UNIVERSITI PUTRA MALAYSIA

EFFECTS OF OVER-EXPRESSING ETHYLENE RESPONSIVE TRANSCRIPTION FACTOR ON EXPRESSION OF SELECTED FRUIT RIPENING RELATED GENES IN OIL PALM (Elaeis guineensis Jacq.) MESOCARP

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FP 2013 77

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By

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

April 2013

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

EFFECTS OF OVER-EXPRESSING ETHYLENE RESPONSIVE TRANSCRIPTION FACTOR ON EXPRESSION OF FRUIT RIPENING GENES IN OIL PALM *(Elaeis guineensis* Jacq.*)* MESOCARP

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April 2013

Chairperson: Siti Nor Akmar Abdullah, Ph.D
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Fruit development, maturation and ripening are important biological processes unique to plants. During the ripening phase, several physiological and biochemical changes take place through differential expression of various genes that are developmentally regulated. Various approaches have been used to understand the complexity of gene expression and interaction in plants. One of the most important mechanisms of gene expression regulation occurs at the transcriptional level through binding of transcription factors to specific DNA sequences.

From a recent study, a transcription factor belonging to the AP2 family members that is highly expressed in mesocarp at the late ripening stage of oil palm fruit designated as *EgDREB* was isolated and sequenced. The present study aimed to identify genes whose expression is regulated by this transcription factor in the mesocarp tissue of oil palm fruits.

Semi-quantitative analysis of *EgDREB* expression by reverse transcriptase (RT)-PCR showed that it is expressed at different stages of mesocarp development but not at the early ripening stage (12 WAA). *EgDREB* is also expressed in vegetative tissues including roots and leaves. Thus, 12 WAA mesocarp was selected to study the effects of over-expression of *EgDREB* in mesocarp tissues on five groups of ripening related cDNAs from oil palm that have been previously identified.

Co-bombardment of 12 WAA mesocarp tissue with recombinant vector construct harboring *EgDREB* and plasmid containing the GFP reporter gene was carried out. Fluorescent detection of GFP microscopically and verification via RT-PCR using GFP-specific primers enabled selection of successfully transformed tissues.

Using transient expression assay, it was demonstrated that over-expression of the *EgDREB* gene in 12 WAA mesocarp tissues results in up-regulation of fruit ripening related genes which are *TCTP*, *MET2a* and *MET2b*. These proteins are categorized under biogenesis of cellular component and protein with binding function or cofactor requirement. More specifically the roles of metallothioneins are in homeostasis of essential metal ions and oxidative stress response. This may suggest that *EgDREB* is involved in regulating cellular processes related to the roles of these three proteins.

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

KESAN PENGEKSPRESAN MELAMPAU TRANSKRIPSI KESAN TINDAKBALAS ETILENA TERHADAP PENGEKSPRESAN GEN TERPILIH BERKAITAN PEMASAKAN BUAH DI DALAM MESOKARPA KELAPA SAWIT (*Elaeis guineensis* Jacq.)

Oleh

NUR ANNIES BINTI ABD HADI

April 2013

Pengerusi: Siti Nor Akmar binti Abdullah, PhD
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Perkembangan, kematangan dan pemasakan buah adalah proses biologi yang penting dan unik kepada tumbuh-tumbuhan. Semasa fasa pemasakan, beberapa perubahan fisiologi dan biokimia mengambil tempat melalui pengekspresan pelbagai gen yang berbeza. Pelbagai pendekatan telah digunakan untuk memahami kerumitan pengekspresan dan interaksi gen dalam tumbuh-tumbuhan. Salah satu mekanisme yang paling penting ialah pengaturan gen yang berlaku di peringkat transkripsi melalui pengikatan faktor transkripsi kepada jujukan DNA tertentu.

Dari kajian baru-baru ini, faktor transkripsi tergolong dalam famili AP2 yang menunjukkan pengekspresan yang tinggi di dalam mesokarpa pada peringkat pemasakan buah kelapa sawit yang dinamakan sebagai *EgDREB* telah dipencilkankan dan dijujukkan. Kajian ini bertujuan untuk mengenal pasti gen yang dikawal oleh faktor transkripsi ini dalam tisu mesokarpa buah kelapa sawit.

Analisis semi kuantitatif pengekspresan *EgDREB* oleh transcriptase berbalik (RT)-PCR menunjukkan bahawa ia diekspres di pelbagai peringkat perkembangan mesokarpa tetapi tidak pada peringkat kematangan awal (12 WAA). *EgDREB* juga di ekspreskan di dalam tisu vegetatif termasuk akar dan daun. Oleh itu, 12 WAA mesokarpa telah dipilih untuk mengkaji kesan lebihan ekspresan daripada *EgDREB* dalam tisu mesokarpa pada lima kumpulan cDNAs yang berkaitan dengan pemasakan buah dari kelapa sawit yang telah dikenal pasti sebelum ini.

Ko-pembedilan 12 WAA mesokarpa tisu dengan vektor rekombinan yang mengandungi *EgDREB* dan gen GFP telah dijalankan. Pengesanan pendarfluor GFP mikroskopik dan pengesahan melalui RT-PCR menggunakan primer GFP khusus membolehkan pemilihan tisu yang berjaya ditransformasikan.

Menggunakan asai pengekspresan transien, ia telah menunjukkan bahawa lebihan pengekspresan gen *EgDREB* dalam 12 WAA mesokarpa tisu menunjukkan keputusan lebihan pengekspresan dalam gen berkaitan dengan pemasakan buah seperti *TCTP*, *MET2a* dan *MET2b*. Protein ini dikategorikan di bawah biogenesis komponen selular dan protein dengan fungsi mengikat atau keperluan kofaktor. Lebih khusus peranan metallothioneins adalah dalam homeostasis ion logam penting dan tindak balas tekanan oksidatif. Ini mungkin menunjukkan bahawa EgDREB terlibat dalam mengawal selia proses selular yang berkaitan dengan peranan ketiga-tiga protein.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude and appreciation to my supervisor Professor Datin Dr. Siti Nor Akmar Abdullah for his enthusiasm towards my work and for giving fruitful suggestions and ideas. To my co-supervisors Associate Professor Dr. Halimi Mohd Saud for his valuable advice, technical guidance, encouragement and thesis improvement.

I would like to dedicate to my family, especially to my parents, Abd Hadi Ibrahim and Mazinah Sulaiman. I also would like to deeply appreciate my husband, Wan Fahmi Wan Mohamad Nazarie for his unconditional sacrifice and love. I wish to acknowledge the guidance and support from my colleagues, especially Vahid Omidvar, Ahmed Al-Shanfari, Mortaza, Farzaneh, Azzreena, Mas and Umaiyal for supplying ideas and guidance for this project.

I wish to extend my appreciation to everyone, although not individually named here, who had contributed directly or indirectly to my project and thesis. Without all of you, it would not be possible for me to complete my project and thesis. Thank you all for your support.

I certify that a Thesis Examination Committee has met on 1st April 2013 to conduct the final examination of Nur Annies Binti Abd Hadi on her thesis entitled "Effects of Over-Expressing Ethylene Responsive Transcription Factor on Expression of Selected Fruit Ripening Related Genes in Oil Palm *(Elaeis guineensis* Jacq.*)* Mesocarp" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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

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DECLARATION

Declaration by graduate student:

I hereby confirm that:

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This is to confirm that:

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%	-	percent
bp	-	base pair
$CaCl_2$	-	calcium chloride
cDNA	-	complementary DNA
^oC	-	degree Celsius
CaMV53S	-	Cauliflower Mosaic Virus 35S promoter
cm	-	centimetre
DEPC	-	diethylpyrocarbonate
DNA	-	deoxyribonucleic acid
dNTP	-	deoxyribonucleotide
GFP	-	Green Fluorescent Protein
g	-	gram
Hg	-	mercury
kb	-	kilobase
KCl	-	potassium chloride
LB	-	Luria-Bertani
LiCl	-	Lithium chloride
M	-	molar
MCS	-	multiple cloning sites
$MgCl_2$	-	magnesium chloride
ml	-	millilitre
mm	-	millimetre
mmHg	-	millimeter of mercury
mM	-	millimolar
mRNA	-	messenger RNA
nm	-	nanometre
µg	-	microgram
µl	-	microlitre
µM	-	micromolar
ng	-	nanogram
PCR	-	polymerase chain reaction
psi	-	per square inch
RT-PCR	-	reverse transcriptase polymerase chain reaction
RNA	-	ribonucleic acid
rpm	-	revolutions per minute
rRNA	-	ribosomal RNA
tRNA	-	transcript RNA
TAE	-	Tris-acetate-EDTA
Tm	-	melting temperature
Tris-Cl	-	tris(hydroxymethyl)amino methane
u	-	unit
V	-	Voltan
v/v	-	volume per volume
WAA	-	weeks after anthesis

CHAPTER 1

INTRODUCTION

The oil palm (*Elaeis guineensis* Jacq.) is the most important industrial crops in Malaysia. It is a monocotyledon tree of the palm family (Arecaceae) (Nasaruddin *et al.,* 2007). There are two main species of oil palm in the genus *Elaeis*, *E. guineensis* and *E. oleifera* (Low *et al.,* 2008). *E. guineensis* has its centre of origin in Africa while *E. oleifera* originated from South American (Fandi *et al*., 2011).

Oil palm is an economically important plant species and provides the world's number one source of edible vegetable oil (Tranbarger *et al.,* 2012). The first oil palm trees planted in Malaysia came from Sumatra, Indonesia. Government promotion of oil palm started in Malaysia in the early 1960s, meanwhile in Indonesia in the late 1960s. Although similar early commercial efforts, it was Malaysia among the two that first promoted successfully oil palm cultivation and diversification. Indonesia's experience became successful, particularly only after 1988 (Rasiah & Shahrin, 2006).

Generally, fruit development, maturation and ripening are important biological processes unique to plants. The molecular basis of oil palm fruit development, maturation and ripening have received less attention. Recent reports indicated that plant hormones play key role in the processes that lead to fruit development (Tranbarger *et al.,* 2011). A series of physical and chemical changes occur in ripening fruits of oil palm (Prada *et al.,* 2011).

The colour change is one of the most significant criteria that can be related to fruit maturity (Alfatni *et al.,* 2008). During the ripening phase, several physiological and biochemical changes take place through differential expression of various genes that are developmentally regulated (Bapat *et al.,* 2010). Various changes happen in the mesocarp during the late period of oil palm fruit ripening including cell wall expansion, oil deposition, vitamin E, carotenoids and ethylene production (Al-Shanfari *et al.,* 2011).

Various approaches have been used to understand the complexity of gene expression and interaction in plants (Low *et al.,* 2008). Understanding the mechanisms of transcriptional regulation underlying the gene expression in its entirety is essential. One of the most important mechanisms of gene expression regulation occurs at the transcriptional level through binding of transcription factors to specific DNA sequences (Laurila & Lahdesmaki, 2009). Transcription appears to be controlled by various transcription factors that mediate the effects of intracellular and extracellular signals. Transcription factors are a group of proteins involve in binding to specific DNA sequences and thereby responsible for either positively or negatively influencing the transcription of specific genes (Phillips & Hoopes, 2008). The analysis of transcription factors is essential for an understanding of mechanisms of gene expression.

The introduction of DNA by particle bombardment had benefitted genetic transformation of recalcitrant and perennial crops, like oil palm, since the technique enables the transfer of any gene to virtually any tissues or cell types (Abdullah *et al.,* 2005). Furthermore the technique enables the analysis of introduced gene(s) in

bombarded tissues via transient expression assay and this serves as a valuable model system to understand transcriptional regulation in different tissues. The use of non-destructive reporter gene such as green fluorescent protein (GFP) enables rapid selection of tissues that have successfully received the bombarded gene constructs (Majid & Parveez, 2007).

From a recent study, gene that encoded a transcription factor belonging to AP2 family that is highly expressed in ripening oil palm fruit mesocarp was isolated and sequenced. Further characterization of this transcription factor will be conducted in order to identify genes whose expression is regulated by this transcription factor in the mesocarp tissue.

Thus, the objectives of this study were;

1) To determine the expression profile of *EgDREB* gene in different developmental stages of oil palm mesocarp, roots and leaves.

2) To bombard expression vector containing *EgDREB* gene into oil palm mesocarp at 12 weeks after anthesis (WAA).

3) To study changes in expression profile of nine selected genes known to be highly expressed in ripening fruit (17 WAA) in the bombarded mesocarp tissues.

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