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INSECTS AS VECTORS FOR ORANGE SPOTTING DISEASE IN OIL PALM

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By

SANTURAKI AHMED ALIYU

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

June 2017

DEDICATION

To late Justice Aliyu Santuraki, late Alh Umarana Santuraki, Hajiya Binta Aliyu, Dr Usman Aliyu, Barister Aishatu Abdullahi Abba, My Sons (Abdullahi and Usman Ahmed), Dr. Yao Hua and Assoc. Prof. Dr. Ganesan Vadamalai.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Science

INSECTS AS VECTORS FOR ORANGE SPOTTING DISEASE IN OIL PALM

By

AHMED ALIYU SANTURAKI

June 2017

Chairman : Associate Professor Ganesan Vadamalai, PhD
Faculty : Agriculture

Virus and viroid plant diseases are responsible for enormous losses worldwide of about USD30-50 billion annually in cultivated and stored crops and are therefore major impediment to effective food production and distribution. Oil palm is one of the most important cultivated crop in Malaysia. Malaysia is one of the two major global suppliers of palm oil. Oil palm is however inflicted with many diseases, among which is an emerging disease called the orange spotting disease caused by Coconut cadang cadang viroid (CCCVd). The epidemiology of this disease is poorly understood in oil palm, including the natural transmission of CCCVd in the field. One possible mode of transmission is through insect vectors but no studies have been conducted to identify the insect vectors in any. This study was conducted to optimize extraction of CCCVd RNA from insect and to screen for possible insect vectors. Insects were collected from oil palm/coconut plantation and reared artificially in the laboratory and glass house for viroid acquisition testing. Four (4) species of insects were collected for the acquisition experiment; *Oryctes rhinoceros (*Beetles), *Metisa plana (*bagworms), *Elaeidobius kamerunicus* (Weevil) and *Cerataphis brasiliensis* (Aphids). Ten (10) oil palm seedling that has already been inoculated with CCCVd were used for feeding the selected insects vector candidates. Prior to feeding the insects, the oil palm seedlings were tested by RT-PCR, cloning and sequencing for the presence of CCCVd. RT-PCR analysis showed the presence of the expected 250 bp band in agarose gel electrophoresis indicating the presence of CCCVd RNA in the inoculated oil palm seedlings. Cloning and sequencing confirmed the presence of three CCCVd variants which had more than 98% sequence similarit with 246 nt CCCV oil palm variant. The insects were allowed to feed on the CCCVd inoculated seedlings in a cage covered with Mushin cloth. Based on their individual feeding method, the insects feed on their specific food part on infected oil palm seedlings (leaflets, inflorescence, etc.). The insects were then tested for presence of CCCVd with molecular detection using RT-PCR assay with CCCVd specific primers. The total nucleic acid extracted from all four species of insects by optimised NETME and Triazol extraction. Analysis nucleic acid samples of all four insect samples using RT-PCR showed no band at the expected size of 250 bp in 1.5% agarose gel electrophoresis. To exclude error in extraction, the

insect samples were extracted with plasmid CCCVd DNA using both NETME and Triazol extraction. RT-PCR analysis of plasmid mixed samples showed a band at 250 bp region but none was observed in the insect samples without the plasmid CCCVd DNA. Futhermore, four species of insects (*Setora nitens, Setothosea asigna, Coptotermas curvignathus, Mahasena corbetti*) collected from oil palm infected with CCCVd in the field also did not show positive bands in the RT-PCR assay. This indicates that there were no CCCVd RNA present in the tested insects and therefore, these insects might not be the vectors for CCCVd.

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

SERANGGA SEBAGAI VECTOR UNTUK MENGESAN PENYAKIT OREN DI DALAMPOKOK KELAPA SAWIT

Oleh

AHMED ALIYU SANTURAKI

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Penyakit tumbuhan virus dan viroid bertanggungjawab untuk kerugian besar di seluruh dunia sebanyak kira-kira USD30-50 bilion setiap tahun dalam tanaman yang ditanam dan disimpan dan oleh itu, halangan utama kepada penghasilan dan pengedaran makanan yang berkesan. Kelapa sawit merupakan salah satu tanaman yang paling penting di Malaysia. Malaysia adalah salah satu daripada dua pembekal utama minyak sawit. Namun, pengeluaran kelapa sawit disekati oleh banyak penyakit, di antaranya adalah penyakit yang sedang muncul iaitu penyakit "orange spotting" yang disebabkan oleh Coconut cadang cadang viroid (CCCVd). Epidemiologi penyakit ini kurang difahami dalam kelapa sawit, termasuk trasmissi semula jadi CCCVd di lapangan. Satu cara transmisi mungkin melalui vektor serangga tetapi tiada kajian telah dilakukan untuk mengenal pasti vektor serangga. Kajian ini dijalankan untuk mengoptimumkan pengekstrakan RNA CCCVd dari serangga dan untuk melihat kemungkinan serranga yang menjadi vektor penyakit. Serangga dikutip dari ladang kelapa sawit / kelapa dan dibela di makmal dan rumah kaca untuk kajian pengambilan viroid. Empat (4) spesies serangga telah dikumpulkan untuk kajian pengambilalihan; Oryctes rhinoceros (Beetles), Metisa plana (bagworms), Elaeidobius kamerunicus (Weevil) dan Cerataphis brasiliensis (Aphids). Sepuluh (10) anak benih kelapa sawit yang telah diinokulasi dengan CCCVd diberi sebagai bahan makanan calon vektor serangga yang dipilih. Sebelum proses pemakanan serangga, anak benih kelapa sawit diuji oleh RT-PCR, kloning dan penjujukan DNA untuk mengenalpasti kehadiran CCCVd. Analisis RT-PCR menunjukkan kehadiran band 250 bp seperti yang dijangkakan dalam elektroforesis gel agarose yang menunjukkan kehadiran RNA CCCVd dalam anak benih kelapa sawit yang ditanam. Pengklonan dan penjujukan mengesahkan kehadiran tiga varian CCCVd yang mempunyai urutan lebih 98% sama dengan varian kelapa sawit CCCV 246 nt. Serangga dibenarkan untuk makan anak benih yang disuntik CCCVd dalam sangkar yang ditutup dengan kain Mushin. Berdasarkan kaedah pemakanan masing-masing, serangga memakan bahagian makanan khusus mereka pada benih kelapa sawit yang dijangkiti (daun, bunga, dan lain-lain). Serangga kemudian diuji untuk kehadiran CCCVd dengan pengesanan

molekul menggunakan ujian RT-PCR dengan primer spesifik CCCVd. Jumlah asid nukleik yang diekstrak dari empat spesies serangga dengan pengekstrakan NETME dan Triazol yang dioptimumkan. Analisis sampel asid nukleik bagi semua empat sampel serangga yang menggunakan RT-PCR menunjukkan tiada band pada saiz yang dijangkakan iaitu 250 bp dalam 1.5% elektroforesis gel agaros. Untuk mengecualikan ralat dalam pengekstrakan, sampel serangga diekstrak dengan DNA CCCVd plasmid menggunakan pengekstrakan NETME dan Triazol. Analisis RT-PCR sampel campuran plasmid dengan serangga menunjukkan sebuah band di 250 bp tetapi tiada dalam sampel serangga tanpa DNA CCCVd plasmid. Lebih-lebih lagi, empat spesies serangga (Setora nitens, Setothosea asigna, Coptotermas curvignathus, Mahasena corbetti) yang dikutip dari minyak sawit yang dijangkiti CCCVd di lapangan juga tidak menunjukkan jalur positif dalam ujian RT-PCR. Ini menunjukkan bahawa tiada RNA CCCVd hadir dalam serangga yang diuji dan oleh itu, serangga ini mungkin tidak menjadi vektor untuk CCCVd.

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I certify that a Thesis Examination Committee has met on 8 June 2017 to conduct the final examination of Santuraki Ahmed Aliyu on his thesis entitled "Insects as Vectors for Orange Spotting Disease in Oil Palm" 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 of Science.

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

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LIST OF ACRONYMS AND ABBREVIATIONS

Acryl	Acrylamide
AMV-RT	avian myeloblastosis virus reverse transcriptase
BLAST	Basic local alignment tool
BP	base pair
CA	Chloroform-Iso-amyl alcohol
cDNA	Complementary deoxyribonucleic acid
CEVd	Citrus exocortis viroid
cRNA	Complementary ribonucleic acid
2D-PAGE	Two-dimensional polyacrylamide gel electrophoresis
dNTP	Deoxy nucleoside-triphosphates
DNA	Deoxyribonucleic acid
DW	Distilled water
EB	Elution buffer
EDTA	Ethylenediamine tetra acetic acid
EtBr	Ethidium bromide
G	Gram
Gb	Giga base
HCl	Hydrochloric acid
Hr	Hour
K	Kilo
L	Littre
M	Molar
M	Meter
U	Micro

M	Milli
Min	Minutes
mRNA	messenger RNA
NaCl	Sodium chloride
Nt	Nucleotide
NTC	non-template control
OS	orange spotting
PAGE	polyacrylamide gel electrophoresis
PCA	phenol-chloroform-iso-amyl alcohol
PCR	polymerase chain reaction
RNA	Ribonucleic acid
RT	Reverse transcription
RT-PCR	Reverse transcriptase polymerase chain
SDS	Sodium dodecyl distilled water
Sec	Second
TBE	Tris-Borate-EDTA
UV	Ultra violet
Vol	Volume
v/v	volume per volume
w/v	weight per volume

CHAPTER 1

INTRODUCTION

The origin of Oil palm (Elaeis guineensis Jacq) is West and Southwest Africa. Oil palm is the major agricultural product in Malaysia and it is the world's second largest producer. In Malaysia, over $30 billion is earned from the export of oil palm with about 14.7 million ha of land of oil palm (MPOB, 2014 FAO, 2011). Oil palm industry is facing challenges from several pests, diseases, and disorder such as pathogenic diseases, nutritional or physiological disorder (Anderson, 2006). Major diseases causing serious economic losses includes, freckle (Cercospora elaeidis), vascular wilt (Fusarium oxysporum f. sp. elaeidis) and basal stem rot (BSR) caused Ganoderma which can cause a loss of about 80%, (Rahamah Bivi et al., 2013). Most often these diseases are not well managed, expensive and difficult to control.

A rising issue in the oil palm industry is the Orange spotting (OS) disease. It is caused by a variant of Coconut cadang cadang viroid (CCCVd), which causes the lethal Cadang Cadang disease of coconut in Philippines (Hanold and Randles, 1991b: Hanold and Randles, 1991; Vadamalai et al., 2006). CCCVd oil palm variants had more than 90% sequence similarity with CCCVd from coconut (Vadamalai et al., 2006; Wu et al., 2013). CCCVd oil palm variants were found in both symptomatic and asymptomatic oil palms (Vadamalai et al., 2006; Wu et al., 2013). CCCVd variants present in much lower concentration in oil palm as compare to coconut therefore, detection of CCCVd variants in oil palm is difficult. In addition, the epidemiology of the OS disease is not properly understood in oil palm (Randles, 1998). CCCVd spreads naturally in the field but the mode of transmission is unclear (Hanold and Randles, 1991a; Randles, 1998).

Vector is an organism which can be defined as a biological agent that transmits pathogens or parasites from one animal or plant to another. Example of pathogens are; bacteria and virus. Organisms that can serve as disease vectors include nematodes, mites and insects (Hogenhout et al., 2008). Vector transmission is a complicated and challenging process; insect vector transmission research includes the issue of vector competence or efficiency in transmission which needs to be carried out regularly over time. Insects transmit most plant pathogens and viruses. About 55% of virus vectors can be attributed to hemipteran insects because their specialized feeding style of piercing and sucking (Chapman, 1998; Yao et al., 1996).

Studies have been conducted in the Philippines to look for insect vector of CCCVd but none of the insects tested were found to be a vector (Randles, 1998). There were no such studies conducted in the oil palm, thus, there are no vectors have been reported to transmit CCCVd variants in oil palm. CCCVd oil palm variants have been reported in Malaysia (Vadamalai, et al., 2006) but there is lack of knowledge in the mode of transmission of this viroid in oil palm. In view of this, the objectives of this study are:

I. To optimize RNA extraction and detection of CCCVd variants from potential insect vectors using RT-PCR with CCCVd specific primers.
II. To screen the insects for their ability to acquire the viroid from CCCVd infected oil palm seedlings.

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