UNIVERSITI PUTRA MALAYSIA

IDENTIFICATION AND CHARACTERIZATION OF DIFFERENTIALLY EXPRESSED GENES IN *GANODERMA BONINENSE*-INFECTED OIL PALMS (*ELAEIS GUINEENSIS*)

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By

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

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IDENTIFICATION AND CHARACTERIZATION OF DIFFERENTIALLY EXPRESSED GENES IN *GANODERMA BONINENSE*-INFECTED OIL PALMS (*ELAEIS GUINEENSIS*)

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Chairman: Associate Professor Faridah Abdullah, PhD

Faculty : Science

Basal stem rot (BSR) caused by *Ganoderma boninense* is the most serious disease of oil palms in Malaysia. Thus, changes in the gene expression patterns of infected palms have gained interests among researchers as a tool to understand the disease. An indication that there are differences in susceptibility to BSR between germplasm materials from different genetic origins have provided hope in generating oil palm varieties with reduced levels of susceptibility by using existing genetic materials. The study of differentially expressed genes may also pave the way towards developing diagnostic tools for early disease detection in oil palms. A novel method combining elements of suppression subtractive hybridization (SSH) permits the efficient and rapid cloning of rarely transcribed differentially expressed genes. The experimental strategy virtually excludes the possibility of isolating false positive clones. This study used SSH and isolated 1,038 differentially expressed cDNAs from the *G. boninense*-inoculated oil palm seedlings (T1) when subtracted from its

uninoculated counterpart (T2), using the basal stem and spear leaf tissues. Sequence data indicated that of the 1,038 clones obtained, 86% showed sequence similarity to proteins already registered in public databases, 10% showed similarity to putative protein sequences and 4% were unknown proteins with no records in public databases. Seven clones harboring genes encoding for defense mechanisms against fungal and insect pathogens in plants were identified. They were pathogenesis-related (PR)-genes and defense-related genes depending on their direct or indirect roles in plant defense against pathogens. Reverse northern analysis of these 7 clones demonstrated that 4 were differentially expressed in T1 but northern analysis showed that only 3 were differentially expressed. Further analysis via reverse transcription-PCR (RT-PCR) confirmed these 3 genes to be differentially expressed in *G. boninense* infected oil palms. They were MAG 43 that codes for serine palmitoyltransferase, MAG 59 for chitinase and MAG 225 for endochitinase.

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

PENGENALPASTIAN DAN PENCIRIAN GEN YANG DIEKSPRESKAN SECARA BERBEZA DI DALAM SAWIT (*ELAEIS GUINEENSIS*) YANG DIJANGKITI OLEH *GANODERMA BONINENSE*

Oleh

ZETTY NORHANA BT. BALIA YUSOF

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Penyakit reput pangkal batang (RPB) yang disebabkan oleh *Ganoderma boninense* merupakan penyakit sawit yang paling serius di Malaysia. Justeru itu, pelbagai kajian biologi molekul terhadap sawit yang dijangkiti oleh *G. boninense* semakin giat dilakukan bagi mengetahui perubahan pengekspresan gen akibat jangkitan. Terdapat juga petunjuk tentang perbezaan kadar jangkitan penyakit RPB yang bergantung kepada kandungan sel daripada keturunan genetik berbeza. Petunjuk ini telah memberi harapan untuk menghasilkan variasi sawit yang mempunyai kadar jangkitan yang rendah dengan mengeksploitasikan unsur-unsur genetik yang sedia ada. Selain itu, kajian terhadap gen yang diekspreskan secara berbeza juga mungkin merupakan satu proses permulaan bagi menghasilkan alat diagnostik untuk mengesan kehadiran patogen di dalam sawit. Kaedah baru yang menggabungkan elemen penindasan, penolakan dan penghibridan membolehkan pengklonan gen yang diekspreskan secara berbeza. Strategi ujikaji sebegini dapat menyingkirkan secara mutlak

kebarangkalian penghasilan klon positif palsu. Potensi kaedah yang digunakan telah dibuktikan dengan pengasingan 1,038 cDNA yang diekspreskan secara berbeza di dalam anak sawit yang telah diinokulasi dengan *G. boninense* (T1) jika dibandingkan dengan anak sawit yang tidak diinokulasi dengan *G. boninense* (T2). Dua jenis tisu yang digunakan di dalam kajian ini ialah tisu pangkal batang dan pucuk sawit. Data jujukan yang diperolehi menunjukkan daripada 1,038 klon yang diperolehi, 86% daripadanya menepati dengan protein yang telah didaftarkan, 10% menunjukkan homologi dengan jujukan protein putatif dan 4% tidak menepati sebarang jujukan di dalam GenBank/EMBL Database. Sebanyak 7 klon yang menepati jujukan gen yang berfungsi di dalam sistem pertahanan daripada serangan kulat dan serangga di dalam tumbuh-tumbuhan telah dikenalpasti. Kesemua 7 klon tersebut mengandungi jujukan protein yang berkaitan dengan patogenesis dan gen-gen yang terlibat di dalam sistem pertahanan tumbuh-tumbuhan. Analisa penghibridan northern berbalik terhadap ketujuh-tujuh gen tersebut menunjukkan hanya 4 daripadanya diekspreskan di dalam sampel T1. Seterusnya, analisa northern membuktikan hanya 3 gen diekspreskan di dalam T1. Kaedah PCR transkripsi berbalik (RT-PCR) dijalankan bagi mengesahkan keputusan yang diperolehi daripada analisa northern ke atas ketiga-tiga gen tersebut. Keputusan RT-PCR mengesahkan bahawa ketiga-tiga gen tersebut adalah diekspreskan secara berbeza di dalam anak sawit yang telah dijangkiti *G. boninense.* Ianya adalah MAG 43 yang membawa jujukan gen serine palmitoyltransferase, MAG 59 yang membawa jujukan gen chitinase dan MAG 225 yang membawa jujukan gen endochitinase.

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I certify that an Examination Committee has met on 13th August 2007 to conduct the final examination of Zetty Norhana Binti Balia Yusof on her Master of Science thesis entitled "Identification and Characterization of Differentially Expressed Genes in *Ganoderma boninense*-infected Oil Palms (*Elaeis guineensis*)" 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 degree of Master of Science.

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

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Date: 15 November 2007

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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ZETTY NORHANA BINTI BALIA YUSOF

Date: 20 September 2007

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	percent
~	about / roughly
<	less than
>	more than
°C	degree Celsius
µg	microgram
µJ	microjoule
µl	microliter
µM	micromolar
^{32}P	radioactive isotope of phosphorus
α	alpha
β	beta
β-NAD	beta-nicotinamide adenine dinucleotide
ATP	adenosine triphosphate
avr-R	avirulence-resistance
BLAST	Basic Local Alignment Search Tool
BSA	bovine serum albumine
BSR	basal stem rot
$Ca2^{+}$	calcium ion
cDNA	complementary DNA

cm	centimeter
cm^2	centimeter square
CPKO	crude palm kernel oil
CPO	crude palm oil
D × P	Dura × Pisifera
dATP	2'-deoxyadenosine 5'-triphosphate
dCTP	2'-deoxycytidine 5'-triphosphate
DEGs	differentially expressed genes
dGTP	2'-deoxyguanosine 5'-triphosphate
dH_2O	distilled water
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
DOA	Department of Agriculture
dTTP	2'-deoxythymidine 5'-triphosphate
EDTA	ethylenediaminetetraacetic acid
EtBr	ethidium bromide
g	gram
GRPs	glycine-rich proteins
GSM	*Ganoderma* selective medium
h	hour

H_2O	water
H_2O_2	hydrogen peroxide
kb	kilo base pairs
KCl	kalium chloride
kDa	kiloDalton
LB	Luria-Bertani
LiCl	lithium chloride
LTPs	lipid-transfer proteins
M	molar
MARDI	Malaysian Agriculture Research and Development Institute
$MgCl_2$	magnesium chloride
min	minute
ml	mililiter
mM	milimolar
MPOB	Malaysian Palm Oil Board
MPOB-UKM	Malaysian Palm Oil Board-Universiti Kebangsaan Malaysia
MPOC	Malaysian Palm Oil Council
MPOCF	Malaysian Palm Oil Wildlife Conservation Fund
mRNA	messenger RNA

NaCl	natrium chloride
ng	nanogram
nm	nanometer
OAS	oral allergy syndrome
OD	optical density
PCR	polymerase chain reaction
PORIM	Palm Oil Research Institue of Malaysia
PR proteins	pathogenesis-related proteins
RIPs	ribosome-inactivating proteins
RNA	ribonucleic acid
rpm	revolutions per minute
rRNA	ribosomal RNA
SAR	systemic acquired resistance
SDS	sodium dodecyl sulfate
SSC	sodium chloride-sodium citrate
SSH	suppression subtractive hybridization
T1	treatment 1
T2	treatment 2
T3	treatment 3
TAE	tris-acetate-EDTA

TL proteins	thaumatin-like proteins
Tris-HCl	tris hydrochloride
U	unit
UV	ultraviolet
V/cm	volt per centimeter
v/v	volume per volume
w/v	weight per volume

CHAPTER 1

INTRODUCTION

Oil palm (*Elaeis guineensis*) is one of the most important oil tree species in tropical regions because of the raw materials it produces, palm oil and palm kernel oil. However, fungal diseases of the oil palm can cause very serious losses in production. Important diseases of oil palms are reported as vascular wilt caused by *Fusarium oxysporum*, basal stem rot (BSR) (*G. boninense*), red ring disease (*Rhadinaphelenchus cocophilus*), sudden wilt (*Phytomonas staheli*) and spear rot (unknown pathogen) (Turner, 1981; Ariffin, 2000). As far as the disease problem to oil palm in Malaysia is concerned, the most serious disease is BSR and it requires an urgent solution.

BSR is not new to Malaysia as it has been known to attack oil palm since the early years when the crop was introduced into this country. The disease was first reported in 1931 (Thompson, 1931). Losses due to BSR is not only through the direct reduction in oil palm stands, but also through a decrease in fruit bunch number and weight from diseased standing palms as well as those with sub-clinical infections (Turner, 1981). The disease can result in the death of more than 80% of the plants by the time they are half-way through their normal economic life and losses reaching 30% have quite frequently occurred (Turner, 1981). A survey indicated that BSR was present in more than 50% of the oil palm fields in Peninsular Malaysia (Idris *et al*., 2001).

The threat of *G. boninense* to the oil palm industry in this country warrants new and more aggressive approaches in finding solution to the disease. It would benefit the industry very much if a rapid and accurate diagnostic technique for detecting *G. boninense* in oil palms at an early stage of infection is available.

Studies by De Franqeville *et al*. in 2001 showed that there were differences in the susceptibility of germplasm materials from different genetic origins to BSR. This provides hope in the screening for oil palm varieties with reduced levels of susceptibility using existing genetic materials. The development of diagnostic tools based on PCR primers have also provided new dimension for early detection of the pathogen in oil palms (Bridge *et al*., 2001). Another new area of investigation is in the altered expression of several classes of genes in plants when infected by pathogens. These include genes associated with cell maintenance and development, genes involved in the biosynthesis of lignin and phenolics and genes implicated in oxidative burst, programmed cell death or hypersensitive response (Schenk *et al*., 2000). Thus, the isolation and characterization of disease resistance or stress responsive genes through molecular biology approach are the initial steps in the commencement of a molecular biology approach towards an understanding of the defense and stress response mechanisms in oil palms.

The discovery of novel genes has traditionally been a laborious task. The conventional breeding techniques to produce disease resistant and stress tolerant lines are limited, time consuming and also laborious. Consequently, the