CHAPTER III

MATERIALS AND METHODS

3.1 Breeding Materials and Site Location

The germplasm used in this study originated from Senegal and Gambia. A random sample of five families from 13 sites originating from Senegal and also Gambia (population; designated as SEN 01, SEN 02, SEN 03, SEN 04, SEN 05, SEN 05, SEN 06, SEN 07, SEN 08, SEN 09, SEN 10, SEN 11, SEN 12, SEN 13 GAM05.02 and GAM05.08) were collected in July-August 1993 by researchers from Malaysian Palm Oil Board (MPOB) from the two countries. The germplasm materials were planted at the MPOB Research Station Kluang, Johor in 1996. The palms were derived from the Independent Completely Randomized Design, where a total number of 415 open-pollinated palms were planted in Trial 0.352 (Senegal materials) and Trial 0.357 (Gambia materials) with two replicates each (41 progenies in replication 1 and 15 progenies in replication 2). For the purpose of this study, available quantitative data on the Senegal and Gambia germplasm were collected at the MPOB headquarter.

3.2 Data Collection

The performance of progenies for yield and oil yield components was assessed based on data on fresh fruit bunch (FFB) yield records, bunch number (BNO) and average bunch weight (ABW), bunch components, vegetative and physiological characters and fatty acid characters.

3.2.1 Yield and Yield Components

Harvesting of oil palm usually begins at 36 months after field planting with subsequent operations carried out at regular intervals of seven to ten days, i.e. three rounds in a month. Data on yield and yield components were evaluated from year 2000 – 2007.

Procedure for Yield Recording

Recordings of bunch weight (BWT) and bunch number (BNO) on individual are carried out during the harvesting rounds in oil palm breeding. Fresh bunch weight (FFB) is the sum of the bunch weight (BWT) while BNO is the total of all the bunch counts and average bunch weight (ABWT) is the quotient between FFB and BNO. The yield components were derived as follows:

FFB (kg/p/yr) $= \sum_{i=1}^{n} BWT_i$

BNO (bunches/p/yr) $= \sum_{i=1}^{n} BNO_i$

ABWT (kg/p/yr) = FFB/BNO

Where n is the number of harvesting rounds.

3.2.2 Bunch Analysis

Quantitative data on the bunch components were evaluated in the 2001 until 2006. Bunch and fruit components were determined using the bunch analysis technique developed by Blaak et al. (1963).

Procedure for Bunch Analysis

Samples of three to five bunches from each palm were analysed. Each bunch was weighed and fruit bearing spikelets chopped off the stalk. The spikelets were randomly segmented for analysis of the fruit to bunch (F/B) and fruit compositions (FC). To ease picking, the F/B portions were kept for two days and the weight of empty spikelets, fertile and parthenocarpic fruits were recorded. Analysis of the fruit compositions was continued following spikelet sampling on the same day. Fruits were detached and randomly sampled, counted, weighed and scraped. The nuts were crack opened and kernel weighed. A 5g sample of minced mesocarp was oil extracted in hexane for 18-24 hours using soxhlet apparatus. The amount of oil in the sample was calculated on weight difference before and after extraction. The components of the bunch were calculated using the below formulae:

F/B = Fruit to Bunch (%) = [(FFWT + PFWT) / SWT] × [(BWT -STKWT) / BWT] × 100

P/F = Parthenocarpic to Fruit (%) = PFWT / (FFWT + PFWT) × 100

M/F = Mesocarp to Fruit (%) = [(FSWT - FNWT) / FSWT] × 100

MC = Moisture Content of mesocarp(%) =100 -[{(TDMWT-TWT)/(FSWT-FNWT)}×100]

O/DM = Oil to Dry Mesocarp (%) = [(ETMWT - ETFWT) / (ETMWT - ETWT)] × 100

O/WM = Oil to Wet Mesocarp (%) = [(100 - MC) × O/DM] / 100

O/B = Oil to Bunch (%) = (F/B × M/F × O/WM) / 10,000

O/Fi = Oil to Fibre (%) = [(ETMWT -ETFWT) / (ETFWT -ETWT)] × 100

K/F = Kernel to Fruit (%) = (KWT / FSWT) × 100

MNW = Mean Nut Weight (g) = FNWT /NOFNUT

MFW =Mean Fruit Weight (g) = FSWT / NOFNUT

P/B = Parthenocarpic to Bunch (%) = (P/F × F/B) / 100

OPY = Oil per Palm per Year (kg) = (O/B × FFB) / 100

KPY = Kernel per Palm per Year (kg) = (K/B × FFB) / 100

Where;

BWT = Bunch weight
STKWT = Stalk weight
SWT = Spikelet weight
FFWT = Fertile fruit weight
PFWT = Parthenocarpic fruit weight
ESPKWT = Empty spikelet weight
FSWT = Fruit sub sample weight
FNWT = Fresh nut weight
NOFNUT = Number of fresh nut
KWT = Kernel weight
TDMWT = Tin + dry mesocarp weight
TWT = Tin weight
ETWT = Extraction thimble weight
ETFWT = Extraction thimble + fibre weight
ETMWT = Extraction thimble + mesocarp weight
FFB = Fresh fruit bunch

3.2.3 Vegetative Measurements and Physiological Characters

Vegetative characters of the oil palm germplasm were pooled eight years after field planting. Frond production was first calculated after the 7th year before other parameters were taken a year after, i.e. year 2004. The physiological characters on the other hand were assessed based on measurements on collective data of bunch yields and bunch quality components, following the methods developed by Squire (1986). Data on the physiological characters were assessed in the year 2007. The various components of the vegetative and physiological parameters were calculated using the below formulae:

FP	= Frond production (no/p/yr)	= number of fronds produced in one year
PCS	= Petiole Cross Sectional Area (cm^2)	= petiole depth × petiole width
RL	= Rachis length (m)	= length from tip of rachis to the first ligule
LL	= Leaflet Length	= (LL1 + LL2 + ...LL6) / 6
LW	= Leaflet Width	= (LW1 + LW2 + ...LW6) / 6
LN	= Leaflet number (no/p/yr)	= (number of fronds on one side of rachis)×2
D	= Trunk diameter (cm)	= diameter of trunk at one meter from ground
HT	= Trunk height (m)	= height of trunk of ground to base of frond 41
HTI	= Height increment	= (HT at year t) / (age at year t - 2)
LA	= Leaf Area (m^2)	$= [(\Sigma (LL_i \times LW_i) \times LN \times 0.57) / 6] / 10{,}000$

LAR = Leaf Area Ratio = (FP × LA) / VDM

LAI = Leaf Index Ratio = (40 fronds × LA × PD^+) / 10,000

LDW = Leaf Dry Weight (kg) = (0.1023 × PCS) + 0.2062

TDW = Trunk Dry weight (kg) = 3.142 × $(D/2)^2$ × (HT / Age in year) × 1,000 × 0.17

FDW = Frond Dry weight (kg) = FP × LDW

FI = Frond Index = LA / LDW

f = Fractional interception = 1 - $\exp^{(-0.47) \times (LAI - 0.3)}$

e = Conversion Efficiency (g/MJ) = TDM / (31 × *f*)

VDM = Vegetative Dry Matter (kg/p/yr) = (FDW + TDW)

BDM = Bunch Dry Matter (kg/p/yr) = 0.53 × FFB

TDM = Total Dry Matter = [(VDM + BDM) × PD^+] / 1,000

BI = Bunch Index = BDM / (BDM + VDM)

NAR = Net Assimilation Rate = TDM / (0.52 × LAI)

TOIL = Total Oil (kg/p/yr) = OPY + (KPY x 0.5*)

TEP = Total Economic Product (kg/p/yr) = OPY + (KPY × $0.6^{\#}$)

Where;

$^+$PD = Planting Density
*0.5 = Kernel Oil Extraction Rate
$^{\#}$0.6 = Relative Price of Palm Kernel Oil to Price of Palm Oil
FFB = Fresh Fruit Bunch (kg/p/yr)
OPY = Oil per Palm Year (kg)
KPY = Kernel per Palm per Year (kg)

3.2.4 Fatty Acid Traits

Fatty acid traits were pooled between years 2000 – 2007. The fatty acid composition was evaluated using the method proposed by Timms (1978) for routine analysis using gas chromatography.

Procedure for Fatty Acid Analysis

The fatty acid analysis was carried out using MPOB method Timms (1978). Fresh bunches were collected from the palm, weighed and chopped. Spikelets samples were selected one each from apical, middle and basal portion of the bunch. The samples were sterilized and fruits from the sterilized spikelets separated. The mesocarps were separated and oven dried at 105 ℃ for a minimum of 3 hours. The dried mesocarp were minced and dried. After mincing, the dried mesocarp was diluted with 300ml hexane and filtered with a spoonful of sodium sulphate. The filtrate was sealed and kept in dry place. Oil and hexane mixture was later separated into oil and hexane, using rotary evaporator by distilling the hexane vapors under vacuum. The extracted oil is then poured in vials, labeled and stored for FAC and carotene analysis.

For the fatty acid methyl determination, 0.05g of the extracted oil was measured and 1.9ml of solvent hexane added and homogenized. Sodium methoxide of 0.1ml was also added until a cloudy solution is formed. After 10 minutes, the clear portion of the methyl ester was pipetted into gas chromatograph vials and covered with Teflon vial caps for analysis. The separation of the FAME by the GC machine was under programmed conditions. The fatty acids components were represented by the peaks on the chromatograph.

3.3 Statistical Analyses

3.3.1 Variability Profile

The quantitative morphological data collected was arranged in Excel Microsoft word. The oil palm accessions were organized according to their family codes. Simple descriptive statistics such as mean, standard deviation, standard error, minimum, maximum and variance for each collected traits were calculated using SPSS statistical tool. This was done in order to know the extent of variation in the germplasm accessions. The average of the quantitative data was also standardized to give equal weight to all measurements using the following formula (Microsoft Office Excel, 2007):

$$Z = X - \mu/\sigma \tag{1}$$

Where, X = Value to standardize

μ = Arithmetic mean

σ = standard deviation of the distribution

3.3.2 Principal Components Analysis (PCA)

PCA simplifies the complex data by transforming number of correlated variables into a smaller number of variables called principal components. The first principal component accounts for maximum variability in the data as compared to each succeeding component. PCA was analyzed using "THE UNSCRAMBLER®X" software (CAMO software version 10.1). Mathematically, PCA involved in the decomposition of original data matrix, X, into a structure part and noise part. In matrix representation, the model with a given number of components as follows:

$$X = TP^T + E \tag{2}$$

where T is the scores matrix, P the loadings matrix (transposed) and E the error matrix. The structured part of data is the combination of scores and loadings in which focused by user in interpretation of PCA results while the remaining part is called error or residual matrix. The ath column of T and ath row of P is represented by vectors of t_a and p_a respectively and both are the vector representations of the ath PC. The number of PCs is denoted by A, while a is the number of PC such as 1, 2, 3 up to A. The maximum number of PCs (A) determined is either I – 1 (number of objects – 1) or J

(number of variables) depending on which give smaller value. Thus, the first scores vector and the first loadings vector are called eigenvectors of the first principal component. Therefore, each successive component is characterized by a pair of eigenvectors for both the scores and loadings (CAMO, 2014).

In contrast, the residual matrix, *E*, represent the fraction of variation that cannot be modeled well. It other words, it cannot be explained by available PCs, yet, useful in the lack-of-fit measurement of model to the original data where small value of *E* indicated the good model and vice-versa (CAMO, 2014). Usually after PCA, the size of each component can be measured and represented by eigenvalue. In PCA, the more significant the components indicated the larger of their size, thus have larger eigenvalue. Therefore, the eigenvalue of a PC is the sum of squares of the scores and represented as follows:

$$g_a = \sum_{i=1}^{I} tij^2 \quad (3)$$

where g_a is the *a*th eigenvalue. The sum of all nonzero eigenvalues for a data matrix equals the sum of squares of the entire data-matrix, so that

$$\sum_{a=1}^{k} g_a = \sum_{i=1}^{I} \sum_{j=1}^{J} x_{ij}{}^2 \quad (4)$$

where *K* is the smaller of *I* or *J* (Brereton, 2003).

3.3.3 Cluster Analysis

Cluster analysis identifies variable which were further clustered into main groups and subgroups using Ward’s method through the “THE UNSCRAMBLER®X” software (CAMO software version 10.1). The general purpose of cluster analysis is to group similar objects or samples into respective classes based on their specified characteristics or variables and it includes different type of algorithms such as joining (tree clustering), two-way joining (block clustering) and *k*-means clustering. The aim of the tree clustering algorithm was to join the objects or samples in each class of themselves together using specified distance measures and linkage rules, thus, forming larger cluster by connecting all the objects at the last step known as hierarchical tree.

The Ward's method (Ward, 1963) used in this study optimizes an objective function; that is, it minimizes the sum of squares within groups and maximizes the sum of squares between groups. Ward's method is similar to the linkage methods in that it begins with *N* clusters, each containing one object, it differs in that it does not use cluster distances to group objects. Instead, the total within-cluster sum of squares (SSE) is computed to determine the next two groups merged at each step of the algorithm. The error sum of squares (SSE) is defined (for multivariate data) as:

$$SSE = \sum_{i=1}^{k} \sum_{j=1}^{n_i} (y_{ij} - \bar{y})^2 \tag{5}$$

where y_{ij} is the j^{th} object in the i^{th} cluster and n_i is the number of objects in the i^{th} cluster.