



UNIVERSITI PUTRA MALAYSIA

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

NURWATTI MD ARIS

FBSB 2007 15



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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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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 applies 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**

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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 dikendalikan bagi mengenalpasti tahap optimum aras penggunaan molekul pengaruh 2, 4-D. Teknik peptida pameran faj telah digunakan dalam membentuk teknik pengasaan 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 diperbanyak 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. Pengasaan 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 ampaiian 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 ampaiian yang mempunyai satu struktur cincin aromatik yang serupa dengan molekul 2, 4-D.

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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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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.

NURWATTI MD ARIS

Date: 15 August 2007



TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	v
ACKNOWLEDGEMENTS	vii
APPROVAL	ix
DECLARATION	xi
LIST OF TABLES	xiv
LIST OF FIGURES	xv
LIST OF ABBREVIATIONS	xvii
CHAPTER	
1 INTRODUCTION	
Introduction	1
2 LITERATURE REVIEW	
2.1 Plant tissues culture	5
2.2 The oil palm tissues culture	10
2.3 Plant growth regulators	14
2.3.1 Auxins	15
2.3.2 Cytokinins	18
2.3.3 Gibberellins, abscisic acid and ethylene	20
2.4 Phage display peptide technology	22
2.4.1 Filamentous bacteriophages (Ff)	28
2.4.2 Phage display system	32
2.4.3 Phage displayed peptide library	34
2.5 Hormone assay development	35
2.5.1 Phage-ELISA	36
3 METHODOLOGY	
3.1 Selection of phage displayed peptides that interact with 2, 4-D	
3.1.1 Media and solutions used in biopanning	38
3.1.2 Biopanning	40
3.1.3 Phage titration	42
3.1.4 Plaque amplification	42
3.1.5 Phage single stranded DNA (ssDNA) extraction	43
3.1.6 Agarose gel electrophoresis	44
3.1.7 DNA sequencing	44
3.1.8 Large scale preparation of fusion M13 bacteriophage	45



3.1.9	Cesium chloride ultracentrifugation	46
3.1.10	ELISA and the optimization of the concentrations of fusion phage, tween 20 and blocking buffer	47
3.2	Phage-dot blot assay	48
3.3	Dot blot assay	49
3.4	Analysis of 2, 4-D in liquid MS medium	
3.4.1	Preparation of Murashige and Skoog (MS) Stock and Friable Callus	50
3.4.2	Suspension culture preparation	52
3.4.3	Phage-ELISA for detection various concentration of purified 2, 4-D, vitamin and in the liquid MS medium containing suspension culture	52
4	RESULTS AND DISCUSSION	
4.1	Biopanning and phage titration	53
4.2	Phage single stranded DNA (ssDNA) extraction and agarose gel electrophoresis	54
4.3	DNA sequencing	57
4.4	Large scale preparation and purification of fusion M13 bacteriophage using cesium chloride ultracentrifugation	64
4.5	Phage-ELISA and the optimization	65
4.6	Phage-dot blot assay and dot blot assay	73
4.7	ELISA for detection various concentration of 2, 4-D	74
4.8	ELISA against actual tissue culture medium	77
4.9	ELISA for detection of 2, 4-D in the liquid MS medium containing suspension culture after being centrifuged at different speed	78
4.10	ELISA against vitamin component on the medium	80
5	SUMMARY AND CONCLUSION	82
	REFERENCES	85
	APPENDICES	95
	BIODATA OF THE AUTHOR	98



LIST OF TABLES

Table		Page
2.1	Genes of the filamentous bacteriophages and their functions	30
3.1	Components for each media and solutions	38
3.2	Parameters optimized in Phage-ELISA	48
4.1	Concentration input and output of phage displayed library after each round of biopanning	54
4.2	Peptides sequences from third and fourth rounds of biopanning against 2, 4-D	61
4.3	Peptide sequences from biopanning against BSA	63
4.4	Peptide sequences from biopanning against Streptavidin	63



LIST OF FIGURES

Figure		Page
2.1	A typical <i>in vitro</i> culture process (George et al., 1993)	6
2.2	<i>Elaies guineensis</i> (Source: www.mpob.gov.my)	10
2.3	Cross breeding between <i>Dura</i> and <i>Pisifera</i> (Source: www.mpob.gov.my)	11
2.4	Oil palm suspension culture in 100 ml Erylenmeyer Flask (Source: www.mpob.gov.my)	13
2.5	Structure of Indole-3-acetic acid (Source: Modified from George, 1993)	16
2.6	Structure of 2, 4-dichlorophenoxyacetic acid (Source: Modified from George, 1993)	17
2.7	Structure of Zeatin (Source: Modified from George, 1993)	19
2.8	Structure of 2-isopentyl adenine (Source: Modified from George, 1993)	19
2.9	Structure of Benzilaminopurin (Source: Modified from George, 1993)	20
2.10	Structure of filamentous bacteriophage (Source: Sindhu, 2001)	24
2.11	Panning methodology	27
2.12	The genome of the M13 filamentous bacteriophages (Webster, 1996)	29
2.13	Phage display system	33
4.1	Blue plaques of M13 recombinants phages	55
4.2	Single Stranded DNA (ssDNA) of M13 phage for clone 4b and 9b	56



4.3	Analysis of amino acid sequence for clone 4b from third round of biopanning	58
4.4	Analysis of amino acid sequence for clone 9b from fourth round of biopanning	59
4.5	Detection of 2, 4-D using different set of peptide concentrations (10^{10} , 10^{11} , 10^{12})	66
4.6	Detection of 2, 4-D using different blocking buffers	68
4.7	Detection of 2, 4-D using different concentrations of Tween 20 (peptides from third round of biopanning)	69
4.8	Detection of 2, 4-D using different concentrations of Tween 20 (peptides from fourth round of biopanning)	70
4.9	Optimisation detection of 2, 4-D using BSA as blocking buffer, 0.1% Tween 20 and 2.0×10^{11} pfu/ml phages from third round of biopanning	71
4.10	Clone C-IPGKPFY-C shows a better signal compared to C-TQANLRT-C	73
4.11	ELISA against different concentrations of 2, 4-D	76
4.12	Detection of 2, 4-D in liquid culture medium using selected peptides	77
4.13	Detection of 2, 4-D in the liquid culture medium at different speed	79
4.14	ELISA against vitamin component in the medium	80
4.15	Structure of both 2, 4-D and thiamine molecule which share same aromatic ring structure.	81



LIST OF ABBREVIATIONS

%	percentage
°C	degree centigrade
2, 4-D	2, 4-dichlorophenoxyacetic acid
A ₄₀₅	absorbance at wavelength 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 ₂ O	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	<i>Puissance hydrogen</i>
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
μ g	micrograms (10^{-6} g)
μ l	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.

