



**UNIVERSITI PUTRA MALAYSIA**

***CHARACTERISATION AND LOCALISATION OF FERTILIZATION  
INDEPENDENT ENDOSPERM AND ENHANCER OF ZESTE-LIKE  
TRANSCRIPTS FROM OIL PALM (ELAEIS GUINEENSIS JACQ.)***

**VASAGI RAMACHANDRAN**

**FBSB 2014 14**



**CHARACTERISATION AND LOCALISATION OF *FERTILIZATION INDEPENDENT ENDOSPERM* AND *ENHANCER OF ZESTE-LIKE* TRANSCRIPTS FROM OIL PALM (*ELAEIS GUINEENSIS* JACQ.)**

By

**VASAGI RAMACHANDRAN**

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

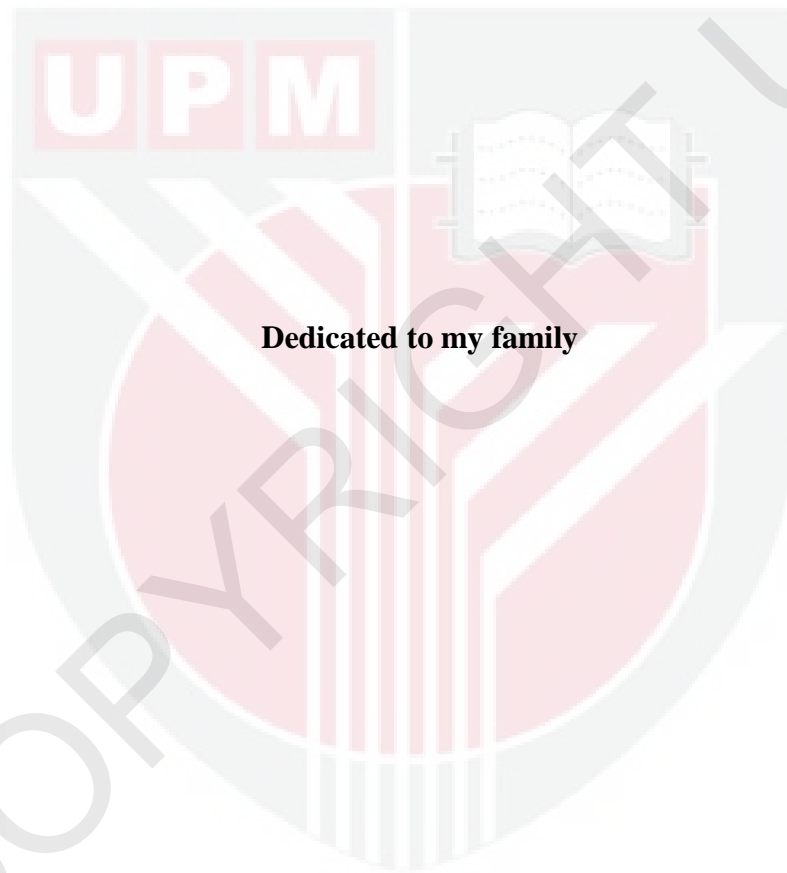
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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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By

**VASAGI RAMACHANDRAN**

**April 2014**

**Chairman : Parameswari Namasivayam, PhD**

**Faculty : Biotechnology and Biomolecular Sciences**

Various studies have proved the epigenetic changes in plants have important phenotypic consequences and significant implication in plant breeding programme. Polycomb group genes are one of the key players for epigenetic changes in plants. However, there are no reports on polycomb group genes of oil palm which plays important role in epigenetics. As such, this has prompted the studies on characterisation and localisation of polycomb group genes, EgFIE (KF430194) and EgEZ1 (KF430195) in oil palm owing to its important role in sexual reproduction. The full length transcripts of EgFIE and EgEZ1 were isolated and their expression were analysed and further localised in tissues via real time PCR and *in situ* hybridisation. The methylation levels in CpG island of each genes were detected by using MSRE-PCR and Southern analysis. The oil palm full length EgFIE cDNA is 1527 bp in length and the ORF is encoding for a protein of 370 amino acids. While, the full length of EgEZ1 transcript is 3200 bp in length with ORF region of 2,757 bp. The expression profile of EgFIE transcript showed that male flower expressed the highest and EgEZ1 transcripts were detected highest in female flower compared to other vegetative and sexual reproductive tissues tested. While for *in vitro* tissue culture materials, cell suspension culture showed the highest expression of EgFIE and EgEZ1 transcripts among the tissues tested. In the case of different developmental stages of fruits, both clonal Tenera and non-clonal Tenera fruits at 5 WAA showed the highest expression among the tissues tested for both transcripts. The *in situ* hybridisation analysis showed that the expression of EgFIE and EgEZ1 transcripts were localised in ovary, stigma and stylar canal region of early development stage of fruit and inflorescences. Based on the expression and localisation studies, EgFIE and EgEZ1 genes play important roles in the oil palm inflorescences and fruits development. Both of these genes might have broad functions in oil palm tree development as their expression was detected in a wide range of tissues. The expression of EgFIE and EgEZ1 genes in *in vitro* tissue culture materials suggest the role of polycomb group complexes as key regulators of

dedifferentiation and differentiation pathways in cell culture. On the other hand, methylation analysis showed the HpaII site of EgFIE CpG island region is methylated in clonal 1 DBA and 1 DAA fruits. However, methylation analysis of EgEZ1 CpG island was a failure. In future, more analyses needed on inflorescences, early stages of developing fruit and cell suspension culture to elucidate their roles. Besides that, more advance tools such as bisulphite sequencing and methylated DNA immunoprecipitation can be employed for methylation analysis.



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**PENCIRIAN DAN PENEMPATAN TRANSKRIP *FERTILIZATION INDEPENDENT ENDOSPERM* DAN *ENHANCER OF ZESTE-LIKE* DARIPADA POKOK KELAPA SAWIT (*ELAEIS GUINEENSIS* JACQ.)**

Oleh

**VASAGI RAMACHANDRAN**

**April 2014**

**Pengerusi : Parameswari Namasivayam, PhD**

**Fakulti : Bioteknologi dan Sains Biomolekul**

Pelbagai kajian telah membuktikan perubahan epigenetik yang berlaku dalam tumbuh-tumbuhan mempunyai kesan fenotip penting dan implikasi yang besar dalam program pembiakan tumbuhan. Gen kumpulan Polycomb adalah salah satu pemain utama bagi perubahan epigenetik dalam tumbuhan. Walau bagaimanapun, tiada laporan dari tisu kelapa sawit mengenai gen kumpulan polycomb yang memainkan peranan penting dalam epigenetik. Oleh itu, ini telah mendorong kajian pencirian dan penempatan gen kumpulan polycomb, EgFIE (KF430194) dan EgEZ1 (KF430195) dari pokok kelapa sawit yang memainkan peranan penting dalam pembiakan seksual. Jujukan lengkap transkrip EgFIE dan EgEZ1 telah dipencil. Pengekspresan jujukan EgFIP dan EgEZ1 dianalisis di dalam tisu melalui tindak-balas berantai polymerase kuantitatif masa sebenar dan hibridisasi secara *in situ*. Tahap metilasi dalam CpG pulau bagi setiap gen dikesan dengan menggunakan MSRE-PCR dan analisis Selatan. Jujukan penuh cDNA EgFIE dari pokok kelapa sawit adalah 1527 bp dan jujukan ORF mengkodkan untuk protein yang terdiri daripada 370 asid amino. Manakala, jujukan penuh transkrip EgEZ1 adalah 3200 bp dengan kawasan ORF terdiri daripada 2,752 bp. Profil pengekspresan bagi transkrip EgFIE menunjukkan pengekspresan yang tertinggi dalam bunga jantan dan transkrip EgEZ1 adalah tinggi dalam bunga betina berbanding dengan tisu vegetatif dan tisu pembiakan seksual yang lain. Manakala untuk kultur tisu *in vitro*, antara tisu yang diuji, kultur ampai sel menunjukkan pengekspresan transkrip EgFIE dan EgEZ1 yang tertinggi. Selain daripada itu, bagi pengekspresan dalam tahap perkembangan biji kelapa sawit yang berbeza, biji buah klon Tenera dan bukan klon Tenera tahap 5 WAA telah menunjukkan pengekspresan yang tertinggi diantara tisu dikaji untuk kedua-dua transkrip. Analisis hibridisasi secara *in situ* menunjukkan pengekspresan transkrip EgFIE dan EgEZ1 telah dijumpai didalam ovari, stigma dan terusan stilar pada peringkat awal perkembangan biji dan bunga kelapa sawit. Berdasarkan kajian ekspresan, EgFIE dan EgEZ1 gen memainkan peranan penting dalam pembungaan dan perkembangan biji kelapa sawit. Kedua-dua gen ini mungkin mempunyai fungsi yang luas dalam perkembangan pokok sawit kerana ekspresasi mereka dikesan

dalam pelbagai tisu. Pengekspresan gen EgFIE dan EgEZ1 di dalam kultur tisu *in vitro* mencadangkan peranan kompleks kumpulan polycomb sebagai pengawal atur bagi tapak jalan perkembangan dan perbezaan dalam kultur sel. Sebaliknya, analisis metilasi menunjukkan tapak HpaII di rantau pulau EgFIE CpG adalah bermetil dalam klon buah 1 DBA dan 1 DAA. Walau bagaimanapun, analisis metilasi pulau EgEZ1 CpG gagal. Dicadangkan analisis yang lebih perlu dijalankan pada pembungaan, peringkat awal perkembangan biji sawit dan kultur ampai sel untuk menjelaskan peranan mereka. Selain itu, teknik yang lebih maju seperti penjujukan bisulphite dan pemendakan immuno DNA bermetil boleh dilaksanakan untuk analisis metilasi.





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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirements for the degree of Master of Science. The members of the Supervisory Committee were as follows:

**Parameswari A/P Namasivayam, PhD**

Associate Professor

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

(Chairman)

**Ho Chai Ling, PhD**

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

(Member)

**Meilina Ong Abdullah, PhD**

Principal Research Officer

Advanced Biotechnology & Breeding Centre

Malaysian Palm Oil Board

(Member)

**Sharifah Shahrul Rabiah Syed Alwee, PhD**

FELDA Biotechnology Centre

FELDA Agriculture Service Sdn Bhd

(Member)

---

**BUJANG BIN KIM HUAT, PhD**

Professor and Dean

School of Graduate Studies

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committee: \_\_\_\_\_

Signature: \_\_\_\_\_  
Name of  
Member of  
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## LIST OF ABBREVIATIONS

%	Percentage
µg	microgram
µl	microliter
$\frac{g}{0^{\circ}C}$	gravitational acceleration degree Centigrade
1 DAA	A day after anthesis
1 DBA	A day before anthesis
bp	base pair
BSA	Bovine Serum Albumin
BLAST	Basic Local Alignment Search Tool
CAP	Contig assembly program
cDNA	complementary DNA
$C_t$	threshold cycle
dATP	2'-deoxy-adenosine-5'-triphosphate
DEPC	diethyl pyrocarbonate
DIG	digoxygen
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
dNTP <sub>s</sub>	deoxynucleotides triphosphates
E value	expected value
EDTA	ethylene diaminetetra acetic acid disodium salt
g	gram
Jacq.	Jacquin
kb	kilo base pair
LB	luria bertani
M	Molar
MgCl <sub>2</sub>	magnesium chloride
mM	milimolar
MPOB	Malaysian Palm Oil Board
mRNA	messenger ribonucleic acid
NaCl	sodium chloride
NaOAc	sodium acetate
ng	Nanogram
PcG	Polycomb group
PCR	polymerase chain reaction
qPCR	quantitative real time reverse transcriptase PCR
RNA	ribonucleic acid
RNase	ribonuclease
RT	room temperature ( $\approx 28^{\circ}C$ )
SDS	sodium dodecyl sulphate
SSC	sodium chloride sodium citrate buffer
TE	tris-EDTA
U	unit
v/v	volume per volume
WAA	week after anthesis
w/v	weight per volume

## CHAPTER 1

### INTRODUCTION

*Elaeis guineensis* Jacq. which is commonly known as the oil palm, is the most important species in the genus *Elaeis* which belongs to the family Palmae. Malaysian palm oil industry has grown tremendously and has maintained its position as one of the world's leading producers country of palm oil (Nasrin *et al.*, 2008). According to Corley (2009), the future demand for vegetable oil will be approximately 240 million tonnes to satisfy the consumption of around 9.2 billion world's population in the year 2050. Malaysia as a palm oil producer and exporter, undoubtedly, will continue to play a major role in meeting the world's oil and fats future needs and demand.

In order to improve the productivity of oil palm, many research studies have been conducted by oil palm industries around the world. The research focus on high yielding and dwarf palms, production of unsaturated oil and high kernel content. Many approaches such as propagation of elite oil palms through tissue culture, recombinant technology, DNA markers and selection breeding have been carried out to improve the production of oil palm. However, at the present oil palm planting materials, DxP seeds, are heterozygous (Rajanaidu and Jalani, 1996). This cause difficulties in making selection on the parents in breeding cycles due to uncontrollable genotype and phenotype variations. As a problem solver, micropropagation of oil palm via *in vitro* tissue culture offers an attractive approach to produce genetically uniform planting materials with desired characteristics through cloning of elite and true to type palms (Paranjothy and Othman, 1982). However, clonal propagation of oil palm is still problematic due to production of somaclonal variation, clonal mantled fruits, floral and vegetative abnormalities of clonal palms (Kushairi *et al.*, 2010). The loss of phenotypic fidelity is now a major obstacle to the development of large scale propagation of clonal oil palm.

Thus, the study of mechanisms that control the gene expression in sexual reproduction will facilitate the understanding of genotype and phenotype variation. Besides that, the finding of mechanisms that responsible for the somaclonal variation and phenotypic variation in tissue culture materials will help us to solve this major problem in the oil palm tissue culture industry.

Many molecular mechanisms contribute to the phenotypic variation and one of it will be epigenetics. Epigenetics is the study of heritable changes in gene expression that occur without a change in DNA sequence. The epigenetic variation commonly regulated by changes in DNA methylation, histone modification or chromatin structure (Makarevitch *et al.*, 2007). The modulation of transcription by chromatin modification involves in the coordination of gene networks regulating development. The chromatin marks deposited by polycomb group (PcG) complexes induces a repressive state of the transcription (Holec and Berger, 2012). There are two PcG complexes which have been studied in animals and plants, polycomb repressive complex 1 (PRC1) and polycomb repressive complex 2 (PRC2). In plants, the PRC2 is composed of E(Z) homologous, FIE, SU(Z)12 homologues and p55 homologues which have been shown to have role in numeral developmental process such as seed

development, flowering, root patterning and regulation of shoot meristem development (Butenko and Ohad, 2011; Guitton and Berger, 2005).

However, the relative important of epigenetic variation especially polycomb group complexes protein in oil palm is not well understood. Besides that, minimal effort has been taken to understand the mechanism of epigenetics regulation in contribution to the genotypic and phenotypic variation in oil palm. As such, this study has been carried out with fundamental investigation on the selected polycomb group complexes with the following objectives:

- I. To isolate the EgFIE and EgEZ1 transcripts
- II. To analyse the expression pattern of the selected transcripts during gametogenesis, sexual reproduction and *in vitro* tissue culture process of oil palm and localisation in developing fruits and inflorescences
- III. To study the methylation pattern of EgFIE and EgEZ1 in developing fruits and inflorescences of oil palm.

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