A STUDY ON AGRONOMIC TRAITS AND GENETIC POTENTIAL OF SELECTED CACAO GERMPLASM COLLECTION OF COCOA RESEARCH AND DEVELOPMENT CENTRE BAGAN DATUK, PERAK

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ABSTRACT – *Malaysian Cocoa Board is the solely agency who responsible for collecting, evaluating and conserving diverse cacao varieties also known as germplasm for various field of study especially in cacao breeding. As an economic importance crop worldwide, enhancement of cacao genetic materials is very crucial in the process of superior planting materials development and crop improvement. In this paper, fourteen traits such as pod length, pod width, pod weight, husk weight, wet bean weight with mucilage, dry bean weight with mucilage, number of bean, wet placenta weight, dry placenta weight, brix, average dry bean weight (ADBW), pod value (PV), bean conversion rate (BCR) and cocoa butter content were evaluated for eight selected cacao genotypes of the Malaysian Cocoa Board's collection in Cocoa Research and Development Centre, Bagan Datuk, Perak. ANOVA result revealed that 64% of the study traits were highly effected significantly by the genotypes at $P \leq 0.01$ and 29% effected significantly at $P \leq 0.05$. Total genetic variation among the eight selected genotypes was accounted for by three Principal Component (PC) axes which ranging from 48.73% (PC1) to 32.47% (PC3). Broad-sense heritability range of 32.59% to 74.63% was observed and 50% of the traits showed high broad-sense heritability. The eight genotype were clustered into two clusters according to studied traits.*

Key words: Cacao, germplasm, broad-sense heritability, genotypes, variation

INTRODUCTION

Theobroma cacao L. also known as cacao is an under storey fruit tree species which grows well in the tropical rainforests. Cacao belongs to the family of Malvaceae and is a diploid species (Mustiga *et al.*, 2018) with 10 pairs of chromosomes (2n = 2x = 20). It is native to South America (Motamayor *et al.*, 2002). Cacao is grown throughout the world for its fruits (pod) and, the seeds (beans) are very important for chocolate and beverages productions as well as cosmetics and pharmaceuticals products.

Growing the cacao is very challenging because of market price fluctuations, pest and disease threats, high input and labour requirements as well as genotype and environment effects. Thus, it is a crucial to develop an outstanding cultivars with special characters and high quality in minimizing those issues.

As the Malaysian Cocoa Board (MCB) is the only research agency to promote cacao crop and, with the biggest cacao collections in the South East Asia, many works have been done and continued to sustain cocoa productivity. The collections also known as cacao germplasm is being collected throughout the world and conserved as genetic resources, which is very important in breeding programs especially for crop improvement. According to Malhotra and Elain Apshara (2017), it is very important to have diversity among the genetic resources for effective improvement of the crop.

The objectives of this paper is to study agronomic traits of the cacao collections in MCB and to reveal their genetic potentials prior to the development of superior planting materials. Potential and promising materials could be then selected, manipulated and used to improve cacao genetic materials while preserving genetic diversity. As breeding programs require a deeper understanding of the genetics controlling each traits, this study would be the key for breeding new outstanding cultivars.

MATERIALS AND METHODS

Planting materials

The source of cacao genetic materials were collected from germplasm collection (plot 18B) of Cocoa Research and Development Centre (CRDC) of Malaysian Cocoa Board (MCB), Bagan Datuk, Perak (3.54 N and 100.51E). Eight cacao clones were selected and used in this study including AMAZ 12, IMC 16, IMC 20, IMC 103, MCBC 5, PNG 296, SLA 16 and UF 12. The trees were maintained at the field with normal agriculture practices for cocoa prior to pods sampling.

Experimental design

The experiment was carried out in nested design and the pods were evaluated for fourteen agronomic traits such as pod length, pod width, pod weight, husk weight, wet bean weight with mucilage, dry bean weight with mucilage, number of bean, wet placenta weight, dry placenta weight, brix, average dry bean weight (ADBW), pod value (PV), bean conversion rate (BCR) and cocoa butter content.

Pod and bean analysis

Three healthy pods were harvested for each genotypes from four individual trees and put in each labelled sack. The harvested pods were measured manually for their length, width and weight. Pods were split to extract the beans and weighted for the husk, wet bean with mucilage, dry bean with mucilage, wet placenta and dry placenta. Number of beans were counted. Total soluble solids as °Brix was measured on the cocoa pulp using digital refractometer (Escalante *et al.*, 2013, Rojas *et al.*, 2020).

Post-harvest process

The wet beans were placed in labeled nylon mesh (5mm x 5mm, mesh size) and tied neatly. Beans were fermented immediately within a mass of bulk cocoa for 5 days with one turning, following the usual fermentation procedure of MCB. The fermented beans were then placed in separate wooden trays and dried in the conventional driers or sun dried to 7% moisture content. The moisture content of the bean must be in the range of 7 – 8 % (Escalante *et al.*, 2013). The fermented dried bean were allowed to cool down to room temperature before weighted. The pod and bean analysis conducted above would determine the following bean parameters (Haya *et al.*, 2007):

Average dried bean weight (ADBW)
ADBW (g) = Weight of dried beans / No. of beans

Pod Value (PV) = *Number of pods required to produce one kilogram of dry beans.*
PV = [No. of pod sampled / Dry bean weight (g)] x 1000

Bean Number per Pod (BNP)
BNP = No. of wet beans / No. of pod sampled

Bean Conversion Rate (BCR) = *Wet to dried bean conversion*
BCR (%) = (Dry bean weight / Wet bean weight) / 100

Determination of cocoa butter content

<u>Extraction procedures</u>: The shell of the fermented and dried cocoa beans were removed to obtain the cocoa nibs. The nibs were then ground to a small particles. The nibs were hydrolysed with dilute hydrochloric acid (25%) and filtered (Whatman filter paper) before extraction processes.

<u>Soxhlet extraction</u>: Cocoa nibs was weighted into the thimble. Then, ethanol (99.5%) added in round bottom flask and refluxed for 6 h using Soxhlet apparatus. The extracts were filtered and concentrated under reduced pressure overnight or until dryness (AOCS, 1998; Roiaini *et al.*, 2016).

The yield of cocoa butter was calculated using the formula below:

Yield (%) = {[Weight of flask (after) – Weight of flask (before)] / Weight of sample} x 100

Statistical analysis

All the data were subjected to the statistical analysis using the Statistical Analysis Software (SAS), version 9.1.3. The analysis of variance (ANOVA) was calculated using the PROC ANOVA procedure in SAS and means were separated using the Duncan's Multiple Range Test (DMRT). ANOVA was used to determine the significance of variation among genotypes and tree within genotypes. Multivariate analysis with principal component analysis (PCA) was used to determine the relative importance of the traits responsible for variation among cacao clones. Cluster analysis with unweighted pair group arithmetic mean average (UPGMA) was used to explore relationships among the studied genotypes according to phenotypic traits.

Heritability

Heritability and components of genetic variation in this clones/genotypes were estimated for the studied traits. The variance components and broad-sense heritability for the cacao genotypes was calculated as follows;

Variance components (from the ANOVA table) includes:

i. Genetic/genotypic variance (σ^2_g) = (MS_G - MS_e)/r
ii. Error/environmental variance (σ^2_e) = MS_e and,
iii. Phenotype/phenotypic variance (σ^2_p) = $\sigma^2_g + \sigma^2_e$

Broad-sense heritability (h^2_B) for the traits measured was estimated using the variance components method suggested by Manga *et al.*, (2018) as:

$$(h^2_B) = (\sigma^2_g / \sigma^2_p) = [\sigma^2_g / (\sigma^2_g + \sigma^2_e)]$$

where, h^2_B = broad-sense heritability, σ^2_g= genotypic variance, σ^2_P = phenotypic variance, σ^2_e = environmental variance, MS_G = mean squares due to genotypes, MS_e = error mean squares, and r = number of trees. Heritability percentage were categorized as low (0-20%), moderate (20-50%) and high (≥50%) as indicated by Elrod and Stanfield (2002) and McWhirter (1979).

RESULTS AND DISCUSSIONS

ANOVA results indicated that thirteen traits effected significantly by the clone or genotype. Pod length, pod width, pod weight, wet bean weight with mucilage, number of bean, wet placenta weight, brix, average dry bean weight (ADBW) and pod value (PV) were highly effected significantly at $P \leq 0.01$ by the clones meanwhile dry bean weight with mucilage, dry placenta weight, bean conversion rate (BCR) and cocoa butter content were effected significantly at $P < 0.05$. Husk weight was not affected by the genotype. This result showed that each studied traits varied among the eight cacao clones except for the husk weight (Table 1). Meanwhile, ten of the studied traits (pod length, pod width, pod weight, husk weight, number of bean, wet placenta weight, dry placenta weight, brix, pod value and cocoa butter content) were not affected by tree within genotype except for wet bean weight with mucilage, dry bean weight with mucilage and bean conversion rate that affected significantly at $P \leq 0.05$. Average dry bean weight was highly affected significantly by tree within genotype at $P \leq 0.01$. Statistical analysis revealed that there was a high variation among the genotypes but less variation between the individual trees for most of the measured traits (Table 1).

The variations showed interesting genotypes characters according to phenotypic studied which could be used for specific objectives in cacao breeding programmes. Among the genotypes, IMC 103, UF 12, SLA 16, AMAZ 12, MCBC 5 and PNG 296 indicated big bean size compared to others which is very important for grading and quality, that is preferable by manufacturers and cacao growers. Genotypes with high number of beans per pod such as IMC 16, IMC 20, IMC 103, UF 12, SLA 16, AMAZ 12 and MCBC 5 are preferable as one of good characteristics for cocoa. Lower pod value is better as less pod needed to produce 1 kg of dried bean weight which cost about RM 6.00 to RM 7.00 per kilogram. Cacao genotypes with low pod value included IMC 16, IMC 20, IMC 103, UF 12, SLA 16 and MCBC 5.

Table 2 indicated mean values comparison among the eight cacao genotypes for each studied traits, using Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$. Pod length size obtained in the range of 14.32 to 16.83 cm, pod width (7.51 – 8.53 cm), pod weight (370.63 – 501.72 g), husk weight (235.57 – 344.26 g), wet bean weight with mucilage (100.98 – 156.57 g), dry bean weight with mucilage (36.88 – 50.40 g), bean number per pod (32.25 – 52.33), wet placenta weight (7.18 – 18.19 g), dry placenta weight (0.93 – 4.00 g), brix (20.54 – 22.86), average dry bean weight (0.96 – 1.26 g), pod value (20.04 – 28.13) and bean conversion rate (32.18 – 38.70%). Meanwhile the percentage of cocoa butter content was in the range of 37.71 to 49.05%.

Statistical analysis conducted revealed that mean of the pod length was 15.73 cm, pod width (8.04 cm), pod weight (437.80 g), husk weight (293.60 g), wet bean weight with mucilage (128.16 g), dry bean weight with mucilage (43.92 g), number of bean (40.49), wet placenta weight (14.94 g), dry placenta weight (2.00 g), brix (21.50), average dry bean weight (1.11 g), pod value (23.70), bean conversion rate (34.67%) and cocoa butter content was 45.46%.

AMAZ 12 indicated the highest mean value for pod length (16.83 cm), IMC 103 for pod width (8.53 cm); wet placenta weight (18.19 g); dry placenta weight (4.00 g), IMC 16 for pod weight (501.72 g); wet bean weight with mucilage (156.57 g); bean number per pod (52.33), PNG 296 for husk weight (344.26 g), UF 12 for dry bean weight with mucilage (50.40 g) and average dry bean weight (1.26 g), SLA 16 for brix (22.86) and MCBC 5 for the highest cocoa butter content (49.05%). IMC 16 and UF 12 had the lowest pod value where only twenty pods needed to produce one kilogram of dried cocoa beans.

Genotypic and phenotypic variances and broad-sense heritability (h^2_B) estimates for studied traits measured on eight cacao genotypes are presented in Table 4. The traits measured among the selected cacao genotypes revealed moderate to high broad-sense heritability, with values ranging from 33.0% to 75.0%. Bean number per pod indicated the most heritable among the traits measured on the genotypes, with heritability of 75.0%, followed by pod width (70.0%), wet bean weight with mucilage (69.0%), average dry bean weight (67.0%), wet placenta weight (60.0%), dry bean weight with mucilage (59.0%), pod value (54.0%), pod length and bean conversion rate with 50% respectively. Moderate estimates of broad-sense heritability were showed by pod weight (46.0%), brix (43.0%), cocoa butter content (42.0%), dry placenta weight (35.0%) and husk weight (33.0%).

Figure 1, the total genetic variation among the eight cocoa clones was accounted for by three Principal Component (PC) axes which ranging from 48.73% (PC1) to 32.47% (PC3). Examination of the first principal component (PC) axis which accounted for 48.73% of total variation (Figure 1a) showed that it was composed mainly of pod length, bean size, pod width, pod weight, husk weight, wet placenta weight, dry placenta weight and cocoa butter content. The second PC axis (Figure 1b) accounting for 45.80% of total variation was mainly composed of pod width, husk weight, bean number per pod, wet placenta weight, dry placenta weight and brix. The third PC axis (Figure 1c) which made up 32.47% of total variation was composed mainly of pod width, pod weight, husk weight, wet placenta weight, dry placenta weight and pod value.

Table 1: Mean squares in ANOVA table for agronomic traits measured on eight cacao genotypes

Source of variation	d.f. †	Mean squares				
		Pod length	**Pod width**	**Pod weight**	**Husk weight**	**Wet bean weight with mucilage**
Genotypes	7	10.43**	2.19**	24572.16**	12973.97 ns	3933.60**
Tree / Genotypes	24	1.76 ns	0.27 ns	5172.48 ns	5917.44 ns	715.19 *
Error	64	2.09	0.21	5591.27	4422.51	401.49
C.V. (%)		9.20	5.74	17.08	22.65	15.64

Source of variation	d.f. †	Mean squares				
		Dry bean weight with mucilage	**Bean number per pod**	**Wet placenta weight**	**Dry placenta weight**	**Brix**
Genotypes	7	280.02 *	505.30**	150.53**	10.67*	7.71**
Tree / Genotypes	24	83.31*	60.65 ns	28.43 ns	3.14 ns	2.20 ns
Error	64	41.50	39.58	21.75	3.36	1.92
C.V. (%)		14.67	15.54	31.21	91.62	6.44

Source of variation	d.f. †	Mean squares			
		Average dry bean weight	**Pod value**	**Bean conversion rate**	**Cocoa butter content**
Genotypes	7	0.19**	100.74**	62.02*	159.86*
Tree / Genotypes	24	0.05**	27.58 ns	23.94*	58.86 ns
Error	64	0.02	17.46	12.50	41.10
C.V. (%)		12.61	17.63	10.20	14.10

† d.f. = Degrees of freedom; C.V. = Coefficient of variation; *, **, ns = significant at $p \leq 0.05$, significant at $p \leq 0.01$, and non-significant, respectively.

Table 2: Mean performance of eight cacao genotypes for the studied traits

GENOTYPE	PL (cm)	PW (cm)	PWT (g)	HWT (g)	WWTWM (g)	DWTWM (g)	BNP	WWP (g)	DWP (g)	Brix	ADBW (g)	PV	BCR (%)	CB (%)
IMC 16	14.32c	8.51a	501.72a	321.00ab	156.57a	50.37a	52.33a	16.19ab	2.40b	21.38bc	0.96d	20.04d	32.18d	47.02a
IMC 20	15.44bc	7.60d	398.74bc	280.09bc	122.25c	43.92b	45.75b	17.09ab	2.26b	22.53ab	0.96d	23.66bcd	35.79abc	37.71b
IMC 103	14.39c	8.53a	446.46ab	285.74abc	140.03ab	44.84b	43.58b	18.19a	4.00a	21.46bc	1.04cd	23.13cd	32.19d	46.38a
UF 12	16.39ab	8.09bc	457.12ab	304.73ab	145.87a	50.40a	40.67bc	13.43b	1.67b	20.83c	1.26a	20.18d	34.66bcd	48.71a
SLA 16	16.16ab	7.63d	417.69bc	273.41bc	125.37bc	41.57bc	36.75cd	16.51ab	1.42b	22.86a	1.14bc	24.39bc	33.36cd	43.32ab
AMAZ 12	16.83a	8.04c	417.83bc	304.01ab	100.98d	36.88c	36.50cd	13.82b	1.97b	21.18c	1.04cd	28.13a	36.63ab	44.81a
MCBC 5	16.05ab	7.51d	370.63c	235.57c	115.32cd	44.34b	36.08cd	7.18	0.93b	21.19c	1.23ab	22.93cd	38.70a	49.05a
PNG 296	16.25ab	8.44ab	492.20a	344.26a	118.85c	39.06bc	32.25d	17.12ab	1.36b	20.54c	1.24ab	27.16ab	33.83bcd	46.65a
Mean	**15.73**	**8.04**	**437.80**	**293.60**	**128.16**	**43.92**	**40.49**	**14.94**	**2.00**	**21.50**	**1.11**	**23.70**	**34.67**	**45.46**
S.E. ‡	**1.62**	**0.61**	**82.97**	**73.69**	**27.22**	**8.35**	**8.90**	**5.74**	**1.96**	**1.55**	**0.20**	**5.11**	**4.36**	**7.37**

PL = pod length, PW = pod width, PWT = pod weight, HWT = husk weight, WWTWM = wet bean weight with mucilage, DWTWM = dry bean weight with mucilage, BNP = number of bean, WWP = wet placenta weight, DWP = dry placenta weight, ADBW average dry bean weight, PV = pod value, BCR = bean conversion rate, CB = cocoa butter
‡ Standard error.
Mean values followed by the same letter in the same column are not significantly different at $p \leq 0.05$, based on DMRT.

Table 3: Genotypic variance (σ^2_G), phenotypic variance (σ^2_P) and broad-sense heritability (h^2_B) for agronomic traits measured on eight cacao genotypes

Agronomic Trait	**Variance, σ^2**		**Heritability, h^2_B (%)**
	Genotypic (σ^2_G)	**Phenotypic (σ^2_P)**	
Pod length (cm)	2.09	4.18	50.0
Pod width (cm)	0.50	0.71	70.0
Pod weight (g)	4745.22	10336.49	46.0
Husk weight (g)	2137.87	6560.38	33.0
Wet bean weight with mucilage (g)	883.03	1284.52	69.0
Dry bean weight with mucilage (g)	59.63	101.13	59.0
Bean number per pod	116.43	156.01	75.0
Wet placenta weight (g)	32.20	53.95	60.0
Dry placenta weight (g)	1.83	5.19	35.0
Brix	1.45	3.37	43.0
Average dry bean weight (g)	0.04	0.06	67.0
Pod value	20.82	38.28	54.0
Bean conversion rate (%)	12.38	24.88	50.0
Cocoa butter content (%)	29.69	70.79	42.0

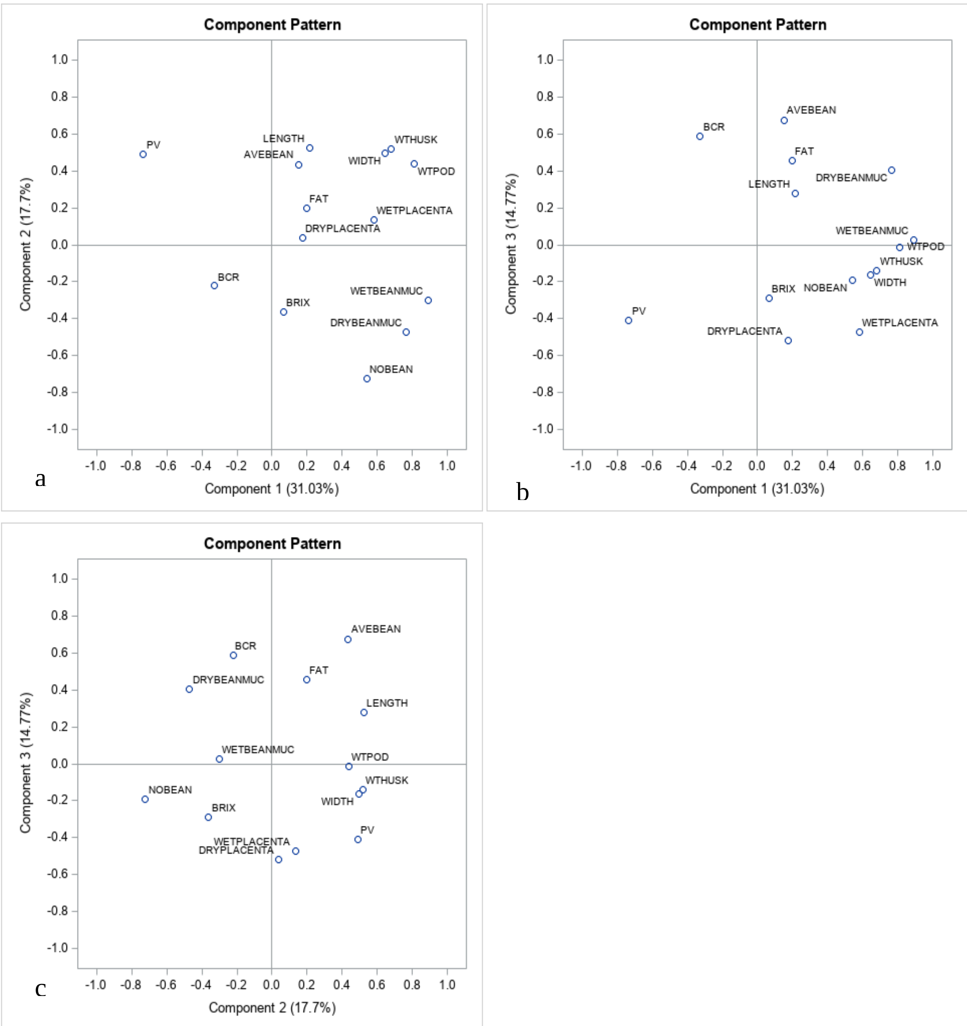

Figure 1: Principal component analysis with studied traits of the eight selected cacao clones

Cluster analysis was used to visualize and describe the relationships among the eight selected cacao genotypes according to fourteen studied traits (Figure 2). Clones with high value of agronomic traits and good quality such as pod length, pod width, pod weight, wet bean weight with mucilage, dry bean weight with mucilage, bean number per pod, brix, butter content and average dry bean weight but low in pod value and bean conversion rate were grouped into clusters.

Dendrogram in Figure 2 revealed that the eight genotypes studied were categorized into two distinct groups (Cluster I and Cluster II) at 1.5 of average distance between clusters. Three genotypes (MCBC 5, PNG 296 and IMC 16) were grouping in Cluster I meanwhile five genotypes (SLA 16, IMC 20, AMAZ 12, UF 12 and IMC 103 were clustering into Cluster II. The dendrogram indicated that the cacao collection of Malaysian Cocoa Board, Bagan Datuk showed distant related relationship among the selected cacao clones. The clones have a wide range of diversity in the agronomic traits study.

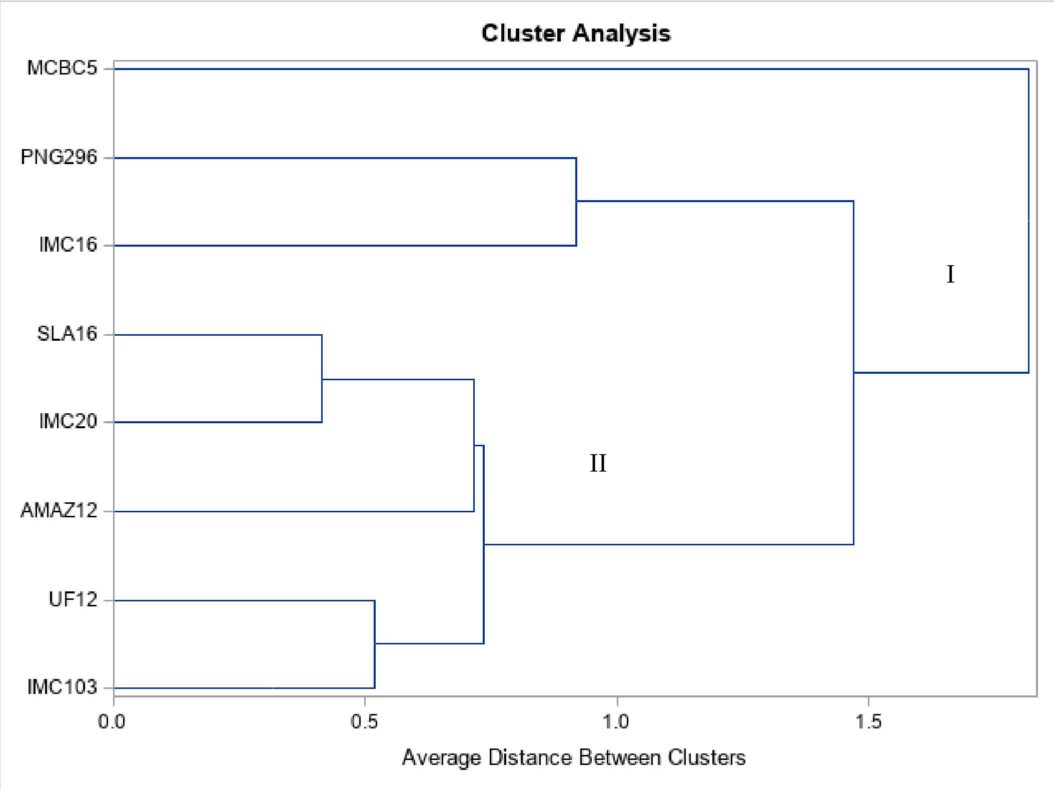

Figure 2: Dendrogram based on the unweighted pair group arithmetic mean average (UPGMA) showing two clusters of cacao clones in Malaysian Cocoa Board collection.

CONCLUSIONS

This paper showed that most of the studied traits were affected significantly by the cacao clones but not by the individual trees within the clones. The traits of the selected clones showed moderate to high broad-sense heritability. The selected clones were clustered into two distinct groups which indicated that the cacao collection of Malaysian Cocoa Board, Bagan Datuk showed distant related relationship among the clones in terms of studied traits. In developing superior planting materials, several agronomic traits and other quality should be emphasized. Moreover, although promising materials have been selected, the genotype and environment condition will affect the crop productivity. In presence of genetic variability would offer a wide range of potential genotypes for selection.

In breeding, promising candidate of planting materials should have high yield, big bean size, most number of beans per pod, low pod value, high and unique flavour, high butter content, tolerance against pest and disease and others. Further study should be done as MCB has many genetic materials in her collection to be explored.

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