UNIVERSITI PUTRA MALAYSIA

TISSUE SPECIFIC LOCALIZATION OF SEVERAL OIL PALM GENES DURING FLOWER DEVELOPMENT

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Flowering is the first introductory step to fruit formation and is a fundamental part of the plants reproduction system. Flowers and fruit are also an integral part of seed production. In most crops, the control of flowering is an important aspect of growth and development. If oil palm flowering could be controlled, yield could be improved by stimulating flowering in accordance to permissive environmental factors. In order to determine the factors that influence flowering of oil palm, the physiological background and the flowering process must be studied. However, oil palm micropropagation had come up against a major difficulty with the discovery of a floral morphogenesis abnormality induced by *in vitro* regeneration (Corley et al., 1986; Toruan-Mathius et al., 1998).

From the examination of both morphology and anatomy of oil palm flower development, 9 key stages of normal and abnormal flower development has been classified to assist in

the study of tissue specific expression of flowering genes. As plant organ systems are composed of anatomically similar cells and tissues, *in situ* hybridization was chosen as a method of determining gene expression based on its sensitivity and ability to determine the specific location of an mRNA. Examinations carried out on 4 oil palm flowering genes provide more information about the processes occurring during normal and abnormal flower formation of oil palm. OPSOC1, an oil palm homologue of AGL20 and OPLFY, the LFY homologue of oil palm, are both expressed throughout flower initiation and development. OPRLK5, a member of the receptor kinase gene family, is expressed throughout flower development. The last gene, OPUIP2, which encodes a UFO-interacting protein, is also expressed throughout flower development but it is not needed during inflorescence meristem development. Combined with other studies in this area, it is hoped that an understanding of the floral abnormality may be within reach in the near future.

Attempts to isolate flower specific genes from an oil palm floral cDNA library however have been unsuccessful. The choice of cDNA library and conventional molecular tools might not be applicable in isolating these types of genes. Nevertheless, with advanced molecular and genetic tools such as yeast one and two-hybrid system that are being developed, isolation and the determination of function of such genes can be achieved.

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TISU SPESIFIKASI SETEMPAT BEBERAPA GEN KELAPA SAWIT SEMASA PERKEMBANGAN BUNGA

Oleh

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Bunga adalah langkah pengenalan pertama bagi penghasilan buah dan ia merupakan asas penting bagi sistem reproduksi tumbuhan. Bunga dan buah juga adalah bahagian penting dalam penghasilan biji benih. Bagi kebanyakan tumbuhan, pengawalan bunga merupakan aspek penting bagi perkembangan pertumbuhan. Jika perkembangan bunga kelapa sawit dapat dikawal, hasilnya boleh ditingkatkan dengan mengstimulasi perkembangan bunga berdasarkan faktor-faktor persekitaran. Bagi menentukan faktor-faktor yang mempengaruhinya, kajian latar belakang fisiologi dan proses-proses perkembangan bunga perlu dijalankan. Walau bagaimanapun, mikropropagasi kelapa sawit telah dilanda masalah berikutan penemuan keabnormalan morfogenasi bunga yang dipengaruhi dari regenerasi *in vitro* (Corley et al., 1986, Toruan-Mathius et al., 1998).

Daripada kajian morfologi dan anatomi perkembangan bunga kelapa sawit, 9 tahap perkembangan bagi kedua-dua bunga normal dan abnormal telah diklasifikasikan untuk

membantu kajian corak ekspresi tisu bagi gen-gen perkembangan bunga. Oleh kerana sistem organ tumbuhan mengandungi sel-sel dan tisu-tisu yang hampir sama anatominya, hibridasi *in situ* dipilih sebagai kaedah penentuan ekspresi gen berdasarkan tahap sensitiviti dan kebolehan kaedah berkenaan untuk menentukan lokasi mRNA yang spesifik. Kajian yang dijalankan ke atas 4 gen perkembangan bunga dapat memberi lebih maklumat tentang proses-proses yang berlaku semasa perkembangan bunga normal dan abnormal bagi kelapa sawit. OPSOC1, "homolog" kelapa sawit bagi AGL20 dan OPLFY, "homolog" LFY bagi kelapa sawit, kedua-duanya menunjukkan ekspresi sepanjang pengenalan dan perkembangan bunga. Manakala 2 lagi gen yang digunakan, OPRLK5, terdiri daripada keluarga gen "receptor kinase" dan OPUIP2, gen yang mengkod protin interaksi-UFO, kedua-duanya juga menunjukkan ekspresi sepanjang perkembangan bunga tetapi OPUIP2 tidak diperlukan semasa perkembangan "inflorescence meristem". Digabung dengan kajian-kajian lain di dalam bidang ini, adalah diharapkan pengetahuan berkenaan keabnormalan bunga boleh dicapai di masa hadapan.

Cubaan pemencilan gen-gen spesifik bunga daripada koleksi cDNA bunga kelapa sawit telah menemui kegagalan. Pemilihan koleksi cDNA dan kaedah molekular konvensional mungkin tidak dapat diaplikasikan di dalam pemencilan gen-gen jenis ini. Walau bagaimanapun, dengan adanya kaedah-kaedah molekular dan genetik lanjutan seperti sistem hibrid yis satu dan dua (yeast one and two hybrid system) yang sedang dikembangkan, pemencilan dan penentuan fungsi bagi gen-gen berkenaan akan dapat dicapai.

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LIST OF ABBREVIATIONS

Symbol	Description
%	Percentage
λ	Lambda
μg	Microgram
μL	Microlitre
μm	micrometer
°C	degree centigrade
AG	AGAMOUS
AGL	AGAMOUS-LIKE
Amp	Ampicillin
AtOH	Acetone
BCIP	5-bromo-4-chloro-3-indolyl-phosphate
bp	base-pair
BSA	Bovine Serum Albumin
cDNA	Copy Deoxyribonucleic Acid
Ci	Curie
Cm	Centimeter
dATP	2'-Deoxy-adenosine-5'-triphosphate
dCTP	2'-Deoxy-cytidine-5'-triphosphate
DEF	DEFICIENS
DEPC	Diethyl Pyrocarbonate
dGTP	2'-Deoxy-guanosine-5'-triphosphate

dH_2O	Distilled water
DIG	Digoxigenin
DNA	Deoxyribonucleic Acid
DTT	Dithiothreithol
dTTP	2'-Deoxy-thymidine-5'-triphosphate
EDTA	Ethylenediaminetetraacetic Acid
EtBr	Ethidium Bromide
EtOH	Ethanol
FLIP	Flower Initiation Process
FLO	FLORICAULA
g	Gram
GLO	GLOBOSA
HCl	Hidrochloric Acid
hr	Hour
ISH	*In Situ* Hybridisation
Jacq.	Jacquin
kb	Kilo base-pair
KCL	Potassium Chloride
LB	Luria-Bertani
LFY	LEAFY
LiCl	Lithium Chloride
MADS	MCM1-AGAMOUS-DEFICIENS-SRF

mg	Milligram
$MgCl_2$	Magnesium Chloride
min	Minute (s)
mm	Millimeter
mM	Millimolar
MPOB	Malaysian Palm Oil Board
mRNA	Messenger Ribonucleic Acid
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
NBT	Nitro Blue Tetrazolium
NTE	Sodium-Tris-EDTA Buffer
OD	Optical Density
OPSOC1	Oil Palm SUPPRESSOR OF CONSTANS OVEREXPRESSION
OPLFY	Oil Palm LEAFY
OPRLK5	Oil Palm Receptor-Like-Kinase Factor 5
OPUIP2	Oil Palm UFO-Interacting Protein
PBS	Phosphate Buffer Saline
RNA	Ribonucleic Acid
RNase	Ribonuclease
rpm	Revolution Per Minute
SDS	Sodium Deodecyl Sulphate
SEM	Scanning Electron Microscope
SSC	Sodium Chloride-Sodium Citrate Buffer

SSC	Sodium Chloride-Sodium Citrate Buffer
TAE	Tris-Acetate-EDTA Buffer
TBS	Tris-Base-Sodium Chloride Buffer
TE	Tris-HCL-EDTA
tRNA	Transfer Ribonucleic Acid
UV	Ultraviolet

CHAPTER 1

INTRODUCTION

Tissue culture of oil palm was initiated in order to provide the oil palm industry with improved, high yielding elite palms by cloning mother palms carrying desirable traits. However, oil palm micropropagation, which began at the start of the 80s, has come up against a major difficulty with the discovery of floral morphogenesis abnormalities induced by *in vitro* regeneration (Corley, et al., 1986, Toruan-Mathius et al., 1998). Corley et al. (1986) discovered the first outbreak of floral abnormalities in 1986 and it is now evident that these abnormalities occur at varying levels at various laboratories.

Flowering is the first introductory step to fruit formation and is a fundamental part of the plants reproduction system. Flowers and fruits also are an integral part of seed production. In most crops, control of flowering is an important aspect of growth and development. And, if oil palm flowering could be controlled, yield could be improved by stimulating flowering in accordance to environmental factors.

In order to identify the factors, which influence flowering in oil palm, both the physiological background and the flowering process must be studied. From the examination of both morphology and anatomy of oil palm flower development, 9 key stages of normal and abnormal oil palm flower development has been identified. These

provide a basis for an examination of tissue specific expression of flowering genes during development.

Since the first report of oil palm floral abnormality, a lot of effort has been made to solve the problem. Genetic mutations in the APETALA 3 locus of *Arabidopsis* and DEFICIENS in *Antirrhinum* are found to produce phenotypes similar to the floral abnormality observed in oil palm. Thus perhaps by studying floral homeotic mutations, organ identity genes and flower development in oil palm, the problems of floral abnormalities can be better understood. Combined with information on gene expression patterns during flower development from other plants such as *Arabidopsis*, *Antirrhinum* and maize, predictions on what types of genes that are expressed at different stages of flowering can be made.

The aim of this project is to study the tissue specific and cell type localization of gene expression patterns in different floral organ and to isolate and characterize full-length homeotic genes that are involved in floral patterning and meristem identity. Full length homeotic genes will contribute to the further understanding of the floral abnormality in oil palm by contributing to the development of a DNA chip for the examination of the floral abnormality as many types of homeotic genes are required to be arrayed onto these chips. This project will lead to a further understanding of the function of flower-specific genes since the characterization technique used allows tissue and cell specific patterns of expression to be examined. This will provide a better view of the differences between normal and abnormal flowers.

CHAPTER 2

LITERATURE REVIEW

2.1 Oil Palm

The oil palm (*Elaeis guineensis*, Jacq.) belongs to the family Palmae where *Elaeis* derived from the Greek word "elaion" or oil while the specific name *guineensis* shows its origin, the Guinea coast. The genus *Elaeis* was founded on palms introduced into Martinique, the oil palm receiving its botanical name from Jacquin in an account of American plants. Its natural habitat is believed to have been restricted to ecosystems such as swamps and riverbanks, with minimal competition from faster growing rainforest species.

Apart from being a large feather-like palm, it is unarmed except for short spines on leaflets on the leaf, which give a characteristic appearance to the palm. The palm is normally monoecious with separate male and female inflorescences on the same plant, but sometimes hermaphrodite flowers do occur (Hartley, 1988). The fruit is a drupe borne on a large compact bunch. The fruit consists of an outer *exocarp* or skin, the fruit pulp or *mesocarp,* which provides the palm oil and the *endocarp* or shell.

2.2 Flowering Habit of Oil Palm

The oil palm is a monoecious plant carrying distinct male and female inflorescence in cycles of varying duration. However, detailed investigation of the flowers showed that each flower primordium is a potential producer of both male and