UNIVERSITI PUTRA MALAYSIA

ANALYSIS OF TRANSCRIPTOMIC CHANGES IN OIL PALM (*Elaeis guineensis* Jacq.) ROOT UPON INOCULATION WITH *Bacillus sphaericus* UPMB10 USING cDNA MICROARRAY

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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 Master of Science

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chairman: Ho Chai Ling, PhD

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Bacillus sphaericus UPMB10 is a plant growth promoting bacteria (PGPB) which enhances plant growth by acting as bioenhancer and biofertilizer. An oil palm cDNA microarray containing 2224 cDNA probes from root (partially derived from cDNA clones generated in this study), 828 from vegetative meristem and 696 from zygotic embryo was generated to investigate the transcriptomic changes in two-month-old *in vitro* oil palm (*Elaeis guineensis* Jacq.) (Deli x Yangambi) roots after inoculation with *B. sphaericus* UPMB10 for 120 h. This study was initiated with the generation of 1824 expressed sequenced tags (ESTs) from the roots of oil palm. A total of 1173 tentative unique genes (TUGs) were assembled from 1566 ESTs with readable sequences. However, only 984 TUGs showed significant matches (*E*-value less than 10^{-5}) to the non-redundant protein database in the GenBank and they were further divided into 13 groups based on their putative functions. Subsequent microarray result showed that 151 and 125 transcripts were significantly up- and down regulated, respectively in the

roots of oil palm inoculated with *B. sphaericus* UPMB10. Although transcripts involved in protein synthesis were increased and the expression level of auxin responsive genes were altered in *B. sphaericus* UPMB10-inoculated oil palms, there was no conclusive result to support the presence of auxin secreted by *B. sphaericus* UPMB10 in the medium. Despite the capability of *B. sphaericus* UPMB10 to fix atmospheric nitrogen, 120 h might not be sufficient for it to establish efficient nitrogen fixation to relieve possible N deficiency in oil palms. The plant-microbe interaction might also have alerted the defense system that led to the up-regulation of transcripts related to synthesis of hydrogen peroxide in oil palm. Verification of microarray result by real time RT-PCR showed that nine out of 11 candidate genes (81.8%) were consistent in their expression patterns. In conclusion, this study has provided a brief understanding of the transcriptomic changes in the oil palm roots inoculated with *B. sphaericus* UPMB10.

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

ANALISASI PERUBAHAN TRANSKRIPTOMIK DI AKAR KELAPA SAWIT (*Elaeis guineensis* Jacq.) SELEPAS DIINOKULASI DENGAN *Bacillus sphaericus* UPMB10 DENGAN MENGGUNAKAN MIKROATUR cDNA

Oleh

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Bacillus sphaericus UPMB10 merupakan bakteria penggalak pertumbuhan pokok (PGPB) yang boleh mempercepatkan pertumbuhan tumbuhan dengan berfungsi sebagai penggalak biologi dan baja biologi. Satu mikroatur cDNA kelapa sawit yang mengandungi 2224 prob cDNA daripada akar (sebahagiannya berasal daripada klon cDNA yang dijanakan dalam kajian ini), 828 daripada meristem vegetatif, dan 696 daripada embrio zigot telah dijanakan untuk menyiasat perubahan transkriptomik akar kelapa sawit (*Elaeis guineensis* Jacq.) (Deli x Yangambi) *in vitro* yang berumur dua bulan selepas diinokulasi dengan *B. sphaericus* UPMB10 selama 120 jam. Kajian ini bermula dengan penjanaan 1824 penanda jujukan terekspres (EST) daripada akar kelapa sawit. Sejumlah 1173 gen unik yang tentatif (TUG) telah dikelompokkan daripada 1566 EST dengan jujukan yang boleh dibaca. Walau bagaimanapun, hanya 984 TUG menunjukkan pemadanan bermakna (nilai E kurang daripada 10^{-5}) dengan jujukan dalam pangkalan data protein yang tidak berulang dan TUG dibahagikan

kepada 13 kumpulan berdasarkan fungsi putatif. Keputusan mikroatur yang seterusnya menunjukkan bahawa 151 dan 125 transkrip mengalami pengawalaturan naik dan turun yang signifikan dalam akar kelapa sawit yang diinokulasi dengan *B. sphaericus* UPMB10. Walaupun transkrip yang terlibat dalam penghasilan protein telah dikawalatur naik dan paras ekspresi gen-gen yang bereaksi terhadap auksin telah berubah dalam kelapa sawit yang diinokulasi dengan *B. sphaericus* UPMB10, tiada keputusan yang muktamad untuk menyokong kehadiran auksin yang dirembeskan oleh *B. sphaericus* UPMB10 dalam medium. Walaupun *B. sphaericus* UPMB10 mempunyai kebolehan untuk mengikat nitrogen daripada udara, tempoh 120 jam mungkin tidak cukup untuk *B. sphaericus* UPMB10 mengwujudkan pengikatan nitrogen yang berkesan untuk membebaskan kelapa sawit daripada kekurangan nitrogen. Interaksi di antara tumbuhan dan bakteria mungkin mengaktifkan pertahanan tumbuhan yang menyebabkan pengawalaturan naik transkrip yang berkaitan dengan penghasilan hidrogen peroksida dalam kelapa sawit. Pengesahan keputusan mikroatur dengan RT-PCR masa-nyata (real time RT-PCR) menunjukkan bahawa corak ekspresi sembilan daripada 11 calon gen (81.8%) adalah konsisten. Kesimpulannya, kajian ini telah memberikan satu pemahaman yang ringkas terhadap perubahan transkriptomik di kelapa sawit yang diinolukasi dengan *B. sphaericus* UPMB10.

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I certify that an Examination Committee has met on 11 June 2008 to conduct the final examination of Lim Kok Ang on his Master of Science thesis entitled "Analysis of Transcriptomic Changes in Oil Palm (*Elaeis guineensis* Jacq.) Root upon Inoculation with *Bacillus sphaericus* UPMB10 Using cDNA Microarray" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Master of Science.

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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.

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LIM KOK ANG

Date: 7 July 2008

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LIST OF ABBREVIATIONS

Δ	Delta
®	Registered trademark
1 x	1 time
10 x	10 times
^{15}N	Nitrogen-15
24:1	Ratio 24 to 1
25:24:1	Ratio 25 to 24 to 1
6 x	6 times
$A_{260/230}$	Absorbance at wavelength of 260 nm over 230 nm
$A_{260/280}$	Absorbance at wavelength of 260 nm over 280 nm
ADP	Adenine diphosphate
aRNA	Amplified RNA
ATP	Adenosine triphosphate
BNF	Biological nitrogen fixation
bp	Base pair
$CaCl_2$	Calcium chloride
CAP3	Contig Assembly Program 3
cDNA	Complementary DNA
cfu	Colonies forming unit
CsCl	Cesium chloride
Ct	Threshold cycle
CTAB	Cetyl trimetyl ammonium bromide
CTP	Cytidine triphosphate
dbEST	Database of EST
dCTP	2'deoxycytidine 5'-triphophate
DEPC	Diethyl pyrocarbonate
DMSO	Dimethylsulfoxide
DNaseI	Deoxyribonuclease I

dNTP	2'-deoxyribonucleoside triphosphate
DRAG	dinitrogenase reductase-activating glycohydrolase
DRAT	Dinitrogenase reductase ADP-ribosyltransferase
DTT	Dithiothreitol
E	Efficiency
e^-	Electron
EDTA	Ethylenediamne tetraacetic acid
EST	Expressed sequence tag
FDR	False discovery rate
Fe	Iron
FELCRA	Federal Land Consolidation and Rehabilitation Authority
FELDA	Federal Land Development Authority
g	Relative centrifugal force (*rcf*)
GTP	Guanosine triphosphate
H^+	Proton
H_2	Hydrogen
HCl	Hydrochloric acid
HEPES	*N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulfonic acid)
IAA	Indole-3-acetic acid
IPTG	Isopropylthio-β-D-galactoside
ISR	Induced systemic resistance
KCl	Potassium chloride
KEGG	Kyoto Encyclopedia of Genes and Genomes
kPa	KiloPascal
LB	Luria-Bertani
LiCl	Lithium chloride
M	Molar
$MgCl_2$	Magnesium chloride
$MgSO_4$	Magnesium sulfate
mM	Milimolar
Mo	Molybdenum

mRNA	Messenger RNA
MS	Murashige and Skoog
N	Nitrogen
N_2	Nitrogen
NAA	Naphthalene acetic acid
NaCl	Sodium chloride
NAD	Nicotinamide adenine dinuceotide
NaOH	Sodium hydroxide
NCBI	National Center for Biotechnology Information
Ndfa	Nitrogen derived from atmosphere
ng	Nanogram
NH_3	Ammonia
NH_4^+	Ammonium
nM	Nanomolar
OD_{600}	Optical density at wavelength of 600nm
PBS	Phosphate-buffered saline
PCR	Polymerase Chain Reaction
Pfu	Plaques forming unit
PGPB	Plant growth promoting bacteria
PGPR	Plant growth promoting rhizobacteria
P_i	Phosphate
PVPP	Polyvinylpolypyrolidone
Real Time RT-PCR	Real time reverse transcription-PCR
RI_{1-5}	Primary roots emitted from a 1 to 5-month-old oil palm
RI_{5-12}	Primary roots emitted from a 5 to 12-month-old oil palm
RISDA	Rubber Industry Smallholders' Development Authority
RNA	Ribonucleic acids
RNase	Ribonuclease
ROS	Reactive oxygen species
Rpm	Revolution per minute
SAM	Significant analysis of microarray

SDS	Sodium dodecyl sulfate
sp.	species
SSC	Standard saline citrate
TAE	Tris-acetate EDTA
Tm	Melting temperature
™	Trade mark
Tris	2-amino-2-hydroxymethyl-1,3-propandiol
TS	Tryptic soy
TUC	Tentative unique contig
TUG	Tentative unique gene
TUS	Tentative unique sequence
U	Unit
UTP	Uridine triphosphate
v/v	Volume per volume
w/v	Weight per volume
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactoside
β-	Beta-
δ	delta
λ	Lambda
µg	Microgram
µL	Microlitre
µM	Micromolar

CHAPTER 1

INTRODUCTION

In the soil surrounding the plant root, the release of root exudates has attracted the colonization of plants by bacteria. The bacteria that are beneficial to plants are collectively known as plant growth-promoting bacteria (PGPB). The major difference between the PGPB and *Rhizobium* is that PGPB do not form a distinct symbiotic relationship with the host plant. Generally, PGPB might establish an endophytic, rhizospheric or associative relationship with the host plant and this depends on the combination of PGPB and host plant. Various mechanisms have been employed by PGPB to improve plant growth, including biological nitrogen fixation, production of plant growth promoting substances which enhance the root development, stimulation of plant defense to suppress the growth of pathogen or combination of modes of mechanisms (Vessey, 2003).

Oil palm (*Elaeis guineensis*) is one of the most important plantation crops in Malaysia and a major source of edible oil in the world. The planting of oil palm requires a high input of chemical fertilizer, thus posing severe threats to the environment such as emission of greenhouse gases, nitrate pollution and acidification of soil. Amir *et al.* (2002) reported oil palm inoculated with a local isolate of PGPB, *Bacillus sphaericus* UPMB10 showed an enhanced growth. Besides reducing the application of chemical fertilizer, inoculation of oil palm with *B. sphaericus* UPMB10 could reduce the production cost, leading toward a more sustainable management.

Amir *et al.* (2002) attributed the enhanced oil palm growth to the ability of *B. sphaericus* to fix atmospheric nitrogen and synthesize plant growth promoting substances such as auxin. However, the detailed understanding of interaction between oil palm and *B. sphaericus* UPMB10 is still lacking. Although PGPB are widely studied, the understanding of their interaction with PGPB and plant is not as good as that between *Rhizobium* and legume. This might be due to the highly diversified PGPB and the lack of specific host plant. A comprehensive transcriptomic study can reveal how the interaction is initiated and developed between oil palm and *B. sphaericus* UPMB10, and can explain mechanism(s) employed by PGPB to promote plant growth. To accelerate the understanding of the interaction between *B. sphaericus* UPMB10 and oil palm, cDNA microarray, a high throughput approach has been used to elucidate the transcriptomic event of oil palm upon inoculation with *B. sphaericus* UPMB10.

Since oil palm is not a model plant and the cDNA sequences in the public domain are limited, expressed sequence tags (ESTs) were generated and used as cDNA probes for cDNA microarray. In this study, the RNA derived from the root of *B. sphaericus* UPMB10-inoculated oil palms and reference controls (without the inoculation with *B. sphaericus* UPMB10) were labeled with fluorescent dyes and co-hybridized to oil palm microarray. Although microarray is a high throughput approach, it is very expensive to use and requires complicated statistic analysis of data. In addition to that, post microarray verification such as real time PCR and Northern analysis is necessary.

The objectives of this study were:

1. To generate ESTs from the oil palm root cDNA library to be used as cDNA probes for cDNA microarray.
2. To study the transcriptomic changes in the oil palm root upon inoculation of *B. sphaericus* UPMB10 by using cDNA microarray approach.
3. To verify the microarray results by using real time PCR.

CHAPTER 2

LITERATURE REVIEW

2.1 Oil Palm

2.1.1 Botany of Oil Palm

The oil palm, *Elaeis guineensis* Jacq is classified together with *Cocos* (the coconut) and other genera under the subfamily Cocosoideae, in the family Aracaceae (formerly known as Palmae) (Corley and Tinker, 2003). The botanical name of *E. guineensis* was given by Jacquin. The genus name *Elaeis* is derived from the Greek word *elaion*, meaning oil while the specific name *guineensis* shows its origin in the Guinea Coast, in West Africa. The genus *Elaeis* consists of three species: *E. guineensis* (African oil palm), *E. oleifera* (American oil palm) and *E. odora* (formerly known as *Barcella odora*) (Corley and Tinker, 2003). *E. guineensis* is found in the tropics, within ±10˚ latitude of the equator, in Africa, South East Asia and South and Central America.

E. guineensis has a solitary columnar stem with the crown consisting of large pinnate leave (Corley and Tinker, 2003). At the base of the stem, there is a prominent bole (bulb) from where the primary roots emerge. *E. guineensis* is normally monoecious, bearing male or female flower (Corley and Tinker, 2003). The fruit is a drupe which consists of a thin epicarp, an oily mesocarp and a nut or seed and generally borne on a large, compact bunch. The seed generally contains one to three embryos surrounding

by a large endosperm (kernel) and hard stone endocarp (shell). The oil palm root will be described in detail in the next section (Corley and Tinker, 2003).

The fruits of oil palm exhibit great variation in either the fruit type or form and these have been used for the classification of the varieties. The oil palm fruit can be divided into two types, *nigrescens* (purple) and *virescens* (green) on the basis of the external color. The thickness of the shell distinguishes the oil palm fruits into three forms: *dura* which produces fruits with a thick shell, *psifera,* which is without a shell in its rare fruits; *tenera* which is a hybrid of *dura* and *psifera* and produces fruits with an intermediate shell. However, Corley and Tinker (2003) indicated that the term variety is inappropriate for *tenera* and other forms since the materials are heterogeneous for most of the characters other than shell thickness.

2.1.2 Root of Oil Palm

The root systems of terrestrial plant play two important primary roles: acquisition of resources especially water and dissolved ions from the soil and anchorage (Fitter, 1991). Other functions of the root system such as storage, synthesis of plant growth regulator and propagation are classified as secondary roles (Fitter, 1991). Root systems have been grouped into three categories based on the root diameters and the development of root hairs: graminoid, magnolioid and intermediate root systems (Ingrouille and Eddie, 2006). Graminoid root systems consist of profuse root hairs and

have very large absorptive area to root volume ratio whereas magnolioid root systems have roots with large diameter and fewer root hairs (Ingrouille and Eddie, 2006).

The oil palm has a fibrous root system. In the juvenile phase of oil palm, six types of root are found: radicle, primary roots emitted from a 1 to 5-month-old oil palm (RI_{1-5}), primary roots emitted from 5 to 12-month-old oil palm (RI_{5-12}), lateral long roots, lateral medium roots and lateral short roots (Figure 2.1) (Jourdan and Rey, 1997).

As the oil palm seed germinates, the radicle emerges and grows continuously in a slightly undulating pattern (Jourdan and Rey, 1997). The roots located at the base of the radicle are known as lateral long root (usually over 10 cm long) and emit very soon after germination (Jourdan *et al.,* 1995). The lateral medium roots that are less than 10 cm in length are distributed over long root and also the entire branched zone of the radicle. They bear the lateral short roots which do not exceed 1.5 cm in length and are non-branching in contrast to the lateral long and medium roots (Jourdan *et al.,* 1995).

One month after germination, the first adventitious primary root (RI_{1-5}) emits and grows in vertical, downward direction (Jourdan and Rey, 1997). Similar to the radicle, the primary roots have the branched structure. The RI_{1-5} and RI_{5-12} are white when young but rapidly turn to dark brown. The lateral roots found in the RI_{1-5} and RI_{5-12} are similar to those of radicle but in different proportions: the proportion of long root increases with a gradual disappearance of medium and short root (Jourdan and Rey, 1997).