UNIVERSITI PUTRA MALAYSIA

EXAMINATION OF COLD-PLAQUE SCREENING TECHNIQUE AS A MEANS TO ISOLATE LOW ABUNDANCE GENES FROM OIL PALM (ELAEIS GUINEENSIS) FLOWERS

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By

LIM CHIN CHING

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Genes that are present at low abundance in cells are difficult to clone by using standard molecular biology techniques such as conventional differential screening. In plants, many of these low abundance genes encode transcription factors or proteins involved in signal tranduction. Therefore in this study, a cold-plaque screening technique was used as a means to enrich for low abundance genes from an oil palm male flower cDNA library.

When a total of 441 non-hybridising plaques (' cold ' plaques) were isolated, 123 clones (*opcp* population) were found to contain inserts with a minimal size of 500 base-pairs and were independent clones. Initial screening of these clones by reverse northern analysis with the same probes used during differential screening and an additional probe from female flower of 6 cm showed that these *opcp* clones could be categorised into five subpopulations based on their tissue-specificity and expression levels. 61.8 % of the 123 clones were expressed at high abundance with all the three probes (Subpopulation A)

whilst 4.1% of the clones were lowly-expressed in both male and female flower tissues (Subpopulation B). 7.3 % of the clones were expressed at medium abundance but were male-predominant (Subpopulation C) while 11.4 % of the clones were expressed at low abundance and were male-predominant (Subpopulation D) and 15.4 % of the clones did not show any detectable expression with any of the probes used (Subpopulation E).

Partial sequencing of all clones from subpopulation B, C, D and E as well as eight clones from subpopulation A showed that *opcp*72 (subpopulation D) is a putative UIP2 (Unusual Floral Organ (UFO) binding protein) homolog, *opcp*144 (subpopulation A) encodes elongation factor-1α, *opcp*327 (subpopulation E) encodes a putative RLK 5 (Receptor-like Protein Kinase) homolog and *opcp*441 (subpopulation A) is a putative fructose 1,6-bisphosphate aldolase gene.

Expression studies on ten *opcp* clones with two representative clones from each subpopulation showed similar expression profiles where hybridisation signals were detected at the early flower development with higher signals in the meristem tissues but no detectable hybridisation signals in 3.5 cm and 6 cm male flower, one of the stages used to make the male flower cDNA library. Southern hybridisation of genomic DNA for clone *opcp*72, *opcp*144 and *opcp*327 showed that these genes are low copy genes.

In conclusion the use of cold-plaque screening techniques can result in the isolation of a variety of clones whose expression ranges from low abundance (undetectable in the Northern blots), to those that are lowly expressed during the stages of floral development used to construct the oil palm male-flower cDNA library.

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KAJIAN KE ATAS TEKNIK PENYARINGAN COLD-PLAQUE SEBAGAI SATU CARA MENDAPATKAN GEN YANG MEMPUNYAI mRNA YANG SEDIKIT DARIPADA BUNGA KELAPA SAWIT (*ELAEIS GUINEENSIS*)

Oleh

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Gen yang mRNAnya hadir pada bilangan yang rendah di dalam sel adalah susah untuk diklonkan dengan menggunakan teknik biologi molekul yang biasa seperti penyaringan berbeza. Di dalam tumbuhan, gen-gen seperti ini adalah faktor transkripsi atau protein yang terlibat di dalam transduksi isyarat. Oleh itu di dalam kajian ini, teknik penyaringan cold-plaque digunakan sebagai satu cara untuk memperkayakan bilangan gen yang mempunyai mRNA yang sedikit daripada koleksi cDNA bunga jantan kelapa sawit.

Apabila sejumlah 441 plak (plaques) yang tidak menunjukkan sebarang penghibridan ('cold plaque') dipilih, 123 klon (populasi *opcp*) didapati mengandungi sisipan cDNA dengan saiz minima 500 bp dan adalah klon individu. Penyaringan pertama ke atas klon-klon ini dilakukan secara Northern Berbalik. Prob-prob yang digunakan adalah seperti semasa penyaringan berbeza biasa dan prob tambahan dari bunga betina pada saiz 6 cm. Keputusan menunjukkan klon-klon *opcp* boleh dikategorikan kepada lima subpopulasi

berdasarkan kepada kespesifikan tisu dan tahap kedalaman tanda penghibridan. 61.8 % daripada 123 klon menunjukkan ekspresi yang tinggi dengan ketiga-tiga prob (subpopulasi A), manakala 4.1 % daripada klon-klon ini hanya menghasilkan sedikit ekspresi pada tisu bunga jantan dan betina (subpopulasi B). 7.3 % daripada klon-klon ini menghasilkan ekspresi sederhana pada bunga jantan sahaja (subpopulasi C); sementara 11.4 % daripada klon-klon itu menghasilkan sedikit ekspresi pada bunga jantan sahaja. 15.4 % daripada klon-klon tersebut pula didapati tidak menunjukkan sebarang ekspresi dengan sebarang prob yang digunakan (subpopulasi E).

Penjujukan separa yang dilakukan ke atas semua klon daripada subpopulasi B, C, D, E dan lapan klon dari subpopulasi A, menghasilkan pemencilan *opcp*72 (subpopulasi D) yang seakan-akan UIP2. *Opcp*144 (subpopulasi A) pula adalah faktor pemanjangan 1-α, *opcp*327 (subpopulasi E) adalah seakan-akan RLK5 dan *opcp*441 (subpopulasi A) adalah fructose1,6-bisphosphate aldolase.

Kajian ekspresi ke atas sepuluh klon *opcp* terpilih dengan dua wakil daripada setiap subpopulasi. Corak ekpresi yang sama didapati untuk kesemua klon di mana tanda penghibridan dikesan pada awal pertumbuhan bunga terutama pada tisu meristem yang memberi tanda penghibridan yang dalam. Namun tiada tanda penghibridan dikesan pada bunga jantan pada saiz 3.5 cm dan 6 cm, satu daripada peringkat-peringkat yang digunakan untuk membuat koleksi cDNA bunga jantan kelapa sawit. Penghibridan Southern organisasi DNA untuk klon-klon *opcp*72, *opcp*144 dan *opcp*327 menunjukkan gen-gen ini adalah gen-gen yang mempunyai salinan yang sedikit.

Kesimpulannya, penggunaan teknik penyaringan cold-plaque membolehkan pelbagai jenis klon dipencilkan; di mana julat ekspresinya adalah dari rendah keseluruhannya kepada rendah pada peringkat pertumbuhan bunga jantan yang digunakan untuk membuat koleksi cDNA bunga jantan kelapa sawit.

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LIST OF ABBREVIATIONS

Symbol	Description
%	percentage
α	alpha
β	beta
λ	lambda
µg	microgram
µl	microliter
µm	micrometer
2,4 -D	2,4-Dichlorophenoxyacetic Acid
2-BE	ethyleneglycolmonobutylether
Amp	Ampicillin
BAP	benzylaminopurine
bp	base-pair
BSA	Bovine Serum Albumin
cDNA	Copy Deoxyribonucleic Acid
cm	centimeter
D X P	Dura X Pisifera
dATP	2' - Deoxy-adenosine-5' - triphosphate
dCTP	2' - Deoxy-cytidine-5'-triphosphate
DEPC	Diethyl Pyrocarbonate
dGTP	2'-Deoxy-guanosine-5'-triphosphate
DNA	Deoxyribonucleic Acid

DTT	Dithiothreithol
dTTP	Thymidine-5'-triphosphate
EDTA	Ethylenediaminetetraacetic Acid
EGTA	Ethylene Glycol Bis- (β-aminoethyle Ether)
EtBr	Ethidium Bromide
g	gram
HCl	Hydrochloric Acid
hr	hour
Jacq.	Jacquin
kb	kilobase-pair
KCl	Potassium Chloride
LB	Luria-Bertani
LiCl	Lithium Chloride
M	Molar
mg	milligram
min	minute (s)
mm	millimeter
mM	millimolar
mRNA	messenger RNA
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
ng	nanogram
O.D.	Optical Density

PORIM	Palm Oil Research Institute of Malaysia
PVP	Polyvinylpyrrolidone
PVPP	Polypolyvinylpyrrolidone
RNA	Ribonucleic Acid
RNase	Ribonuclease
rRNA	Ribosomal Ribonucleic Acid
rpm	Revolution Per Minute
SDS	Sodium Dodecyl Sulphate
TAE	Tris Acetate EDTA
UV	Ultraviolet
°C	degree Centigrade

CHAPTER I

INTRODUCTION

Palm oil is one of the leading vegetable oils traded in the world market. In order to fulfill the increasing demand for palm oil, research and development is needed to fulfill the need for quality improvement. One area of research that has received interest is the flowering habits of the crop since palm oil is obtained from the fruits and flowering is an important introductory step to fruit formation.

Flower development is determined by both genetic and environmental factors. Although the process is responsive to environmental influence, it is primarily under genetic control (Gasser et al., 1989). This has been demonstrated in plants such as *Arabidopsis thaliana* and *Antirrhinum majus* where the formation and function of flowers are well-conserved. Therefore a detailed understanding of the mechanisms regulating gene expression in the flower

Oil palm flower

such as biochemistry, cytology and molecular biology (Shahrul, 1998). Nevertheless a logical approach to understanding floral development would be to identify the various genes that are expressed in the various tissues, to characterise their regulation and to determine the nature of their products. Hence cloning of these genes will provide molecular markers for the analysis of flower development as well as facilitate the basis of their tissue-specific regulation.

As a first step towards addressing this issue, a cDNA library has been constructed from the tissues of 4-6cm male flowers and was used for differentially screening. However only four flower-predominant cDNA clones were identified (Shahrul, 1998). Conventional differential screening technique is limited by its low sensitivity and precludes the detection of low abundance genes. (Gasser et al., 1989).

Low abundance genes represent about 30% of the different mRNA sequences in the mRNA population (Sabelli, 1996). The low transcript abundance could be the result of expression being confined to a single cell type in a complex tissue or organ (Galau et al., 1977; Hodge et al., 1992). They are the manifestation of a detailed programme of structural gene regulation (ibid.). Nevertheless, the low transcript abundance could also be due to developmentally regulated expression. Developmentally regulated genes are present in very small quantities, and often for only short periods of time (Sargent & Dawid, 1983; Gasser et al., 1988; Smith et al., 1990). Most of them encode transcription factors, signal transduction components and membrane receptors (Yoshida et al.,1994; Huang, 1996; Scutt, 1997; Schmidt et al ,1997; Li et al.,1998; Frugier et al, 1998) which play key roles in establishing structures, patterns and regulating developmental processes. It is likely that members of such genes are uniquely expressed in 4-6cm oil palm male flowers at low abundance.

Several improved and highly sensitive techniques have been developed to enrich for low abundance genes, such as subtractive library screening (Duguid et al., 1990; Rubeinstein et al., 1990; Sive and St. John, 1988) and differential display reverse-transcription polymerase chain reaction (DDRT - PCR) (Goormachtig et al., 1995;

Heidstra et al., 1997). However all these techniques seem to isolate low abundance genes that are tissue- or organ specific (Hodge et al., 1992). Cold-plaque screening technique (Hodge et al., 1992) serves as an alternative means to isolate low abundance genes that are the result of all of the above processes. This technique utilises polymerase-chain reaction (PCR) to enrich for low abundant transcripts. It is simple and yet powerful especially when coupled with high throughput automated sequencing. It has been used successfully in the isolation of low- or medium abundant transcripts from various cDNA libraries (Ng et al., 1996; Schmidt et al., 1997; Frugier et al., 1998).

This study looks into the potential and efficiency of cold-plaque screening as a means to isolate low abundance cDNA clones from an oil palm male flower cDNA library. The isolation of these genes may facilitate the study of oil palm flower development.

CHAPTER II

LITERATURE REVIEW

Flower Development

Molecular Biology of Flowering

It has been well established that the modulation in expression of genes control floral development (Jordan et al., 1993). Thus studies on the molecular aspects of floral development is essential to facilitate the understanding of floral development.

Flowers are determinate sporophyll-bearing shoots that hold the organ for gametogenesis and fertilization. The flowering process is complex and it involves the transition of the shoot apex from vegetative to reproductive growth (Jordan et al., 1993). O'Neill in 1993 summarised and classified the flowering process into four sequential component stages : (i) floral induction; (ii) transduction of the induced state to the meristem; (iii) floral evocation of the meristem ; (iv) organogenesis. These stages can be considered as homeotic changes as they involve the replacement of the meristem or organs (Jordan et al., 1993). Homeotic genes have been identified which control both transition as well as organogenesis of floral development (Coen, 1991; Coen & Meyerowitz, 1991). These genes are involved in the spatial arrangement of cells and tissues within the organism.

Flowering begins with an inductive process that occurs in the vegetative shoot apex. Through a translocatable stimulus, floral evocation in the shoot meristem is

triggered. According to Herdenberger (1990), these processes are believed to be controlled by several biochemical and physiological systems. Initiation of a floral meristem then marks the beginning of the floral developmental process. At this time, floral meristem identity genes are activated to promote flower- meristem fate (Jordan et al., 1993). These genes are involved in the establishment of floral meristem identity and the inactivation of these genes causes partial transformation of flowers into inflorescence shoots.

Meristem Identity Genes

Flower-meristem identity genes have been identified in *Antirrhinum majus* and *Arabidopsis thaliana*. These genes have been cloned and their expression pattern analyzed. In *Antirrhinum*, these genes are FLORICAULA (FLO) and SQUAMOSA (SQUA) (Coen et al., 1990; Huijser et al., 1992). LEAFY (LFY) (Weigel et al., 1992) and APETALA 1 (AP1) are Arabidopsis orthologs (functional homolog) of FLO and SQUA. FLO and LFY are single-copy genes and their putative protein possess amino acids motifs that suggest a transcriptional regulatory function (Coen et al., 1990; Weigel et al., 1992), whereas SQUA and AP1 are members of the MADS- box family (Huijser et al., 1992; Mandel et al., 1992). The MADS -box, in reference to the four founding proteins (MCM1, AG, DEFA and SRF) is a highly conserved motif within the N-terminus region that contains activities that are sufficient for DNA-binding and transcriptional activation (Christ and Tye, 1991). The pair of floral meristem identity genes LFY/FLO and AP1/SQUA are activated independently and in each species, at least two meristem identity genes act together to promote flower development. For example, in *Antirrhinum*,