UNIVERSITI PUTRA MALAYSIA

EFFECTS OF COLONIZATION OF AN ENDOPHYTIC FUNGUS, Hendersonia toruloidea ON THIAMINE BIOSYNTHESIS IN OIL PALM SEEDLINGS (Elaeis guineensis Jacq.)

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By

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October 2017

Chairman : Zetty Norhana Binti Balia Yusof, PhD
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Thiamine, or vitamin B1 plays an indispensable role in many metabolic reactions. Besides that, thiamine is also associated with the induction of systemic acquired resistance (SAR) in plants and having a role in boosting plant's immunity and defense system. In Malaysia, oil palm productivity is hampered by basal stem rot disease caused by a pathogenic fungus, *Ganoderma boninense* and proper disease management have yet to be discovered. Application of endophytes as biocontrol agent is a promising measure to prevent the disease. *Hendersonia toruloidea* is an endophytic fungus originally isolated from oil palm roots which have been shown to have excellent biocontrol activity in oil palm seedlings. Previous studies showed that this endophyte is able to suppress *G. boninense* infection in oil palm seedlings. This work aimed to investigate the responses in oil palm seedlings, specifically on the expressions of thiamine biosynthesis genes upon application of *H. toruloidea*. Seven months old oil palm seedlings were inoculated with *H. toruloidea* and microscopy analyses were carried out to visualize the colonization of the fungus. Total RNA was extracted from oil palm leaves at day 1, 7, 15 and 30 post inoculation. Quantitative real-time PCR (qPCR) was performed to measure the level of expression of four key thiamine biosynthesis genes, namely *THI4*, *THIC*, *TH1* and *TPK*. The results showed of up to 12-fold of increase in the expression of all gene transcripts at day 1 post inoculation. At subsequent days of day 7, day 15 and 30 post inoculation, the relative expression of these genes were shown to be downregulated. Thiamine accumulation was observed via HPLC analysis at day 7 post inoculation and subsequently attenuated until day 30. This work provides first evidence of enhancement of thiamine biosynthesis by endophytic colonization in oil palm and suggesting the role of thiamine in stress protection in oil palm seedlings.

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

KESAN KOLONISASI KULAT ENDOFIT *Hendersonia toruloidea* TERHADAP BIOSINTESIS VITAMIN B1 DALAM KELAPA SAWIT (*Elaeis guineensis* Jacq.)

Oleh

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Vitamin B1 memainkan peranan yang penting dalam metabolisma dalam semua kehidupan. Selain itu, vitamin B1 juga terlibat dalam induksi rintangan sistemik dalam tumbuhan dan memainkan peranan penting dalam immuniti dan pertahanan tumbuhan. Di Malaysia, produktiviti kelapa sawit adalah terjejas disebabkan oleh penyakit pereputan pangkal akar yang disebabkan oleh kulat *Ganoderma boninense*. Namun, pengawalan terbaik dan efektif terhadap kulat *Ganoderma boninense* belum dikenalpasti. Pengawalan penyakit BSR telah dilakukan dengan menggunakan agen kawalan biologi iaitu melalui penggunaan endofit terhadap anak pokok kelapa sawit. Kulat *Hendersonia toruloidea* merupakan kulat endofit yang telah dijumpai dalam akar kelapa sawit dan telah menunjukkan keberkesanan yang memberangsangkan terhadap kawalan penyakit BSR. Oleh itu, kajian ini bertujuan untuk menyelidiki kesan kolonisasi kulat endofit *H. toruloidea* terhadap biosintesis vitamin B1 di dalam anak pokok kelapa sawit. Kulat endofit *H. toruloidea* telah diinokulasi ke atas anak benih kelapa sawit berusia 7 bulan di nurseri. Analisa mikroskopi telah dijalankan untuk mengkaji kolonisasi kulat endofit di dalam akar kelapa sawit. RNA telah diekstrak daripada daun kelapa sawit pada hari ke 1, 7, 15 dan 30 hari usai inokulasi. Tindak balas rangkaian polimerase secara secara kuantitatif masa sebenar (qPCR) telah dijalankan untuk melihat pengkspresan empat gen biosintesis B1 yang utama iaitu *THI4*, *THIC*, *TH1* dan *TPK*. Hasil penemuan menunjukkan pengekspresan gen biosintesis vitamin B1 meningkat sehingga 12 kali ganda. Selepas 15 dan 30 hari usai inokulasi, pengekspresan gen vitamin B1 telah menurun. Analisa HPLC menunjukkan kolonisasi kulat endofit telah menyebabkan pengumpulan metabolit vitamin B1 di dalam daun kelapa sawit. Hasil kajian ini berjaya menunjukkan kolonisasi *H. toruloidea* meningkatkan biosintesis vitamin B1 di dalam pokok dan mencadangkan peranan vitamin B1 dalam perlindungan kelapa sawit terhadap tekanan.

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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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LIST OF ABBREVIATIONS

°C	Degree celsius
%	Percentage
A_{260nm}	Optical density at wavelength 260 nanometer
A_{280nm}	Optical density at wavelength 280 nanometer
µL	Microliter
µm	Micrometre
µmoles	Micromoles
mg	Milligram
ATP	Adenosine triphosphate
bp	Base pair
cDNA	Complementary deoxyribonucleic acid
CTAB	Cetyl trimethyammonium bromide
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
g	Gram
HCl	Hydrochloric acid
kb	Kilobase
L	Litres
LB	Luria Bertani
M	Moles
mRNA	Messenger RNA
nm	Nanomoles
NaOH	Sodium hydroxide

PDA	Potato dextrose agar
PCR	Polymerase chain reaction
qPCR	Quantitative real-time PCR
RNA	Ribonucleic acid
RT	Reverse trancriptase

CHAPTER 1

INTRODUCTION

Plants are sessile organisms that are inevitably exposed to unfavourable biotic and abiotic stresses, namely salinity, drought, and also microbial pathogens. Climate changes and attack by pathogenic diseases can severely hamper the productivity of important crop plants, including oil palm. Oil palm is one of Malaysia's important commodity. However, the productivity of oil palm is threatened by basal stem rot disease caused by a fungus, *Ganoderma boninense* which resulted in major economic losses (Paterson, 2007; Rees *et al.,* 2009). Current research in oil palm is accelerating towards finding ways to control the disease and is focusing on the detailed molecular mechanism in the plant-pathogenic interaction (Ho and Tan, 2014). Recently, studies of plant and microorganism interactions are significantly attracting interest because it has been demonstrated that microorganisms such as endophytes play a role in alleviating stresses in their host plants (Boivin *et al.,* 2016).

Endophytes are microorganisms that colonize the insides of plant tissues without causing any disease (Wilson, 1995). It is widely documented that endophytes formed a beneficial mutualistic relationship with plants (Hernández-Montiel *et al.*, 2013; Seerangan and Thangavelu, 2014). It is suggested that plant-endophyte mutualism is formed through direct and indirect mechanisms. Direct mechanism include antibiosis, and indirect is through production of biochemical compounds that are associated to alleviating stresses through induced systemic resistance (ISR), thereby enhancing plant's immune system and preventing pathogenic attack (Alquéres *et al.*, 2010; Gao *et al.*, 2010).

Relatively, no studies have been done on the role of thiamine in stress protection in oil palm. Thiamine or vitamin B1, an enzymatic cofactor in metabolic reactions, is involved in plant adaptation and alleviation of biotic and abiotic stresses in plants (Tunc-Ozdemir *et al.*, 2009; Rapala-Kozik *et al.*, 2008; Goyer, 2010). It was observed that there was an accumulation of thiamine when the plants were subjected to salinity stress, oxidative stress and pathogenic attack (Rapala-Kozik *et al.*, 2008; Tunc-Ozdemir *et al.*, 2009; Zhou *et al.*, 2013). It is now understood that thiamine formed an indirect role in enhancing anti oxidative capacity in the plants, which is important in defense responses (Zhou *et al.*, 2013). Yet, the exact mechanism of biosynthesis of thiamine in response to stresses is still poorly understood.

Thiamine is involved in adaptation to biotic and abiotic stresses and application of endophytes enhance the synthesis of defence metabolites that is associated with ISR/SAR (Zheng *et al*.2015). This led to a hypothesis that thiamine biosynthesis in oil palm will be upregulated by colonisation by endophytes. In this study, the endophytic fungus *Hendersonia toruloidea* was chosen as the strain of choice due to its excellent

colonization ability and also its ability to suppress *Ganoderma boninense* disease infection in oil palm (Idris *et al.,* 2013).

1.1 Objectives

The objectives of this study are:

1. To determine the localization and colonisation of endophytic fungus in oil palm by scanning electron microscopy and transmission electron microscopy
2. To determine the level of expression of thiamine biosynthesis genes upon colonisation of endophytic fungus by quantitative PCR
3. To quantify the total thiamine accumulation in oil palm upon colonisation by endophytic fungus by HPLC

The first objective was to perform microscopy analysis to study the colonisation and morphological pattern of the endophytic fungus. It was expected that the endophytic *H. toruloidea* will actively colonizing oil palm root tissues. Scanning electron microscopy (SEM) revealed the morphology of the *H. toruloidea* while transmission electron microscopy (TEM) was carried out to examine the structure and localisation of *H. toruloidea* in the oil palm root.

The second objective was to determine the expressions of thiamine biosynthesis genes in oil palm upon successive colonisation of *H. toruloidea*. The successful colonization of endophytic fungus was hypothesized to cause an upregulation of the thiamine biosynthesis genes in oil palm. The gene expression study was performed using quantitative real time-PCR (qRT-PCR), a simple, high throughput technology that enable us to measure gene expression in real time. The expression of thiamine biosynthesis genes was examined over a time course of 1, 7, 15 and 30 days post inoculation and the result will reflect the changes in transcript abundances of thiamine biosynthesis genes upon colonization of *H. toruloidea*.

The third objective of this study was to measure total thiamine content in oil palm upon colonization of the endophytic fungus. The upregulation of thiamine biosynthesis genes was expected to cause the increase in total thiamine and its intermediates accumulation overall. It was performed using High Performance Liquid Chromatography (HPLC). Since gene expressions are not necessarily translated into functional protein, the measurement of total thiamine and its intermediate content will verify that thiamine biosynthesis genes are expressed to synthesise total thiamine or the synthesis of its intermediates might be involved in other mechanisms in the overall metabolic pathways.

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