UNIVERSITI PUTRA MALAYSIA

ISOLATION AND CHARACTERISATION OF GENES EXPRESSED IN ZYGOTIC EMBRYOS AND SUSPENSION CULTURES OF OIL PALM (ELAEIS GUINEENSIS JACQ.)

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By

PARAMESWARI NAMASIVAYAM

Thesis submitted in Fulfilment of the Requirements for the Degree of Master of Science in the Faculty of Food Science and Biotechnology Universiti Putra Malaysia

May 2000

Specially Dedicated
to My Late Mother

Om Sharavana Bhava Namaha

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

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Chairman : Assoc. Prof. K. Harikrishna, Ph.D.

Faculty : Food Science and Biotechnology

The bottleneck of the current oil palm tissue culture technique is the low rate of embryogenesis and the development of viable embryoid lines. Therefore, it is vital to increase the efficiency of callusing, embryogenesis, germination and proliferation of embryoids so that the number of subcultures per line can be reduced without effecting the number of shoots produced *in-vitro*. Thus, it is necessary to elucidate and understand the molecular processes that are involved during somatic embryogenesis of oil palm particularly those involved in specifying embryogenic competence. Based on the role of cell division cycle (*cdc/cdk*) genes and *cyclins* in cell division cycle control of other eukaryotes, it is likely that *cyclins* are also partially involved in the regulation of somatic embryogenesis.

Hence, an attempt to isolate *cyclin* genes from the oil palm zygotic embryo cDNA library was made using heterologous *cyclin* cDNA probes from *Arabidopsis*. 32 putative clones designated as *OPZE*, were isolated from screening. A preliminary characterisation was carried out on these clones in order to identify clones with

sequences related to the cell division cycle. This was achieved by hybridising the PCR amplified *OPZE* clones with amplified cDNA from suspension cultures and mature leaves separately. The *OPZE* clones were categorised into 3 subpopulations according to their tissue-specific expression pattern: a, b and c. Randomly selected clones from these subpopulations were sequenced partially and used for sequence homology searches using DNA sequence databases.

Most clones did not have any significant homology to any known sequences in the database, thus they were designated as novel clones. Three clones *OPZE1A*, *OPZE3A* and *OPZE5A* that had significant homology to oleosin, calmodulin and tumour suppressor protein respectively were selected for northern analysis. From the northern analysis studies, it was found that *OPZE1A* (oleosin) is zygotic embryo specific and both *OPZE3A* and *OPZE5A* are ubiquitously expressed in all evaluated tissues. In order to complement this study, a partial length homeobox gene, *OPHb1 (Knotted1*-like) from oil palm was isolated and was found to be expressed specifically in meristematic tissues. However, the specific functions of these genes during oil palm embryogenesis are still unknown.

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

PEMENCILAN DAN PENCIRIAN GEN-GEN YANG DIEKSPRES DALAM EMBRIO ZIGOTIK DAN KULTUR AMPAIAN KELAPA SAWIT (*ELAEIS GUINEENSIS* JACQ.)

Oleh

PARAMESWARI NAMASIVAYAM

Mei 2000

Pengerusi : Prof. Madya K. Harikrishna, Ph.D.

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Masalah semasa di dalam teknik kultur tisu kelapa sawit ialah kadar embriogenesis yang rendah dan kurangnya pembentukan generasi embroid yang boleh hidup dengan baik. Maka, adalah penting untuk meningkatkan kecekapan pengkalusan, kadar embriogenesis, percambahan dan kadar pembahagian embroid supaya bilangan subkultur untuk setiap generasi boleh dikurangkan tanpa menjejaskan penghasilan jumlah bilangan tumbuhan kelapa sawit *in-vitro*. Ini juga menunjukkan yang kita perlu memahami proses–proses molekular yang terlibat semasa proses embriogenesis somatik terutamanya langkah yang mengspesifikasikan ciri embriogenik. Berdasarkan peranan yang dimainkan oleh gen-gen yang terlibat dalam kitaran sel seperti gen *cdc/cdk* dan gen *cyclin* di dalam pengawalaturan kitaran sel organisma eukariot, maka diandaikan bahawa *cyclin* juga memainkan peranan yang serupa dalam pengawalaturan proses embriogenesis somatik kelapa sawit.

Salah satu langkah yang telah diambil dalam kajian ini ialah untuk memencilkan gen-gen *cyclin* daripada koleksi cDNA embrio zigotik kelapa sawit. Pemencilan telah dilakukan dengan menggunakan prob heterologos iaitu gen-gen *cyclin* daripada *Arabidopsis*. 32 klon telah berjaya dipencilkan daripada proses penyaringan koleksi cDNA dan ia telah dinamakan sebagai *OPZE*. Suatu langkah penyaringan awal telah dilakukan untuk mengidentifikasi klon-klon yang berkaitan dengan kitaran sel. Ini telah dilakukan dengan mempergandakan DNA klon-klon *OPZE* melalui tindakbalas rantai polimerase diikuti dengan pemblotan dan penghibridan dengan cDNA daripada kultur ampaian kelapa sawit dan cDNA daripada daun-daun sawit yang tua secara berasingan. Berdasarkan keputusan yang diperolehi daripada eksperimen ini, klon-klon *OPZE* telah dikategorikan kepada 3 subpopulasi (a, b dan c) mengikut spesifisiti pengekspresannya kepada tisu. Daripada 3 kategori ini klon-klon telah dipilih secara rawak untuk penjujukan secara sehala. Keputusan penjujukan DNA bagi klon-klon tersebut digunakan untuk pencarian jujukan homologi melalui databes jujukan DNA yang sedia ada di Internet.

Kebanyakan klon-klon tidak mempunyai homologi yang bererti terhadap sebarang jujukan yang diketahui di dalam databes, maka klon-klon ini digelar sebagai klon novel. Hanya 3 klon iaitu *OPZE1A*, *OPZE3A* dan *OPZE5A* yang masing-masing menunjukkan homologi yang bererti dengan gen oleosin, 'calmodulin' dan protein penindas barah. Ketiga-tiga klon ini telah dipilih untuk penganalisaan Northern. Kajian Northern menunjukkan bahawa *OPZE1A* (oleosin) adalah spesifik kepada

embrio zigotik manakala *OPZE3A* dan *OPZE5A* menunjukkan pengekspresan di dalam semua tisu yang diuji. Bagi mengkomplemenkan kajian ini, gen homeobox yang separa panjang, *OPHb1* (homolog *Knotted1*) telah berjaya dipencilkan daripada tisu kelapa sawit dan pengekspresannya hanya didapati pada tisu meristematik. Walaubagaimanapun, fungsi gen-gen yang telah dipencilkan ini masih tidak diketahui lagi.

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I certify that an Examination Committee met on 3rd May, 2000 to conduct the final examination of Parameswari Namasivayam on her Master of Science thesis entitled "Isolation and Characterisation of Genes Expressed in Zygotic Embryos and Suspension Cultures of Oil Palm (*Elaeis guineensis* Jacq.)" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows :

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This thesis was submitted to the Senate of Universiti Putra Malaysia and was accepted as fulfilment of the requirements for the degree of Master of Science.

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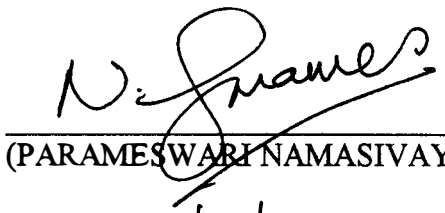
Date : 8 JUN 2000

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 instituitions.



(PARAMESWARI NAMASIVAYAM)

Date : 10/5/2000

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α	alpha
β	beta
λ	lambda
%	percentage
ABA	Abscissic acid
Amp	ampicillin
2-BE	Ethyleneglycolmonobutylether
bp	base pair
BLAST	Basic Local Alignment Search Tool
BSA	bovine serum albumin
CycA2	*CyclinA2*
CycB1	*CyclinB1*
cpm	counts per minute
DNA	Deoxyribonucleic acid
cDNA	copy 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
D X P	*dura* x *pisifera*

EDTA	ethylene glycol bis-(β-aminoethyl ether)
EGTA	Ethylene Glycol Bis- (β-aminoethyle ether)
g	gram
HCl	hydrochloric acid
hr	hours
Jacq.	Jacquin
LB	Luria-Bertani
k	Kilo
Kan	kanamycin
kb	Kilobase
Kn1	*Knotted1*
KCl	potassium chloride
kDa	Kilodalton
L	liter
LD	Long distance
LiCl	Lithium chloride
M	Molar
mg	milligram
min	minute
ml	milliliter
mm	millimeter
mM	Millimolar
MMLV	Maurine Moloney Leukemia Virus
$MgCl_2$	Magnesium chloride

mRNA	Messenger RNA
min	minute(s)
n	Chromosome number
NaCl	sodium chloride
NaOAc	Sodium Acetate
ng	nanogram
OD	Optical density
ORF	open reading frame
OPZE	oil palm zygotic embryo
OPHb1	oil palm homeobox-1
PCI	Phenol:chloroform:isoamylalcohol
PCR	Polymerase Chain Reaction
pfu	plaque forming unit
PORIM	Palm Oil Research Institute of Malaysia
pI	isoelectric point
PVP	Polyvinylpyrrolidone
PVPP	Polypolyvinylpyrrolidone
RNA	Ribonucleic acid
rRNA	ribosomal RNA
RNase	Ribonuclease
rpm	revolution per minute
RT	Reverse transcriptase
sscDNA	single-stranded copy DNA
SDS	sodium dodecyl sulfate

STM	Shoot meristemless gene
TAE	Tris Acetate EDTA
TBE	Tris Borate EDTA
TEMED	N,N,N',N'-tetramethylethylenediamine
U	Unit
UFO	Unusual Floral Organs
ug	microgram
ul	microliter
UV	Ultraviolet
v/v	volume per volume
WAA	weeks after anthesis
w/v	weight per volume

CHAPTER I

INTRODUCTION

On the scale of world production, palm oil has recorded the highest average annual growth rate compared to other vegetable oils. Based on the demand for palm oil and oil palm seeds, it is believed that there is a ready market for more than 100 million tissue culture plantlets world wide.

Since oil palm is the most productive oil bearing crop in Malaysia and has great economic value, a high priority has been given to intensify the research in this area particularly in improving the existing tissue culture technique. Research areas which are currently viewed seriously by PORIM and the industry are issues related to clonal abnormality, performance of oil palm clones in the field and its implication on the production of oil palm clones for commercial production.

The current technique employed by PORIM and most of the oil palm industry depend largely on the use of polyembryonic (PE) cultures for shoot production. Several reports have indicated that PE cultures maintained for prolonged periods via subculturing, showed a higher frequency of abnormality (Corley et al., 1986). Since the frequency and severity of abnormalities increased with culture time and one way to limit this occurring to reduce the number of culture cycles. However, this will consequently reduce the number of shoots produced. Therefore, it is vital to increase