

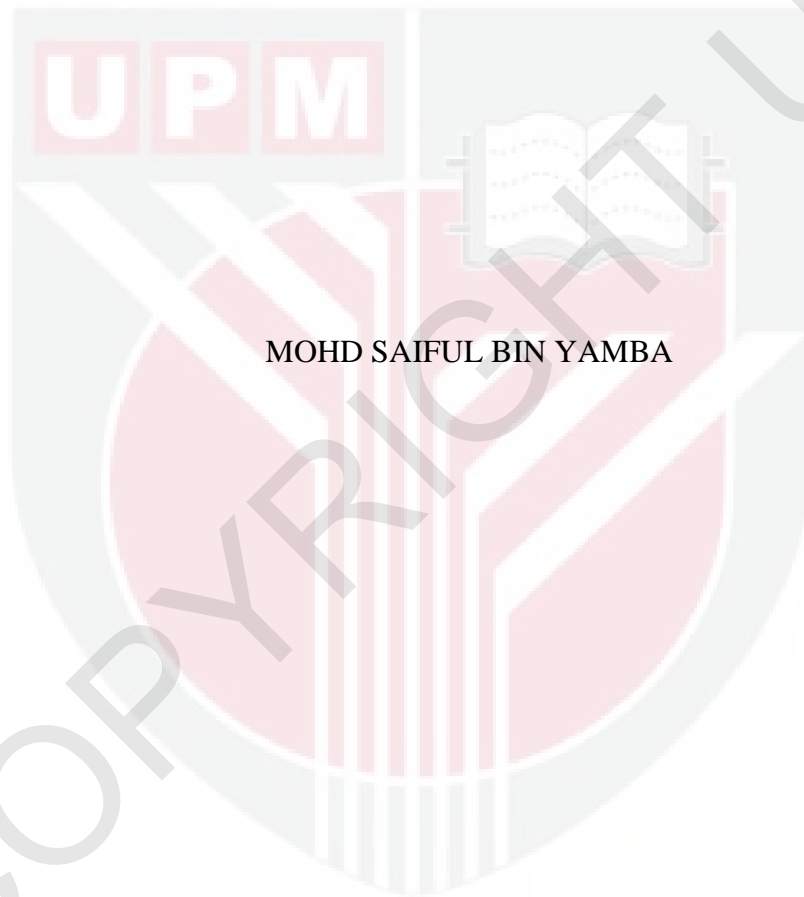


***ISOLATION AND CLONING OF THE PROMOTER SEQUENCE OF VITAMIN
E BIOSYNTHESIS GENE (HOMOGENTISATE PHYTYLTRANSFERASE)
FROM OIL PALM***

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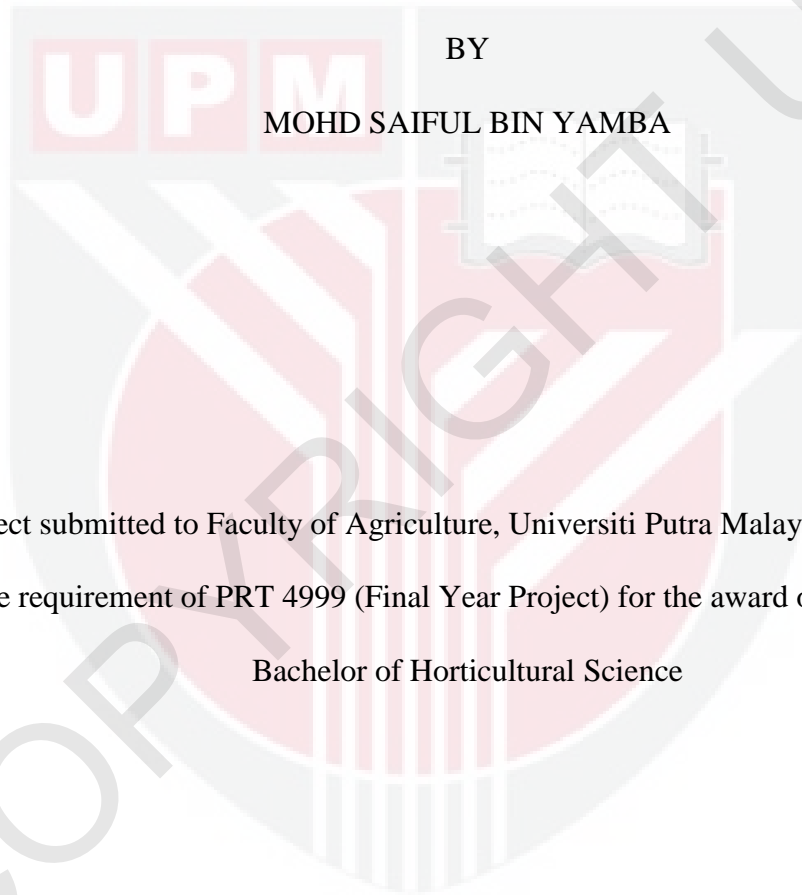
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FACULTY OF AGRICULTURE

UNIVERSITI PUTRA MALAYSIA

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OIL PALM



BY

MOHD SAIFUL BIN YAMBA

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 Horticultural Science

FACULTY OF AGRICULTURE

UNIVERSITI PUTRA MALAYSIA

2015/2016

CERTIFICATION

This project paper entitled “**Isolation and Cloning of The Promoter Sequence of Vitamin E Biosynthesis Gene (Homogentisate Phytlytransferase) from Oil Palm**” is prepared by Mohd Saiful Bin Yamba 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 Horticultural Science.

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ABBREVIATIONS

CI	Chloroform isoamyl
CI	Chloroform isoamylalcohol
CTAB	Cetyltrimethyl Ammonium Bromide
DNA	Deoxyribonucleic acid
FAO	Food and Agriculture Organization of the United Nations
GFP	Gentisate Phytyltransferase
HPT	Homophytyltransferase
PCI	Phenol chloroform isoamyl
PCR	Polymerase Chain Reaction
PE	Phycoerythrin
SDSC	San Diego Supercomputer Centre
TE	Tris-EDTA

ABSTRACT

The oil palm tissue contains vitamin E in the form of tocotrienols and tocopherols. The combination of prenyldiphosphate and homogentisic acid (HGA) is the first committed step in vitamin E production. The reaction is catalyzed by homogentisate phytyltransferase (HPT). The oil palm genomic sequence containing the HPT gene is used to study the expression of HPT gene in oil palm tissues. The objective of this study is to isolate and clone the HPT promoter sequence. To obtain the result from this study, some molecular work was done, including DNA extraction by DNA extraction kit and the cetyltrimethylammonium bromide (CTAB) method, designing the primer for HPT promoter isolation, running the polymerase chain reaction (PCR) to get the HPT promoter sequence and performing agarose gel purification to purify the HPT promoter sequences. After obtaining the amplified HPT promoter sequence, the HPT promoter was introduced into an entry vector and then to destination vector forming the expression clone.

ABSTRAK

Tisu kelapa sawit mengandungi vitamin E dalam bentuk tokotrienol dan tokoferol. Gabungan asid prenildiphosphate dan homogentisic acid (HGA) adalah langkah pertama yang berlaku dalam pengeluaran vitamin E. Tindak balas ini dimangkinakan oleh homogentisate phytyltransferase (HPT). Genom kelapa sawit mengandungi urutan gen HPT. Setiap gen dikawal oleh urutan promoter. Promoter gen HPT akan digunakan untuk mengkaji pengekspresan HPT dalam tisu kelapa sawit. Objektif kajian ini adalah untuk mengasingkan dan mengklonkan urutan promoter HPT. Untuk mendapatkan hasil dari kajian ini, kaedah biologi molekul telah digunakan, termasuk pengestrakan DNA oleh kit pengestrakan DNA atau dengan kaedah cetyltrimethylammonium bromida (CTAB), mereka bentuk pencetus untuk pemencilan HPT promoter, menjalankan tindak balas rantai polymerase (PCR) untuk mendapatkan urutan promoter HPT dan penulinan gel agarose serpihan DNA promoter. Selepas mendapat urutan promoter HPT, promoter HPT yang telah diamplifikasikan dimasukkan ke dalam vektor dan seterusnya dipindahkan ke vektor destinasi untuk membentuk klon pengekspresan.

CHAPTER 1

INTRODUCTION

Oil palm originated from Africa. There are two well-known species of oil palm, *Elaeis guineensis* and *Elaeis oleifera* (Arunachalam, 2012). Oil palm also has different types of variety. The tenera variety from *Elaeis guineensis* is the well known commercial variety. It is now planted in large hectarage in certain countries in South East Asia. Oil palm industries continue to expand in the South East Asian countries, especially in Indonesia and Malaysia. According to Food and Agriculture Organization of the United Nation (FAO) in 2013, Indonesia is the leader in oil palm fruit production in the world, followed by Malaysia. Increasing progress in world oil palm industry causes increases in the production of oil palm-based edible oils.

Vitamin E is synthesized through plant secondary metabolic pathway (Eitenmiller & Lee, 2005). Plant vitamin E consists of tocotrienols and tocopherols (Hofius and Sonnewald, 2003). Vitamin E has specific and various functions in the human body and plant tissues. It was first discovered during research on leafy vegetables (Musa, 2012). Vitamin E can be synthesized naturally in plant and also by chemical synthesis to form artificial vitamin E. The research on vitamin E focuses on extraction from vegetable oils such as from soy bean and oil palm. They are several steps in the biosynthesis pathway to produce vitamin E (Collakova & DellaPenna, 2003b). The pathway contains several biosynthesis enzymes (Collakova & DellaPenna, 2003b).

Plant biotechnology research is advancing aggressively including in the field of plant genetic engineering. Genetic engineering is the way to improve the plant characteristics by modification of the gene of interest and expressing the introduced foreign gene in plant to form transgenic plant (Izadfard, 2009). Genetic engineering can produce hybrid and new cultivar without having to wait for a long time unlike the traditional breeding approach (Izadfard, 2009). Transient gene expression and promoter reporter gene analysis are part of plant genetic engineering.

Promoter sequences of the gene is located in front of gene sequence. Promoter used in this study is homogentisate phytyltransferase (HPT) promoter. HPT is one of the enzyme involved in the biosynthesis of tocopherol. In this study, there are some experimental work including DNA extraction, agarose gel electrophoresis, designing of primer, PCR and gel extraction in order to isolate the promoter sequence.

After the HPT promoter sequence is isolated from oil palm, the promoter will be cloned to form expression clone by a Gateway Cloning strategy. Gateway Cloning basically has two reactions which are BP reaction, where a the promoter sequence and a donor vector is combined and introduced into an entry clone. The second is LR reaction, where the entry clone is mixed with a destination vector to form expression clone.

The objectives of this study are:

1. To isolate HPT promoter sequence from oil palm DNA.
2. To clone HPT promoter using a Gateway Cloning strategy.

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