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MOLECULAR DISSECTION AND QTL MAPPING OF RICE BLAST DISEASE RESISTANCE USING SIMPLE SEQUENCE REPEAT MARKERS

SADEGH ASHKANI

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MOLECULAR DISSECTION AND QTL MAPPING OF RICE BLAST DISEASE RESISTANCE USING SIMPLE SEQUENCE REPEAT MARKERS

By

SADEGH ASHKANI

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

December 2011

DEDICATIONS

To My Dear Wife, Fatemeh Azad with Innermost and Everlasting Affection and Love

To My Dear Children, Hasti and Mobin, the Little Angels Bringing Us Joy and Happiness

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman: Associate Professor Mohd Rafii binYusop, PhD

Faculty: Agriculture

DNA marker technology and the mapping of the major genes have played important roles in rice (*Oryza sativa* L.) disease improvement. Blast caused by *Magnaporthe oryzae* is an important disease of rice in Malaysia and all over the world. Its frequent appearance during all the stages of plant growth greatly decreases yield and grain quality. Quantitative trait loci (QTL)-based resistance for rice blast disease control is becoming increasingly important in breeding rice programs. This study used molecular marker approaches in order to analyse molecular genetics of resistance in segregating populations and to identify QTL conferring resistance against two different pathotypes of *M. oryzae*, namely, P7.2 and P5.0, in F_3 families derived from the cross of Pongsu Seribu 2 (resistant) variety and Mahsuri (susceptible) cultivar. One hundred and twenty five micro-satellite markers closely linked to the blast resistant genes (*Pi*-genes) distributed over 12 chromosomes of the rice genome were chosen and used in the study to determine polymorphism and potential association with blast resistance. Twenty three of polymorphic markers were used to identify blast resistant segregation ratios in 320 individuals of F_2 population. Eleven 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 families were grown in a greenhouse and challenged with two blast pathotypes, P7.2 and P5.0. In analysis of Chi-square tests for single gene model, two independent genes and possible different gene models of epistasis effect different segregation ratio (3R:1S) and (15R:1S) were observed for pathotypes P7.2 and P5.0 in blast lesion trait respectively. Pongsu Seribu 2 was resistant against both blast pathotypes tested. Pathotype P7.2 was found to be a high virulence blast pathotype. The plants resistant to blast pathotype P7.2 from F_3 population were good linked with genotypes of four SSR markers, RM413, RM1233, RM8225 and RM5961, with observed segregation ratio of (1:2:1) for single dominant gene model. These markers were found as suitable SSR markers for use in marker assisted selection of blast resistant genes conferring resistance to *Magnaporthe oryzae* pathotype, P7.2. A total of 188 F_3 families derived from the cross between, Pongsu Seribu 2 and Mahsuri were used in this experiment to identify QTLs for resistance to local *Magnaporthe oryzae* pathotypes, P7.2 and P5.0. There was a positive correlation for the three traits (BLD, BLT and % DLA) for each isolate. A trait distribution analysis did not show continuous variation with normal distribution. For both pathotypes, the distributions of disease severity (DS) were skewed towards resistance. Sixty three polymorphic SSR markers were used to construct a linkage map in 188 F_3 families. The 63 SSR markers covered 3989.9 cM of the rice genome. Single marker analysis (SMA), interval mapping (IM) and composite interval mapping with permutation analysis was used for Quantitative Trait Loci (QTL) analysis. Twenty eight independent QTLs were detected to be associated with blast resistance on chromosomes 1, 2, 3, 5, 6, 8, 10, 11 and 12. Six putative QTL (qRBr-

1.2, qRBr-2.1, qRBr-5.1, qRBr-6.1, qRBr-11.1 and qRBr-11.2) with Logarithmic of Odds (LOD) > 3.0.and 7 suggestive QTLs (qRBr-1.1, qRBr-3.1, qRBr-6.2, qRBr-10.1, qRBr-10.2, qRBr-11.3 and qRBr-12.1, LOD < 3.0) were detected for pathotype P7.2. Meanwhile, three putative QTLs (qRBr-6.1, qRBr-11.4, and qRBr-12.1) with Logarithmic of Odds (LOD) > 3.0 and 12 suggestive QTLs (qRBr-1.2, qRBr-2.1, qRBr-4.1, qRBr-5.1, qRBr-6.2 qRBr-6.3, qRBr-8.1, qRBr-10.1, qRBr-10.2, qRBr-11.1, qRBr-11.2 and qRBr-11.3, LOD < 3.0) were detected for pathotype P5.0. Likelihood Ratio Statistics (LRS) for the association of the traits with locus at $p \leq 0.05$ ranged from 4.0 to 32.4. However, only 9 putative QTLs were found with a single marker analysis and either interval mapping or composite interval mapping (at LRS up to15) on chromosomes1, 2, 5, 6, 11 and 12. The individual locus found in the population F_3 for traits studied, explained 2-16% of the total phenotypic variance in resistance against blast pathotypes. QTLs that had effects on both of the two blast isolates, such QTLs may be commonly involved in the defense response against a broad range of pathogens infections and others may only be involved in limited defense responses, thus showing degrees of race specificity. Some of our identified QTL were mapped to region where previously some *Pi* genes for blast resistance have been reported. Such quantitative resistance genes might be defeated major R-genes. In conclusion, from this research it was found that resistance to blast in Pongsu Seribu 2 is very complex and is composed of a combination of *Pi* genes as well as some unknown genes, and major and minor effects of multiple loci that appear to contribute to partial resistance to local blast isolates.

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

DISEKSI MOLEKUL DAN PEMETAAN QTL KERINTANGAN PENYAKIT KARAH PADI MENGGUNA PENANDA ULANGAN JUJUKAN RINGKAS

Oleh

SADEGH ASHKANI

Disember 2011

Pengerusi: Profesor Madya Mohd Rafii bin Yusop, PhD

Fakulti: Pertanian

Teknologi penanda DNA dan pemetaan gen memainkan peranan penting dalam pembiakbakaan penyakit tanaman padi (*Oryza sativa* L.). Karah yang disebabkan oleh *Magnaporthe oryzae* adalah penyakit padi yang penting di Malaysia dan seluruh dunia. Kekerapan kemuculannya di semua peringkat tumbesaran pokok mengakibatkan penurunan ketara hasil dan kualiti bijian padi. Pencirian lokus kuantitatif (QTL) kerintangan yang mengawal penyakit karah padi telah meningkat kepentingannya di dalam program pembiakbakaan padi.. Kajian ini menggunakan pendekatan penanda molekul bagi menganalisa genetik molekul yang rintang di dalam populasi yang bersegregasi dan untuk mengenalpasti QTL yang rintang terhadap dua patotip *M oryzae* yang berbeza, P7.2 dan P5.0 dalam famili F_3 hasil dari kacukan antara varieti Pongsu Seribu 2 (rintang) dan Mahsuri (rentan). Satu ratus dua puluh lima penanda mikrosatelit yang berkait rapat dengan gen rintang penyakit karah (*Pi* gen) meliputi kesemua 12 kromosom genom padi dipilih dan digunakan dalam kajian menentukan polimorfisme dan potensi hubungannya dengan

kerintangan kepada penyakit karah. Dua puluh tiga penanda polimorfik digunakan bagi mengenalpasti nisbah kerintangan karah dalam 320 individu dalam populasi F_2. Sebelas penanda menunjukkan kecocokan yang baik dengan kadar segregasi yang dijangka (1:2:1) untuk model gen tunggal (df=1.0, $p \leq 0.05$). Sementara penanda yang lain segregasi tidak mengikut nisbah jangkaan segregasi Mendel. Famili F_3 telah di tanam di rumah hijau dan dilakukan rawatan saringan dengan patotip karah, P7.2 dan P5.0. Dalam analisa ujian khi kuasadua untuk model satu gen, model dua gen bebas dan kemungkinan model gen yang berbeza kesan epistasis, beberapa nisbah segregasi (3R: 1S) dan (15R:1S) telah diperolehi masing-masing bagi patotip P7.2 dan P5.0. Pongsu Seribu 2 di dapati ringtang terhapap kedua-dua patotip. Patotip P7.2 didapati patotip karah yang paling virulen. Pokok yang rintang kepada patotip P7.2 daripada populasi F_3 didapati mempunyai kaitan baik dengan empat genotip penanda SSR, RM413, RM1233, RM8225 dan RM5961, dimana nisbah segregasi dicerap adalah (1:2:1) untuk model satu gen dominan. Penanda-penanda SSR ini didapati sesuai digunakan di dalam pemilihan bantuan penanda gen rintang penyakit karah terhadap *Magnaporthe oryzae* patotip P7.2. Sejumlah 188 famili F_3 hasil kacukan Pongsu Seribu 2 dan Mahsuri telah digunakan dalam kajian mengenalpasti QTL rintang kepada *Magnaporthe oryzae* tempatan patotip P7.2 dan P5.0. Perhubungan yang positif diperolehi untuk ketiga-tiga ciri (BLD, BLT and %DLA) bagi setiap patotip. Analisa taburan sesuatu ciri didapati tidak memberikan variasi beterusan dan taburannya tidak secara normal. Ujian distribusi keterukan penyakit (DS) didapati, kedua-dua patotip condong ke arah rintang. Enam puluh tiga penanda SSR yang polimorfik digunakan untuk membina peta tautan dalam kromosom bagi 188 famili F_3. Enam puluh tiga penanda SSR tersebut meliputi 3989.9 cM genom padi. Analisa penanda tunggal (SMA), pemetaan interval (IM) dan pemetaan komposit *interval*

(CIM) dengan analisa permutasi digunakan dalam analisa QTL. Dua puluh lapan QTL berasingan telah dikesan mempunyai hubungan dengan kerintangan karah pada kromosom 1, 2, 3, 5, 6, 8, 10, 11 and 12. Enam putatif QTL (qRBr-1.2, qRBr-2.1, qRBr-5.1, qRBr-6.1, qRBr-11.1 and qRBr-11.2) dengan *Logarithmic of Odds* (LOD) > 3.0 dan 7. sugestif QTL (qRBr-1.1, qRBr-3.1, qRBr-6.2, qRBr-10.1, qRBr-10.2, qRBr-11.3 dan qRBr-12.1, LOD < 3.0) dikesan bagi patotip P7.2. Manakala, 3 putatif QTL (qRBr-6.1, qRBr-11.4, and qRBr-12.1) dengan *Logarithmic of Odds* (LOD) > 3.0 and 12 jangkaan QTLs (qRBr-1.2, qRBr-2.1, qRBr-4.1, qRBr-5.1, qRBr-6.2 qRBr-6.3, qRBr-8.1, qRBr-10.1, qRBr-10.2, qRBr-11.1, qRBr-11.2 and qRBr-11.3 LOD < 3.0) dikesan bagi patotip P5.0. Statistik nisbah kebarangkalian (LRS) untuk perhubungan ciri dengan lokus pada $p \leq 0.05$ adalah dari julat 4.0 hingga 32.4. Walaubagaimanapun, hanya 9 *putative* QTL diperolehi dengan Analisa penanda tunggal dan samada pemetaan interval dan pemetaan komposit *interval* (pada LRS lebih daripada 15). Lokus individu yang dijumpai dalam populasi F_3 bagi ciri-ciri ynag dikaji, menerangkan 2-16% daripada jumlah variasi fenotip kerintangan terhadap patotip karah. QTL yang mempunyai kesan ke atas kedua patotip, kemungkinan terlibat dalam gerakbalas pertahanan terhadap jangkitan pathogen dan yang lain mungkin terlibat pada gerakbalas pertahanan yang terhad, menunjukkan terdapat spesifisiti terhadap ras patotip. Sebahgian QTL yang ditemui dipeta dalam rantau Pi-gen yang telah dilaporkan oleh penyelidik sebelum ini. Gen rintang kuantitatif tersebut mungkin disebabkan oleh R-gen utama. Kesimpulannya, dari penyelidikan ini didapati kerintangan terhadap karah dalam Pongsu Seribu 2 adalah sangat komplek, dan ianya dibentuk dari satu kombinasi beberapa *Pi* gen serta gen yang tidak diketahui, dan kesan major dan minor pelbagai lokus yang menyumbang kepada separa rintang terhadap isolasi karah tempatan.

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Sincerely

SADEGH ASHKANI

I certify that a Thesis Examination Committee has met on 15 December 2011 to conduct the final examination of Sadegh Ashkani on his thesis entitled " MOLECULAR DISSECTION AND QTL MAPPING OF RICE BLAST DISEASE RESISTANCE USING SIMPLE SEQUENCE REPEAT MARKERS " 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.

Members of the Thesis Examination Committee were as follows:

Mihdzar Bin Abdul Kadir, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Maziah binti Mahmood, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Maheran Abd. Aziz, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Muhammad Ashraf, PhD
Professor
Faculty of Sciences
University of Agriculture, Faisalabad
Pakistan
(External Examiner)

_____

SEOW HENG FONG, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Mohd Rafii Bin Yusop, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Sariah Meon, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Siti Nor Akmar Abdullah, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Rusli Ibrahim, PhD
Principal Researches
Agrotechnology & Bioscience Division
Agensi Nuklear Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institutions.

SADEGH ASHKANI

Date: 15 December 2011

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