UNIVERSITI PUTRA MALAYSIA

ILLEGITIMACY IN OIL PALM (Elaeis guineensisJacq.) HALF-SIB FAMILIES AND COMPARATIVE MOLECULAR MARKER MAPPING ASSOCIATED WITH BASAL STEM ROT DISEASE USING SINGLE LOCUS DNA MICROSATELLITE MARKERS

EMAD OMER HAMA-ALI

FBSB 2013 14

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By

EMAD OMER HAMA-ALI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirement for the Degree of Doctor of Philosophy

December 2013

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Specially dedicated to

My parents
My wife
My son

For their love, patience and support

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

ILLEGITIMACY IN OIL PALM (*Elaeis guineensis*Jacq.) HALF-SIB FAMILIES AND COMPARATIVE MOLECULAR MARKER MAPPING ASSOCIATED WITH BASAL STEM ROT DISEASE USING SINGLE LOCUS DNA MICROSATELLITE MARKERS

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Chairman: Professor Tan Soon Guan- PhD

Faculty: Biotechnology and Biomolecular Sciences

The oil palm *Elaeis guineensis*Jacq., is a source of commercial planting material that makes oil palm an important oil crop in the world. Oil palm breeding has been progressing very well in Southeast Asia, especially in Malaysia and Indonesia. Despite the progress, there are still problems due to the difficulty of controlled crossing in oil palm. Contamination/illegitimate progeny has appeared in some breeding programs which causes a waste of money, time and labor once it is detected by the traditional method. Also, oil palm is badly affected by basal stem rot (BSR) disease in Southeast Asia. BSR disease is caused by the fungus*Ganoderma boninense*, which is a major threat to oil palm compared with other *Ganoderma* sp. Breeders' information suggested that there is no error in the assignment of parents to these breeding families (Family-1, Family-2, Family-3 and Family-4). As such, the use of molecular markers is necessary for breeding program management especially for perennial crops like oil palm and the use of molecular markers associated with BSR disease will accelerate the identification of resistant planting materials.

The goals of these studies were to establish a procedure for sibship assignment, detection of illegitimacy and examination of a possible association between *Ganoderma* disease incidences (GDI) in an oil palm breeding program by using microsatellite markers. In the first study, four half-sib families (Family-1, Family-2, Family-3 and Family-4) were investigated, each with 50 offsprings with their candidate parents using 69 microsatellite loci. Among the 69 polymorphic microsatellite loci tested, 30 were selected based on high polymorphic information content (PIC) values and absence of null allele, for parental and sib-ship assignments. The parental palms stated by the breeder in the first study are not the true parents as revealed by the microsatellite loci gel patterns. The results of the parental assignments using the CERVUS program showed negative LOD score for all candidate parents FD6 (-37.5), FD8 (-31.1), FD10 (-34.6), FD 1/224 (-9.98),

FP1/28 (-14.2) and FP1/10 (-9.63). These negative LOD scores revealed that these candidate parents were not the true parents for all progeny tested.

The COLONY analysis results showed that 16 loci were sufficient for obtaining correct family assignments by using short run and pair likelihood-score (PLS) methods in the four half-sib families. The COLONY results gave two half-sib dyads. A probability of one in the first dyad resulted in three half-sib families namely, Family-1, Family-2, and Family-3, in which they shared the same father. The second dyad gave four half-sib families Family-1 (offspring ID 1 to 50), Family-2 (offspring 52 to 100, but not including offspring ID 74 and 97), Family-3 (offspring ID 101 to 150) and family-4 (offspring ID 151 to 200, not including offspring ID 180) with a probability of one as their fathers were sibs. In addition, correct pedigree reconstructions were done by COLONY from offspring genotypic data. The best configuration output gave four mothers for each family and one father for all the families. Furthermore, three (1.5%) illegitimate offsprings (offspring ID 51, 97 and 180) were detected among the 200 offsprings in this study by COLONY.

The STRUCTURE software results presented four pure clusters (families), which is the same as the COLONY results and in 100% agreement with the breeders' documentation. In addition, all illegitimate offsprings (offsprings ID 51, ID 97, and ID 180) were detected among the progeny of the controlled crosses as admixed individuals in the clusters, and offspring ID 74 was assigned to the correct family. Moreover, from the STRUCTURE analyses, the sources of the illegitimate offsprings were detected. Illegitimate offspring ID 51 and ID 97 wereproduced during pollination (hybridization) time. Offspring ID 180 was caused by this seedling being mixed with those of other families in the nursery stage.

In addition, possible associations between *Ganoderma* disease incidences (GDI) in three oil palm progeny types (KA4G1, KA4G8, and KA14G8) and 58 microsatellite markers were examined. The results of GDI showed that KA4G1 is a resistant progeny type against *G. boninense*, whereas KA4G8 and KA14G8 are susceptible progeny types. The microsatellite markers produced 319 alleles in the three oil palm progeny types, and the average as 5.51 alleles per locus. Five markers, mEgCIR0793:180, mEgCIR0894:200, mEgCIR03295:210, mEgCIR3737:146, and mEgCIR3785:299, were found to be associated with *Ganoderma* disease with P-valuesof 0.018, 0.033, 0.037, 0.034 and 0.037, respectively, in the single progeny analysis. In the pooled data (KA4G1, KA4G8, and KA14G8), 89 alleles from 46 loci were associated with GDI. Among the 89 significantalleles, 59 alleles showed significance at $P < 0.01$and 30 alleles had significance at $P < 0.05$.

Abstrak tesis yang dikemukakan kapada SenatUniversiti Putra Malaysia sebagai memenuhi keperluran untuk ijazah Doktor Falasfah

PENCEMARAN DALAM KELUARGA SEPARUH-BERADIK KELAPA SAWIT (*Eliaeis guineensis* Jacq.) DAN PEMETAAN PENANDA MOLEKUL BANDINGAN BERKAITAN DENGAN PENYAKIT REPUT PANGKAL BATANG DENGAN MENGGUNAKAN PENANDA MIKROSATELIT LOKUS TUNGGAL DNA

Oleh

EMAD OMER HAMA-ALI

Disember 2013

Pengerusi: Professor Tan Soon Guan- PhD

Fakulti:Bioteknologi dan Sains Biomolekul

Minyak sawit *Elaeis guineensis* Jacq., merupakan sumber bahan-bahan penanaman komersil yang menjadikan kelapa sawit tanaman minyak yang penting di dunia. Pembiakan kelapa sawit telah berkembang dengan baik di Asia Tenggara, terutama di Malaysia dan Indonesia.Walaupun dengan kemajuan ini, masih terdapat masalah kerana kesukaran mengawal kacukan silang di kelapa sawit. Pencemaran progeni telah muncul dalam beberapa program pembiakan yang menyebabkan pembaziran masa dan tenaga sebaik sahaja ia dikesan oleh kaedah tradisional. Juga, kelapa sawit terjejas teruk akibat penyakit reput pangkal batang (BSR) di Asia Tenggara. Penyakit BSR adalah disebabkan oleh kulat *Ganoderma boninense*, yang merupakan ancaman utama kepada kelapa sawit berbanding dengan *Ganoderma* sp. yang lain. Oleh itu, penggunaan penanda molekul adalah perlu untuk pengurusan program pembiakan terutamanya untuk tanaman seperti kelapa sawit dan penggunaan penanda molekul yang dikaitkan dengan penyakit BSR akan mempercepatkan pengenalpastian balan yang rintang.

Matlamat kajian ini adalah untuk mewujudkan prosedur untuk tugasan saudara kandung, pengesanan kelancunganyang tercemar dan pemeriksaan kemungkinan pembiakan antara kjadianpenyakit Ganoderma (GDI) dalam program pembiakan kelapa sawit dengan menggunakan penanda mikrosatelit.Dalam kajian pertama, empat keluarga ‘half-sib’ (Keluarga-1, Keluarga-2, Keluarga-3 dan Keluarga-4) telah kaji, dengan 50 keturunan masing-masing dengan induk calon mereka menggunakan 69 lokus mikrosatelit. Antara 69 lokus mikrosatelit polimorfik yang diuji, 30 telah dipilih berdasarkan kandungan yang tinggi maklumat polimorfik (PIC) nilai-nilai dan ketiadaan alel ‘null’, untuk induk dan dan ‘sib-ship’. Induk sawit yang dinyatakan oleh pedadas dalam kajian pertama bukan ibu bapa yang sebenar seperti yang didedahkan oleh lokus mikrosatelit corak gel.Keputusan tugasan untuk induk menggunakan program CERVUS menunjukkan skor LOD negatif untuk semua calon

induk FD6 (-37.5), FD8 (-31.1), FD10 (-34.6), FD1/224 (-9.98), FP1/28 (-14.2) and FP1/10 (-9.63).Skor LOD negatif ini menunjukkan bahawa calon induk yang dinyatakan ini bukan induk yang sebenar untuk semua keturunan diuji.

Keputusan analisis COLONY menunjukkan bahawa 16 lokus adalah memadai untuk mendapatkan penentuan keluarga yang betul dengan menggunakan kaedah pasangan kemungkinan skor (PLS) jangka pendek dalam empat keluarga 'half-sib'. Keputusan COLONY memberikan dua 'half-sib' 'dyad'. Ada kemungkinan untuk satu antara pasangan 'dyad' yang pertama menghasilkan tiga keluarga 'half-sib' iaitu , Keluarga-1 , Keluarga-2, dan Keluarga-3, di mana mereka berkongsi induk jantan yang sama. 'Dyad' yang kedua memberikan empat keluarga 'half-sib'- keluarga-1 (ID keturunan 1 hingga 50) , Keluarga-2 (anak 52-100, tetapi tidak termasuk anak-anak ID 74 dan 97) , Keluarga-3 (ID keturunan 101-150) dan keluarga-4 (ID keturunan 151-200, tidak termasuk anak-anak ID 180) dengan kebarangkalian bersamaan dengan satu kerana induk jantan mereka adalah 'sibs'. Di samping itu, pembinaan semula keturunan yang betul telah dilakukan oleh COLONY daripada data genotip anak.Output konfigurasi terbaik memberikan empat induk betina bagi setiap keluarga dan satu induk jantan untuk semua keluarga. Tambahan pula, tiga (1.5%) keturunan tercemar (keturunan ID 51, 97 dan 180) telah dikesan di kalangan 200 keturunan dalam kajian ini dengan COLONY.

Keputusan perisian STRUCTURE menunjukkan empat kelompok tulen (keluarga), adalah sama seperti keputusan COLONY dan 100% tepat dengan dokumentasi alili pembaik biak tananan sawah.Di samping itu, semua keturunan yang tercemar (keturunan ID 51, 97 ID dan ID 180) telah dikenalpasti di kalangan keturunan kawalansilang sebagai individu tercampur dalam kelompok, dan keturunan ID 74 telah diberikan kepada keluarga yang betul. Selain itu, daripada analisis STRUCTURE, sumber keturunan tercemar telah dikesan.Keturunan tercemar ID 51 dan ID 97 terjadi ketika waktu pendebungaan (penghibridan). Progeni ID 180 adalah disebabkan oleh anak benih ini yang bercampur dengan keluarga-keluarga yang lain di peringkat nurseri. Di samping itu, kemungkinan perkaitan antara penyakit *Ganoderma* insiden (GDI) dalam tiga jenis keturunan kelapa sawit (KA4G1, KA4G8 dan KA14G8) dan 58 penanda mikrosatelit telah diperiksa.Keputusan menunjukkan bahawa GDI KA4G1 adalah jenis progeni yang rintang terhadap *G. boninense*, manakala KA4G8 dan KA14G8 mempunyai kerintangan yang rendah.Penanda mikrosatelit yang dihasilkan 319 alel dalam tiga jenis keturunan kelapa sawit, dan puratanya adalah 5.51 alel per lokus. Lima penanda, mEgCIR0793:180, mEgCIR0894:200, mEgCIR03295:210, mEgCIR3737:146 dan mEgCIR3785: 299, telah didapati mempunyai kaitan dengan penyakit *Ganoderma* dengan nilai-P bersamaan 0.018, 0.033, 0.037, 0.034 dan 0.037, masing-masing, dalam analisis keturunan tunggal. Dalam data terkumpul (KA4G1, KA4G8 dan KA14G8), 89 daripada 46 alel lokus dikaitkan dengan GDI.Antara 89 alel penting, 59 alel menunjukkan kepentingan di $P < 0.01$ dan 30 alel mempunyai signifikan di $P < 0.05$.

ACKNOWLEDEGEMENTS

First and foremost, I would like to thank almighty ALLAH for all He has done in my life, and I praise him for everything.

I would like to take this opportunity to thank Prof. Dr. Tan Soon Guan, chairman of the supervisory committee for all his help, inspiration and invaluable guidance along the way.

I wish to express my deepest appreciation to the committee members: Prof. Dr. Jothi Malar Panandam for giving valuable time to discuss and for suggesting valuable information during the study, Dr. Sharifah Shahrul Rabiah Syed Alwee, who made it possible for me to complete this work at the FELDA Biotechnology Centre and for providing research facilities, plant materials and her support for the research, Assoc Prof. Dr. Ho Chai Lingfor providing ESTs and her support for the research, Assoc Prof. Dr. Parameswari Namasivayam for her support for the research and Dr. Hoh Boon Peng for his valuable suggestions in during the study. Thank you again for all your comments, suggestions and for editing the paper manuscripts.

My special thanks to Dr. Tan Joon Sheong from FELDA for all inputs and discussion on the project. I express my cordial thanks to FELDA Biotechnology Centre staff, especial to those in the Molecular Biology Lab. Also, my special thanks to Assoc Prof. Dr. Subha Bhassu and her student Ms. Sarasvathi Easwvara who helped me with the CERVUS program.

And most importantly, thanks to my family at home. It is because of you, your support and your prayers that I finally got here. Last but not least, I would like to express my deepest thanks to my wife and my son for your support, prayers, and patience, without you the study would not have been successfully completed.

I certify that a Thesis Examination Committee has met on 27 December 2013to conduct the final examination of Emad Omer Hama-Ali on his thesis entitled "Illegitimacy in Oil Palm (*Elaeis guineensis* Jacq.) Half-Sib Families and Comparative Molecular Marker Mapping Associated with Basal Stem Rot Disease Using Single Locus DNA Microsatellite Markers"in accordance with Universitiesand University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March1988. The Committee recommends that the candidate be awarded the Doctor of Philosophy.

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirements for the degree of the Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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DECLARATION

Declaration by graduate student

I hereby confirm that:

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This is to confirm that:

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