INCIDENCE OF ORANGE SPOTTING AND CHARACTERIZATION OF COCONUT CADANG-CADANG VIROID VARIANTS IN SELANGOR AND SABAH OIL PALM PLANTATIONS, MALAYSIA

CHEONG LI CHU

ITA 2012 12
INCIDENCE OF ORANGE SPOTTING AND CHARACTERIZATION OF COCONUT CADANG-CADANG VIROID VARIANTS IN SELANGOR AND SABAH OIL PALM PLANTATIONS, MALAYSIA

CHEONG LI CHU

MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA

2012
INCIDENCE OF ORANGE SPOTTING AND CHARACTERIZATION OF COCONUT CADANG-CADANG VIROID VARIANTS IN SELANGOR AND SABAH OIL PALM PLANTATIONS, MALAYSIA

By

CHEONG LI CHU

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

November 2012
ORANGE SPOTTING AND CHARACTERIZATION OF COCONUT CADANG-CADANG VIROID VARIANTS IN SELANGOR AND SABAH OIL PALM PLANTATIONS, MALAYSIA

By

CHEONG LI CHU

November 2012

Chair: Ganesan Vadamalai, PhD

Institute: Tropical Agriculture

Orange Spotting (OS) is a disorder of oil palm (Elaeis guineensis Jacq.) that has been associated with coconut cadang-cadang viroid (CCCVd), where CCCVd variants characterized from oil palms had more than 90% sequence similarity with CCCVd in coconut. These CCCVd oil palm variants pose a threat to the oil palm industry in Malaysia because CCCVd has caused extensive losses to coconut production in the Philippines. Routine screening and detection methods for CCCVd are radioactive based methods, which are expensive and hazardous. In view of this, the objectives of this study are (a) to survey for OS incidence in Selangor and Sabah oil palm plantations and to sequence the CCCVd variants in oil palm, (b) to develop a non-radioactive RPA using DIG-labelled probe for detection of CCCVd variants in oil palm. Preliminary survey of OS incidence was done in Sabah (Papar and Tawau...
areas) and Selangor (Kuala Selangor area) from 2009 until 2010 by symptom observation. A minimum of 500 palms were randomly observed from the study plots for the assessment of disease incidence (DI) and disease severity. The visual observation was then supported by molecular analysis and sequence characterization. Regarding to the experiment results from molecular diagnosis, palms with OS mild (1-30%) were excluded from the measurement of incidence. Therefore, OS incidence in Sabah ranged from 13-17% of total surveyed palms, while in Selangor the OS incidence was slightly higher at 25%. This is the first report survey of OS incidence conducted in commercial oil palm plantations in Selangor and Sabah. The surveys do not reflect actual OS incidence rate of an entire estate or plantation due to the limited number of palms surveyed. Larger scale field survey in oil palm plantations in Malaysia is recommended for future research together with a consistent OS monitoring system in order to quantify the actual OS incidence. Of total 27 tested palms, approximately 48% of palms presented positive signal to CCCVd on molecular diagnosis using dot blot assay and about 19% of tested palms were characterized and sequenced. A new oil palm CCCVd variant (OP<sub>252</sub>-SBK88 and OP<sub>252</sub>-SBTW122) was discovered in Sabah that is being reported for the first time compared to those CCCVd variants reported from previous studies in Malaysian oil palm showed over 95% sequence similarity to the 246 nucleotides form of CCCVd in coconut palm. Non-radioactive RPA using digoxigenin (DIG)-labelled CCCVd full length cRNA probes was developed for detection of CCCVd from oil palm with OS moderate and severe symptoms. Two out of four selected positive palms from dot blot screening (OP<sub>SBK88</sub> and OP<sub>SBTW122</sub> from Sabah) were detected positive for CCCVd variants, produced three protected fragments approximately 175, 125 and 50 nucleotides after RNAse digestion similar to the
positive control (OP$_{SRDS}$). In this study, the sensitivity of DIG-labelled RPA was lesser compared to the previous study with radioactive-based detection; however, all of the practical advantages of non-radioactive RPA were over radioactivity in term of cost and time consuming, labor intensive, handling safety and environmental health.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk mendapat Ijazah Sarjana

KEJADIAN PENYAKIT ORANGE SPOTTING DAN PENCIRIAN VARIAN COCONUT CADANG-CADANG VIROID DARI PERLADANGAN KELAPA SAWIT DI SELANGOR DAN SABAH, MALAYSIA

Oleh

CHEONG LI CHU

November 2012

Pengerusi: Ganesan Vadmalai, PhD
Institut: Pertanian Tropika

Orange spotting (OS) adalah penyakit kelapa sawit (Elaeis guineensis Jacq.) yang bertalian rapat dengan coconut cadang-cadang viroid (CCCVd), dimana varian CCCVd pada pokok kelapa sawit telah dicirikan dan mencapai persamaan jujukan lebih daripada 90% berbanding dengan CCCVd_{246} pada pokok kelapa. Varian CCCVd pada kelapa sawit boleh menjadi satu ancaman yang serius kepada perindustrian kelapa sawit di Malaysia kerana serangan CCCVd pada pokok kelapa telah mengakibatkan kerugian padah bagi pengeluaran kelapa di negara Filipina. Pengesanan dan saringan harian CCCVd secara biasanya dilakukan dengan meggunakan kaedah radioaktif yang mahal malang juga merbahaya. Dengan demikian, objektif utama kajian ini adalah (a) untuk meninjau kejadian OS di perladangan kelapa sawit di Selangor dan Sabah dan pencirian varian CCCVd dalam
kelapa sawit, (b) untuk membangunkan ribonuclease protection assay (DIG-labelled RPA) yang tidak berasaskan unsur radioaktif sebagai kaedah pengesanan varian CCCVd. Tinjauan OS telah dijalankan dalam kawasan Papar dan Tawau di Sabah dan Kuala Selangor di Selangor sejak 2009 sehingga 2010 melalui simptom permerhatian. Sekurang-kurangnya 500 pokok kelapa sawit diambilkira secara rawak dari plot yang terpilih untuk kajian selidik OS kejadian dan tahap kecedearaan. Hasil pemerhatian kemudian disahkan dengan data sokongan yang terdapat daripada molekular análise. Mengikut keputusan molekular, kelapa sawit yang simptom ringan dikecualikan dan tidak diambil kira sebagai OS. Dengan sedemikian, kadar kejadian OS di Sabah adalah merangkumi 13-17%, manakala kejadian OS di Selangor setinggi 25%. Ini merupakan laporan pertama bagi tinjauan OS di perladangan kelapa sawit di Selangor dan Sabah. Walau bagaimanapun, kajian selidik ini tidak mencerminkan kadar sebenar OS di ladang secara menyeluruh disebabkan oleh kuantiti pokok kelapa sawit adalah terhad untuk ditinjau. Kajian selidik yang berskala besar perlu dianjurkan untuk menentukan kadar sebenar OS di Malaysia. Daripada jumlah 27 pokok sawit yang teruji dari Sabah dan Selangor, 48% daripada sampel tersebut menunjukkan tanda positif kepada CCCVd-like RNAs melalui kaedah molekular dengan menggunakan saringan dot blot, namun hanya 19% daripadanya dapat dicirikan menggunakan jujukan RNA. Penemuan varian baru CCCVd pada sampel kelapa sawit dari Sabah (OP_{252-SBK88} and OP_{252-SBTW122}) dengan persamaan jujukan melebihi 95% berbanding dengan CCCVd_{246} dalam pokok kelapa. RPA dengan DIG-labelled probes telah dibangunkan dan berjaya mengesan CCCVd varian pada pokok sawit yang bersimptom OS serderhana dan OS parah. Dua daripada empat pokok sawit (OP_{SBK88} and OP_{SBTW122}) yang terpilih daripada kajian dot blot menunjukkan tanda positif kepada varian CCCVd dengan...
menghasilkan tiga serpihan terlindung. Jalur-jalur yang terhasil dari sampels kira-
kiranya 175, 125 dan 50 nukleotida selepas penghadaman oleh RNAses, dimana ia 
seriras dengan jalur-jalur pada pokok kawalan (OPSRD6). Dalam kajian ini, kepekaan 
DIG-labelled RPA didapati lemah berbanding dengan kajian awal yang terpapar 
dengan menggunakan kaedah radioaktif. Namun sedemikian, RPA berasaskan unsur 
tanpa radioaktif adalah lebih manfaat secara praktikal daripada kaedah radioaktif dari 
segi kos dan masa, tenaga pekerja, keselamatan dan kesiapan alam persekitaran.
ACKNOWLEDGEMENTS

I am thankful and express special gratitude to my main supervisor, Dr. Ganesan Vadamalai as well as my co-supervisors, Prof. Dr. Sariah Meon and Dr. Idris Abu Seman for their patience, concern, encouragement and critical discussion throughout committee meetings and the findings of my study. Special thanks are also due to Prof. John Randles from The University of Adelaide, Australia for his valuable advices and comments during his laboratory visit.

I also acknowledge to the Malaysia Palm Oil Board (MPOB) for their financial support and sampling of oil palm leaves from Sabah. Special thanks also the oil palm plantations in Selangor and Sabah for their kindness and co-operation for allowing survey and sampling of oil palm materials. My appreciation to Institute of Tropical Agriculture (ITA), UPM for providing good working conditions for my laboratory studies. In addition, all the staffs from ITA, UPM were very helpful and supportive.

Best regards also to all my dearest laboratory partners and friends who supported me in many ways: Roslina, Sathis, Chee Yong, Lee Chuan, Carmen, Jackie, Sze Ling, Roger, Yee Min and particularly Dr. Hendry Joseph and Ying Hooi for their assistance during the field survey for OS incidence. Besides that, I would like to thank Mr. Reza, Dr. Naghmeh Nejat, and Dr. Kong Lih Ling for sharing and critical discussions during the course of my study. To my beloved family and husband; your patience, understanding and endless support and love are greatly appreciated.
I certify that a Thesis Examination Committee has met on 8 November 2012 to conduct the final examination of Cheong Li Chu on her thesis entitled "Incidence of Orange Spotting and Characterization of Coconut Cadang-Cadang Viroid Variants in Selangor and Sabah Oil Palm Plantations, Malaysia" 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 Master’s Degree.

Members of the Thesis Examination Committee were as follows:

**Zainal Abidin bin Mior Ahmad, PhD**  
Professor Madya Dr.  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Chairman)

**Jugah bin Kadir, PhD**  
Prof Madya Dr.  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Internal Examiner)

**Lau Wei Hong, PhD**  
Lecturer  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Internal Examiner)

**Sepiah Muid, PhD**  
Lecturer  
Faculty of Technology and Science Resources  
Universiti Malaysia Sarawak  
Malaysia  
(External Examiner)

---

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

Date: 23 January 2013
This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirements for the degree of Master. The members of the Supervisory Committee were as follows:

Ganesan A/L Vadomalai, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

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

Idris Abu Seman, PhD
Senior Lecturer
Ganoderma and Disease Research of Oil Palm Unit
Malaysia Palm Oil Board
(Member)

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

Date:
DECLARATION

I declare that the thesis is my original work except for the 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 institution.

__________________
CHEONG LI CHU

Date: 8 November 2012
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td></td>
<td>ABSTRAK</td>
<td>v</td>
</tr>
<tr>
<td></td>
<td>ACKNOWLEDGEMENTS</td>
<td>viii</td>
</tr>
<tr>
<td></td>
<td>APPROVAL</td>
<td>ix</td>
</tr>
<tr>
<td></td>
<td>DECLARATION</td>
<td>xi</td>
</tr>
<tr>
<td></td>
<td>LIST OF TABLES</td>
<td>xiv</td>
</tr>
<tr>
<td></td>
<td>LIST OF FIGURES</td>
<td>xv</td>
</tr>
<tr>
<td></td>
<td>LIST OF ABBREVIATIONS</td>
<td>xvii</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>INTRODUCTION</th>
<th>1</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>LITERATURE REVIEW</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Orange spotting (OS) of oil palm</td>
<td>4</td>
</tr>
<tr>
<td>2.1</td>
<td>Symptoms and effects of OS on growth</td>
<td>5</td>
</tr>
<tr>
<td>2.1.1</td>
<td>Distribution, spread and epidemiology</td>
<td>8</td>
</tr>
<tr>
<td>2.2</td>
<td>Viroids</td>
<td>8</td>
</tr>
<tr>
<td>2.2.1</td>
<td>Biological properties</td>
<td>9</td>
</tr>
<tr>
<td>2.2.2</td>
<td>Viroid classification and structure</td>
<td>10</td>
</tr>
<tr>
<td>2.3</td>
<td>Coconut cadang-cadang viroid (CCCVd)</td>
<td>15</td>
</tr>
<tr>
<td>2.3.1</td>
<td>Variants of CCCVd</td>
<td>15</td>
</tr>
<tr>
<td>2.3.2</td>
<td>Host range</td>
<td>17</td>
</tr>
<tr>
<td>2.3.3</td>
<td>Ecology and control</td>
<td>17</td>
</tr>
<tr>
<td>2.3.4</td>
<td>Diagnostic methods for CCCVd</td>
<td>18</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>GENERAL METHODOLOGY</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Materials</td>
<td>22</td>
</tr>
<tr>
<td>3.1.1</td>
<td>Collection of leaf samples</td>
<td>22</td>
</tr>
<tr>
<td>3.1.2</td>
<td>Biochemical and miscellaneous chemicals</td>
<td>22</td>
</tr>
<tr>
<td>3.1.3</td>
<td>Gels, buffers, solvents and bacterial media</td>
<td>23</td>
</tr>
<tr>
<td>3.1.4</td>
<td>Kits used in this study</td>
<td>23</td>
</tr>
<tr>
<td>3.2</td>
<td>Methods</td>
<td>23</td>
</tr>
<tr>
<td>3.2.1</td>
<td>Nucleic acid extraction</td>
<td>23</td>
</tr>
<tr>
<td>3.2.2</td>
<td>Gel electrophoresis</td>
<td>24</td>
</tr>
<tr>
<td>3.2.3</td>
<td>Ethidium bromide (EtBr) stain</td>
<td>25</td>
</tr>
<tr>
<td>3.2.4</td>
<td>In-vitro transcription of digoxigenin (DIG)-labelled CCCVd full length antisense cRNA probes</td>
<td>25</td>
</tr>
<tr>
<td>3.2.5</td>
<td>Hybridization assay</td>
<td>27</td>
</tr>
<tr>
<td>3.2.6</td>
<td>Dot blot hybridization</td>
<td>27</td>
</tr>
<tr>
<td>3.2.7</td>
<td>Northern blot hybridization</td>
<td>28</td>
</tr>
</tbody>
</table>
4 SURVEY FOR DISEASE INCIDENCE AND DISEASE SEVERITY OF OS, PRELIMINARY SCREENING AND CHARACTERIZATION OF CCCVd VARIANTS IN OIL PALM

4.1 Introduction 37
4.2 Materials and methods 38
4.2.1 Survey locations 38
4.2.2 Data collections 38
4.2.3 Sampling of surveyed palms 39
4.2.4 Preliminary screening for CCCVd-like RNAs by dot blot assay 40
4.2.5 RT-PCR amplification 41
4.2.6 Cloning and sequencing 41
4.3 Results 42
4.4 Discussion 63

5 DEVELOPMENT OF NON-RADIOACTIVE RPA FOR THE DETECTION OF CCCVd OIL PALM VARIANTS

5.1 Introduction 68
5.2 Leaf materials 70
5.3 Methods 70
5.3.1 Nucleic acid extraction 71
5.3.2 Non-radioactive RPA using DIG-labelled probes 71
5.3.3 Detection of CCCVd oil palm variants from RPA products 72
5.3.4 Immunological detection of DIG-labelled RPA 73
5.4 Results 73
5.5 Discussion 80

6 SUMMARY, GENERAL CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH 84

REFERENCES 86
APPENDICES 93
BIODATA OF STUDENT 108