IDENTIFICATION OF AMPLIFIED FRAGMENT LENGTH POLYMORPHISM FRAGMENTS LINKED TO FRUIT SKIN COLOUR OF OIL PALM (*Elaeis guineensis* Jacq.)

By

SENG TZER YING

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

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Dedicated to my parents

Mr. SENG HOI LUM & Mrs. SING MOOI

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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Chairman: **Faridah Qamaruz Zaman, PhD**

Faculty: **Institute of Bioscience**

Oil palm (*Elaeis guineensis* Jacq.) is an important commercial oil crop. It can be classified by its fruit colour into two types: a) *nigrescens* (*Nig*) type which is dark violet (unripe) and turning to reddish violet (ripe), and b) *virescens* (*Vir*) type which changes from green colour (unripe) to orange (ripe). *Vir* and *Nig* are monofactorially inherited, with *Vir* dominant over *Nig*. The different degrees of ripeness during harvesting of the fruits would influence the quantity and quality of oil in the mesocarp. The oil palm harvesting system is based on a minimum ripeness standard whereby the workers use the number of fruits detached from a bunch as a measure of its ripeness. Most of the oil palms grown commercially are the *Nig* type, being the more common type in the wild. However, *Vir* is an economically important trait as it is much easier to determine the degree of ripeness in *Vir* fruits. Molecular markers are powerful tools with the potential to influence plant breeding. Segregating populations for fruit colour CBP line which is *dura* x *tenera* cross and NPC1 line, *tenera* x *tenera* cross were obtained from Pamol Plantation. Obtaining high quality

DNA from mature leaves was difficult. Therefore, a protocol of DNA isolation was developed in this study after 12 different extraction methods were attempted. The aim of this project was to identify Amplified Fragment Length Polymorphism (AFLP) primer combinations and markers that have the potential to distinguish the fruit skin colour trait of oil palm by using the AFLP-based Bulked Segregant Analysis (BSA) technique. Of the 64 primer combinations, 10 primer combinations for CBP line and four primer combinations for NPC1 line were selected. In study 1, only three bands that showed 100% specificity to fruit colour differentiation which are regarded as fruit skin colour-specific markers were obtained for both lines, respectively. In study 2, there were four and eight specific bands which showed >80% probability of significant association to the fruit skin colour trait in CBP and NPC1, respectively. Primer B12 (E-ACT/M-CAT) generated fragments 142.13 bp and 355.76 bp as *Vir*-specific markers for CBP line and NPC1 line, respectively. It gave 83.30% (CBP) and 83.70% (NPC1) accuracy to distinguish *Vir* in study 2. Primer combination B13 (E-ACT/M-CTA) targeted 253.79 bp as the *Nig*–specific marker for both lines. It gave 76.50% (CBP) and 75.70% (NPC1) of confidence in differentiating the trait in study 2. The sequences of the AFLP markers were considered to be too short for reliability as specific markers. For an efficient MAS, it is therefore highly desirable to convert the linked markers into sequence-specific primers, such as STS, SCAR, STMS or CAP. Dice similarity coefficient (Nei and Li 1979) chosen to estimate the genetic similarity of the progenies studied, which gave average similarities of the CBP progenies of 0.790±0.057 and the NPC1 progenies of 0.761±0.089. The values derived from this study were almost similar showing that the progenies are variable only at their segregating trait. The dendrograms generated by cluster analysis using NJ based on similarity coefficients indicated the

applicability and reliability of AFLP polymorphism for distinguishing the two varieties in both lines.

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

IDENTIFIKASI SERPIHAN AFLP YANG MEMAUTKAN PADA WARNA KULIT BUAH KELAPA SAWIT (*Elaeis guineensis* Jacq.)

By

SENG TZER YING

Desember 2005

Chairman: **Faridah Qamaruz Zaman, PhD**

Faculty: **Institut Biosains**

Kelapa sawit (*Elaeis guineensis* Jacq.) merupakan suatu tanaman minyak kormesial yang penting di dunia ini. Kelapa sawit boleh diklasifikasi berasaskan warna kulit buahnya kepada dua jenis: a) *nigrescens* (*Nig*) berwarna ungu gelap (muda) dan bertukar kepada warna kemerahan ungu (masak) dan b) *virescens* (*Vir*) berwarna hijau (muda) dan menjadi warna oren (masak). *Vir* dan *Nig* diwaris secara monofaktor, di mana *Vir* dominan ke atas *Nig*. Darjah perbezaan perihal masaknya buah semasa penuaian buah akan mempengaruhi kuantiti and kualiti minyak dalam mesokarp. Sistem penuaian kelapa sawit adalah berdasarkan piawai minimum dalam penentuan perihal masaknya di mana pekerja menggunakan bilangan buah yang tertanggal dari jambak sebagai suatu ukuran. Kebanyakan kelapa sawit yang ditanam untuk tujuan kormesil adalah jenis *Nig*, sebagai jenis yang paling biasa di habitat semulajadi dan yang telah diperbaikkan untuk biakbaka. Adalah lebih mudah menentukan darjah masak buah bagi buah *Vir*, maka *Vir* adalah suatu sifat ekonomi yang mustahak. Petanda molekul adalah merupakan teknik yang berpengaruh dengan mempunyai potensi demi mempengaruhi bidang pembiakbakaan tumbuhan. Populasi

segregasi ciri untuk warna kulit buah telah didapati dari Perladangan Pamol yang terdiri daripada CBP iaitu kacukan *dura* x *tenera* dan NPC1, kacukan *tenera* x *tenera*. DNA yang berkualiti adalah sukar diekstrakan daripada daun yang matang. Dengan itu, satu protokol pengekstrakkan DNA telah dibangunkan dalam kajian ini setelah menguji 12 kaedah yang berbeza. Tujuan utama bagi projek ini ialah mengidentifikasi kombinasi primer dan penanda Amplified Fragment Length Polymorphism (AFLP) yang dapat membezakan warna kulit buah kelapa sawit dengan menggunakan teknik AFLP berdasarkan '*Bulked Segregant Analysis*' (BSA). Daripada 64 kombinasi primer, 10 kombinasi primer telah dipilih untuk CBP dan empat kombinasi primer untuk NPC1. Dalam kajian 1, tiga serpihan yang menonjolkan 100% dalam membezakan sifat warna kulit buah dianggap sebagai petanda khas bagi warna kulit buah telah didapati bagi dua populasi yang dikaji. Dalam kajian 2, terdapat empat dan lapan serpihan menunjukkan >80% signifikan demi membezakan warna kulit buah bagi CBP dan NPC1. Primer B12 (E-ACT/M-CAT) menghasilkan serpihan 142.13 bp (CBP) and 355.76 bp (NPC1) sebagai petanda khas–*Vir*. Penanda – penanda khas ini memberi keyakinan sebanyak 83.30% (CBP) dan 83.70% (NPC1) untuk membezakan sifat *Vir* dalam kajian 2. Kombinasi primer B13 (E-ACT/M-CTA) menyasar 253.79 bp sebagai petanda khas–*Nig* untuk dua populasi. Ia memberikan keyakinan sebanyak 76.50% (CBP) dan 75.70% (NPC1) dalam tujuan untuk membezakan sifat warna dalam kajian 2. Jujukan-jujukan penanda AFLP ini telah dipertimbangkan terlalu pendek untuk menjadi penanda khas. Tujuan bagi pemilihan biakbaka berpandukan penanda (Marker-assisted selection, MAS), adalah disyorkan pertukaran penanda berpaut tersebut kepada primer berjujukan khas seperti STS, SCAR, STMS atau CAP. Koeffisien Pendekatan Dice (Nei and Li 1979) telah dipilih untuk menganggar pendekatan

genetik progeni dalam kajian ini, dengan purata pendekatan bagi progeni CBP ialah 0.790±0.057 dan NPC1 ialah 0.761±0.089. Nilai-nilai yang didapati daripada kajian ini yang seakan sama telah menunjukkan progeni-progeni mempunyai sedikit perbezaan kerana segregasi pada satu ciri sahaja. Dendrogram yang dijanakan oleh analisis berkelompok dengan kaedah '*Neighbor-Joining*' telah menunjukkan aplikasi polimorfisme AFLP dan boleh dipercayai untuk membezakan dua variasi. Primer tersebut mungkin mempunyai petanda AFLP yang berpotensi sebagai kaedah untuk membezakan variasi.

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I certify that an Examination Committee met on 30th December 2005 to conduct the final examination of Seng Tzer Ying on her Master thesis entitled "Identification of Amplified Fragment Length Polymorphism Fragments Linked to Fruit Skin Colour of Oil Palm (*Elaeis guineensis* Jacq.)" 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 requirements for the degree of Master of Science. The members of the Supervisory 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.

_____

SENG TZER YING

Date :

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