UNIVERSITI PUTRA MALAYSIA

THE EFFECT OF OSMOTIC STRESS TOWARDS THE EXPRESSIONS OF THIAMINE BIOSYNTHESIS GENES (THIC & THI1/THI4) IN OIL PALM (*Elaeis guineensis*)

WONG SOOK YEE

FBSB 2015 86

THE EFFECT OF OSMOTIC STRESS TOWARDS THE EXPRESSIONS OF THIAMINE BIOSYNTHESIS GENES (THIC & THI1/THI4) IN OIL PALM (*Elaeis guineensis*)

WONG SOOK YEE

162345

DEPARTMENT OF BIOCHEMISTRY

FACULTY OF BIOTECHNOLOGY AND BIOMOLECULAR SCIENCES

UNIVERSITI PUTRA MALAYSIA

2015

PENGESAHAN

Dengan ini adalah disahkan bahawa tesis projek yang bertajuk "The effect of osmotic stress towards the expressions of thiamine biosynthesis genes in oil palm" telah disiapkan serta dikemukakan kepada Jabatan Biokimia, Fakulti Bioteknologi dan Sains Biomolekul, Universiti Putra Malaysia oleh Wong Sook Yee (162345) sebagai syarat untuk kursus BCH4999 Projek.

Disahkan oleh,

..... Tarikh: .....
(Dr. Zetty Norhana Balia Yusof)
Penyelia projek
Jabatan Biokimia
Fakulti Bioteknologi dan Sains Biomolekul
Universiti Putra Malaysia

..... Tarikh: .....
(Prof. Dato' Dr. Abu Bakar Salleh)
Ketua Jabatan Biokimia
Fakulti Bioteknologi dan Sains Biomolekul
Universiti Putra Malaysia

ABSTRACT

Thiamine or vitamin B_1 composes of a pyrimidine moiety and a thiazole moiety. Thiamine pyrophosphate (TPP), the active form of thiamine, acts as a cofactor for various major enzymes for example transketolase (TK), α-ketoglutarate dehydrogenase (KGDH) and pyruvate dehydrogenase (PDH). In this study, THIC and THI1/THI4 gene transcripts, the first two enzymes in thiamine biosynthesis pathway were identified and amplified in oil palm. Primers were designed based on sequence comparison of the genes from *Arabidopsis thaliana*, *Zea mays*, *Oryza sativa* and *Alnus glutinosa*. The responses of oil palm through the expression profiles of thiamine biosynthesis genes (THIC and THI1/THI4), in response to polyethylene glycol (PEG) which induce osmotic stress were examined. This is due to the suggestion that TPP has another important role, which is protecting plants against abiotic and biotic stresses. The expression of gene transcripts were detected using reverse transcriptase polymerase chain reaction (RT-PCR) and from the 8 sets of primers designed, primer 3 (F3) for THIC gene and primer 8 (F8) for THI1/THI4 successfully amplified the transcripts. The results showed that THIC gene expression increases (200% of increase in treated plant compared to non-treated plant) in the presence of 1% PEG. The expression of THI1/THI4 gene caused by stress showed similar results to that of THIC gene but in accumulation of no more than 2.0-fold (100% of increase in treated plant compared to non-treated plant). The results agreed with the suggestion that thiamine may play important function in plant defence against stresses as these findings may lead to an overexpression of thiamine in general.

ABSTRAK

Thiamina atau vitamin B_1 dikomposisikan dengan gegelang pirimidina dan gegelang tiazol. Thiamina dalam bentuk aktifnya, thiamina pirofosfat (TPP) berfungsi sebagai kofaktor bagi beberapa enzim utama seperti dehidrogenase piruvat, dehidrogenase α-ketoglutarate dan transketolase. Dalam kajian ini, gen transkrip THIC dan THI1/THI4, kedua-dua gen yang pertama wujud dalam laluan biosintesis thiamina telah dikenalpasti dan diamplifikasi dalam kelapa sawit. Primer telah direka berdasarkan perbandingan jujukan gen daripada *Arabidopsis thaliana*, *Zea mays*, *Oryza sativa* dan *Alnus glutinosa*. Tindak balas kelapa sawit melalui profil ekpresi gen biosintesis thiamina (THIC dan THI1/THI4) dari segi potensi osmotik apabila dikenakan tekanan yang disebabkan oleh polietilena glikol (PEG) telah dikaji juga. Ini disebabkan oleh cadangan bahawa TPP memainkan peranan yang penting, iaitu merintang terhadap tekanan abiotik dan biotik dalam tumbuh-tumbuhan. Ekpresi gen telah dikesan dengan menggunakan RT- PCR dan daripada 8 pasangan primer, primer 3 (F3) bagi gen THIC dan primer 8 (F8) bagi gen THI1/THI4 menunjukkan peningkatan ekpresi dalam kelapa sawit yang mengalami tekanan. Hasil kajian menunjukkan peningkatan ekpresi bagi gen THIC (200% lebih tinggi ekpresi dalam kelapa sawit yang telah diberi tekanan berbanding dengan kelapa sawit yang tidak diberi tekanan) dalam aplikasi 1% PEG. Perubahan akibat tekanan dalam ekpresi bagi gen THI1/THI4 sama dengan gen THIC tetapi menunjukkan peningkatan tidak melebihi 2.0 kali ganda (100% lebih tinggi ekpresi dalam kelapa sawit yang telah diberi tekanan berbanding dengan kelapa sawit yang tidak diberi tekanan). Keputusan yang diperolehi menyokong cadangan bahawa thiamina mungkin memainkan peranan yang penting dalam pertahanan tumbuhan terhadap tekanan kerana ianya menjurus kepada ekpresi thiamina yang berlebihan secara umum.

ACKNOWLEDGEMENT

First of all, I would like to thank God for His blessings that enable me to complete my thesis successfully. I would also like to take this opportunity to express my profound gratitude and deep regards to my beloved supervisor, Dr. Zetty Norhana Balia Yusof for her exemplary guidance, monitoring and constant encouragement throughout the time of my final year project research .

Besides that, I also want to take this opportunity to express my deep sense of gratitude to all Lab 230 members, Mus, Tahjul, Syuhadah and Zainor for their cordial support, valuable information and guidance, and helped me in completing my trial through various stages. Special thanks to Prof. Maziah Mahmood for generously lending me chemicals and machines, Dr. Noor Azmi Shaharuddin for lending me his lab and also Dr. Siti Aqlima Ahmad for lending me the PCR machine.

At last, I wish to dedicate my thesis to my parent as they always supported me mentally and financially. Their love, belief, support and blessings have made it possible for me to complete my thesis successfully.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

°C	Degree Celsius
%	Percent
bp	Base Pair
g	Gram
µl	Microliter
µg	Microgram
mg	Milligram
ml	Millilitre
mM	Millimolar
M	Molar
ng	Nanogram
nm	Nanometre
AIR	5-Aminoimidazole Ribonucleotide
CTAB	Cetyltrimethylammonium Bromide
cDNA	Complementary DNA
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic Acid
EtBr	Ethidium Bromide
HET-P	4-Methyl-5-(2-Hydroxyethyl)-Thiazole Phosphate
HMP-P	4-Amino-2-Methyl-5-Hydroxymethylpyrimidine Monophosphate
HMP-PP	4-Amino-2-Methyl-5-Hydroxymethylpyrimidine Pyrophosphate
LiCl	Lithium Chloride
NaCl	Sodium Chloride
NAD^+	Nicotinamide Adenine Dinucleotide
NCBI	National Centre for Biotechnology
PEG	Polyethylene Glycol
PVP	Polyvinylpyrrolidone
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SAM	S-Adenosylmethionine
SAR	Systemic Acquired Resistance
SDS	Sodium Dodecyl Sulphate
TAE	Tris-Acetate-EDTA
TMP	Thiamine Monophosphate
TPP	Thiamine Pyrophosphate
TTP	Thiamine Triphosphate
Tris-HCl	Tris-Hydrochloride Acid

CHAPTER 1

INTRODUCTION

Thiamine or vitamin B_1 is important for all living organism as it serves vital functions in carbohydrate metabolism, nicotinamide adenine dinucleotide phosphate (NADPH) and adenosine triphosphate (ATP) synthesis and also nucleic acids formation (Nosaka, 2006). Thiamine pyrophosphate (TPP), the active form of thiamine acts as a cofactor for various key enzymes for example pyruvate dehydrogenase, transketolase and α-ketoglutarate dehydrogenase (Frank *et al.*, 2007). Animals and humans must consume thiamine through their diets because they cannot manufacture it themselves while plants and microorganism can biosynthesize it *de novo* (Moulin *et al.*, 2013). Therefore, study of thiamine biosynthesis in plants is very important as it is crucial to meet human nutrition and also for the plant to function efficiently.

Plants synthesize TPP from elementary precursors via biosynthetic pathways that are analogous to both bacteria and yeast (Goyer, 2010). The initial phases of TPP biosynthesis involve two pathways. One is alike to the mechanism found in bacteria which the pyrimidine branch of thiamine (4-amino-2-methyl-5-hydroxymethylpyrimidine monophosphate, HMP-P) is synthesized from 5-aminoimidazole ribonucleotide (AIR) and is catalysed by an enzyme which is encoded by THIC gene and has been identified in *Arabidopsis thaliana* (Raschke *et al.*, 2007). The other pathways is similar to the mechanism found in yeast (Chatterjee *et al.*, 2008) which the thiazole branch of thiamine (4-methyl-5-(2-hydroxyethyl)-thiazole phosphate, HET-P) is synthesized from glycine, nicotinamide adenine

dinucleotide (NAD^+) and a sulphur donor protein. THI1 gene, which has been identified in *Zea mays* and Arabidopsis (Machado *et al*., 1996) and its orthologues THI4 gene which has been recognized in bacteria encodes the main enzyme that synthesized HET-P.

THIC and THI1/THI4 are the genes that encode the first enzymes of pyrimidine (4-amino-2-methyl-5-pyrimidyl) and thiazole (4-methyl-5-β-hydroxyethylthiazolium) moieties of the thiamine biosynthesis pathway. These genes play a crucial role in thiamine biosynthesis yet they seem to have a non-cofactor function in DNA damage tolerance induced by abiotic and biotic stresses in plants (Goyer, 2010). Studies on THI4 gene in yeast also proved that it has dual functions, in thiamine biosynthesis and also in DNA damage tolerance when subjected to abiotic stresses (Machado *et al*., 1997). Research by Rapala-Kozik *et al.* (2008) revealed the homeostasis of thiamine metabolism in *Zea mays* seedlings under abiotic stress. Recently, experimentation by Tunc-Ozdemir *et al.* (2009) proved that under oxidative stress, hypoxia, high salinity and sugar deprivation, THI1 gene was accumulated. During different abiotic stress conditions, thiamine and TPP act as essential stress-response molecules that lessen oxidative stress. Since the previous studies showed that thiamine can improve the immune system of plants, it is believed that sustained accumulation of thiamine can make plants immune to severe diseases for example basal stem rot and upper stem rot.

Oil palm is economically valuable for its oil and has become one of the leading oil crops in the world. Malaysia, the world's second largest producer and exporter of palm oil and its by-products, produced nearly 18 million tons in 2011. This crop produces palm kernel oil and palm oil which has given rise to a range of commercial products ranging from cooking oils to soap and detergents. The steadily increasing

world population will thus make an escalating demand on fats and oils. According to the Malaysian Palm Oil Board (MPOB), the growth of oil palm must increase to approximately 24.6% by 2020 as to balance the demand for oil palm. To fulfil the demand of an ever growing population, an improvement both in yield and quality of palm oil is necessary (Sambanthamurthi *et al.*, 2009). Therefore, there is an urgent need to manage oil palm in adapting to a fast-changing environment and also to exploit the potential for genetic engineering in oil palm fully.

Abiotic stresses have negative influences on oil palm survival, palm oil production and crop yield. Common environmental stresses in Malaysia include water deficit, high temperature and salinity. Among the abiotic factors, water deficit is the most common stress that restricts oil palm growth, survival, distribution and productivity. As sessile organisms facing abiotic stresses, plants have strong adaptation at molecular, cellular and physiological levels toward environmental changes. Therefore, understanding plant tolerance toward osmotic stress mimic the condition of dry soil due to water deficit is necessary. Under osmotic stresses for example high salinity and drought, various genes functioning in stress response and tolerance are activated (Bartels and Sunkar, 2005).

An array of plant growth regulators are known to control growth and development of most plants under stress conditions. Thiamine is one of the plant growth factors needed for growth and differentiation in some plant species (Rapala-Kozik *et al.*, 2012). Studies have shown that thiamine biosynthesis is induced during plant adaptation responses to persistent abiotic stress conditions for example salting and flooding (Ribeiro *et al*., 2005), cold, heat, drought (Ferreira *et al.*, 2006; Wong *et al*., 2006) and oxidative stress (Tunc-Ozdemir *et al*., 2009).

In this study, the expression changes of thiamine biosynthetic genes (THIC and THI1/THI4) in oil palm when subjected to polyethylene glycol (PEG)-induced osmotic stress was analysed. It is hypothesized that THIC and THI1/THI4 genes will show an increase in expression in treated palms compared to non-treated palms. This supports the suggestion that thiamine may play an important function in plant protection against stress as the increase of gene expressions of THIC and THI1/THI4 may lead to an overexpression of thiamine in general. It is postulated that overexpression of thiamine will contribute to a more stress-tolerant oil palm variety. This is a very interesting topic as in Malaysia no similar research has been done so far.

The objectives for this study are:

1. To identify and sequence thiamine biosynthesis gene transcripts (THIC and THI1/THI4) in oil palm.
2. To study the effect of osmotic stress induced by PEG on the expression of thiamine biosynthesis genes (THIC and THI1/THI4) in oil palm.
3. To compare the level of expression of thiamine biosynthesis genes (THIC and THI1/THI4) in treated and non-treated palms.

The specific objectives for this study include:

1. To mine data and design primers of thiamine biosynthesis gene transcripts (THIC and THI1/THI4) in oil palm.
2. To extract and quantitate total RNA from oil palm spear leaves.
3. To amplify thiamine biosynthesis gene transcripts (THIC and THI1/THI4) using RT-PCR and to analyse the level of gene expression using ImageJ.

REFERENCES

Ahmad, S., Ahmad, R., Ashraf, M. Y., Ashraf, M., and Waraich, E. A. (2009). Sunflower (*Helianthus annuus* L.) response to drought stress at germination and seedling growth stages. *Pakistan Journal of Botany*, *41*, 647–654.

Ahn, I. P., Kim, S., and Lee, Y. H. (2005). Vitamin B_1 functions as an activator of plant disease resistance. *Plant Physiology*, *138*, 1505–1515.

Ajjawi, I., Rodriguez Milla, M. A., Cushman, J., and Shintani, D. K. (2007). Thiamin pyrophosphokinase is required for thiamin cofactor activation in Arabidopsis. *Plant Molecular Biology*, *65*, 151–162.

Baena-González, E. (2010). Energy signaling in the regulation of gene expression during stress. *Molecular Plant*, *3*, 300–313.

Balia Yusof, Z. N., Borhan, F. P., Mohamad, F. A., and Rusli, M. H. (2015). The effect of *Ganoderma boninense* infection on the expressions of thiamine (vitamin B_1) biosynthesis genes in oil palm. *Journal of Oil Palm Research*, *27*, 12–18.

Bartels, D., and Salamini, F. (2001). Desiccation tolerance in the resurrection plant *Craterostigma plantagineum*. A contribution to the study of drought tolerance at the molecular level. *Plant Physiology*, *127*, 1346–1353.

Bartels, D., and Sunkar, R. (2005). Drought and salt tolerance in plants. *Critical Reviews in Plant Sciences*, *24*, 23–58.

Bayoumi, T. Y., Eid, M. H., and Metwali, E. M. (2008). Application of physiological and biochemical indices as a screening technique for drought tolerance in wheat genotypes. *African Journal of Biotechnology*, *7*, 2341–2352.

Belanger, F. C., Leustek, T., Chu, B., and Kriz, A. L. (1995). Evidence for the thiamine biosynthetic pathway in higher-plant plastids and its developmental regulation. *Plant Molecular Biology*, *29*, 809–821.

Bocobza, S., Adato, A., Mandel, T., Shapira, M., Nudler, E., and Aharoni, A. (2007). Riboswitch-dependent gene regulation and its evolution in the plant kingdom. *Genes & Development*, *21*, 2874–2879.

Bocobza, S. E., and Aharoni, A. (2008). Switching the light on plant riboswitches. *Trends in Plant Science*, *13*, 526–533.

Chang, A. K., and Duggleby, R. G. (1997). Expression, purification and characterization of *Arabidopsis thaliana* acetohydroxyacid synthase. *Biochemical Journal*, *327*, 161–169.

Chatterjee, A., Jurgenson, C. T., Schroeder, F. C., Ealick, S. E., and Begley, T. P. (2007). Biosynthesis of thiamin thiazole in eukaryotes: Conversion of NAD to an advanced intermediate. *Journal of the American Chemical Society*, *129*, 2914–2922.

Chatterjee, A., Schroeder, F. C., Jurgenson, C. T., Ealick, S. E., and Begley, T. P. (2008). Biosynthesis of the thiamin-thiazole in eukaryotes: Identification of a thiazole tautomer intermediate. *Journal of the American Chemical Society*, *130*, 11394–11398.

Chen, W., Provart, N. J., Glazebrook, J., Katagiri, F., Chang, H.-S., Eulgem, T., and Zhu, T. (2002). Expression profile matrix of Arabidopsis transcription factor genes suggests their putative functions in response to environmental stresses. *The Plant Cell*, *14*, 559–574.

Edem, D. O. (2002). Palm oil: Biochemical, physiological, nutritional, hematological and toxicological aspects: A review. *Plant Foods for Human Nutrition*, *57*, 319–341.

Ferreira, S., Hjernø, K., Larsen, M., Wingsle, G., Larsen, P., Fey, S., and Salomé Pais, M. (2006). Proteome profiling of *Populus euphratica* Oliv. upon heat stress. *Annals of Botany*, *98*, 361–377.

Fleige, S., and Pfaffl, M. W. (2006). RNA integrity and the effect on the real-time qRT-PCR performance. *Molecular Aspects of Medicine*, *27*, 126–139.

Frank, R. A. W., Leeper, F. J., and Luisi, B. F. (2007). Structure, mechanism and catalytic duality of thiamine-dependent enzymes. *Cellular and Molecular Life Sciences*, *64*, 892–905.

Funk, C. (1912). The etiology of the deficiency diseases. *The Journal of State Medicine*, *20*, 341–368.

Gangolf, M., Wins, P., Thiry, M., El Moualij, B., and Bettendorff, L. (2010). Thiamine triphosphate synthesis in rat brain occurs in mitochondria and is coupled to the respiratory chain. *The Journal of Biological Chemistry*, *285*, 583–594.

Gopal, J., and Iwama, K. (2007). In vitro screening of potato against water-stress mediated through sorbitol and polyethylene glycol. *Plant Cell Reports*, *26*, 693–700.

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

Habib, S. H., Saud, H. M., and Kausar, H. (2014). Efficient oil palm total RNA extraction with a total RNA extraction kit. *Genetics and Molecular Research: GMR*, *13*, 2359–2367.

Henson, I. E., and Harun, M. H. (2005). The influence of climatic conditions on gas and energy exchanges above a young oil palm stand in north Kedah, Malaysia. *Journal of Oil Palm Research*, *17*, 73–91.

Henson, I. E., Noor, M. R. M., Harun, M. H., Yahya, Z., and Mustakim, S. N. A. (2005). Stress development and its detection in young oil palms in north Kedah, Malaysia. *Journal of Oil Palm Research*, *17*, 11–26.

Imbeaud, S., Graudens, E., Boulanger, V., Barlet, X., Zaborski, P., Eveno, E., and Auffray, C. (2005). Towards standardization of RNA quality assessment using user-independent classifiers of microcapillary electrophoresis traces. *Nucleic Acids Research*, *33*, 1–12.

Jones, L. H. (1997). The effects of leaf pruning and other stresses on sex determination in the oil palm and their representation by a computer simulation. *Journal of Theoretical Biology*, *187*, 241–260.

Julliard, J.-H., and Douce, R. (1991). Biosynthesis of the thiazole moiety of thiamin (vitamin B_1) in higher plant chloroplasts. *Proceedings of the National Academy of Sciences of the United States of America*, *88*, 2042–2045.

Knight, H., and Knight, M. R. (2001). Abiotic stress signalling pathways: Specificity and cross-talk. *Trends in Plant Science*, *6*, 262–267.

Kong, D., Zhu, Y., Wu, H., Cheng, X., Liang, H., and Ling, H.-Q. (2008). At THIC, a gene involved in thiamine biosynthesis in *Arabidopsis thaliana*. *Cell Research*, *18*, 566–576.

Leclere, S., Rampey, R. A., and Bartel, B. (2004). IAR4, a gene required for auxin conjugate sensitivity in Arabidopsis, encodes a pyruvate dehydrogenase E1a homolog. *Plant Physiology*, *135*, 989–999.

Li, Z., and Trick, H. N. (2005). Rapid method for high-quality RNA isolation from seed endosperm containing high levels of starch. *BioTechniques*, *38*, 872–876.

Lonsdale, D. (2006). A review of the biochemistry, metabolism and clinical benefits of thiamin(e) and its derivatives. *Evidence-Based Complementary and Alternative Medicine*, *3*, 49–59.

López, R., Aranda, I., and Gil, L. (2009). Osmotic adjustment is a significant mechanism of drought resistance in *Pinus pinaster* and *Pinus canariensis*. *Investigacion Agraria: Sistemas Y Recursos Forestales*, *18*, 159–166.

Machado, C. R., de Oliveira, R. L. C., Boiteux, S., Praekelt, U. M., Meacock, P. A., and Menck, C. F. M. (1996). THI1, a thiamine biosynthetic gene in *Arabidopsis thaliana*, complements bacterial defects in DNA repair. *Plant Molecular Biology*, *31*, 585–593.

Machado, C. R., Praekelt, U. M., de Oliveira, R. C., Barbosa, A. C. C., Byrne, K. L., Meacock, P. A., and Menck, C. F. M. (1997). Dual role for the yeast THI4 gene

in thiamine biosynthesis and DNA damage tolerance. *Journal of Molecular Biology*, *273*, 114–121.

Mohamad, H. (2000). Utilization of oil palm biomass for various wood-based and other products. In *Advances in Oil Palm Research, Malaysian Palm Oil Board*. pp 1346-1412.

Moulin, M., Nguyen, G. T. D. T., Scaife, M. A., Smith, A. G., and Fitzpatrick, T. B. (2013). Analysis of Chlamydomonas thiamin metabolism *in vivo* reveals riboswitch plasticity. *Proceedings of the National Academy of Sciences of the United States of America*, *110*, 14622–14627.

Munns, R., Passioura, J. B., Guo, J., Chazen, O., and Cramer, G. R. (2000). Water relations and leaf expansion: Importance of time scale. *Journal of Experimental Botany*, *51*, 1495–1504.

Naher, L., Ho, C.-L., Tan, S. G., Yusuf, U. K., and Abdullah, F. (2011). Cloning of transcripts encoding chitinases from *Elaeis guineensis* Jacq. and their expression profiles in response to fungal infections. *Physiological and Molecular Plant Pathology*, *76*, 96–103.

Nair, K. P. P. (2010). Oil palm (*Elaeis guineensis* Jacquin). In *The Agronomy and Economy of Important Tree Crops of the Developing World*. pp 209–36.

Nam, D. K., Lee, S., Zhou, G., Cao, X., Wang, C., Clark, T., and Wang, S. M. (2002). Oligo(dT) primer generates a high frequency of truncated cDNAs through internal poly(A) priming during reverse transcription. *Proceedings of the National Academy of Sciences of the United States of America*, *99*, 6152–6156.

Narberhaus, F. (2010). Translational control of bacterial heat shock and virulence genes by temperature-sensing mRNAs. *RNA Biology*, *7*, 84–89.

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

Papini-Terzi, F. S., da Silva Galhardo, R., Farias, L. P., Menck, C. F. M., and Van Sluys, M.-A. (2003). Point mutation is responsible for Arabidopsis tz-201 mutant phenotype affecting thiamin biosynthesis. *Plant and Cell Physiology*, *44*, 856–860.

Pourcel, L., Moulin, M., and Fitzpatrick, T. B. (2013). Examining strategies to facilitate vitamin B_1 biofortification of plants by genetic engineering. *Frontiers in Plant Science*, *4*, 1–8.

Rapala-Kozik, M., Olczak, M., Ostrowska, K., Starosta, A., and Kozik, A. (2007). Molecular characterization of the THI3 gene involved in thiamine biosynthesis in *Zea mays*: cDNA sequence and enzymatic and structural properties of the recombinant bifunctional protein with 4-amino-5-hydroxymethyl-2-

methylpyrimidine (phosphate) kinase and thiamine monophosphate synthase activities. *The Biochemical Journal*, *408*, 149–159.

Rapala-Kozik, M., Kowalska, E., and Ostrowska, K. (2008). Modulation of thiamine metabolism in *Zea mays* seedlings under conditions of abiotic stress. *Journal of Experimental Botany*, *59*, 4133–4143.

Rapala-Kozik, M., Gołda, A., and Kujda, M. (2009). Enzymes that control the thiamine diphosphate pool in plant tissues. Properties of thiamine pyrophosphokinase and thiamine-(di)phosphate phosphatase purified from *Zea mays* seedlings. *Plant Physiology and Biochemistry*, *47*, 237–242.

Rapala-Kozik, M., Wolak, N., Kujda, M., and Banas, A. K. (2012). The upregulation of thiamine (vitamin B_1) biosynthesis in *Arabidopsis thaliana* seedlings under salt and osmotic stress conditions is mediated by abscisic acid at the early stages of this stress response. *BMC Plant Biology*, *12*, 1–14.

Raschke, M., Bürkle, L., Müller, N., Nunes-Nesi, A., Fernie, A. R., Arigoni, D., and Fitzpatrick, T. B. (2007). Vitamin B_1 biosynthesis in plants requires the essential iron-sulfur cluster protein, THIC. *Proceedings of the National Academy of Sciences of the United States of America*, *104*, 19637–19642.

Ribeiro, D. T., Farias, L. P., de Almeida, J. D., Kashiwabara, P. M., Ribeiro, A. F. C., Silva-Filho, M. C., and Van Sluys, M.-A. (2005). Functional characterization of the THI1 promoter region from *Arabidopsis thaliana*. *Journal of Experimental Botany*, *56*, 1797–1804.

Rindi, G., and De Giuseppe, L. (1961). A new chromatographic method for the determination of thiamine and its mono-, di- and tri-phosphates in animal tissues. *The Biochemical Journal*, *78*, 602–606.

Roje, S. (2007). Vitamin B biosynthesis in plants. *Phytochemistry*, *68*, 1904–1921.

Rychlik, W., Spencer, W. J., and Rhoads, R. E. (1990). Optimization of the annealing temperature for DNA amplification in vitro. *Nucleic Acids Research*, *18*, 6409–6412.

Sambanthamurthi, R., Singh, R., Kadir, A. P. G., Abdullah, M. O., and Kushairi, A. (2009). Opportunities for the oil palm via breeding and biotechnology. In *Breeding Plantation Tree Crops: Tropical Species*. pp 377–421.

Sayed, S. A., and Gadallah, M. A. A. (2001). Effects of shoot and root application of thiamin on salt-stressed sunflower plants. *Plant Growth Regulation*, *00*, 1–10.

Schneider, C. A., Rasband, W. S., and Eliceiri, K. W. (2012). NIH image to ImageJ: 25 years of image analysis. *Nature Methods*, *9*, 671-675.

Smith, A. M., Fuchs, R. T., Grundy, F. J., and Henkin, T. M. (2010). Regulation of gene expression by direct monitoring of a physiological signal. *RNA Biology*, *7*, 104–110.

Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994). CLUSTALW: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, *22*, 4673–4680.

Thore, S., Leibundgut, M., and Ban, N. (2006). Structure of the eukaryotic thiamine pyrophosphate riboswitch with its regulatory ligand. *Science (New York, N.Y.)*, *312*, 1208–1211.

Tunc-Ozdemir, M., Miller, G., Song, L., Kim, J., Sodek, A., Koussevitzky, S., and Shintani, D. (2009). Thiamin confers enhanced tolerance to oxidative stress in Arabidopsis. *Plant Physiology*, *151*, 421–432.

Wachter, A., Tunc-Ozdemir, M., Grove, B. C., Green, P. J., Shintani, D. K., and Breaker, R. R. (2007). Riboswitch control of gene expression in plants by splicing and alternative 3' end processing of mRNAs. *The Plant Cell*, *19*, 3437–3450.

Wachter, A. (2010). Riboswitch-mediated control of gene expression in eukaryotes. *RNA Biology*, *7*, 67–76.

Wahid, M. B., Abdullah, S. N. A., and Henson, I. E. (2004). Oil Palm – Achievements and potential. In *New Directions for a Diverse Planet*. pp 1–13.

Wang, G., Ding, X., Yuan, M., Qiu, D., Li, X., Xu, C., and Wang, S. (2006). Dual function of rice OsDR8 gene in disease resistance and thiamine accumulation. *Plant Molecular Biology*, *60*, 437–449.

Wang, L., and Stegemann, J. P. (2011). Extraction of high quality RNA from polysaccharide matrices using cetlytrimethylammonium bromide. *Biomaterials*, *31*, 1–14.

Wong, C. E., Li, Y., Labbe, A., Guevara, D., Nuin, P., Whitty, B., … Moffatt, B. A. (2006). Transcriptional profiling implicates novel interactions between abiotic stress and hormonal responses in Thellungiella, a close relative of Arabidopsis. *Plant Physiology*, *140*, 1437–1450.

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.