



UNIVERSITI PUTRA MALAYSIA

**CHARACTERIZATION OF NOVEL OIL PALM DEFENSE-RELATED
TRANSCRIPTION FACTORS DURING BIOTROPHIC AND
NECROTROPHIC INFECTION PHASES OF *Ganoderma boninense***

NURSHAFIKA BINTI MOHD SAKEH

IPTSM 2020 4



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By

NURSHAFIKA BINTI MOHD SAKIH

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfillment of the Requirement for the Degree of Doctor of Philosophy**

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TO MY LOVELY PARENTS.

**You both have sacrificed through blood, sweat and tears to nurture me
into whom I have become today.**

May Allah rewards both of you Jannatul Firdaus.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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Basal stem rot (BSR) disease, caused by *Ganoderma boninense* has been pinpointed to be one of the major factors that contribute to the decline in yield of oil palm. Hemibiotroph pathogen such as *G. boninense* manipulates the host defense mechanisms to strategically infect host plant by switching from biotrophic to necrotrophic phase. Recognizing the early infection phase of *G. boninense* by identifying phase-specific oil palm transcription factors (TFs) may offer opportunities to tackle or attenuate the progress of infection. The fragmentary information on molecular interactions between plant and hemibiotroph should be explicated to identify the key molecular mechanisms of pathogenesis and plant immunity. Thus, this study is an attempt to recognize specific TFs as 'key' biomarkers in identifying oil palm defense mechanisms during biotrophic and necrotrophic infection phases of *G. boninense*. Artificial infection of *G. boninense* on oil palm was performed by using *Ganoderma*-inoculated rubber wood blocks (RWBs) and bare RWBs serves as mock-treatment presenting abiotic stress. In order to identify oil palm defense response during early interaction with *G. boninense*, transcriptomic analysis of root tissues at different time points of 3, 7 and 11 days post inoculation (d.p.i) was carried out. High-throughput RNA-seq data analysis has revealed two distinguishable expression profiles of oil palm genes that formed the basis for deducing biotrophic phase at early interaction (3 d.p.i) which switched to initiation of necrotrophic phase at later stage (11 d.p.i) of infection. Based on the findings, the present study focused on identifying differentially expressed genes (DEGs) encoding TFs from the generated RNA-seq data. A total of 106 upregulated and 108 downregulated DEGs of TFs were identified. There are four established defense-related pathways that have been presented whereby reported genes involved in cell wall modification, reactive oxygen species (ROS)-mediated signaling, programmed cell death (PCD) and plant innate immunity were differentially expressed. The genes were found to be either upregulated or downregulated during the two distinct infection phases. Multiplex semi-quantitative RT-PCR was conducted to screen for defense-related TFs independent of abiotic stress. Normalized band intensity of *Ganoderma*-treated (GT) samples were compared to the mock-treated (MT) samples to estimate the mRNA expression level between groups. The expression patterns of eight candidate TFs genes including *EgJUB1*, *EgERF113*,

EgTCP15, *EgNAC29*, *EgEIN3*, *EgMYC2*, *EgNAC83* and *EgMYB122* were further quantified via quantitative Real-Time PCR (qPCR). Both *EgJUB1* and *EgERF113* were found to be specifically upregulated under biotic stress at all time points. The findings discovered upregulation of *EgJUB1* during biotrophic phase while *EgERF113* demonstrated prominent upregulation as oil palm switches to defense against necrotrophic phase. Characterization of *EgJUB1* and *EgERF113* was performed via *in vivo* Yeast One-Hybrid (Y1H) assay and *in vitro* electrophoretic mobility shift assay (EMSA). JUB1 has been reported to bind to NAC binding site (NACBS) motif during abiotic stress. The present study is the first report on the binding activity of *EgJUB1* to secondary wall NAC binding element 1 (SNBE1) which was present in the promoter region of *EgHSFC-2b* having similar expression profile as *EgJUB1*. SNBE1 motifs with single nucleotide change at either 5th or 18th position have been found in the promoter regions of a few TFs that co-expressed with *EgJUB1*, including *EgHSFB-4b* and *EgGAMYB X2*. Meanwhile *EgERF113* binds to GCC-box and DRE/CRT motifs promoting plasticity in upregulating downstream defense-related genes against *G. boninense* attack. Sequence analysis revealed the presence of NAC DNA binding domain (DBD) in *EgJUB1*. Amino acid change from phenylalanine (F) to tryptophan (W) at 14th position of *EgERF113* DBD proved the binding specificity to both GCC-box and DRE/CRT motifs. Prolonged treatment revealed oil palm seedlings succumbing to the *G. boninense* infection. Mature basidiomata of *G. boninense* was observed at 24 weeks post inoculation (w.p.i) and the infection culminated in plant death. Overall, our findings propose *EgJUB1* and *EgERF113* as key TFs in orchestrating the oil palm defense mechanisms during biotrophic and necrotrophic infection phases of hemibiotrophic *G. boninense*, respectively.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

**PENCIRIAN FAKTOR TRANSKRIPSI BERKAITAN PERTAHANAN POKOK
KELAPA SAWIT YANG BAHARU SEMASA JANGKITAN FASA BIOTROFIK
DAN NEKROTROFIK OLEH *Ganoderma boninense***

Oleh

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Penyakit reput pangkal batang (BSR), disebabkan oleh *Ganoderma boninense* telah ditunjukkan sebagai salah satu faktor utama yang menyumbang kepada penurunan hasil kelapa sawit. Patogen hemibiotrof seperti *G. boninense* memanipulasi mekanisme pertahanan perumah untuk menjangkiti perumah tumbuhan secara strategik dengan menukar fasa dari biotrofik kepada nekrotrofik. Mengenali fasa awal jangkitan *G. boninense* dengan mengenal pasti faktor transkripsi (TFs) kelapa sawit yang berfasa spesifik boleh menawarkan peluang untuk menangani atau melemahkan perkembangan jangkitan. Maklumat serpihan terhadap interaksi molekular antara tumbuhan dan hemibiotrof harus diperincikan untuk mengenalpasti mekanisme molekular yang utama daripada patogenesis dan imuniti tumbuhan. Oleh itu, kajian ini adalah percubaan untuk mengenal TFs spesifik sebagai 'kunci' penanda bio dalam mengenal pasti mekanisme pertahanan kelapa sawit semasa jangkitan fasa biotrofik dan nekrotrofik *G. boninense*. Jangkitan buatan *G. boninense* ke atas kelapa sawit telah dilakukan menggunakan blok kayu getah (RWB) terjangkit inokulum *G. boninense* dan RWB kosong sebagai rawatan olokkan mewakili tekanan abiotik. Untuk mengenal pasti respon pertahanan kelapa sawit semasa interaksi awal dengan *G. boninense*, analisis transkriptomik pada tisu akar pada titik masa yang berlainan iaitu 3, 7, dan 11 hari-selepas-inokulasi (d.p.i) telah dijalankan. Analisis data RNA-seq keupayaan celusan tinggi telah mendedahkan dua profil ekspresi yang jelas daripada gen kelapa sawit yang membentuk asas kepada mendeduksi fasa biotrofik pada jangkitan awal (3 d.p.i) yang mana ditukar kepada permulaan fasa nekrotrofik pada peringkat kemudian (11 d.p.i). Berdasarkan kepada penemuan-penemuan ini, kajian kini memfokuskan pada mengenal pasti gen diekspres secara ketara (DEGs) yang mengekod TFs dari data RNA-seq yang terhasil. Sejumlah 106 DEGs dikawal naik dan 108 DEGs dikawal turun daripada TFs telah dikenalpasti. Terdapat empat laluan berkaitan pertahanan yang kukuh telah dibentangkan yang mana gen yang dilaporkan terlibat dalam pengubahsuaian dinding sel, isyarat spesis oksigen reaktif (ROS)-perantara, kematian sel terprogram, dan keimunan semulajadi tumbuhan telah diekspres secara ketara. Gen ini didapati samada dikawal naik atau dikawal turun dalam dua fasa jangkitan yang berbeza tersebut. RT-PCR semi-kuantitatif multipleks telah dijalankan untuk menyaring TFs berkaitan pertahanan yang bebas tekanan abiotik.

Keamatan jalur ternormalkan daripada sampel terawat *Ganoderma* (GT) dibanding dengan sampel olokan (MT) untuk mengganggu tahap ekspresi mRNA antara kumpulan. Pola ekspresi lapan calon gen TFs termasuk *EgJUB1*, *EgERF113*, *EgTCP15*, *EgNAC29*, *EgEIN3*, *EgMYC2*, *EgNAC83* dan *EgMYB122* selanjutnya diukur melalui PCR-masa nyata kuantitatif (qPCR). Kedua-dua *EgJUB1* dan *EgERF113* telah dikesan dikawal naik secara spesifik di bawah tekanan biotik pada semua titik masa. Dapatan-dapatan tersebut menunjukkan kawalan naik *EgJUB1* semasa fasa biotrofik sementara *EgERF113* menunjukkan kawalan naik yang ketara apabila kelapa sawit menukar pertahanan menentang fasa nekrotrofik. Pencirian *EgJUB1* dan *EgERF113* telah dijalankan melalui asai Yis Satu-Hibrid (Y1H) *in vivo* dan asai syif pergerakan elektroforesis (EMSA) *in vitro*. *JUB1* telah dilaporkan mengikat kepada motif tapak ikatan NAC (NACBS) semasa tekanan abiotik. Kajian ini adalah laporan pertama ke atas aktiviti ikatan *EgJUB1* kepada dinding sekunder element ikatan 1 NAC (SNBE1) yang mana berada dalam kawasan promoter *EgHSFC-2b* dengan profil ekspresi yang sama seperti *EgJUB1*. Motif SNBE1 dengan pertukaran nukleotida tunggal samada pada kedudukan kelima atau kelapan telah ditemukan dalam kawasan promoter beberapa TFs yang terekspres bersama *EgJUB1*, termasuk *EgHSFB-4b* dan *EgGAMYB X2*. Sementara itu *EgERF113* mengikat pada kotak GCC dan motif DRE/CRT yang menggalakkan keplastikan dalam mengawal naik gen berkaitan pertahanan hilir melawan serangan *G. boninense*. Analisis jujukan menunjukkan kehadiran domain ikatan DNA (DBD) NAC dalam *EgJUB1*. Perubahan asid amino daripada fenilalanina kepada triptofan pada kedudukan ke-14 DBD *EgERF113* membuktikan kespesifikan ikatan pada kedua-dua kotak GCC dan motif DRE/CRT. Rawatan berpanjangan menyebabkan anak benih kelapa sawit alah kepada jangkitan *G. boninense*. Basidiomata matang *G. boninense* telah diperhati pada 24 minggu selepas inokulasi (w.p.i) dan jangkitan tersebut berakhir dengan kematian tumbuhan. Kesimpulannya, penemuan kami mencadangkan *EgJUB1* dan *EgERF113* sebagai TFs utama dalam mengatur mekanisme pertahanan kelapa sawit masing-masing semasa fasa jangkitan biotrofik dan nekrotrofik oleh hemibiotrofik *G. boninense*.

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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 Doctor of Philosophy. The members of the Supervisory Committee were as follow:

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DRE/CRT: Dehydration response element/C-repeat; EIN3: Ethylene insensitive 3; ET: Ethylene; ETI: Effector-triggered immunity; HR: Hypersensitive response; HSFs: Heat stress transcription factors; HSP: Heat shock protein; JA: Jasmonic acid; NPR1: NONEXPRESSOR OF PATHOGENESIS-RELATED GENE 1; PR: Pathogenesis-related; PRRs: Pattern recognition receptors; PTI: PAMP-triggered immunity; SNBE: Secondary wall NAC binding element; SA: Salicylic acid; TSS: Transcription start site.



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LIST OF ABBREVIATIONS

A	Alanine
AbA	Aureobasidin A
AbA ^r	Aureobasidin A resistance gene
ABA	Absciscic acid
ANOVA	Analysis of variance
APS	Ammonium persulfate
AP2/ERF	APETALA2/ethylene responsive factor
ATA	Aurin tricarboxylic acid
Avr	Avirulence
BCA	Biological control agent
bHLH	basic Helix-loop-helix
BLAST	Basic Local Alignment Search Tool
bZIP	basic leucine zipper
Ca ²⁺	Calcium ion
CAMTA	Calmodulin-binding transcription activator
cm	Centimeter
⁶⁰ Co	Cobalt-60
CTR1	CONSTITUTIVE TRIPLE RESPONSE 1
bp	Base pair
BSR	Basal stem rot
CaCl ₂	Calcium chloride
cDNA	complementary Deoxyribonucleic acid
CDPK	Calcium dependent protein kinase
CDS	Coding sequence
CESA	Cellulose synthase
CML	Calmodulin-like
COR	Coronatine
CSL	Cellulose synthase-like
C _T	Threshold cycle
Cu	Copper
CWDE	Cell wall degrading enzyme
d	Day
Da	Dalton
DAMP	Damage-associated molecular pattern
DBD	DNA-binding domain
DEG	Differentially expressed gene
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
d.p.i	Days post inoculation
DREB	Dehydration responsive element-binding
DRE/CRT	Dehydration-Responsive Element/C-Repeat
E	Glutamic acid
EBNA	Epstein-Barr Nuclear Antigen
EDS1	Enhanced Disease Susceptibility 1
EDTA	Ethylenediaminetetraacetic acid
EIN3	Ethylene-insensitive 3

EIL	EIN3-like
EMSA	Electrophoretic Mobility Shift Assay
ENA	European Nucleotide Archive
ERE	Ethylene-Response Element
ERF	Ethylene Responsive Factor
EST	Expressed Sequence Tag
ET	Ethylene
ETI	Effector-triggered immunity
EU	European Union
F	Phenylalanine
Fe	Iron
fmole	femtomole
Felda	Federal Land Development Authority
FFB	Fresh fruit bunch
G	Glutamic acid
GanoDROP	Ganoderma and Disease Research of Oil Palm
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
gDNA	Genomic deoxyribonucleic acid
GDP	Gross Domestic Product
GO	Gene Ontology
GST	Glutathione S-transferase
GT	<i>Ganoderma</i> -treated
Gy	Gray
h	hour
ha	hectare
HLH	Helix-loop-helix
HR	Hypersensitive response
HSF	Heat stress transcription factor
HSP	Heat shock protein
Ile	Isoleucine
IPTG	Isopropyl β -d-1-thiogalactopyranoside
JA	Jasmonic acid
JAMA	Japan Automobile Manufacturers Association
JAZ	Jasmonic acid-ZIM domain
JUB1	JUNGBRUNNEN 1
kb	kilobase
LB	Luria-Bertani
LD-PCR	Long-distance Polymerase Chain Reaction
Leu	Leucine
LiAc	Lithium acetate
LiCl	Lithium chloride
M	Molar
MAMP	Microbe-associated molecular pattern
MAPK	Mitogen-activated protein kinase
MC	Metacaspase
MEA	Malt extract agar
ME	Mercaptoethanol
min	Minute
MgCl ₂	Magnesium chloride
MIC	Minimal Inhibitory Concentration

MITC	Methyl isothiocyanate
Mn	Manganese
MPOB	Malaysian Palm Oil Board
MSD	Manganese superoxide dismutase
MT	Mock-treated
MTI	MAMP-triggered immunity
MW	Molecular weight
MYB	Myeloblastosis
MYC	Myelocytomatosis
N	Total sample size
n	Sample size
NaCl	Sodium chloride
NADH	Nicotinamide adenine dinucleotide dehydrogenase
NaOH	Sodium hydroxide
NAC	No apical meristem (NAM), Arabidopsis transcription activation factor (ATAF1/2), and cup-shaped cotyledon (CUC2)
NACBS	NAC binding site
NCBI	National Centre of Biotechnology Information
NGS	Next Generation Sequencing
NIH	National Institute of Health
NLS	Nuclear localization signals
nm	Nanometer
nM	Nanomolar
NPR1	NONEXPRESSOR OF PATHOGENESIS-RELATED GENE 1
ns	Not significant
NST	NAC SECONDARY WALL THICKENING PROMOTING FACTOR1
NTC	Non-template control
OD	Optical density
ORF	Open reading frame
pmole	picomole
PAMP	Pathogen-associated molecular pattern
PDA	Potato dextrose agar
PEG	Polyethylene glycol
pI	Isoelectric point
PlantTFDB	Plant Transcription Factor Database
POFA	Palm oil fuel ash
PCD	Programmed cell death
PR	Pathogenesis-related
PRR	Pattern recognition receptor
PTI	PAMP-triggered immunity
qPCR	Quantitative Real-Time Polymerase Chain Reaction
R	Resistance
R ²	Regression coefficient
RAP2.6	RELATED TO APETALA 2.6
RAP2.6L	RELATED TO APETALA 2.6-LIKE
RAV	Related to Absciscic Acid Insensitive 3/Viviparous 1
RBOH	Respiratory burst oxidase homolog protein

ROS	Reactive Oxygen Species
RT	Room temperature
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
RWB	Rubber wood block
s	second
SA	Salicylic acid
SAGE	Serial Analysis of Gene Expression
SAR	Systemic acquired resistance
SBS	Sequencing by synthesis
SBL	Sequencing by ligation
SD	Synthetic dropout
SDS	Sodium dodecyl sulfate
Si	Silicon
SEM	Standard error of mean
SNAC	Stress-responsive NAC
SNBE	Secondary wall NAC binding element
S.O.C	Super Optimal broth with Catabolite repression
SOD	Superoxide dismutase
t	tonne
TAE	Tris-Acetate-EDTA
Tb	Terabase
TBE	Tris-borate-EDTA
TCP	Teosinate branched 1, Cycloidea, Proliferating Cell Factor
TdT	Terminal deoxynucleotidyl transferase
TE	Tris-EDTA
TEMED	N,N,N',N'-tetramethylethane-1,2-diamine
TESH	Thermally enhanced sustainable hybrid
TF	Transcription factor
TIR-NBS- LRR	Toll/interleukin-1 receptor-nucleotide-binding site-leucine-rich repeat
TPM	Transcript per kilobase million
TPL	TOPLESS
TPLR	TOPLESS-related
TSS	Transcription start site
U	Unit
UBQ	Ubiquitin- β -chain
URA	Uracil
V	Voltan
VND	VASCULAR-RELATED NAC-DOMAIN
W	Tryptophan
w.p.i	Weeks post inoculation
YPDA	Yeast Extract Peptone Dextrose Adenine
Y1H	Yeast One-Hybrid
μ	Micro
x g	Gravities (Unit for relative centrifugal force)
$^{\circ}\text{C}$	Degree Celsius

CHAPTER 1

INTRODUCTION

Oil palm (*Elaeis guineensis*) is economically important for countries especially in Southeast Asian region. Malaysia has become the world second largest palm oil exporter accounting 44% of world's export and contributing 39% of world palm oil production (Malaysian Palm Oil Council, 2017). The country achieved the highest export revenue in 2017 from palm oil and palm oil products of approximately USD18.5 billion (Malaysian Palm Oil Board, 2018). Oil palm has been recognized as one of the world's largest edible oil source as well as precursor of biodiesel fuel (Kurnia et al., 2016; Stichnothe et al., 2014). To date, oil palm is the most efficient oil-bearing crop with 5 to 9 times higher yield per ha yearly than other vegetable oil crops such as soybean, sunflower and rapeseed (Lam et al., 2019).

However, the sustainable production of palm oil is hampered severely due to basal stem rot (BSR) disease caused by pathogenic and destructive fungus, *Ganoderma boninense*. Malaysia and Indonesia have suffered highest economic loss from the disease ranging from RM225 million to RM1.5 billion (up to USD 361 million) per year in both countries (Morel et al., 2016, Arif et al., 2011). The vast spread of BSR from one oil palm plantation to another in both countries is so serious and almost uncontrollable. *G. boninense* is a basidiomycete soil-borne pathogen which is the prevalent species causing BSR in oil palm (Ishaq et al., 2014; Hushiarian et al., 2013). The spreading of *G. boninense* in oil palm plantation involves dikaryotic mycelium spreading through root-to-root contact, dispersal of basidiospore and the presence of secondary inoculum in the soil (Isaac et al., 2018; Chong et al., 2017a).

The main constraint in managing the BSR disease is the failure in identifying *Ganoderma*-infected palm trees at the earliest stage of infection since there is no visible symptoms. The pathogenic fungus is categorized as hemibiotroph wherein initial biotrophic lifestyle is needed for a period of time before switching to subsequent necrotrophic phase (Chong et al., 2017a). The dynamic intermediate lifestyle of hemibiotroph made it possible for *G. boninense* to adapt and manipulate the host plant defense mechanisms which almost ultimately result in the plant succumbing to the infection. During the biotrophic phase (early infection), *G. boninense* survives by parasitically extracting nutrients from the living host plant while keeping it viable. The earliest symptoms are only visible on the foliage as the infection progresses up to 60-70%, reaching the later stage of necrotrophic phase with half of the stem base already ravaged (Chong et al., 2017a). BSR is the most common manifestation of *G. boninense* infection whereby it affects and decays the bole and produces multiple spears as well as fruiting bodies (Hushiarian et al., 2013).

Cultural practices including soil mounding, sanitation and trenching have been commonly applied as one of BSR disease management strategies although Chong et al. (2017b) has highlighted the risks of disease recurrence. Chemical control using

fungicides is the next choice of controlling BSR in oil palm. Studies on systemic fungicide such as hexaconazole have shown strong inhibition towards *Ganoderma* growth (Maluin et al., 2019; Mustafa et al., 2018). The application of fungicides on fields using trunk injection technique was also shown to have positive impacts in reducing the BSR incidence (Jelani et al., 2018). However, growing concerns on the negative impacts of fungicides on beneficial soil microbes, health and environmental hazards as well as development of fungicides resistance results in the intense need for green sustainable alternatives. As a result, anti-fungal compounds synthesized by bacteria (termed as biofungicides) are actively being studied as continuous efforts in reducing the use of synthetic fungicides (Lee et al., 2018; Pramudito et al., 2018). Alternatively, biocontrol agents (BCA) of soil-borne *Trichoderma* spp. have been numerous proven to exhibit antagonistic effect on *G. boninense* growth while promoting plant resistance against the fungal infection (Musa et al., 2018; Alizadeh et al., 2014; Priwiratama and Susanto, 2014). However, little is known about the oil palm defense mechanisms during different infection phases staged by this hemibiotroph pathogen, *G. boninense*.

The most promising molecular approach in managing *Ganoderma*-infected palm trees as well as minimizing the disease progress caused by *G. boninense* is most likely through identifying the infection at earlier stage (Kushairi et al., 2019). Phytohormones crosstalk of salicylic acid (SA) and jasmonic acid- ethylene (JA-ET) signalling has been widely studied to determine the fine-tuning defense response against biotrophic and necrotrophic infection phases, respectively (Häffner et. al., 2015). However, there is poor molecular information available explaining the defense regulation by transcription factors (TFs) during transition from biotrophic to necrotrophic state. The fragmentary information on molecular interactions between plant and hemibiotroph should be explicated to identify the key molecular mechanisms of both pathogenesis and plant immunity. A recent high-throughput RNA-seq analysis has pointed out the counter-act defense mechanisms executed by plant during transition from biotrophic to necrotrophic phase (Bahari et al., 2018). In the attempt of finding novel phase-specific biomarkers, this study focus on the regulation of defense-related TFs.

TFs are the ‘master switches’, which regulate the downstream genes transcription processes. Alteration of the genes expression by the TFs may either over-express or suppress different stress-related genes (Ng et al., 2018; Tsuda and Somssich, 2015). These pathogen-defensive genes are responsible in the production of antimicrobial secondary metabolites and antifungal proteins (Pandey et al., 2016; Alves et al., 2014). There are six major families of TFs recognized to regulate defense response against pathogens including NAC, bHLH, MYB, WRKY, AP2/ERF and bZIP (Ng et al., 2018). To date, no study has reported comprehensive transcriptomic profiling of oil palm defense-related TFs against *G. boninense*. Recognizing the infection phase-specific TFs may enables researchers to underpin the regulation of downstream target genes and offer opportunities to tackle or attenuate the progress of infection. The TFs can be used as biomarkers in plant breeding and engineering to develop *Ganoderma*-resistant palms. Thus, this study aims to recognize specific oil palm TFs as ‘key’ biomarkers associated with biotrophic and necrotrophic infection phases of hemibiotroph *G. boninense*.

The objectives of this study are;

1. To perform high-throughput transcript analysis via Next Generation Sequencing (NGS) and to determine differentially expressed genes (DEGs) of TFs during early infection of *G. boninense*
2. To identify oil palm TFs regulated specifically under biotic stress via quantitative Real-Time PCR (qPCR)
3. To carry out molecular characterization of an oil palm TF with potential in regulating defense response during the biotrophic and necrotrophic infection phase of *G. boninense*, respectively.



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LIST OF PUBLICATIONS

Journal

Mohamad Nazri Abdul Bahari, **Nurshafika Mohd Sakeh**, Siti Nor Akmar Abdullah, Redzyque Ramza Ramli and Saied Kadkhodaei. (2018). Transcriptome profiling at early infection of *Elaeis guineensis* by *Ganoderma boninense* provides novel insights on fungal transition from biotrophic to necrotrophic phase. *BMC Plant Biology*, 18(1): 377 – **(Joined first author)**

Nurshafika Mohd Sakeh, Siti Nor Akmar Abdullah, Mohamad Nazri Abdul Bahari, Azzreena Mohamad Azzeme, Noor Azmi Shaharuddin, Idris Abu Seman. (2020). EgJUB1 and EgERF113, master regulators of defense response in *Elaeis guineensis* against hemibiotroph *Ganoderma boninense*. *BMC Plant Biology*. Manuscript is under revision.

Nurshafika Mohd Sakeh, Siti Nor Akmar Abdullah, Mohamad Nazri Abdul Bahari, Azzreena Mohamad Azzeme, Noor Azmi Shaharuddin, Idris Abu Seman. (2020). Putative role of *EgNAC83* as a hub gene in abiotic and biotic responses. *Journal of Oil Palm Research*. Manuscript is submitted for publication.

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Nurshafika Mohd Sakeh, Siti Nor Akmar Abdullah and Mohammad Nazri Abdul Bahari. (2017). Characterization of Transcription Factors Involved During Early Interaction of Oil Palm (*Elaeis guineensis* Jacq.) with *Ganoderma boninense*. Global Conference on Plant Science and Molecular Biology (GPMB) held in Valencia, Spain.

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Nurshafika Mohd Sakeh, Siti Nor Akmar Abdullah, Mohamad Nazri Abdul Bahari, Azzreena Mohamad Azzeme, Noor Azmi Shaharuddin, Idris Abu Seman. (2019). Expression Patterns of Oil Palm Defense-related Transcription Factors During Different Infection Phases of *Ganoderma Boninense*. 4th International Conference on Crop Improvement (ICCI) held in Universiti Putra Malaysia (pp 77-81). Perpustakaan Negara Malaysia **ISBN 978-967-960-466-5**

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Poster

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