



UNIVERSITI PUTRA MALAYSIA

***COMPARISON OF THE PROMOTER SEQUENCES OF
HOMOGENISATE GERANYLGERANYL TRANSFERASE (HGGT)
FROM HIGH & LOW VITAMIN E POPULATIONS OF TANZANIAN
OIL PALM (*Elaeisguineensis*)***

MUHAMMAD FARIS FAZDHLI BIN MOHD NOOR

FP 2014 16

COMPARISON OF THE PROMOTER SEQUENCES OF HOMOGENITISATE
GERANYLGERANYL TRANSFERASE (HGGT) FROM HIGH & LOW VITAMIN E
POPULATIONS OF TANZANIAN OIL PALM (*Elaeisguineensis*)

UPM BY
MUHAMMAD FARIS FAZDHLI BIN MOHD NOOR

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

Faculty of Agriculture
Universiti Putra Malaysia

2013/2014

CERTIFICATION

This project report entitled Comparison of The Promoter Sequences of HomogentisateGeranylgeranylTransferase (HGGT) from High & Low Vitamin E Populations of Tanzanian Oil Palm, (*Elaeisguineensis*)is prepared by Muhammad FarisFazdhli Bin Mohd Noor and submitted to the Faculty of Agriculture in fulfilment of the requirement of PRT4999 (Final Year Project) for the award of the degree of Bachelor of Agriculture Science.

Student's name:

Muhammad FarisFazdhli Bin Mohd Noor

Student's signature:

.....

Certified by:

.....
(Prof. Datin Dr. Siti Nor AkmarBinti Abdullah)

Project Supervisor,

Department of Agriculture Technology,

Faculty of Agriculture,

Universiti Putra Malaysia,

Serdang Selangor

Date:

2013

ACKNOWLEDGEMENT

Alhamdulillah praises to Allah S.W.T for giving me strength, passion, ability and capability in finishing my final year project paper entitled Comparison of The Promoter Sequences of HomogentisateGeranylgeranylTransferase (HGGT) from High & Low Vitamin E Populations of Tanzanian Oil Palm(*Elaeisguineensis*)

First of all, I would like to convey my deepest appreciation and sincere gratitude to my supervisor, Prof. Datin Dr. SitiNorAkmarBinti Abdullah for her guidance and patience in enabling me to complete my study towards supporting the biomolecular technology method for oil palm improvement.

Special appreciation goes to Madam Mas Munirah who had always assisted and guided me in my laboratory works. I also would like to thank all my friends for their help and support during the preparation of this project paper.

Last but not least, special thanks to my beloved parents; Mohd Noor Bin HjMuhamad and RubiahBinti Abdullah and also to my siblings for their endless love, prayers and encouragement. To those who indirectly contributed to this research, your kindness is meaningful to me. Thank you very much for your support.

CONTENT

	PAGES
ACKNOWLEDGEMENT	i
CONTENT	ii
LIST OF TABLES	v
LIST OF FIGURES	vi
LIST OF PLATES	vii
LIST OF APPENDICES	viii
ABSTRACT	ix
ABSTRAK	x
CHAPTER	1
1. INTRODUCTION	1
2. LITERATURE REVIEW	
2.1 Vitamin E	
2.1.1 Introduction to vitamin E	4
2.1.2 Tocotrienols	7

2.2	Oil palm	
2.2.1	Origin of oil palm	8
2.3	Benefits of vitamin E	9
2.4	Vitamin E Biosynthesis Pathway	11
2.5	Molecular Marker	14
2.6	Single Nucleotide Polymorphisms (SNPs)	14
3.	MATERIALS AND METHODS	
3.1	Polymerase Chain Reaction (PCR)	15
3.2	Electrophoresis of PCR product	16
3.3	Gel extraction	17
3.4	Ligation Using 2X Rapid Ligation Buffer	18
3.5	Transformation	20
3.6	White Colony	20
3.7	Stock for Plasmid Purification	21
3.8	Plasmid Purification	21
3.9	DNA sequencing	22

4.	RESULTS AND DISCUSSION	
4.1	Concentration and purity of the DNA samples	23
4.2	Amplified PCR Product	25
4.3	Multiple Sequence Alignment of the HGGT Promoter	27
	Sequence of Low and High Vitamin E Tanzanian Palms and Commercial <i>Elaeisguineensis</i>	
4.4	Comparison of HGGT promoter sequences between Low vitamin E and commercial palms	29
5.	CONCLUSION	31
6.	REFERENCES	32
7.	APPENDICES	35

LIST OF TABLES

Table	Pages
1. Table 1 DNA concentration, DNA purity and vitamin E content of the twodifferentTanzanianoil palmsamples	25



LIST OF FIGURES

Figure	Pages
1. Figure 1 The structure of tocopherols and tocotrienols which differs at its carbon phytyl tail.	6
2. Figure 2 The source of tocotrienols and tocopherols in crop which are divided into their four isomers.	7
3. Figure 3 Vitamin E biosynthesis pathway	14

LIST OF PLATES

Plates	Pages
1. Plate 1 DNA gel electrophoresis image of the PCR products of the Tanzanian oil palms. Lanes 1, 2, represent oil palm number 0.256/819 (high vitamin E), 0.256/193 (low vitamin E).	27
2. Plate 2 comparison between commercial oil palm HGGT promoter DNA sequence, high and low vitamin E Tanzanian palm HGGT promoter DNA sequence.	29
3. Plate 3 comparison between commercial oil palm HGGT promoter DNA sequence and low vitamin E Tanzania palm HGGT promoter DNA sequence.	31

LIST OF APPENDICES

Appendix		Page
1. APPENDIX 1	HGGT Promoter Sequence of Commercial Palm	36
2. APPENDIX 2	HGGT Promoter Sequence of High Vitamin E37 Tanzanian Palm	
APPENDIX 3	HGGT Promoter Sequence of Low Vitamin E Tanzanian Palm	39
APPENDIX 4	Comparison between HGGT Promoter DNA Sequence of High and Low Vitamin E Tanzanian Palms.	41

ABSTRACT

Oil palm is widely used in various food applications. Palm oil is rich in vitamin E essential for human consumption. Variability in the level of vitamin E exists in the oil palm germplasm materials. Tocotrienols and tocopherols are two important forms of vitamin E. In fact, tocotrienols content in *Elaeis guineensis* are higher compared to tocopherols. Tocotrienol synthesis requires homogentisylgeranyltransferase (HGGT), which catalyzes the condensation of homogentisate and the unsaturated C₂₀ isoprenoidgeranyldiphosphate (GGDP). Tocopherol differs from tocotrienol because there are three double bonds in the side chain of the tocotrienol. The aim of the study is to detect sequence variation in the form of single nucleotide polymorphism (SNP) by comparing individuals from the Tanzania population that exhibited low and high vitamin E contents. The project involves analyzing the promoter sequence and detecting for variation that falls within important promoter motifs. The genomic structures of the HGGT gene in the germplasm materials were determined. Variation in the gene structure may be exploited for developing PCR-based marker in marker assisted selection to improve vitamin E content of planting materials.

ABSTRAK

Minyak kelapa sawit digunakan secara meluas dalam pelbagai aplikasi makanan. Minyak kelapa sawit kaya dengan vitamin E yang berguna untuk keperluan manusia. Kepekannya tahap vitamin E wujud di dalam material germplasm kelapa sawit. Tokoferol dan tokotrienol adalah dua bentuk penting dalam vitamin E di mana kandungan tokotrienol adalah lebih tinggi berbanding dengan tocopherols dalam *Elaeis guineensis*. Sintesis tokotrienol memerlukan homogenisasi geranylgeranyl transferase (HGGT), yang dimangkin oleh pemeluwapan homogenisasi dan C20 difosfat geranylgeranyl isoprenoid tak tepu (GGDP). Tocopherols berlainan dengan tokotrienol kerana terdapat tiga ikatan kembar dalam rantaian samping tokotrienol. Tujuan kajian ini adalah untuk mengesan variasi jujukan dalam bentuk Polimorfisme nukleotid tunggal (SNP) dengan membandingkan individu dari populasi Tanzania yang mempamerkan kandungan vitamin E yang rendah dan tinggi. Projek ini melibatkan menganalisis urutan promoter dan mengesan variasi yang terdapat dalam motif promoter penting. Struktur genomik gen HGGT dalam material germplasm ditentukan. Perbezaan dalam struktur gen ini boleh dieksploitasi untuk membangunkan penanda berasaskan PCR bagi pemilihan penanda berbantu untuk memperbaiki kandungan vitamin E dalam bahan tanaman.

CHAPTER 1

INTRODUCTION

Elaeis guineensis is a tropical forest palm native to the West and Central Africa. It has been introduced and cultivated in the humid tropics which cover from 16°N to 16°S. Another important species of oil palm is *Elaeis oleifera* (American Oil Palm) which is less planted as plantation crop compared with *E. guineensis*. The main product that can be derived from oil palm is palm oil. It can produce 3-8 times more oil in a given area than other tropical or temperate oil crops. Oil can be extracted from both the fruit and the seed, crude palm oil (CPO) from the outer mesocarp and palm-kernel oil (PKO) from the endosperm. Crude palm oil has important role in food industry especially as cooking oil and food production. Meanwhile, palm-kernal oil is used in various non-edible products, such as detergents, cosmetics, plastics, surfactants, herbicides, as well as a broad range of other industrial and agricultural chemicals.

Palm oil contains a high amount carotenoids and vitamin E was discovered mainly due to the major interest in its ability to act as antioxidant and potential to prevent atherosclerosis and cancer (Schneider,2005). Vitamin E consists of 2 major molecules which are tocopherols and tocotrienols. Tocopherol (vitamin E) is a lipid-soluble antioxidant synthesized only by photosynthetic organism (Shintani and DellaPenna,1998). Vitamin E is a natural biological

antioxidant, also known to have anti-inflammatory activity, which has been extensively used to improve biostability and biocompatibility of different biomaterials (Rizziet *al.*,2012). Compared to other vegetable oil, palm oil is the richest source of tocotrienols where the concentration in crude palm oil (CPO) is at 600-1000ppm (Terzaghi and Cashmore., 1995). This shows that palm oil has the high potential to be a good resource of vitamin E and at the same time it can increase the market value of palm oil. Vitamin E also plays a role in inhibition of platelet aggregation and neurological functions (Atkinson *et al.*,2008). Then, the productivity of oil palm which has high vitamin E content needs to be increased for market demand. So, scientific research is one step to improve the content of vitamin E in the commercial oil palm in Malaysia.

There are more than 26 populations of *Elaeis guineensis* with different content of vitamin E and each of the population shows high variability in vitamin E content (Terzaghi and Cashmore., 1995). One of the *Elaeis guineensis* population that has a high diversity in its vitamin E content is the Tanzanian population. So, through discovering the variation of homogentisate geranylgeranyl transferase (HGGT) promoter sequence of *Elaeis guineensis* of the Tanzania population may help to identify the single nucleotide polymorphism (SNPs) which control the production of tocotrienols in oil palm. The first committed step for tocotrienol biosynthesis is catalyzed by HGGT hence it is a key regulatory enzyme. The gene structure of HGGT was analysed as this may help in developing molecular tools for marker assisted selection in breeding programmes.

The objective of the project were

1. To detect variation of the promoter sequence of HGGT in palms with high and low vitamin E content from Tanzanian population
2. To select for SNPs effecting important promoter motifs.



REFERENCES

Atkinson, R.F and Epand, R.M (2008). Tocopherols and tocotrienols in membranes: a critical review. *Free radical biology & medicine* 44 (5): 739–64.

Bagali, P.G., Prabhu, P.D., Raghavendra, K., Bagali, P.G., and Vadivelu, J.S. (2010) Application of Molecular Markers in Plant Tissue Culture. Proceedings Asia Pacific Conference on Plant Tissue and Agribiotechnology (APaCPA)27 (3) : 85-87

Buring J.E and Hennekens C.H (1997) Antioxidant vitamins and cardiovascular disease. *Nutr Rev.* 55, 53-60.

Carotech.Inc, November 23, (2007). Source of Tocotrienols <http://www.tocotrienol.org>. 23 November 2012.

Discover Many Function of Vitamin E. May (2009) Statement on Vitamin E. http://www.naturalnews.com/032323_vitamin_E_antioxidants.html. 4 April 2012.

DellaPenna, D.(2005). A Decade of Progress in Understanding Vitamin E Synthesis in Plant. *Plant physiology.* 162.: 729-737.

Gasser S.M., Amati B.B., Cardenas M.E., and Hofmann J.F.X (1989) Studies on scaffold attachment sites and their relation to genome function. *Intnatl Rev Cyto* 119:57-96

Kushairi, A. (1992) Prestasi baka kelapa sawit *dura x pisifera* di Malaysia.M.Sc. Thesis submitted to Universiti Kebangsaan Malaysia, Bangi, Selangor, Malaysia.

Li Y., Zhou Y., Wang Z., Sun X.F., and Tang K.X. (2010). Engineering Tocopherol Biosynthesis Pathway in *Arabidopsis* Leaves and its Effect on Antioxidant Metabolism. *Plant Science*. 178 (1) : 312-320

Neugebauer, K.M. (2002). On the Importance of Being Cotranscriptional. *J. Cell Sci*. 115 : 3865-3871.

Prestridge, D.S. (1991) SIGNAL SCAN: A computer program that scans DNA sequences for eukaryotic transcriptional elements. *CABIOS* 7, 203-206.

Pursglove, J.W. (1972). Tropical crop monocotyledons. John Wiley & Sons, New York.

Rizzi, M., Reno.F and Cannas, M. (2012) Vitamin E (α -tocopherol) addition modifies P(d.l)LA sponge degradation and protein release. *J.Biomater.Appl.*,27(2) 165-170

RocheFord, T.R., Wong, J.C, Egesel, C.O., and Lambert, R.J. (2002). Enhancement of Vitamin E Levels in Corn. *Journal of the American College of Nutrition*. 21(3) : 191-198

Sanders S.K., Morgan J.B., Wulf D.M., Tatum J.D., Williams S.N. and Smith G.C. (1997) Vitamin E supplementation of cattle and shelf-life of beef for the Japanese market. *Journal of animal science*75 : 2634-2640

Schneider, C. (2005). Chemistry and biology of Vitamin E. *Molecular Nutrition of and Food Research* 49:7-30

Semagn, K., Bjornstad. A., and Ndjioudjop, M. N. (2006). An overview of molecular marker method for plants. *African Journal of Biotechnology* 5(25) : 2540-2568.

Shintani, D.K and DellaPenna, D (1998). Elevating the vitamin E content of plant through metabolic engineering. *Science* 282:2098-2100

Soll, J and Schultz, G. (1979). Comparison of Geranylgeranyl and Phytyl Substituted Methylquinols in the Tocopherol Synthesis of Spinach Chloroplast. *Biochemistry Biophyslogy Res Community*. 91. 715-720

Southworth, A. (1985). Palm Oil and Palm Kernels. *Journal of the American Oil Chemists' Society*. 62: 250-254.

Terzaghi W.B and. Cashmore AR (1995) Light-regulated transcription. *Annu Rev Plant Physiol Plant Mol Biol* 46:445-474