



UNIVERSITI PUTRA MALAYSIA

***ORANGE SPOTTING, POTASSIUM CONTENT AND VERTICAL
TRANSMISSION OF COCONUT CADANG-CADANG VIROID VARIANTS
IN OIL PALM***

ROSLINA BINTI SULAIMAN

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OIL PALM**

By

ROSLINA BINTI SULAIMAN

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

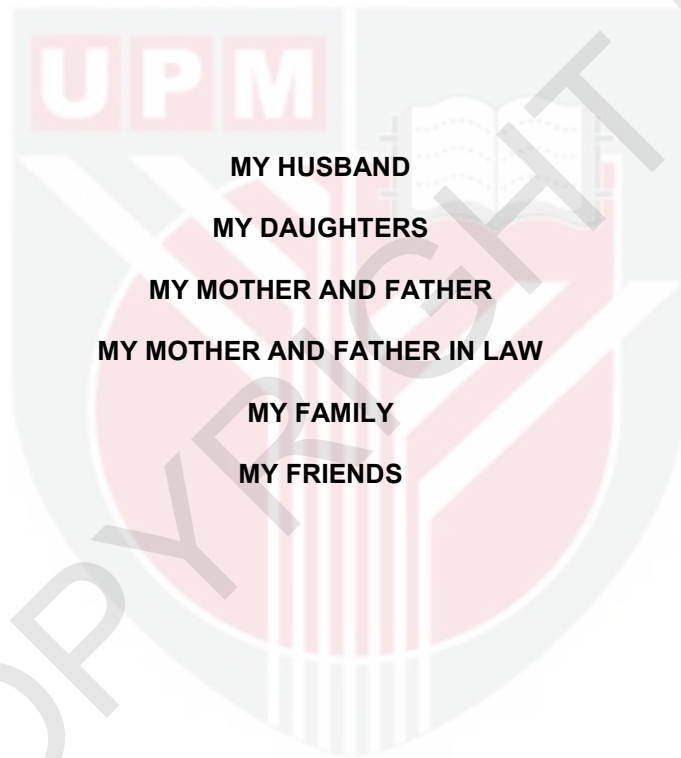
January 2019

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This thesis is dedicated to



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MY DAUGHTERS

MY MOTHER AND FATHER

MY MOTHER AND FATHER IN LAW

MY FAMILY

MY FRIENDS

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

ORANGE SPOTTING, POTASSIUM CONTENT AND VERTICAL TRANSMISSION OF *Coconut Cadang-cadang Viroid* VARIANTS IN OIL PALM

By

ROSLINA BINTI SULAIMAN

January 2019

Chair : Ganesan Vadamalai, PhD
Institute : Tropical Agriculture and Food Security

Oil palm, (*Elaeis guineensis* Jacq.) is a golden crop for Malaysia. It was first introduced to Malaysia as an ornamental plant and has become the most important commodity crop nowadays. Generally, Malaysian oil palms were free from diseases. However, with greatly increasing acreages in oil palm plantations, certain diseases and various seedling diseases became prominent. Orange Spotting (OS) is an emerging disease that has been reported in oil palm. It is caused by *Coconut cadang-cadang Viroid* (CCCVd). CCCVd is the causal agent of the lethal Cadang-cadang disease of coconut in the Philippines and the variants can be detected in nucleic acid extracts of both in symptomatic and asymptomatic oil palms in Malaysia. However, the epidemiology of CCCVd variants in oil palm is poorly understood. Potassium deficiency and CCCVd variant infection induced OS. Nevertheless, the symptoms are always being misled. The purpose of this study was to identify and characterize CCCVd variants from oil palm and to discuss the severity of orange spotting disease caused by CCCVd and the level of potassium content through leaf potassium analysis. The study was carried out in three main experiments which is survey and sampling, detection of CCCVd and potassium analysis. Through survey and sampling 30% OS disease incidence was recorded and 60 samples were collected. Detection of CCCVd through molecular cloning and sequencing found 48.3% samples contained CCCVd variants and five new CCCVd variants were characterized (Accession no. MF579860-MF579864). This study also found that OS symptom is not depending on or related directly to the level of potassium in oil palm leaves. The research was extended to determine vertical transmission of CCCVd variants through seeds. This objective was done

through embryo culture technique and germinated seedlings. The oil palm embryos were excised then were transplanted on Murashige and Skoog media and incubated for 60 days. The plantlet and seedling were extracted for total RNA and amplified using CCCVd specific primers. These experiments found that a CCCVd variant existed in the seed's embryo and germinated seedlings. These studies demonstrate that the germination rate for embryo and seedlings was 28.9% and 37.8% respectively and frequency of CCCVd variant transmission for embryo and seedlings was 20% and 11.1% respectively, thus confirming that CCCVd is vertically transmitted. The thesis was further investigating host and gene interaction through the last objective which is to examine the effect of CCCVd infection in host gene expression. *Non-radioactive Differential Display Reverse Transcriptase Polymerase Chain Reaction* (DDRT-PCR) was used to fulfill this objective. The result found that non-radioactive DDRT-PCR was a fast and simple technique that permitted detection of genes whose expression level is up-regulated as well as those that are down-regulated, however, it is not escaped from high production of false positive. The experiment found that only one band was successfully re-amplified with the same primer pairs and was identical as *formin binding protein 1* from FNBP 1 protein family.

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

ORANGE SPOTTING, KANDUNGAN KALIUM DAN PENYEBARAN VERTIKAL BAGI VARIAN *Coconut Cadang-cadang Viroid* PADA KELAPA SAWIT (*Elaeis guineensis* Jacq.)

Oleh

ROSLINA BINTI SULAIMAN

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Kelapa sawit, (*Elaeis guineensis* Jacq.) adalah tanaman berharga pada Malaysia. Ia mula diperkenalkan di Malaysia sebagai tumbuhan hiasan dan menjadi tanaman komoditi yang paling penting masa kini. Secara amnya, kelapa sawit di Malaysia bebas daripada penyakit. Walau bagaimanapun, dengan pembukaan banyak kawasan baru, pelbagai penyakit telah tersebar. Orange Spotting (OS) adalah penyakit baru yang dilaporkan dalam kelapa sawit. Ia disebabkan oleh varian *Coconut cadang-cadang Viroid* (CCCVd). CCCVd adalah agen penyebab penyakit Cadang-cadang pada pokok kelapa di Filipina dan variannya dapat dikesan dalam ekstrak nukleik asid pada pokok kelapa sawit yang mempunyai simptom dan pokok yang bebas simptom di Malaysia. Walau bagaimanapun, epidemiologi varian CCCVd dalam kelapa sawit masih kurang difahami. Kekurangan kalium dan jangkitan CCCVd boleh menghasilkan simptom OS. Oleh sebab itu, simptom tersebut sangat mengelirukan. Tujuan kajian ini adalah untuk mencari dan mencirikan varian CCCVd dalam kelapa sawit dan membincangkan tahap serangan OS dengan kandungan kalium dalam kelapa sawit. Kajian ini dijalankan dalam tiga eksperimen utama iaitu tinjauan dan pensampelan, pengesanan varian CCCVd dan analisis kalium. Melalui tinjauan dan persampelan, 30% kejadian penyakit OS telah direkod dan 60 sampel telah berjaya dikumpulkan. Sebanyak 48.3% sampel telah dikesan mengandungi varian CCCVd melalui pengklonan dan penjujukan molekul. Melalui eksperimen ini, lima varian CCCVd baru telah dikenalpasti (Accession no. MF579860-MF579864). Kajian ini juga mendapati bahawa simptom OS tidak bergantung atau berkaitan langsung dengan paras kalium dalam daun kelapa sawit. Kajian juga ini diperluas bagi menentukan

cara penyebaran varian CCCVd melalui biji. Objektif ini dilakukan melalui teknik kultur embrio dan semaian anak pokok. Embrio kelapa sawit telah dikeluarkan dan dipindahkan ke media Murashige dan Skoog dan diinkubasi selama 60 hari. Embryo yang bercambah telah diekstrak untuk mendapatkan RNA dan menggandakannya menggunakan primer khusus varian CCCVd. Eksperimen-eksperimen ini mendapati bahawa CCCVd wujud dalam embrio dan anak pokok. Kajian ini mendapati kadar pertumbuhan embrio dan anak pokok adalah 28.9% dan 37.8% dan kekerapan penghantaran gen kepada embrio adalah 20% dan kepada anak benih adalah 11.1% seterusnya mengesahkan bahawa CCCVd boleh tersebar melalui transmisi vertikal. Tesis ini diteruskan lagi dengan mengkaji interaksi gen dengan perumah melalui objektif terakhir yang iaitu kesan jangkitan varian CCCVd dalam ekspresi gen perumah. *Non-radioaktif Reverse Transcriptase Polymerase Chain Reaction* (DDRT-PCR) digunakan untuk memenuhi objektif ini. Kajian mendapati bahawa DDRT-PCR bukan radioaktif adalah teknik yang cepat dan mudah yang boleh mengesan perubahan regulasi gen namun ia juga menghasilkan banyak positif palsu. Eksperimen mendapati bahawa hanya satu jujukan Berjaya diampifikasi dengan menggunakan pasangan primer yang sama diklon dan dikenali sebagai protein pengikat formin 1 dari keluarga protein FNBP 1.

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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:

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LIST OF ABBREVIATIONS

µg	Microgram
g	Gram
µl	Microliter
L	Liter
ml	Milliliter
mg	Miligram
µg	Microgram
nm	Nanometer
min	Minute
°C	degree Celsius
%	percent
w/w	weight per weight
mg/ml	Milligram per milliliter
µg/ml	Microgram per milliliter
rpm	rotation per minute
UV	ultraviolet light
v/v	Volume per volume
w/v	Weight per volume
PDA	Potato dextrose agar
PDB	Potato dextrose broth
bp	Base pair
dNTP	Mixture of deoxynucleoside-triphosphate in equimolar amounts
DNA	Deoxyribonucleic acid
cDNA	complementary deoxyribonucleic acid
EDTA	Ethylenediamine tetra acetic acid
EtBr	Ethidium bromide
kb	kilo base
h	hour
M	Molar
TAE	Tris-acetic acid
U	unit
PCR	Polymerase chain reaction
PCIAA	Phenol:Chloroform:Isoamyl-alcohol
CIAA	Chloroform:Isoamyl-alcohol
DDRT-PCR	Differential display reverse transcriptase polymerase chain reaction
µg	Microgram
g	Gram
µl	Microliter
L	Liter
ml	Milliliter
mg	Miligram
µg	Microgram
nm	Nanometer
min	Minute
°C	degree Celsius
%	percent
w/w	weight per weight

mg/ml	Milligram per milliliter
µg/ml	Microgram per milliliter
rpm	rotation per minute
UV	ultraviolet light
v/v	Volume per volume
w/v	Weight per volume
PDA	Potato dextrose agar
PDB	Potato dextrose broth
bp	Base pair
dNTP	Mixture of deoxynucleoside-triphosphate in equimolar amounts
DNA	Deoxyribonucleic acid
cDNA	complementary deoxyribonucleic acid
EDTA	Ethylenediamine tetra acetic acid
EtBr	Ethidium bromide
kb	kilo base
h	hour
M	Molar
TAE	Tris-acetic acid
U	unit
PCR	Polymerase chain reaction

CHAPTER 1

GENERAL INTRODUCTION

The oil palm (*Elaeis guineensis* Jacq.) originated from West Africa and later was developed into an agricultural crop. The first commercial palm oil cultivation in Malaysia was done in Tennamaran, Selangor in 1917 (Azhar, 2009). In the early 1960s, palm oil cultivation increased significantly to reduce Malaysia's dependency on rubber and tin. Since then, Malaysia has become the world's second-largest producer of the commodity after Indonesia (Norhidayu et al., 2017). In 2017, the export value of palm oil and palm-based products in Malaysia was valued at approximately 77.81 billion ringgit (Statista, 2019).

Oil palm yield in Malaysia has been stagnating for the last few years due abiotic factors such as weather changes, drought and fertilizers and biological stress on the trees that can limit the productivity (MPOC, 2009). Historically, Malaysian oil palms were remarkably free from diseases (Turner and Bull, 1967). However, with increasing acreages in oil palm plantations including replanting coconut and rubber, certain diseases particularly basal stem rot and various seedling diseases became prominent (Turner and Bull, 1967). Through the development of certain diseases, it has become evident that diseases are an important issue in the oil palm industry nowadays and in the future.

Orange Spotting (OS) is an emerging disease that has been reported in commercial oil palm plantations in Southeast Asia and the South Pacific including Malaysia (Hanold and Randles, 1991; Vadamalai, 2005; Cheong, 2012; Selvaraja et al., 2012). OS is of a concern as recent study verified that the presence of *Coconut cadang-cadang viroid* (CCCVd; family Pospiviroidae, genus Cocadviroid) variants in nucleic acid extracts of both symptomatic and asymptomatic oil palms (Vadamalai, 2005; Vadamalai et al., 2006; Wu et al., 2013). CCCVd is the causal agent of the lethal Cadang-cadang disease of coconut in the Philippines that have killed more than 40 million coconut palms (Hanold and Randles, 1991). OS-infected palms yield has been reported with yield reduction of 25-50% compared to the nearest healthy palms (Randles, 1998).

Four CCCVd variants have been identified in oil palm (Vadamalai, 2005; Vadamalai et al., 2006; Wu et al., 2013). Selection of variants may occur through a host or environmental factor. One or more of the variants may predominate in the infection and the variants are relevant as single nucleotide changes may have major effects on symptom expression (Domigo et al., 1996) and diversity of this pathogen (Gago et al., 2009). Internal recombination events within the same viroid, generating terminal repeats, have also been reported for some nuclear-replicating viroids, such as CCCVd and CEVd (Fadda et al., 2003; Haseloff et al., 1982; Szychowski et al., 2005). Passage

through a host or environmental factors may result in selection of variants and impact disease management strategies, hence the existence and characterization of CCCVd variants in oil palm still needs to be studied.

The orange spotting caused by CCCVd variants can easily be misled with "confluent orange spotting" caused by deficiency of K element previously known as "genetic orange spotting" (Turner, 1981). Although Broeshart et al (1957) found that OS is not nutrient deficiency, and Gascon and Munier (1979) suggested that OS may be caused by a biotic agent, the relationship between CCCVd infection and potassium uptake in oil palm is not clearly established yet. The epidemiology of OS is still poorly understood, especially the natural spread and transmission of CCCVd through seeds. The viroid has been detected in the husk and embryo of the nuts and can be seed transmitted, but only at low frequencies (Hanold and Randles, 1991).

Current effective control methods for viroid diseases include detection and eradication as well as cultural control (Natalia et al., 2014). Many host-plants lack natural resistance to viroid infection and host factors play a key role in viroid spread and pathogenicity (Natalia et al., 2014). Furthermore, the information regarding host gene expression after CCCVd infection is still poorly explored. Therefore, this research was conducted to elucidate the epidemiology of OS in oil palm due to CCCVd infection with the following objectives: 1) to identify and characterize CCCVd variants from oil palm, 2) to discuss the severity of orange spotting disease caused by CCCVd and the level of potassium content through leaf potassium analysis 3) to determine the vertical transmission of CCCVd variants through seeds and 4) to examine the effect of CCCVd infection in host gene expression.

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