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THE EFFECT OF SALINITY STRESS TOWARDS EXPRESSION OF THIAMINE BIOSYNTHESIS GENES (THIC AND THI1/THI4) IN OIL PALM (ELAIES GUINEENSIS)

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By

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APPROVAL

This is confirmed that the report entitled "The Effect of Salinity Stress towards Expression of Thiamine Biosynthesis Genes (THIC and THI1/THI4) in Oil Palm (*Elaies Guineensis*)" has been completed and sent to the Department of Biochemistry by Nur Syuhadah binti Abdul Rahman as a requirement for BCH4999 project from Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia.

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ABSTRACT

Thiamine or also known as vitamin B_1, it is the first water soluble B-complex vitamin to be identified which plays an important role as a cofactor and as a non-cofactor as well. As a cofactor, it is important for various types of enzymes involved in central metabolism such as pyruvate decarboxylase, pyruvate dehydrogenase, α-ketoglutarate dehydrogenase and transketolase in its active form, thiamine pyrophosphate (TPP). As a non-cofactor, it has been shown to have a role in plant protection against stress. Salinity stress is one of the most critical abiotic stresses that affects the productivity and growth of a plant. Plus, Malaysia is now the second largest producer and exporters of palm oil but the oil palm plantations recently facing problems due to the plant stresses. Thus, we investigated what happens to the first two enzymes involved in thiamine biosynthesis pathway, THIC and THI1/THI4 when subjected to salinity stress induced by sodium chloride (NaCl) in oil palm. Eight pairs of primer were designed based on consensus sequence of other genes obtained from *Oryza sativa, Zea mays, Arabidopsis thaliana* and *Alnus glutinosa.* Total RNA was extracted from spear leaf tissue samples that being treated with 0 mM, 50 mM, 150 mM and 200 mM of sodium chloride solution for 3 days, 7 days and 30 days. Then, RT-PCR was conducted to amplify both gene transcripts. As for THIC, the highest level of expression was observed on day 7 for 50 mM NaCl treated oil palm seedling with an increase of up to 331% level of expression compared to the untreated seedling with 100%. For THI1/THI4 gene transcript, the highest level of expression was 723% on day 3 for 200 mM NaCl treated oil palm seedling when compared to the untreated palm with 100%. The results showed that relatively higher levels were observed in THI1/THI4 for its additional role in protecting DNA from damage compare to TH1C. Sequence verification was conducted to confirm the amplification of THIC and THI1/THI4 gene transcripts. This study supports the finding suggesting that thiamine may play a role in plant protection against stress as it may lead to an overexpression of thiamine in general.

ABSTRAK

Thiamina atau dikenali sebagai vitamin B_1, adalah vitamin B-kompleks larut air pertama yang dikenal pasti. Ia dapat memainkan peranan penting sebagai kofaktor dan juga sebagai bukan kofaktor. Sebagai kofaktor, ia adalah penting untuk pelbagai jenis enzim yang terlibat dalam metabolisme pusat seperti dicarboxylase piruvat, piruvat dehidrogenase, α-ketoglutarate dehidrogenase dan transketolase dalam bentuk aktifnya, pirofosfat thiamina (TPP). Sebagai bukan kofaktor, ia dilihat mempunyai fungsi dalam melindungi tumbuhan daripada tekanan. Tekanan kemasinan adalah salah satu daripada tekanan abiotik yang paling kritikal yang memberi kesan kepada produktiviti dan pertumbuhan tumbuhan. Tambahan, Malaysia kini merupakan pengeluar kedua terbesar dan pengeksport minyak sawit tetapi ladang-ladang kelapa sawit baru-baru ini menghadapi masalah akibat tekanan tumbuhan. Oleh itu, kami menyiasat apa yang berlaku terhadap dua enzim pertama yang terlibat dalam biosintesis laluan thiamina, THIC dan THI1/THI4 apabila dikenakan tekanan kemasinan yang disebabkan oleh natrium klorida (NaCl) dalam kelapa sawit. Lapan pasang primer telah direka berdasarkan urutan consensus kedua-dua gen tersebut yang diperolehi daripada *Oryza sativa, Zea Mays, Arabidopsis thaliana* dan *Alnus glutinosa*. RNA telah diekstrak daripada sampel tisu lembing daun yang telah dirawat dengan 0 mM , 50 mM , 150 mM dan 200 mM larutan natrium klorida selama 3 hari, 7 hari dan 30 hari. Kemudian, RT-PCR dijalankan untuk mengamplikasi kedua-dua transkrip gen. Untuk THIC, tahap tertinggi peningkatan diperhatikan pada Hari 7 untuk anak benih kelapa sawit yang diberi 50 mM NaCl dengan peningkatan 331% berbanding anak benih yang tidak diberi NaCl dengan peningkatan 100%. Untuk gen transkrip THI1 / THI4, tahap tertinggi peningkatan adalah 723% pada hari ke-3 untuk anak benih kelapa sawit yang diberi 200 mM NaCl berbanding sawit yang tidak diberi NaCl dengan peningkatan 100%. Hasil kajian menunjukkan bahawa tahap yang lebih tinggi diperhatikan dalam THI1/THI4 disebabkan peranan tambahannya dalam melindungi DNA daripada kerosakan berbanding TH1C. Penyemakan urutan telah dilakukan bagi mengesahkan urutan gen transkrip THIC dan THI1/THI4. Secara keseluruhan, penyelidikan ini menyokong pernemuan cadangan yang menyatakan bahawa thiamina dapat memainkan peranan dalam melindungi tumbuhan daripada tekanan kerana ia membawa kepada ekspresi thiamina secara berlebihan secara amnya.

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%	Percent
~	About
v/v	Volume/Volume
°C	Degree Celsius
µg	Microgram
µl	Microlitre
µM	Micromolar
α	Alpha
A	Absorbance
ATP	Adenosine Triphosphate
BLAST	Basic Local Alignment Search Tool
bp	Base Pair
cDNA	Complementary DNA
D x P	Dura x Pisifera
DEPC	Diethyl Pyrocarbonate
dH_2O	Distilled Water
DI water	Deionised Water
DNA	Deoxyribonucleic Acid
dNTP	Deoxyribonucleotide Triphosphate
EDTA	Ethylenediaminetetraacetic Acid
EtBr	Ethidium Bromide
F, R	Forward, Reverse
g	Gram
G, C	Guanine, Cytosine
L	Litre
LiCl	Lithium Chloride
M	Molar
Min	Minute
mg	Milligram
ml	Millilitre
mM	Millimolar
mRNA	Messenger RNA
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NCBI	National Centre For Biotechnology
PCR	Polymerase Chain Reaction
qPCR	Real-time PCR
RT-PCR	Reverse-transcriptase PCR
RNA	Ribonucleic Acid
S	Svedberg
TAE	Tris-Acetate-EDTA
UV	Ultraviolet

CHAPTER 1

INTRODUCTION

Oil palm (*Elaeis guineensis*) was originated from Africa and it is a crop that growth in rainy tropical lowlands. In the early 20^{th} century, it evolved dramatically after being introduced to Sumatera and Peninsular Malaysia. In Malaysia, oil palm acts as an important plantation crop to fulfill the world wide demand for palm oil (Law *et al.,* 2012). This is due to the oil palm efficiency in terms of oil yield, land usage and distribution of asset compared to the other oil crops such as olive and coconut (Murphy, 2014). Besides palm oil, oil palm products also consist of palm kernel oil, palm kernel cake, oleochemicals and biodiesel that contributed to the expansion of oil palm plantations. Basically, 80% of palm oil was estimated goes into food and another 20% is used in the non-food sector (Jamek *et al.,* 2010).

Currently, productivity and growth of the oil palm being limited that influenced by the important environmental factors from biotic and abiotic stresses. One of the most critical abiotic stress that adversely affect the growth and production of yields are salinity caused by poor control of irrigation and deficiency of proper drainage, saline water irrigation and changes of vegetation that lead to growing groundwater tables (Rewald *et al.,* 2013). The physiological, biochemical, molecular level and productivity of plants were adversely affected by salt stress which associated to ionic toxicity and osmotic potential (Khan *et al.,* 2014). However, the accumulation of thiamine in plants has been shown in various studies in responses to abiotic and biotic stress conditions to protect the cells (Pourcel *et al.,* 2013).

Thiamine or also known as B_1 vitamin, is a water-soluble compound with several important roles in animals and plants (Sylvander *et al.,* 2012). In its active form, thiamine pyrophosphate (TPP), it acts as an essential cofactor for various types of enzymes involved in central metabolism such as glycolysis, pentose phosphate pathway and Krebs cycle (Khan *et al.,* 2014). In human body, thiamine *de novo* cannot be synthesized as the enzymes presence in the human intestine can only hydrolyse thiamine monophosphate derivatives to form thiamine (Roje, 2007). So, thiamine is a dietary requirement for human and animals which biologically resources from plants and microorganisms (Pourcel *et al.,* 2013). In plant, thiamine have been shown to have non cofactor roles by trigger defense responses in plants (Balia Yusof *et al.,* 2015) and helps in DNA damage tolerance caused by biotic and abiotic stress (Tun-Ozdemir *et al.,* 2009).

The pathway of biosynthesis of thiamine involves the formation of thiamine monophosphate (TMP) by coupling of the pyrimidine and thiazole moieties, which were formed separately (Gerdes *et al.,* 2012). This formation occurs in the chloroplast of plants and it is identical to the bacteria and yeast which the pyrimidine moiety, hydroxymethylpyrimidine pyrophosphate (HMP-PP) are condensed and phosphorylated with hydroxyethylthiazole phosphate (HET-P), the thiazole moiety that catalysed by THID/THIE enzyme. As both moieties were synthesised separately, in the first step under pyrimidine branch, hydroxymethylpyrimidine phosphate (HMP-P) requires a complex chemical arrangement of aminoimidazole ribonucleotide (AIR) that catalysed by phosphomethylpyrimidine synthase (THIC) and THID/THIE enzymes. As for thiazole moiety formation, hydroxyethyl thiazole phosphate (HET-P) require glycine, sulphur donor and reduced nicotinamie (NAD^+) that was catalysed by THI4 and THIM enzymes. Then, the thiamine being synthesised from TMP which catalyse by thiamine pyrophosphatase (TPPH). Finally, phosphorylation of thiamine to form active cofactor thiamine pyrophosphate (TPP), catalysed by the enzyme thiamine pyrophosphokinase (TPK) occurs in the cytosol (Pourcel *et al.,* 2013).

As THIC and THI1/THI4 were claimed to be major first the key enzymes of the thiamine biosynthesis pathway, controlling the modulation would seem to be a significant way of dealing with thiamine content determination in plants. Differ from THIC that was detected in green tissue of plants, THI1/THI4 have additional roles which is not only required in thiamine biosynthesis but also helps in mitochondrial DNA damage tolerance (Machado *et al.*, 1997) as it existing have been observed in both chloroplast and mitochondria.

The problem statement for this study is no studies on the effect of salinity stress in oil palm towards the expression of thiamine biosynthesis genes have been done so far.

As for hypothesis for this research, it is suggested that thiamine will accumulate in the plant upon the induction of stress and this will result in enhanced tolerance towards stress.

The objectives of this project are:

1) To identify and amplify the THIC and THI1/THI4 gene transcripts which encode for the first two enzymes of thiamine biosynthesis pathway in oil palm.
2) To investigate what happens to THIC and THI1/THI4 genes expression in oil palm when subjected to salinity stress induced by sodium chloride.

3) To compare the expression level of THIC and THI1/THI4 gene transcripts in treated and untreated oil palm seedlings under salt stress.

The specific objectives of this study are:

1) To design primer for THIC and THI1/THI4 gene transcripts from various species of plants via Genbank of NCBI database using Primer 3 website.
2) To amplify thiamine biosynthesis gene transcripts (THIC and THI1/THI4) in oil palm using RT-PCR.
3) To analyse the level of expression of THIC and THI1/THI4 in untreated and treated oil palm seedlings using ImageJ software.

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