UNIVERSITI PUTRA MALAYSIA

THE EFFECT OF OXIDATIVE STRESS TOWARDS THE EXPRESSIONS OF THIAMINE BIOSYNTHESIS GENES (THIC & THI1/THI4) IN OIL PALM (*ELAEIS GUINEENSIS* JACQ.)

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Project In Department Of Biochemistry
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PENGESAHAN

Dengan ini adalah disahkan bahawa laporan projek "The Effect of Oxidative Stress Towards the Expressions of Thiamine Biosynthesis Genes (THIC & THI1/THI4) in Oil Palm *(Elaeis Guineensis* Jacq.*)*" telah disiapkan serta dikemukakan kepada Jabatan Biokimia oleh ZAINOR HAFISAH BINTI CHE IDRIS sebagai syarat untuk kursus BCH4999 Projek.

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ABSTRACT

Thiamine is known to be important in human diet and its cofactor is required for the vital metabolic processes such as acetyl-CoA biosynthesis, amino acid biosynthesis, and Krebs and Calvin cycle. The active cofactor is thiamine pyrophosphate (TPP). There are findings about TPP which can act as a non-cofactor function which is as stress-response molecule. In this study, the level of expression of THIC and THI1/THI4 gene transcripts upon induction of oxidative stress in oil palm was observed. The gene transcripts for the first two enzymes in the biosynthesis pathway, THIC and THI1/THI4 were successfully identified and amplified. Primers were designed based on the comparison of different plants such as *Arabidopsis thaliana*, *Zea mays*, *Oryza sativa*, and *Alnus glutinosa*. Also, the expression of these thiamine biosynthesis genes were investigated in response to the stress given to the oil palm. The gene transcripts' expressions were analyzed via RT-PCR over a time point after the application of paraquat. As the oxidative stress induced the expression of the THIC gene transcript was increased by 20 % and the production of THI1/THI4 gene transcript was increased by 10 % upon the induction of oxidative stress. Thus, these findings support the suggestion that thiamine may play a role in plant protection against stress as it may lead to the overexpression of thiamine in general.

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ABSTRAK

Tiamina dianggap penting dalam diet manusia dan adalah merupakan kofaktor yang diperlukan untuk proses metabolisma seperti sintesis asetil-koA, sintesis asid amino dan kitaran Krebs dan Calvin. Kofaktor yang aktif adalah tiamina pirofosfat (TPP). TPP juga mempunyai fungsi bebas-kofaktor yang mana boleh bertindak sebagai molekul yang beertindak balas terhadap tekanan. Di dalam kajian ini, tahap ekspresi THIC dan THI1/THI4, dua enzim pertama di dalam tapak jalan biosintesis tiamina terhadap tekanan oksidatif dalam kelapa sawit dikaji. Transkrip gen bagi dua enzim iaitu THIC dan THI1/THI4 telah berjaya dikenal pasti dan diamplifikasi. Primer telah direkabentuk berdasarkan perbandingan dengan tumbuh-tumbuhan seperti *Arabidopsis thaliana, Zea mays, Oryza sativa* dan *Alnus glutinosa*. Selain itu, ekspresi kedua-dua gen tersebut telah disiasat jika terdapat sebarang tindak balas terhadap tekanan yang diberikan kepada kelapa sawit. Ekspresi transkrip gen telah dianalisa melalui RT-PCR mengikut masa yang telah ditetapkan selepas tekanan oksidatif yang digalakkan oleh paraquat dikenakan ke atas kelapa sawit. Penghasilan transkrip bagi gen THIC telah meningkat sebanyak 20 % dan sebanyak 10 % peningkatan bagi transkrip gen THI1/THI4 setelah masing-masing dikenakan tekanan oksidatif. Oleh itu, penemuan ini menyokong cadangan mengenai tiamina yang mungkin memainkan peranan dalam melindungi pokok terhadap tekanan, ia akan membawa kepada ekspresi tambahan tiamina secara keseluruhan.

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LIST OF ABBREVIATIONS

%	Percentage
°C	Celcius
µL	microliter
$^1O_2$	Singlet oxygen
·OH	Hydroxyl radical
A	Absorbance
AIR	5-aminoimidazole ribonucleotide
Blastn	Nucleotide blast
CTAB	Cecyl trimethylammoniumbromide
cDNA	Complementary DNA
DNA	Dioxyribonucleic acid
dsDNA	Double strand DNA
EDTA	Ethylenediaminetetraacetic acid
EtBr	Ethidium bromide
F	Forward
g	gram
gDNA	Genomic DNA
H_2O_2	Hydrogen peroxide
ha	hectare
HDL	High density lipoprotein
HET-P	4-methyl-5-β-hydroxyethylthiazolephosphate
HMP-P	4-amino-2-methyl-5-hydroxymethylpyrimidine monophosphate
HMP-PP	4-amino-2-methyl-5-hydroxymethylpyrimidine diphosphate
LDL	Low density lipoprotein
LiCl	Lithium chloride
M	Molar
mg	miligram
mL	mililiter
mM	milimolar
NaCl	Sodium chloride
NAD^+	Nicotinamide adenine dinucleotide
NCBI	National Center for Biotechnology Information
nm	nanometer
$O_2^{\cdot-}$	Superoxide radical
PCR	Polymerase chain reaction
PQ	Paraquat
PVPP	Polyvinylpyrrolidone
R	Reverse
RCBD	Randomized complete block designed
RNA	Ribonucleic acid
ROS	Reactive oxygen species

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RT	Reverse transcriptase
RT-PCR	Reverse transcriptase-Polymerase chain reaction
SDS	Sodium dodecyl sulphate
ssDNA	Single strand DNA
TAE	Tris-Acetate-EDTA
thiL	Thiamine monophosphate in bacteria
THIC	HMP-P synthase
THI1/THI4	HET-P synthase
THID	HMP-P kinase
THIE	TMP synthase
THIM	HET-P kinase
TMP	Thiamine monophosphate
TPK	Thiamine pyrophosphokinase
TPP	Thiamine pyrophosphate
TPPH	a phosphatase
Tris-HCl	Tris-hydrochloride
UV	Ultra violet
v/v	Volume per volume
v/w	Volume per weight
volt	Voltan

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CHAPTER 1

INTRODUCTION

Thiamine is a very important vitamin because it provides cofactor for vital metabolic process such as acetyl-CoA biosynthesis, amino acid biosynthesis, and Krebs and Calvin cycle (Pourcel*et al.*, 2013; Tunc-Ozdemir *et al.*, 2009). Previously, researchers have also found the non-cofactor function of thiamine which is as stress-response molecule. The stress usually caused by biotic stress or abiotic stress. Biotic stress is something that involved the damage of plant which are caused by living organisms such as animals, bacteria, virus, and parasite. However, abiotic stress is due to the environmental factor like drought, high salinity, and oxidative stress which will affect the plant growth, development, and productivity of the plant (Kumar and Venkateswarlu, 2011). This experiment focuses on oxidative stress induced by the application of paraquat towards the oil palm (*Elaeis guineensis*) and to check on the upregulation of thiamine genes as thiamine has been suggested to increase upon stress application to protect the plant from the stress.

Generally, the thiamine biosynthesis in all organisms is made due to the condensation of 5-hydroxyethyl-4 methylthiazole phosphate (HET-P) and 4-amino-5-hydroxymethyl-2-methylpyrimidine pyrophosphate (HMP-PP) by the action of TMP synthase (THIE). For the pyrimidine moiety, AIR is converted to 4-amino-2-methyl-5-hydroxymethylpyrimidine monophosphate (HMP-P) by HMP-P synthase (THIC) and the further is phosphorylated to 4-amino-2-methyl-5-hydroxymethylpyrimidine diphosphate (HMP-PP) by HMP-P kinase (THID) (Rapala-Kozik *et al*., 2008). The enzyme that catalyzed the phosphorylation of HMP-PP to TMP, is known as TMP synthase (THIE) (Rapala-Kozik *et al.*, 2008; Pourcel *et al.*, 2013). Futhermore, NAD^+, glycine, and a sulfur is catalyzed by HET-P synthase (THI1/THI4) and then THIM to produce HET-P. HET-P synthase can function as mitochondrial DNA damage tolerance (Machado *et al*., 1997), modulated against heat stress (Ferreira *et al.*, 2006; Tunc-Ozdemir *et al.*, 2009), and disease resistance (Wang *etal*., 2006; Tunc-Ozdemir *et al*., 2009). Then, HMP-PP and HET-P will be condensed together by TMP synthase (THIE) to produce TMP. TMP is

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dephosphorylated to free thiamine by enzyme phosphatase (TPPH). The pyrophosphorylation of thiamine to produce active cofactor of thiamine pyrophosphate (TPP) is by the reaction of thiamine pyrophosphokinase (TPK) enzyme.

Oil palm is widely planted in Malaysia and the commercial product that is produced from oil palm is palm oil and palm kernel oil. Palm oil is versatile and also sustainable. It is because it acts as the cheapest traded edible oil and can be used for food and non-food purposes like cooking oil, shortenings and margarine, palm kernel oil, which is a raw material is used in soaps, detergents, toiletries, cosmetics and candles. Recently, palm oil is also known a potentially good as biofuel.

However, the production of palm oil in Malaysia has met a great challenge as it needs to counter with the biotic and abiotic stress. The abiotic stress is that caused by the environment or by the farming techniques like salinity stress, osmotic stress, and oxidative stress. Oxidative stress is mainly caused by bad farming techniques and also through the application of pesticides.

THIC and THI1/THI4 are the first two enzymes in thiamine biosynthesis pathway. This project investigated the expression of THIC and THI1/THI4 gene transcripts when the application of paraquat, an oxidative stress inducer. It is very interesting to look at the thiamine biosynthesis genes after the application of paraquat towards oil palm as this will give an insight of the role that thiamine may have in plant protection against stress. In Malaysia, this kind of research has not been done yet, thus it is a great opportunity for discovery of new things.

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The general objectives of this project are:

1. To identify and amplify the THIC (HMP-P Synthase) and THI1/THI4 (HET-P synthase) gene transcripts in oil palm (*Elaeis guineensis* Jacq.).
2. To study the effect of oxidative stress induced by paraquat on the expression of THIC and THI1/THI4 gene transcripts in oil palm (*Elaeis guineensis* Jacq.).
3. To verify the sequence of THIC and THI1/THI4 gene transcripts in oil palm (*Elaeis guineensis* Jacq.).

The specific objectives of this project are:

1. To identify and amplify the sequence of THIC and THI1/THI4 gene transcripts by designing primers using Primer3 software.
2. To study the expression of the THIC and THI1/THI4 gene transcripts upon paraquat application using RT-PCR and imageJ software.
3. To verify the sequence of the amplified THIC and THI1/THI4 gene transcripts by using nucleotide blast (blastn) in the NCBI.

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