



UNIVERSITI PUTRA MALAYSIA

***EARLY DETECTION OF ORANGE SPOTTING DISEASE IN OIL PALM
USING RED EDGE PARAMETERS***

KAMLESH GOLHANI

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**EARLY DETECTION OF ORANGE SPOTTING DISEASE IN OIL PALM
USING RED EDGE PARAMETERS**

By

KAMLESH GOLHANI

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

July 2018

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DEDICATION

Dedicated to Lord Sri Venkatesha”



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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

EARLY DETECTION OF ORANGE SPOTTING DISEASE IN OIL PALM USING RED EDGE PARAMETERS

By

KAMLESH GOLHANI

July 2018

Chairman : Associate Professor Siva Kumar Balasundram, PhD
Faculty : Agriculture

Viroids are single-stranded, low molecular weight, circular RNA between 246 and 401 nucleotides that lacks a protective protein coat. Viroids have only been found in the plants. Coconut cadang-cadang viroid (CCCVd; Genus Cocadviroid, Family Pospiviroidae) is one of the known species of viroids that has been reported to cause Orange Spotting (OS) disease in the oil palm (*Elaeis guineensis* Jacq.; Areaceae). OS disease is an emerging oil palm disease in Malaysia. Variants of CCCVd have been reported in both symptomatic and asymptomatic oil palm. Currently, there is no direct control measure reported that can be recommended to control OS disease. Replacement of the infected plants is the only measure to reduce losses. Molecular marker techniques are used to detect CCCVd-infected plants. But these techniques are destructive and typically take a longer time in molecular analysis and sequence characterisation. Therefore, for early disease detection, visible-near infrared spectroscopy was employed for the first time at the leaf scale to screen oil palm seedlings using a hand-held spectroradiometer. Glasshouse experiment was conducted on three-month-old inoculated and healthy oil palm seedlings for a duration of four months in two different years, 2015 and 2017. In this research, oil palm seedlings were inoculated with a CCCVd oil palm variant (OP246), and an ASD spectroradiometer was employed to measure reflectance from inoculated and control seedling. In particular, the red edge region (680-780 nm), which has been frequently shown to indicate plant stress, was investigated for selection of red edge wavebands, red edge indices, and development of the Orange Spotting Disease Index (OSDI) using red edge parameters. Firstly, using a standard foreoptic with a 25° Field of View (FOV), two red edge wavebands (i.e. 680 nm and 754 nm) were identified. Their reflectance sensitivity was also examined. Secondly, using a contact probe, two other red edge wavebands (i.e., 700 nm and 768 nm) and a red edge index (i.e., Enhanced Vegetation Index 2) were identified. Finally, a simple ratio, i.e. the sum of the first derivative spectra of right side – Red Edge Point (REP) to the sum of the first derivative spectra

of left side – REP of the red edge region, was developed as an OSDI. The OSDI values between experiment batch of 2015 and 2017 demonstrated a strong correlation ($r = 0.96$). The OSDI is a first spectral index developed for early detection of OS disease at the leaf scale and can be tested at canopy scale in the future. This study has proved that OS disease can be detected at an early stage using a hand-held spectroradiometer.



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

PENGESANAN AWAL PENYAKIT TOMPOK JINGGA DI KELAPA SAWIT MENGUNAKAN PARAMETER KELEBIHAN MERAH

Oleh

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Viroid adalah organisma yang sangat kecil mengadungi satu rantai, berat molekul yang rendah, size RNA diantara 246 dan 401 nukleotida dan tidak mempunyai lapisan protein pelindung. Viroid hanya ditemui di tumbuh-tumbuhan. Coconut cadang-cadang viroid (CCCVd; Genus *Cocadviroid*, Family Pospiviroidae) adalah salah satu spesies viroid yang diketahui yang menyebabkan Penyakit Orange Spotting (OS) dalam kelapa sawit (*Elaeis guineensis* Jacq., *Arecaceae*). Penyakit OS dalam kelapa sawit adalah penemuan terbaru di Malaysia. Varian CCCVd telah dilaporkan dalam kedua-dua kelapa sawit simptomatik dan asimptomatik. Pada masa ini, tiada langkah kawalan langsung yang dilaporkan yang boleh disyorkan untuk mengawal penyakit OS. Penggantian tumbuhan yang dijangkiti hanyalah langkah untuk mengurangkan kerugian. Teknik molekul digunakan untuk mengesan tumbuhan yang dijangkiti CCCVd. Tetapi teknik ini merosakkan dan biasanya mengambil masa yang lebih lama dalam analisis molekul dan jujukan rantai DNA. Oleh itu, untuk pengesanan penyakit awal, spektroskopi inframerah yang kelihatan pada masa dahulu digunakan pada skala daun untuk menanam benih kelapa sawit menggunakan spectroradiometer tangan. Dalam kajian ini, biji benih kelapa sawit disuntik dengan varian kelapa sawit CCCVd (OP₂₄₆) dan spectroradiometer ASD digunakan untuk mengukur anak benih yang disuntik. Anak kelapa sawit diletak di kaca rumah dilakukan selama lebih dari tiga bulan dalam dua tahun yang berlainan, iaitu tahun 2015 dan 2017. Khususnya, kawasan gelombang merah (680-780 nm), yang sering ditunjukkan untuk menunjukkan tekanan tumbuhan, disiasat diikuti oleh pemilihan pada kawasan tepi gelombang merah, indeks kelebihan merah, dan perkembangan Indeks Penyakit Bercahaya Orange (OSDI) dengan menggunakan parameter tepi merah. Pertama, menggunakan 25° foreoptic, dua helai kelebihan merah (iaitu 680 nm dan 754 nm) telah dikenal pasti berdasarkan titik permulaan dan titik cerun curam, masing-masing. Kepekaan and kejituan juga diperiksa. Kedua, menggunakan penyelidikan tumbuhan, dua helai pinggir merah lain (iaitu, 700 nm dan 768 nm) dan indeks kelebihan merah (iaitu, Indeks Peningkatan Tanaman 2) telah dikenalpasti. Nisbah mudah, iaitu jumlah

spektra derivatif pertama sebelah kanan Red Edge Point (REP) kepada jumlah spektra derivatif pertama sebelah kiri - REP dari kawasan pinggir gelombang merah, telah dicatatkan sebagai OSDI. Nilai OSDI antara kumpulan percubaan pada tahun 2015 dan 2017 telah menunjukkan korelasi yang kuat ($r = 0.96$). OSDI adalah indeks spektrum pertama yang dicatatkan untuk pengesanan awal penyakit OS pada skala daun dan boleh diuji pada skala kanopi pada masa akan datang. Kajian ini telah membuktikan bahawa penyakit OS dapat dikesan pada peringkat awal menggunakan spectroradiometer tangan.



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I certify that a Thesis Examination Committee has met on 9 July 2018 to conduct the final examination of Kamlesh Golhani on his thesis entitled "Early Detection of Orange Spotting Disease in Oil Palm Using Red Edge Parameters" 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 Doctor of Philosophy.

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LIST OF ABBREVIATIONS

ANN	Artificial Neural Network
ANOVA	Analysis of Variance
ANS	Automated Network Search
ARS	Agriculture Research Service
ASD	Analytical Spectral Devices
BPNN	Back-Propagation Neural Network
CCCVd	Coconut cadang-cadang viroid
CI	Confidence Interval
CIRE	Chlorophyll Index Red Edge
CChMVd	Chrysanthemum chlorotic mottle viroid
CLSI	Cercospora Leaf Spot- Index
CNN	Convolutional Neural Network
Dai	Days after inoculation
DSWI	Disease –Water Stress Index
ELM	Extreme Learning Machine
EVI 2	Enhanced Vegetation Index 2
FAPAR	Fraction of Absorbed Photosynthetically Active Radiation
FDR	First Derivative of Reflectance
FAO	Food and Agriculture Organization
FOV	Field-of-View
GIS	Geographic Information Systems
GOS	Genetic Orange Spotting
GPS	Global Positioning Systems
GVF	Green Vegetation Fraction

HD	High Definition
HI	Healthy Index
ICTV	International Committee on Taxonomy of Viruses
IR/R	Infrared/Red
LAI	Leaf Area Index
LDA	Linear Discriminant Analysis
LRDSI	Leaf Rust Disease Severity Index
LS-SVM	Least Squares Support Vector Machine
Lw	Laurel wilt
MLP	Multi-Layer Perceptron
MLPNN	Multi-Layer Perceptron Neural Network
MPOB	Malaysian Palm Oil Board
MSA	Multivariate Statistical Analysis
NCBI	National Center for Biotechnology Information
NDVI	Normalized Difference Vegetation Index
NETME	Natrium Chloride EDTA Tris-HCL Mercaptoethanol
NIR	Near Infrared
NLRHI	Normalized Leaf Rust Healthy Index
NN	Neural Network
NPP	Net Primary Productivity
OS	Orange Spotting
OSDI	Orange Spotting Disease Index
PAGE	Poly-acrylamide gel electrophoresis
PAR	Photosynthetically Active Radiation
PC	Principal Component

PC	Peach Calico
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PLS	Partial Least Squares
PMI	Powdery Mildew- Index
PNN	Probabilistic Neural Network
PLMVd	Peach latent mosaic viroid
RBF	Radial-Basis Function
RD	Reflectance Difference
REIP	Red Edge Infection Point
RENDVI	Red Edge Normalized Difference Vegetation Index
REP	Red Edge Point
RF	Random Forest
RNA	Ribonucleic Acids
RPA	Ribonuclease Protection Assay
RS	Reflectance Sensitivity
RT-PCR	The Reverse Transcription-Polymerase Chain Reaction
RT-LAMP	Reverse Transcription Loop-mediated Isothermal Amplification
RVI	Ration Vegetation Index
RWC	Relative Water Content
SAIL	Scattering by Arbitrarily Inclined Leaves
SAVI	Soil Adjusted Vegetation Index
SBRI	Sugar Beet Rust-Index
SD	Shifting Distance
SDI	Spectral Disease Index

SE	South East
SE	Standard Error
SLP	Single-Layer Perceptron
SOM	Self-Organising Map
SR	Simple Ratio
SRI	Simple Ratio Index
SVI	Spectral Vegetation Index
SVM	Support Vector Machine
SWIR	Short-Wave Infrared
TMV	Tobacco Mosaic Virus
UPM	Universiti Putra Malaysia
USDA, FAS	United States Department of Agriculture, Foreign Agriculture Service
VI	Vegetation Index
VNIR	Visible/Near-infrared

CHAPTER I

INTRODUCTION

Oil palm industry is the backbone of Malaysian economy. Malaysia is one of the largest producers and exporters of oil palm in the world. In 2017, Malaysia contributed greatly to the world palm oil industry accounting for approximately 29% of the production and 37% of the exports. In 2017 alone, the total exports of oil palm products has been increased from 23.29 million tonnes to 23.97 million tonnes, which is 2.9% higher than that of 2016 (MPOB, 2018). In the recent years, the Malaysian oil palm industry has been facing significant constraints from crop disease outbreaks leading to significant yield decline.

Orange Spotting (OS) is an emerging disease of oil palm (*Elaeis guineensis* Jacq.; Arecaceae) which has been associated with Coconut cadang-cadang viroid (CCCVd; Genus, *Cocadviroid*, Family, *Pospiviroidae*). Over 40 million coconut palms have been killed by CCCVd since first being described 1914 (Hanold and Randles, 1991a, Randles and Rodriguez, 2003). CCCVd variants characterised from oil palm (i.e. OP₂₄₆, OP₂₉₇, OP₂₉₃, and OP₂₇₀) had more than 90% sequence similarity with a 296-nt form of CCCVd variant in coconut palm (Vadamalai et al., 2006; Wu et al., 2013). Detection of CCCVd based on molecular biology techniques are destructive, expensive and time consuming. Instead, non-imaging hyperspectral remote sensing is a new indirect diagnostic approach that provides a rapid and non-destructive means to investigate crop disease (Sankaran et al., 2010).

1.1 Background

Recently, non-imaging hyperspectral remote sensing has emerged as a powerful technique for diagnosing a wide range of plant diseases. Non-imaging hyperspectral data are basically multivariate in nature, combining more than hundred contiguous spectral wavebands within the electromagnetic spectrum. Particularly, there has been an ongoing interest in non-imaging Visible/Near-infrared (VNIR) spectroscopy. VNIR spectroscopy employs wavebands between visible and Near-infrared (NIR) range (400-1050 nm) of the spectrum. This is a fast emerging technique that provides an optical means for non-destructive and cost-effective crop diagnosis.

A spectral index that is developed from a ratio of disease sensitive wavebands is called Spectral Disease Index (SDI). This technique involves selection of inflection points (i.e. peaks and troughs) which are found to be sensitive to plant disease. A disease sensitive waveband can indicate crop stress caused by a disease. No study has previously reported on the use of VNIR spectroscopy to diagnose OS disease at the leaf scale (nursery stage). This study aimed at developing Orange Spotting Disease Index (OSDI), which would become the first SDI for OS disease. For this, twenty novel red edge parameters (Li et al., 2016, Liu et al., 2007) were derived from red

edge region (680-780 nm) of the electromagnetic spectrum and tested. The red edge region, which is located between the far red and NIR range of the spectrum, has been frequently shown to indicate plant stress.

1.2 Research problems

1. Infection of CCCVd variants which causes bright orange non-necrotic spots (size about 2-3 mm) in oil palm frond, has been reported in commercial plantations in South East (SE) Asia and South West Pacific region (Randles et al., 1980; Imperical et al., 1985; Hanold and Randles, 1991b). Evidence of horizontal and vertical spread of CCCVd in commercial plantations has been seen in recent years. Viroids detected in DxP hybrids in Oceania and SE Asia has revealed a close association between CCCVd and a specific type of OS in oil palm. In addition, commercial oil palm with OS symptoms was reported to show stunting over a period of 5-15 years. OS disease was found to be irreversible and very lethal during this period (Randles et al., 2009).
2. In recent years, for detecting CCCVd in oil palm, many diagnostic assays have been evaluated such as the Reverse Transcription-polymerase Chain Reaction (RT-PCR) (Wu et al., 2013; Vadamalai et al., 2006), Ribonuclease Protection Assay (RPA) (Vadamalai et al., 2009), and Reverse Transcription Loop-mediated Isothermal Amplification (RT-LAMP) (Thanarajoo et al., 2014). These techniques have confirmed presence of low concentration of CCCVd variants in oil palm. However, due to low concentration of CCCVd, these techniques are neither consistent nor sensitive and not able to quantify the viroid concentrations (Thanarajoo, 2014).
3. Symptom expression is not a necessary outcome of CCCVd infection. Previously reported CCCVd oil palm variants, OP₂₉₇, OP₂₉₃ and OP₂₇₀, were all obtained from an asymptomatic oil palm in Malaysia (Vadamalai et al., 2006). In a recent investigation, an oil palm variant (CCCVd₂₉₃ OP) showed low accumulation of viroid load with no symptoms one year after inoculation (Thanarajoo, 2014).

1.3 Problem Statement

OS disease has similar foliar symptoms to that of potassium deficiency in oil palm. Symptomatic separability between OS disease and potassium deficiency is very difficult to achieve via visual assessment (Selvaraja et al., 2013). Asymptomatic infection of CCCVd even makes detection process much harder. So far, real-time and on the spot detection of OS disease has not been possible using any molecular techniques. To resolve this problem, oil palm seedlings can be screened using a non-imaging hand-held spectroradiometer. The spectroradiometer measures solar radiation reflected from the plants. On the basis of percentage reflectance, healthy and diseased plants can be differentiated. In this research, reflectance of OS infected seedlings will be measured using a spectroradiometer, pre-processed by pre-processing techniques, and analysed by multivariate analyses.

1.4 Scope and relevance

Application of hyperspectral ground sensor (hand-held spectroradiometer) for plant disease diagnosis is gaining much prominence due to its non-destructive feature. To date, there has only been one recent documentation on the use of spectroradiometer for detection of OS disease by Selvaraja et al. (2013) at the canopy scale. They used a spectroradiometer to discriminate between reflectance spectra of OS disease and potassium deficiency, both of which manifest symptoms that are similar.

A hand-held spectroradiometer can be used to screen oil palm seedlings prior to confirmation by any molecular techniques. On the basis of spectral signatures and OSDI values, oil palm seedlings can be differentiated between infected and healthy seedlings. This technique can also be considered as a promising tool for mechanical control of OS disease in order to eliminate infected seedlings. Randles et al. (2009) reported that CCCVd spreads naturally by unknown means. Its eradication has not yet been successful. Currently, it can only be managed by physical removal of affected palms.

The use of spectral signatures and OSDI to detect infected oil palm seedlings is a plausible idea. Mahlein et al. (2012a) studied different spectral signatures on host-pathogen (sugar beet-fungi) interaction for early detection of fungal leaf diseases of sugar beet (*Beta vulgaris*). Mahlein et al. (2013) have developed SDIs and Healthy Index (HI) for screening sugar beet foliage disease using a single waveband and normalized waveband differences. The works of Mahlein et al. (2012a; 2013) have motivated this work of developing a spectral index for OS disease. Diagnosis of plant diseases caused by fungi, bacteria and viruses using hand-held spectroradiometer is not new. However, the application of a hand-held spectroradiometer to study plant disease caused by viroids is new, particularly in oil palm.

In this research, oil palm seedlings were inoculated with a CCCVd oil palm variant (OP₂₄₆). This research was designed to observe the spectral changes between CCCVd-inoculated and healthy oil palm seedlings followed by development of spectral signatures, selection of red edge wavebands, selection of red edge indices and development of the OSDI using red edge parameters.

It is further noted that every new technique has its own merits and demerits. Every oil palm grower cannot be an expert on sensor application, data processing and data interpretation. Another concern is one needs to be proficient in developing indices from spectral wavebands. Several researchers have proposed several indices for disease detection rather than completely rely on spectral signatures. A spectral index plays an important role in data-dimensionality reduction and data analysis. Nonetheless, a long distance yet to cover for popularising the use of spectroradiometer for OS disease detection. The major issues are availability, affordability and applicability of the spectroradiometer.

1.5 Objectives

1. To measure spectral reflectance from CCCVd-inoculated and healthy oil palm seedlings and select optimal red edge wavebands and indices that offer maximum information for diagnosis of OS disease
2. To develop OSDI based on the red edge parameters for discriminating between diseased and healthy oil palm seedlings
3. To verify OSDI values in a repeated experiment and test the performance and efficacy of OSDI in diagnosing OS disease



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