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CDNA CLONING AND EXPRESSION ANALYSIS OF TOCOPHEROL CYCLASE GENE FROM VARIOUS OIL PLAM TISSUES (*Elaeis guineensis* and *Elaeis oleifera*)

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By

LAI MUN SEONG

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

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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

cDNA CLONING AND EXPRESSION ANALYSIS OF TOCOPHEROL CYCLASE GENE FROM VARIOUS OIL PALM TISSUES (*Elaeis guineensis* and *Elaeis oleifera*)

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Chair: Associate Professor Datin. Siti Nor Akmar Abdullah, PhD

Faculty: Faculty of Agriculture

Tocopherol cyclase (TC) which catalyses the formation of γ-tocopherol from reduced (quinol form) 2,3-dimethyl-6-phytyl-1,4-benzoquinone (DMPBQ) is one of the key enzymes in tocopherol biosynthetic pathway. The aim of this study is isolate the cDNA clone encoding TC and to study its expression profile in different oil palm tissues and different developmental stages in the mesocarp. A partial cDNA 0.5 kb in length was amplified using gene-specific primer from an oil palm expressed sequence tags sequence and found to have high sequence identity with other plant species TC such as *Oryza sativa* (81%) and *Helianthus annus* (76%) at the amino acid level. Rapid amplification of cDNA ends (RACE) and end to end PCR successfully amplified the cDNA for the full mature protein of TC from two oil palm species (*E. guineensis* and *E. oleifera*) of 1. 233 kb in length encoding 411 amino acids. The amino acid identity between the *E. guineensis* and *E. oleifera* sequence was 99.6%. Both sequences have three long stretches of hydrophobic residues and 30 carboxyl-terminal amino acid unique for plants (not found in cynobacteria TC). However, the

reported conserved 5 amino acid carboxyl domain KPPGL in plant TC has 1 variant amino acid in both oil palm sequences (RPPGL). Quantitative gene expression analysis performed using Real-time PCR showed expression of *E. guineensis* TC in mesocarp was moderately high at 7 w.a.a., dropped at 10 w.a.a then increased steadily from 10 w.a.a and reached a maximum level at 17 w.a.a and the expression level dropped significantly at 19 w.a.a. The overall expression levels in leaves were lower than mesocarp with young leaves having a higher expression level than mature leaves. Real-time gene copy number analysis indicated only one copy of TC exists in the oil palm genome. The isolated mature protein sequence of oil palm TC can be used in functional study to understand the vitamin E biosynthetic process in oil palm and plants in general. It can also be used in genetic engineering efforts to modify vitamin E content and composition in plants.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGKLONAN cDNA DAN ANALISIS PENGEKSPRESAN GEN TOKOFEROL SIKLASE DARIPADA PELBAGAI JENIS TISU KELAPA SAWIT
(*Elaeis guineensis* dan *Elaeis oleifera*)

Oleh

LAI MUN SEONG

JUN 2010

Pengerusi: Profesor Madya Datin Dr Siti Nor Akmar Abdullah

Fakulti: Fakulti Pertanian

Tokoferol siklase (TC) yang memangkinkan tindak balas penghasilan "γ-tocopherol" daripada "2,3-dimethyl-6-phytyl-1,4-benzoquinone" (DMPBQ) adalah salah satu enzim yang penting dalam tapak biosintesis tokoferol. Matlamat kajian ini adalah untuk memencilkan cDNA yang megkodkan tokoferol siklase and menganalisis corak pengekspresan TC dalam pelbagai peringkat tisu mesokarpa. Jujukan cDNA bersaiz 0.5 kb telah diamplifikasi dengan mengunakan pencetus spesifik yang direka daripada tag mendedahkan urutan dan didapati mempunyai peratusan persamaan jujukan yang tinggi di peringkat asid amino dengan tumbuhan lain seperti *Oryza sativa* (81%) dan *Helianthus annus* (76%). Amplifikasi cepat hujung cDNA (RACE) dan PCR hujung ke hujung berjaya mengamplifikasikan cDNA untuk protein matang daripada dua spesies kelapa sawit (*Elaeis guinenesis* dan *Elaeis oleifera*) dengan kepanjangan 1,233 kb dan mengkodkan 411 asid amino. Peratus kesamaan jujukan asid amino TC antara *E. guineensis* dengan *E. oleifera* adalah 99.6%. Kedua-dua jujukan

mengandungi tiga turutan panjang residu hidrofobik dan 30 asid amino di bahagian hujung karboksil yang unik pada tumbuhan (tidak dijumpai dalam TC cyanobacteria). Namun, terdapat satu variasi asid amino yang dijumpai dalam jujukan TC bagi kedua-dua spesis kelapa sawit (RPPGL) berbanding dengan 5 asid amino pada hujung karboksil domain (KPPGL) yang dilaporkan terpulihara dalam TC tumbuhan yang lain. Analisis ekspresi gen secara kuantitatif dilaksanakan dengan menggunakan tindak-balas rantai polimerase "masa sebenar" menujukkan ekspresi TC *E. guineensis* adalah sederhana tinggi dalam tisu mesokarpa 7 minggu selepas pendebungan (w.a.a), menurun pada minggu ke-10 w.a.a dan beransur naik sehingga mencapai ekspresi yang maksima pada minggu ke-17 w.a.a dan menurun dengan signifikan pada minggu ke 19 w.a.a. Secara keseluruhan, ekspresi TC dalam tisu daun adalah rendah berbanding dengan tisu mesokarpa; dan daun muda mempunyai ekspresi yang lebih tinggi berbanding dengan daun matang. Analisis salinan gen menggunakan "masa sebenar" menunjukkan hanya terdapat satu salinan TC wujud dalam genom kelapa sawit. Jujukan protein matang yang dipencilkan daripada kelapa sawit boleh digunakan dalam kajian kefungsian untuk memahami proses biosintesis vitamin E dalam kelapa sawit dan tumbuhan lain secara umum. Ia juga boleh digunakan dalam kejuruteraan genetik untuk memanipulasi kandungan dan komposisi vitamin E.

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I certify that a Examination Committee has met on 23th Dec 2010 to conduct the final examination of Lai Mun Seong on his master thesis entitled "cDNA Cloning and Expression Analysis of Tocopherol Cyclase Gene from Various Oil Palm Tissues (*Elaeis guineensis* and *Elaeis oleifera*)" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U(A) 106]. The Committee recommends that the student be awarded the relevant degree.

Members of the Thesis Examination Committee were as follows:

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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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DECLARATION

I declare that the thesis my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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LAI MUN SEONG

Date: 23 December 2010

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