UNIVERSITI PUTRA MALAYSIA

AN EXAMINATION OF OIL PALM FLOWERING : MOLECULAR BIOLOGY AND ANATOMICAL ANALYSIS

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By

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IN THE NAME OF ALLAH MOST GRACIOUS MOST MERCIFUL

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LIST OF ABBREVIATIONS

BAP	benzylaminopurine
2-BE	ethyleneglycolmonobutylether
BSA	bovine serum albumin
cDNA	copy DNA
dATP	2' - deoxy-adenosine-5'- triphosphate
dCTP	2'- deoxy-cytidine-5'- triphosphate
dGTP	2'- deoxy- guanosine-5'-triphosphate
dTTP	thymidine- 5'- triphosphate
DEPC	diethyl pyrocarbonate
D X P	dura X pisifera
2,4-D	2,4-Dichlorophenoxyacetic acid
DTT	dithiothreithol
EDTA	ethylenediaminetetraacetic acid
EGTA	ethylene glycol bis- (β-aminoethyl ether)
HCl	hydrochloric acid
hr	hours
IEF	isoelectric focusing
Jacq.	Jacquin

KCl	potassium chloride
kD	kilodalton
LiCl	lithium chloride
$MgCl_2$	magnesium chloride
mRNA	messenger RNA
min	minute (s)
NaCl	sodium chloride
Na_2CO_3	sodium carbonate
NaN3	sodium azide
$(NH_4)_2SO_4$	ammonium sulfate
ORF	open reading frame
PAGE	polyacrylamide gel electrophoresis
PORIM	Palm Oil Research Institute of Malaysia
pI	isoelectric point
PVP	polyvinylpyrrolidone
PVPP	polypolyvinylpyrrolidone
rpm	revolution per minute
SDS	sodium dodecyl sulfate
SEM	scanning electron microscopy
TEMED	N,N,N',N'-tetramethylethylenediamine
UV	ultraviolet

Abstract of dissertation submitted to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Doctor of Philosophy

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By

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Chairman Dr Suhaimi Napis

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Flowering is an important part of oil palm crop production The ability to control flowering will be economically beneficial to the oil palm industry Before control can be exerted, factors that influence flowering must be studied at the level of physiology, biochemistry and molecular biology The morphological aspects of oil palm flower development were examined by light and electron microscopy In

doing so, oil palm flower development was able to be partially staged for future reference to determine the expression pattern of flower-predominant genes.

Protein analysis was carried out to identify unique proteins that are expressed in the vegetative meristem or the floral meristem. Three proteins were identified, the presence or absence of which could be associated with the conversion of a vegetative meristem into an inflorescence meristem. Histological studies made on the flowers produced by the inflorescence meristem revealed that these flowers were not true flowers. These flower-like structures were not found to have any reproductive structures and only contained bracts. This reflected that *in vitro,* under high cytokinin growth condition, the conversion of inflorescence meristem to a floral meristem is disrupted.

Flower-predominant clones were isolated from an oil palm male flower cDNA library by a differential screening procedure. Four out of 10 floral-predominant clones were characterized. Clone 68 has homology to cytochrome P450 while clone 65, 70 and 72 do not have any significant homology with any known functional genes but their characteristics showed that clones 65 and 70 could be a glycoprotein and clone 72, a protein kinase. However, the functions of these genes in oil palm flowering are still unconfirmed.

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

KAJIAN KE ATAS PROSES PENGHASILAN BUNGA POKOK KELAPA SAWIT: BIOLOGI MOLIKUL DAN ANALISA ANATOMI

Oleh

SHARIFAH SHAHRUL RABIAH BT. SYED ALWEE

FEBRUARI 1998

Pengerusi : Dr. Suhaimi Napis

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Pembungaan adalah satu proses yang penting dalam penghasilan tanaman kelapa sawit. Kemampuan untuk mengawal penghasilan bunga dapat memberikan faedah ekeonomi yang lebih baik kepada industri kelapa sawit. Walaubagaimanapun, sebelum pembungaan dapat dikawal, faktor yang mempengaruhi pembungaan perlu dikaji dan ini meliputi aspek fisiologi, biokimia dan biologi molikul.

Aspek morfoloji dalam penghasilan bunga kelapa sawit dikaji dengan menggunakan mikroskop cahaya dan elektron. Dengan cara ini, penghasilan bunga kelapa sawit dapat diperingkatkan sebagai panduan dalam menentukan corak ekspresi gen-gen pembungaan.

Analisa protein dilakukan untuk mengenalpasti perbezaan protein-protein yang diekspres di dalam meristem vegetatif dan meristem bunga. Tiga protein dapat dikenalpasti yang mempunyai kaitan dengan penukaran meristem vegetatif kepada meristem infloresen. Walaubagaimanapun, hasil kajian anatomi ke atas bunga yang dihasilkan oleh infloresen itu menunjukkan bahawa bunga yang dihasilkan adalah bunga yang tidak lengkap. Bunga-bunga ini didapati tidak mengandungi sistem reproduksi. Ini menunjukkan bahawa dalam keadaan *in vitro* dan pertumbuhan pucuk di dalam media yang mempunyai hormon cytokinin yang tinggi, penukaran meristem infloresen kepada meristem bunga terganggu.

Klon-klon pembungaan telah dipencilkan dari cDNA librari bunga jantan kelapa sawit dengan menggunakan kaedah penyaringan berbeza (differential screening). Empat klon telah dikenalpasti. Klon 68 berkemungkinan mengkodkan cytochrome P450 manakala klon 65, 70 dan 72 tidak mempunyai persamaan dengan sebarang gen berfungsi tetapi sifat-sifat proteinnya menunjukkan bahawa protein-protein bagi klon 65 dan 70 ini mungkin berhubungkait dengan glikoprotein dan protein bagi klon 72 pula mungkin berfungsi sebagai protein kinase.

Walaubagaimanapun, fungsi sebenar klon- klon ini di dalam proses pembungaan kelapa sawit masih tidak dapat dikenalpasti.

CHAPTER I

INTRODUCTION

The palm family is generally recognized as a diverse family of woody monocotyledons. There are about 212 genera and about 2500 species that grow in the tropics and subtropics, many of which provide a variety of food and raw materials. The oil palm particularly, is an important species in world trade as a major economic crop. It also has wide usage in the food and non-food industries. Currently, Malaysia is the largest palm oil producer in the world. The production of palm oil has increased tremendously from 0.1 million tonnes in 1960 to 6 million tonnes in 1990 and is predicted to increase to 8 million tonnes by the year 2000. Due to the increase in demand for palm oil, we are always in search of methods to increase the yield of oil palm. Breeding and molecular biology techniques have been used to facilitate increases in yield of oil palm and many other annual oil crops. One area that has not been looked at thoroughly is the control of flowering in oil palm.

Flowers play an essential part in oil palm crop production. As with oil palm and all other fruit crops, the fruit is the final product, but flowers are an important introductory step to fruit formation Flowers and fruits also are an integral part of seed production

Control of flowering, whether positively, by promoting or negatively, by preventing flowering is an important aspect in the growth of most crops In oil palm, if flowering could be controlled or manipulated, yield could be improved by stimulating flowering in accordance to environmental factors resulting in the prevention or reduction of floral abortion

In order to learn which factors influence flowering in oil palm, the physiological background of the action of these factors and the flowering process itself must be studied This is a much more difficult problem and various scientific disciplines such as biochemistry, physiology and molecular biology are needed to provide a better understanding of flowering biology

Since the appearance of fruit and flower abnormalities in clonal palms was first brought to attention in 1986, a lot of effort has been placed towards solving this problem Genetic mutations in the APETALA 3 locus of *Arabidopsis* and DEFICIENS in *Antirrhinum* are found to produce phenotypes similar to the floral abnormality in oil palm Thus by studying floral homeotic mutations, organ identity genes and flower development of oil palm, the problems of floral abnormalities can be better understood and possibly solved

The objective of this thesis is to understand oil palm flowering from the aspects of protein analysis, anatomy and molecular biology. The meristematic behavior of the oil palm shoots in relation to floral abnormalities has been studied. Alterations in protein profile during the development of a vegetative meristem into an inflorescence meristem are examined. The anatomy of oil palm flower during its development is also documented to assist in staging of oil palm flowering. Finally, flower-predominant genes were isolated using differential screening methods. This combination of protein analysis, anatomical studies and molecular biology will provide us with the groundwork needed for a more comprehensive study of oil palm flowering.

CHAPTER II

LITERATURE REVIEW

Botany of Oil Palm

The oil palm, *Elaeis guineensis* Jacq., is classified under the family Palmae in the order Palmales. Based on its anatomy it is grouped with Cocos, Coroza and other genera under the tribe Coconeae. The name *Elaeis* is derived from the Greek word 'elaion' meaning oil while the specific name *guineensis* shows that it originates from the Guinea Coast.

Elaeis guineensis is a large feather-palm having a solitary columnar stem with short internodes. It has short spines on the leaf base and within the fruit bunch. The leaflets are set irregularly on the leaf thus giving the palm its characteristic appearance.

An inflorescence is initiated in the axil of every leaf but some inflorescences abort prior to emergence. Each inflorescence is a compound spike carried on a stout peduncle of about 12-18 inches in length. The spikelets are spirally arranged on a central rachis in a similar manner to the arrangements of the leaves. The inflorescence is tightly enclosed by an inner and outer spathe until about a month after it has fully emerged. Six weeks before anthesis, the outer spathe opens and 2-3 weeks later, the inner spathe opens. Both spathes disintegrate as the inflorescence pushes its way through (Corley et al., 1976).

The fruit is a drupe borne on spikelets that are spirally arranged to form a large compact bunch. The pericarp of the fruit is composed of three typical layers, namely the exocarp (external skin), the mesocarp (outer pulp which contains the palm oil) and the endocarp (a hard shell enclosing the kernel). The kernel is packed with kernel oil and other food reserves for the young embryo. The seed consists of an embryo embedded within the kernel that is externally covered by a testa (seed coat). The fruit type can be divided into three forms, dura, pisifera and tenera, according to the shell thickness. The shell thickness of the oil palm fruit is controlled by a single gene (Beirnaert and Vanderweyen, 1941). The homozygote dura (sh^+, sh^+) has a thick shell while the pisifera (sh^-, sh^-) is shell-less. When a dura crosses with a pisifera, the tenera, a heterozygote with a thin shell surrounded by a ring of fibers in the mesocarp is produced. This results in more oil-bearing mesocarp in the tenera in comparison with the thick-shelled dura fruit.