Basal stem rot disease (BSR) is a common disease that affects the Malaysian oil palm. The disease devastates thousands of hectares of oil palm plantings in Southeast Asia every year. It is caused by the fungus *Ganoderma boninense,* which infects the oil palm trees, causing loss of yield and finally killing the trees. In the present study, gene expression and proteomic investigations were carried out on the root tissues of the oil palm infected with *G. boninense.* While the gene expression data obtained from this study may be used in future work on the development of resistant or tolerant oil palm varieties against this fatal infection, the proteomics data can be used to develop protein biomarkers that may be used for the early detection of the fungal infection. Three different plant genes related to response to fungal infection, comprising those that express polygalacturonase-inhibiting protein (PGIP), lipid transfer protein (LTP) and pathogen related protein 10 (PR10), were identified in the oil palm, based on conserved sequences of the same genes of other monocots. The three identified gene sequences demonstrated high similarities with their counterparts from the other monocots and up to 100% identity with those of rice. When expression of the genes was studied in the oil palm roots, the highest levels of expression for all three genes were detected in uninfected palms for all the three genes. The levels of expression of the genes significantly decreased subsequent to an infection with *G. boninense* for all treatment timeframes studied (2, 4, 6 and 8 weeks post infection). Collectively, the gene expression investigation that was performed in this study demonstrated the coordinated down-regulated expression of defence related genes PGIP, LTP and PR10 in the oil palm roots during the early stages of infection with *G. boninense.* This differential expression may provide some indication as to how the fungus actively suppresses the host response and/or escape being recognized by the host system allowing

fection. In an attempt to identify proteins that may be used detection of *G. boninense* infection of the oil palm, a proteomics study was performed on proteins extracted from the infected and non-infected root tissues of the oil palm plant. The study allowed for the investigation of the global response of the oil palm genome to the pathogen during the early stages of infection. When profiled by 2-dimensional gel electrophoresis, 61 protein spots were initially detected to be differentially expressed between the uninfected control and infected root tissues. Among the differentially expressed proteins, 22 spots that showed highest differential expression were chosen for identification. This included 13 proteins that were significantly down-regulated and 9 that were significantly up-regulated subsequent to the *G. boninense* inoculation. Analysis by mass spectrometry and database search generated 21 protein hits, with 11 of them considered putatively identified on the basis of MASCOT scores of more than 55. However, among these 11 proteins, two were of unknown functions, while the remainder included enolase, fructokinase, caffeoyl-CoA O-methyltransferase, caffeic acid O-methyltransferase, aminopeptidase, enoyl-acyl carrier protein reductase, pyridoxal 5-phosphate (PLP)-dependent enzyme, malate dehydrogenase and ATP synthase. While the altered expression of these proteins may have some physiological relevance to the plant, such as the need to change its metabolism or being involved in its defence mechanism, these proteins may also be exploited for their potential use as biomarkers for oil palm root infection. The analysis of activation and synthesis of infection/stress related proteins identified can potentially generate a set of biomarkers to discriminate between different defence-related strategies, as diagnostic tools and in the prognosis monitoring of basal stem rot infection.

Penyakit Basal Stem Rot (BSR) adalah penyakit biasa yang menjangkiti pokok kelapa sawit di Malaysia. Setiap tahun, penyakit ini telah dilaporkan mengakibatkan kerugian pada beribu-ribu hektar ladang kelapa sawit di Asia Tenggara. Penyakit ini berpunca daripada sejenis kulat, *Ganoderma boninense* yang menjangkiti kelapa sawit, mengakibatkan hasil kelapa sawit berkurangan dan akhirnya membunuh pokok tersebut. Di dalam kajian ini, penyiasatan berkaitan ekspresi gen dan proteomik telah dijalankan pada tisu akar kelapa sawit yang telah dijangkiti oleh *G. boninense*. Maklumat ekspresi gen yang diperolehi daripada kajian ini boleh digunakan untuk kajian-kajian akan datang bagi menghasilkan variati kelapa sawit yang mempunyai daya tahan terhadap penyakit ini. Manakala maklumat proteomik boleh digunakan dalam kajian-kajian akan datang bagi mengenalpasti protein bio-penanda untuk pengesanan awal jangkitan kulat.

Tiga gen tumbuhan yang berhubung kait dengan tindak balas terhadap jangkitan kulat, iaitu gen yang mengekspres protein penghalang polygalacturonase (PGIP), protein pemindahan lipid (PLT) dan protein 10 berkaitan patogen (PR10) dikenalpasti di dalam sistem pokok kelapa sawit, berdasarkan jujukan serupa daripada gen yang sama yang terdapat pada tumbuhan monokot yang lain. Ketiga-tiga gen ini menunjukkan persamaan yang tinggi dengan gen dari tumbuhan monokot yang berkait rapat dengan kelapa sawit dan juga menunjukkan 100% identiti dengan beras. Apabila ekspresi gen ini dikaji pada akar kelapa sawit, ketiga-tiga gen menunjukkan kadar ekspresi yang tinggi pada pokok kelapa sawit yang tidak dijangkiti. Manakala, kadar ekspresi gen didapati berkurangan secara signifikan apabila dijangkiti oleh *G. boninense* untuk semua sampel tanpa mengira tempoh jangkitan (2, 4, 6 atau 8 minggu selepas jangkitan). Secara keseluruhannya, siasatan

i dalam kajian ini menunjukkan ekspresi gen yang berkaitan PGIP, LTP dan PR10, menurun secara koordinasi semasa peringkat awal jangkitan *G. boninense* pada akar pokok kelapa sawit. Perbezaan ekspresi ini berkemungkinan memberi pentunjuk tentang bagaimana kulat menghalang tindak balas hos dan/atau terlepas daripada dikenalpasti oleh sistem pertahanan hos sekaligus membolehkan jangkitan berlaku. Di dalam usaha untuk mengenalpasti protein yang boleh digunakan sebagai biopenanda untuk pengesanan awal jangkitan *G. boninense* pada kelapa sawit, kajian proteomik telah dijalankan pada protein yang diekstrak daripada tisu akar pokok kelapa sawit yang telah dijangkiti dan yang tidak dijangkiti. Kajian ini membolehkan siasatan tentang tindak balas umum genom kelapa sawit pada patogen semasa peringkat awal jangkitan. Apabila pemprofilan dilakukan menggunakan elektroforesis gel 2 dimensi, 61 bintik protein yang dikenalpasti daripada tisu akar yang dijangkiti dan tisu akar kawalan yang tidak dijangkiti telah menunjukkan ekspresi yang berbeza pada peringkat awal. Daripada protein-protein yang telah diekspresikan itu, 22 bintik protein yang menunjukkan ekspresi protein yang tertinggi telah dipilih untuk tujuan identifikasi. Ini termasuk 13 protein yang menunjukkan penurunan dan peningkatan yang signifikan berikutan inokulasi *G. boninense.* Analisa menggunakan spektrometri jisim dan carian pangkalan data menjana 21 hasil carian, 11 daripadanya telah diambilkira sebagai wujud berdasarkan skor MASKOT yang melebihi 55. Bagaimanapun, daripada 11 protein ini, 2 daripadanya tidak dapat dikenalpasti fungsinya manakala yang selebihnya merupakan enolase, fruktokinase, kafeoil-CoA O-metiltransferase, asik kafeik O-metiltransferase, aminopeptidase, protein pembawa enoil-asil reductase, piridoksil 5-fosfat (PLP)-enzim dependen, malat dehidrogenase dan ATP sintase. Sementara perubahan ekspresi protein-protein tersebut mungkin mengakibatkan kesan fisiologi pada tumbuhan, seperti keperluan untuk mengubah

ibatan di dalam mekanisme pertahanan, protein-protein ini bagi potensi penggunaannya sebagai bio-penanda untuk jangkitan akar pokok kelapa sawit. Analisa aktivasi dan sintesis protein yang berkaitan jangkitan/stres yang dikenalpasti boleh menjana suatu set bio-penanda untuk membezakan strategi yang berkaitan pertahanan, sebagai alat diagnostik dan semasa prognosis pengawasan jangkitan BSR.

ement

In the name of ALLAH, the Most Gracious and the Most Merciful

Prayers and peace be upon His kind Messenger Mohammad Bin Abdullah, his family members, all his companions and true followers until the Day of Judgment.

Alhamdulillah, all praises to ALLAH for the strengths and His blessing in completing this thesis.

Foremost, I would like to express my sincere gratitude to my supervisor Prof. Dr. Rofina Yasmin Binti Othman for the continuous support of my PhD study and research, for her patience, motivation, enthusiasm, and immense knowledge. Her guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor for my PhD study. It has been an honor to be her PhD student.

I gratefully acknowledge my second supervisor Prof. Dr. Onn Bin Haji Hashim for his advice, supervision, and crucial contribution, which made him a backbone of this research and so to this thesis. His involvement with his originality has triggered and nourished my intellectual maturity that I will benefit from, for a long time to come.

My sincere thanks also goes to Dr. Yusmin Mohd Yusuf, for her continues help and supports during this long journey. I especially thank her for her encouragement and her accurate comments which were of critical importance, during this work. Our cooperation was truly an inspiring experience.

I also would like to dedicate this work to my labmates, Wei wei, Pei see, Puan Marina, Puan Maria , Dr Teh ,Dr Syarifa , Su yen ,Yee song, Kim hain, Dr. Sew Hwa.

esearch Sdn Bhd (grant no. 55-02-03-1038) for funding this

nples. Not to forget to thank the University of Malaya for providing me the IPPP grant No.PSP424-2010A which supported this project.

Last but not least, my deepest gratitude goes to my beloved parents; Mr. Rabee Jameel Al-Obaidi and and Mrs.Hayfaa Abdulmunim Al-Obaidi and also to my wife, brother and my sisters for their endless love, prayers and encouragement, (Eda, Gaith, Salwa and Roaa) I love you so much. To those who indirectly contributed in this research, your kindness means a lot to me. Thank you very much.

Wasslamualikum

Jameel Rabee Al-Obaidi

CONTENTS

ONS

1st D	first dimension
2-DE	two-dimensional electrophoresis
ACN	acetonitrile
APS	ammonium persulphate
ATP	adenosine triphosphate
BLAST	basic local alignment search tools
BSA	bovine serum albumin
BSR	basal stem rot disease
CCoAOMT	caffeoyl-CoA O-methyltransferase
cDNA	complementary deoxribonucleic acid
CHAPS	3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate
COMT	caffeic acid O-methyltransferase
CTAB	cetyltrimethylammonium bromide
CWDE	cell wall degrading enzymes
DEPC	diethyl pyrocarbonate
DNA	deoxribonucleic acid
dNTP	deoxyribonucleotide triphosphate
DTT	dithiothreitol
EB	elution buffer
EDTA	ethylenediaminetetraacetic acid
ENR	enoyl-acyl carrier protein reductase
EtBr	ethidium bromide
IDD	iodo acetamide
IEF	isoelectric focusing

	ized pH gradient
	c acid
LTP	lipid transfer protein
MALDI-TOF	matrix assisted laser desorption ionization - time of flight
MDH	malate dehyrogenase
MPOB	Malaysian Palm Oil Board
mRNA	messenger ribonucleic acid
MS	mass spectrometry
NaoAC	sodium acetate
NIFOR	Nigerian Institute for Oil Palm Research
PCR	polymerase chain reaction
PE	buffer from Qiagen
PG	polygalacturonase
PGIP	polygalacturonase-inhibiting protein
pI	isoelectric point
PBI	Phosphorus Buffer Index
PLP	pyridoxal 5- phosphate
PR10	pathogen related protein-10
PVPP	polyvinylpolypyrrolidone
Q-PCR	quantitative- polymerase chain reaction
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
RT-PCR	reverse transcription- polymerase chain reaction
sdH_2O	sterile distilled water
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis

	e ethylenediaminetetraacetic acid
	ylenediaminetetraacetic acid
TEM	transmission electron microscopy
TEMED	N,N,N',N' ï tetramethyl-ethylenediamine
UV	ultraviolet

RES

LES