UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT AND APPLICATION OF EXPRESSED SEQUENCE TAGS AND DNA MICROARRAY FOR SOMATIC EMBRYOGENESIS IN OIL PALM

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By

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

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Doctor of Philosophy

DEVELOPMENT AND APPLICATION OF EXPRESSED SEQUENCE TAGS AND DNA MICROARRAY FOR SOMATIC EMBRYOGENESIS IN OIL PALM

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February 2009

Chairman : Professor Raha Abdul Rahim, PhD

Faculty : Biotechnology and Biomolecular Sciences

Oil palm (*Elaeis guineensis* Jacq.) is one of the most important oil bearing crops in the world. However, genetic improvement of oil palm through conventional breeding is extremely slow and costly, as the breeding cycle can take up to 10 years. This has brought about interest in vegetative propagation of oil palm. Since the introduction of oil palm tissue culture in the 1970s, clonal propagation has proven to be useful in producing uniform planting materials. However, despite considerable progress in improving the tissue culture techniques, the callusing and embryogenesis rates from proliferating callus cultures remain very low. Thus, understanding the gene diversity and expression profiles during somatic embryogenesis is critical in increasing the efficiency of these processes. To achieve this, a total of six standard cDNA libraries, representing three developmental stages (non-embryogenic callus, embryogenic callus and embryoids) in oil palm tissue

culture, were generated in this study. Random sequencing of clones from the embryogenic callus cDNA libraries generated 2,716 expressed sequence tags (ESTs). These ESTs were combined with 14,883 ESTs available in MPOB's EST programme. The 17,599 ESTs were analysed, annotated and assembled to generate 9,584 putative unigenes distributed in 3,268 consensi and 6,316 singletons. These unigenes were assigned putative functions based on similarity and gene ontology annotations. A subset of these ESTs were selected and spotted on cDNA microarrays. Both the EST and microarray data analysis were able to identify expression profiles that could differentiate non-embryogenic callus from embryogenic samples. The *in silico* EST data analysis identified 52 unigenes that showed potential to be developed as candidate markers for embryogenesis. The microarray experiment identified 76 unigenes that could differentiate non-embryogenic callus from embryogenic callus, embryoids and shoots from polyembyoids. The EST and microarray data analysis revealed that lipid transfer proteins were highly expressed in embryogenic tissues. The results also showed that glutathione S-transferases were highly expressed in non-embryogenic callus. This study has provided an overview of genes expressed during oil palm tissue culture and real-time PCR analysis identified four genes (pOP-EA00703, pOP-EA01249, pOP-EA01117, pOP-SFB01045) that had the potential to be developed as molecular markers for embryogenesis.

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

PEMBANGUNAN DAN APLIKASI PENANDA JUJUKAN TERUNGKAP AND DNA MIKROATUR DALAM KAJIAN EMBRIOGENESIS SOMATIK KELAPA SAWIT

Oleh

LESLIE LOW ENG TI

February 2009

Pengerusi : Profesor Raha Abdul Rahim, PhD

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Kelapa sawit (*Elaeis guineensis Jacq.*) merupakan salah satu tanaman penghasil minyak yang terpenting di dunia. Proses pembaikan genetik kelapa sawit melalui kaedah konvensional mengambil masa yang lama dan memerlukan kos yang tinggi kerana satu kitaran pembiak-bakaan mengambil masa sehingga 10 tahun. Faktor ini telah mengalih perhatian industri kelapa sawit kepada proses propagasi vegetatif. Semenjak penggunaan proses kultur tisu kelapa sawit pada tahun 1970an, propagasi klonal didapati berkesan dalam penghasilan pokok kelapa sawit yang seragam. Walaupun pelbagai penyelidikan telah dijalankan dalam pembaikan teknik proses kultur tisu kelapa sawit, kadar penghasilan kalus dan embriogenesis masih rendah. Oleh itu, pemahaman terhadap kepelbagaian dan profil pengekspresan gen semasa embriogenesis somatik adalah kritikal untuk meningkatkan kecekapan proses tersebut. Untuk mencapai objektif ini, sebanyak enam perpustakaan cDNA daripada tiga peringkat perkembangan (kalus tidak embriogenik, kalus embriogenik dan embriod) proses kultur tisu telah

dihasilkan. Penjujukan klon secara rawak daripada perpustakaan cDNA kalus embriogenik telah menghasilkan 2,716 penanda jujukan terungkap (EST). Jujukan-jujukan EST ini telah digabungkan dengan 14,883 jujukan EST yang terdapat di bawah program EST MPOB. Analisa pergabungan 17,599 jujukan EST menemui 3,268 jujukan konsensi dan 6,316 singleton. Penentuan fungsi putatif ke atas unigen tersebut adalah berdasarkan anotasi kesamaan dan ontologi gen. Sebahagian daripada koleksi EST ini juga telah dicetak di atas mikroatur DNA. Analisa data EST dan mikroatur telah mengenalpasti profil pengekspresan gen yang dapat membezakan kalus tidak embriogenik daripada sampel-sampel embriogenik. Bardasarkan analisa data '*in silico*' EST, sebanyak 52 gen mempunyai potensi untuk dibangunkan sebagai penanda molekul bagi proses embriogenesis. Eksperimen mikroatur pula menemui 76 gen yang dapat membezakan kalus tidak embriogenik daripada kalus embriogenik, embriod dan pucuk daripada poliembriod. Analisa '*in silico*' EST dan DNA mikroatur menunjukkan bahawa pengekspresan protein pemindah lipid adalah tinggi di dalam tisu embriogenik. Manakala glutation S-transferase menunjukkan pengekspresan yang tinggi di dalam kalus tidak embriogenik. Kajian ini telah memberikan gambaran menyeluruh mengenai gen-gen yang diekspres semasa kultur tisu kelapa sawit. Teknik 'real-time PCR' telah mengenalpasti empat gen (pOP-EA00703, pOP-EA01249, pOP-EA01117, pOP-SFB01045) yang mempunyai potensi untuk dibangunkan sebagai penanda molekul bagi proses embriogenesis.

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I certify that a Thesis Examination Committee has met on 22th February 2009 to conduct the final examination of Leslie Low Eng Ti on his thesis entitled "Development and Application of Expressed Sequence Tags (ESTs) and DNA Microarray for Somatic Embryogenesis in Oil Palm" in accordance with the Universities and Universiti 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.

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DECLARATION

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

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LESLIE LOW ENG TI

Date: 9 June 2008

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ABBREVIATIONS

%	Percentage
α	Alpha
β	Beta
λ	Lambda
°C	Degree Celsius
μg	Microgram
μl	Microliter
μM	Micromolar
A	Adenine
ABA	Abscisic Acid
AFLP	Amplified Fragment Length Polymorphism
AGL15	Agamous-like 15
ANOVA	Analysis of Variance
aRNA	antisense RNA
ASP	Automated Slide Processor
BBM	Baby Boom
BLAST	Basic Local Alignment Search Tool
bp	Base Pair
C	Cytosine
cDNA	complementary DNA
Ci	Curie
cm	Centimetre
CTAB	Cetyltrimethylammonium Bromide
cps	Counts Per Second

C_t	Threshold Cycle
Cy3	Cyanine 3
Cy5	Cyanine 5
D x P	*Dura* x *Pisifera*
dATP	2'-deoxy-adenosine-5'-triphosphate
dCTP	2'-deoxy-cytidine-5'-triphosphate
DEPC	Diethyl Pyrocarbonate
DGE	Differential Gene Expression
dGTP	2'-deoxy-guanosine-5'-triphosphate
dH_2O	Deionized Water
DMSO	Dimethylsulphonyl Oxide
DNA	Deoxyribonucleic Acid
DNase 1	Deoxyribonuclease 1
dNTP	Deoxynucleotide Triphosphates
dTTP	2'-deoxy-thymidine-5'-triphosphate
EC	Embryogenic Callus
EDTA	Ethylenediaminetetraacetatic Acid
EGTA	Ethylene glycol bis-(β-aminoethylene ether)
EMB	Embryoid
EC/EMB	Embryogenic Cultures (Embryogenic Callus and Embryoid)
ERE	Ethylene Responsive Element
ESTs	Expressed Sequence Tags
EtBr	Ethidium Bromide
FDR	False-Discovery Rate

Flourophores	Fluorescent Dyes
FRET	Fluorescence Resonance Energy Transfer
FUS3	Fusca3
g	Gram
G	Guanine
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase
GO	Gene Ontology
GSH	Tripeptide glutathione
GST	Glutathione S-transferase
GUI	Graphical User Interface
H_2O_2	Hydrogen Perokside
HCl	Hydrochloride Acid
hr	Hours
i.e.	that is
INF	Inflorescence
IPTG	Isopropyl-β-D-thiogalactoside
Jacq.	Jacquin
K	Potassium
k	Kilo
kb	Kilobase
kDA	Kilodalton
KOH	Potassium Hydroxide
L	Liter
LB	Luria Bertani
LEA	Late Embryogenesis Abundant

LEAF	Spear Leaf
LEC1	Leafy Cotyledon 1
LEC2	Leafy Cotyledon 2
LiCl	Lithium Chloride
LOWESS	Locally Weighted Scatterplot Smoothing
LUS	Lucidea™ Universal ScoreCard™
M	Molar
Mb	Megabase
MES	Mesocarp
$MgCl_2$	Magnesium Chloride
$MgSO_4$	Magnesium Sulphate
MIDAS	TIGR Microarray Data Analysis System
min	Minute
mL	Milliliter
mm	Millimeter
mM	Millimolar
MMLV-RT	Maurine Moloney Leukemia Virus Reverse Transcriptase
mmol	Millimole
MPOB	Malaysia Palm Oil Board
mRNA	Messenger RNA
MT	Metallothionein
mW	Milliwatt
NaCl	Sodium Chloride
NaOAc	Sodium Acetate
NaOH	Sodium Hydroxide

NEC	Non-Embryogenic Callus
ng	Nanogram
nt	Nucleotide
nr	Non-Redundant Protein
O_2	Oxygen
OD	Optical density
ORF	Open Reading Frame
PAGE	Polyacrylamide Agarose Gel Electrophoresis
PAS	p-aminosalicylic acid
PCR	Polymerase Chain Reaction
PEG	Polyethylene Glycol
pfu	Plaque Forming Unit
pmol	Picomole
Poly(A)$^+$ RNA	Polyadenylated Rna
PORIM	Palm Oil Research Institute Of Malaysia
PPO	2,5-Diphenyloxazole
PVP	Polyvinylpyrrolidone
PVPP	Polyvinylpolypyrrolidone
QTL	Quantitative Trait Loci
qRT-PCR	Quantitative Real-Time PCR
R-I plot	Ratio-Intensity Plot
RFLP	Restriction Fragment Length Polymorphism
RFU	Relative Fluorescent Units
RGs	Reference Genes
RN	Reverse Northern

RNA	Ribonucleic Acid
RNase	Ribonuclease
ROS	Reactive O_2 Species
rRNA	Ribosomal RNA
RT	Room Temperature
SAGE	Serial Analysis Of Gene Expression
SAM	Significance Analysis of Microarray
SAP	Shrimp Alkaline Phosphatase
sarkosyl	Sodium *N*-lauroyl sarcosine
SD	Standard Deviation
SDS	Sodium Dodecyl Sulphate
sec	Seconds
SERK	Somatic Embryogenesis Receptor Kinase
SNPs	Single Nucleotide Polymorphisms
SOD	Superoxide Dismutase
SOTA	Self Organisation Tree Algorithm
SSR	Simple Sequence Repeat
LEAF	Spear Leaf
SSC	Sodium Saline Citrate
SSPE	Saline Sodium Phosphate EDTA
SSR	Simple Sequence Repeat
ST	Shoot from polyembryoids
STE	Sodium-Tris-EDTA
T	Thiamine
TAE	Tris-Acetate-EDTA