UNIVERSITI PUTRA MALAYSIA

GENETIC DIVERSITY AMONG OIL PALM PARENTAL GENOTYPES REVEALED BY MICROSATELLITE POLYMORPHISM AND ITS RELATIONSHIP TO PROGENY PERFORMANCE

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Abstract of this thesis presented to the senate of University Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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By

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October 2008

Chairman: **Assoc. Prof. Mohd. Rafii bin Yusop, PhD**

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Oil palm (*Elaeis guineensis* Jacq.) is a perennial crop. One complete cycle of selection takes about 10 to 15 years and due to that, breeding and selection of the crop is slow. By developing marker assisted selection for this species, the time needed for breeding and selection could be decreased to almost half compared to that through conventional method. This study involves investigating the genetic relationship between the parental palms (*dura* and *pisifera*) and their progenies based on microsatellite markers. The general objectives of this study are to estimate genetic diversity between *Dura* and *Pisifera* parental combinations using microsatellite markers and to investigate the association between genetic diversity and progeny performance. Nine microsatellite markers were used to screen selected parental palms (15 parental *duras* and 4 parental *pisiferas*) and their progenies (16 DxP crosses). Data were scored and analysed using Biosys-1 software to calculate the genetic distance values and subsequently constructing the dendrogram. A total of 29 polymorphic bands were generated. The genetic distances among progenies ranged from 0.444 to 0.746. Considerable polymorphism of 94.5% was observed in DxP progenies. Cluster analysis based on genetic distances revealed associations among progenies which were closely in agreement with the pedigree data. The performance of 16 *Dura* x *Pisifera* progenies was evaluated for quantitative characters. A large variation among the genotypes was detected in these DxP progenies for yield and yield components. Based on *pisifera* components, shell to fruit ratio (S/F) exhibited the highest heritability (58.18%) among the traits examined whereas for *duras* within *pisifera,* the highest heritability correspond to palm height (HT) and rachis length (RL). Correlation analyses between genetic distances and progeny performance were estimated by simple correlation coefficient. The correlation values of genetic distances with progeny performance were mostly non-significant, except for mean nut weight (MNW) and leaf number (LN). However, the correlation of genetic distances with these characters is too low to be used as predictive value. These results indicate that genetic distances based on the microsatellite markers used in this study may not be useful for predicting progeny performance in oil palm.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

KEPELBAGAIAN GENETIK KELAPA SAWIT GENOTIP INDUK BERDASARKAN POLIMORFISM PENANDA MIKROSATELIT DAN HUBUNGANNYA DENGAN PRESTASI PROGENI

Oleh

NORZIHA BINTI ABDULLAH

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Pengerusi: **Prof. Madya Mohd. Rafii bin Yusop, PhD**

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Kelapa sawit (*Elaeis guineensis* Jacq.) merupakan sejenis tanaman saka. Satu kitar lengkap pemilihan mengambil masa lebih kurang 10 hingga 15 tahun dan ini mengakibatkan proses biakbaka dan pemilihan bagi tanaman ini adalah perlahan. Dengan meluaskan penggunaan ‘penanda membantu pemilihan’ bagi spesis ini, masa yang diperlukan untuk pemilihan dan pembiakbakaan boleh dikurangkan separuh daripada masa yag diperlukan apabila menggunakan cara ‘lama’. Projek ini melibatkan kajian tentang hubungan di antara induk kelapa sawit (*dura* dan *pisifera*) dan progeni yang dihasilkan berdasarkan penanda mikrosatelit. Objektif umum kajian ini adalah untuk menganggarkan kepelbagaian genetik antara kombinasi-kombinasi pokok induk *dura* dan *pisifera* menggunakan penanda mikrosatelit dan mengkaji hubungan antara kepelbagaian genetik induk kelapa sawit dan prestasi progeni. Sembilan penanda mikrosatelit digunakan untuk menyaring induk yang terpilih (15 induk *dura* dan 4 induk *pisifera*) serta progeni yang terhasil (16 kacukan DxP). Data yang diambil kemudiannya dianalisis menggunakan program Biosys-1 untuk mengira nilai jarak genetik dan seterusnya membina dendrogram. Sebanyak 29 jalur polimorfisme telah dihasilkan. Jarak genetik antara progeni antara 0.444 hingga 0.746. Polimorfisme bernilai 94.5% terdapat pada progeni DxP. Analisis kelompok berdasarkan jarak genetik menunjukkan hubungan antara progeni dan sangat bertepatan dengan data pedigree. Prestasi 16 progeni *Dura* x *Pisifera* dikaji untuk ciri-ciri kuantitatif. Variasi yang tinggi antara genotip dikesan di dalam progeni ini bagi ciri hasil dan komponen-komponen hasil. Berdasarkan komponen *pisifera,* nisbah tempurung ke buah (S/F) menunjukkan keterwarisan yang paling tinggi (58.18%) antara ciri-ciri yang dikaji manakala untuk *dura* dalam *pisifera*, keterwarisan yang paling tinggi ialah ketinggian pokok (HT) dan panjang pelepah (RL). Analisis korelasi antara jarak genetik dan prestasi progeni dianggarkan dengan menggunakan pekali korelasi mudah. Nilai korelasi antara jarak genetik dan prestasi progeni kebanyakannya tidak bererti selain purata berat biji (MNW) dan jumlah daun (LN). Walau bagaimanapun, nilai korelasi adalah sangat rendah untuk menjadi nilai penentuan. Keputusan kajian ini menunjukkan bahawa jarak genetik berdasarkan penanda mikrosatelit tidak boleh digunakan untuk menjangka prestasi progeni dalam tanaman sawit.

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I certify that an Examination Committee has met on 24th September, 2008 to conduct the final examination of Norziha binti Abdullah on her Master of Science entitled “” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the degree Master of Science.

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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.

NORZIHA BINTI ABDULLAH

DATE:

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LIST OF ABBREVIATIONS

ABW	Average bunch weight
AVROS	Algemene Vereniging van Rubber-planters ten Oostkust van Sumatra
BNO	Bunch number
df	Degree of freedom
DIA	Diameter
DNA	Deoxyribonucleic acid
EMS	Expected mean squares
EST	Expressed sequence tag
F/B	Fruit to bunch
FFB	Fresh fruit bunch
FP	Frond production
h^2_N	Narrow-sense heritability
HT	Palm height
K/B	Kernel to bunch ratio
K/F	Kernel to fruit ratio
KY	Kernel yield
LA	Leaflet area
LAI	Leaf area index
LAR	Leaf area ratio
LL	Leaflet length
LN	Leaflet number
LW	Leaflet width
M/F	Mesocarp to fruit ratio
MPOB	Malaysian Palm Oil Board
NCM 1	North Caroline Mating Design 1
O/B	Oil to bunch ratio
O/DM	Oil to dry mesocarp ratio
O/WM	Oil to wet mesocarp ratio
OY	Oil yield
PCS	Petiole cross-section
r	Correlation coefficient
RL	Rachis length
S.E	Standard error
S/F	Shell to fruit ratio
TEP	Total economic product
TOIL	Total oil

CHAPTER 1

INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) cultivation has expanded tremendously in recent years and it is now the leading vegetable oil in the world, taking over the top position from soybean in 2006 (MPOB, 2008). For the last few years, Southeast Asia is the dominant region of production for palm oil with Malaysia being the leading producer and exporter. To date, Indonesia has overtaken Malaysia both in terms of planted area and production (Rajanaidu, *et al*., 2007). In 2007, Malaysia produced approximately 15.8 million tonnes and exported 13.74 million tonnes of palm oil (MPOB, 2008).

Oil palm plays significant role in the socio-economic development of the country especially in rural areas by providing employment and raising income levels. It is an important contributor to Malaysia's Gross Domestic Product (GDP) and also foreign exchange earnings which contributed considerably to bolster the country's economy (MPOB, 2008). The success of oil palm industry in Malaysia is attributed to many factors, among them are favorable climatic conditions, well-established infrastructure, improved management and technologies for oil palm cultivation and establishment of estate type plantations.

In 2007, the total oil palm planted area in Malaysia increased by 3.4% and to 4.3 million hectares. Sabah remained the largest oil palm planted state with 1.27 million hectares or 30% of the total planted area (MPOB, 2008). In Malaysia, oil palm planted areas can be divided into large estates which are managed by public listed companies, smaller independent estates, independent smallholders and government smallholder settler schemes.

The oil palm remained as a remarkable competitor compared to other vegetable oil crops in terms of oil yield per hectare. The oil from a properly maintained oil palm plantation can be six times higher than those from commercially grown rapeseed (Ernst and Thomas, 1999). Besides that, researches have shown that palm oil contains antioxidants for human diet such as vitamin A and E, as well as other properties which assist in sustaining lower cholesterol levels. Through latest blending and separating technologies, palm oil has good potential as biofuel (Yusof, 2005).

In order to stay in the forefront, Malaysia has launched vast number of technologies in oil palm. Among others are new planting materials to meet the future needs of the industry. Since 1994, 12 planting series and breeding populations have been introduced. For improvement of oil yield, most breeders will consider oil and kernel yields per hectare as the most important characters when producing planting material for commercial release. Other agronomic traits such as short frond, long stalk and high carotene were also emphasized.

Selection of best parental combinations is essential to ensure maximum gain of genetic improvement. It is therefore of greatest importance to obtain the basic knowledge on the diversity, inheritance and genetic pattern of the materials used in oil palm breeding and selection. The information may be useful in designing breeding programme for oil palm improvement.

The development of molecular markers provides tools for assessing the genetic diversity at the DNA level in plant species (Melchinger and Gumber, 1998). Genetic distances based on molecular markers has been used for grouping of similar germplasm as a first step in identifying diverse parents for promising heterotic expression (Melchinger, 1999).

Besides that, molecular data have frequently been included in several methods of hybrid performance prediction. They have been integrated into yield prediction models in several ways. Among others are for calculation of parental genetic distances and correlation analysis with progeny yield (Lee *et al*., 1989; Boppenmaier *et al*., 1992; Ajmone *et al*., 1998), generation of covariates for specific combining abiltiy (SCA) in the distance and factorial regression interaction models (Charcosset *et al*., 1998), and calculation of the coefficients of parentage (Bernado, 1993) to be used as covariance between single-crosses in the best linear unbiased prediction (BLUP) method (Bernado, 1995; Charcosset *et al*., 1998).

Molecular breeding is well suited to a perennial crop like oil palm, in which the economic products are not produced until several years after planting. They offer promising tools in plant breeding, particularly, in identifying and eliminating poorer parents in the early stage. Molecular markers can be used to evaluate the genetic distance among the parental palms. Since hybrid vigor is contributed by genetic complementation between divergent parents, it can be assumed that parents with high genetic distance coefficients have the tendency to produce more vigorous hybrids (Gupta and Varshney, 2000).

Therefore, molecular markers maybe useful in helping breeders to choose the most promising parental combinations to be tested. Besides that, a number of traits in oil palm such as shell thickness, external fruit colour and fatty acid composition can eventually be tracked using molecular markers. These will fast-track the production of new and improved oil palm planting materials (Rajinder and Cheah, 2005).

The study is composed of two main experiments.

Experiment 1: Genetic variation among oil palm parental genotypes and their progenies based on microsatellite markers

Experiment 2: Genetic inheritance and performance of oil palm DxP progenies

The objectives of this study are 1) to estimate genetic diversity between *dura* and *pisifera* parental combinations using microsatellite markers and 2) to investigate the association between genetic diversity and progeny performance.

CHAPTER II

LITERATURE REVIEW

2.1 Origin, Botany and Suitable Environment of Oil Palm

2.1.1 Origin

Oil palm exists in wild, semi-wild and cultivated part of the tropic, within 10^o N and 10^o S of the equator in Africa, South East Asia and Central America (Hartley, 1998). Generally, the African oil palm (*Elaeis guineensis* Jacq.), the name was given by Jacquin in 1763, originated from the tropical rain forest region of West Africa. The Latin American oil palm, *E. oleifera* Cortes, is endemic to Central and South America.

Commercially, *E. guineensis* is more important than *E. oleifera*. However, *E. oleifera* that has been hybridized with the *E. guineensis*, has several desirable attributes such as short, slow growth, high unsaturated fatty acid profiles and resistance to fatal yellowing disease (Meunier and Hardon, 1982). The two oil palm species were presumed to have diverged when the American and African continents drifted apart in prehistoric times (Zeven, 1965).

2.1.2 Botany

Oil palm belongs to Palmae or Arecaceae family, subfamily Cocoideae, tribe Cocoineae and the genus *Elaeis*. The genus *Elaeis* consists of two species namely the African oil palm, *E. guineensis* Jacq. and the Latin American oil palm, *E. oleifera* Cortez (Moore, 1973). Another species, *E. odora* which was previously placed under the genus *Elaeis* has been reclassified in the genus Barcella (Uhl and Dransfield, 1987). The term *Elaeis* comes from the Greek word, elaion, meaning oil while the word *guineensis* was attributed to the Guinea coast, the expected origin of the oil palm (Hartley, 1998). It is a monocotyledonous plant and a diploid species with chromosome number of 2n=2x=32 (Rival *et al*., 1997).

As described by Hishamudin *et al*. (1985), the oil palm is a monoecious plant which grows to a height of 20-30 meters and lives up to 200 years. The trunk is unbranched and crowned with normally 35 to 40 fronds per year. The fronds are arranged around the trunk in two spirals which may be right-handed or left-handed (Latiff, 2000).

Male and female inflorescences normally occur in different cycles, reducing the chances of self pollination (Turner and Gillbank, 1982). At anthesis, the male and female flowers have a strong smell similar to aniseed. As a cross-pollinated species, the pollination is mainly carried out by insects such as the weevil, *Eleidobius kamerunicus.* This species was imported to Malaysia from Cameroons, plays a dominant role in oil palm pollination (Syed, 1979).

Following the pollination, the female inflorences develop into a fruit bunch. Fruit bunches ripe 5-6 months after pollination. The number of fruits for each bunch varies with age, normally containing more than 1000 fruits and 10-60 kg in bunch weight. The fruit is a sessile drupe varying in shape from nearly spherical to ovoid or elongated with sizes between 2-5 cm and weight ranging from 3 to 40 gram (Turner and Gillbank, 1982). The outer fruits are normally fully formed because of ample space for their development as compared to the inner fruits, inside the bunch, which tend to be somewhat flattened, smaller and less pigmented. Oil palm bunches bear about 1000-3000 fruits borne on 100-120 spikelets attached to a peduncle from the axil of a frond (Yusof and Chan, 2004). The individual fruit is made up of an outer skin (the exocarp), a pulp (mesocarp), shell (endocarp) and kernel.

2.1.3 Cultivation Requirement

According to Hartley (1998) oil palm needs continuous sunlight about 5-7 hours everyday. It is now grown as a plantation crop in most countries with high rainfall (minimum 1 600 mm/yr) in tropical climates within 10° of the equator. It is suitable to be planted in loose-textures soil on flat land, grown on a wide range of soil types, provided good drainage and pH between 4 and 7 with no stone or gravel layer in the first 1.2 meter below the surface (Hishamudin *et al*., 1985). The average maximum temperature which is suitable for oil palm ranged from 22 to 24 ^{0}C. Oil palm is a perennial crop with life span reaching 200 years old (Purseglove, 1975). However, the economic life span of oil palm commercial plantation is about 25 to 35 years (Ng, 1972).

2.2 Types of Oil Palm

The oil palm fruit has distinct variations of the external appearance (colour of fruits) and thickness of shell.

2.2.1 Fruit Types Based on Colors

The variation in colors which is controlled by monogenetic inheritance is due to marked differences in the carotene content of the mesocarp (Latiff, 2000). There are three types of fruit which can be distinguished by colour; *nigrescens*, *virescens* and *albescens* (Figure 2.1). *Nigrescens* fruits are the most common in oil palm due to high carotenoid contents. These fruits are dark purple or almost black before ripening. The color of the *nigrescens* fruit varies to some extent on ripening, to either entirely red, or black over the upper half but red at the base (Hartley, 1998). When ripe, two types of fruit form are recognized which are described as *rubro-nigrescens* and *rutilo-nigrescens*. *Rubro-nigrescens* is ripe fruit with deep reddish orange in colour and brown cap on upper half.

On the other hand, *rutilo-nigrescens* is ripe fruit of pale orange and black cap on upper half. Another fruit colour type known as *virescens* (relatively uncommon) is green with yellowish cap before ripening. At maturity, the colour of the fruit changes to bright reddish orange. An extremely rare fruit, *albescens,* is characterized by the very small quantity of carotene in the mesocarp. It is pale yellow or ivory when ripe and very dark brown when young (Hishamudin *et al*., 1985).

2.2.2 Fruit Forms Based on Shell-Thickness

Thickness of oil palm fruit shell has significant overwhelming agronomic importance (Hartley, 1998). The inheritance of shell thickness (internal structure) was elucidated by Beirnaert and Vanderweyen (1941). There are three naturally occurring forms of the oil palm fruit, termed as *dura*, *tenera*, and *pisifera* based on shell thickness (Figure 2.2). The *dura* palm is homozygous dominant ($sh^+ sh^+$) for thick shell (2-8mm) trait that give mesocarp to fruit ratio of about 60% while the *pisifera* palm is shell-less recessive homozygote ($sh^- sh^-$) which is usually female sterile that give mesocarp to fruit ratio about 90%. The shell is absent in the *pisifera*