

**CONSTRUCTION OF RFLP AND AFLP GENETIC LINKAGE MAPS FOR
OIL PALM (*ELAEIS GUINEENSIS* JACQ.) USING A
DELI *DURA* X YANGAMBI *PISIFERA* CROSS**

By

CHUA KIA LING

**Thesis Submitted to the School of Graduate Studies, University Putra
Malaysia, in Fulfillment of the Requirement for the Degree of Master
Science**

February 2006

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

**CONSTRUCTION OF RFLP AND AFLP GENETIC LINKAGE MAPS
FOR OIL PALM (*ELAEIS GUINEENSIS* JACQ.) USING A
DELI *DURA* X YANGAMBI *PISIFERA* CROSS**

By

CHUA KIA LING

February 2006

Chairman: Professor Tan Soon Guan, PhD

Faculty: Science

Conventional oil palm improvement using traditional breeding is a slow and expensive process. If markers linked to a useful trait, such as yield, shell thickness and embryogenesis rate, can be identified, marker-assisted selection (MAS) can be carried out, which can reduce the time taken for conventional breeding. Generating a linkage map is the first step towards marker-assisted selection. In this study, two oil palm maps were generated based on 87 F₁ progeny of a controlled cross (*Deli dura* x *Yangambi pisifera*). A total of 106 RFLP markers and 171 AFLP markers were identified and scored. Of the 277 markers scored, 28 markers (10.1%) were deviated from expected Mendelian ratio ($p < 0.05$). *Pseudo*-testcross strategy was used to generate two parental maps. The *dura* map consisted of 18 linkage groups and covered a total map distance of 584.1cM. The *pisifera* map resolved into 19 linkage groups and covered a total map distance of 1099.3cM. Of all the markers analyzed, 16.9% of the *dura* markers and 25.1% of the *pisifera*

markers remained unlinked. RFLP marker although difficult to develop, proved very useful because only a small fraction is deviated from the expected Mendelian ratio. Furthermore, about 80% of the RFLP markers can be mapped on both parental maps. More markers will be needed to reduce the number of linkage groups of both parental maps to the haploid chromosome number of oil palm ($n=16$). Five homologous regions between the *dura* and the *pisifera* maps were identified by comparing the co-dominant RFLP markers. The orders of the homologous markers were conserved and the overall distances were nearly the same in both varieties, although a small difference was observed in one homologous region on linkage group D3 and P5. This difference might be due to unequal recombinations that occurred at that particular region.

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

**PENJANAAN PETA GENETIK BERDASARKAN RFLP DAN AFLP BAGI
KELAPA SAWIT (*ELAEIS GUINEENSIS* JACQ.) DENGAN
MENGUNAKAN KACUKAN DELI *DURA* X YANGAMBI *PISIFERA***

Oleh

CHUA KIA LING

Februari 2006

Pengerusi: Professor Tan Soon Guan, PhD

Fakulti: Sains

Pembaikan ciri-ciri penting kelapa sawit melalui kaedah pembiakbakaan konvensional adalah proses yang memakan masa dan mahal. Sekiranya penanda yang berkaitan dengan ciri-ciri penting seperti hasil yang tinggi, ketebalan tempurung buah dan kadar embriogenesis yang tinggi dapat dikenalpasti, maka pemilihan berasaskan penanda dapat dijalankan dan ini dapat menjimatkan masa yang diperlukan dalam pembiakbakaan konvensional. Langkah pertama ke arah merealisasikan penggunaan kaedah pemilihan berasaskan penanda adalah melalui penghasilan peta genetik. Di dalam kajian ini, dua peta genetik kelapa sawit telah dihasilkan berdasarkan maklumat yang diperolehi daripada 87 progeni F_1 bagi kacukan terkawal (*Deli dura* x Yangambi *pisifera*). Sejumlah 106 penanda RFLP dan 171 penanda AFLP telah dikenalpasti. Daripada jumlah ini, 28 penanda (10.1%) didapati tersisih daripada nisbah Mendel ($p < 0.05$). Untuk penghasilan peta genetik, strategi 'pseudo-testcross' telah digunakan. Peta *dura* terdiri daripada 18

lingkaran genetik dan meliputi jarak peta sebanyak 584.1cM. Manakala peta pisifera pula mengandungi 19 lingkaran genetik dan meliputi jarak peta sebanyak 1099.3cM. Berdasarkan semua penanda yang telah dianalisis, didapati sebanyak 16.9% penanda dura dan 25.1% penanda pisifera tidak dapat dipetakan. Walaupun penanda RFLP sukar untuk dibangunkan, namun ianya lebih berguna memandangkan hanya sebilangan kecil daripada penanda ini tersisih daripada nisbah Mendel. Tambahan pula, sejumlah 80% penanda RFLP telah berjaya dipetakan ke atas peta genetik. Untuk mengurangkan bilangan lingkaran genetik di atas peta genetik agar sejajar dengan bilangan kromosom haploid kelapa sawit ($n=16$), lebih banyak penanda diperlukan. Lima bahagian homologi antara peta dura dan pisifera telah dikenalpastikan dengan membuat perbandingan dengan menggunakan penanda-penanda RFLP yang bersifat co-dominant. Jujukan penanda di semua bahagian homologi adalah terpelihara dan jarak antara satu sama lagi juga terpelihara antara dua variety ini. Jujukan penanda antara linkaran D3 dan P5 telah didapati berbeza sedikit antara satu sama lain. Perbezaan ini mungkin disebabkan oleh ketidaksamaan rekombinasi yang telah berlaku di bahagian itu.

ACKNOWLEDGEMENTS

I would like to thank Prof. Dr. Tan Soon Guan for his guidance and constructive advice through out the project. His comments and patient while reading through my master piece are highly appreciated.

A deep bow of appreciation also goes to Dr. Cheah Suan Choo and Mr. Rajinder Singh from Malaysian Palm Oil Board (MPOB) for giving me the opportunity to become part of the players in the Malaysian-MIT Biotechnology Partnership Program (MMBPP). My journey through this project was full with buds of new knowledge. Furthermore, their remarkable guidance and knowledge sharing has enriched me with scientific issues in the oil palm industry.

Bunches of thanks also go to Dr. Maizura Ithnin from MPOB. Her constructive advice and thinking as a breeder has blended my thesis with more meaningful information. I am very grateful for her countless efforts in checking through my thesis.

Additional thanks to Ms. Leslie Ooi Cheng Li and Ms. Rahimah Abdul Rahman for their technical assistance in the laboratory. Their willingness to share the secret recipes of the technical skill has expedited my work progress.

I certify that an Examination Committee has met on 20.02.2006 to conduct the final examination of Chua Kia Ling on his Master thesis entitled “Construction of RFLP and AFLP Genetic Linkage Maps for Oil Palm (*Elaeis Guineensis* Jacq.) Using Deli *Dura* x Yangambi *Pisifera* Cross” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Siti Shapor Hj. Siraj, PhD

Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Nor Aini Mohd. Fadzillah, PhD

Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Faridah Qamaruz Zaman, PhD

Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Wickneswari Ratnam, PhD

Professor
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
(External Examiner)

HASANAH MOHD. GHAZALI, PhD

Professor/ Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 18th May 2006

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

Tan Soon Guan, PhD

Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Cheah Suan Choo, PhD

Advanced Biotechnology and Breeding Centre
Malaysian Palm Oil Board
(Member)

Maizura Ithnin, PhD

Advanced Biotechnology and Breeding Centre
Malaysian Palm Oil Board
(Member)

AINI IDERIS, PhD

Professor/ Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 8th June 2006

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.

CHUA KIA LING

Date: 10th May 2006

TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	iv
ACKNOWLEDGEMENTS	vi
APPROVAL	vii
DECLARATION	ix
LIST OF TABLES	xii
LIST OF FIGURES	xiii
LIST OF ABBREVIATIONS	xiv
CHAPTER	
I INTRODUCTION	1
II LITERATURE REVIEW	4
Oil Palm	4
Biology of Oil Palm	4
Shell-thickness	5
Fruit Color	6
<i>Elaeis oleifera</i> (HBK) Cortés	7
OxG Interspecific Hybrid	8
Breeding History of <i>Deli Dura</i> Populations	10
Serdang Avenue <i>Deli Dura</i>	11
Elmina <i>Deli Dura</i>	12
Ulu Remis <i>Deli Dura</i>	12
Johore Labis <i>Deli Dura</i>	13
Breeding History of <i>Pisifera</i> Populations	14
Yangambi <i>Pisifera</i>	14
AVROS <i>Pisifera</i>	15
Binga <i>Pisifera</i>	15
Molecular Markers	16
Restriction Fragment Length Polymorphism (RFLP)	17
Amplified Fragment Length Polymorphism (AFLP)	18
Simple Sequence Repeat (SSR)	21
Random Amplified Polymorphic DNA (RAPD)	22
Genetic Linkage Mapping in Plants	24
Linkage Map of Oil Palm	24
Linkage Map of Loblolly Pine	29
Linkage Map of Eucalyptus	32
III MATERIALS AND METHODS	35
Plant Material	35
Genomic DNA Extraction	35
Restriction Fragment Length Polymorphism (RFLP)	37
Probe Preparation	37

Nomenclature	39
Southern Blot	40
Southern Hybridization	41
Probe-enzyme Screening and Genotyping	42
Amplified Fragment Length Polymorphism (AFLP)	43
Preparation of Double Stranded Adapters	43
AFLP Analysis Using <i>TaqI</i> and <i>HindIII</i> restriction site	43
Data Analysis	45
Linkage Map Construction	46
IV RESULTS AND DISCUSSION	49
Single Marker Analysis	49
Source of RFLP Probe	52
Polymorphism and Segregation of RFLP Markers	53
Polymorphism and Segregation of AFLP Markers	56
Linkage Map	59
Comparison of <i>Dura</i> and <i>Pisifera</i> Genome	64
V CONCLUSIONS	70
REFERENCES	72
APPENDICES	82
BIODATA OF THE AUTHOR	115
LIST OF PUBLICATIONS	126