



UNIVERSITI PUTRA MALAYSIA

***POST-TRANSCRIPTIONAL GENE SILENCING (PTGS) IN OIL PALMS
INFECTED WITH COCONUT CADANG-CADANG VIROID (CCCVd)
VARIANTS***

WU YING HOOI

ITA 2012 13

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VARIANTS**

By

WU YING HOOI

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

August 2012

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DEDICATION

**To my beloved parents, sisters, brothers, supervisor and friends for their
inspiration, love and support**

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

**POST-TRANSCRIPTIONAL GENE SILENCING (PTGS) IN OIL PALMS
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August 2012

Chairperson: Ganesan A/L Vadamalai, PhD

Institute : Institute of Tropical Agriculture

Post-transcriptional gene silencing (PTGS), also known as RNA silencing, is a natural host defense mechanism that regulates gene expression in eukaryotes and results in the sequence-specific degradation of single stranded RNAs (ssRNAs) from genetic elements of internal or foreign origin. Based on the detection of short interference RNA (siRNAs), several viroids have been shown to induce PTGS. Recently, *Coconut cadang-caang viroid* (CCCVd) variants had been detected in commercial oil palm plantations in Malaysia. CCCVd is the causal agent of the lethal Coconut cadang-cadang disease in the Philippines. Losses over 30 million coconut palms have been estimated since the disease was first reported. CCCVd variants in oil palm were reported to be present at much lower concentrations than of CCCVd in coconut palm. Reports suggested that the low concentrations of the oil palm variants could be due to PTGS. In view of this, these studies were conducted to examine the presence of PTGS targeting against CCCVd variants in oil palm, optimize the detection method and also sequence the CCCVd

variants in oil palm with PTGS. Samples were collected from commercial oil palm plantations in Malaysia. Large amount of nucleic acid extractions, polyacrylamide gel electrophoresis, electroblotting, hybridization and immunological detections were optimized for detection of siRNA and the CCCVd variant. The results show that combination of Phenol-SDS extraction, 15% denaturing polyacrylamide gel electrophoresis, electroblotting and autoradiography showed the best result for the detection of CCCVd variants. siRNA were absent from the oil palm isolates that were sampled in this study, it could be due to undetectable, suppression from host or quasi species. CCCVd variants were sequenced from four symptomatic palms and one asymptomatic oil palm with Reverse-transcriptase polymerase chain reaction (RT-PCR), cloning and sequencing. The expected amplicons of about 200-300 bp were successfully amplified, cloned and sequenced. The sequence showed 99% sequence homology to CCCVd (246 nt) from coconut variants.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

POST-TRANSCRIPTIONAL GENE SILENCING (PTGS) PADA KELAPA SAWIT YANG DIJANGKITI OLEH VARIAN COCONUT CADANG CADANG VIROID (CCCVd)

Oleh

WU YING HOOI

Ogos, 2012

Pengerusi: Ganesan A/L Vadamalai, PhD

Institut : Institut Pertanian Tropika

Post-transcription gene silencing (PTGS), juga dikenali sebagai *RNA silencing*, mekanisme PTGS adalah satu pertahanan perumah semulajadi daripada unsur-unsur genetik dalaman atau asing. PTGS dapat mengawal ekspresi gen dalam eukarot dan menyebabkan degradasi mRNAs kepada unsur-unsur yang pendek (siRNAs). Baru-baru ini, *Coconut cadang-cadang viroid* (CCCVd) telah dikesan di ladang kelapa sawit komersial di Malaysia. CCCVd adalah penyebab maut penyakit cadang-cadang pada pokok kelapa di Filipina. Anggaran kerugian oleh penyakit cadang-cadang pada pokok kelapa adalah melebihi 30 juta pohon-pohon kelapa sejak penyakit ini mula-mula diiktiraf. Walau bagaimanapun, varian CCCVd dalam kelapa sawit yang didapati berada pada kepekatan yang jauh lebih rendah daripada CCCVd kelapa. Laporan mencadangkan bahawa kepekatan rendah varian CCCVd pada pokok kelapa sawit mungkin disebabkan oleh PTGS. Disebabkan fakta-fakta ini, kajian ini telah dijalankan bertujuan untuk

memeriksa kehadiran PTGS dari varian CCCVd dalam kelapa sawit, mengoptimalkan kaedah pengesanan CCCVd dan siRNANYA, dan juga untuk mengesan urutan varian CCCVd dalam kelapa sawit dengan PTGS. Sampel kelapa sawit dikutip dari ladang-ladang komersial di Malaysia. Kaedah pengekstrakan asid nukleik, elektroforesis gel polyacrylamide, electroblotting, hibridisasi dan pengesanan imunologi telah dioptimumkan untuk pengesanan siRNA dan varian CCCVd. Kesimpulan mendapati gabungan pengekstrakan Fenol-SDS, 15% denaturing polyacrylamide gel, electroblotting dan autoradiography memberikan hasil yang terbaik untuk mengesan varian CCCVd. siRNA tidak dapat dikesan di dalam sampel kelapa sawit yang telah diekstrak. Urutan varian CCCVd dapat diujuk dari empat pokok bersimptom dan satu pokok kelapa sawit yang sihat dengan reaksi songsang-transcriptase rantaian polimerase (RT-PCR), pengklonan dan penjujukan. Amplicons yang dijangka kira-kira 200-300 bp telah berjaya diperbanyakkan, klon dan jujukan. Urutan yang didapati menunjukkan urutan homologi 99% untuk CCCVd daripada varian kelapa.

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

Ganesan A/L Vadamalai, PhD

Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Sariah Meon, PhD

Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Lau Wei Hong, PhD

Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

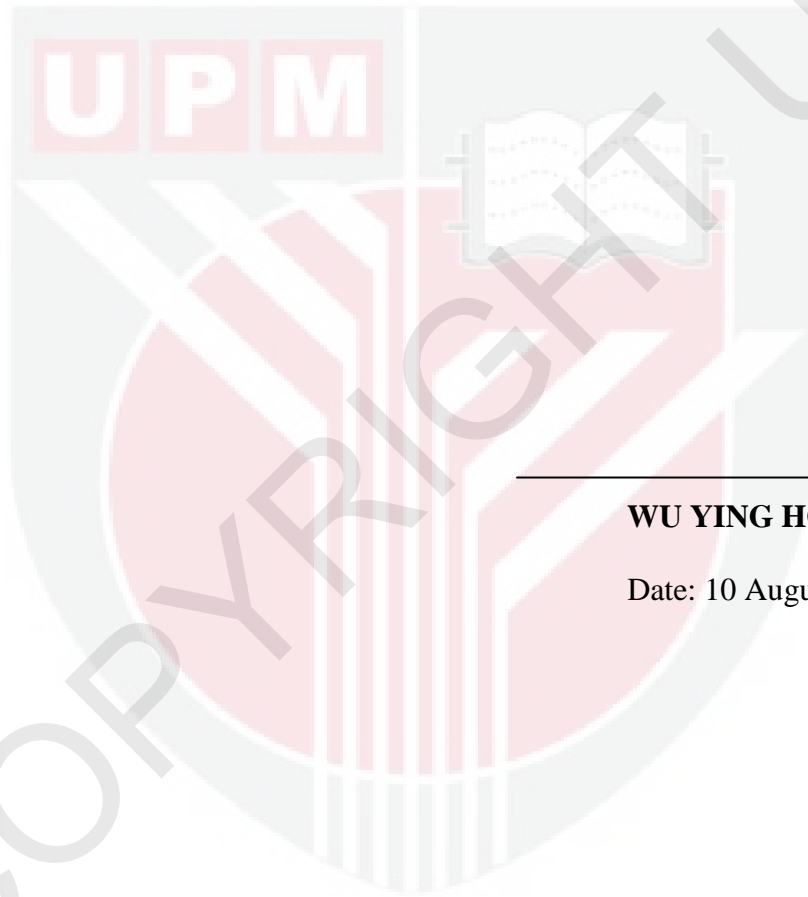
BUJANG BIN KIM HUAT, PhD

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 University Putra Malaysia or at any other institution.



WU YING HOOI

Date: 10 August 2012



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