IDENTIFICATION OF QUANTITATIVE TRAIT LOCI FOR TISSUE CULTURE AMENABILITY TRAITS IN A DURA X PISIFERA F_1 OIL PALM POPULATION

By

TING NGOOT CHIN

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

December 2006
DEDICATION

To my loving family

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 : Professor Tan Soon Guan, PhD

Faculty : Science

Tissue culture is employed for the large scale production of elite planting materials in the oil palm plantation industry of Malaysia. The advantage of this technique is the homogeneous quality that can be produced across the clones. However, the production cost is high compared to conventional seedlings due to some unpredictable constraints. One of the major problems that has been reported is the average low rate of regeneration among the clones. Inconsistencies have been reported in that both callogenesis and embryogenesis varied among the lines. Attempts were made to generate restriction fragment length polymorphism (RFLP) and amplified fragment length polymorphism (AFLP). These DNA markers were used for constructing genetic linkage maps for two commercial palms namely, Ulu Remis Deli dura (ENL48) and Yangambi pisifera (ML161). Both maps were used as frameworks to detect quantitative trait loci (QTL) associated with the traits of tissue culture amenability. The generation of DNA markers involved the genotyping of 87 F_1 palms using 70 cDNA probes and 16 *Eco*RI/*Mse*I and 8 *Pst*I/*Mse*I primer-pairs. This produced a total of 36 RFLPs and 43 AFLPs for

ENL48 and 66 RFLPs and 58 AFLPs for ML161. The data were integrated into the existing database for the mapping analysis by using JoinMap®3.0 at LOD 4.0 and recombination frequency θ <0.400. The ENL48 linkage map was constructed by using 87 RFLPs and 123 AFLPs. These markers were grouped into 25 linkage groups covering a total genetic distance of 1,136.3cM. A denser map of ML161 was obtained with 147 RFLPs and 225 AFLPs which resulted in 16 linkage groups with a total map length of 1,755.7cM. The QTL for tissue culture amenability were determined using interval mapping and multiple quantitative mapping (MQM) with the help of the MapQTL® 4.0 software. The LOD thresholds were estimated using the permutation test. The QTLs for the callusing rate (CR) were detected at linkage groups D9 and P7 on the ENL48 and the ML161 maps, respectively. For embryogenesis rate (Er_ex), QTL was only detected on the ML161 genetic map at linkage group P13. All the detected QTLs were confirmed in a second experiment where the tissue culture data was collected using different media compositions. These improved confidence in the QTLs detected for tissue culture amenability.

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

PENGESANAN LOKUS BERCIRI KUANTITATTIF (QTL) UNTUK KEBOLEHAN KULTUR TISU DALAM DURA X PISIFERA F_1 POPULASI KELAPA SAWIT

Oleh

TING NGOOT CHIN

Disember 2006

Pengerusi : Profesor Tan Soon Guan, PhD

Fakulti : Sains

Kultur tisu digunakan dalam penghasilan bahan tanaman elit secara besar-besaran di kebanyakan ladang sawit di Malaysia. Kelebihan kaedah ini adalah penghasilan klon-klon yang berkualiti homogenus. Walau bagaimanapun, kos pengeluaran adalah tinggi berbanding dengan kaedah tradisional disebabkan masalah-masalah di luar jangkaan. Salah satu masalah yang dilaporkan ialah purata kadar regenerasi yang rendah di antara klon. Kalogenesis dan embriogenesis diperhatikan tidak konsistan di antara kultur. Kajian awal telah dibuat untuk menghasilkan penanda-penanda RFLP dan AFLP. Penanda DNA ini telah digunakan untuk membina peta rangkaian genetik bagi dua jenis pokok sawit komersial iaitu Ulu Remis Deli dura (ENL48) dan Yangambi pisifera (ML161). Kedua-dua peta tesebut seterusnya digunakan dalam penentuan lokus berciri kuantitatif (QTL) yang berkaitan dengan kebolehan kultur tisu. Dalam penghasilan penanda DNA, sebanyak 70 prob cDNA, 16 pasangan primer *Eco*RI/*Mse*I dan 8 *Pst*I/*Mse*I digenotip dengan menggunakan 87 pokok F_1. Ini telah menghasilkan sejumlah 36 penanda RFLP dan 43 AFLP bagi ENL48, dan 66 RFLP dan 58 AFLP bagi ML161. Data kemudiannya

diintegrasikan ke dalam pangkalan data sedia ada untuk analisis pemetaan yang menggunakan JoinMap® 3.0 pada LOD4.0 dan kekerapan rekombinasi (θ)<0.400. Peta ENL48 telah dihasilkan dengan pemetaan 87 RFLP dan 123 AFLP. Penanda-penanda ini telah dikumpulkan kepada 25 kumpulan rangkaian dengan jumlah jarak genetik 1,136.3cM. Peta ML161 yang lebih padat telah dihasilkan dengan 147 RFLP dan 225 AFLP yang menghasilkan 16 kumpulan rangkaian dengan jumlah jarak genetik 1,755.7cM. QTL bagi kebolehan kultur tisu telah dikenalpasti melalui pemetaan "Interval' dan "MQM" dengan bantuan perisian MapQTL® 4.0. Nilai ambang LOD dianggarkan dengan menggunakan ujian pilih atur. QTL bagi kadar pembentukan kalus (CR) telah dikesan pada kumpulan rangkaian D9 dan P7 masing-masing pada peta ENL48 dan ML161. Bagi kadar embriogenesis (Er_ex), QTL hanya dikesan pada kumpulan rangkaian P13 pada peta ML161. Semua QTL yang dikesan telah disahkan dalam eksperimen kedua di mana data kultur tisu diperolehi dengan menggunakan komposisi media yang berlainan. Ini telah menambah keyakinan dalam pengesanan QTL untuk kebolehan kultur tisu.

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I certify that an Examination Committee met on 20 December 2006 to conduct the final examination of Ting Ngoot Chin on her degree thesis entitled "Identification of quantitative trait loci for tissue culture amenability traits in a dura x pisifera F_1 oil palm population'' 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.

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TING NGOOT CHIN

Date: 13 FEBRUARY 2007

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