ENDOPHYTIC Trichoderma virens TRIGGERS INDUCED SYSTEMIC RESISTANCE IN OIL PALM SEEDLINGS

FARAH AMIRA BT MD PAUDZAI

FBSB 2018 52

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By

FARAH AMIRA BT MD PAUDZAI

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

March 2018

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

ENDOPHYTIC *Trichoderma virens* TRIGGERS INDUCED SYSTEMIC RESISTANCE IN OIL PALM *(Elaeis guineensis Jacq.)*

By

FARAH AMIRA BINTI MD PAUDZAI

March 2018

Chair : Mohd Termizi Yusof, PhD
Faculty : Biotechnology and Biomolecular Sciences

Basal stem rot disease caused by *Ganoderma* spp. is a crucial disease of oil palm (*Elaeis guineensis* Jacq.) and a major economic concern in Southeast Asian countries. The disease causes death of oil palm by slowly rotting the trunk base of oil palm. Fungicide or herbicide has long been the temporary solution for the disease control, however, planters prefer for natural solution using biological control agent such as endophytic microorganisms. *Trichoderma* is a well-known biological control agent (BCA) for plant disease and has shown to be effective against basal stem rot (BSR) infection. *Trichoderma* possesses control mechanism through mycoparatism, antibiosis, and also trigger induced systemic resistance (ISR) in plant. *Trichoderma* produces compounds and cell-wall degrading enzymes during the colonization of plant roots, thus, limiting the growth of pathogenic fungi. Thus, this study attempts to investigate endophytic *Trichoderma* ability as an antagonistic biocontrol against *Ganoderma boninense* and its ability to prime the immune system of the host through ISR. Two potential endophytic *Trichoderma* isolates 7b and 159c were identified using universal primer pairs TW81 and AB28. The amplification of internal transcribed (ITS) region produced a fragment 600bp. Sequence analysis of 7b and 159c isolates revealed that the isolates showed 99 % percent identical to *Trichoderma virens* strains available in Genbank database. Phylogenetic analysis showed that *T.* virens isolate7b and 159c are grouped in same clade with other *T. virens* and *Hypocrea virens.* The potential of both *T. virens* isolates 7b and 159c to be used as BCA were assessed through *in vitro* assays by percentage inhibition of radial growth (PIRG) and potential in dual culture (PIRG: 65.33% ± 1.42 and 67.20% ± 2.19) and poison agar assay (PIRG: 91.06% ± 9.63 and 58.82% ± 8.64). Observation using scanning electron microscope (SEM) detected severe mycelia deformation such as shriveling, clumping, and shrinking of *Ganoderma boninese* hyphae in the presence of *T. virens* isolate 7b and 159c. The zone of interaction between the pathogen and *T. virens* isolates 7b and 159c detected mycoparatism activity to which breaking and coiling of BSR pathogen by *T,virens* was observed. Oil palm seedlings inoculated with combination of *T. virens* isolates 7b and 159c have increased in vegetative parameters such as height, girth, frond count and chlorophyll content. However, disease suppression was significantly the highest in seedlings treated with *T. virens* isolate 159c

having the disease severity of 16.98% when compared to other treatments. Seedlings inoculated with *T. virens* isolate 159c significantly reduced the disease development measured as the area under disease progression curve (AUDPC) with disease reduction of 64.04% ($P<0.05$). External and internal symptom of BSR development in oil palm seedlings were correlated each other. Production of peroxidase, polyphenol oxidase, phenylalanine lyase, superoxide dismutase, chitinase, and β-1,3-glucanase were measured using enzymatic assay from leaf tissue upon inoculation of *T. virens* isolates 7b and 159c. Treatment of *T. virens* enhanced the levels of plant defense-related enzymes in oil palm seedlings. This study showed that the inoculation of *T. virens* isolates 7b and 159c triggered the induced systemic resistance in oil palm seedlings through induction of defense enzymes. Application of endophytic *T. virens* to oil palm seedlings is effective to control the development of BSR disease in oil palm.

KEYWORDS: Oil palm, *Ganoderma boninense*, endophytic *Trichoderma*, induced systemic resistance

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

ENDOFITIK *Trichoderma virens* MENGAKTIFKAN PENINGKATAN RINTANGAN SISTEMIK PADA KELAPA SAWIT (*Elaeis guineensis* Jacq.)

Oleh

FARAH AMIRA MD PAUDZAI

Mac 2018

Pengerusi : Mohd Termizi Yusof, PhD
Fakulti : Bioteknologi dan Sains Biomolekular

Penyakit reput pangkal kelapa sawit (*Elaeis guineensis* Jacq.) yang disebabkan oleh *Ganoderma* spp. merupakan penyakit yang menjadi kebimbangan ekonomi di Negara-negara Asia Tenggara. Penyakit ini menyebabkan kelapa sawit mereput secara perlahan dan akhirnya menyebabkan kematian. Racun kulat dan/atau racun rumpai telah lama menjadi penyelesaian untuk mengawal penyakit ini, walau bagaimanapun, petani memilih untuk menggunakan penyelesaian semula jadi iaitu agen kawalan biologi (BCA) seperti mikroorganisma endofitik. *Trichoderma* merupakan agen kawalan biologi yang terkenal untuk mengawal pelbagai jenis penyakit tumbuhan dan didapati berkesan terhadap penyakit reput pangkal. *Trichoderma* mempunyai mekanisme kawalan penyakit tumbuhan melalui mikoparatisma, antibiosis dan juga mengaktifkan peningkatan rintagan sistemik (ISR). *Trichoderma* menghasilkan sebatian dan enzim pengurai dinding sel semasa pengkolonian akar pokok kelapa sawit, sekaligus menyekat pertumbuhan kulat patogenik. Oleh itu, kajian ini dijalankan untuk menyelidik keupayaan endofitik *Trichoderma* sebagai agen kawalan biologi terhadap *Ganoderma boninense* dan kebolehannya untuk mengaktifkan sistem imun pokok kelapa sawit melalui penigkatan rintangan sistemik. Dua isolate endofitik *Trichoderma* yang berpotensi, isolat 7b dan 159c telah dikenalpasti menggunakan pasangan primer universal TW81 dan AB28. Amplifikasi kawasan ITS menghasilkan fragmen 600bp. Analisa jujukan isolat 7b dan 159c menunjukkan isolat-isolat ini mempunyai 99% kesamaan dengan *Trichdoerma virens* yang didapati dari pangkalan data Genbank. Analisa filogenetik menunjukkan *T. virens* isolat 7b dan 159c berada di kumpulan yang sama dengan *T. virens* dan *Hypocrea virens*. Potensi kedua-dua *T. virens* isolate 7b dan 159c sebagai BCA dinilai melalui ujian *in vitro* melalui peratusan perencatan pertumbuhan radial (PIRG) dan menunjukkan kesan antagonistik pada kultur dual (PIRG: 65.33% ± 1.42 and 67.20% ± 2.19) dan ujian agar beracun (PIRG: 91.06% ± 9.63 and 58.82% ± 8.64). Pemerhatian melalui imej mikroskop elektron pengimbasan (SEM) mengesan kecacatan miselium yang teruk seperti pengecutan, pengumpulan dan pengecilan hifa *G. boninense* dengan kehadiran *T. virens* isolat 7b dan 159c. Zon interaksi antara pathogen BSR dan *T. virens* isolat 7b dan 159c mengesan aktiviti mikoparatisma di mana hifa patogen kelihatan putus dan dilingkari oleh *T. virens.* Anak

benih kelapa sawit yang diinokulasi dengan kombinasi *T. virens* isolat 7b dan 159c didapati mempunyai peningkatan pada parameter vegetatif seperti ketinggian, lilitan batang anak benih, kiraan pelepah dan kandungan klorofil. Walau bagaimanapun, perencatan penyakit adalah paling tinggi secara signifikan pada anak benih yang dirawat dengan *T. virens* isolat 159c yang mempunyai keterukan penyakit sebanyak 16.98% jika dibandingkan dengan rawatan lain. Pengurangan perkembangan penyakit diukur sebagai kawasan di bawah kurva perkembangan penyakit (AUDPC) menunjukkan anak benih yang diinokulasi dengan *T. virens* isolat 159c mengurangkan perkembangan penyakit secara signifikan sebanyak 64.04% ($P<0.05$). Simptom luaran dan dalaman perkembangan BSR pada benih kelapa sawit adalah berkait antara satu sama lain. Penghasilan peroksida, polifenol oksida, fenilalanin lyase, superoksida dismutase, kitinase dan β-1,3-glukanase diukur daripada tisu daun yang diinokulasi *T. virens* isolat 7b dan 159c menggunakan ujian enzimatik. Rawatan *T. virens* meningkatkan tahap enzim berkaitan dengan pertahanan tanaman pada benih kelapa sawit. Kajian ini menunjukkan bahawa inokulasi *T. virens* isolat 7b dan 159c mengaktifkan peningkatan rintangan sistemik pada benih kelapa sawit melalui induksi enzim pertahanan. Aplikasi endofitik *T. virens* kepada anak benih kelapa sawit adalah berkesan untuk mengawal perkembangan penyakit BSR.

KATA KUNCI: Kelapa sawit, *Ganoderma boninense*, endofitik *Trichoderma*, peningkatan rintangan sistemik

ACKNOWLEDGEMENTS

I would first like to express my deepest gratitude to my supervisor, Dr. Shamala Sundram (MPOB) for her excellent guidance, caring and patience. The door to her office is always open whenever I have problems to solve. She has shown me how great experimental studies are done and her passion in doing research is truly an inspiration.

I am also grateful for the patience and motivation given by Dr. Termizi Yusof (UPM). His positive and 'can-do' attitude always be my motivation to sail through any research hiccups. To the rest of the committee members: Dr. Amalia Mohd Hashim and Prof. Datin Dr. Siti Nor Akmar Abdullah for willing to accept me as student.

Many thanks to Erhan Abdul Hak. Nor Shazwana Hamdan, Mohd Al-Hafiz Rosli, Noor Haliza Jamaluddin and other workers from GanoDrop unit in MPOB. My research would not have been possible without their help. With special mention, Angel Lee, Raimathy and Hazlina, it was fantastic to share this enjoyable moment with all of them.

A very special gratitude goes out to Malaysian Palm Oil Board for giving me the opportunity to be part in GSAS programme and providing funding for the research. I am grateful to have the opportunity to work majority of my research in the facilities.

Finally, I must express my profound gratitude to my family for providing me with unfailing support and continuous encouragement throughout my years of study. To Mursyid Hamzah, you came at the right time and I am grateful for that.

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:

Mohd Termizi bin Yusof, PhD
Senior Lecturer
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Amalia binti Mohd Hashim, PhD
Senior Lecturer
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Datin Siti Nor Akmar binti Abdullah, PhD
Professor
Institute of Tropical Agriculture
Universiti Putra Malaysia
(Member)

Shamala A/P Sundram, PhD
Principal Research Officer
Malaysian Palm Oil Board
(Member)

_____
ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
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Signature: _____
Name of Member of Supervisory Committee: Amalia binti Mohd Hashim

Signature: _____
Name of Member of Supervisory Committee: Datin Siti Nor Akmar binti Abdullah

Signature: _____
Name of Member of Supervisory Committee: Shamala A/P Sundram

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LIST OF ABBREVIATIONS

Abs	Absorbance
AUDPC	Area Under Disease Progressive Curve
BCA	Biocontrol agent
bp	Base pair
BLAST	Basic Local Alignment Search Tool
BSR	Basal stem rot
Cm	centimetre
CPD	Critical point drying
CWDE	Cell wall degrading enzyme
^{o}C	Degree Celsius
DNA	Deoxyribonucleic acid
dNTP	Deoxynuceotide phosphate
DS	Disease Severity
DSI	Disease severity Index
EDTA	Ethylenediaminetetraacetic acid
ET	Ethylene
G	Gram
H	Hour
Hpi	Hour post inoculation
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulfuric acid
JA	Jasmonic acid
LTGA	Ligninthioglycolic acid
PAL	Phenylalanine lyase

PGPF	Plant-growth promoting fungi
pH	Potential of hydrogen
%	Percent
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PER 71	*Ganoderma boninense*
PIRG	Percentage inhibition radial growth
POX	Peroxidase
PPO	Polyphenol oxidase
PR	Pathogenesis-related protein
pv.	Pathovar
L	Litre
min	minute
MEA	Malt extract agar
µL	Microlitre
µm	micrometer
mL	Milimetre
mM	Milimolar
mm	Milimetre
M	Molar
mRNA	Messenger ribonucleic acid
MPOB	Malaysia Palm Oil Board
MPOC	Malaysia Palm Oil Council
N	Normality
NaOH	Sodium hydroxide
Ng	nanogram

Nm	nanometre
NCBI	National Center for Biotechnology Information
ISR	Induced systemic resistance
ITS	Internal transcribef spacer
R^2	Correlation coefficient
RCBD	Randomized Completely Block Design
ROS	Reactive oxygen species
Rpm	revolution per minute
SA	Salicyclic acid
SAR	Systemic acquired resistance
Sec	Seconds
SEM	Scanning Electron Microscopy
spp.	Species
SOD	Superoxide dismutase
TMV	Tobacco mosaic virus
USDA	United States Department of Agriculture
U	Unit
UV	Ultra violet

CHAPTER 1

INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is an important commodity crop in Malaysia that plays major role in Malaysia economy. Malaysia as the second largest oil palm producer in the world is currently accounting for 30% of world's palm oil production in 2014 (MPOB, 2016). Oil palm planted areas in Malaysia grew from approximately 640,000 hectares in 1975 to 5.74 million hectares in 2016 (MPOB, 2016).

However, oil palm industry is facing a serious threat of fungal disease basal stem rot (BSR) that cause very serious losses in palm oil production and requiring an urgent solution. BSR is a serious disease in oil palm resulting in substantial losses worldwide caused by pathogenic fungi *Ganoderma boninense* (Khairudin, 1990; Rao, 1990). This disease is lethal; not only to old oil palm but also younger oil palm (Singh, 1991). The emergence of first symptom of the disease on oil palm indicates extensive internal tissue decay and the application of disease control at this stage would be ineffective (Hushiarian, Yusof, Dutse, 2013). Losses due to BSR can be seen through reduction of mature oil palm stand as well as the number and weight of fruit bunches from infected palm (Turner, 1981).

Application of chemical treatments usually used to treat or control BSR but it is impracticable and costly, therefore, biological method is among the options to control this disease to suppress or control the development of BSR disease. Studies by several researchers on biological control agents showed that beneficial microorganism such as bacteria, fungi and actinomycetes have proven their ability to control plant disease (Susanto, Sudharto, Purba, 2005; Sundram, Abdullah, Ahmad, Yusuf. 2008; Gajera, Savaliya, Patel, Golakiya, 2015). *Trichoderma* has been widely used as biocontrol agent for plant diseases since it was first recognized in the early 1930 (Weindling, 1934). Many studies had shown *Trichoderma* is one of the most effective beneficial microorganisms for controlling plant disease (Papavizas, 1985; Benítez, Rincón, Limón, Codón, 2004; Harman, Howell, Viterbo, Chet, Lorito, 2004; Chowdappa, Mohan Kumar, Jyothi Lakhsmi, Upreti, 2013). Mycoparatism is the direct mechanism of biocontrol activity by *Trichoderma* in which it attaches to pathogenic fungi by physical interaction such as coiling and strangulation of the pathogen (Howell, 2003).

Although *Trichoderma* is a common choice for controlling plant diseases, their efficacies however depend mainly on environmental conditions (Hadar, 1984; Benítez, Rincón, Limón, Codón, 2004). Furthermore, biocontrol activity by *Trichoderma* is sometimes unpredictable due to unheritable and irreproducible resistance. For this reason, understanding their control mechanism within host plant can exert positive effect on plant-defense stimulation. *Trichoderma* is also found to trigger induced systemic resistance in plant (Pieterse *et al*., 2014). During the colonization of plant root, *Trichoderma* will produce compounds and cell-wall degrading enzymes such as chitinase, glucanase, peroxidase, polyphenol oxidase, superoxide dismutase and/or phenylalanine (Harman et al., 2004). The accumulation of these compounds stimulate

localized and systemic plant defense responses, limiting the growth of pathogenic fungi (Benítez et al., 2004). Thus, the hypothesis for this study is the potential endophytic *Trichoderma* have the ability to suppress the development of BSR disease and enhance the induced systemic resistance in oil palm seedlings.

The objectives of this study are:

1. To identify potential endophytic *Trichoderma* against *Ganoderma boninense* from oil palm root and its effect of pathogenicity on the pathogenic *Ganoderma boninense*.
2. To determine the vegetative enhancement of *Trichoderma virens* in oil palm seedlings.
3. To determine the level of induced systemic resistance of oil palm triggered by endophytic *Trichoderma*.

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