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SCREENING FOR ACYL CARRIER PROTEIN (ACP) GENE OF OIL PALM (ELAEIS QUINEENSIA) USING A SYNTHETIC ACP DNA PROBE

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By

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IN THE NAME OF ALLAH, MOST GRACIOUS, MOST MERCIFUL.

Dedicated To :

My Wife : Nor Cahaya Idris

My Parents : Abd. Manaf and Perubisah

My Brothers and Sisters

My Teachers and Friends .....

Tidak suatu bencana pun yang menimpa di bumi dan (tidak pula) pada dirimu sendiri melainkan telah ditulis di dalam kitab (Lauhul Mahfuzh) sebelum Kami menciptakannya. Sesungguhnya yang demikian itu adalah mudah bagi Allah. Kami jelaskan yang demikian itu supaya jangan kamu berdukacita terhadap apa yang luput dari kamu dan supaya kamu jangan terlalu gembira terhadap apa yang diberikanNya kepadamu. Dan Allah tidak menyukai setiap orang yang sombong lagi membangga diri.

(57 : 22 - 23)

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TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

The following abbreviations were used in the text :

ACP	Acyl carrier protein
Ala (A)	Alanine
Arg (R)	Arginine
Asn (N)	Asparagine
Asp (D)	Aspartic acid
ATP	Adenosine triphosphate
BCCP	Biotin carboxyl carrier protein
cDNA	Complimentary deoxyribonucleic acid
CoA	Coenzyme A
Cys (C)	Cysteine
ddNTP	Dideoxynicotinamide triphosphate
dNTP	Deoxynicotinamide triphosphate
EDTA	Ethylenediaminetetra acetic acid
EGTA	Ethyleneglycol bis ß aminoethyl ether tetra acetic acid
FAS	Fatty acid synthetase
Gln (Q)	Glutamine
Glu (E)	Glutamic acid
Gly (G)	Glycine
His (H)	Histidine

Ile (I)	Isoleucine
IPTG	Isopropyl-ß-D-thiogalactopyranoside
Leu (L)	Leucine
Lys (K)	Lysine
Met (M)	Methionine
mRNA	Messenger ribonucleic acid
$NADP^+$	Nicotinamide adenine dinucleotide phosphate (oxidized form)
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
PAS	4-amino salicylic acid
PC	Phosphatidylcholine
Phe (F)	Phenylalanine
Pro (P)	Proline
SDS	Sodium dodecyl sulfate
Ser (S)	Serine
Thr (T)	Threonine
TNAS	Tri-isopropylnapthalene foric acid
Trp (W)	Tryptophan
Tyr (Y)	Tyrosine
Val (V)	Valine
xGAL	5-Bromo-4-chloro-3-indoyl-ß-D-galactopyranoside

Abstract of thesis submitted to the Senate of Universiti Pertanian Malaysia in fulfilment of the requirement for the degree of Master of Science.

SCREENING FOR ACYL CARRIER PROTEIN (ACP) GENE OF OIL PALM (*Elaeis guineensis*) USING A SYNTHETIC ACP DNA PROBE

By
MOHAMAD ARIF B. ABD. MANAF
April 1994

Chairman : Assoc. Prof. Abdullah Sipat, Ph. D.
Faculty : Science and Environmental Studies.

A cDNA library was constructed in phagemid vector pT7T3 using messenger RNA (mRNA) extracted from 15-week oil palm mesocarp as template. The cDNA inserts were detected from agarose gel analysis and ranged in length from 130 to 1300 base pairs. This library was screened with a synthetic ACP probe. Of the 2,000 clones screened using colony hybridisation analysis, 550 clones which gave high intensity of signal were then further screened using phagemid blot hybridisation analysis. This method yielded 184 clones which showed a high intensity of signal. The phagemids from these clones were then screened by

Southern hybridisation analysis using a synthetic ACP DNA probe. Two phagemid samples gave positive signal and were arbitrarily named pOP-MA79 and pOP-MA94. The length of insert cDNA in both phagemids was 338 bases. DNA sequencing of these insert cDNA was performed using the positive single strand DNA as template. Cross hybridisation, Northern blot analysis and DNA sequencing have revealed that both putative ACP clones are the same gene. Comparison of the DNA sequences of pOP-MA79 and pOP-MA94 with the sequence of ACP genes from other plants was carried out using the Microgenie Sequence Analysis Program. The degree of similarity between this putative oil palm ACP clone and spinach ACP-I gene in nucleotide level was found to be 50% whilst in amino acid level was 22%.

Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia sebagai memenuhi syarat untuk mendapatkan Ijazah Master Sains.

PENYARINGAN GEN PROTEIN PEMBAWA ASIL (ACP) DARIPADA KELAPA SAWIT (Elaeis guineensis) MENGGUNAKAN PROB DNA ACP SINTETIK

Oleh
MOHAMAD ARIF B. ABD. MANAF
April 1994

Pengerusi : Prof. Madya Abdullah Sipat, Ph. D.
Fakulti : Sains dan Pengajian Alam Sekitar

Himpunan cDNA dibina di dalam vektor fajmid pT7T3 menggunakan RNA pengutus (mRNA) sebagai templat. RNA pengutus diekstrak daripada mesokap kelapa sawit berusia 15 minggu. Sisip cDNA telah dikesan di dalam analisis gel agarosa. Julat saiz sisip cDNA adalah di antara 130 hingga 1300 pasangan bes. Himpunan cDNA telah disaring dengan prob sintetik yang ditulenkan daripada pPB269. Penyaringan melalui penghibridan koloni terhadap 2000 koloni menghasilkan sebanyak 550 koloni yang memberikan isyarat keamatan yang tinggi. Sejumlah 550 koloni tersebut seterusnya disaring melalui penghibridan titik. Sebanyak 184 titik yang setiap satunya mewakili fajmid daripada koloni

tertentu menghasilkan isyarat keamatan yang tinggi. Sisip cDNA daripada koloni-koloni tersebut kemudiannya disaring melalui penghibridan Southern. Sebanyak 2 klon menghibrid dengan prob ACP sintetik. Kedua-dua klon ini dinamakan pOP-MA79 dan pOP-MA94. Sisip cDNA di dalam kedua-dua klon ACP putatif bersaiz 338 pasangan bes. Penjujukan DNA dilakukan dengan menggunakan DNA bebenang tunggal positif sebagai templat. Penghibridan silang, analisis Northern dan penjujukan DNA membuktikan bahawa kedua-dua klon putatif tersebut merupakan klon yang sama. Perbandingan di antara klon ACP putatif dengan gen-gen ACP daripada tumbuhan lain dilakukan menggunakan Program Analisis Jujukan Microgenie. Darjah persamaan di antara klon ACP putatif dan gen ACP-I bayam pada peringkat nukleotida adalah 50% sementara pada peringkat asid amino adalah 22%.

CHAPTER 1

INTRODUCTION

Vegetable oil production is an important sector of modern agriculture. The world total production of vegetable oil in 1991 was 54 million metric tonnes (MT), with a market value of 39 billion US dollars. The major oil crops contributing to this output are oil palm, soyabean, rapeseed and sunflower (PORLA, 1993). The importance of biotechnology in the high world oil output is undeniable. As an example, in 1987 up to 2.6 billion US dollars of the annual return has been contributed to advances in agricultural biotechnology (Holbrook and Scowcroft, 1988).

Edible and industrial vegetable oils can thus be exploited through the use of biotechnology. Perhaps one of the major areas of applications of biotechnology is in the modification of oilseeds for specific market use. This area would involve studies in seed triacylglycerol synthesis and compartmentation (Holbrook and Scrowcroft, 1988). Such studies will facilitate efforts to modify the fatty acid composition of plant lipids. Therefore, there is

substantial interest in the understanding of the biochemistry of the synthesis of oil and the regulation of its composition.

The oils that are found in plants consist largely of mixtures of triacylglycerols. Triacylglycerols differ according to the type and placement of their three fatty acid residues within the glyceryl backbone of the molecule. Chain elongation of fatty acids is affected by a series of C_2 condensation from malonyl CoA. This reaction is catalysed by a fatty acid synthase complex consisting of malonyl-CoA-ACP transacylase, ß-ketoacyl-ACP synthase, ß -ketoacyl-ACP reductase, ß-hydroxyacyl-ACP-dehydrase, enoyl-ACP reductase and palmitoyl thioesterase.

One of the proteins involved in the fatty acid synthase is an acyl carrier protein (ACP). The long flexible phosphopantetheine chain of ACP transfers the substrates between the various catalytic sites of fatty acid synthase complex. Therefore, it is important as a cofactor in fatty acid synthesis. In the assembly of C_{16} and C_{18} fatty acids, ACP serves to

the next while preserving the high energy or activated state inherent in a thioester bond. For example it carries the acyl chain for ß-ketoacyl-ACP synthase I which is involved in synthesizing fatty acids up to C_{16} and ß- ketoacyl ACP synthase II which is responsible for the conversion of C_{16} and C_{18}.

The oil palm, Elaeis guineensis, is one of the most important edible oils and fats producer in the world. By the year 2000, palm oil is likely to meet about 21% of world demand (Mielke, 1993). Palm oil is mainly made up of 39% oleic acid and 44% palmitic acid and has an iodine value (IV) of 56. A report by a Task Force set up by Palm Oil Research Institute of Malaysia (PORIM) to study various aspects of oil composition in oil palm recommended the production of palm oil with an IV of more than 72 and having an oleic acid content of more than 60% and that of palmitic acid less than 25%. It is envisaged that such an oil will facilitate palm oil's entry into the liquid oil market as well as providing oleic acid feedstock for the oleochemical industry.

In an attempt to alter the composition of oil palm to meet the above specifications , a strategy

focussing on the enzymes controlling the synthesis of oleic acid in the oil palm is employed. For example, the palmitate can be diverted to form oleate in the pathway by chain elongation followed by desaturation.

Thus, understanding the role of ACP in fatty acid synthesis is relevant in efforts to modify the oil palm lipid composition. There is as yet no published report on the successful isolation of ACP gene from oil palm although report on its isolation from other plants such as barley, spinach and rapeseed have been published. This study on screening of ACP gene of oil palm using a synthetic DNA probe is thus the first step towards the isolation of ACP gene from oil palm.

The objectives of this study, are :

1) To construct a cDNA library from the mesocarp RNA of oil palm *Elaeis guineensis*,
2) To screen this library for the putative ACP clones using a synthetic DNA probe,
3) Sequencing of putative ACP clones and
4) Analysis of nucleotide and predicted amino acid sequences.

CHAPTER 2

LITERATURE REVIEW

The Importance of Lipids

Lipids are a heterogenous class of compounds which is soluble in organic solvents such as chloroform but not in water or polar solvents. The major classes of lipids are fatty acids, triacylglycerols, glycerophospholipids, sphingolipids and cholesterol.

Plant lipids are present in the fruit or seeds as fats or oils. Fats and oils differ in that the former are solid while the later are liquid at room temperature. Field crops such as peanuts, flax, rapeseed, sunflower, and palm contained approximately 45 % of the seed weight as oil (Slabas and Fawcett, 1992).

Plant oil is currently commercially produced in excess of 60 million metric tons annually (Battey et al., 1989). It is used for human consumption as well in the manufacturing industries, particularly for the