UNIVERSITI PUTRA MALAYSIA

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

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By

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

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November 2012

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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_{252}-SBK88 and OP_{252}-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_{SBK88} and $OP_{SBTW122}$ 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_{SRD6}). 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

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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 análisis. 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} dan $OP_{SBTW122}$) yang terpilih daripada kajian dot blot menunjukkkan 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 (OP_{SRD6}). 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 pratikal daripada kaedah radioaktif dari segi kos dan masa, tenaga pekerja, keselamatan dan kesihatan alam persekitaran.

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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.

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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.

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CHEONG LI CHU

Date: 8 November 2012

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