

UNIVERSITI PUTRA MALAYSIA

DETERMINATION OF GENETIC DIVERSITY AND INBREEDING LEVEL IN DELI DURA AND AVROS ADVANCED BREEDING MATERIALS IN OIL PALM (ELAEIS GUINEENSIS JACQ.) USING MICROSATELLITE MARKERS

TAY CHEE CHUN

FBSB 2016 10



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By

TAY CHEE CHUN

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

June 2016

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

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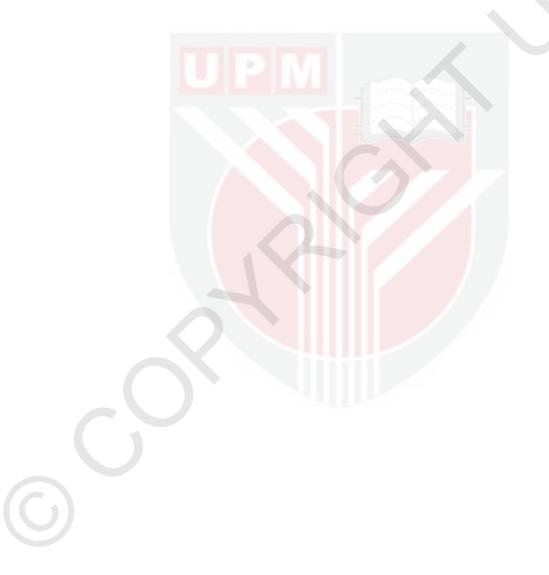
June 2016

Chairman: Professor Tan Soon Guan, PhD

Faculty: Biotechnology and Biomolecular Sciences

Intensive selections in breeding have led to a narrowed genetic diversity in oil palm. Although uniformity is favorable to breeders, loss of genetic diversity may lead to deleterious consequences. Thus, background knowledge on genetic diversity in advanced breeding material of oil palm (Elaeis guineensis Jacq.) is critical for crop improvement. The objectives of the study are to evaluate the genetic diversity in the advanced breeding materials, Deli dura and AVROS populations, and to determine the levels of inbreeding in these populations using CIRAD's Simple Sequence Repeat (SSR) markers. The parental populations evaluated here were 186 palms from 8 DxD/D-selfs Deli dura and 188 palms from 8 TxT/T-selfs AVROS progenies sourced from Agency 1 and Agency 2. Ekona population was included as control. Genotyping was done by using 32 SSR markers which produced 230 alleles among the 17 oil palm progenies. The number of alleles scored per SSR primer ranged between 4 to 11. The average number of alleles per locus was 7.1875. The expected heterozygosity (He) and observed heterozygosity (Ho) among the populations were 0.7063 and 0.5270, respectively. Genetic distance values were calculated. The dendogram resulting from the Neighbour-Joining clustering analysis revealed that the 17 populations were clustered into 3 main clusters namely Deli dura, AVROS and Ekona populations. The AVROS cluster was further divided into two sub-clusters according to Agency. The Deli dura cluster was sub-clustered based on the cross type i.e. DxD and D-selfs. The two different generations of AVROS provided an overview of the inbreeding level in the AVROS population. AVROS from Agency 2 had inbreeding coefficient of 0.0336 compared to AVROS from Agency 1 which was -0.0810. The inbreeding value indicated that Agency 2 AVROS had lower heterozygosity compared to Agency 1. The genetic variability revealed via SSR markers on the advanced breeding populations

provide a better understanding to the breeder and the information can guide them in decision making during the inbred lines development.



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

PENENTUAN KEPELBAGAIAN GENETIK DAN TAHAP PEMBIAKBAKAAN DALAM BAHAN PEMBIAKAN MAJU DELI DURA DAN AVROS BAGI KELAPA SAWIT (*Elaeis guineensis* Jacq.) MENGGUNAKAN PENANDA MIKROSATELIT

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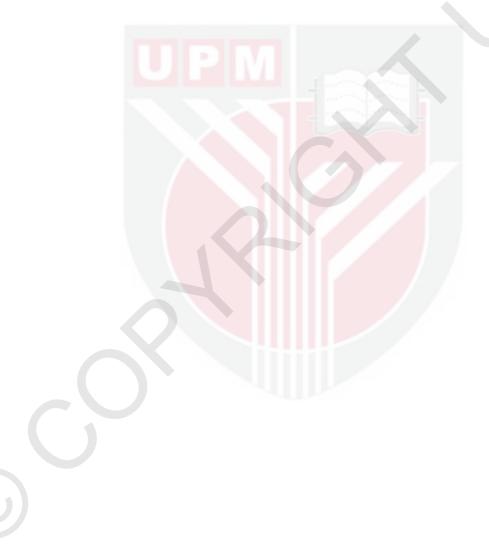
TAY CHEE CHUN

Jun 2016

Pengerusi: Professor Tan Soon Guan, PhD

Fakulti: Biotechnology and Biomolecular Sciences

Pemilihan yang intensif dalam pembiakbakaan kelapa sawit telah membawa kepada kepelbagaian genetik yang semakin sempit. Walaupun keseragaman penting untuk peladang, tetapi kehilangan kepelbagaian genetik boleh membawa kepada akibat yang parah. Oleh itu, pengetahuan latarbelakang ke atas kepelbagaian genetik dalam bahan pembiakbakaan maju kelapa sawit (Elaeis guineensis Jacq.) adalah kritikal untuk penambahbaikan tanaman. Objektif kajian ini adalah untuk menilai kepelbagaian genetik dalam bahan-bahan pembiakbakaan maju, Deli dura dan AVROS, dan untuk menentukan tahap pembiakbakaan dalam populasi ini menggunakan penanda molekul (SSR) dari CIRAD. Populasi yang dinilai terdiri daripada 186 pokok Deli dura dari 8 progeni DxD / D-selfs dan 188 pokok AVROS dari 8 progeni TxT / T-selfs diperoleh daripada Agensi 1 dan Agensi 2. Populasi Ekona telah digunakan sebagai kawalan. Penjenisan gen telah dilakukan dengan menggunakan 32 penanda molekul SSR yang menghasilkan 230 alel antara 17 populasi kelapa sawit. Bilangan alel untuk setiap SSR berbilang antara 4 hingga 11. Purata bilangan alel setiap lokus adalah 7.1875. Heterozigositi dijangka (He) dan heterozigositi diperhati (Ho) di kalangan populasi adalah 0.7063 dan 0.5270, masing-masing. Nilai jarak genetik telah dikira. Dendogram dari analisis kelompok, Neighbour-Joining telah menunjukkan bahawa 17 populasi telah dikelompokkan ke dalam 3 kelompok utama iaitu Deli dura, AVROS dan Ekona populasi. Progeni AVROS telah dikelompokkan kepada 2 sub-kelompok mengikut agensi. Kelompok Deli dura telah bercabang dengan lanjut berdasarkan jenis jujukan iaitu DxD dan D-selfs. Kedua-dua generasi yang berbeza bagi AVROS telah memberi gambaran keseluruhan tahap pembiakbakaan dalam populasi AVROS. AVROS daripada Agensi 2 mempunyai pekali pembiakbakaan 0.0336 berbanding AVROS daripada Agensi 1 yang mempunyai -0.0810. Nilai pembiakbakaan ini menunjukkan bahawa AVROS daripada Agensi 2 mempunyai heterozigositi yang lebih rendah berbanding dengan Agensi 1. Kesimpulannya, kepelbagaian genetik yang didedahkan melalui penggunaan penanda molekul (SSR) terhadap populasi pembiakbakaan maju telah memberi peladang kefahaman yang lebih baik dan informasi ini dapat memberi garis panduan kepada meraka semasa membuat keputusan untuk pembangunan pembiakbakaan.



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I certify that a Thesis Examination Committee has met on 29th June 2016 to conduct the final examination of TAY CHEE CHUN on his thesis entitled "Determination of Genetic Diversity and Inbreeding Level in Deli Dura and AVROS Advanced Breeding Materials in Oil Palm (*Elaeis guineensis* Jacq.) Using Microsatellite Markers " in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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LIST OF ABBREVIATIONS

°C	Desma selaine
°C	Degree celsius
AFLP	Amplified fragment length polymorphism
AVROS	Algemene Vereniging van Rubberplanters ter Oostkust van Sumatra
bp	Base pairs
CE	Capillary electrophoresis
CIRAD	Centre International de Reseaux Agriculture and Development
cm	centimeter
СРО	Crude palm oil
D × P	dura × pisifera
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleoside triphosphate
Gb	Gigabase
GBS	Genotyping-by-sequencing
GDP	Gross domestic product
GENP	Genting Plantations
На	Hectare
H _e	Expected heterozygosity
H _o	Observed heterozygosity
HWE	Hardy-Weinberg Equilibrium
MAS	Marker assisted selection
MgCl ₂	Magnesium chloride
mL	milliliter
mM	millimole
МРОВ	Malaysian Palm Oil Board
MRRS	Modified reciprocal recurrent selection
μL	microliter
μΜ	micromole
Na	Observed number of alleles
NCM1	North Carolina Model 1

(C)

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NGS	Next generation sequencing
PCR	Polymerase chain reaction
PIC	Polymorphic information content
PORIM	Palm Oil Research Institute of Malaysia
PORLA	Palm Oil Registration and Licensing Authority
QTL	Quantitative trait loci
R&D	Research and development
RAPD	Randomly Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeats
Т	tenera
U	Units (of an enzyme)
URT	Ulu Remis Tenera
UV	Ultra Violet
v	volts

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CHAPTER 1

INTRODUCTION

The African oil palm (*Elaeis guineensis* Jacq.) is an oil crop that is native to Africa. As the world's leading oil crop, oil palm produces 29.8% of the total vegetable oils and fats surpassing soybean 22.5% (Oil World 2013). It yields 5 to 15 times more oil per hectare as compared to rapeseed or soybean. In 2012, the estimated global oil palm planted area was 14.8 million ha that produced 53.67 million tonnes of crude palm oil (CPO). In 2014, the oil palm planted area in Malaysia was 5.39 million ha producing 19.67 million tonnes of palm oil. The great economic value generated by palm oil export has changed the oil palm from its ornamental status to that of a cash crop.

In Malaysia, oil yield has increased fourfold in the last 50 years, half of this being attributable to genetic improvement of the planting material (Lee *et al.*, 1990). However, the study showed that selection for this improvement reduced the coefficient of variation for fruit bunch yield by nearly half. Intensive breeding from a narrow genetic base is leading to limited variation within the elite material, potentially reducing the rate of future breeding progress. Efforts to broaden the genetic variability of the current oil palm advanced breeding populations are crucial to enable the breeders to exploit the genetic potentials in order to produce planting materials that are high yielding with better oil quality and high tolerance to pests and diseases. Musa and Gurmit (2008) has indicated great potential for improvement through the introgression of selected palms from the MPOB germplasm into the advanced dura and tenera lines which reduced palm height (Isa *et al.*, 2008) and improved oil palm yield and fruit qualities (Junaidah *et al.*, 2008).

Conventional breeding takes several years for oil palm improvement due to the fact that it is perennial oil crop with a long generation cycle of breeding. These led to slow progress and hamper the release of high yielding planting materials. However, there are various molecular techniques that are available today which provide a powerful tool to facilitate crop improvement and selection. Molecular markers have been exploited in oil palm research over the last two decades in areas such as DNA fingerprinting using restriction fragment length polymorphism (RFLP) (Cheah *et al.*, 1996). According to Corley (2005), the employment of molecular markers has allowed illegitimate crosses to be identified and legitimate individuals to be determined. In addition, molecular markers are able to detect genetic variability (Billotte *et al.*, 2005), genome mapping and Quantitative Trait Loci (QTL) detection for Marker Assisted Selection (MAS) (Billotte *et al.*, 2010).

With the advancement of biotechnology and oil palm genome sequencing, molecular markers were developed and are available in the public domain. Microsatellite marker

or Simple Sequence Repeat (SSR) is one of the molecular markers that are in abundance which is well utilised for genetic diversity studies (Norziha *et al.*, 2008; Singh *et al.*, 2008). It has great advantages over other markers systems due to its high polymorphism and wide distribution of the loci within the genome (Vignal *et al.*, 2002). Besides, SSR is a co-dominant marker which is ideal for diversity measurements and is highly reproducible through the Polymerase Chain Reaction (PCR). Hamblin *et al.* (2007) reported that SSRs performed better to assign the inbreds to sub-populations as compared to Single Nucleotide Polymorphism (SNPs) in their research to evaluate the genetic diversity in a set of public maize inbreds. Besides microsatellite markers, there are reports of other molecular markers such as restriction fragment length polymorphism (RFLP) of ribosomal DNA (Rajanaidu *et al.*, 1989), random amplified polymorphism (AFLP) (Kularatne, 2000).

The Malaysian advanced breeding materials particularly the Deli population and AVROS population have been subjected to extensive selection since the 1930s (Maizura *et al.*, 2009). Breeding and selection for crop improvement using these populations by breeders had led to narrowed genetic diversity as shown by various reports (Lee *et al.*, 1990; Hayati *et al.*, 2004; Maizura *et al.*, 2006). The majority of the studies were carried out using germplasms and not many focused on advanced breeding materials. Besides, no comparison of different generations of materials was carried out previously. Thus, an understanding on the genetic diversity of the advanced breeding populations at the molecular level is of importance for breeders to exploit the remaining genetic potential for crop improvement. The findings from this study could serve as supplementary information for the breeders during their crossing design enabling more precise decisions on the selection of parental materials for seed production and inbred line development.

The objectives of the current study were:

- 1. To determine the genetic diversity in the advanced breeding materials, Deli and AVROS populations, for future breeding and improvement.
- 2. To determine the levels of inbreeding across different generations in the advanced breeding populations.
- 3. To utilise SSR markers as a tool to investigate the occurrence of illegitimate palms among the progenies of specific crosses.

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BIODATA STUDENT

Tay Chee Chun was born on 24th February 1986 in Johor, Malaysia. He obtained his BSc. in Biotechnology and Management from National University of Malaysia (UKM). During his final year project, he carried out research on gene cloning of Fatty Acid Thioesterase (FAT) from Capsicum frutescens with aim to identify the component of capsaicinoid that contribute the pungency as the presence of the FAT is correlated to the degree of pungency. After earning his bachelor's degree, Chee Chun joined ACGT in June 2008 and later was seconded to Genting AgTech Sdn Bhd (GAT), a subsidiary company of Genting Plantations Berhad (GENP). Since joining GAT, Chee Chun has been involved in projects related to linkage mapping and population genetic studies for oil palm. His interest includes molecular genetics and oil palm breeding. He is currently involved in oil palm breeding and marker assisted selection activities. In 2014, he was given the opportunity to pursue a Master's degree in Plant Biotechnology at Universiti Putra Malaysia (UPM) under the sponsorship of GENP. He has spent two years studying the genetic diversity and inbreeding level of different generation of oil palm advanced breeding materials under the supervision of Prof. Tan Soon Guan and Assoc. Prof. Dr. Mohd. Puad Abdullah.

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