UNIVERSITI PUTRA MALAYSIA

# TRANSCRIPTIONAL CHANGES IN RESPONSE TO SINGLE AND COMBINE INOCULATION OF MYCORRHIZA AND *GANODERMA* IN OIL PALM (*Elaeis guineensis Jacq*.) ROOTS

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**MASTER OF SCIENCE**
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**2009**

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**TEE SUE SEAN**

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**TEE SUE SEAN**

**October 2008**

**Chairman : Ho Chai Ling, PhD**

**Faculty : Biotechnology and Biomolecular Sciences**

Mycorrhiza is a symbiotic fungus that aids in nutrient uptake, enhances root development and promotes plant growth in most of the vascular flowering plants. Under natural conditions, oil palm is often colonized by arbuscular mycorrhizal (AM) fungi. AM was also proposed to mitigate *Ganoderma* infection, which leads to basal stem rot (BSR) disease in oil palm. In this study, cDNA microarray approach was used to examine the transcript profile of oil palm roots during the development of AM symbiosis and upon *Ganoderma* infection. Besides, the role of AM as a biocontrol agent against BSR disease was also investigated. The analysis of microarray results using LIMMA (Linear Model for Microarray Analysis) revealed that different sets of genes were expressed upon different interactions. A total of 183 genes, 123 genes and 391 genes were up- or down-regulated in oil palm in response to mycorrhizal inoculation, *Ganoderma* infection and *Ganoderma*-mycorrhizal inoculation, respectively. Among the differentially expressed genes, defense and stress related genes formed the largest category in all three treatments, such as putative beta 1,3-glucanase, early methionine labeled polypeptide, metallothionein-like protein and type 2 ribosome-inactivating protein cinnamomin III precursor. These transcripts

were regulated differently in different experimental conditions. Isoflavone reductase homolog was found to be differentially expressed in *Ganoderma* infected root, implying the involvement of isoflavonoid phytoalexin in oil palm defense system against BSR disease. The results also showed that different types of protein kinase and calmodulin were differentially expressed in mycorrhizal symbiosis and *Ganoderma* infection. The WRKY and bHLH transcription factors were among the transcription factors which have been identified in oil palm mycorrhizal and *Ganoderma* interaction studies. In addition, a number of cell wall related proteins such as plasma membrane proteins were differentially expressed in mycorrhizal and *Ganoderma*-mycorrhizal treatment inferring the importance of cell wall proteins during mycorrhizal inoculation. Although the mycorrhization of oil palm was unable to suppress *Ganoderma* infection, the transcription profiles of oil palm *Ganoderma*-mycorrhizal interaction showed that many defense related transcripts were induced indicating that mycorrhiza might induce systemic resistance of oil palm against *Ganoderma* infection. In conclusion, the findings of this study have provided new insights into the molecular events that happened during symbiotic and pathogenic associations of fungi with oil palms.

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

**PERUBAHAN TRANSKRIPSI TERHADAP INOKULASI TUNGGAL DAN CAMPURAN ANTARA MIKORIZA DAN *GANODERMA* PADA AKAR KELAPA SAWIT (*Elaeis guineensis Jacq*.)**

Oleh

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Mikoriza adalah kulat simbiotik yang membantu dalam proses penyerapan nutrisi, memperkuatkan perkembangan akar dan mempromosikan tumbesaran dalam kebanyakan tumbuhan bunga vaskular. Dalam keadaan semulajadi, kelapa sawit sering dijangkiti oleh kulat mikoriza arbuskul (MA). MA juga dicadangkan dapat mengatasi masalah jangkitan *Ganoderma* yang menyebabkan penyakit reput pangkal batang (RPB) pada kelapa sawit. Dalam kajian ini, pendekatan mikroatur cDNA telah digunakan untuk mengkaji corak transkripsi pada akar kelapa sawit samasa perkembangan simbiosis MA dan juga semasa jangkitan *Ganoderma*. Selain itu, peranan MA sebagai agen pengawal biologi terhadap penyakit RPB juga dikaji. Analisis keputusan mikroatur dengan menggunakan LIMMA (Linear Model for Microarray Analysis) menunjukkan bahawa gen yang berlainan diekspres dalam interaksi yang berlainan. Sejumlah 183 gen, 123 gen dan 391 gen yang ekspresinya dipertingkatkan atau dikurangkan dalam akar kelapa sawit yang terdedah kepada inokulasi mikoriza, jangkitan *Ganoderma* dan inokulasi *Ganoderma*-mikoriza masing-masing telah dikenalpasti. Di antara gen yang ekspresinya berlainan, gen yang berkaitan dengan pertahanan dan tekanan membentuk kategori yang terbesar

dalam kesemua tiga rawatan, gen-gen tersebut adalah seperti ‘putative beta 1,3-glucanase’, ‘early methionine labelled polypeptide’, ‘metallothionein-like protein’ dan ‘type 2 ribosome-inactivating protein cinnamomin III precursor’. Transkrip ini mempunyai ekspresi yang berlainan dalam keadaan eksperimen yang berlainan. ‘Isoflavone reductase homolog’ didapati mempunyai ekspresi yang berlainan dalam akar kelapa sawit yang dijangkiti oleh *Ganoderma*. Ini menunjukkan penglibatan ‘isoflavone phytoalexin’ di dalam sistem pertahanan kelapa sawit terhadap penyakit RPB. Keputusan ini juga menunjukkan bahawa jenis ‘protein kinase’ dan ‘calmodulin’ yang berlainan diekspreskan dalam simbiosis mikoriza dan jangkitan *Ganoderma*. Faktor transkripsi WRKY dan bHLH adalah di antara faktor transkripsi yang telah dikenalpastikan di dalam kajian mikoriza kelapa sawit dan interaksi *Ganoderma*. Tambahan pula, sejumlah protein yang berkaitan dengan dinding sel seperti membran plasma protein yang diekspreskan dalam rawatan mikoriza dan *Ganoderma*-mikoriza menunjukkan kepentingan protein dinding sel semasa inokulasi mikoriza. Walaupun mikoriza tidak dapat mengurangkan jangkitan *Ganoderma*, corak transkripsi interaksi *Ganoderma*-mikoriza dalam kelapa sawit telah menunjukkan banyak transkrip berkaitan dengan pertahanan. Ini menunjukkan bahawa mikoriza mungkin meningkatkan pertahanan sistemik kelapa sawit terhadap jangkitan *Ganoderma*. Kesimpulannya, hasil kajian semasa penglibatan kulat secara simbiotik dan patogenik dengan kelapa sawit telah memberikan pandangan baru dari segi aspek molekular.

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I certify that an Examination Committee met on 30th October 2008 to conduct the final examination of Tee Sue Sean on her Master of Science thesis entitled "Transcriptional Changes in Response to Single and Combine Inoculation of Mycorrhiza and *Ganoderma* in Oil Palm (*Elaeis guineensis Jacq.*) Roots" in accordance with University Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Putra Malaysia (Higher Degree) Regulations 1981. The committee recommends that the student be awarded the degree of Master Science.

Members of the Examination Committee were as follows:

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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 Master Science. The members of the Supervisory Committee were as follows:

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Date: 12 February 2009

## DECLARATION

I hereby 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.

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**Tee Sue Sean**

Date: 30 December 2008

## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF ABBREVIATIONS

| | |
|---|---|
| ABA | absisic acid |
| AM | arbuscular mycorrhiza |
| aRNA | antisense riboxynucleic acid |
| ARM | arbutoid mycorrhiza |
| BBI | Bowman-Birk serine protease inhibitor |
| BSR | basal stem rot |
| C | carbon |
| cDNA | complementary deoxyribonucleic acid |
| C:I | chloroform:isoamylalcohol |
| Ct | threshold cycle |
| CTAB | cetyltrimethylammonium bromide |
| Cy3 | cyanine-3 |
| Cy5 | cyanine-5 |
| DEPC | diethypyrocarbonate |
| DNA | deoxyribonucleic acid |
| dATP | deoxyadenosine triphosphate |
| dCTP | deoxycytidine triphosphate |
| dGTP | deoxyguanosine triphosphate |
| dNTP | deoxyribonucleoside triphosphate |
| DTT | dithiothreitol |
| dUTP | 2'-deoxyuridine 5'-triphosphate |
| EDTA | ehylenediaminetriacetic acid |
| ERM | ericoid mycorrhiza |
| EST | expressed sequence tag |
| gpr | GenePix result |
| HCl | hydrochloric acid |
| HEPES | N-2-hydroxyehylpiperazine-N-2-ethanesulfonic acid |
| IVT | *in vitro* transcription |
| JA | jasmonic acid |
| K | potassium |
| KOH | potassium hydroxide |
| LiCl | lithium chloride |
| Limma | linear models for microarray data |
| LimmaGUI | linear models for microarray data graphic user interphase |
| LRR | leucine rich repeat |
| MAPK | mitogen-activated protein kinase |
| MM | monotropoid mycorrhiza |
| N | nitrogen |
| NaCl | sodium chloride |
| NaOH | nitrogen hydroxide |
| OM | orchid mycorrhiza |
| P | phosphorus |
| P:C:I | phenol:chloroform:isoamylalcohol |
| PDA | potato dextrose agar |
| PMT | photomultiplier tube |
| PR | pathogenesis related |
| PVPP | polyvnylpolypyrolydone |
| QRT-PCR | real-time quantitative polymerase chain reaction |

| | |
|---|---|
| RCBD | randomized complete block design |
| RFLP | restriction fragment length polymorphism |
| RIP | ribosome inactivating protein |
| RNA | riboxynucleic acid |
| SAM | S-adenosyl-L-methionine synthethase |
| SDS | sodium dodecyl sulphate |
| VAM | vesicular arbuscular mycorrhiza |
| v/v | volume/volume |
| w/v | weight/volume |
| WAK2 | wall associated kinase 2 |
| Zn | zinc |

# CHAPTER 1

# INTRODUCTION

Oil palm has been the golden crop for Malaysia, since the past few decades. To date, the total oil palm planted area has increased to 4.17 million hectares (MPOB, 2007). The oil palm industry has generated great revenue from the export of crude oil. In year 2006, the total export revenue of oil palm products including palm oil, palm kernel oil, palm kernel cake, oleochemical products, finished products and others was RM 31.85 billion (MPOB, 2007). Thus, improvement of palm oil production is currently the main agenda for palm oil industry and oil palm plantation. This can be achieved by supplying sufficient and appropriate fertilizers to the palms. Besides that, decreasing the rate of diseases that are detrimental to the palms is one of the efforts to improve palm oil production.

Mutualitistic associations (known as mycorrhizae) between plant roots and fungi, occurring in 83% of dicotyledonous and 79% of monocotyledonous plants, are ubiquitous in terrestrial ecosystems throughout the world (Trappe, 1987). The term 'mycorrhiza' originated from a combination of two Greek words *mykes*, meaning fungus, and *rhiza*, meaning root (Read, 2001). There are many types of mycorrhizal symbioses, and arbuscular mycorrhizal (AM) symbiosis is the most important and common (Smith and Read, 1997). As an outcome of this symbiosis, the fungi obtain carbon supply to support their growth and reproductive system from their host. On the other hand, the plants gain enhanced nutrient supplies (e.g. phosphorous and nitrogen) that are captured by fungi from soil, and acquire enhanced root

development as well as increased resistance to disease. Association between mycorrhiza and oil palm has led to enhanced growth development as compared to oil palm without mycorrhizal association (Rini, 2001). In addition, better root system development aids in nutrient uptake. The development of symbiosis requires significant alterations in both symbionts that are proposed to be coordinated via reciprocal signal exchange. There is evidence for mobile fungal signals that elicit changes in plant gene expression before contact with the root (Kosuta *et al.*, 2003). The hypothesis of this study is: mycorrhizal symbiosis in oil palm is related to growth enhancement and nutrient uptake. If this hypothesis is correct, differential expression of genes related to these two processes should be demonstrated by their transcriptional profiles.

*Ganodermataceae* are cosmopolitan basidiomycetes that cause white rot of hardwoods, such as oak, maple and ash by decomposing lignin as well as cellulose and related polysaccharides (Blanchette, 1984). Some *Ganoderma* species are wood-rotting fungi, with a number being pathogenic and thus harmful on economically important trees and perennial crops. The basal stem rot (BSR) disease is the most serious disease in oil palm. In addition, BSR disease also attacks other plantation crops, such as coconut, tea and rubber. The hypothesis of this study is: *Ganoderma* is an oil palm pathogen which related to basal stem rot during the infection. If this hypothesis is correct, differential expression of genes related to defense- and pathogenesis-related genes should be revealed in the transcriptional profiles of Ganoderma infected oil palm.

Mycorrhiza might play a role in mitigating BSR infections in oil palm. Relatively little is known about the interaction between mycorrhiza-*Ganoderma*, and oil palm. Thus, it is important to investigate the role of mycorrhiza as a biocontrol agent through the analysis of gene expression profiles of oil palm in contact with both fungi. The hypothesis of this study is: inoculation of mycorrhiza in *Ganoderma* infected oil palm is able to mitigate *Ganoderma* infection in Ganoderma infected oil palms. If this hypothesis is correct, the level of disease severity should be reduced and differential expression of genes related to systemic acquired resistance in oil palm will be demonstrated in their expression profile.

cDNA microarray approach is a powerful tool for transcript profiling and gene identification (Duggan *et al.*, 1999). Microarray can be hybridized simultaneously with combined fluorescent-labeled targets representing an experimental and a control condition. Thus, thousands of gene expressions for both samples can be monitored and the expression patterns can be compared in one single experiment.

The objectives of this study are as follow:

1. To profile the transcripts of mycorrhiza inoculated oil palm seedlings after 6 and 9 weeks treatment.

2. To profile the transcripts of *Ganoderma* infected oil palm seedlings after 3 and 6 weeks treatment.

3. To profile the transcripts of *Ganoderma* and mycorrhiza treated oil palm seedlings after 3 and 6 weeks treatment.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Oil Palm

The oil palm (*Elaeis guineensis*, Jacq.) is one of the most important perennial oil producing crops in the world. It is widely cultivated in tropical and humid countries such as Brazil, South East Asia and Africa. Oil palm tree originated from the tropical rain forest region of West and Central Africa (Hartley, 1977a). Four oil palm seedlings were first introduced to South East Asia in 1848 and planted in Buitenzorg (now Bogor) Botanical Garden. The oil palm industry started with the establishment of the first large commercial plantation in Sumatra in 1911. In Malaysia, oil palms were cultivated as a commercial oil crop in 1917 when the first oil palm estate was established in the Selangor state (Corley and Tinker, 2003a).

Nowadays, palm oil has been used extensively in the food industries to produce margarine and cooking oil. Besides that, it is used for producing non-edible products such as soaps, resins, candles, glycerol, fatty acids and cosmetics. In addition, oleochemicals production is the most recent and important usage of palm oil. Oil palm biomass can be used for many general purposes like paper making and as general solid fuel. Attempts have been made to produce biodiesel from palm for diesel substitute (Corley and Tinker, 2003b).

Oil palm is a monoecious plant of which both female and male flower are growing on the same tree. Approximately 2500-3000 fruits can be borne on 100-120 spikelets of the female bunch (Hartley, 1977b). The oil palm fruit is spherical, elongated or oval in shape while the colour is dark purple before ripen and orange red when ripe. The oil palm fruit has a center seed portion which is called palm kernel and is surrounded by a fresh pulp called palm mesocarp. The oil palm fruit starts to produce oil as early as two and a half year after planting. The production life of oil palm can be up to 25 to 30 years until the palms are too tall for the fruits to be harvested economically (Wahid *et al.*, 2004).

### 2.1.1 Oil Palm Root System

Plant root, often referred as the "hidden half" of a plant, is one of the vital and indispensable organs of a plant. The root is the underground organ with complex pattern of development and architecture. The major function of the root is to absorb nutrients and water for its growth and survival. Other than that, the root serves as an anchorage system for the plant. In some plants, the root acts as a storage system for water and nutrients. Another important feature of root is its ability to secrete root exudates with growth regulatory properties into the rhizosphere; the root-soil contact surface (Bertin *et al.*, 2003).

The oil palm has a root system consisting of four different orders of roots; primary, secondary, tertiary and quaternary root. This four degree of roots can be further divided into different morphological types including primary vertical and horizontal roots, secondary horizontal, upward growing vertical and downward growing