UNIVERSITI PUTRA MALAYSIA

AN EXAMINATION OF EMBRYOGENIC AND NON-EMBRYOGENIC CULTURES OF OIL PALM (ELAEIS GUINEENSIS JACQ.)

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AN EXAMINATION OF EMBRYOGENIC AND NON-EMBRYOGENIC CULTURES OF OIL PALM (*ELAEIS GUINEENSIS* JACQ.)

By

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Thesis is submitted in Fulfilment of the Requirement for the Degree of Doctor of Philosophy in the Faculty of Food Science and Biotechnology Universiti Putra Malaysia

March 2001

Specially Dedicated
To the
ONGs and ALWIs

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

AN EXAMINATION OF EMBRYOGENIC AND NON-EMBRYOGENIC CULTURES OF OIL PALM (*ELAEIS GUINEENSIS* JACQ.)

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Chairman : Associate Professor Dr. K. Harikrishna, Ph.D

Faculty : Food Science and Biotechnology

Somatic embryogenesis of crop plants such as oil palm has generated considerable research interest. However, the main obstacle that hinders the development of an economically viable propagation system is the low frequency of embryogenesis. Currently, most local tissue culture laboratories are reporting embryogenesis rates of approximately 6%. Due to the lack of knowledge about oil palm somatic embryogenesis, it would be difficult to understand or even to attempt to improve the process. Hence, this study has been tailored to understand the fundamental processes that could occur during embryogenesis by analyzing differences between embryogenic (EC) and non-embryogenic (NEC) *in vitro* cultures of oil palm.

The initial studies concentrated on elucidating the differences found between EC and NEC at the microscopical level. Proembryo (PE) structures were predominantly found in ECs. The phenomenon of isolation of cells, as a prerequisite to embryogenesis, was observed in the formation of PEs. It was hypothesized that the surrounding cells at the periphery of each PE structure could have gone through programmed cell death (PCD) hence creating the condition of 'isolation of cells'.

The hypothesis that PCD could play an important role in the embryogenesis process was further supported by studies carried out at the physiological and molecular level. ECs were found to be metabolically more active than NECs, thus indicating that ECs would need an efficient system to overcome the accumulation of reactive oxygen species (ROS), a toxic byproduct of aerobic metabolism. With the isolation of the embryogenic tissue specific OPEm1, which encodes for an antioxidant known as peroxiredoxin, it is believed that it functions by protecting the proembryos from being damaged by the ROS but killing the cells surrounding them.

OPEm1 represents the first peroxiredoxin to be isolated from a palm and has potential to be exploited as a molecular marker for embryogenic potential of *in vitro* cultures. In addition to this, with the knowledge of the physiological state of embryogenic and non-embryogenic cultures, a non-destructive method for the detection of embryogenic potential can now be devised by taking advantage of the reaction mechanism of oxidative dyes in culture media.

Besides this, attempts were also made to isolate other embryogenic related genes by means of a rapid cloning method for differentially expressed cDNAs. This technique is better known as Suppression Subtractive Hybridization. Out of a total 595 clones screened, only 66 were found to be embryogenic specific. Amongst these clones, one of them was characterized and shown to be closely related to a class IV chitinase EP3. This clone was designated as OPSSH1. There is some evidence to suggest from the northern analysis study, that different subsets of class IV endochitinase EP3 were being detected, as two differently sized transcripts were observed. It is possible that they encode proteins that have differing functions. However, due to the

generally short fragments being produced through this technique, it is still too early to propose a functional role for endochitinase(s) in the oil palm system.

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

KAJIAN KE ATAS KULTUR EMBRIOGENIK DAN TAK-EMBRIOGENIK KELAPA SAWIT (*ELAEIS GUINEENSIS* JACQ.)

Oleh

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Mac 2001

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Embriogenesis somatik tumbuh-tumbuhan, seperti kelapa sawit, telah menjana minat yang mendalam terhadap penyelidikan. Walau bagaimanapun, batu penghalang bagi perkembangan sesuatu sistem pembiakan yang berdaya maju dari sudut ekonomi ialah kadar embriogenik yang rendah.

Pada masa ini, kebanyakan makmal kultur tisu tempatan melaporkan kadar embriogenesis dalam lingkungan 6%. Oleh kerana pengetahuan yang terhad mengenai embriogenesis somatik kelapa sawit, maka proses tersebut sukar difahami, apatah lagi untuk memperbaiki tarafnya. Justru itu, kajian ini telah disesuaikan untuk memahami proses-proses asas yang mungkin berlaku semasa embriogenesis dengan menganalisa perbezaan di antara kultur *in vitro* kelapa sawit yang embriogenik (EC) dan tak-embriogenik (NEC).

Kajian awalan menumpukan kepada penjelasan tentang perbezaan antara EC dan

NEC pada peringkat mikroskopikal. Sebahagian besar proembrio (PE) boleh didapati pada EC. Fenomena di mana pengasingan sel-sel adalah syarat untuk embriogenesis, dapat diperhatikan semasa PE terbentuk. Hipotesis dibuat bahawa sel-sel pinggiran sekeliling setiap struktur PE mungkin melalui proses kematian sel yang telah dirancang ('Programmed Cell Death', PCD), yakni menghasilkan keadaan 'isolation of cells'. Hipotesis di mana PCD memainkan peranan yang penting dalam proses embriogenesis selanjutnya disokong oleh kajian-kajian di peringkat fisiologi dan molekular. EC didapati lebih aktif secara metabolik jika dibandingkan dengan NEC, oleh itu EC memerlukan satu sistem yang efisien untuk mengatasi pengumpulan spesis oksigen reaktif ('Reactive Oxygen Species', ROS) iaitu hasil sampingan toksik daripada metabolisme aerobik. Adalah dipercayai bahawa OPEm1 yang khusus kepada tisu embriogenik, yang mengekod untuk sejenis antioksidan yang dikenali sebagai 'peroxiredoxin', mungkin bertindak dengan cara melindungi proembrio terhadap kerosakan akibat tindakan ROS tetapi membiarkan sel-sel disekelilingnya mati.

OPEm1 merupakan 'peroxiredoxin' yang pertama yang telah dipencilkan daripada palma dan ia berupaya untuk dieksploitasikan sebagai petanda molekular untuk mengesan keupayaan embriogenik dalam kultur *in vitro*. Tambahan pula, dengan pengetahuan keadaan fisiologi kultur embriogenik dan tak-embriogenik, satu kaedah untuk mengesan keupayaan embriogenik tanpa mengorbankan kultur dapat diperkembangkan dengan menggunakan mekanisme tindakbalas pewarna oksidatif dalam kultur media.

Selain daripada itu, pemencilan gen embriogenik yang lain juga telah dijalankan dengan menggunakan kaedah pengklonan segera untuk cDNA yang mempunyai pengekspresan yang berbeza. Teknik ini lebih dikenali sebagai 'Suppression Subtractive Hybridization'. Daripada jumlah klon sebanyak 595 yang telah disaring, hanya 66 didapati tisu embriogenik terkhusus. Di antara klon-klon tersebut, satu daripadanya telah dihuraikan dan klon tersebut didapati berkait rapat dengan 'class IV chitinase EP3'. Klon ini telah dinamakan sebagai OPSSH1. Terdapat bukti daripada penganalisaan Northern yang mencadangkan bahawa terdapat beberapa subset 'class IV chitinase EP3' yang berlainan telah dikesan dari pemerhatian dua transkrip yang berlainan saiz. Kemungkinan transkrip-transkrip tersebut mengekod protein-protein yang berlainan fungsi. Olehkerana penghasilan kebanyakan serpihan melalui kaedah ini adalah pendek, maka ianya terlalu awal untuk mencadangkan satu peranan fungsi kepada 'endochitinase' dalam sistem kelapa sawit.

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy.

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α	alpha
β	beta
λ	lambda
%	percentage
°C	degree centigrade
A	angstrom
Arg	arginine
2-BE	ethyleneglycolmonobutylether
bp	base pair
BDMA	n-benzyl dimethyl amine
BLAST	Basic Local Alignment Search Tool
BSA	bovine serum albumin
Ci	curie
CO_2	carbon dioxide
C-terminal	carboxyl terminal
Cys	cysteine
1-D	one dimensional
2-D	two dimensional
2,4-D	2,4- dichlorophenoxy acetic acid
DDSA	dodecenyl succinic anhydride
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
DMF	dimethyl fluoride
DMSO	dimethylsulphonyl oxide
DTT	dithiothreitol
D X P	*dura* x *pisifera*
EtBr	ethidium bromide
EDTA	ethylenediaminetetraacetic acid
EGTA	ethylene glycol bis- (β-aminoethyle ether)
g	gram
HCl	hydrochloric acid
His	histidine
H_2O_2	hydrogen peroxide
hr	hours
IPTG	isopropyl-β-D-thiogalactoside
Jacq.	Jacquin
LB	luria-bertani
k	kilo

kb	kilobase
KCl	potassium chloride
kDa	kilodalton
L	liter
LiCl	lithium chloride
M	molar
mA	milliampere
mg	milligram
min	minute
ml	milliliter
mm	millimeter
mM	millimolar
mmol	millimole
MMLV	Maurine Moloney Leukemia Virus
$MgCl_2$	magnesium chloride
$MgSO_4$	magnesium sulphate
MNA	methyl nadic anhydride
MOPS	3-(N-morpholino) propane-sulphonic acid
MPOB	Malaysian Palm Oil Board
mRNA	messenger RNA
MW	molecular weight
N	normal
NAA	napthylacetic acid
NaCl	sodium chloride

NaOAc	sodium acetate
ng	nanogram
NO	nitrite oxide
N-terminal	amino terminal
OD	Optical density
ORF	open reading frame
OPZE	oil palm zygotic embryo
OPEm	oil palm embryogenic
OPSSH	oil palm suppression subtractive hybridization
PAGE	polyacrylamide gel electrphoresis
PCR	polymerase chain reaction
pfu	plaque forming unit
pI	isoelectric point
Poly A^+RNA	polyadenylated RNA
PVP	Polyvinylpyrrolidone
PVPP	Polypolyvinylpyrrolidone
RNA	ribonucleic acid
rRNA	ribosomal RNA
RNase	ribonuclease
rpm	revolution per minute
RT	reverse transcriptase
SDS	sodium dodecyl sulfate
TAE	tris acetate EDTA
TBE	tris borate EDTA

TEMED	N,N,N',N'-tetramethylethylenediamine
U	unit
µg	microgram
µl	microliter
µm	micrometer
UPM	Universiti Putra Malaysia
UV	ultraviolet
v/v	volume per volume
WAA	weeks after anthesis
w/v	weight per volume
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranose