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

ONG LI MEI

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

By

ONG LI MEI

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.)

By

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March 2001

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

ONG LI MEI

Mac 2001

Pengerusi : Profesor Madya Dr. K. Harikrishna, Ph.D.

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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

OPEm1 merupakan ‘peroxiredoxin’ yang pertama yang telah dipencilkan daripada palma dan ia berupaya untuk dieksploitasi 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.
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No words will be able to describe my heartfelt gratitude and appreciation to Assoc. Prof. K. Harikrishna for his constant guidance, invaluable advise, stimulating discussions and ideas throughout the course of this project although it may not have been his ‘pet-project’. There has never been a time that he is without an encouraging word when in need. He has been not only a supervisor but also a mentor and friend. Special thanks are extended to other members of my committee, Drs. Tan Siang Hee and Ruslan Abdullah for their advice, comments and guidance whenever sought.

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I certify that an Examination Committee met on 23\textsuperscript{rd} March 2001 to conduct the final examination of Ong Li Mei on her Doctor of Philosophy thesis entitled “An Examination of Embryogenic and Non-Embryogenic Cultures of Oil Palm (\textit{Elaies 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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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.

(ONG LI MEI)

Date: 4/4/2001
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LIST OF ABBREVIATIONS

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
CO₂  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
dH₂O distilled water
DEPC diethyl pyrocarbonate
DMF dimethyl fluoride
DMSO dimethylsulphonyl oxide
DTT dithiothreitol
DXP *dura x pisifera*
EtBr ethidium bromide
EDTA ethylenediaminetetraacetic acid
EGTA ethylene glycol bis- (β-aminoethyle ether)
g gram
HCl hydrochloric acid
His histidine
H₂O₂ hydrogen peroxide
hr hours
IPTG isopropyl-β-D-thiogalactoside
Jacq. Jacquin
LB luria-bertani
k kilo

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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>NaOAc</td>
<td>sodium acetate</td>
</tr>
<tr>
<td>ng</td>
<td>nanogram</td>
</tr>
<tr>
<td>NO</td>
<td>nitrite oxide</td>
</tr>
<tr>
<td>N-terminal</td>
<td>amino terminal</td>
</tr>
<tr>
<td>OD</td>
<td>Optical density</td>
</tr>
<tr>
<td>ORF</td>
<td>open reading frame</td>
</tr>
<tr>
<td>OPZE</td>
<td>oil palm zygotic embryo</td>
</tr>
<tr>
<td>OPEm</td>
<td>oil palm embryogenic</td>
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<tr>
<td>OPSSH</td>
<td>oil palm suppression subtractive hybridization</td>
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<td>PAGE</td>
<td>polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>pfu</td>
<td>plaque forming unit</td>
</tr>
<tr>
<td>pI</td>
<td>isoelectric point</td>
</tr>
<tr>
<td>Poly A⁺RNA</td>
<td>polyadenylated RNA</td>
</tr>
<tr>
<td>PVP</td>
<td>Polyvinylpyrrolidone</td>
</tr>
<tr>
<td>PVPP</td>
<td>Polypolyvinylpyrrolidone</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>rRNA</td>
<td>ribosomal RNA</td>
</tr>
<tr>
<td>RNase</td>
<td>ribonuclease</td>
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<tr>
<td>rpm</td>
<td>revolution per minute</td>
</tr>
<tr>
<td>RT</td>
<td>reverse transcriptase</td>
</tr>
<tr>
<td>SDS</td>
<td>sodium dodecyl sulfate</td>
</tr>
<tr>
<td>TAE</td>
<td>tris acetate EDTA</td>
</tr>
<tr>
<td>TBE</td>
<td>tris borate EDTA</td>
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TEMED: N,N,N',N'-tetramethylethylenediamine

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