UNIVERSITI PUTRA MALAYSIA

ASSESSMENT OF *GANODERMA* INFECTION IN OIL PALM (*ELAEIS GUINEENSIS* JACQ.) BY PRE-INOCULATION OF ARBUSCULAR MYCORRHIZA FUNGI AND ENDOPHYTIC BACTERIA

SHAMALA SUNDRAM

ITA 2012 2

ASSESSMENT OF *GANODERMA* INFECTION IN OIL PALM (*ELAEIS GUINEENSIS* JACQ.) BY PRE-INOCULATION OF ARBUSCULAR MYCORRHIZA FUNGI AND ENDOPHYTIC BACTERIA

By

SHAMALA SUNDRAM

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

August 2012

Dedicated to my dearest amma, hubby and kutties

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor Philosophy

ASSESSMENT OF *GANODERMA* INFECTION IN OIL PALM (*ELAEIS GUINEENSIS* JACQ.) BY PRE-INOCULATION OF ARBUSCULAR MYCORRHIZA FUNGI AND ENDOPHYTIC BACTERIA

By

SHAMALA SUNDRAM

August 2012

Chairperson: Sariah Meon Professor, PhD

Faculty : Institute of Tropical Agriculture

Basal stem rot of oil palm (*Elaeis guineensis* Jacq.) caused by *Ganoderma* spp is of major economic concern and it is the predominant disease of oil palm in Southeast Asia. The disease is a “white rot” process involving growth of the fungus within the oil palm as the lignin and cellulose are biodegraded. There is yet an effective measure to control the disease. Endophytic microorganisms such as arbuscular mycorrhizal fungi (AMF) and endophytic bacteria (EB) have been previously identified as potential biocontrol agents especially for soil borne diseases. These endophytes are able to suppress diseases with various mechanisms such as antibiosis, lysis, and production of antifungal compounds and induced systemic resistance (ISR). The use of endophytes is also preferred as they reside within the plant system avoiding rhizosphere competence

such as competition for space and nutrient between other soil microbes. Therefore this study was designed with the specific objectives to (i) investigate the antagonistic potential and compatibility between both endophytes, (ii) explore the potential disease suppression and vegetative growth enhancement in pre-inoculated oil palm seedlings challenged with *G. boninense*, (iii) determine the biochemical responses and gene expression profile in pre-inoculated seedlings challenged with *G. boninense,* and (iv) evaluate the effect of single and a consortium of endophytic microorganisms in field trial using the seedling baiting technique. AMF (*Glomus intraradices* UT 126 and *G. clarum* BR152B) and EB (*Pseudomonas* strain UPMP3 and *Burkholderia* strain UPMB3) represent the endophytic microbes tested in this study. Symbiotic interactions were observed between AMF species; *Glomus intraradices* UT 126 and *G. clarum* BR152B with *Pseudomonas* strain UPMP3 and *Burkholderia* strain UPMB3. Both EB strains significantly increased germination and hyphal length of AMF spores by 180 to 240%, respectively. This is a novel finding as the EB strains were never reported as potential mycorrhizal helper bacteria (MHB). Antagonistic effect of EB strains was demonstrated by the percentage inhibition of radial growth of *G. boninense* at >86% and >74% by UPMP3 and UPMB3, respectively. Scanning electron micrographs revealed severe morphological deformities such as shrivelling, flattening and shrinking of *G. boninense* hyphae in the presence of UPMP3 and UPMB3 strains. In addition, production of POX, PPO, chitinase and β-1, 3-glucanase during pre and post infection were enhanced in pre-inoculated seedlings and reassessed

with gene expression analysis. It is also proposed that PPO acts as a potential biomarker for induced resistance against *G. boninense*. The single application of UT126 (T2) was found to be superior in increasing the vegetative parameters such as height, girth, leaf area, frond count and fresh weight of leaf and root with significant difference at $P<0.05$. However, disease suppression was highest in treatment that had the application of both AMF and UPMP3 with a disease reduction of 57.53% ($P<0.05$). The disease incidence in treatment with both AMF and UPMP3 (T4) was delayed by 8 weeks indicating effective suppression of BSR infection. Similar treatments were subjected to field trial using seedling bait technique. Disease suppression in T4 was significantly highest with reduced disease severity of 4.33% ($P<0.05$) when compared to other treatments (ranging from 7 to 14% o infection), 20 months after planting. This is the first report of field seedling baiting technique to be successfully implemented in testing microbial pre-inoculation for disease suppression. Pre-inoculation with AMF and UPMP3 was most effective in reducing disease severity in oil palm either in nursery or field conditions.

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

PENILAIAN JANGKITAN *GANODERMA* PADA KELAPA SAWIT (*ELAEIS GUINEENSIS JACQ*.) MELALUI PRE-INOKULASI KULAT MIKORIZA ARBUSKUL DAN BAKTERIA ENDOFIT

Oleh

SHAMALA SUNDRAM

Ogos 2012

Pengerusi : Sariah Meon, Profesor, PhD

Fakulti : Institut Pertanian Tropika

Penyakit reput pangkal batang kelapa sawit (*Elaeis guineensis* Jacq.) yang disebabkan oleh *Ganoderma* spp telah menjadi kebimbangan ekonomi utama kerana ia merupakan penyakit paling serius menjangkiti kelapa sawit di Asia Tenggara. Penyakit ini adalah "reput putih" melibatkan proses pertumbuhan kulat dalam kelapa sawit sebagai pengurai lignin dan selulosa. Sehingga kini belum ada langkah yang berkesan untuk mengawal penyakit ini. Mikroorganisma endofit seperti kulat arbuskul mikoriza (AMF) dan bakteria endofit (EB) telah dikenalpasti sebagai agen kawalan biologi yang berpotensi terutamanya untuk penyakit-penyakit bawaan tanah. Mikrob endofit mampu menindas penyakit dengan pelbagai mekanisme seperti antibiosis, lisis, dan pengeluaran sebatian anti-kulat dan peningkatan keresistenan sistemik

(ISR). Penggunaan mikrob endofit juga diutamakan kerana habitat melibatkan sistem dalaman tumbuhan dan dapat mengelakkan halangan persekitaran seperti persaingan ruang dan nutrien sesama mikrob tanah yang lain. Oleh itu kajian ini telah direka dengan objektif khusus untuk (i) mengkaji potensi antagonistik dan keserasian di antara kedua-dua microorganisma endofit, (ii) menilai kebolehan penindasan penyakit dan peningkatan pertumbuhan vegetatif anak benih kelapa sawit yang diinokulat sebelum dijangkiti *G. boninense*, (iii) menentukan tindak balas biokimia dan profil ekspresi gen dalam anak benih yang diinokulat sebelum dan dijangkiti *G. boninense*, dan (iv) menilai keberkesanan inokulasi individu dan konsortium mikroorganisma endofit dalam kajian lapangan menggunakan menggunakan teknik umpan anak benih. AMF (*Glomus intraradices* UT 126 dan *G. clarum* BR152B) dan EB (*Pseudomonas* isolat UPMP3 dan *Burkholderia* isolat UPMB3) telah digunakan sebagai mikrob endofit dalam kajian ini. Interaksi simbiotik dikenalpasti di antara spesis AMF; *Glomus intraradices* UT 126 dan *G. clarum* BR152B dengan *Pseudomonas* strain UPMP3 dan *Burkholderia* strain UPMB3 Kedua-dua strain EB meningkatkan percambahan dan panjang hifa AMF masing-masing sebanyak 180 hingga 240%. Ini adalah keputusan novel strain EB ini yang belum pernah dilaporkan sebagai bakteria berpotensi pembantu mikoriza (MHB). Manakala kesan bakteria endofit ditunjukkan oleh perencatan peratusan pertumbuhan miselium *G. boninense* sebanyak 86.12% dan 74.35% masing-masing oleh UPMP3 dan UPMB3. Pengimbasan elektron mikrograf telah menunjukkan kecacatan morfologi yang teruk pada hifa *G. boninense* bila dikultur

bersama UPMP3 an UPMB3. Di samping itu, aktiviti POX, PPO, chitinase dan β-1, 3-glucanase telah meningkat sebelum dan selepas jangkitan *G. boninense*. Adalah dicadangkan PPO boleh bertindak sebagai penanda-bio ISR yang berpotensi terhadap *G. boninense*. Aplikasi tunggal UT126 (T2) telah didapati meningkatkan secara bererti ($P<0.05$) parameter vegetatif seperti ketinggian, lilitan batang, luas daun, kiraan pelepah dan berat segar daun dan akar anak benih. Walau bagaimanapun, pengawalan penyakit yang tertinggi dalam rawatan (T4) yang mempunyai aplikasi kedua-dua AMF dan UPMP3 dengan pengurangan penyakit 57.53% ($P <0.05$). Insiden penyakit di T4 juga tertangguh 8 minggu dan ini menunjukkan pengurangan jangkitan BSR yang berkesan. Rawatan yang sama telah diaplikasikan pada anak benih untuk kajian lapangan menggunakan teknik umpan anak benih. Pengurangan penyakit dalam T4 adalah paling berkesan dengan nilai keparahan penyakit sebanyak 4.33% ($P <0.05$) berbanding dengan rawatan-rawatan lain (diantara 7 – 14%). Ini juga adalah laporan pertama kajian lapangan yang meilbatkan teknik umpanan anak benih yang berjaya dilaksanakan untuk ujian pra-inokulasi mikrob bagi pengawalan penyakit. Pra-inokulasi dengan AMF dan UPMP3 paling berkesan dalam pengawalan penyakit kelapa sawit sama ada di tapak semaian atau lapangan.

ACKNOWLEDGEMENTS

By the grace of the Almighty, I have finally completed my PhD studies. It has been a long four years wait and I would like to thank everyone who is involved in the completion of this study. I would like to first express my utmost gratitude to my PhD supervisor, Professor Dr Sariah Meon. She has been an inspirational and a dedicated supervisor throughout the completion of this study. Without her guidance and supervision, I would not have completed the studies as intended. “Thank you very much Prof”. I would like to take this opportunity to thank my supervisory committee members, Associate Professor Dr Radziah Othman, Associate Professor Datin Dr Siti Nor Akmar and Dr Idris Abu Seman for their valuable advice and guidance. This study would not have been concluded according to the time frame without the assistance of En Erhan Hak, Cik Noorhaliza Jamaludin and Mohd AlHafiz. My sincere appreciation to you guys. I would like to take this opportunity to also thank Dr Ooi Siew Eng for tirelessly taking the time to tutor me on the molecular analysis of qPCR. Last but not least, to my beloved family. Thank you to my dearest mother, Madam Kanakammah for teaching me to believe in myself. You are the pillar of my strength. Prajiv, my beloved husband, I thank you for your understanding and motivation throughout this study. To my lovely daughters, Prameesha and Pratheasha....amma has finally completed her homework. My sincere appreciation also goes to my mother in law, Madam Geetha who has been very supportive, both mentally and morally. Naa...thank you to you too.

I certify that a Thesis Examination Committee has met on **13 August 2012** to conduct the final examination of Shamala Sundram on her thesis entitled **Assessment of *Ganoderma* Infection in Oil Palm (*Elaeis guineensis* Jacq.) by Pre-inoculation of Arbuscular Mycorrhiza Fungi and Endophytic Bacteria** 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 Degree of Doctor Philosophy.

Members of the Thesis Examination Committee were as follows:

Mohd Rafii Yusop, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Zulkifli Hj. Shamsuddin, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Zainal Abidin Mior Ahmad, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Richard M. Cooper, PhD
Professor
Reader in Plant-Microorganism Interactions
Department of Biology and Biochemistry
University of Bath
United Kingdom
(External Examiner)

SEOW HENG FONG, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

The thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of **Doctor of Philosophy.** The members of Supervisory Committee were as follows:

Sariah Meon, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairperson)

Siti Nor Akmar Abdullah, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia

Radziah Othman, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia

Idris Abu Seman, PhD
Senior Principle Research Officer
Ganoderma and Disease Research of Oil Palm (GANODROP)
Malaysian Palm Oil Board

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 Universiti Putra Malaysia or at any other institution.

_____

SHAMALA SUNDRAM

Date:

TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	vi
ACKNOWLEDGEMENTS	ix
APPROVAL	x
DECLARATION	xii
LIST OF TABLES	xvi
LIST OF FIGURES	xviii
LIST OF ABBREVIATIONS	xxii
CHAPTER	
1 INTRODUCTION	1.1
2 LITERATURE REVIEW	2.1
2.1 Oil Palm (*Elaeis guineensis* Jacq.)	2.1
2.1.1 Origin of oil palm	2.1
2.1.2 Importance of oil palm	2.1
2.1.3 Botany	2.2
2.1.4 Oil Palm Diseases	2.5
2.2 Basal Stem Rot (BSR)	2.7
2.2.1 Causal Pathogen	2.8
2.2.2 Symptoms of BSR	2.9
2.2.3 Epidemiology and spread of BSR	2.11
2.2.4 Control of BSR	2.13
2.3 Endophytic microorganisms	2.20
2.3.1 Fungal endophytes – Mycorrhiza	2.21
2.3.2 Ectomycorrhiza (ECM)	2.22
2.3.3 Endomycorrhiza	2.23
2.4 Arbuscular Mycorrhiza Fungi (AMF)	2.24
2.4.1 General biology and characteristics	2.25
2.4.2 Hyphae	2.26
2.4.3 Arbuscules	2.27
2.4.4 Vesicles	2.27
2.4.5 Spores	2.28
2.5 Benefits of AMF	2.28
2.5.1 Nutrient Uptake and Plant Growth	2.28
2.5.2 Plant Water Relation	2.30
2.5.3 Root Morphology	2.32
2.5.4 AMF as biological control agent	2.33
2.6 Endophytic bacteria (EB)	2.37
2.6.1 General characteristics	2.37
2.6.2 Endophytic bacteria as biological control agent	2.41

3 DETERMINATION OF SYMBIOTIC INTERACTION BETWEEN ENDOPHYTIC BACTERIA AND ARBUSCULAR MYCORRHIZA FUNGI AND ITS ANTAGONISTIC EFFECT ON *GANODERMA BONINENSE* 3.1

3.1 Introduction 3.1
3.2 Materials and Methods 3.3
3.2.1 Compatibility between Arbuscular Mycorrhiza Fungi and Endophytic Bacteria 3.3
3.2.2 Antagonistic Assay – Endophytic Bacteria and *Ganoderma boninense* PER 71 3.6
3.2.3 Statistical Analysis 3.9
3.3 Results 3.10
3.3.1 Compatibility between Arbuscular Mycorrhiza Fungi and Endophytic Bacteria 3.10
3.3.2 Antagonistic Test – Endophytic Bacteria and *Ganoderma boninense* PER 71 3.16
3.4 Discussion 3.22

4 EFFECT OF ARBUSCULAR MYCORRHIZA FUNGI AND ENDOPHYTIC BACTERIA ON VEGETATIVE GROWTH AND BSR DISEASE SUPPRESSION IN OIL PALM SEEDLINGS 4.1

4.1 Introduction 4.1
4.2 Materials and Methods 4.3
4.2.1 Effect of Endophytic Microorganism on Oil Palm Seedlings Vegetative Growth 4.3
4.2.2 Effect of Arbuscular Mycorrhiza Fungi (AMF) and Endophytic Bacteria (EB) on BSR Disease Suppression 4.10
4.2.3 Statistical Analysis 4.20
4.3 Results 4.21
4.3.1 Effect of Endophytic Microorganism on Oil Palm Seedlings Vegetative Growth 4.21
4.3.2 Effect of Arbuscular Mycorrhiza Fungi (AMF) and Endophytic Bacteria (EB) on Basal Stem Rot (BSR) Disease Suppression 4.30
4.4 Discussion 4.38

5 BIOCHEMICAL RESPONSES AND GENE EXPRESSION PROFILE INDUCED BY PRE-INOCULATION WITH ENDOPHYTIC MICROORGANISMS IN OIL PALM SEEDLINGS 5.1

5.1 Introduction 5.1
5.2 Materials and Methods 5.3
5.2.1 Experimental Design 5.3
5.2.2 Inoculum Preparation – Arbuscular Mycorrhiza Fungi (AMF) and Endophytic Bacteria (EB) 5.3
5.2.3 Preparation of Seedlings and Pre-Inoculation of Arbuscular Mycorrhiza Fungi (AMF) and Endophytic Bacteria (EB) 5.4
5.2.4 Sampling for Enzyme Activity Assay and Gene

Expression Study 5.4
5.2.5 Effect of Arbuscular Mycorrhiza Fungi (AMF) and Endophytic Bacteria (EB) on Inducible Defense Related Enzymes 5.5
5.2.6 Effect of Arbuscular Mycorrhiza Fungi (AMF) and Endophytic Bacteria (EB) on Defense Related Genes 5.8
5.2.7 Statistical Analysis 5.17
5.3 Results 5.18
5.3.1 Effect of Arbuscular Mycorrhiza Fungi (AMF) and Endophytic Bacteria (EB) on Inducible Defense Related Enzymes 5.18
5.3.2 Effect of Arbuscular Mycorrhiza Fungi (AMF) and Endophytic Bacteria (EB) on Defense Related Genes 5.23
5.4 Discussion 5.29

6 SUPRESSION OF BASAL STEM ROT DISEASE USING ARBUSCULAR MYCORRHIZA FUNGI AND ENDOPHYTIC BACTERIA ON OIL PALM SEEDLINGS IN THE FIELD TRIAL VIA SEEDLING BAIT TECHNIQUE 6.1
6.1 Introduction 6.1
6.2 Materials and Methods 6.3
6.2.1 Experimental Design 6.3
6.2.2 Selection of Experimental Palms 6.5
6.2.3 Preparation of Inoculum – Arbuscular Mycorrhiza Fungi and Endophytic Bacteria 6.5
6.2.4 Planting of Seedlings and Treatment with Arbuscular Mycorrhiza Fungi and Endophytic Bacteria 6.9
6.2.5 Field Site Preparation 6.9
6.2.6 Planting of Pre-inoculated Seedlings as “Seedling Baits” in the Field 6.11
6.2.7 Effect of Arbuscular Mycorrhiza Fungi and Endophytic Bacteria on Seedling Baits 6.11
6.2.8 Statistical Analysis 6.16
6.3 Results 6.17
6.3.1 Effect of Arbuscular Mycorrhiza Fungi and Endophytic Bacteria on Vegetative Growth of Oil Palm Seedlings 6.17
6.3.2 Effect of Arbuscular Mycorrhiza Fungi and Endophytic Bacteria on Disease Suppression in Oil Palm 6.22
6.4 Discussion 6.25

7 GENERAL DISCUSSION AND CONCLUSIONS 7.1

REFERENCES R1
APPENDICES A1
BIODATA OF THE STUDENT B1