THE EFFECT OF *Ganoderma boninense* INFECTION ON THE EXPRESSIONS OF THIAMINE (vitamin B1) BIOSYNTHESIS GENES IN OIL PALM

ZETTY NORHANA BALIA YUSOF*; FARRAH PAYYADHAH BORHAN*; FATIMATUL AZMA MOHAMAD* and MOHD HEFNI RUSLI**

ABSTRACT

Thiamine pyrophosphate *(TPP), an active form of thiamine plays a fundamental role as an enzymatic cofactor in universal metabolic pathways including glycolysis and pentose phosphate pathway. We identified and amplified gene transcripts of the first two enzymes in the pathway, THI1/THI4 and THIC. Primers were designed based on sequence comparison of the genes from Arabidopsis, rice and maize. We also investigated the relationship between expression of thiamine biosynthesis genes and stress. This is due to findings suggesting that TPP has functions other than as a cofactor in response to stress in plants. The response of THI1/THI4 and THIC gene transcripts towards the infection of* Ganoderma boninense *on oil palm was observed. The gene transcripts' expressions were analysed via RT-PCR over a time course after infection. For THI1/THI4, the percentage of level of expression for healthy oil palm is 29.79% and an increase up to 70.21% was observed in artificially infected oil palm seedlings. For THIC gene, in healthy oil palm, the level of expression was 45.83% but when infected, it increased slightly up to 54.17%. Our results support the suggestion that thiamine may play an important role in the protection of cells against stress as it leads to an overexpression of thiamine in general.*

Keywords: thiamine, vitamin B1, *Ganoderma boninense*, oil palm, stress.

Date received: 17 February 2014; **Sent for revision:** 21 April 2014; **Received in final form:** 12 August 2014; **Accepted:** 5 January 2015.

INTRODUCTION

Thiamine is required as a vitamin because it is the precursor to the enzyme cofactor, thiamine pyrophosphate (TPP). TPP is a coenzyme for several enzymes involved in carbohydrate metabolism, including pyruvate dehydrogenase and α-ketoglutarate dehydrogenase, which are involved in metabolism of carbohydrates. It is also a coenzyme for transketolase, which is a key player in the pentose phosphate pathway, a major route for the biosynthesis of the pentose sugars deoxyribose and ribose. The enzyme thiamine pyrophosphokinase (TPK) catalyses the conversion of free thiamine to TPP in plants and other eukaryotic organisms.

Thiamine Biosynthesis

The thiamine biosynthetic pathway is absent in animals, which is why they must obtain it in their diet. In contrast, plants, fungi, algae and bacteria can synthesise the vitamin but the pathway is not identical in all organisms. However, in all cases, the thiazole and pyrimidine moieties are synthesised in separate branches of the pathway and coupled

* Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
Email: zettynorhana@upm.edu.my

** Malaysian Palm Oil Board, 6 Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia.

to form thiamine monophosphate (TMP). A final phosphorylation by a specific kinase gives TPP, the active form of the cofactor. In bacteria, the enzyme encoded by the *ThiL* gene, directly phosphorylates TMP to TPP. In contrast, in eukaryotes, TMP is first dephosphorylated to thiamine, and then TPK converts this to TPP.

The best studied thiamine biosynthetic pathways are those of *Escherichia coli* and *Bacillus subtilis*, which utilise very similar pathways, yet differ in some notable ways. Like bacteria, thiamine biosynthesis in eukaryotes also consists of two branches to produce HMP-PP and HET-P but uses different sources to produce these moieties (Kowalska and Kozik, 2008). The understanding of the enzymatic activities of thiamine-dependent genes is still very limited in yeasts and fungi. None of the bacterial genes have homologues in the yeast genome (Croft *et al.*, 2006; Rodionov *et al.*, 2002). To date, only a gene family called *THI5* or *NMT1* has been discovered for the HMP-PP production in yeasts and fungi (Nosaka, 2006).

Interestingly, algae and higher plants use the bacterial route to produce HMP-PP but the same route as yeast and fungi to produce HET-P (Croft *et al*., 2007). Sequence comparisons have confirmed that bacterial THIC homologs and the fungal THI4 homologs are found in algae and higher plants (Rodionov *et al.*, 2002; Raschke *et al.*, 2007; Kong *et al.*, 2008). In addition, the bacterial THIM homologs were also found in *Chlamydomonas reinhardtii* and *Arabidopsis thaliana.* In bacteria, THIM is only required for salvage of exogenous HET but in *C. reinhardtii,* THIM was demonstrated to be essential for the HET-P production (Rodionov *et al*., 2002; Croft *et al*., 2006). Besides that, unlike bacteria, algae and higher plants use single bifunctional enzyme, THID/E (in *C. reinhardtii*), BTH1 (in *Brassica napus*), THI3 (in *Zea mays*), and THI1 (in *A. thaliana*) for the phosphorylation of HMP-P to HMP-PP and condensation of HMP-PP and HET-P to form TMP (Croft *et al*., 2007; Goyer, 2010).

The THI1/THI4 and THIC are the genes encoding the first enzymes of the thiazole and pyrimidine branches, respectively, of the thiamine biosynthesis pathway in studied algae and higher plants (*Figure 1*) (Balia Yusof, 2012). THI1/THI4 and THIC play an important role in thiamine biosynthesis but they seem to also have a non-cofactor role and importance in DNA damage tolerance caused by biotic and abiotic stresses in plants (Goyer, 2010). Additionally, modulation of thiamine metabolism was reported in *Z. mays* seedlings under conditions of abiotic stress (Rapala-Kozik *et al*., 2008). Machado *et al*. (1997) also reported that studies on THI4 in yeast proved that it has a dual role. First, in thiamine biosynthesis and the other one is in DNA damage tolerance when subjected to abiotic stress. Besides that, thiamine has recently been shown to have functions in response to abiotic and biotic stress in plants. Studies by Tunc-Ozdemir *et al*. (2009) showed that under oxidative stress, sugar deprivation, high salinity and hypoxia, THI1 was accumulated. Studies by Ahn *et al*. (2005) and Zhang *et al*. (1998) also showed that thiamine affects the defence-related genes or systemic acquired response (SAR)-related genes expression in plants such as tobacco, Arabidopsis and wheat (Friedrich *et al.*, 1996). The SAR-related genes were rapidly and strongly expressed in the thiamine treated plants. According to Tunc-Ozdemir *et al*. (2009), thiamine and TPP function as important stress-response molecules that alleviate oxidative stress during different abiotic stress conditions. Accumulation of thiamine and TPP was observed in Arabidopsis when subjected to abiotic stress conditions, such as high light, cold, osmotic, salinity, and oxidative treatments and also enhanced expression of transcripts encoding thiamine biosynthetic enzymes. Enhanced tolerance to oxidative stress was observed when plants were supplemented with exogenous thiamine. Research by Rapala-Kozik *et al*. (2008) suggests a role of thiamine metabolism in the plant response to environmental stress. In the study, *Z. mays* seedlings were used as a model system and subjected to conditions of drought, high salt, and oxidative stress. The total thiamine compound content in the maize seedling leaves increased under each stress condition applied and it was associated with changes in the relative distribution of free thiamine, TMP, and thiamine diphosphate (TDP).

Apart from that, a research programme has been recently launched to create rice with higher levels of vitamin B1 to make it more nutritious and at the same time, resistant to crop-damaging diseases (Goyer, 2010). Since previous studies showed that vitamin B1 can boost the immune system of plants, including rice, cucumber and tobacco, it is hoped that sustained accumulation of thiamine can make plants immune to important diseases.

Malaysia currently accounts for 39% of world palm oil production and 44% of world exports. However, there is a common disease that affects palm oil production in Malaysia called the basal stem rot (BSR). This disease is caused by *Ganoderma boninense* and it is the most fatal disease of oil palm in South-east Asia, and it causes very serious losses in palm oil production (Ariffin, 2000).

In this study, the relationship between thiamine biosynthesis in oil palm and stress was investigated. This is due to the finding suggesting that there is some evidence for protection of DNA by THI4 in mitochondria in plants (Machado *et al*., 1997). The response of the expression of thiamine biosynthesis genes specifically the first two enzymes on each thiazole and pyrimidine branch in thiamine biosynthesis pathway, THI1/THI4 and THIC when infected with *G. boninense* was carried out.

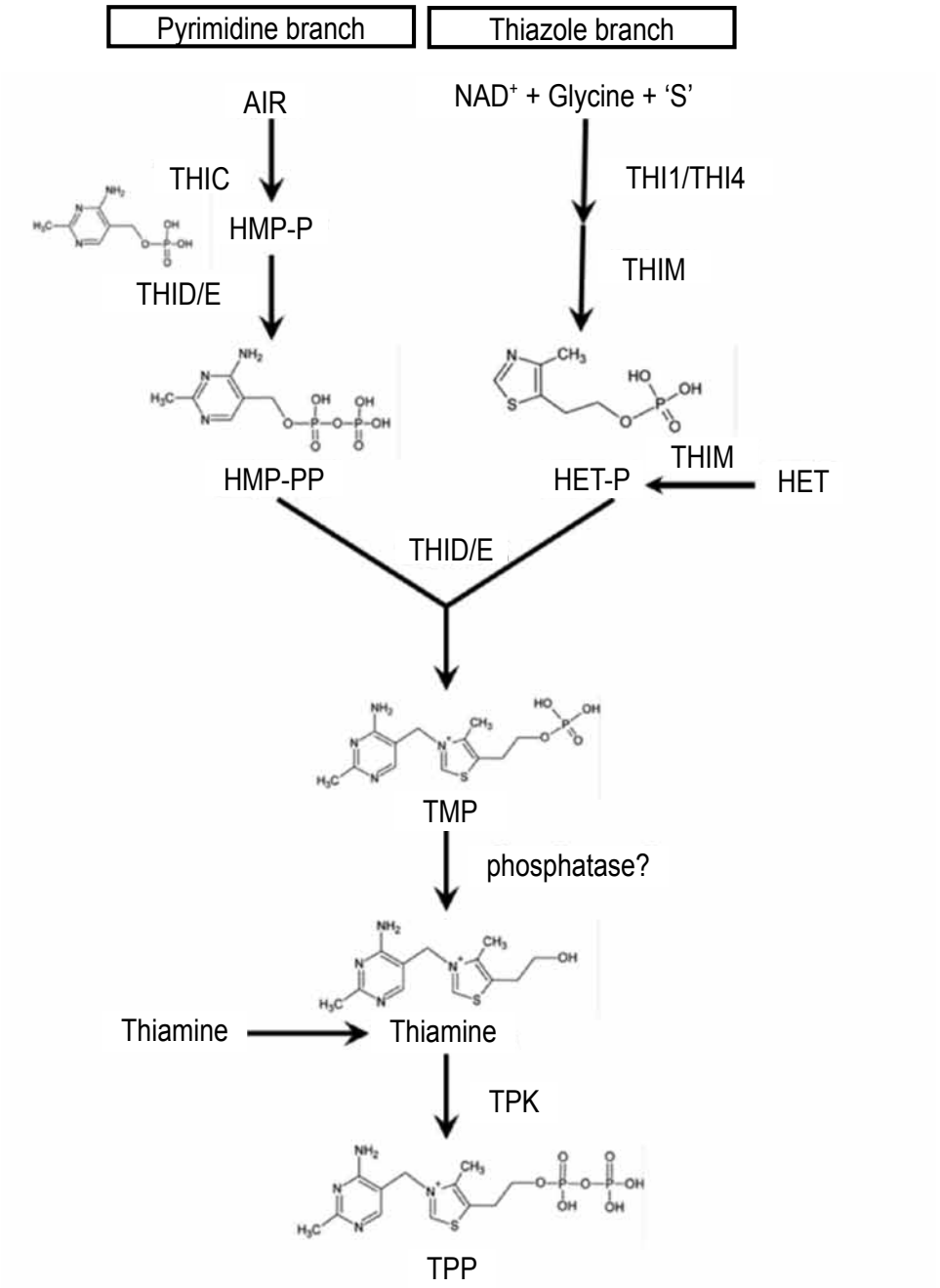

Figure 1. Thiamine biosynthesis in algae and higher plants. These organisms use the bacterial route to produce HMP-PP but the same route as yeasts and fungi to produce HET-P. Algae and higher plants use a bifunctional enzyme that catalyse the HMP-P phosphorylation and the thiamine monophosphate (TMP) formation steps (Balia Yusof, 2012).

MATERIALS AND METHODS

Fungal Strain

Ganoderma boninense isolate (PER 71) was used for infection of oil palm seedlings at the Malaysian Palm Oil Board (MPOB) – Universiti Kebangsaan Malaysia (UKM) nursery. It was isolated from an infected oil palm in United Plantations, Perak on *Ganoderma* selective medium (GSM) (Ariffin and Seman, 1991) and was maintained on potato dextrose agar as described by Balia Yusof (2007).

Plant Materials

Six-month old oil palm seedlings used for infection studies were commercial Deli x *Pisifera* crosses supplied by the Pathology Laboratory, MPOB. Palms were maintained under glasshouse conditions following the normal nursery practices.

Artificial Inoculation of *G. boninense*

Artificial inoculation of *G. boninense* was carried out based on the method by Idris *et al*. (2001), using

the single root inoculation technique. Preparation of *G. boninense*-colonised rubber woodblock was carried out by the Plant Pathology Group, MPOB. For the artificial inoculation process, a small incision was made on the side of the polybag through which a primary root was allowed to protrude. The primary roots chosen for inoculation were brown to brownish black and the secondary and tertiary roots were removed using a scalpel. The end of the root was aseptically cut and the root immediately inserted into the *G. boninense*-colonised rubber woodblock. About 3.5 cm length of the root was allowed to remain inside the woodblock and the whole block was then put in a small polybag filled with soil. The uninoculated seedlings were used as healthy tissue sample.

Sampling

Sampling was scheduled at two weeks and four weeks after inoculation with the pathogen. Spear leaves and basal stem were taken as tissue samples from three palms at each sampling. All spear leaves and the whole basal stem were taken. The spear leaves were cleaned and dissected before being frozen in liquid nitrogen and stored at -80°C until needed. The basal stem was washed with water to remove adhering soil, surface sterilised with 5% Clorox for 10 min, re-washed with sterile distilled water and then blotted dry with sterile tissue paper. The basal stem was cut into small cubes of approximately 0.5 cm x 0.5 cm x 0.5 cm before being frozen in liquid nitrogen and stored at -80°C until needed.

Data Mining and Primer Designing

Data mining included the gathering of all the nucleotide and amino acid sequences for the first two enzymes in thiamine biosynthesis pathway (THI1/THI4 and THIC) from different plant species and bacteria from GenBank of NCBI Database. The selected sequences were aligned by using Vector NTI Advance v11 software (Invitrogen) and the consensus regions from different plants and bacteria species were identified and used for designing PCR primers.

Total RNA Extraction

Total RNA was isolated by using modified RNA extraction protocol by Presscott and Martin (1987). The tissues used were basal stem and spear leaves from two weeks and four weeks after *G. boninense* inoculation and from non-inoculated seedlings. For each treatment and different tissue parts, a total of three replicates were carried out. The quality of the total RNA was quantified using NanoDrop UV-Vis spectrophotometer and was kept at -80°C.

Amplification of THI1/THI4 and THIC Genes

The method was performed by using a two-tube reaction, QuantiTech® Reverse Transcription Kit (Qiagen, USA). Complementary-DNA (cDNA) was prepared by mixing 20 μl reaction mixture of Reverse Transcription master mix (Quantiscript Reverse Transcriptase that consist of RNase inhibitor), 4 μl of 5 × Reverse Transcription buffer (includes Mg^{2+} and dNTPs), 1 μl of RT Primer Mix and 14 μl of the template RNA preparation (from the entire genomic DNA elimination reaction) which consisted of 5 μg of RNA for each reaction. The reverse transcription master mix contained all components required for first strand cDNA synthesis except template RNA. Then, template RNA from previous step was added to each tube containing reverse-transcription master mix. The mixtures was mixed and stored on ice. Next, the mixture was mixed and incubated at 42°C for 15 min. The tube was then incubated at 95°C for 3 min to stop the reverse transcriptase activity and chilled on ice. The cDNA formed was then used to proceed with PCR reactions.

The 10 sets of working solution of the forward and reverse primer was 10 pmol μl^{-1}, diluted by using distilled water. Then, PCR reaction was carried out. A 20 μl of reaction was prepared in a 50 μl PCR tube consisting of 1 μl of first-strand cDNA reaction, 0.5 μl of 10 μM dNTP mixture, 1 μl of 1.25 mM $MgCl_2$, 2.0 μl of 5 × Taq DNA polymerase buffer, 1 μl of 10 pmol of forward primer, 1 μl of 10 pmol of reverse primer, 13 μl sterile distilled water topped up to 20 μl and lastly 0.5 μl of *Taq* DNA Polymerase was added. The mixture was mixed and placed inside the thermocycler (Biometra). Reactions were cycled at 95°C for 2 min for one cycle, 28 repetitive cycles of denaturation at 95°C for 45 s, annealing temperature at 55°C for 45 s, extension at 72°C for 1 min and a final cycle of extension at 72°C for 5 min, and then held at 4°C. The PCR product was kept in the -20°C freezer until further use.

Analysis of PCR Products

PCR products were analysed via gel electrophoresis and calculated using ImageJ software (http://rsbweb.nih.gov/ij/).

RESULTS AND DISCUSSION

Data Mining and Primer Designing

Primers were designed based on sequence alignments of various plant species and bacteria as shown in *Table 1*. *Table 2* shows the primers designed in this study.

TABLE 1. SELECTED PLANT SPECIES AND BACTERIA USED FOR THI1/THI4 AND THIC PRIMERS DESIGNING

Gene	Data from Genbank	Accession No.
THI1/THI4	*Zea mays* thiamine biosynthesis1 (thi1)	NM_001112226.1
	Oryza sativa Japonica group mRNA for thiamine biosynthetic enzyme	AB110170.1
	Arabidopsis thaliana thiazole biosynthetic enzyme (THI1) mRNA	NM_124858.3
	Arabidopsis thaliana Thi1 protein mRNA, complete cds	U17589.1
THIC	*Arabidopsis thaliana* thiamine biosynthesis protein ThiC (THIC) mRNA, complete cds	NM_001202705.1
	Arabidopsis thaliana thiamine biosynthesis protein ThiC (THIC) mRNA complete cds	NM_128517.3
	Arabidopsis thaliana thiamine biosynthesis protein ThiC (THIC) mRNA, complete cds	NM_179804.2
	Bacillus subtilis sub sp. *subtilis* str. RO-NN-1 chromosome, complete genome	NC_017195.1

TABLE 2. PRIMERS DESIGNED FOR THE AMPLIFICATION OF THI1/THI4, THIC AND ACTIN GENE TRANSCRIPTS

Description	Primer name	Sequence
THI1/THI4	THI4 F1	5′- CAAGAGGCCTACGTTGTGGT- 3′
	THI4 R1	5′- CGTGTCGTGGTTCAGAGACA- 3′
	THI4 F2	5′- TGTCTCTGAACCACGACACG- 3′
	THI4 R2	5′- CGTCAATAGCATTCGGCAGC- 3′
	THI4 F3	5′- CCAGCTTTTGGCTCGTCCTA- 3′
	THI4 R3	5′- GTCAACCGCACAATAGCGTC- 3′
	THI4 F4	5′- GACGCTATTGTGCGGTTGAC- 3′
	THI4 R4	5′- TCCGTCAATAGCATTCGGCA- 3′
	THI4 F5	5′- TTCCGCTGAGATCGTGGATG- 3′
	THI4 R5	5′- ACGCATTACGAAGGAACGGA- 3′
THIC	THIC F6	5′- CAACCATGTGGGGAGCTGAT- 3′
	THIC R6	5′- TCAGCACTTTTTCAAGCGCC- 3′
	THIC F7	5′- GGGATCATAACTGAGGCGGG- 3′
	THIC R7	5′- ATCAGCTCCCCACATGGTTG- 3′
	THIC F8	5′- AGGAAGTTGGGCAACCATGT- 3′
	THIC R8	5′- GCAGTGCAAAACTCCAGCA- 3′
	THIC F9	5′- CATAACTGAGGCGGGCGATT- 3′
	THIC R9	5′- ACATGGTTGCCCAACTTCCT- 3′
	THIC F10	5′- CCATGAGACACGTGAGTGGA- 3′
	THIC R10	5′- ATGGATCCTCCCTTGGCAGT- 3′
Actin	ACT1S1	5′- CTTGCTCCAAGCAGCATGA- 3′
	ACT1AS1	5′- AGAAGCACTTCCGGTGCACG- 3′

Detection of PCR Product

The level of expression is shown visually in *Figure 2* where both THI1/THI4 and THIC were amplified successfully in healthy oil palm tissues using the primers designed. Bands at approximately 500 bp could be detected clearly which showed the amplification of THIC while bands at approximately 300 bp showed the amplification of THI1/THI4 gene. This finding proved that the first two enzymes in thiamine biosynthesis pathway gene transcripts were successfully amplified from oil palm tissue sample.

In this study, we hypothesised that the level of expression of THI1/THI4 and THIC gene transcripts will increase in infected oil palm tissue samples. The difference in the level of expressions could be seen where there was a slight increase in the infected tissue samples for both gene transcripts as shown in *Figure 3*.

In spear leaf tissue sample, the expression of THIC in healthy tissue sample was at 45.83% but an increase to 54.17% was observed in infected tissue sample. Approximately, the same amount of increment was observed in basal stem tissue sample for THIC gene expression with 42.95% expression

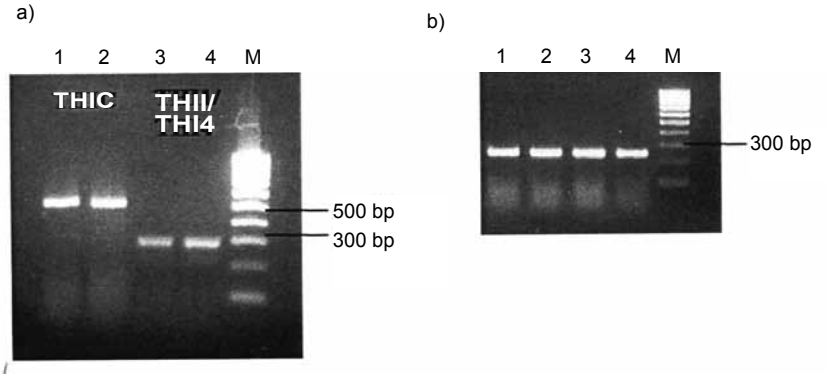

Figure 2. Amplification of THIC and THI1/THI4 (a) and actin gene transcripts (b) from healthy seedlings. Lanes 1 and 3 represent the spear leaf tissue samples and lanes 2 and 4 represent the basal stem tissue samples. M represents HyperLadder IV.

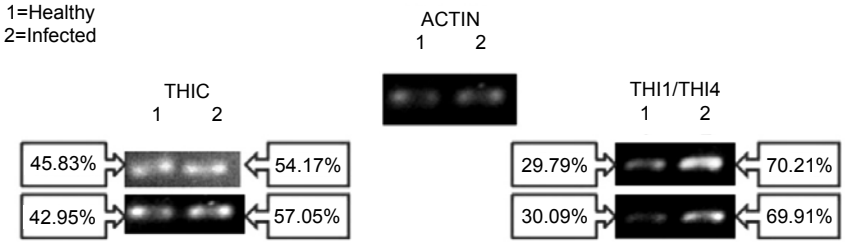

Figure 3. The level of expressions of THIC and THI1/THI4 in healthy and infected oil palm spear leaf tissue samples analysed using ImageJ software. Lane 1 represents healthy oil palm spear leaf (top gel) and basal stem (bottom gel) tissue sample while lane 2 represents infected oil palm spear leaf (top gel) and basal stem (bottom gel) tissue sample.

in healthy tissue sample and an increment to 57.05% in infected tissue sample. For THI1/THI4 gene transcript, in spear leaf tissue sample, the expression was 29.79% in healthy tissue sample and an obvious increase to 70.21% was observed. Again, approximately the same amount of increment was observed in basal stem tissue sample where healthy tissue sample showed 30.09% expression while infected tissue sample showed 69.91% expression. The gel was run alongside with the actin gene as control.

These results show that indeed, the level of expression of both gene transcripts were elevated in the infected tissue samples even though there was a huge difference between both THI1/THI4 and THIC in terms of the percentage of increment. For THIC gene transcript, even though the difference is small when compared with the increase of expression of THI1/THI4, an increase was observed.

For future works, sequence verification still need to be done and determination of level of thiamine and its intermediates need to be carried out to verify the results obtained. Besides that, expression of other genes encoding enzymes involved in thiamine biosynthesis pathway in oil palm will be useful in order to elucidate the regulation of the making of the vitamin. It is hoped that overexpression of thiamine or the specific enzymes in thiamine biosynthesis pathway will lead to the production of a more stress tolerant oil palm variety.

CONCLUSION

From this study, a total of 10 primer pairs were designed for amplification of THI1/THI4 and THIC gene transcripts in oil palm. Both THI1/THI4 and THIC gene transcripts were successfully amplified from oil palm tissue samples. RT-PCR was carried out and showed an increased expression of these two genes in *G. boninense* infected palms. For the THI1/THI4 gene, the highest percentage level of expression for healthy oil palm is 29.79% and for infected oil palm is 70.21%. For THIC gene, the result shows that in healthy oil palm, the percentage of expression is 45.83% but in the infected oil palm, it is slightly increased to 54.17%. Our results support the suggestion that thiamine may play an important role in the protection of cells against stress as it leads to an overexpression of thiamine in general.

This project was carried out in order to see if stress has contributed in the overexpression of this vitamin and/or its intermediates in diseased oil palm. If so, an overexpression of the genes will increase the production of thiamine overall. In the long run, overexpression of thiamine will open up the potential to engineer oil palm with high level of thiamine so that they will be more stress tolerant and at the same time producing thiamine-rich oil.

ACKNOWLEDGEMENT

The first author would like to thank Dr Syahida Ahmad and Dr Siti Aqlima Ahmad for chemicals and laboratory equipment, the students who had carried out the experiments in the laboratory, Farrah Payyadhah Borhan and Fatimatul Azma Mohamad, and to all the members of the Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia.

REFERENCES

AHN, I P; KIM, S and LEE, Y H (2005). Vitamin B1 functions as an activator of plant disease resistance. *Plant Physiology, 138*: 1505-1515.

ARIFFIN, D (2000). Major diseases of oil palm. *Advances in Oil Palm Research* (Yusof, B; Jalani, B S; Chan, K W eds.). MPOB, Bangi. p. 596-622.

ARIFFIN, D and SEMAN, I A (1991). *A Selective Medium for the Isolation of* Ganoderma *from Diseased Tissues*. PORIM, Bangi.

BALIA YUSOF, Z N (2007). *Identification and Characterization of Differentially Expressed Genes in* Ganoderma boninense*-Infected Oil Palms* (Elaeis guineensis). M. Sc. thesis, Universiti Putra Malaysia.

BALIA YUSOF, Z N (2012). *Regulation of Thiamine Biosynthesis in Chlamydomanes reinhardtin*. Ph.D thesis, University of Cambridge United Kingdom.

CROFT, M T; MOULIN, M; WEBB, M E and SMITH, A G (2007). Thiamine biosynthesis in algae is regulated by riboswitches. *Proc. Natl. Acad. Sci. USA 104*: 20770-20775.

CROFT, M T; WARREN, M J and SMITH, A G (2006). Algae need their vitamins. *Eukaryotic Cell, 5(8)*: 1175-1183.

FRIEDRICH,L; LAWTON, K; RUESS, W; MASNER, P; SPECKER, N; RELLA, MG; MEIER, B; DINCHER, S S; STAUB, T and UKNES, S(1996) A benzothiadiazole derivative induces systemic acquired resistance in tobacco. *Plant J 10*: 61-70.

GOYER, A (2010). Thiamine in plants: Aspects of its metabolism and functions. *Phytochemistry, 71*: 1615-1624.

IDRIS, A S; ARIFFIN, D; SWINBURNE, T R and WATT, T A (2001). Distribution of species of *Ganoderma* basal stem rot of oil palm in relation to the environmental conditions in Peninsular Malaysia. *Proc. of the PIPOC 2001 International Palm Oil Congress - Agriculture Conference*. p. 385-394.

IDRIS, A S; KUSHAIRI, A; ISMAIL, S and ARIFFIN, D (2004). Selection for partial resistance in oil palm progenies to *Ganoderma* basal stem rot. *J. Oil Palm Res. Vol. 16*: 12-18.

KONG, D; ZHU, Y; WU, H; CHENG, X; LIANG, H and LING, H Q (2008). AtTHIC, a gene involved in thiamine biosynthesis in *Arabidopsis thaliana*. *Cell Research, 18*: 566-576.

KOWALSKA, E and KOZIK, A (2008). The genes and enzymes involved in the biosynthesis of thiamin and thiamin diphosphate in yeasts. *Cellular & Molecular Biology Letters, 13(2)*: 271-282.

MACHADO, C R; PRAEKELT, U M; OLIVEIRA, R C; DE BARBOSA, A C; BYRNE, K L and MEACOCK, P A (1997). Dual role for the yeast *THI4* gene in thiamine biosynthesis and DNA damage tolerance. *J. Molecular Biology, 273(1)*: 114-21.

NOSAKA, K (2006). Recent progress in understanding thiamin biosynthesis and its genetic regulation in *Saccharomyces cerevisiae*. *Applied Microbiology and Biotechnology, 72(1)*: 30-40.

PRESSCOTT, A and MARTIN, C (1987). A rapid method for the quantitative assessment of levels of specific mRNAs in plants. *Plant Molecular Biology Reporter, 4*: 219-224.

RAPALA-KOZIK, M; KOWALSKA, E and OSTROWSKA, K (2008). Modulation of thiamine metabolism in *Zea mays* seedlings under conditions of abiotic stress. *J. Exp. Bot*., *59*: 4133-4143.

RASCHKE, M; BüRKLE, L; MüLLER, N; NUNES-NESI, A; FERNIE, A R and ARIGONI, D (2007). Vitamin B1 biosynthesis in plants requires the essential iron sulfur cluster protein, THIC. *Proc. Natl. Acad. Sci. USA 104(49)*: 19637-19642.

RODIONOV, D A; VITRESCHAK, A G; MIRONOV, A A and GELFAND, M S (2002). Comparative genomics of thiamin biosynthesis in procaryotes. New genes and regulatory mechanisms. *The Journal of Biological Chemistry, 277(50)*: 48949-48959.

TUNC-OZDEMIR, M; MILLER, G; SONG, L; KIM, J; SODEK and KOUSSEVITZKY, S (2009). Thiamin confers enhanced tolerance to oxidative stress in *Arabidopsis*. *Plant Physiology, 151(1)*: 421-432.

ZHANG, W; HAN, D Y; DICK, W A; DAVIS, K R and HOITINK, H A J (1998). Compost and compost water extract-induced systemic acquired resistance in cucumber and *Arabidopsis*. *Phytopathology, 88*: 450-455.