UNIVERSITI PUTRA MALAYSIA

DIVERSITY OF *CORYNESPORA CASSIICOLA* ISOLATES AND CHANGES IN RUBBER (*HEVEA BRASILIENSIS)* LEAF PROTEIN PROFILES IN RESPONSE TO PATHOGEN INOCULATION

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By

NGUYEN ANH NGHIA

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

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Thành quả này xin kính dâng

Hương hồn Ba, Ông Nguyễn Văn Sửu

Mẹ Kính Yêu, Bà Nguyễn Thị Bé

Và Gia Đình Thân Yêu

Vì Sự Hy Sinh Lớn Lao Cho Cuộc Đời Tôi

This Thesis is Specially Dedicated to

The Memory of My Late Adored Father, Mr. Nguyen Van Suu

My Dearest Mother, Mrs. Nguyen Thi Be

And Also to My Beloved Family

Their Sacrifice and Infinite Love Led Me to Present Achievements

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 : Suhaimi Napis, PhD

Faculty : Biotechnology and Biomolecular Sciences

Corynespora leaf fall, caused by *Corynespora cassiicola*, is one of the most important diseases in rubber (*Hevea brasiliensis*) plantations. A study was conducted to analyse the diversity among *C. cassiicola* isolates and to investigate the changes in rubber leaf protein profiles in response to this pathogen. Inter Simple Sequence Repeat (ISSR) and rDNA-ITS sequence markers along with morphological characteristics and detached leaf assay were employed to analyse 21 isolates of *C. cassiicola* collected from different rubber clones grown in several states of Malaysia. Variations in morphological features were observed within and among isolates with no inclination to either clonal or geographical origins of the isolates. The ISSR and rDNA-ITS sequence analyses segregated the studied isolates into two distinct groups. Group 1 includes 12 isolates from the states of Johor and Selangor (this group was split into 2 subgroups 1A and 1B, subgroup 1B includes a unique isolate, CKT05D); and group 2 includes 9

isolates obtained from the other states. AMOVA analysis showed 84% of total genetic variation was attributed to variation between two groups with highly significant difference. The detached leaf assay performed on selected rubber clones grouped the isolates in subcluster 1A into Race 1; the isolates in cluster 2 into Race 2 while the pathogenicity of the isolate CKT5D was dissimilar to either Race 1 or Race 2. Two Single Nucleotide Polymorphisms (SNPs) were discovered from the rDNA-ITS region of the studied isolates. They are correlated to the races that were identified in Malaysia. The BLAST search results revealed that the nucleotide sequences in the rDNA-ITS region of *C. cassiicola* fungus are highly conserved. Seven SNPs and two indels were detected in the rDNA-ITS region of the studied and deposited *C. cassiicola* isolates obtained from several countries on diverse hosts and their presence may be correlated with the race of this fungus. The changes in the leaf protein profiles of two rubber clones RRIM 600 and PB 260 in response to inoculation with the spores of two isolates representing two races of this fungus were analysed using two-dimensional gel electrophoresis (2-DE). Several differentially expressed proteins were detected at different time points after inoculation. Dissimilarities in expression patterns were observed within and among the four clone/isolate interaction systems. The number of differentially expressed proteins was also different among the systems. These proteins differed in their estimated isoelectric points (pI) and molecular weights (MW) with the exception of three detected identical proteins.

In conclusion, morphological analysis could identify but not differentiate the races of *C. cassiicola*; ISSR markers proved useful to distinguish the races while rDNA-ITS sequence markers could not only identify but could also infer the races of this fungus. This study confirmed that at least two distinct groups of *C. cassiicola* infect rubber trees in Malaysia. The changes in the 2-DE protein profiles of the rubber leaf proteomes in response to inoculation with *C. cassiicola* are highly dependent on the compatibility reactions of the rubber clone to a particular isolate. Differences in protein profiles implied the complexity of the interactions.

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

KEPELBAGAIAN ISOLAT C*ORYNESPORA CASSIICOLA* DAN PERUBAHAN PROFIL PROTEIN DAUN GETAH (*HEVEA BRASILIENSIS*) TERHADAP PATHOGEN TERSEBUT

Oleh

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Penyakit luruhan daun yang disebabkan oleh kulat *Corynespora cassiicola* mengakibatkan kemudaratan pada tanaman getah (*Hevea brasiliensis*). Kajilidikan ini dilakukan untuk menganalisa kepelbagaian isolat-isolat *C. cassiicola* dan menyelidik perubahan profil protein daun getah selepas diperlakukan dengan kulat pathogen tersebut. Penanda Inter Simple Sequence Repeat (ISSR) dan rDNA-ITS, pencirian morfologi serta pengasaian daun in vitro digunakan untuk menganalisa 21 isolat kulat *C. cassiicola* yang diperolehi daripada klon-klon getah yang ditanam di beberapa negeri di Semenanjung Malaysia. Perbezaan ciri-ciri morfologi dicerap di antara konidia-konidia daripada isolat yang sama dan juga di antara isolat tetapi perbezaan ini tidak dapat dikaitkan dengan klon hos atau lokasi isolat-isolat diperolehi. Analisa jujukan ISSR dan rDNA-ITS membahagikan isolat-isolat tersebut kepada dua kumpulan yang berlainan. Kumpulan 1 merangkumi 12 isolat yang diperolehi dari Johor

dan Selangor (kumpulan ini berpecah kepada 2 kumpulan kecil iaitu 1A dan 1B, kumpulan kecil 1B mempunyai isolat unik, CKT05D); manakala kumpulan 2 termasuk 9 isolat yang diperolehi daripada negeri-negeri lain. Analisa AMOVA menunjukkan bahawa 84% daripada keseluruhan variasi genetik ditentukan oleh variasi di antara kedua-dua kumpulan tersebut. Pengasaian daun in vitro yang dilakukan pada klon tertentu pula mengklasifikasikan kumpulan kecil 1A ke dalam ras 1; isolat-isolat daripada kumpulan 2 tergolong ke dalam ras 2; kepatogenan isolat CKT5D tidak menyerupai ras 1 atau 2. Dua polimorfisma nukleotid tunggal (SNP) ditemui pada kawasan rDNA-ITS isolat-isolat yang dikaji; dan ia menunjukkan korelasi dengan ras yang dikenalpasti di Malaysia. Analisa BLAST menunjukkan bahawa jujukan nukleotid di dalam kawasan rDNA-ITS kulat *C. cassiicola* adalah sangat terpelihara. Tujuh SNP dan 2 indel dikesan pada kawasan rDNA-ITS isolat *C.cassiicola* yang dikaji dan isolat daripada pelbagai hos yang terdapat di pangkalan data, perbezaan ini mungkin mempunyai korelasi dengan ras kulat ini. Perubahan profil protein klon-klon RRIM 600 dan PB260 selepas perlakuan dengan spora 2 isolat daripada ras yang berlainan dianalisa menggunakan teknik elektroforesis 2-dimensi (2-DE). Sebilangan tompok protein dicerap menunjukkan pola pengekspresan yang berubah mengikut masa selepas perlakuan; Perbezaan pola pengekspresan juga dicerap sesama dan di antara sistem interaksi 4 klon/isolat. Bilangan protein yang dikesan diekspres secara berbeza juga berlainan di antara sistem. Kesemua protein-protein ini berbeza dari aspek titik iso-elektrik (pI) dan berat

molekul kecuali 3 tompok protein yang sama yang diekspres secara berbeza di dalam kesemua sistem.

Kesimpulannya, analisa morfologi dapat digunakan untuk tujuan pengenalpastian sepsis *C. cassiicola* secara umum tetapi penanda ISSR berguna untuk melakukan pencirian isolat kepada dalam ras-ras yang diketahui; penanda jujukan rDNA-ITS pula boleh digunakan untuk tujuan pengenalpastian dan kajian lanjutan ras kulat ini. Kajian ini juga mengesahkan bahawa kulat *C. cassiicola* yang menjangkiti tanaman getah di Malaysia terdiri daripada 2 kumpulan yang berlainan. Perubahan profil 2-DE protein ke atas proteome daun getah yang diperlakukan dengan spora kulat *C. cassiicola* didapati sangat bergantung kepada keserasian di antara klon getah terhadap isolat tertentu. Pola pengekspresan yang berlainan dalam setiap sistem juga menggambarkan kompleksiti respon pokok getah terhadap kulat *C. cassiicola*.

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I certify that a Thesis Examination Committee has met on 3rd June 2009 to conduct the final examination of Nguyen Anh Nghia on his thesis entitled "Diversity of *Corynespora cassiicola* Isolates and Changes in Rubber (*Hevea brasiliensis)* Leaf Protein Profiles in Response to Pathogen Inoculation" 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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DECLARATION

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

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NGUYEN ANH NGHIA

Date:

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%Vol	percentage volume of protein spots
µg	microgram
µL	microlitre
µm	micrometre
µM	micromolar
2-DE	two-dimensional gel electrophoresis
AFLPs	Amplified Fragment Length Polymorphisms
AMOVA	analysis of molecular variance
ANOVA	analysis of variance
APS	ammonium persulfate
Avr gene	avirulence gene
BLAST	Basic Local Alignment Search Tool
bp	base pairs
BSA	Bovine Serum Albumin
CMI	Commonwealth Mycological Institute
CAD	cinnamyl alcohol dehydrogenase
CHAPS	3-[(3-cholamidopropyl)dimethylammonio]-1-propane sulfonate
CLF	Corynespora leaf fall disease
CRD	completely randomised design
CTAB	hexacetyltrimethyl ammonium bromide
DIGE	fluorescence 2-D difference gel electrophoresis
DNA	deoxyribonucleic acid
dNTPs	deoxynucleotides