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

LIM KOK ANG

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By

LIM KOK ANG

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

June 2008
ANALYSIS OF TRANSCRIPTOMIC CHANGES IN OIL PALM (*Elaeis guineensis* Jacq.) ROOT UPON INOCULATION WITH *Bacillus sphaericus* UPMB10 USING cDNA MICROARRAY

By

LIM KOK ANG

June 2008

Chairman: Ho Chai Ling, PhD

Faculty: Faculty of Biotechnology and Biomolecular Sciences

*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

LIM KOK ANG

Jun 2008

Pengerusi: Ho Chai Ling, PhD

Fakulti: Fakulti Bioteknologi dan Sains Biomolekul

*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 membeaskan 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*. 
ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to Assoc. Prof. Dr. Ho Chai Ling and Prof. Zulkifli Hj. Shamsuddin for their advice and guidance; Dr. Tan Siang Hee and Ng Wai Har for cDNA library and cDNA clones of root from oil palm; Dr. Harikrishna Kulaveerasingam, Dr. Meilina Ong Abdullah, Lee Yang Ping, Kwan Yen Yen, and Tee Jin Meng for cDNA clones of vegetative meristem and zygotic embryo; Dr. Maheran Abu Bakar and Aw Khoo Teng for preparation of \textit{in vitro} oil palm seedling; Irni Suhayu Bt. Sapian for microarray scanning and Dr. Premalatha Pakirisamy and all laboratory members especially Siti Habshah, Tee Sue Sean, Teo Chin Jit, Teo Swee Sen, Teoh Seddon and Yong Sock Hwa for their assistance and support throughout this study. This work was financially supported by grant IRPA 01-04-03-T0045-TC2 from the Ministry of Science, Technology and Innovation of Malaysia.
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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Date : 14 August 2008
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.

LIM KOK ANG

Date: 7 July 2008
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<tr>
<td>Δ</td>
<td>Delta</td>
</tr>
<tr>
<td>®</td>
<td>Registered trademark</td>
</tr>
<tr>
<td>1 x</td>
<td>1 time</td>
</tr>
<tr>
<td>10 x</td>
<td>10 times</td>
</tr>
<tr>
<td>15N</td>
<td>Nitrogen-15</td>
</tr>
<tr>
<td>24:1</td>
<td>Ratio 24 to 1</td>
</tr>
<tr>
<td>25:24:1</td>
<td>Ratio 25 to 24 to 1</td>
</tr>
<tr>
<td>6 x</td>
<td>6 times</td>
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<tr>
<td>A_{260/230}</td>
<td>Absorbance at wavelength of 260 nm over 230 nm</td>
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<td>A_{260/280}</td>
<td>Absorbance at wavelength of 260 nm over 280 nm</td>
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<tr>
<td>ADP</td>
<td>Adenine diphosphate</td>
</tr>
<tr>
<td>aRNA</td>
<td>Amplified RNA</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>BNF</td>
<td>Biological nitrogen fixation</td>
</tr>
<tr>
<td>bp</td>
<td>Base pair</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>Calcium chloride</td>
</tr>
<tr>
<td>CAP3</td>
<td>Contig Assembly Program 3</td>
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<tr>
<td>cDNA</td>
<td>Complementary DNA</td>
</tr>
<tr>
<td>cfu</td>
<td>Colonies forming unit</td>
</tr>
<tr>
<td>CsCl</td>
<td>Cesium chloride</td>
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<tr>
<td>Ct</td>
<td>Threshold cycle</td>
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<td>CTAB</td>
<td>Cetyl trimethyl ammonium bromide</td>
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<td>CTP</td>
<td>Cytidine triphosphate</td>
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<td>dbEST</td>
<td>Database of EST</td>
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<td>dCTP</td>
<td>2’deoxyctydine 5’-triphosphate</td>
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<td>DEPC</td>
<td>Diethyl pyrocarbonate</td>
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<td>DMSO</td>
<td>Dimethylsulfoxide</td>
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<td>DNaseI</td>
<td>Deoxyribonuclease I</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>dNTP</td>
<td>2’-deoxyribonucleoside triphosphate</td>
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<td>DRAG</td>
<td>dinitrogenase reductase-activating glycohydrolase</td>
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<tr>
<td>DRAT</td>
<td>Dinitrogenase reductase ADP-ribosyltransferase</td>
</tr>
<tr>
<td>DTT</td>
<td>Dithiothreitol</td>
</tr>
<tr>
<td>E</td>
<td>Efficiency</td>
</tr>
<tr>
<td>$e^-$</td>
<td>Electron</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediamine tetraacetic acid</td>
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<td>EST</td>
<td>Expressed sequence tag</td>
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<tr>
<td>FDR</td>
<td>False discovery rate</td>
</tr>
<tr>
<td>Fe</td>
<td>Iron</td>
</tr>
<tr>
<td>FELCRA</td>
<td>Federal Land Consolidation and Rehabilitation Authority</td>
</tr>
<tr>
<td>FELDA</td>
<td>Federal Land Development Authority</td>
</tr>
<tr>
<td>g</td>
<td>Relative centrifugal force ($rcf$)</td>
</tr>
<tr>
<td>GTP</td>
<td>Guanosine triphosphate</td>
</tr>
<tr>
<td>H$^+$</td>
<td>Proton</td>
</tr>
<tr>
<td>H$_2$</td>
<td>Hydrogen</td>
</tr>
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<td>HCl</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>HEPES</td>
<td>N-(2-hydroxyethyl)piperazine-$N'$-(2-ethanesulfonic acid)</td>
</tr>
<tr>
<td>IAA</td>
<td>Indole-3-acetic acid</td>
</tr>
<tr>
<td>IPTG</td>
<td>Isopropylthio-$\beta$-D-galactoside</td>
</tr>
<tr>
<td>ISR</td>
<td>Induced systemic resistance</td>
</tr>
<tr>
<td>KCl</td>
<td>Potassium chloride</td>
</tr>
<tr>
<td>KEGG</td>
<td>Kyoto Encyclopedia of Genes and Genomes</td>
</tr>
<tr>
<td>kPa</td>
<td>KiloPascal</td>
</tr>
<tr>
<td>LB</td>
<td>Luria-Bertani</td>
</tr>
<tr>
<td>LiCl</td>
<td>Lithium chloride</td>
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<td>M</td>
<td>Molar</td>
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<td>MgCl$_2$</td>
<td>Magnesium chloride</td>
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<td>MgSO$_4$</td>
<td>Magnesium sulfate</td>
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<td>Mo</td>
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<td>mRNA</td>
<td>Messenger RNA</td>
</tr>
<tr>
<td>MS</td>
<td>Murashige and Skoog</td>
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<tr>
<td>N</td>
<td>Nitrogen</td>
</tr>
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<td>N₂</td>
<td>Nitrogen</td>
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<td>Naphthalene acetic acid</td>
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<td>Sodium chloride</td>
</tr>
<tr>
<td>NAD</td>
<td>Nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>NaOH</td>
<td>Sodium hydroxide</td>
</tr>
<tr>
<td>NCBI</td>
<td>National Center for Biotechnology Information</td>
</tr>
<tr>
<td>Ndfa</td>
<td>Nitrogen derived from atmosphere</td>
</tr>
<tr>
<td>ng</td>
<td>Nanogram</td>
</tr>
<tr>
<td>NH₃</td>
<td>Ammonia</td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>Ammonium</td>
</tr>
<tr>
<td>nM</td>
<td>Nanomolar</td>
</tr>
<tr>
<td>OD&lt;sub&gt;600&lt;/sub&gt;</td>
<td>Optical density at wavelength of 600nm</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate-buffered saline</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>Pfu</td>
<td>Plaques forming unit</td>
</tr>
<tr>
<td>PGPB</td>
<td>Plant growth promoting bacteria</td>
</tr>
<tr>
<td>PGPR</td>
<td>Plant growth promoting rhizobacteria</td>
</tr>
<tr>
<td>P&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Phosphate</td>
</tr>
<tr>
<td>PVPP</td>
<td>Polyvinylpolypyrrolidone</td>
</tr>
<tr>
<td>Real Time RT-PCR</td>
<td>Real time reverse transcription-PCR</td>
</tr>
<tr>
<td>RI&lt;sub&gt;1-5&lt;/sub&gt;</td>
<td>Primary roots emitted from a 1 to 5-month-old oil palm</td>
</tr>
<tr>
<td>RI&lt;sub&gt;5-12&lt;/sub&gt;</td>
<td>Primary roots emitted from a 5 to 12-month-old oil palm</td>
</tr>
<tr>
<td>RISDA</td>
<td>Rubber Industry Smallholders’ Development Authority</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acids</td>
</tr>
<tr>
<td>RNase</td>
<td>Ribonuclease</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>Rpm</td>
<td>Revolution per minute</td>
</tr>
<tr>
<td>SAM</td>
<td>Significant analysis of microarray</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>SDS</td>
<td>Sodium dodecyl sulfate</td>
</tr>
<tr>
<td>sp.</td>
<td>species</td>
</tr>
<tr>
<td>SSC</td>
<td>Standard saline citrate</td>
</tr>
<tr>
<td>TAE</td>
<td>Tris-acetate EDTA</td>
</tr>
<tr>
<td>Tm</td>
<td>Melting temperature</td>
</tr>
<tr>
<td>TM</td>
<td>Trade mark</td>
</tr>
<tr>
<td>Tris</td>
<td>2-amino-2-hydroxymethyl-1,3-propanediol</td>
</tr>
<tr>
<td>TS</td>
<td>Tryptic soy</td>
</tr>
<tr>
<td>TUC</td>
<td>Tentative unique contig</td>
</tr>
<tr>
<td>TUG</td>
<td>Tentative unique gene</td>
</tr>
<tr>
<td>TUS</td>
<td>Tentative unique sequence</td>
</tr>
<tr>
<td>U</td>
<td>Unit</td>
</tr>
<tr>
<td>UTP</td>
<td>Uridine triphosphate</td>
</tr>
<tr>
<td>v/v</td>
<td>Volume per volume</td>
</tr>
<tr>
<td>w/v</td>
<td>Weight per volume</td>
</tr>
<tr>
<td>X-gal</td>
<td>5-bromo-4-chloro-3-indolyl-β-D-galactoside</td>
</tr>
<tr>
<td>β-</td>
<td>Beta-</td>
</tr>
<tr>
<td>δ</td>
<td>delta</td>
</tr>
<tr>
<td>λ</td>
<td>Lambda</td>
</tr>
<tr>
<td>μg</td>
<td>Microgram</td>
</tr>
<tr>
<td>μL</td>
<td>Microlitre</td>
</tr>
<tr>
<td>μM</td>
<td>Micromolar</td>
</tr>
</tbody>
</table>
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 Cocosideae, 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 (RI1-5), primary roots emitted from 5 to 12-month-old oil palm (RI5-12), 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 (RI1-5) emits and grows in vertical, downward direction (Jourdan and Rey, 1997). Similar to the radicle, the primary roots have the branched structure. The RI1-5 and RI5-12 are white when young but rapidly turn to dark brown. The lateral roots found in the RI1-5 and RI5-12 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).