



UNIVERSITI PUTRA MALAYSIA

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

AMIRAH NOR BT. KAMARUDIN

FBSB 2018 6



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By

AMIRAH NOR BT. KAMARUDIN

**Thesis Submitted to School of Graduate Studies, Universiti Putra Malaysia, in
Fulfilment of the Requirements for the Degree of Master of Science**

October 2017

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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October 2017

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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*, *THI* 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.)**

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Vitamin B1 memainkan peranan yang penting dalam metabolisme 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 kuantitatif masa sebenar (qPCR) telah dijalankan untuk melihat pengespresan empat gen biosintesis B1 yang utama iaitu *THI4*, *THIC*, *TH1* dan *TPK*. Hasil penemuan menunjukkan pengespresan gen biosintesis vitamin B1 meningkat sehingga 12 kali ganda. Selepas 15 dan 30 hari usai inokulasi, pengespresan 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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TABLE OF CONTENTS

	Page
ABSTRACT	i
ABSTRAK	ii
ACKNOWLEDGEMENTS	iii
APPROVAL	v
DECLARATION	vi
LIST OF TABLES	xi
LIST OF FIGURES	xii
KIST OF APPENDICES	xvi
LIST OF ABBREVIATIONS	xvii
 CHAPTER	
1 INTRODUCTION	1
1.1 Objectives	2
2 LITERATURE REVIEW	3
2.1 Oil palm industry and its economic importance in Malaysia	3
2.2 Basal stem rot disease (BSR) and disease management	3
2.3 Endophytes and its role in plant protection	3
2.3.1 Mechanism of endophyte-mediated tolerance to biotic stress is through induced systemic resistance	4
2.3.2 Transcriptomic analysis on plant response to fungal endophytes	8
2.3.3 Endophytic microbes in oil palm	8
2.3.4 Endophytic fungus <i>Hendersonia toruloidea</i>	8
2.4 Overview of thiamine	9
2.4.1 The thiamine biosynthesis pathway in plants	9
2.4.2 Role of thiamine in biotic and abiotic stresses in plants	11
2.4.3 Role of thiamine in plant growth	14
2.5 Endophytic <i>H. toruloidea</i> as inducer of thiamine biosynthesis in oil palm	14
3 MATERIALS AND METHODS	15
3.1 Plant Materials	15
3.2 Fungal Materials	15
3.3 Inoculation procedure	17
3.4 Sampling	17
3.5 Colonization of <i>Hendersonia toruloidea</i> in oil palm roots	18
3.5.1 Reisolation of endophytic fungus <i>H. toruloidea</i> in oil palm roots	18
3.5.2 Scanning electron microscopy analysis	18
3.5.3 Transmission electron microscopy	18
3.6 Quantitative real time PCR	19

3.6.1	Total RNA isolation	19
3.6.2	RNA purification	20
3.6.3	Data mining and primer designing	20
3.6.4	Agarose gel electrophoresis	20
3.6.5	cDNA synthesis	21
3.6.6	Amplification Efficiency Test	21
3.6.7	Cloning and plasmid isolation	21
3.6.8	Sequencing of DNA and sequence analysis.	22
3.6.9	Amplification of thiamine biosynthesis genes by quantitative real-time PCR (qPCR)	22
3.7	HPLC analysis for determination of thiamine, thiamine monophosphate and thiamine pyrophosphate	23
3.7.1	Sample preparation of crude plant extract	23
3.7.2	Standard preparation of thiamine, thiamine monophosphate and thiamine pyrophosphate	23
3.7.3	Sample and standard derivatization to thiochromes derivatives of thiamine	23
3.7.4	Chromatography settings	24
3.8	Statistical analyses	24
4	RESULTS AND DISCUSSION	25
4.1	Colonization of <i>Hendersonia toruloidea</i> in oil palm root	25
4.1.1	Scanning electron microscopy analysis	27
4.1.2	Transmission electron microscopy	28
4.2	Quantitative real-time PCR analysis (qPCR)	32
4.2.1	Total RNA isolation and purification	32
4.2.2	Data mining and primer designing	34
4.2.3	Testing for amplification efficiency	39
4.2.4	Cloning and analysis of insert with EcoR1	44
4.2.5	Sequencing analysis	44
4.2.6	Validation of reference genes	45
4.2.7	The effects of colonization of <i>H. toruloidea</i> on expression of thiamine biosynthesis genes in oil palm seedlings	47
4.3	HPLC analysis of total thiamine, thiamine monophosphate and thiamine pyrophosphate	52
4.3.1	Simultaneous determination of thiamine in oil palm leaves sample using HPLC with fluorescent detection (HPLC-FLD)	52
4.3.2	The effect of colonization of <i>H. toruloidea</i> on total content of thiamine and thiamine pyrophosphate in oil palm leaves	55
5	CONCLUSION AND RECOMMENDATION FOR FUTURE RESEARCH	57
5.1	Conclusion	57
5.2	Recommendation for future research	57

REFERENCES	59
APPENDICES	68
BIODATA OF STUDENT	81
LIST OF PUBLICATIONS	82



LIST OF TABLES

Table	Page
2.1 Biotic stress tolerance conferred by fungal endophytes in different host plants	6
2.2 Biochemical function of thiamine biosynthesis enzymes	10
3.1 Ratio of acetone and resin mixture and the duration of infiltration with the specimens	18
3.2 Gradient elution settings to manipulate the concentration of mobile phase eluting the column bed	24
4.1 Assessment of nucleic acid quality using NanoDrop. A good quality total RNA was successfully isolated as shown on the A260/A280 and A260/A30 values of (1.8-2.0)	32
4.2 Selected plants and microalgae species obtained from GenBank used for primer designing	35
4.3 List of primers designed using Primer Premier™ software	38
4.4 Amplification efficiency tests. Amplification efficiency tests were evaluated through r^2 value and efficiency (%). All primers met the criteria of $r^2 > 0.98$ and efficiency ranging from 90-110%	42
4.5 BLAST analysis of the sequencing results of the PCR fragments significantly reflected high similarity of the alignment with <i>Elaies guineensis</i> as indicated by the E value score and sequence similarity (%)	45

LIST OF FIGURES

Figure		Page
2.1	The thiamine biosynthesis pathway in plants. HMP-P is hydroxymethylpyrimidine phosphate, HMP-PP is hydroxymethylpyrimidine pyrophosphate, HET-P is hydroxyethylthiazole phosphate. THIC is hydroxymethylpyrimidine-synthase, THI4 is thiazole biosynthetic protein, TH1 is hydroxymethylpyrimidine phosphate kinase, TH2 is thiamine monophosphate phosphatase and TPK is thiamine pyrophosphate kinase. Adapted from Guan <i>et al.</i> (2014)	11
2.2	The transgenic rice that has a knocked-out OsDR8 gene which is homologs of THI1/THI4 showed an apparent disease symptoms caused by <i>Xanthomonas oryzae</i> pv. <i>Oryzae</i> and <i>Magnaporthe grisea</i>	13
3.1	Sequential steps of preparation of <i>H. toruloidea</i> bioformulation. Pure cultures of <i>H. toruloidea</i> was encapsulated in an alginate formulation to maintain a steady release of fungal inoculum	16
4.1	Establishment of <i>H. toruloidea</i> in oil palm seedlings	26
4.2	SEM images of 10 mm root sections of oil palm seedlings inoculated with <i>H. toruloidea</i> revealed fungal ultrastructures after ten days of incubation. A) Invasive growth of hyphae (arrow) B) Arrow shows proliferation of conidiospore C) Appresorium-like structure forming clamp connection (arrow). D) Arrow shows ascospores formation. Bar size 10 μ m	27
4.3	Light micrographs of transverse section of oil palm root stained with toluidine blue. A) Transverse section of uninoculated seedling B) Transverse section of oil palm root colonized by <i>H. toruloidea</i> at day 30 post colonization. Arrow shows direct penetration of hyphae through epidermal and cortical cells. Bar size 0.02 mm	29
4.4	A) and B) Transmission electron microscopy of transverse section of oil palm root colonized by <i>H. toruloidea</i> at day 30 post colonization. Arrow showed appresorium-like structures in cortical cells at day 30 of colonization. Bar size is 5000 nm	30

4.5	A) and B) Transmission electron microscopy of oil palm root colonized by <i>H. toruloidea</i> at day 30 post colonization. Red arrows showing hyphal coils in cortical cells. Dark cell wall aspositions were apparent as indicated by orange arrows. Bar size is 5000 nm	31
4.6	Assessment of RNA integrity by visualizing RNA on 1% agarose gel electrophoresis. Lane M: 1kb ladder (NEB), Lane 1: Total RNA isolated from oil palm spear leaves showing 2 intact bands of 28S and 18S rRNA	33
4.7	Sequential effects of RNA integrity after each steps of DNase treatment. Lane M is 100bp Ladder (GeneRuler, Thermoscientific); Lane 1 is RNA at Room temperature, Lane 2 is RNA at heat treatment at 37 °C, Lane 3 is RNA at heat treatment at 37 °C and addition of EDTA, Lane 4 is RNA at heat treatment 37 °C, addition EDTA, and lastly heat treatment at 65 °C. DNase treatment successfully removed genomic DNA and maintained the integrity of RNA	33
4.8	Alignment of THIC gene sequences from <i>Arabidopsis thaliana</i> (NM_001202705), <i>Elaeis guineensis</i> (XM_010924818.1), <i>Poa secunda</i> (AF264021.1) and <i>Zea mays</i> (EU972242.1) using MSA tool from EMBL Clustal W (http://www.ebi.ac.uk/Tools/msa/). Residues which are similar or identical were denoted by asterisks. The consensus sequence was utilized for primer designing using Primer Premier 6.0	37
4.9	Phases of PCR amplification curve. qPCR amplification curves of seven 2-fold dilutions of cDNA generated from cDNA of control oil palm seedlings in triplicates. Each amplification curves represent the four different phase: the baseline, the exponential phase, the log linear phase and finally the plateau phases. Y axis represents reference fluorescence units (RFU), which is a fluorescence value of an experimental reaction minus the Rn value of baseline signal generated by Biorad CFX 96. X-axis represents the cycle number. (A) represents the threshold at which Delta Rn reached the linear phase of amplification of all samples	40
4.10	Standard curves generated from cDNA of control oil palm seedlings. Seven 2-fold dilutions, starting with 100ng template copies of cDNA and ending with 3.125 ng copies. Y-axis represents the threshold cycle number. X-axis represents the log starting quantity of the cDNA template. A line representing the best fit was calculated for the standard curve using the least squares method of linear regression. A slope of -3.54 (91.3% efficiency), a y-intercept of 33 and r^2 of 0.988 was obtained	41

4.11	Amplification of gene fragments from cDNA derived from oil palm seedlings. The gene fragments corresponded to the expected fragment length. Lane 1 is <i>TUB</i> , lane 2 is <i>GAD</i> , lane 3 is <i>THI4</i> , lane 4 is <i>THIC</i> , lane 5 is <i>THI</i> , and lane 6 is <i>TPK</i> . Lane M is 50bp DNA ladder (GeneRuler, ThermoScientific)	42
4.12	Melt curve analysis. qPCR amplification was performed on 2-fold serial dilutions of cDNA template. Y-axis represents the rate of change of fluorescence versus temperature. X-axis represents the melting temperature of the PCR product at approximately 82.5 °C. A single peak on the melt curve analysis indicates a single PCR product has melted. No amplification was found in no template control (NTC)	43
4.13	Clones with inserts were identified by digestion of plasmid by EcoR1. Lane 1, 3, 5, 7, 9, and 11: Undigested plasmids containing inserts of <i>THI4</i> , <i>THIC</i> , <i>THI</i> , <i>TPK</i> , <i>TUB</i> , and <i>GAD</i> . Lane 2, 4, 6, 8, and 10: Digested plasmids containing inserts of <i>THI4</i> , <i>THIC</i> , <i>THI</i> , <i>TPK</i> , <i>TUB</i> , and <i>GAD</i> . Lane M is 100 bp DNA ladder (Invitrogen)	44
4.14	The changes in copy number of tubulin and GAPDH in the experimental samples. Bar represents mean Ct values of control and treated samples. Error bars indicated standard deviation (SD) of three independent experiments. No significant difference between all samples (p value<0.05). C1 is untreated control day 1, C7 is untreated control day 7, C15 is untreated control day 15, C30 is untreated control day 30, T1 is Ht-inoculated day 1, T7 is Ht- inoculated day 7, T15 is Ht-inoculated day 15, T30 is Ht-inoculated day 30	46
4.15	Changes in transcript abundance of <i>THI4</i> and <i>THIC</i> , the first enzymes in the thiamine biosynthesis pathway. (A) & (B) qPCR analysis was conducted on <i>THI4</i> and <i>THIC</i> transcripts of 7 months oil palm seedlings that were inoculated with <i>H. toruloidea</i> at day 1, 7, 15 and 30. Error bars denote standard deviation (SD). Asterisks denote significant difference at $p<0.05$ compared to control seedlings	50
4.16	Changes in transcript abundance of <i>TPK</i> and <i>THI1</i> . (C) & (D) qPCR analysis of <i>TPK</i> and <i>THI</i> transcripts of 7 months old oil palm seedlings inoculated with <i>H. toruloidea</i> at day 1, 7, 15, and 30. Error bars denote standard deviation (SD). Asterisks denote significant difference at $p<0.05$ compared to control seedlings	51
4.17	Chromatograms of thiochrome derivatives of thiamine standards (A) and oil palm leaves sample (B), where 1 is TPP ($R_t=3.04$), 2 is TMP ($R_t=3.35$) and 3 is TF ($R_t=7.22$)	53

- 4.18 Calibration curves of standard thiamine pyrophosphate (A), thiamine monophosphate (B) and thiamine (C). 10 ppm, 2 ppm, 1 ppm, 0.4 ppm and 0.2 ppm of standards were injected 54
- 4.19 HPLC analysis of thiamine content (TPP, TMP and TF) in 7 months old oil palm seedlings in control and inoculated seedlings T1 is *H. toruloides* inoculated at day 1, T7 is *H. toruloides* inoculated at day 7, T15 is *H. toruloides* inoculated at day 15, and T30 is *H. toruloides* inoculated at day 30. In each analysis, 5g of fresh oil palm leave was used. Data are means \pm SE of three replicates. Asterisks denotes significant difference between control and inoculated seedlings. Significance level is at p value <0.05 56

LIST OF APPENDICES

Appendix		Page
1	Preparation of buffers and chemicals	68
2	Sequencing results	70
3	Melt curve analysis	74
4	Calculation for HPLC analysis	77



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 transcriptase



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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