CHARACTERIZATION AND PROFILING OF CELL WALL LIPIDS FROM *Ganoderma boninense*-INFECTED OIL PALM ROOTS

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I hereby declare that this thesis is based on my original work except for citations, quotations, and equations which have been duly acknowledged. I also declare that no part of this thesis has been previously or concurrently submitted for a PhD at any other university.

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CERTIFICATION

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Arnnyitte Alexander
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ABSTRACT

Basal stem rot disease (BSR) is the most devastating fungal disease in oil palm (OP) caused by *Ganoderma boninense,* and is one of the most commercially catastrophic diseases in Southeast Asia. This disease has resulted significant decreases in OP yield. On the basis of the importance of early detection for controlling the BSR disease, studies on the mechanism of interaction between OP and *G. boninense* through metabolomic approaches is currently still on-going. Information on response of OP to BSR is still scarce, particularly concerning changes in the plant membrane-cell wall continuum as the ultimate consequence of biological systems to pathogenesis responses. This interconnection borders most likely a reservoir in signal transduction, defense and antimicrobial metabolites, involving lipids and its derivatives released during the pathogen attack. Therefore this study focuses on lipid components in oil palm roots cell wall towards understanding the pathogenesis and defense response mechanism involved during the OP-*G. boninense* interaction. In this study, eight-months old OP seedlings were artificially inoculated with *G. boninense* using rubber wood blocks. Establishment of *G. boninense* infection was accessed after three and six-months post inoculation referred as first (T1) and second interval (T2), respectively. The cell wall preparation with the highest degree of cytosol component release was produced by lyophilization with homogenization for both OP roots and *G. boninense* mycelium. The optimum condition on extraction of lipid from cell wall components was investigated using Central Composite Design (CCD). A high lipid extraction yield (3.36%) was obtained under the following extraction conditions: 10 mL of solvent mixture (chloroform: methanol (2:1, v/v)) per gram tissue for 120 min with gentle agitation at 30˚C of extraction temperature. The changes of cell wall-lipid profiles in infected OP (IN) against healthy root in healthy OP (H), at T1 and T2 were investigated via High Performance-Thin Layer Chromatography (HP-TLC) and Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Statistical analysis including Principal Components Analysis (PCA), Partial Least Square-Discriminant Analysis (PLS-DA) and metabolic pathway analysis were performed using Metaboanalyst. The study observed that glycerolipid, linoleic acid, pyruvate and glycerophospholipid metabolism and glycolysis or gluconeogenesis are the most perturbed pathways at T1. Meanwhile, pyruvate metabolism and glycolysis or gluconeogenesis pathways are the most perturbed at T2. Global metabolite pathways found steroid biosynthesis, pyruvate metabolism and glycolysis or gluconeogenesis are the most perturbed pathways in both T1 and T2. There are many lipid metabolites in the OP cell wall are significantly differentially regulated during *G. boninense*-infection. Using a statistical biomarker validation analysis, PC(6:0/0:0), PC(2:0/2:0), methyl (6E,9E,12E)-6,9,12-octadecatrienoate, PA(18:4(6Z,(Z,12Z,15Z)/0:0), methyl palmitoleate, PA(14:0/ 0:0), γ-linolenic acid and stigmasterol were the metabolites found to be most useful in discriminating infected OP from healthy OP. The results distinguished metabolites present and

correspond with *G. boninense* infection are crossed-reference with the pathogen cell wall-lipid profiles to identify biomarker of the pathogen presence. Ergosterol, 5-lanost-8-en-3-ol, methyl tridecanoate and methyl 9-eicosenoate could be exploited as biomarkers for *G. boninense* presence. The identified biomarkers may serve as potential biomarkers for *G. boninense*-infection and assist in the early diagnosis and preventive treatment of BSR disease. The related pathways provide novel insights into developing strategies for better BSR management and resistant OP breed in the future.

Keywords: oil palm, *Ganoderma boninense,* basal stem rot, cell wall-lipids, biomarkers, pathway

ABSTRAK

PENCIRIAN DAN PEMPROFILAN LIPID DINDING SEL DARIPADA AKAR POKOK KELAPA SAWIT DIJANGKITI Ganoderma boninense

Penyakit Reput Pangkal Batang (BSR) merupakan penyakit pokok kelapa sawit (OP) yang paling memudaratkan. Penyakit ini disebabkan oleh kulat Ganoderma boninense, dan merupakan penyakit paling membinasakan di Asia Tenggara. Penyakit ini menyebabkan penurunan ketara ke atas hasil OP. Untuk mengawal penyakit RPB, pengesanan jangkitan pada peringkat awal amat penting, dan untuk tujuan ini kajian ke atas mekanisma interaksi di antara OP dan G. boninense melalui pendekatan metabolomik masih diteruskan. Informasi terhadap tindak balas OP terhadap BSR masih sedikit, khususnya merujuk kepada perubahan di dalam kontinum membran-dinding sel sebagai kesan sistem biologi terhadap tindak balas jangkitan. Sempadan perhubungan ini merupakan takungan pemindahan dan penghantaran isyarat, pertahanan dan metabolit antimikrob, yang melibatkan lipid dan derivatifnya yang mana di rembeskan semasa serangan patogen. Oleh itu kajian ini memfokuskan komponen lipid di dalam dinding sel akar OP dalam memahami patogenesis dan tindak balas pertahanan terlibat semasa interaksi OP-G. boninense. Dalam kajian ini, anak pokok OP yang berumur lapan-bulan telah di dijangkitkan secara inokulasi tiruan dengan G. boninense menggunakan bongkah kayu getah. Pengenalpastian jangkitan G. boninense dinilai pada tiga dan enam bulan selepas inokulasi tiruan, masing-masing dirujuk sebagai selang pertama (T1) dan selang kedua (T2). Penyediaan dinding sel OP dan G. boninense yang mempunyai darjah pembebasan komponen sitosol tertinggi dihasilkan dengan liofilisasi dan homogenisasi. Keadaan optimum ke atas pengekstrakan lipid dari dinding sel dikaji menggunakan rekabentuk komposit central (RSM). Hasil pengekstrakan lipid tertinggi (3.36%) telah diperolehi menggunakan kaedah pengekstrakan berikut: 10 mL campuran pelarut (chlorofom: methanol (2:1, v/v)) per gram tisu dengan pengacauan lembut selama 120 min pada suhu 30˚C. Perubahan profil dinding sel-lipid pada akar OP yang dijangkiti dan sihat pada T1 dan T2 disiasat menggunakan Kromatograpi lapisan nipis-berprestasi tinggi (HP-TLC) dan kromatograpi gas dan spetrometri jisim (GC-MS). Analisa statistik termasuk Analisa komponen berprinsip (PCA), Analisa diskriminan-kawasan paling kurang separa (PLS-DA) dan analisa laluan metabolik dibuat menggunakan Metaboanalyst. Kajian mendapati metabolisme gliserolipid, linolik asid, piruvat, gliserofosfolipid dan glikolisis atau glukoneogenesis adalah laluan metabolik paling terganggu semasa T1. Manakala, metabolisme piruvat dan glikolisis atau glukoneogenesis merupakan laluan metabolik paling terganggu semasa T2. Secara keseluruhan, laluan metabolik yang paling terganggu pada T1 dan T2 adalah biosintesis steroid, metabolik piruvate dan glikolisis atau glukoneogenesis. Terdapat banyak metabolit lipid di dalam dinding sel OP yang terubah dan berbeza secara

signifikan semasa jangkitan G. boninense. Dengan menggunakan analisa statistik pengesahan penanda-bio, PC(6:0/0:0), PC(2:0/2:0), metil (6E,9E,12E)-6,9,12-oktadecatrienoate, PA(18:4(6Z,(Z,12Z,15Z)/0:0), metil palmitoleate, PA(14:0/ 0:0), γ- asid linolenik and stigmasterol merupakan metabolit yang paling berguna dalam mendiskriminasikan OP yang dijangkiti daripada OP sihat. Hasil keputusan ke atas kehadiran metabolit berbeza berkaitan dengan jangkitan G. boninense di rujuk-silang dengan lipid pada dinding sel kulat tersebut untuk mengenalpasti penanda-bio kehadiran G. boninense. Ergosterol, 5-lanost-8-en-3-ol, metil tridekanoate and metil 9-eikosenoate boleh diekploitasi sebagai penanda-bio kehadiran G. boninense. Penanda-bio yang dikenalpasti boleh digunakan sebagai penanda-bio untuk jangkitan G. boninense dan membantu dalam pengenalpastian awal dan pencegahan jangkitan BSR. Laluan metabolik berkaitan memberikan pemerhatian nobel di dalam pembangunan strategi untuk menguruskan BSR dan baka OP yang tahan-rintang di masa hadapan.

Kata kunci: *kelapa sawit, Ganoderma boninense, reput pangkal batang, lipid-dinding sel, penanda-bio, laluan metabolik*

TABLE OF CONTENTS

LIST OF SYMBOLS AND ABBREVIATIONS

±	-	plus-minus
+	-	plus
-	-	minus
=	-	equals to
≥	-	more or equal to
/	-	divide by
%	-	percentage
ºC	-	degree Celcius
α	-	alpha
β	-	beta
γ	-	gamma
µg	-	microgram
g	-	gram
kg	-	kilogram
L	-	litre
m	-	meter
µl	-	microlitre
µM	-	micromolar
pM	-	picomolar
mg	-	milligram
ml	-	millilitre
mm	-	millimeter
µg/mL	-	microgram per millilitre
mg/mL	-	milligram per millilitre
µg/g	-	Microgram per gram
U	-	unit
pH	-	power of hydrogen
m/z	-	mass to charge ratio
g	-	g force
rpm	-	revolution per minute
h	-	hour

min	-	minute
e.g.	-	*exemplii gratia* (example)
UV	-	ultraviolet
OD	-	optical density
SD	-	standard deviation
PCA	-	principal component analysis
PLSDA	-	partial least square discriminant analysis
CPD	-	critical point dryer
SEM	-	scanning electron microscopy
FTIR	-	fourier infrared spectroscopy
IR	-	infrared
VIS	-	visible light
RSM	-	response surface methodology
GCMS	-	gas chromatography-mass spectrometry
HPTLC	-	high performance thin layer chromatography
HPLC	-	high performance liquid chromatography
ESI	-	electron spray ionization
SPE	-	solid phase extraction
CW	-	cell wall
FA	-	fatty acid
FAME	-	fatty acid methyl ester
ROS	-	reactive oxygen species
BCA	-	biological control agent
RWB	-	rubber wood block
GSM	-	*Ganoderma* selective media
AI	-	artificial inoculation
CMR	-	chloroform methanol ratio
ROCCET	-	ROC curve explorer and tester

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES