UNIVERSITI PUTRA MALAYSIA

BIOPANNING FOR PEPTIDES THAT INTERACT WITH 2, 4-DICHLOROPHENOXYACETIC ACID AND THEIR DETECTION IN THE MEDIUM OF OIL PALM SUSPENSION CULTURE.

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By

NURWATTI MD ARIS

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

June 2007

This thesis is dedicated to my family especially to my dad, mum, grandpa, grandma, uncle and auntie,

Friends & to the beloved one EZERI FITRI RAMLI

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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June 2007

Chairman: Associate Professor Tan Wen Siang, PhD

Faculty: Biotechnology and Biomolecular Sciences

2, 4- dichlorophenoxyacetic acid (2, 4-D) is one of the commonly used plant growth regulators in somatic embryogenesis of oil palm cultures. However, high concentrations of 2, 4- D have been associated with abnormalities deriving from these cultures. Therefore, to discern the optimal concentration of 2, 4-D that can be safely used in oil palm tissue culture, a phage-ELISA (Enzyme Linked Immunosorbent Assay) was developed. Biopanning was carried out for four rounds, by incubating a pool of disulfide constrained phage displayed peptide library with 2, 4-D that had been immobilized onto a microtiter plate. The unbound phages were washed away while the bound phages were eluted and amplified in *Escherichia coli*. The ssDNAs of phages from the 3rd and 4th rounds of biopanning were extracted and sequenced. Two phages bearing the

C-TQANRLT-C and C-IPGKPFY-C sequences interacted with 2, 4-D in ELISA. A capture dot blot assay, which applys colorimetry in the detection of 2, 4-D, was established and the peptide C-IPGKPFY-C gave a better colour formation compared to C-TQANRLT-C peptide. The intensity of the colour formed is indicative of the binding capacity of the peptide. The phages were used as capturing antigens for the development of the phage-ELISA and the sensitivity of the assay was 1-10 ng/ml. The selected peptides were used for detecting 2, 4-D in actual Murashige & Skoog (MS) liquid medium containing oil palm suspension culture. Difference in absorbance value in the positive and negative culture was only shown in the media that have been centrifuged at 5000 rpm. The C-IPGKPFY-C phage was found to cross react with thiamine molecule that has similar aromatic ring structure with 2, 4-D, in the suspension culture.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENYARINGAN PEPTIDA YANG BERINTERAKSI DENGAN MOLEKUL 2, 4- DICHLOROPHENOXYACETIC ACID DAN PENGESANAN DI DALAM MEDIA KULTUR AMPAIAN KELAPA SAWIT

Oleh

NURWATTI MD ARIS

Jun 2007

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Fakulti: Bioteknologi dan Sains Biomolekul

2, 4- dichlorophenoxyacetic acid (2, 4-D) adalah sejenis molekul pengaruh tumbuhan yang banyak digunakan di dalam kultur tisu kelapa sawit terutamanya sel embrio somatik. Namun begitu, di dalam aras yang tinggi, molekul ini mampu mengaruh ketidaknormalan terhadap kultur tisu kelapa sawit. Asai immunojerapan berpaut enzim (ELISA) telah dikendalikankan bagi mengenalpasti tahap optimum aras penggunaan molekul pengaruh 2, 4-D. Teknik peptida pameran faj telah digunakan dalam membentuk teknik pengasaian ELISA untuk mengesan kehadiran molekul 2, 4-D. Proses penyaringan terhadap molekul 2, 4-D telah dilakukan sehingga 3-4 pusingan dengan menggunakan perpustakaan peptida pameran faj yang terbatas secara disulfida. Faj yang tidak terikat pada molekul 2, 4-D akan dibuang manakala

yang terikat dengan kuat akan disimpan dan diperbanyakkan dengan menggunakan *Escherichia coli* sebagai hos. Rantai bebenang tunggal faj dari proses penyaringan pusingan 3 dan 4 telah diekstrasi dan dilakukan proses penjujukan. Dua faj yang membawa jujukan asid amino C-TQANRLT-C dan C-IPGKPFY-C berinteraksi dengan 2, 4-D apabila diuji dengan ELISA. Pengasaian dot blot secara tangkapan telah dilakukan untuk menentusahkan pengikatan faj terpilih terhadap sasaran. Jujukan asid amino C-IPGKPFY-C telah memberi signal yang kuat berbanding faj yang membawa jujukan C-TQANRLT-C. Faj yang membawa peptida tertentu telah digunakan untuk mengesan antigen dan tahap sensitiviti adalah di antara 1-10 ng/ml. Peptida yang terbaik telah digunakan untuk mengesan kehadiran 2, 4-D di dalam medium MS bersama kultur ampaian kelapa sawit. Perbezaan nilai bacaan penyerapan antara kultur positif dan negatif dapat dilihat selepas diempar pada 5000 rpm. Peptida yang membawa jujukan asid amino C-IPGKPFY-C didapati bertindak balas dengan molekul thiamin di dalam kultur ampaian yang mempunyai satu struktur cincin aromatik yang serupa dengan molekul 2, 4-D.

ACKNOWLEDGEMENTS

It is my great pleasure to express my appreciation to all my supervisors, Associate Professor Dr. Tan Wen Siang (UPM), Dr. Janna Ong Abdullah (UPM), Dr. Ahmad Tarmizi Hashim (MPOB) and Dr. Meilina Ong Abdullah (MPOB) for their invaluable guidance and support.

I would like to express my gratitude to Professor Datin Dr. Khatijah Yusoff from the Virology Laboratory, Department of Microbiology, Faculty of Biotechnology and Biomolecular Sciences for allowing me to conduct experiments in her laboratory.

A lot of thanks to Max for helping me with many of things. Thank you so much for your understanding. For me, you are such a good friend and a good sister.

My sincere thank goes to the member of the Virology Laboratory in Faculty of Biotechnolgy and Biomolecular Sciences: Tan Geok Hun, Tang Kah Fai, Tan Yan Peng, Siti Salwa Hasmoni, Puan Raha Raus, Tang Kie Hie, Ho Chin Woi, Andrew Lee Lin Kiat, Sim Han Koh, Michelle Ng Yeen Tan, Ong Swee Tin, Mas Jaffri Masarudin and others.

I am grateful to all staff members of the Tissue Culture Laboratory, Advance Biotechnolgy and Breeding Center (ABBC), Biology Division, Malaysia Palm Oil Board (MPOB): Puan Zaiton, Puan Fajariah, Puan Mimi, Puan Siti Ajar, Puan Miza, Cik Faizah and others.

Deepest appreciation to Malaysia Palm Oil Board (MPOB) and Universiti Putra Malaysia (UPM) for the financial support and also the research facilities provided throughout the course of this study.

My appreciation also goes to all my friends especially to Norfakhrana Iskandar and Azean Iza Ahmad Anuar. Last but not least to my family and to my beloved one Ezeri Fitri bin Ramli for his encouragement and never ending love.

I certify that an Examination Committee has met on 18 June 2007 to conduct the final examination of Nurwatti Md Aris on her Master of Science thesis entitled "Biopanning For Peptides That Interact With 2, 4- Dichlorophenoxyacetic Acid And Their Detection In The Medium Of Oil Palm Suspension Culture" 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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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master Science. The members of the Supervisory Committee are as follows:

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Date: 13 September 2007

DECLARATION

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.

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NURWATTI MD ARIS

Date: 15 August 2007

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LIST OF ABBREVIATIONS

%	percentage
°C	degree centrigrade
2, 4-D	2, 4-dichlorophenoxyacetic acid
A_{405}	absorbance at wavelenght 405 nm
ABA	abscisic acid
ABTS	2, 2′-Azino-bis [3-ethylbenzothiazoline-6-sulfonic acid] diammonium salt
BSA	bovine serum albumin
C- terminus	carboxyl terminus
dH_2O	distilled water
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetate
ELISA	enzyme-linked immunoabsorbent assay
g	gram
gp3 & gp8	product of M13 genes 3 and 8
h	hour
HRP	horseradish peroxidase
IAA	indole-3-acetic acid
IPTG	isopropyl-β-D-thiogalactopyranoside
l	litre

LB	luria bertani
M	molar
mAb	monoclonal antibody
mg	milligram (10^{-3} g)
min	minute
ml	millilitre (10^{-3} l)
mm	millimetre (10^{-3} m)
mM	millimolar (10^{-3}M)
MS	Murashige and Skoog
MW	molecular weight
NAA	naphthalene acetic acid
NC	nitrocellulose
N-terminus	amino terminus
OD	optical density
PEG	polyethylene glycol
pfu	plaque forming unit
pH	*Puissance hydrogen*
pmol	picomol
rpm	revolutions per minute
s	second
ssDNA	single-stranded DNA
TBE	tris-buffered EDTA solution

TBS	tris-buffered saline
TE	tris-EDTA buffer
UV	ultraviolet
v	volt
V	volume
w/v	weight/volume
x *g*	centrifugal force
X-gal	5-bromo-4-chloro-3-indole-β-D-galactopyranoside
α	alpha
β	beta
µg	micrograms (10^{-6} g)
µl	microlitre (10^{-6} l)

Amino Acid Abbreviations

	One letter code	**Three letter code**
Alanine	A	Ala
Arginine	R	Arg
Asparagine	N	Asn
Aspartic acid	D	Asp
Cysteine	C	Cys
Glutamic acid	E	Glu

Glutamine	Q	Gln
Glycine	G	Gly
Histidine	H	His
Isoleucine	I	Ile
Leucine	L	Leu
Lysine	K	Lys
Methionine	M	Met
Phenylalanine	F	Phe
Proline	P	Pro
Serine	S	Ser
Threonine	T	Thr
Tryptophan	W	Trp
Tyrosine	Y	Tyr
Valine	V	Val

CHAPTER 1

INTRODUCTION

The improvement of crop plants is largely due to various researches in plant genetics, breeding and tissue culture (Rohani *et al.*, 1994). Clonal propagation of oil palm by the tissue culture method has been developed by various groups. However, there are still a lot of basic studies that need to be conducted in order to understand the tissue culture system. One of them is to quantify the hormonal levels in oil palm cultures. Plant hormone research has been carried out basically on the hormones themselves, their syntheses, and distribution within tissue, displacement and physiological effects.

Plant hormone or plant growth substances play a major role in regulating growth. These compounds, which are generally active at very low concentrations, are produced in one tissue and transported to another where they cause physiological responses. Synthetic chemicals with similar biological properties with plant hormones have been known as plant growth regulators. Both the natural and synthetic hormones can influence a single aspect of plant

growth and development, and particular responses by changing the ratios of the hormones.

2, 4- dichlorophenoxyacetic acid (2, 4-D) is a herbicide that is most widely used to control weed growth. Besides that, as a synthetic compound, it also can be applied in tissue culture system to regulate the growth of culture especially in the development of callus. However, clonal propagation of oil palm by the tissue culture method has led to the production of ramets (clonal offsprings) exhibiting various abnormalities even though they have been supplemented with the same concentration of 2, 4-D. Assaying for the hormones is attempted to understand the level of 2, 4-D that might can cause the abnormalities. Hormonal profiles related to the growth development have shown that cultures established from normal palms had different levels of endogenous hormones compared to abnormal palms (Ng, 1994).

Monoclonal antibodies have been proven to be useful sources of specific diagnostic reagents for plant diseases (Torrance, 1995) and plant hormones (Franek *et al.*, 1994). However, the procedures for their production and maintenance have some disadvantages because it is costly for an established specialized mammalian cell culture system as well as storage of cell lines in liquid nitrogen.