

RESPONSE OF OIL PALM TO GANODERMA BONINENSE, TRICHODERMA HARZIANUM AND GLOMUS ETUNICATUM INTERACTIONS

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By

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

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RESPONSE OF OIL PALM TO Ganoderma boninense, Trichoderma harzianum AND Glomus etunicatum INTERACTIONS

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April 2012

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Knowledge on the microbial relationship and molecular mechanism during oil palmfungi interactions is of primary importance for the development of diagnostic tools for early detection of basal stem rot disease caused by *Ganoderma* species. Mycelial growth rate of different *Ganoderma* species were examined on various culture media to develop a medium for rapid growth of *Ganoderma* species. Rubber or oil palm wood waste was sufficient to improve growth of *Ganoderma* species; hence could be a useful renewable source for the early detection of *Ganoderma* disease. The density of soil microfungal community and growth profile of oil palm seedlings were investigated in oil palm artificially inoculated with pathogenic fungus *Ganoderma boninense* and the symbiotic fungi *Trichoderma harzianum and Glomus etunicatum*. Densities of the soil microfungal community increased significantly in oil palm inoculated with *T. harzianum* and *G. etunicatum*, while oil palm inoculated with *G. boninense* showed a significant decrease in the density of the soil microfungal community. Thus, the density of soil microfungal community could be useful for early detection and control of Ganoderma disease in oil palm. Fatty acid (FA) signaling is emerging as an important mechanism in plant response during interaction with microbial organisms. Furthermore plants have several antioxidant scavenging mechanisms which are induced in response to biotic and abiotic stresses. For a comprehensive evaluation of key genes involved in FA pathway and antioxidant reactive oxygen species (ROS) scavenger genes during oil palm-G boninense and -T. harzianum interactions, a lane-based array analysis of gene expression in artificially inoculated oil palm seedlings was performed. The results obtained demonstrated that acetyl-CoA carboxylase, β -ketoacyl-ACP synthases II and III, Δ^9 -stearoyl-acyl carrier protein desaturase, palmitoyl-ACP thioesterase, oleoyl-ACP thioesterase and glycerol-3-phosphate acyltransferase showed identical response in root and leaf tissues for the same fungi. Oil palm-G. boninense interaction up-regulated the expression of these genes in both root and leaf tissues and induced plant defense responses at 21 days postinoculation (dpi). Thereafter the production of physical symptoms occurred at 42 and 63 dpi concomitantly with suppression of expression of these genes. In oil palm-T. harzianium interaction increase in the expression level of these genes was observed in both tissues which correlated with the colonization of roots and promotion of plant growth at 3-63 dpi. The results from transcript level analysis of antioxidant ROS scavenger genes, metallothionein type 3 (MT3-A and MT3-B) revealed a different pattern of gene expression. Expression of MT3-A in roots was significantly up-regulated in G. boninense inoculated seedlings at 21 dpi. While the transcripts of *MT3-A* and *MT3-B* genes were synthesized in *G. boninense* inoculated leaves at 42 dpi,

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and the analyses did not show detectable expression of these genes before 42 dpi. In *T. harzianum* inoculated seedlings, the *MT3-A* expression was significantly up-regulated in roots at 3 dpi and thereafter were maintained at this level. The expression levels of *MT3-A* and *MT3-B* were induced in leaves at 3 dpi and subsequently maintained at same levels until 63 dpi. Differences in the expression profiles of the FA biosynthetic pathway genes during pathogenic and symbiotic interaction demonstrated their role in plant resistance mechanism and growth promotion by *T. harzianum* and could be the basis for molecular marker development for early detection of infection. The antioxidant ROS scavenger genes expressed in leaves and root tissues during oil palm-fungi interactions led to the discovery of their potential use as marker for the detection of oxidative stress.

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

PENYELIDIKAN MENGENAI TINDAK BALAS KELAPA SAWIT TERHADAP INTERAKSI DENGAN Ganoderma boninense, Trichoderma harzianum AND Glomus etunicatum

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Pengetahuan mengenai hubungan mikrob dan mekanisme molekul semasa interaksi kelapa sawit dengan kulat adalah sangat penting bagi tujuan penghasilan alat diagnostik untuk pengesanan awal penyakit reput pangkal batang yang disebabkan oleh spesies *Ganoderma*. Kadar pertumbuhan miselium spesies *Ganoderma* yang berlainan telah diuji di atas pelbagai kultur media bagi tujuan penghasilan satu media yang mampu menggalakkan pertumbuhan pesat spesies *Ganoderma*. Bagi meningkatkan pertumbuhan spesis *Ganoderma*, penggunaan sisa kayu getah dan kelapa sawit adalah mencukupi, dengan itu sisa-sisa kayu tersebut boleh digunakan sebagai sumber yang boleh diperbaharui untuk pengesanan awal penyakit yang disebabkan oleh *Ganoderma*. Ketumpatan komuniti kulat mikro tanah dan profil pertumbuhan anak pokok kelapa sawit dikaji dalam kelapa sawit yang diinokulat secara artifisial dengan patogen kulat *Ganoderma boninense* dan kulat simbiotik; *Trichoderma harzianum* dan *Glomus*



etunicatum. Ketumpatan komuniti kulat mikro tanah meningkat dengan ketara pada anak pokok kelapa sawit yang diinokulat dengan T. harzianum dan G. etunicatum, manakala anak pokok kelapa sawit yang diinokulat dengan G. boninense menunjukkan penurunan yang signifikan dalam ketumpatan komuniti kulat mikro tanah. Oleh itu, ketumpatan komuniti kulat mikro tanah boleh menjadi penanda bagi tujuan pengesanan awal dan kawalan penyakit *Ganoderma* dalam kelapa sawit. Pengisyaratan asid lemak sebagai mekanisme penting dalam tindakbalas tumbuhan semasa berinteraksi dengan mikrob kian menjadi tumpuan. Tambahan pula, tumbuh-tumbuhan mempunyai mekanisme antioksidan yang dihasilkan akibat daripada tekanan biotik dan abiotik. Untuk penilaian secara komprehensif ke atas interaksi kelapa sawit-G. boninense dan kelapa sawit-T. harzianum, analisis pengekspresan secara jaluran gen-gen utama dalam tapak jalan asid lemak dan gen antioksidan penghapus spesis oksijen reaktif dijalankan pada anak pokok kelapa sawit yang diinokulat secara artifisial. Keputusan yang diperolehi menunjukkan bahawa acetyl-CoA carboxylase, β-ketoacyl-ACP synthases II and III, Δ^9 -stearoyl-acyl carrier protein desaturase, palmitoyl-ACP thioesterase, oleoyl-ACP thioesterase dan glycerol-3-phosphate acyltransferase mempamirkan tindak balas yang serupa dalam tisu akar dan daun bagi kulat yang sama. Interaksi anak pokok kelapa sawit dan G. boninense meningkatkan pengekspresan gen-gen ini dalam akar dan tisu daun serta merangsang tindak balas pertahanan pokok pada 21 selepas inokulasi (si). Selepas itu pengeluaran gejala-gejala fizikal berlaku pada 42 dan 63 si, selari dengan perencatan ekspresi gen-gen tersebut. Dalam interaksi anak pokok kelapa sawit dengan T. harzianium, peningkatan kadar pengekspresan gen-gen ini telah diperhatikan dalam kedua-dua tisu seiring dengan penyebaran pada akar serta peningkatan

pertumbuhan pokok pada 3-63 si. Keputusan analisis tahap transkripsi gen antioksidan penghapus spesis oksijen reaktif, metallothionein jenis 3 (MT3-A dan MT3-B) menunjukkan penghasilan corak pengekspresan gen yang berbeza. Pengekspresan MT3-A dalam akar meningkat secara tinggi pada anak pokok kelapa sawit yang diinokulat dengan G. boninense pada 21 si. Sementara itu, gen-gen transkrip MT3-A dan MT3-B disintesis pada daun yang telah diinokulat dengan G. boninense pada 42 si, dimana pengekspresan gen-gen ini tidak dikesan sebelum 42 si. Dalam anak pokok yang diinokulat dengan T. harzianum, pengekspresan gen MT3-A meningkat secara tinggi dalam akar pada 3 si dan ia kekal pada tahap yang sama selepas itu. Pengekspresan gen MT3-A dan gen MT3-B teraruh dalam daun pada 3 si dan kekal pada tahap yang sama sehingga 63 si. Perbezaan dalam profil pengekspresan gen asid lemak semasa interaksi patogenik dan simbiotik menunjukkan peranan mereka dalam mekanisme pertahanan dan penggalak pertumbuhan oleh T. harianum dan boleh menjadi asas bagi penghasilan penanda molekul untuk pengesanan awal jangkitan penyakit. Gen antioksidan penghapus spesis oksijen reaktif yang diekspresi dalam daun dan tisu akar semasa interaksi anak pokok kelapa sawit dan kulat telah membawa kepada penemuan penanda yang berpotensi bagi pengesanan tekanan oksidatif.

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Last and foremost, my deepest admiration goes out to the late Professor Dr. Faridah Abdullah for her encouragement and guidance. To all of you, thank you so much. I certify that a Thesis Examination Committee has met on 3 April 2012 to conduct the final examination of Fahimeh Alizadeh on her thesis entitled "Response of oil palm to *Ganoderma boninense*, *Trichoderma harzianum* and *Glomus etunicatum* interactions" 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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DECLARATION

I declare that the thesis is original work except for quotation 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 any other institutions.



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LIST OF ABBREVIATIONS

ACC	Acetyl–CoA carboxylase		
ACP	Acyl carrier protein		
ACS	1-aminocyclopropane-1-carboxylate synthase		
ACT	Glycerol–3–phosphate acyltransferase		
AMF	Arbuscular mycorrhizal fungi		
ATCC	American Type Culture Collection		
AVR protein	Avirulent protein		
BSR	Basal stem rot		
CABI	Centre for Agricultural Bioscience International		
CDPKs	Calcium-dependent protein kinases		
CFU	Colony forming units		
CoA	Coenzyme A		
Cys	Cysteine		
DAG	Diacylglycerol		
DAMPs	Damage-associated molecular patterns		
DGDG	Digalactosyldiacylglycerol		
dpi	days postinoculation		
EFB	Empty fruit bunches		
ET	Ethylene		
ETI	Effector triggered immunity		
FA	Fatty acid		
FAD	FA desaturase		
FAS	Fatty acid synthase		
G	G. boninense inoculated		
GL	Glycerolipids		
Glo	G. etunicatum inoculated		
GloU	G. etunicatum uninoculated		
G-protein	Guanine nucleotide-binding protein		

G3P	Glycerol–3–phosphate
GSM	Ganoderma Selective Medium
GU	G. boninense uninoculated
HR	Hypersensitive response
IAC	Internal amplification control
ISR	Induced systemic resistance
JA	Jasmonic acid
К	Kinase
KAS	β -ketoacyl-ACP synthases
MAMPs	Microbe-associated molecular patterns
MAPK	Mitogen-activated protein kinase
MEA	Malt extract agar
MGDG	Monogalactosyldiacylglycerol
MPOB	Malaysian Palm Oil Board
MT	Metallothionein
NC	Untreated negative control
NCBI	National Center for Biotechnology Information
OP	Oil palm
OPWEA	Oil palm wood extract agar
OTE	Oleoyl-ACP thioesterase
Р	Peptone
PA	Posphatidic acid
PAMPs	Pathogen-associated molecular patterns
PAS	Periodic acid-schiff
PDA	Potato dextrose agar
PG	Phosphatidylglycerol
PP	Phosphatase
PRRs	Pattern recognition receptors
PTE	Palmitoyl-ACP thioesterase
PTI	Patterns-triggered immunity
R	Rubber

RGR	Relative growth rate
R protein	Resistance protein
ROS	Reactive oxygen species
RT-PCR	Reverse transcription polymerase chain reaction
RWB	Rubber wood block
RWEA	Rubber wood extract agar
SA	Salicylic acid
SAD	Δ^9 -stearoyl-acyl carrier protein desaturase
SAR	Systemic acquired resistance
SL	Sulfolipid
Т	T. harzianum inoculated
TAG	Triacylglycerol
TE	Acyl-ACP thioesterase
TU	T. harzianum uninoculated
VLCFA	Very long-chain fatty acid
WP	Wood powder

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CHAPTER 1

INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is the most profitable oil-bearing tropical crop. The extracted oil is widely used for diverse industrial applications including food, cosmetics, oleo-chemicals and bio-fuel. One of the important factors contributing to significant economic losses in oil palm industry is basal stem rot (BSR) caused by the fungi of *Ganoderma* species (Paterson *et al.*, 2009).

Plants employ a wide range of defense mechanisms both performed and induced responses to protect themselves against pathogens. However when performed defense is suppressed, inducible defense is triggered by recognition of the pathogen/microbe (Kachroo and Kachroo, 2009). In addition to the major phytohormone-mediated defense pathways, fatty acid (FA) pathways play significant roles in pathogen defense and abiotic stress (Venugopal *et al.*, 2009a). Historically, plant FAs are believed to play passive roles in plant defense such as serving as precursors of the phytohormone jasmonic acid and cuticular wax components. Recent evidence demonstrates direct roles for FAs and their degradation products in plant defenses (Kachroo and Kachroo, 2009; Brown *et al.*, 2009).

To avoid potential damage of reactive oxygen species (ROS), plants contain several protective antioxidant scavenging systems such as production of metallothioneins

(MTs) that protect plant DNA from oxidative damage of ROS. The expression of *MT* genes is regulated by some important developmental processes and in response to various abiotic and biotic stresses (Dauch and Jabaji-Hare, 2006; Obertello *et al.*, 2007).

Studying the genotypic and phenotypic diversity and the dynamics of pathogen populations can be achieved by recovery and isolation of plant pathogens (Amiri *et al.*, 2009). Isolation of *Ganoderma* species is difficult due to the presence of bacterial, yeast, and several fast-growing fungal species. The nature of a particular culture medium has a major role to play in the growth of fungi (Zhao and Shamoun, 2006). For this reason, it is possible to develop a substantial number of alternative rapid culture media to produce results more quickly for various biotechnological applications.

Since the BSR disease occurs as the result of interaction between plant roots and the soil environment (Mazliham *et al.*, 2007), understanding the microbial relationship of oil palm-*Ganoderma boninense* interactions may be useful for the development of early detection and respective preventive measures. It is important to advance knowledge on soil microfungal communities as biological indicators in oil palm cultivated area. Based on plant-microbe interactions, it is possible to develop microbial inoculants for use in agricultural biotechnology.

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Molecular responses to *G. boninense* in oil palm can be a useful tool to design appropriate strategies to produce palms resistant to *Ganoderma*. As *G. boninense*, *Trichoderma harzianum* and *Glomus etunicatum* undergo pathogenic or symbiotic development at different points in their infection cycle; both pathogenic and symbiotic fungi are uniquely suitable for studies aimed at uncovering unifying principles underlying both types of interaction.

General objective:

To investigate microbial and molecular relationship of oil palm-G. boninense, T. harzianum and G. etunicatum interactions.

Specific objectives:

- 1. To improve culture media for *Ganoderma* and to find a medium that would allow for rapid colony growth.
- 2. To analyze density of soil microfungal community as well as growth profile of plant during oil palm-*G. boninense*, *T. harzianum* and *G. etunicatum* interactions.
- 3. To investigate the expression profiles of defense-associated and antioxidant ROS scavenger genes in oil palm tissues during oil palm-G. boninense and T. harzianum interactions.
- 4. To assess the potential role for FA biosynthetic pathway genes in oil palm defense response during oil palm-*G. boninense* and *T. harzianum* interactions.



Thus this study was undertaken with the ultimate goal to develop a medium for rapid growth of *Ganoderma* species and finding the probable microbial indicator and molecular markers in oil palm.

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BIODATA OF STUDENT

Fahimeh Alizadeh enrolled at Islamic Azad University and obtained her first degree in Bachelor of Biology-Microbiology. She continued her education in Master of Biology-Microbiology. After that, she worked as a lecturer in Islamic Azad University for 5 years. She enrolled at Universiti Putra Malaysia for PhD in the field of Microbial Biotechnology in 2007 under the guidance of Assoc. Prof. Datin Dr. Siti Nor Akmar Abdullah, Assoc. Prof. Dr. Chong Pei Pei and Prof. Dr. Umi Kalsom Yusuf. The title of her research was "Response of oil palm to *Ganoderma boninense*, *Trichoderma harzianum* and *Glomus etunicatum* interactions"

LIST OF PUBLICATIONS

Alizadeh, F., Siti Nor Akmar, A., Khodavandi, A., Faridah, A., Umi Kalsom, Y. and Chong, P.P. (2011). Differential expression of oil palm pathology genes during interactions with *Ganoderma boninense* and *Trichoderma harzianum*. *Journal of Plant Physiology*. 168(10): 1106-1113.

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