dalam populasi bersegregasi dan mengenal pasti QTL yang memberikan kerintangan terhadap dua biotip berbeza bena perang, iaitu, Biotip 2 dan 3, dalam famili generasi F_3 yang diperolehi daripada kacukan kultivar Rathu Heenati (rintang BPH) dan MR276 (rentan BPH). Seratus sepuluh pasang penanda SSR berkaitan dengan gen kerintangan bena perang (gen-*Bph*) meliputi 12 kromosom genom padi telah dipilih dan digunakan untuk penanda SSR bagi menganalisis potensi hubungannya dengan kerintangan *Bph*. Lima puluh tujuh penanda polimorfik telah digunakan untuk mengenal pasti nisbah segregasi rintangan *Bph* dalam 176 individu populasi F_2. Tiga puluh lima penanda memberikan padanan yang baik dengan nisbah segregasi yang dijangka (1: 2: 1) untuk model gen tunggal (df = 1.0, $p \leq 0.05$). Penanda selebihnya tidak menunjukkan padanan nisbah segregasi dijangka Mendel. Generasi famili F_3 telah ditanam di rumah hijau dan didedahkan dengan serangan dua biotip bena perang, Biotip 2 dan 3. Analisis Chi-kuasa dua menunjukkan padanan yang baik pada nisbah fenotip 3:1 untuk segregasi kerintangan dan kerentanan BPH Biotip 2 dan 3. Enam penanda SSR, RM401, RM5953, RM217, RM210, RM242 dan RM1103 didapati mempunyai perkaitan bererti dengan kerintangan untuk biotip 2 dan 3 BPH ke atas padi. Penanda ini menunjukkan ketepatan pemilihan yang tinggi untuk pokok rintang dengan pengesahan kesan kerintangan sebanyak 17 dan 20% masing-masing untuk variasi fenotip, dan boleh digunakan dalam MAS untuk gen kerintangan. Penanda gen rintang yang diperolehi ini adalah berguna dan dapat membantu ahlibiakbaka dan genetik padi untuk pemilihan penanda-berbantu gen rintangan serangga BPH. Sebanyak 150 generasi famili F_3 dari kacukan antara Rathu Heenati dan MR276 telah digunakan dalam eksperimen ini untuk mengenal pasti QTL untuk kerintangan pada biotip BPH 2 dan 3. Analisis serakan ciri menunjukkan variasi secara selanjar dengan bertaburan normal. Dua puluh QTL bebas berkaitan dengan kerintangan BPH telah dikesan pada sembilan kromosom. Lima QTL putatif (qBph-1-1, qBph-3-1, qBph-6-1, qBph-7-1 dan qBph-3-1) dengan Logarithmic of Odds (LOD) > 3.0 dan lima QTL yang dicadang (qBph -5-1, qBph-11-1, qBph-6-1, qBph-9-1 dan qBph-12-1, LOD <3.0) telah dikesan untuk Biotip 2. Sementara itu, dua QTL putatif (qBph-3-1 dan qBph-6-1) dengan LOD > 3.0 dan lapan QTL yang dicadang (qBph-1-1,

qBph-7-1, qBph-6-1, qBph-9-1, qBph-3-1, qBph-6-1, qBph-10-1 dan qBph-12-1, LOD <3.0) telah dikesan untuk Biotip 3. Locus individu yang terdapat dalam populasi F_3 untuk sifat dinilai, menjelaskan 7 hingga 24% daripada jumlah varians fenotip dalam rintangan terhadap biotip BPH. Kesimpulannya, kajian QTL ini diharapkan dapat membantu ahli biakbaka dalam program pemilihan penanda-berbantu bagi menghasilkan varieti padi dengan ciri kerintangan BPH.

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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 Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
AXT	Antixenosis Test
BPH	Brown Planthopper
CIM	Composite Interval Mapping
cM	Centimorgan
EST	Expressed Sequence Tag
ET	Effector-Triggered
HRM	High Resolution Melt
HT	Honeydew Test
INDELS	Insertions/Deletions
LOD	Log Of Odd
MAS	Marker Assisted Selection
Mb	Million base pair
Mha	Million hectare
Mt	Million tonnes
QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorphic Dna
RFLP	Restriction Fragment Length Polymorphism
RH	Relative Humidity
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeat
SSST	Standard Seedbox Screening Test
TN1	Taichung Native 1
χ^2	Chi-square

CHAPTER 1

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important staple food crop for more than two billion people in the world. Brown planthopper (BPH), *Nilaparvata lugens* Stål, is one of the most serious and destructive monophagous insect pests for rice throughout Asia. The brown planthopper damages plants not only causes by sap-sucking, but also acts as a vector of rice stripe virus (RSV), rice grassy stunt (RGSV) and rice ragged stunt virus (RRSV), which often cause significant yield loss in epidemic years.

Application of host-plant resistance cultivars become the major control methods for brown planthopper but their long-term stability is threatened because of the evolution of prolific biotypes (Saxena and Barrion, 1987). Numerous rice cultivars, such as 'IR26', 'IR64', Mudgo and IR46, were found to be resistant against BPH; it reveals that, in some cases, provided important short-term protection against BPH. In some areas BPH populations adapted to new developed resistant rice cultivars, sometimes they are unstable and tend to become susceptible in as little as 3 years of release. Breeding for rice cultivars with BPH resistance, varieties carrying polygenes provide a more durable resistance must be incorporated into individual varieties.

Knowledge about the biotypes of BPH and function of the insect-resistance gene and use them to develop resistant rice varieties is important for controlling this insect pest. Therefore, detailed characterization of the resistant genes against the BPH need to be developed to increase long-term stability resistance, giving security for a long period of time and over a broad geographic region.

Indiscriminate use of pesticides leading to the loss of animal genetic resources, elimination of natural enemies of pests, genetic resistance of BPH to pesticides, chemical contamination and environmental pollution (Chelliah and Gunathilagaraj, 2011). Therefore large efforts have been made to identifying resistance gene resources varieties that help guard against the negative impacts of BPH. Development of broad spectrum resistance capabilities is necessary for crop improvement, therefore use of DNA markers derived from the fine mapped position of the genes and marker assisted selection (MAS) genes for important biological or agronomic traits will provide opportunities for breeders to develop higher yield potential, improved grain quality, and durable resistance rice cultivars.

To develop a sustainable pest management system, it is important to find the right balance between breeding and management strategies to reduce the ecological fitness of BPH and to keep the pest under economic threshold levels. Host-plant resistance is the most practical and economical approach to control insect pests. A very few

information is available about resistance to BPH in Malaysian rice cultivars. The information regarding brown planthopper resistance genes (*Bph* genes) and QTLs existed in local cultivars might be helped in marker assisted selection. So the result of this project will be come up with first SSR-based QTL map of BPH resistance of rice in Malaysia.

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