UNIVERSITI PUTRA MALAYSIA

DETERMINATION OF THE GENOMIC STRUCTURE OF HOMOGENTISATE GERANYLGERANYL TRANSFERASE GENE OF OIL PALM

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BY

MOHD FAUZE HAZIS BIN ABDULLAH

A project submitted to Faculty of Agriculture, Universiti Putra Malaysia, in fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agriculture Science

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CERTIFICATION

This project paper entitled **"Determination of The Genomic Structure of Homogentisate Geranylgeranyl Transferase Gene of Oil Palm"** is prepared by Mohd Fauze Hazis Bin Abdullah and submitted to the Faculty of Agriculture in fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agriculture Science.

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TABLE OF CONTENTS

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LIST OF TABLES

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LIST OF FIGURES

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ABBREVIATION

DNA	deoxyribonucleic acid
PE	Phycoerythrin
EB	Elution buffer
QG	QIAGEN
UV	Ultraviolet
TAE	Tris-Acetate -EDTA
PCR	Polymerase Chain Reaction
RNA	Ribonucleic acid
TE	Tris-EDTA
PCI	Phenol chloroform isoamyl
CI	Chloroform isoamyl
CTAB	Cetyltrimethyl ammonium bromide
DOA	Department of Agriculture
HMG-CoA	3-hydroxy-3-methylglutaryl-coenzyme A
HGGT	Homogentisate geranylgeranyl transferase
HPT	Homogentisate phytyltransferase

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PDP	Phytyl diphosphate
g	gram
ml	milliliter
nm	nanometre
bp	base pair
µl	microliter
º C	degree Celsius
γ	gamma
α	alpha
mg/kg	milligram/kilogram
%	percentage

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ABSTRACT

Elaeis guineensis originated from West Africa is the only species that was planted in the Malaysian plantation as a commercial oil palm. Since 1971, Malaysia has been the largest producer and exporter of oil palm. Oil palm is rich in vitamin E that consists of two major molecules which are tocopherols and tocotrienols. Oil palm is the richest source of tocotrienol. Homogentisate geranylgeranyl transferase (HGGT) is the enzyme that initiates tocotrienol synthesis in monocot seeds such as oil palm. The main objective of this study was to compare the genomic structure of HGGT gene of oil palm with other genomic structure of HGGT obtained from the previous study by using polymerase chain reaction (PCR) based method. Young leaves of oil palm were collected from the plantation area at Universiti Putra Malaysia, Serdang, Selangor, Malaysia and the leaves were extracted by using modified cetyltrimethyl ammonium bromide (CTAB). From this study, the genomic structure obtained was not exactly the same with the previous study due to the sequencing errors or the presence of introns in the sequence. From the alignment between genomic and the cDNA sequence, the introns that separate the coding sequence was able to be determined.

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ABSTRAK

Elaeis guineensis yang berasal dari Afrika Barat adalah satu-satunya spesies yang ditanam di ladang Malaysia sebagai kelapa sawit komersial. Sejak 1971, Malaysia telah menjadi pengeluar dan pengimport terbesar kelapa sawit. Kelapa sawit yang kaya dengan vitamin E yang terdiri daripada dua molekul utama iaitu tokoferol dan tokotrienol. Kelapa sawit adalah sumber terkaya tocotrienol. *Homogentisate Geranylgeranyl Transferase* (HGGT) adalah enzim yang memulakan sintesis tocotrienol dalam biji monokot seperti kelapa sawit. Objektif utama kajian ini adalah untuk membandingkan struktur genomik gen HGGT kelapa sawit dengan struktur genomik gen HGGT yang diperoleh dari kajian yang lepas dengan menggunakan kaedah berasaskan tindak balas rantaian polymerase (PCR). Daun muda kelapa sawit dikumpulkan dari ladang di Universiti Putra Malaysia, Serdang, Selangor, Malaysia dan daun ini diekstrak dengan menggunakan penyelesaian CTAB yang diubahsuai. Daripada kajian ini, struktur genomik yang diperoleh adalah tidak sama dengan kajian yang lepas disebabkan oleh kesilapan pengurutan atau kehadiran intron dalam jujukan. Daripada penjajaran antara jujukan genom dan cDNA, intron yang memisahkan urutan pengekodan dapat ditentukan.

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CHAPTER 1

INTRODUCTION

Oil palm is the most productive oil-bearing crop. Although it is planted on only 5% of the total world vegetable oil acreage, palm oil accounts for 33% of vegetable oil and 45% of edible oil worldwide, but increased cultivation competes with dwindling rainforest reserves. The first evidence of palm oil consumption in human diets dates back as far as 3000 BC, with a long history of use in Western Africa (Kiple and Ornelas., 2000). Oil palm trees were introduced to the West (Brazil, West Indies) in the 15th century by the Portuguese and to the East (Indonesia) by the Dutch in the 19th century (Sundram *et al*., 2003).

Commercial cultivation began in the early twentieth century and despite the long breeding cycle (10 to 12years) and large land requirement for field trials (Mayes *et al.,* 1997), high yield breeding materials (up to 12 tonnes per hectare per year (t/ha−1yr−1) (Corley and Tinker*.,* 2003) have been developed in less than 100 years. As such, the largely undomesticated oil palm is an ideal candidate for genomic-based tools including expressed sequence tags (ESTs) (Jouannic*.,* 2005) (Ho*.,* 2007) (Low*.,* 2008) and transcriptome sequencing of the oil palm fruit during development, maturation and ripening (Tranbarger*.,* 2011) to harness the potential of this remarkably productive crop. Furthermore, oil palm is rich with vitamin E that consists of two major molecules which are tocopherols and tocotrienols. In the biosynthesis of vitamin E, both molecules are synthesized through two important converging pathways that fused the side chain building block and head group together (Schnieder., 2005).

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Homogentisate geranylgeranyl transferase (HGGT) is the enzyme that initiates tocotrienol synthesis in monocot seeds such as oil palm. This enzyme is related to homogentisate phytyltransferase (HPT), which catalyzes the prenylation step in tocopherol synthesis. The application of DNA markers could greatly improve precision and efficiency of selection, leading to accelerated development of new yielding planting materials. While in breeding, the use of DNA markers are based on the knowledge of the relation between genotypic and phenotypic variation. Compare to other vegetable, oil palm is the richest source of tocotrienols in Vitamin E and it can enhance the palm oil commercial value in the world market. Therefore, the improvement of Vitamin E of Malaysian commercial oil palm needs to be done through scientific research. The objective of this study is to determine the gene structure of oil palm HGGT gene. The gene structures used from previous study were used as guideline to obtain the best gene structure. Hence, the information generated can assist in the development of molecular markers for Vitamin E improvement.

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