



UNIVERSITI PUTRA MALAYSIA

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

NGUYEN ANH NGHIA

FBSB 2009 15

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CHANGES IN RUBBER (*HEVEA BRASILIENSIS*) LEAF PROTEIN
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By

NGUYEN ANH NGHIA

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
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of Philosophy**

June 2009



*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

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

By

NGUYEN ANH NGHIA

June 2009

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 *CORYNESPORA CASSIICOLA* DAN PERUBAHAN PROFIL PROTEIN DAUN GETAH (*HEVEA BRASILIENSIS*) TERHADAP PATHOGEN TERSEBUT

Oleh

NGUYEN ANH NGHIA

Jun 2009

Pengerusi : Suhaimi Napis, PhD

Fakulti : Bioteknologi dan Sains Biomolekul

Penyakit luruhan daun yang disebabkan oleh kulat *Corynespora cassiicola* mengakibatkan kemudaratan pada tanaman getah (*Hevea brasiliensis*). Kajiilidikan 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 pengasaan 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. Pengasaan 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 tempok 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.

Members of the Thesis Examination Committee were as follows:

Shuhaimi Mustafa, PhD

Associate Professor

Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Tan Soon Guan, PhD

Professor

Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Sariah Meon, PhD

Professor

Institute of Tropical Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Hj. Mohd Azib Salleh, PhD

Professor

Research and Innovation Management Centre
Universiti Malaysia Sarawak
(External Examiner)

BUJANG KIM HUAT, 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 requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Suhaimi Napis, PhD

Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Mohd. Puad Abdullah, PhD

Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
(Member)

Jugah Kadir, PhD

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Sunderasan Elumalai, PhD

Research Officer
Malaysian Rubber Board
(Member)

HASANAH MOHD. GHAZALI, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:



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.

NGUYEN ANH NGHIA

Date:

TABLE OF CONTENTS

		Page
	ABSTRACT	iii
	ABSTRAK	vi
	ACKNOWLEDGEMENTS	ix
	APPROVAL	xi
	DECLARATION	xiii
	LIST OF TABLES	xvii
	LIST OF FIGURES	xix
	LIST OF APPENDICES	xxii
	LIST OF ABBREVIATIONS	xxiv
 CHAPTER		
1	INTRODUCTION	1
2	LITERATURE REVIEW	5
	<i>Corynespora cassiicola</i> (Berk. & Curt.) Wei	5
	Taxonomy and Morphology	5
	Geographical Distribution, Host Range and Pathogenicity	8
	<i>Corynespora</i> Leaf Fall Disease on Rubber	13
	Diversity Analysis Using DNA-based Techniques	27
	Random Amplified Polymorphic DNA (RAPD)	28
	Inter Simple Sequence Repeats (ISSR)	30
	Ribosomal DNA-ITS (rDNA-ITS) Sequencing	34
	Single Nucleotide Polymorphisms (SNPs)	38
	Plant Defence Responses to Pathogens	43
	Nonhost and Host Resistance	43
	Local Induced Resistance	44
	Oxidative Burst Process	46
	Hypersensitive Response (HR)	47
	Structural and Biochemical Barriers	48
	Systemic Acquired Resistance (SAR)	48
	Proteomics Studies on Plant Defence Responses to Pathogens	49
	Techniques in Proteomics	50
	Application of Proteomics to Study Plant Defence Response to Pathogens	54
3	DIVERSITY ANALYSIS OF <i>Corynespora cassiicola</i> ISOLATES	57
	Introduction	57
	Materials and Methods	59
	Isolates of <i>Corynespora cassiicola</i>	59



	Colony Morphology	61
	Conidial Morphology	62
	Fungal DNA Isolation	63
	Inter Simple Sequence Repeat (ISSR) PCR Analysis	64
	Ribosomal DNA-ITS Sequence Analysis	67
	Pathogenesis of the Representative <i>Corynespora cassiicola</i> Isolates on Detached Leaves of Different Rubber Clones	69
	Results	71
	Colony Morphology	71
	Conidial Morphology	75
	Genomic DNA Extraction	78
	Diversity Analysis of <i>Corynespora cassiicola</i> Isolates Based On ISSR Markers	79
	Identification and Diversity Analysis of <i>Corynespora cassiicola</i> Based On rDNA-ITS Sequence Markers	83
	Pathogenesis of the Representative <i>Corynespora cassiicola</i> Isolates on Detached Leaves of Different Rubber Clones	89
	Discussion	92
	Conclusion	99
4	THE CHANGES IN LEAF PROTEIN PROFILES OF SELECTED RUBBER CLONES IN RESPONSE TO INOCULATION WITH ISOLATES OF DIFFERENT <i>Corynespora cassiicola</i> RACES	102
	Introduction	102
	Materials and Methods	104
	Rubber Clones, Pathogens and Plant Materials	104
	Inoculation of Plants	105
	Leaf Sampling for Protein Analyses	105
	Chemicals	105
	Protein Analyses	106
	Results	112
	The Changes in Leaf Protein Profiles of Rubber Clones RRIM 600 in Response to Inoculation with Isolate CKT05B (Races 1) of <i>Corynespora cassiicola</i>	112
	The Changes in Leaf Protein Profiles of Rubber Clones RRIM 600 in Response to Inoculation with Isolate CLN16 (Races 2) of <i>Corynespora cassiicola</i>	115



The Changes in Leaf Protein Profiles of Rubber Clones PB 260 in Response to Inoculation with Isolate CKT05B (Races 1) of <i>Corynespora cassiicola</i>	118
The Changes in Leaf Protein Profiles of Rubber Clones PB 260 in Response to Inoculation with Isolate CLN16 (Races 2) of <i>Corynespora cassiicola</i>	121
Analysis of Identical Proteins Which Were Differentially Expressed in Two Rubber Clones in Response to Inoculation with Isolates CKT05B and CLN16 Representing Two Races of <i>Corynespora cassiicola</i>	122
Discussion	125
Conclusion	132
5 SUMMARY, GENERAL CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH	134
REFERENCES	140
APPENDICES	164
BIODATA OF STUDENT	184
LIST OF PUBLICATIONS	185



LIST OF TABLES

Table		Page
3.1	Sources of <i>Corynespora cassiicola</i> isolates used in the study	60
3.2	List of ISSR primers, their annealing temperatures and DNA polymorphisms which were employed to differentiate <i>Corynespora cassiicola</i> isolates	76
3.3	Visual characteristics of the <i>Corynespora cassiicola</i> cultures seven days after incubation	73
3.4	The conidia size and number of pseudoseptate of the <i>Corynespora cassiicola</i> isolates, which were measured on 100 conidia/isolate except that for isolate CSB16, observations were made on 82 conidia	77
3.5	Analysis of molecular variance (AMOVA) of two <i>Corynespora cassiicola</i> population isolates from rubber trees growing in Malaysia, using Inter Simple Sequence Repeat (ISSR) markers data	82
3.6	Extractive BLAST search results and GenBank data of 29 similar deposited isolates with expectation value (E value) equal to zero (0) located on top of the BLAST hit lists of three representative studied isolates	87-88
3.7	Description of Single Nucleotide Polymorphisms (SNPs) detected from the rDNA-ITS region of 49 <i>Corynespora cassiicola</i> isolates	89
3.8	Pathogenicity of the representative <i>Corynespora cassiicola</i> isolates on different rubber clones using detached leaf assay	90
4.1	List of differentially expressed protein spots detected from leaf protein profiles of rubber clone RRIM 600 inoculated with isolate CK05B (Race 1) of <i>Corynespora cassiicola</i>	113
4.2	List of differentially expressed protein spots detected from leaf protein profiles of rubber clone RRIM 600 inoculated with isolate CLN16 (Race 2) of <i>Corynespora cassiicola</i>	116



4.3	List of differentially expressed protein spots detected from leaf protein profiles of rubber clone PB 260 inoculated with isolate CKT05B (Race 1) of <i>Corynespora cassicola</i>	119
4.4	List of differentially expressed protein spots detected from leaf protein profiles of rubber clone PB 260 inoculated with isolate CLN16 (Race 2) of <i>Corynespora cassicola</i>	123



LIST OF FIGURES

Figure		Page
1.1	Conidia and conidiophore of <i>Corynespora cassiicola</i> (all those pictures were taken from one colony) (Source: Ellis and Holliday, 1971)	7
3.1	Map of Malaysia showing the locations of rubber plantations where the <i>Corynespora cassiicola</i> isolates were obtained. Numbers indicate the isolates of the fungus listed in Table 3.1	61
3.2	Variability in colony morphology among <i>Corynespora cassiicola</i> isolates seven days after incubation on PSA observed from top and bottom of the Petri dishes. Note on the top of each picture is the name of the isolate and the rubber clone from which the isolate was obtained is in parenthesis	74
3.3	Variations in conidia shapes and sizes among and within isolates of <i>Corynespora cassiicola</i> . Note on the top of each picture is the name of the isolate and the rubber clone from which the isolate was obtained is given in parenthesis	76
3.4	Distribution in percentage (%) of conidia contour (A) and shape (B) of <i>Corynespora cassiicola</i> isolates which were observed on 100 conidia/isolate except that for isolate CSB16, observations were made on 82 conidia	78
3.5	Gel electrophoresis of genomic DNA products extracted from mycelia of 21 <i>Corynespora cassiicola</i> isolates using modified CTAB extraction method. The 1st and the last lane (MW) are 1kb DNA Ladder (Promega). The numbers below DNA bands describe A260/A280 ratio of the appropriate band	79
3.6	Gel electrophoresis of amplification products from <i>Corynespora cassiicola</i> genomic DNA obtained by 4 ISSR primers (UBC 826, UBC 835, Mj3 and Mj5) using the ISSR-PCR technique. The 1 st and the last lanes (MW) are 2-Log DNA Ladder (BioLabs). Lanes 1 to 12 represent the isolates from Johor and Selangor. Lanes 13 to 21 represent the isolates from the other states	81



3.7	Dendrogram derived from UPGMA cluster analysis, using Nei & Li's coefficient based on 106 ISSR bands, showing the genetic relationships among 21 <i>Corynespora cassiicola</i> isolates	82
3.8	Gel electrophoresis of amplification products obtained from 21 <i>Corynespora cassiicola</i> isolates using ITS1 and ITS4 primers. The 1st lane (MW) is a 2-Log DNA Ladder (BioLabs)	84
3.9	Single nucleotide polymorphisms located at two nucleotide positions (100 and 135) in the ITS1 region of 21 <i>Corynespora cassiicola</i> isolates. Isolates 1 to 12 were from Johor and Selangor. Isolates 13 to 21 were from other states. The numbers on the top show the position of nucleotide counted from the 5' end of the sequence	84
3.10	Pictures showing necrotic lesions that reflect on the levels of infection of the representative <i>Corynespora cassiicola</i> isolates from subcluster 1A, 1B and cluster 2 on detached leaves of different rubber clones. Note on the top of each picture is the name of the isolate and notes at the bottom are the names of the rubber clones	91
4.1	Silver-stained 2-DE gels of leaf proteins of rubber clone RRIM 600 inoculated with isolate CKT05B (Race 1) of <i>Corynespora cassiicola</i> . (A) Sample collected at 72 hours after inoculation. Names and positions of differentially expressed protein spots are indicated. (B) Enlarged gels in combination with histograms illustrate the variations of representative spots at different time points (a, b: 0 hour; c, d: 24 hours; e, f: 48 hours; and g, h: 72 hours)	114
4.2	Silver-stained 2-DE gels of leaf proteins of rubber clone RRIM 600 inoculated with isolate CLN16 (Race 2) of <i>Corynespora cassiicola</i> . (A) Sample collected at 72 hours after inoculation. Names and positions of differentially expressed protein spots are indicated. Lines with + sign show the putative position of spots that do not exist in gel. (B) Enlarged gels in combination with histograms illustrate the variations of representative spots at different time points (a, b: 0 hour; c, d: 24 hours; e, f: 48 hours; and g, h: 72 hours)	117



- 4.3 Silver-stained 2-DE gels of leaf proteins of rubber clone PB 260 inoculated with isolate CKT05B (Race 1) of *Corynespora cassiicola*. (A) Sample collected at 72 hours after inoculation. Names and positions of differentially expressed protein spots are indicated. Lines with + sign show the putative position of spots that do not exist in gel. (B) Enlarged gels in combination with histograms illustrate the variations of representative spots at different time points (a, b: 0 hour; c, d: 24 hours; e, f: 48 hours; and g, h: 72 hours) 120
- 4.4 Silver-stained 2-DE gels of leaf proteins of rubber clone PB 260 with isolate CLN16 (Race 2) of *Corynespora cassiicola*. (A) Sample collected at 72 hours after inoculation. Names and positions of differentially expressed protein spots are indicated. Lines with + sign show the putative position of spots that do not exist in gel. (B) Enlarged gels in combination with histograms illustrate the variations of representative spots at different time points (a, b: 0 hour; c, d: 24 hours; e, f: 48 hours; and g, h: 72 hours) 124



LIST OF APPENDICES

Appendix		Page
A1	Binary matrix data of 106 DNA bands generated from 21 <i>Corynespora cassiicola</i> isolates using 8 ISSR primers	164-165
A2	Nei and Li's similarity matrix of 21 <i>Corynespora cassiicola</i> isolates calculated from binary matrix data of 106 ISSR bands	166
A3	Percent disease intensity (PDI) and arcsine square-root transformed data of selected rubber clones inoculated with three representative <i>Corynespora cassiicola</i> isolates using detached leaf assay	167
A4	Genomic DNA concentrations extracted from mycelia of 21 <i>Corynespora cassiicola</i> isolates using modified CTAB extraction method ($\mu\text{g/mL}$ extracted sample)	168
A5	Protein concentrations of the extracted samples measured using Bradford protein assay method ($\mu\text{g/mL}$)	168
B1	Reference gel containing protein sample and 2-D SDS-PAGE Standards (Cat# 161 0320) (Bio-Rad, Hercules, USA)	169
B2	Histograms of differentially expressed protein spots detected from leaf protein profiles of rubber clone RRIM 600 inoculated with isolate CKT05B (Race 1) of <i>Corynespora cassiicola</i>	169
B3	Histograms of differentially expressed protein spots detected from leaf protein profiles of rubber clone RRIM 600 inoculated with isolate CLN16 (Race 2) of <i>Corynespora cassiicola</i>	170
B4	Histograms of differentially expressed protein spots detected from leaf protein profiles of rubber clone PB 260 inoculated with isolate CKT05B (Race 1) of <i>Corynespora cassiicola</i>	171
B5	Histograms of differentially expressed protein spots detected from leaf protein profiles of rubber clone PB 260 inoculated with isolate CLN16 (Race 2) of	172



	<i>Corynespora cassiicola</i>	
C1	Analysis of variance (ANOVA) and Duncan's multiple range test of colony growth rates of 21 <i>Corynespora cassiicola</i> isolates	173
C2	Analysis of variance (ANOVA) and Duncan's multiple range test of colony sizes of 21 <i>Corynespora cassiicola</i> isolates	174
C3	General linear model analysis (GLM) and Duncan's multiple range test of conidia length of 21 <i>Corynespora cassiicola</i> isolates	175
C4	General linear model analysis (GLM) and Duncan's multiple range test of conidia width of 21 <i>Corynespora cassiicola</i> isolates	176
C5	General linear model analysis (GLM) and Duncan's multiple range test of conidia pseudoseptate of 21 <i>Corynespora cassiicola</i> isolates	177
C6	Analysis of variance (ANOVA) and Duncan's multiple range test of arcsine square-root of percent disease intensity (PDI) of selected rubber clones inoculated with <i>Corynespora cassiicola</i> isolates CKT05B using detached leaf assay	178
C7	Analysis of variance (ANOVA) and Duncan's multiple range test of arcsine square-root of percent disease intensity (PDI) of selected rubber clones inoculated with <i>Corynespora cassiicola</i> isolates CLN16 using detached leaf assay	179
C8	Analysis of variance (ANOVA) of arcsine square-root of percent disease intensity (PDI) of selected rubber clones inoculated with <i>Corynespora cassiicola</i> isolates CKT05D using detached leaf assay	180
D	Formulation of media and solutions	181-183



LIST OF ABBREVIATIONS

%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