

CHAPTER 3

MATERIALS AND METHODS

3.1 Breeding Materials and Site Locations

A random sample of five families from 13 sites (populations) originating from Senegal was collected in July-August 1993 by researchers from Malaysian Palm Oil Board (MPOB). At each site, five to ten palms were sampled. A total of 104 samples were collected and only *dura* palms were encountered. This could be due to the low rainfall and differential survival of *duras* and *teneras* in harsh environment (Rajanaidu and Jalani, 1994b). The germplasm material was planted at Kluang under the MPOB Research Station Kluang, Johor in June 1996. Kluang is characterized by Rengam soil series and rainfall of about 2500 mm/yr. A total of 415 open-pollinated palms were planted in trial 0.352 (Figure 1) using Independent Completely Randomized Design (ICRD) with two replicates; 41 progenies in replicate 1 and 15 progenies in replicate 2. The materials were evaluated for the bunch yield, bunch quality, vegetative and physiological traits. A total of 35 traits were evaluated in this study.

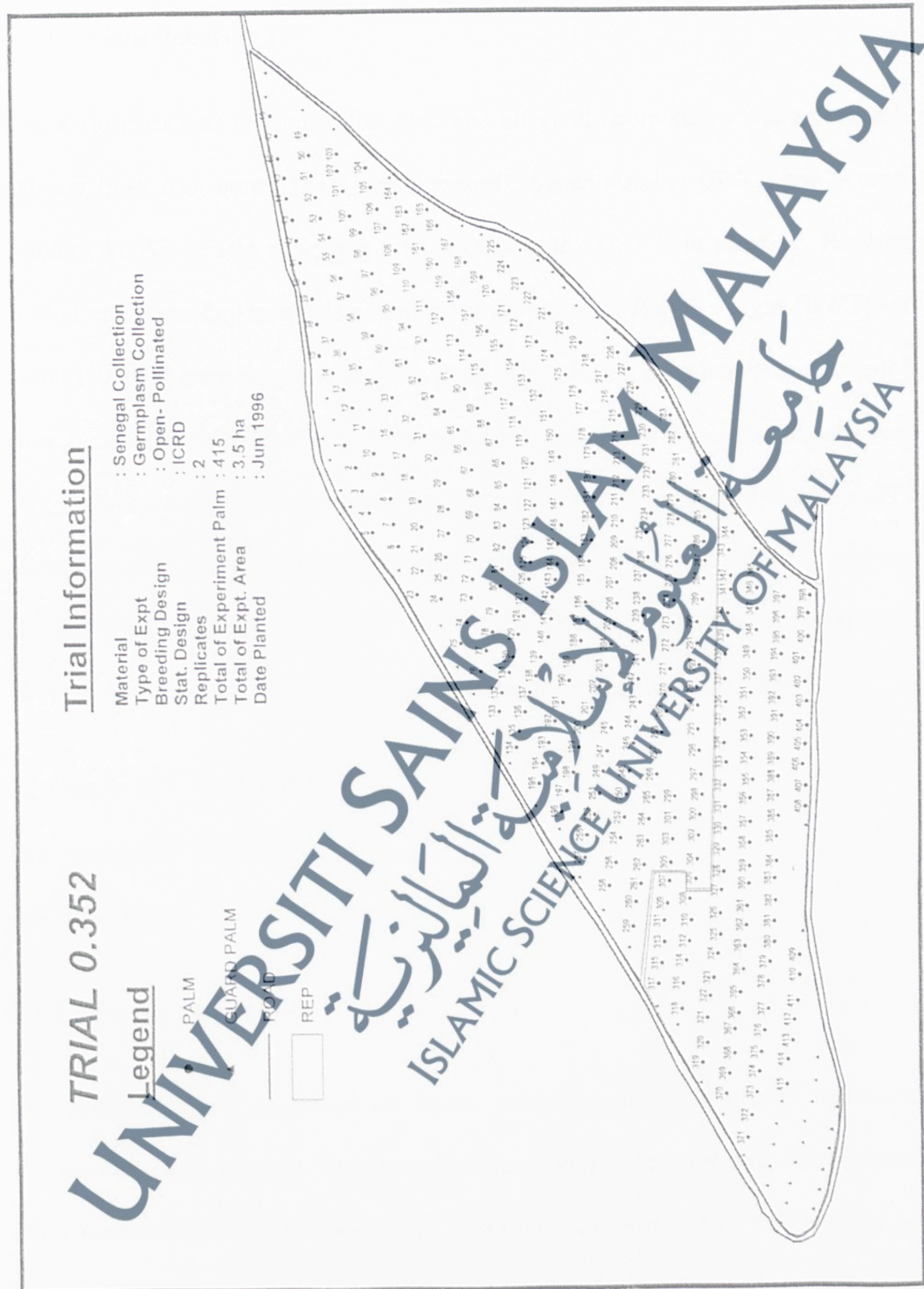


Figure 1: Trial Map and Information of Senegal Population

3.2 Data Collection

3.2.1 Yield Recording

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). Yield recording started 36 months after field planting. Harvesting was carried out at ten-day intervals or three rounds a month. Bunch weight (BWT) and bunch number (BNO) were recorded on individual palm basis at each harvesting round. The data were summarized within a twelve month period, January to December in each year.

In this trial, the number and weight of harvested fresh fruit bunches (FFB) from each palm were recorded during each harvesting round for seven years starting from 2000 to 2007. FFB yield was the total of BWT for each palm, BNO was the total bunch count obtained from each palm and ABW was the ratio between FFB and BNO.

$$\text{FFB (kg/p/yr)} = \sum \text{BWT}$$

$$\text{BNO (no./p/yr)} = \sum \text{BNO}$$

$$\text{ABW (kg/p/yr)} = \text{FFB/BNO}$$

3.2.2 Bunch Analysis

The performance of progenies for bunch analysis and bunch quality components was assessed based on 14 traits. Bunch analysis method developed at Nigerian Institute for Oil Palm Research (NIFOR) (Blaak et al., 1963 and Kushairi, 1998) was used to determine the bunch and fruit quality components. The formulae for the analysis of bunch quality components are presented in (APPENDIX A). For bunch analysis, three to five bunches

were sampled from each palm. Bunch analysis for the trial was carried out from 2001 to 2006. To avoid seasonal variation, ripe bunches with one to ten loose fruits were randomly sampled between intervals of at least three months from previous sampling from the same palm.

Bunch samples were taken to the laboratory. Each bunch was weighed separately before being chopped to separate the fruit bearing spikelets from the stalk. The spikelets were then separated randomly using compartment boxes. One portion was used for analysis of fruit composition (FC) and the other half for fruit to bunch analysis (F/B). For the (F/B), it will be separated and weighted into empty spikelets, fertile and parthenocarpic fruits.

Analysis of FC was done on the same day as spikelets samplings. The fruits detached from the spikelets were randomly sampled, counted, weighed and depericarped. The nuts and mesocarp were weighed separately before a 24 hour oven drying at 40°C and 105°C, respectively. The dried nuts were then cracked and the kernel weighed. A five gram sample of minced and sieved mesocarp was used for oil extraction. Finally, a solvent (hexane) is used to extract the oil from the dry mesocarp sample. The amount of oil in the sample was then calculated on weight differences before and after extraction. The characters for fruit components were calculated as follows:

3.2.2.1 Fruit to bunch (F/B) (%)

Fruit to bunch (F/B) is a ratio of fruits to bunch by weight. It is an indicator of pollination efficiency. It was calculated from a random sample of spikelets. The spikelets taken were

weighed and the normal or fertile and parthenocarpic fruits separated from the spikelets were weighed separately. F/B was calculated using the following formula:

$$\left[\frac{(\text{Fertile fruit wt.} + \text{Parthenocarpic fruit wt.})}{\text{Spikelet wt.}} \right] \times \left[\frac{(\text{Bunch wt.} - \text{Stalk wt.})}{\text{Bunch wt.}} \right] \times 100$$

3.2.2.2 Mesocarp to Fruit (M/F) (%)

It is a weight ratio of fresh mesocarp to fruit (M/F). The mesocarp was scrapped from the fruit samples manually using a sharp knife. The weight of the fresh mesocarp was determined by subtracting the weight of fresh nuts from the fruit sub-sample weight. M/F is calculated as follows:

$$\text{Mesocarp to fruit (\%)} = \left[\frac{(\text{Fruit sub sample wt.} - \text{fruit nut wt.})}{\text{Fruit sub sample wt.}} \right] \times 100$$

3.2.2.3 Mean Fruit Weight (MFW) (g)

Mean fruit weight (MFW) is the average weight of the fruitlets in the fruit sub-sample weight. Normally 30 - 40 fruits are selected randomly for fruit sub-sample weight. The MFW is calculated as follows:

$$\text{Mean Fruit Weight (g)} = \frac{\text{Fruit sub sample weight}}{\text{Number of fruit}}$$

3.2.2.4 Oil to Dry Mesocarp (O/DM) (%)

The fresh mesocarp obtained after depericarping the fruit samples were dried in the oven at 105°C overnight. The dried mesocarp was then weighed before being minced. The oil to dry mesocarp is calculated as follows:

$$\frac{(\text{Thimble} + \text{mesocarp wt.}) - (\text{Thimble} + \text{fibre wt.})}{(\text{Thimble} + \text{mesocarp wt.}) - (\text{Thimble wt.})} \times 100$$

3.2.2.5 Oil to Wet Mesocarp (O/WM) (%)

It is a weight ratio of solvent extract oil to wet mesocarp, calculated as follows:

$$\text{Oil to wet mesocarp (\%)} = \frac{[\text{Oil to dry mesocarp} \times (100 - \text{moisture content})]}{100}$$

3.2.2.6 Mean Nut Weight (MNW) (g)

Mean nut weight (MNW) is the average weight of the nuts obtained after scrapping the mesocarp of the fruit sub-sample. The MNW is calculated as follows:

$$\text{Mean Nut Weight (g)} = \frac{\text{Fresh nut weight}}{\text{Number of fresh nut}}$$

3.2.2.7 Kernel to Fruit (K/F) (%), Shell to Fruit (S/F) (%), Kernel to Bunch (K/B) (%) and Kernel Yield (kg)

The nuts obtained after the removal of mesocarp or depericarping are kept in the oven at 105°C overnight to facilitate cracking of the nuts. The shell is then removed and the kernels are weighed to calculate the kernel to fruit ratio (K/F). Shell weight and shell to

fruit (S/F) is calculated by difference between kernel weight and nut weight. Kernel to fruit (K/F), shell to fruit (S/F), kernel to bunch (K/B) and kernel yield are calculated as follows:

$$\text{Kernel to fruit (\%)} = \frac{\text{Kernel weight}}{\text{Fruit sub sample wt.}} \times 100$$

$$\text{Shell to fruit (\%)} = \frac{\text{Fruit nut wt.} - \text{Kernel wt.}}{\text{Fruit sub sample wt.}} \times 100$$

$$\text{Kernel to bunch (\%)} = \frac{(\text{Kernel to fruit} \times \text{Fruit to bunch})}{100}$$

$$\text{Kernel yield (kg)} = \frac{(\text{Kernel to bunch} \times \text{Fresh fruit bunch})}{100}$$

3.2.2.8 Oil to Bunch (O/B) (%) and Oil Yield (OY) (kg)

Oil to bunch (O/B) depends on F/B, M/F and O/WM. Oil is produced in two components of fruits, mesocarp and kernel. The main components of oil to bunch (O/B) are the mesocarp to fruit (M/F), oil to wet mesocarp (O/WM) and fruit to bunch (F/B). O/B and OY is calculated as follows:

$$\text{Oil to bunch (\%)} = \frac{\text{Fruit to bunch} \times \text{Mesocarp to fruit} \times \text{Oil to wet mesocarp}}{10\ 000}$$

$$\text{Oil yield (kg)} = \frac{\text{Oil to bunch} \times \text{Fresh fruit bunch}}{100}$$

3.2.2.9 Total Economic Product (TEP) (kg/p/yr)

In oil palm fruits, the oil is derived from the two main portions which are the mesocarp and the kernel. The calculation of TEP involves three components that is oil yield (OY), kernel yield (KY) and a correction factor of 0.6. The correction factor is the relative price of palm kernel oil to price of palm oil.

Total economic product (kg/p/yr) = Oil yield + (Kernel yield $\times$ 0.6)

3.2.3 Vegetative Measurements

Vegetative measurements for the palms were carried out in 2004. The vegetative data was measured on frond 17 as proposed by Corley and Breure (1981). The vegetative measurement included rachis length (RL), leaflets number (LN), leaflet length (LL), leaflet width (LW), petiole width (PW) and petiole depth (PD), petiole cross-section (PCS), leaf area (LA) and leaf area index (LAI). Marking the first frond will be done by painting it with bright colour. It is done during the seventh year of planting and vegetative measurements commence at the eight years of planting. The first frond is identified by looking at the young frond that already breaks formation among other young fronds.

3.2.3.1 Frond Production (FP) (no./p/yr)

The annual frond production (FP) was based on frond 1 marked 12 months earlier. The number of fronds produced after one year was taken as frond production based on the painted frond 1 the previous year. Total production of new frond is useful to estimate the monthly and yearly frond production of the palm.

Frond Production (no/p/yr) = Number of fronds produced in one year

3.2.3.2 Palm Height (HT) (m) and Height Increment (HTI) (cm/yr)

Reducing height has been the interest of oil palm breeders because of the high cost of harvesting tall palm. Palm height (HT) was measured from ground level to the base of frond 41. Since stem is not apparent in the first two years of growth, the annual height increment was the height at year t divided by $t-2$, where t is the number of years after planting (Breure and Powell, 1988).

Palm Height (m) = Height of trunk of ground to base of frond 41

Height Increment (cm/yr) = $\frac{\text{Trunk Height at year } t}{\text{Age at year } t-2}$

3.2.3.3 Petiole Cross-section Area (PCS) (cm²)

The petiole cross-section (PCS) at the junction of the rachis and petiole is nearly triangular and its width and depth can be measured accurately. Corley et al. (1971) found a high correlation between PCS and total dry weight of the leaf and this relationship is useful for estimating leaf dry weight without destroying the palms. Petiole cross-section (PCS) was measured at the first rudimentary leaflet. Petiole width (PW) and depth (PD) were measured using calipers. Their product (PW × PD) is the PCS area.

Petiole cross-section area (cm²) = Petiole depth × Petiole Width

3.2.3.4 Rachis Length (RL) (cm)

Rachis length (RL) is used as a guide for the determination of planting density. Shorter rachis length is preferred in planting at higher density and may increase the total yield per hectare. Rachis length (RL) was measured from the first rudimentary leaf at the petiole to the tip of the rachis.

Rachis Length (cm) = Length from tip of rachis to the first ligule

3.2.3.5 Trunk Diameter (DIA) (m)

The diameter of the trunk (DIA) was measured using a big caliper. The caliper was placed one meter above from the ground.

Trunk diameter (m) = diameter of trunk at one meter from the ground

3.2.3.6 Leaflet Number (LN) (no./p/yr)

The leaflet number (LN) was counted from the first rudimentary leaflet on the petiole to the last leaflet at the tip of the frond. Only one side of the frond leaflets was counted. Doubling the number of the leaflets on one side of the frond gave the leaflet number per frond.

Leaflet number (no./p/yr) = (number of leaflets on one side of rachis) $\times$ 2

3.2.3.7 Leaflet Width (LW) and Leaflet Length (LL)

Six leaflets near the ligule were measured for leaflet width (LW) and leaflet length (LL).

The LW was measured at the middle point by folding the leaflet into two.

$$\text{Leaflet width (cm)} = \frac{(\text{LW1} + \text{LW2} + \text{LW3} + \text{LW4} + \text{LW5} + \text{LW6})}{6}$$

$$\text{Leaflet Length (cm)} = \frac{(\text{LL1} + \text{LL2} + \text{LL3} + \text{LL4} + \text{LL5} + \text{LL6})}{6}$$

3.2.3.8 Leaf Area (LA) (m²)

Dry matter is produced by photosynthesis, conversion of carbon dioxide and water to carbohydrate which occurs in the leaves. Rate of increase in dry weight is usually expressed by unit leaf area (net assimilation rate) thus increase in total leaf area will increase leaf area index (LAI) to maximize dry matter production (Corley, 1973a). The non-destructive method developed by Hardon et al. (1969) is used to measure leaf area. Leaflet area (LA) is the product of mean of the six leaflets area, leaflet number and correction factor of 0.56.

$$\text{Leaf Area (m}^2\text{)} =$$

$$\left[\sum_{i=1}^6 (\text{leaflet length } i \times \text{leaflet width } i) \times (\text{leaflet number} \times 0.57) \right] / 10\,000$$

3.2.3.9 Leaf Area Index (LAI)

The leaf area index (LAI) is an important crop parameter together with the extinction coefficient (K) to determine light interception (f). Supply of water, carbon dioxide (CO_2) and solar energy is essential to maximize dry matter production. We can increase the efficiency of these resources by increasing either the total leaf area or rate of light conversion (e) per unit area (Corley, 1973a).

LAI depends on the mean area of individual fronds, the number of fronds per palm and the planting density. LAI is the product of fronds (40), leaf area and planting density.

$$\text{Leaf area index} = \frac{(\text{L40 fronds} \times \text{leaflet area} \times \text{planting density})}{10\,000}$$

3.2.3.10 Fractional Interception of Light (f)

Interception of light (f) is dependent on leaf area index (LAI) and the canopy extinction coefficient, K . Oil palm has a K value of 0.47 with respect to photosynthetically active radiation (PAR) so a high LAI is required for maximal light interception (Squire, 1986).

$$\text{Interception of light } (f) = 1 - \text{EXP} [-0.47 \times (\text{LAI} - 0.3)]$$

3.2.4 Physiological Traits

The oil palm yield and bunch oil content are found to be influenced by physiological traits at various stages of development. Oil production can be increase by increasing the total dry matter (TDM) production by increasing leaf area (LA) and light saturated rate of photosynthesis, reducing the total respiratory loss, increasing bunch index (BI) and increasing oil to bunch ratio (O/B). Breure (1986) showed that selection of parent palms for high LAR is an effective method of improving progeny yield.

Total dry matter production (TDM) is dependent on bunch dry matter (BDM) which is the fraction of dry matter directed into bunches and partitioning of dry matter within the bunch and vegetative dry matter (VDM) between the organs for nutrient and

water absorption (roots), support (trunk) and carbon assimilation rate (fronds). Bunch index (BI) is the proportion of dry matter incorporated in fruit bunches. The photosynthetic conversion coefficient (e) can be estimated from total dry production. The level of (e) depends between photosynthesis and respiration.

Physiological traits were measured using a model suggested by Squire (1986). The model is based on the use of solar energy that can be limited by other climatic factors such as temperature, water and soil nutrients. The physiological parameters were calculated as follows:

$$\text{Bunch Dry Matter (BDM) (t/ha/yr)} = \frac{(0.53 \times \text{Mean FFB} \times \text{Planting Density})}{1000}$$

$$\text{Vegetative Dry Matter (VDM) (t/ha/yr)} = \frac{(\text{Frond dry wt.} + \text{Trunk dry wt.}) \times \text{Planting Density}}{1000}$$

$$\text{Total Dry Matter (TDM) (t/ha/yr)} = \text{BDM} + \text{VDM}$$

$$\text{Bunch Index (BI)} = \text{BDM} / (\text{VDM} + \text{BDM})$$

$$\text{Conversion Efficiency (} e \text{)} = \text{TDM} / (31 \times f)$$

$$\text{Net Assimilation Rate (NAR)} = \text{TDM} / (0.52 \times \text{LAI})$$

3.3 Statistical Analysis

Due to dead palms, only 358 palms were used in this study. The data analysis was divided into two parts, genetic variability among populations and genetic variability among progenies. For genetic variability among populations, 9 populations with 242 palms from replication 1 and 92 palms from replication 2 were evaluated. The 9 populations were SEN 01, SEN 02, SEN 03, SEN 04, SEN 05, SEN 06, SEN 07, SEN 10 and SEN 12.

For genetic variability among progenies, due to unequal number of progenies between the two replications only 224 palms were evaluated among 14 progenies with 132 palms from replication 1 and 92 palms from replication 2. The 14 progenies were SEN 01.03, SEN 02.06, SEN 02.09, SEN 03.07, SEN 04.02, SEN 04.03, SEN 05.01, SEN 05.03, SEN 05.08, SEN 06.08, SEN 07.03, SEN 10.03, SEN 10.05 and SEN 12.03.

The analysis of variance (ANOVA) for all the characters was computed using the general linear model procedure (PROC GLM) using SAS 9.2 statistical analysis. Missing palms were common in field trials involving perennial crops and oil palm is no exception. The number of palms per replication was no longer constant and the F-test was therefore only an approximate. Due to missing palms, these losses were confined to the lowest level of the hierarchy: each of the population progeny was represented by at least 3 palms in each replicate. In Analysis of Variance (ANOVA) the missing values were accommodated by reducing the degree of freedom (df) of the seedlings item and the initial values of n (actual number of palms) were adjusted using harmonic mean n' . The harmonic mean can be obtained by the following formula (Steel and Torrie, 1981):

$$\frac{1}{H} = \frac{1}{n_i} \sum \left(\frac{1}{Y_i} \right)$$

where

H = n' = harmonic mean for palms in progeny/population

n_i = number of palms in ith progeny/population

Y_i = total number of palms in ith progeny/population

3.3.1 Performance of Populations

The ANOVA table for population is given in Table 2 and the test of significance is as follows:

Table 2: ANOVA and Expected Mean Squares for Populations Analysis

Source	df	MS	EMS
Replications (R)	(r-1)	MS1	$\sigma_w^2 + n_1\sigma_{pr}^2 + n_3p\sigma_r^2$
Populations (P)	(p-1)	MS2	$\sigma_w^2 + n_1\sigma_{pr}^2 + n_2r\sigma_p^2$
P×R	(r-1)(p-1)	MS3	$\sigma_w^2 + n_1\sigma_{pr}^2$
Within palms	rp(n-1)	MS4	σ_w^2

where

n₁ = harmonic mean of all the n_i where n_i is the number of seedlings in the ith population

n₂ = harmonic mean for palms in populations

n₃ = harmonic mean for palms in replicate

σ_w² = variance within palms

σ_{pr}² = variance replicate × population

σ_p² = variance population

σ_r² = variance replicate

MS = mean squares

EMS = expected mean squares

The tests of significance for each source of variations are as follows:

Populations × Replications (P × R) : MS3/MS4

Replications (R) : MS1/MS3

Populations (P) : MS2/MS3

The variance components were estimated from the estimated mean squares in Table 2.

$\sigma^2_w = MS4$

$\sigma^2_{pr} = \frac{MS3 - MS4}{n}$

$\sigma^2_p = \frac{MS2 - MS3}{n \cdot r}$

3.3.2 Performance of Progenies

3.3.2.1 Progeny Means and ANOVA

The ANOVA table for progenies is given in Table 3 and the test of significance is as follows:

Table 3: ANOVA and Expected Mean Squares for Progenies Analysis

Source	df	MS	EMS
Replications (R)	(r-1)	MS1	$\sigma^2_w + n_1\sigma^2_{gr} + n_3g\sigma^2_r$
Progenies (G)	(g-1)	MS2	$\sigma^2_w + n_1\sigma^2_{gr} + n_2r\sigma^2_g$
G×R	(r-1)(g-1)	MS3	$\sigma^2_w + n_1\sigma^2_{gr}$
Within palms	rg(n-1)	MS4	σ^2_w

where

- n_1 = harmonic mean of all the n_i where n_i is the number of seedlings in the i^{th} progeny
- n_2 = harmonic mean for palms in progenies
- n_3 = harmonic mean for palms in replicate
- σ^2_w = variance within palms
- σ^2_{gr} = variance replicates × progenies
- σ^2_g = variance progenies
- σ^2_r = variance replicates
- MS = mean squares
- EMS = expected mean squares

The tests of significance for each source of variation are as follows:

Progenies x Replications (G×R) : MS3/MS4

Replication (R) : MS1/MS3

Progeny (G) : MS2/MS3

The variance analysis for the estimation of broad-sense heritability was processed through the procedure variance component procedure (PROC VARCOMP) using SAS. The variance components were estimated from the expected mean squares in Table 3 as follows:

$$\sigma^2_w = MS4$$

$$\sigma^2_{gr} = \frac{MS3 - MS4}{n'}$$

$$\sigma^2_g = \frac{MS2 - MS3}{n'r}$$

3.3.2.2 Heritability Estimates for Each Trait

Heritability value for each trait was calculated based on intra-class correlation coefficient (t_g) as follows:

$$t_g = \frac{\sigma^2_g}{\sigma^2_w + \sigma^2_{gr} + \sigma^2_g}$$

$$h^2_B = 2t$$

2t equals to broad sense heritability (h^2_B) (Falconer and McKay, 1996).

3.3.3 Phenotypic Correlation Studies

The intensity of correlation between the cause and the effect can be measured by correlation coefficient, (r). r is use to determine the degree to which the two related variable can vary together. The correlation coefficient ranges from -1 to +1, r = -1 means there is 100% correlation between X and Y but both vary in opposite direction (negative correlation). The coefficient of determination was computed by squaring the correlation coefficient for deeper investigation of the association between characters (Jawahar, 1995). Simple correlation coefficient can be determined using the following formula:

$$r_{xy} = \frac{\sum[(X_i - \bar{X})(Y_i - \bar{Y})]}{\sqrt{\sum[(X_i - \bar{X})^2 \times (Y_i - \bar{Y})^2]}}$$

where

r_{xy}	=	correlation coefficient between traits X and Y
X_i	=	value of traits X to i
$\bar{X}$	=	mean value for traits X
Y_i	=	value for trait Y to i
$\bar{Y}$	=	mean value for trait Y

The correlation coefficients (r) were determined by the use of correlation procedure of SAS (PROC CORR) using 222 individual palms.