UNIVERSITI PUTRA MALAYSIA

CHARACTERIZATION OF DIFFERENTIALLY EXPRESSED TRANSCRIPTS OF OIL PALM (*ELAEIS GUINEENSIS* JACQ.) SUSPENSION CELLS

SITI HABSAH ROOWI

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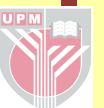

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By

SITI HABSAH ROOWI

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

April 2009

Dedicated to my daughter, Tengku Sofia Tengku Adnin

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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April 2009

Chairman : Associate Prof. Dr. Suhaimi Napis, PhD

Faculty : Biotechnology and Biomolecular Sciences

Oil palm tissue culture through somatic embryogenesis remains a problem because the production cost is still high and the process is very labour-intensive. The average callogenesis rate per ortet is only 20% with embryogenesis rate averages 6%. In this study, oil palm suspension cultures were initiated by transferring the gel-like friable embryogenic tissue onto liquid medium supplemented with auxin(s). The aim of this study was to understand and identify the underlying factors that are involved in the induction of somatic embryo through suspension cells cultured in different auxin(s) concentrations. Transcripts that were differentially expressed in oil palm suspension cells cultured at different auxin(s) were examined by using suppression subtractive hybridization (SSH). The four different hormone combinations examined were: T1 (0.1 mg/l 2,4-D and 1 mg/l NAA), T2 (0.4 mg/l 2,4-D and 1 mg/l NAA), T3 (1 mg/l NAA) and T4(0.4 mg/l 2,4-D). The first and second subtractions were performed using samples T1 and T2 in forward and reverse order. The other two subtractions were forward and reverse subtractions of T3 and T4, respectively. A total of 2019 cDNAs were cloned and isolated from these SSH libraries. Reverse northern analyses showed

that 82 clones were isolated from library 1, 64 clones from library 2, 72 clones from library 3 and 76 clones from library 4. Among the 294 cDNA clones that were sequenced, 61 contigs (assembled from 165 sequences) and 129 singletons were obtained. Among the 61 contigs, 10 contigs consisted of sequences from treatment T1, 8 contigs from treatment T2, 10 contigs from treatment T3 and 13 contigs contain sequences from treatment T4, respectively. Northern analyses of 5 transcripts that were shown to be differentially expressed by reverse northern analysis revealed that transcripts 16A1 (a putative lignostilbene-α,β dioxygenase, *Eg*LSD) and 16H12 (a putative ethylene responsive 6, *Eg*ER6) were differentially expressed in oil palm suspension cells treated with different levels of auxin. The full length cDNA sequence of *Eg*LSD is 1801 bp with 58 bp of 5' untranslated region and 119 bp of 3' non coding region. The full length cDNA sequence of *Eg*ER6 is 810 bp with 120 bp of 5' untranslated region and 88 bp of 3' non coding region. The gene expression of these two candidates was further monitored by real-time quantitative RT-PCR. The transcripts of *Eg*LSD were present throughout cell maturation from 0 day to 25 days with higher increase in ABA treated samples compared to the control without ABA treatment. On the other hand, the transcript level of *Eg*ER6 decreased drastically from 1 to 0.0001 for ABA treated samples. The results showed that these two genes respond to ABA during maturation stage in oil palm suspension cultures. The current finding showed that there is a crosstalk between ABA and Ethylene, whereby sugar acts as a switch to control the expression of *Eg*LSD and *Eg*ER6. These genes can be used as a marker for somatic embryogenesis of oil palm through cell suspension culture.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah.

PENCIRIAN TRANSKRIP YANG TEREKSPRES SECARA BERBEZA DARIPADA SEL TERAMPAI KELAPA SAWIT (*ELAEIS GUINEENSIS* JACQ.)

Oleh

SITI HABSAH ROOWI

April 2009

Pengerusi : Prof. Madya Dr. Suhaimi Napis, PhD

Fakulti : Bioteknologi dan Sains Biomolekul

Tisu kultur kelapa sawit melalui pembentukan embrio somatik masih mengalami masalah kerana kos pengeluaran yang tinggi dan proses penghasilannya menggunakan tenaga kerja yang ramai. Purata penghasilan kalus adalah sebanyak 20% dengan kadar penghasilan kalus embriogenik sebanyak 6%. Dalam kajian ini, kultur terampai kelapa sawit dimulakan dengan memindahkan tisu embriogenik mudah lerai ke medium pertumbuhan yang mengandungi auksin. Tujuan kajian ini adalah untuk memahami dan mencari faktor-faktor yang terlibat dalam pembentukan embrio somatik. Transkrip-transkrip yang terekspres secara berbeza di dalam sel ampaian kelapa sawit yang di rawat menggunakan kombinasi auksin berbeza kepekatan di kaji menggunakan strategi ‘suppression subtractive hybridization (SSH)’. Empat jenis kepekatan auksin yang digunakan adalah T1 (0.1 mg/l 2,4-D dan 1 mg/l NAA), T2 (0.4 mg/l 2,4-D dan 1 mg/l NAA), T3 (1 mg/l NAA) dan T4(0.4 mg/l 2,4-D). Penyingkiran ke hadapan dan ke belakang adalah mengunakan sampel T1 dan T2. Seterusnya, dua sample untuk penyingkiran ke hadapan dan ke belakang juga menggunakan sampel T3 dan T4.

Sebanyak 2019 koleksi cDNA telah diperolehi menggunakan strategi SSH. Analisa 'northern' berbalik menunjukkan 82 klon daripada koleksi cDNA adalah daripada rawatan T1, 64 klon daripada rawatan T2, 72 klon daripada rawatan T3 dan 76 klon daripada rawatan T4. Sejumlah 294 klon cDNA telah dijujuk. Daripada jujukan tersebut, 61 kontig dan 129 'singleton' telah dihasilkan. Daripada 61 kontig yang terhasil, 10 kontig mengandungi jujukan daripada rawatan T1, 8 kontig daripada rawatan T2, 10 kontig daripada rawatan T3 dan 13 kontig daripada rawatan T4. Analisa 'northern' di jalankan ke atas 5 transkrip yang didapati terekspres secara berbeza. Keputusan analisa menunjukkan transkrip-transkrip *Eg*LSD dan *Eg*ER6 terekspres secara berbeza di dalam sel terampai kelapa sawit yang mengandungi kepekatan auksin berlainan. Susunan lengkap *Eg*LSD adalah sepanjang 1801 bp termasuk 58 bp adalah '5' untranslated region' dan 119 bp adalah '3' non coding region'. Susunan lengkap *Eg*ER6 adalah 810 bp termasuk 120 bp adalah '5' untranslated region' dan 88 bp adalah '3' non coding region'. Pengekspressan kedua-dua gen ini seterusnya di analisa menggunakan 'real-time quantitative RT-PCR'. Transkrip *Eg*LSD dikesan meningkat sepanjang proses mematangkan sel dari 0 hari hingga 25 hari dan menunjukkan peningkatan yang lebih tinggi untuk sel yang di rawat menggunakan ABA. Transkrip *Eg*ER6 didapati menurun serta merta untuk sel yang di rawat menggunakan ABA. Keputusan menunjukkan kedua-dua gen bertindakbalas terhadap ABA semasa peringkat mematangkan sel. Ini menunjukkan terdapat interaksi antara ABA dan ethylene, di mana gula juga bertindak mengawal pengekspresan *Eg*LSD dan *Eg*ER6. Kedua-dua gen ini boleh digunakan sebagai penanda untuk pembentukan embrio somatik daripada kultur ampaian kelapa sawit.

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I certify that a Thesis Examination Committee has met on 28th April 2009 to conduct the final examination of Siti Habsah binti Roowi on her thesis entitled "Characterization of Differentially Expressed Transcripts of Oil Palm (*Elaeis guineensis* JACQ.) Suspension Cells" 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 Doctor of Philosophy.

Members of the Thesis Examination Committee are as follows:

Mohd. Arif Syed, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Nor Aini Abd.Rahman, PhD
Professor
Faculty of Forestry
Universiti Putra Malaysia
(Internal Examiner)

Maheran Abdul Aziz, PhD
Associate Professor
Faculty of Agricultural
Universiti Putra Malaysia
(Internal Examiner)

R. Wickneswari a/p V Ratnam, PhD
Professor
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
(External Examiner)

____

BUJANG KIM HUAT, Ph.D.
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 18 June 2009

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

Suhaimi Napis, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Ho Chai Ling, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Meilina Ong Abdullah, PhD
Advanced Biotechnology and Breeding Centre,
Research Division of Biology,
Malaysian Palm Oil Board (MPOB)
(Member)

Sharifah Shahrul Rabiah Bte Syed Alwee, PhD
Felda Biotechnology Centre,
Felda Agricultural Services Sdn.Bhd,
(Member)

____
HASANAH MOHD.GHAZALI, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 9 July 2009

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledge. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institution.

SITI HABSAH ROOWI

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LIST OF ABBREVIATIONS

ABA	Abscisic acid
α	alpha
β	beta
bp	base pair
BLAST	Basic Local Alignment Search Tool
BSA	bovine serum albumin
^{o}C	degree centigrade
%	percentage
Ci	curie
CTAB	hexadecyl (or cetyl) trimethyl ammonium bromide
2,4-D	2,4- dichlorophenoxy acetic acid
DNA	deoxyribonucleic acid
DNase 1	deoxyribonuclease 1
cDNA	complementary DNA
dNTPs	Deoxynucleotides
dATP	2'-deoxy-adenosine-5'-triphosphate
dCTP	2'- deoxy-cytidine-5'-triphosphate
dGTP	2'- deoxy-guanosine-5'-triphosphate
dTTP	thymidine-5'-triphosphate
dH_2O	distilled water
DEPC	diethyl pyrocarbonate
DMSO	dimethylsulphonyl oxide

DTT	dithiothreitol
D X P	dura x pisifera
*Eg*LSD	*Elaeis guineensis* lignostilbene α,β dioxygenases
*Eg*ER6	*Elaeis guineensis* ethylene responsive 6
EtBr	ethidium bromide
EDTA	ethylenediaminetetraacetic acid
EGTA	ethylene glycol bis- (β-aminoethyle ether)
Fe	Ferum
FELDA	Federal Land Development and Authority
FET	friable embryogenic tissue
g	gram
GA	Gibberelic acid
HCl	hydrochloric acid
hr	hours
IAA	Indole acetic acid
IBA	Indole butyric acid
Jacq.	Jacquin
kb	kilobase
LB	luria-bertani
L	liter
LiCl	lithium chloride
M	molar
mg	milligram

min	minute
ml	milliliter
mm	millimeter
mM	millimolar
mmol	millimole
$MgCl_2$	magnesium chloride
$MgSO_4$	magnesium sulphate
mRNA	messenger RNA
MW	molecular weight
NAA	Naphthalene acetic acid
NaCl	sodium chloride
NaOAc	sodium acetate
NCBI	National Center for Biotechnology Information
NH_4OAc	Ammonium acetate
NCED	9-cis-epoxycarotenoid dioxygenase
ng	nanogram
nm	nanometer
OD	Optical density
ORFs	Open reading frames
PCI	Phenol : chloroform : isoamyl alcohol
PCR	polymerase chain reaction
PEM	polyembryogenic masses
Poly A^+RNA	polyadenylated RNA

PVP	Polyvinylpyrrolidone
PVPP	Polypolyvinylpyrrolidone
RNA	ribonucleic acid
rRNA	ribosomal RNA
RNase	ribonuclease
rpm	revolution per minute
RT	reverse transcriptase
SAAP	streptavidin-alkaline phosphatase conjugate
SDS	sodium dodecyl sulfate
SSC	standard saline citrate
TAE	tris acetate EDTA
TBE	tris borate EDTA
TE	Tris-EDTA
Tris	tris[hydroxymethyl]aminomethane
Tris-HCl	tris hydrochloride
U	unit
µg	microgram
µl	microliter
µm	micrometer
µM	micromolar
UV	ultraviolet
VP14	*VIVIPAROUS14*
V	Volts

v/v	volume per volume
w/v	weight per volume
X	times

CHAPTER 1

INTRODUCTION

The biological characteristics of oil palm do not allow vegetative propagation of this plantation crop through use of conventional means. The viable alternative is to clonally propagate elite oil palm is by inducing somatic embryogenesis on callus derived from various tissue sources. There is a ready market for more than hundred million oil palm tissue cultured plantlets in the world (Corley and Tinker, 2003; Basri et al., 2004). There are at least 10 companies or organizations that are producing elite palms extensively for field evaluation of clonal performance and fidelity in Malaysia (Basri et al., 2004). Having to cope with large scale production of oil palm ramets, improving the tissue culture processes is the main agenda for those involved in producing oil palm planting materials. The potential advantages of somatic embryogenesis in oil palm for breeding purposes and production of synthetic seed have been widely investigated (Besse et al., 1992; Duval et al., 1995; Morcillo et al., 2001).

The oil palm tissue culture process involves ortet selection, sampling of explants, callogenesis and embryogenesis, embryo proliferation, shoot regeneration, rooting and acclimatization of ramets and field evaluations of clones. Embryo proliferation and shoot regeneration usually occur concurrently in the same polyembryoid clusters. Separation of the regenerated shoots allows polyembryoid to continue proliferating in subsequent subcultures. This process is inefficient and not cost effective because the handling of individual shoots

requires substantial amount of labour and relies heavily on the skill and experience of the operating personnel. Thus, there is a need to find ways to improve the efficiency of the oil palm tissue culture process. Besides, clonal propagation is used in producing good palms with high oil yield for future planting materials and also used for the production of monoclonal and biclonal seed for crop improvement program. One possible strategy to overcome this problem is to use liquid culture. The liquid suspension culture system is more efficient than the conventional method which uses solidified gelled system with respect to labour, skill and culture space, large and constant source of cultures, culture duration, production schedule and cost. Oil palm cell suspension is a viable alternative for mass production.

Auxin is considered to be the most important hormone in regulating somatic embryogenesis *in vitro* (Cooke et al., 1993). The hormone 2,4-dichlorophenoxyacetic acid (2,4-D) and naphthalene acetic acid (NAA) are common auxins used in oil palm tissue culture (Wong et al., 1999; Tarmizi et al., 2004; Gorret et al., 2004). Exogenous application of auxin(s) to various plant parts (immature leaf, mature and immature zygotic embryo, inflorescence and roots) of oil palm has proven to be the most efficient treatment of embryogenesis. The development of somatic embryo has been achieved by reducing or removing auxin from the culture media (Teixera et al., 1994 and 1995; Maheran et al., 1995; Wong et al., 1999). Benzylaminopurine (BA) and zeatin riboside are the cytokinins that have been used for regeneration in oil palm (Aberlenc-Bertossi et al., 1999). However, prolonged culture in a cytokinin supplemented media could