UNIVERSITI PUTRA MALAYSIA

GENETIC DIVERSITY OF MPOB-SENEGAL OIL PALM GERMPLASM BASED ON QUANTITATIVE TRAITS AND MICROSATELLITE MARKERS

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By

KHIN AYE MYINT

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

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DEDICATION

This thesis is dedicated to my beloved parents (U Tet Tun and Daw Aye) for their boundless love, understanding, encouragement, support and sacrifice throughout my study.

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

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KHIN AYE MYINT

December 2019

Chairman : Professor Mohd Rafii Yusop, PhD
Institute : Institute of Tropical Agriculture and Food Security

Understanding the genetic structure of different vegetative, yield and yield component traits plays a major role in oil palm breeding program. Evaluation of genetic diversity based on morphology and physiology is insufficient to get comprehensive information because of low polymorphism, long juvenile phase, and vulnerability to environmental effects like oil palm. Based on this background, this study was carried out to evaluate the genetic diversity and heritability among 26 families of MPOB-Senegal oil palm germplasm using quantitative traits (yield, bunch quality, and vegetative traits) and microsatellite (SSR) molecular markers, in order to identify superior families for future oil palm breeding program and development of D×P planting materials. Data on yield components, bunch quality, and vegetative traits were collected for eight consecutive years and were subjected to analysis of variance and multivariate analysis. The analysis of variance showed significant variability among the families for most of the traits evaluated. The mean nut weight and rachis length had high broad-sense heritability value of 62.15% and 61.06%, respectively while the rest of the traits ranged from moderate to low. The 26 families were grouped into six major clusters at the similarity coefficient of 0.72 based on quantitative traits with the aid of unweighted pair group method with arithmetic mean (UPGMA) dendrogram. This finding revealed that MPOB-Senegal germplasm exhibited the important economic traits namely, high kernel to fruit (KTB), kernel to bunch (KTB) and kernel yield (KY), small petiole cross section (PCS) and short rachis length (RL) which are vital for oil palm improving program. Based on the results, the three families SEN02.05, SEN05.02 and SEN06.01 were identified as prospective families for high kernel content which could be the most essential sources of lauric acids for oleochemical industry. Moreover, five families namely, SEN05.02, SEN03.07, SEN02.05, SEN07.05 and SEN10.03 were also recognized as prospective families which could be utilized to breed for the compact palm materials. In the molecular characterization, out of 72, a total 35 highly

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polymorphic and reproducibility markers were selected for genotyping study. The percentage of polymorphism (P=96.26%), number of effective allele (Ne = 2.653), observed heterozygosity (Ho = 0.584), expected heterozygosity (He = 0.550), total heterozygosity (H_T = 0.666), and rare alleles (54) were observed which indicates that MPOB-Senegal germplasm has a broad genetic variation. Among the SSR markers employed, sMo00053 and sMg00133 were recommended as the most informative markers for the MPOB-Senegal oil palm germplasm due to discrimination power in detection of both the highest private and rare alleles. The prevalence of private alleles was high in the SEN05.03, SEN12.03, SEN 3.07 and SEN10.3 families. High rare alleles was observed in SEN07.05, SEN12.03, SEN03.07, SEN06.08 and SEN10.03 families, while the highest observed heterozygosity (Ho) and expected heterozygosity (He) was recorded in SEN06.08 and SEN05.05, respectively. The oil palm populations with high rare alleles and He should be taken into account in selection and conservation, because these populations may have interesting and unique genes to be exploited further. This study identified that MPOB-Senegal germplasm had a broad genetic variation and revealed that the oil palm genetic materials from this germplasm possess very important and useful genetic resources which can broaden the narrow genetic base of current planting materials in terms of high kernel content.

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Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KEPELBAGAIAN GENETIK GERMPLASMA KELAPA SAWIT MPOB-SENEGAL BERDASARKAN CIRI KUANTITATIF DAN PENANDA MIKROSATELIT

Oleh

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Memahami struktur genetik yang berbeza bagi ciri vegetatif, hasil dan komponen hasil yang berbeza memainkan peranan penting dalam program pembiakbakaan kelapa sawit. Penilaian kepelbagaian genetik berdasarkan morfologi dan fisiologi tidak mencukupi untuk mendapatkan maklumat yang komprehensif kerana polimorfisme yang rendah, fasa remaja yang panjang, dan sangat dipengaruhi oleh persekitaran seperti tanaman kelapa sawit. Berdasarkan maklumat ini, kajian ini telah dijalankan untuk menilai kepelbagaian genetik dan heritabiliti di kalangan 26 famili germplasma kelapa sawit MPOB-Senegal menggunakan ciri-ciri kuantitatif (ciri-ciri hasil, kualiti tandan dan vegetatif) dan penanda molekul mikrosatelit (SSR) bagi mengenalpasti pokok unggul untuk program pembiakbakaan kelapa sawit di masa hadapan dan pembangunan bahan tanaman D×P. Data komponen hasil, kualiti tandan, dan ciri vegetatif dikumpulkan selama lapan tahun berturut-turut dan analisis varians dan analisis multivarians telah dijalankan. Analisis varians menunjukkan kepelbagaian yang ketara di kalangan famili tersebut bagi kebanyakan ciri-ciri yang telah dinilai. Purata berat isirong dan panjang pelepah mempunyai nilai heritabiliti yang tinggi iaitu 62.15% dan 61.06% masing-masing, manakala ciri-ciri yang lain mempunyai nilai antara sederhana hingga rendah. Dua puluh tujuh famili yang dinilai ini telah diklusterkan ke dalam enam kumpulan utama pada pekali persamaan 0.72 berdasarkan ciri-ciri kuantitatif dengan bantuan kaedah kumpulan pasangan yang tidak berwajaran dengan dendrogram purata aritmetik (UPGMA). Hasil kajian menunjukkan bahawa germplasma MPOB-Senegal mempunyai ciri-ciri ekonomi penting seperti isirong ke tandan (KTB), hasil isirong (KY) yang tinggi, keratan rentas pelepah yang kecil (PCS) dan kepanjangan pelepah (RL) yang pendek di mana ia adalah penting untuk program pembaikan kelapa sawit. Keputusan kajian mendapati, tiga famili, SEN02.05, SEN05.02 dan SEN06.01 telah dikenalpasti sebagai famili berpotensi bagi menghasilkan kandungan isirong tinggi yang merupakan sumber asid laurik yang paling penting untuk industri oleokimia. Sebagai

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tambahan, lima famili iaitu SEN05.02, SEN03.07, SEN02.05, SEN07.05 dan SEN10.03 juga dikenalpasti sebagai famili berpotensi di kalangan germplasma MPOB-Senegal yang boleh digunakan untuk pembiakbakaan bahan tanaman sawit bagi penanaman padat. Dalam pencirian molekul, sejumlah 297 penanda SSR telah digunakan untuk menyaring koleksi germplasma ini dan 72 penanda didapati polimorfik. Tahap polimorfisme yang tinggi (P = 96,26%), jumlah alel yang efektif (Ne = 2,653), keheterozigotan yang dicerap (Ho = 0.584), keheterozigotan yang dijangka (He = 0.550), jumlah keheterozigotan (H_T = 0.666), dan alel luarbiasa (54) yang didapati menunjukkan bahawa germplasma MPOB-Senegal mempunyai variasi genetik yang luas. Antara penanda SSR yang digunakan, sMo00053 dan sMg00133 adalah disyorkan sebagai penanda yang paling bermaklumat untuk germplasma kelapa sawit MPOB-Senegal ini untuk kuasa diskriminasi dalam mengesan kedua-dua alel peribadi dan alel luarbiasa yang tertinggi. Kelaziman alel peribadi telah ditunjukkan dalam famili SEN05.03, SEN12.03, SEN 3.07 dan SEN10.3. Alel luarbiasa yang tinggi diperhatikan dalam famili SEN07.05, SEN12.03, SEN03.07, SEN06.08 dan SEN10.03 manakala keheterozigotan (Ho) dicerap dan jangkaan keheterozigotan (He) yang tertinggi masing-masing direkodkan dalam SEN06.08 dan SEN05.05. Pemilihan dan pemuliharaan perlu mengambil kira populasi kelapa sawit dengan alel luarbiasa dan He kerana populasi ini mungkin mempunyai gen yang menarik dan unik untuk dimanfaatkan dengan lebih lanjut. Kajian ini telah mengenalpasti bahawa germplasma MPOB-Senegal mempunyai variasi genetik yang luas dan membuktikan bahawa bahan genetik kelapa sawit dari germplasma ini mengandungi sumber genetik yang sangat penting dan berguna yang dapat memperluaskan asas genetik bahan penanaman semasa yang terhad terutama bagi kandungan isirong yang tinggi.

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LIST OF ABBREVIATIONS

"E	East
"N	North
˚S	South
%	Percentage
%P	Percentage of polymorphism
˚C	Degree Celsius
AFLP	Amplified fragment length polymorphism
AMOVA	Analysis of Molecular Variance
ANOVA	Analysis of variance
bp	base pair
cm	Centimetre
cm^2	Square centimetre
CTAB	Cetyltrimethylammonium bromide
CV	Coefficient of variation
DNA	Deoxyribonucleic acid
dNTPs	Deoxyribonucleoside triphosphate
EDTA	2'-Deoxynucleoside 5'-triphosphate Ethylenediamine tetra-acetic acid
EDTA	Ethylene Diamine Tetra-acetic Acid
EU	Europe
F_{is}	Fixation indices
GCV	Genotypic coefficient of variation
h^2B	Broad sense heritability
ha	Hectare
H_e	Expected heterozygosity
H_o	Observed heterozygosity

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H_T	Total heterozygosity
I	Shannon's information index
ml	Millilitre
MSE	Mean square error
Na	Number of observed alleles
Ne	Number of effective alleles
ng	Nanogram
NJ	Neighbour-Joining
NTYSYS	Numerical taxonomy multivariate analysis system
OD	Optical density
PCA	Principal component analysis
PCR	Polymerase chain reaction
PCV	Phenotypic coefficient of variation
PS	Plant Series
PVP	Polyvinylpyrrolidone
QTL	Quantitative Trait Loci
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
REML	Restricted maximum likelihood
RNAse	Ribonuclease
SE	Standard error
SEN	Senegal
HSD	honestly significant difference
MS	Malaysian Standard
SSR	Simple sequence repeat
Std.	Standard deviation

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TAE	Tris-acetate-EDTA
Taq	Thermas aquaticus
UPGMA	Unweighted pair group method using arithmetic averages
USDA	United States Department of Agriculture
UV	Ultraviolet light
µl	Microliter (s)
SAS	Statistical Analysis System

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CHAPTER 1

INTRODUCTION

1.1 General introduction

The oil palm belongs to the genus Elaeis under the family Arecaceae that consists of two species; *Elaeis guineensis* and *Elaeis oleifera*, which originated from Central-Western Africa and Central America, respectively. Oil palm is the most productive oil-bearing crop in the world and a good source of high-value products such as palm oil, palm kernel oil, and palm kernel cake. Palm oil is derived from mesocarp while kernel oil extracted from kernel. Due to their different distinctive chemical properties, palm oil is used for edible purposes whereas palm kernel oil is mostly used for non-edible uses such as soaps, cosmetics, and detergents. In favorable growing condition, oil palm is able to produce palm oil and kernel oil on average about 4.5 and 0.50 tons/year/hectare, respectively. This yield is almost three times higher than coconut oil yields and 10 times higher than that of soybean (Robblelen *et al*.,1989; Byerlee, *et al*., 2017; Rajanaidu, *et al*., 2017). Kushairi (2019) illustrated that palm oil is the only product to meet the requirements of global oil consumption.

According to USDA-FSA (USDA,2017), world vegetable oil production is estimated to reach 187 million tonnes for 2016/2017. Palm oil and palm kernel oil produced 70.3 million tons which accounted for 37.6% of global oil production (USDA, 2017). In 2018, palm oil production accounted for 32% of the 230.08 million tons of oils and fats produced worldwide and contributed 57% of the 88.04 million tons of global oils and fats exported. Palm oil is the world's largest produced and traded vegetable oil in oils and fats market. The major consumer countries of palm oil are Indonesia, India, the EU, and China. Malaysia still holds the world's second-largest position of palm oil producer and exporter in 2018. Malaysia produced 19.52 million tons of the total 72.08 million tons of palm oil production, representing 33% of global palm oil exports (Kushairi, 2019).

Oil consumption is growing with an increasing world population. In order to meet the increasing demand for global vegetable oil consumption and multipurpose of the oil palm products, oil palm cultivation is being expanded. However, the availability of cultivated land is limited to oil palm area expansion. Therefore, it should be considered to increase yield per unit land area coupled with breeding methods of improved planting materials (Murphy, 2014).

However, a significant feature of the oil palm breeding programs is the very narrow genetic basis and Malaysian Palm Oil Board (MPOB), formerly known as Palm Oil Research Institute of Malaysia (PORIM) had been collected wild germplasm from its origin in West Africa. The germplasm collections are maintained *ex situ* in the field gene bank of MPOB Research Station in Kluang, Johore for evaluation, conservation, selection and utilization in oil palm breeding program (Rajanaidu, 1985, 1994).

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Genetic diversity study in natural population is very important for the breeders to develop new and cultivars with desirable characters. Therefore, MPOB oil palm germplasms were evaluated for yield and agronomic traits to determine the genetic diversity for commercial utilization and posterity. Primarily, MPOB oil palm germplasms have been investigated based on morphological and physiological traits (Rajanaidu, 1980), fatty acid composition (Arasu, 1985), and yield parameters (Rao, 1987). Populations of MPOB-Nigerian germplasm were evaluated for quantitative traits Rajanaidu *et al*. (1989). The results exhibited that they have high genetic resources for the improvement of oil palm breeding program. These results have been proven by developing new and improved planting materials by MPOB namely, high yielding dwarf (PS1), high iodine value (P2), (Kushairi, 1999 a,b), large kernel (PS3) (Rajanaidu, 1996), high carotene (PS4 and PS11) (Mohd Din *et al*., 2002; 2006), thin-shell *teneras* (PS5) (Kushairi *et al*., 2003a), large-fruit *duras* (PS6) (Kushairi, 2003b), high bunch index (PS7) (Junaidah *et al*., 2004), high vitamin E (PS8) (Kushairi *et al*., 2004), long stalk (PS10) (Noh *et al*., 2005), high oleic acid (P12) (Isa *et al*., 2006), low lipase (P13) (Maizura *et al*., 2008) and high protein kernel (P14) (Noh *et al*., 2015).

Nevertheless, evaluation of genetic diversity based on quantitative traits is influenced by environmental factors. Hence, it is insufficient to get comprehensive information based on quantitative evaluation due to vulnerability as a result of environmental effects. Mondini *et al*. (2009) described that the applications of molecular markers in the genetic diversity was more efficient because they are stable and detectable in all stages of plant growth, development effect, and can overcome environmental effects. Several researchers have investigated MPOB germplasms from 11 African countries of using different molecular techniques; by isoenzyme analysis (Hayati *et al*., 2004), Amplified Fragment Length (AFLP) markers (Kularante, 2001), Restriction Fragment Length Polymorphism (RFLP) (Maizura *et al*., 2006), and Simple Sequence Repeat markers (SSR) (Bakoume' *et al*., 2009, 2015; Zulkifili *et al*., 2012).

Consequently, Maizura *et al*. (2006) stated that oil palm breeders should investigate the valuable genes from the higher level of diversity of unused germplasm in order to improve the oil palm breeding program. Ting *et al*. (2010) also pointed that palms from natural collections possess a valuable genetic resource to improve the current planting materials. Similarly, Zukifili *et al*. (2012) suggested that individual population of germplasm from each country among MPOB collections should be evaluated. Through evaluation results from 11 oil palm germplasm collected by MPOB, most of MPOB-Senegal populations were found to possess rare alleles (Hayati *et al*., 2004; Maizura *et al*., 2006; Zulkifili *et al*., 2012; Bakoume´*et al*., 2015) and Hayati *et al*. (2004) described that population 2 of Senegal had high genetic diversity and could be utilized for oil palm improvement program. According to Rajora *et al*. (2000), Senegal oil palm populations may possess desired traits related to low rainfall and dry weather for its rare allele occurrence. The results of molecular characterization of MPOB germplasms using Simple Sequence Repeated markers by Singh *et al*. (2008), Ting *et al*. (2010), Zulkifili *et al*. (2012), and Bakoume' *et al*. (2015) showed that SSR markers are one of the most promising

molecular systems for understanding the genetic diversity and structure of oil palm and its relatives.

1.2 Problem statement

The genetic background of commercial oil palm in Malaysia is narrow due to the fact that the current breeding materials are derived from four palms planted at Bogor Botanical Garden, Indonesia in 1848 (Hartley, 1988). The consequence of restricted gene pool brings the slow selection progress in oil palm. MPOB made the collections of oil palm from 11 African countries, the center of the origin from West Africa to expand the gene pool. The natural palm collections have been evaluated for morphology in the field as well as molecular methods to explore the genetic variability and to seek the valuable traits. Breeders are now emphasizing not only on oil yield but also for better adaption to drought, heat and other abiotic stress to meet future demands. The MPOB-Senegal oil palm germplasm is adapted to dry weather condition from its original site of collection which implies that it may possess drought-tolerant characteristics. However, the germplasm yet to be evaluated to cover all the populations. Therefore, comprehensive evaluation among and within the populations of MPOB-Senegal germplasm should be carried out to access any promising traits to broaden the current breeding programme. This is the first assessment of genetic variation study in MPOB-Senegal germplasm which cover all the populations. Hence, this study is carried out with the following objectives;

1.3 Objectives

The objectives of this study are as follow:

i. To quantify genetic variability and heritability values on quantitative traits.
ii. To determine the traits contributing to the overall variation in the yield and important agronomic characteristics.
iii. To quantify genetic diversity by SSR markers, and
iv. To select superior families for further improvement and development of D×P planting materials for commercial cultivation.

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Journal

Myint, K. A., Amiruddin, M. D., Rafii, M. Y., Samad, M. Y. A., Ramlee, S. I., Yaakub, Z., & Oladosu, Y. (2019). Genetic diversity and selection criteria of MPOB-Senegal oil palm (Elaeis guineensis Jacq.) germplasm by quantitative traits. *Industrial Crops and Products*, *139*, 111558. (Published)

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