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Morphological Characterization and Genetic Diversity Analysis of Cacao (*Theobroma cacao* L.) Genotypes from Sierra Leone

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Abstract

Cacao (*Theobroma cacao* L.) contributes to 60% of income of smallholder cocoa farmers and to the gross domestic product (GDP) of Sierra Leone. Despite its huge importance to the economy, production efficiency has been sub-optimal due primarily to the low yields, a consequence of the severe dearth of improved planting materials. Dearth of information on extent of genetic variability and genetic parameter estimates in cocoa limits its genetic improvement. This study assessed the genetic diversity and relationships using agro-morphological markers to determine the genetic parameter estimates of cocoa genotypes in Sierra Leone. Morphological classification categorized the germplasm into four main cluster groups. Findings showed that leaf length (LL), leaf width (LW), leaf area (LA), stem diameter (SD), pod diameter (PDIA), pod set percent (PSP), number of pods per tree (NPT), pod length (PL), pod size or area (PSA), number of beans per pod (NBP), dry bean weight per pod (DBWP), and estimated dry yield per tree (EDYT) had high heritability, pod width (PW) and pod thickness (PT) had intermediate heritability. High genetic advances, high phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were also recorded. The PCV values were higher than GCV, indicating the influence of the environment in the studied traits. Significant and strong positive correlations were observed between pod size (PS) and pod width (PW) (r = 0.91**), DBWP and NPT (r = 0.73**), EDYT and NPT (r = 0.87**), LA and LL (r = 0.90*) and between LA and LW (r = 0.89**), LW and LL (r = 0.61**), SD and EDYT (r = 0.68**), SD and NPT (r = 0.75**), EDYT and DBWP (r = 0.84**), SD and DBWP (r = 0.62**); and intermediate positive correlations between PST and NTP (r = 0.60**), DS and PSP (r = 0.56**), and between PSP and EDYT (r = 0.52**). Significant and strong negative correlations were also found between LL and PW (r = −0.70**), LA and PL (r = −0.80**), PSP and NFT (r = −0.70**). Findings are useful for conservation and genetic improvement of the crop. This is the first report of cocoa genotypes performance evaluation in Sierra Leone that could be utilized in future cocoa improvement efforts.

Keywords

Phenotypic Coefficient of Variation, Genotypic Coefficient of Variation, Genetic Advance, Germplasm, Selection Gain

1. Introduction

The cocoa tree has been classified to belong to the genus *Theobroma* which is a group of small trees found in the wild in the Amazon basin and other areas that have tropical climates in the South and Central America [1]. The genus *Theobroma* has over twenty species but cocoa tree is the only one that is cultivated widely and belongs to the species called *cacao*, hence *Theobroma cacao* L. From amongst the twenty or more species of *T. cacao*, commercial plantations are notable in Cote D'Ivoire, Ghana, Nigeria, Sierra Leone, Cameroon, Bolivia, Ecuador, Indonesia and India [2]. Even though history has shown that the cocoa tree has its origin from South and Central America, the "headwaters" of the Amazon basin have been said to be the precise origin of the tree. However, Wood and Lass [3] suggested that it is more appropriate to describe the origin as the primary centre of diversity, since, it is an area known for great variation in morphological and physiological characters of the cocoa tree, pod, flowers, bean structure, organoleptic character etc. *Theobroma cacao* is mainly cultivated for its beans which has commercial value in the chocolate and beverage industries. The domesticated *T. cacao* cultivars such as Criollo, Forastero and Trinitario are known to be morphologically diverse which is why classification was based on their morphology especially pod and bean characters [4].

Commercial cultivation of cocoa in Sierra Leone started in the early 1960's, few years after the introduction of West African Cocoa Research Institute (WACRI) Series I and II materials. Amongst the three cultivars introduced, Forastero has over the years gained prominence in farmer fields and large-scale plantations by companies such as Tropical Harvest and place of wealth (OFIR) Commercial cultivation of cocoa in Sierra Leone occurs in the South and Eastern regions and part of Tonkolili district in the North that borders the Southern region. One of the reasons that may be attributed to cocoa production and productivity in the southern and eastern regions of Sierra Leone is rainforest vegetation and soil type that is similar to the areas of origin of the crop.

In 2007, agricultural export in Sierra Leone was estimated at US $13.67 million, representing a modest increase from US $12.8 million in 2006. Of this figure, cocoa alone accounted for US $11.36 million or 85 percent of agricultural export earnings in 2007 [5]. Comparatively, 2003 and 2007 recorded 11,000 and 12,000 Mt of cocoa production on 30,250 and 33,000 ha lands respectively compared to 14,000 Mt on 38,000 ha area of land in 2006. Export of cocoa in 2003 was estimated at 4600 tons which was a fraction of levels in the past [6]. Trade figures in 2011 showed that about 198,000 Mt of cocoa was exported by Sierra Leone, with economic dividend of about US $400 million [7]. These are clear indications that Cocoa has the potential to become major cash and export crop in Sierra Leone. Most production is under smallholder farming on plots of 1 to 6 ha with average yields between 100 - 200 $kg{\cdot}ha^{-1}$, which are very low even by African standards [8] largely due to lack of improved planting materials.

Sierra Leone once had a successful cocoa genetic improvement program during the colonial days. Prior to independence in 1961, Sierra Leone, Ghana and Nigeria were served with parent materials of cocoa clones by the WACRI established by the British Colonial Administration. However, cocoa improvement program from field gene bank and seed gardens were destroyed during the decade long war. This has complicated and compromised the integrity of the gene bank collections and seed garden plots to deliver improved planting materials. Coupled with loss of tags, field map and loss of information on the fields, evaluation of genotypes of WACRI materials in the field gene bank is imperative and critical to the development of cocoa improvement programs for the country. Evaluation of cocoa genotypes for pod and bean characters serves as the prerequisite to the identification of high yielding genotypes.

Selection, evaluation and improvement of cultivars is possible by the use of genetic diversity within the plant species present [9]. A good knowledge of the extent of genetic variability of key morpho-agronomic traits of cocoa genotypes is imperative for genetic conservation and utilization of the crop in breeding programs targeted at improving the high beans quality, yield and related traits required by the global, regional and local demands for cocoa beans. The detection of significant variations in associated traits serves as a premise for selection and improvement of cocoa genotypes. Agro-morphological characterization is the first step in the assessment of genetic variability and identification of desirable traits of interest.

The research questions that prompted the present study included the following: 1) are there existing useful genetic variability within the cocoa germplasm for genetic improvement of the crop? 2) are the genetic parameter estimates high to guide selection of desired trait values of the crop? and 3) are there useful genetic relationships among traits? Thus, the objective of the present study was to assess the genetic diversity, genetic parameter estimates and relationships within cocoa germplasm using agro-morphological markers.

2. Materials and Methods

2.1. Experimental Site

The experiment was conducted at three sites comprising Pendembu and Kpuwabu. that lie within Longitude 10˚40'28.896"W and Latitude 8˚5'22.524"N with an elevation of 176 m representing eastern region agro-ecological zone, while Njala, that represents the southern region, lies within Longitude 10˚46'42.81"W and Latitude 8˚5'34.29"N and an elevation of 150 m. This study was conducted in 2023 and 2024 cocoa production seasons for morphological characterization of the earlier introduced parents.

Variations in temperature at experimental sites are modest with an estimated annual high between 26.1˚C and 36.1˚C and a low from 17.6˚C to 22.1˚C. Relative Humidity at these sites has a similar trend, ranging between 49% and 94% annually. The region experiences most rain from June to October, when monthly rainfall amounts rise from 166 mm to 319 mm. In contrast, the dry season months of January to February exhibit precipitation of 8 mm to 12 mm [10]. Fluctuations in daylight hours vary from 11.6 to 12.6 h. However, the amount of sunshine experiences larger swings, with December and January reaching up to 9.7 h, while July and August exhibit 3 h per day [11]. The soils possess low structural stability, compact subsoil, acidic and associated aluminum toxicity which are among major crop production constraints in the country. Organic matter is often utilized to boost soil health and fertility, as well as serve as a reservoir of nitrogen, phosphorus, and Sulphur for cation exchange capacity and promoter of aggregate structural stability [11].

2.2. Experimental Materials, Design, Layout and Management

The experimental materials utilized in this study were botanical seeds of cocoa. Records show that earlier introductions were made possibly by the Portuguese who are known to have introduced the coconut palm and pineapples into Sierra Leone in the 15th and 16th centuries. Prior to the WACRI introductions in 1974, two introductions were already made from the Gold Coast (now Ghana) in 1905 and 1912 to the Agricultural experimental farm at Njala. However, these introductions and those from 1909, and 1911 failed due to drought until the 1974 introductions that brought in cloned materials from which planting materials were indiscriminately obtained and given out to farmers. These clones since their introductions had never been characterized for yield and yield contributing factors. Twenty cocoa genotypes that were introduced by WACRI from Brazil in 1974 were selected based on their yield performance at the gene banks in Pendembu and Kpuwabu. The experimental design used was independent complete randomized design (ICRD) as described by Rafii *et al.* [9] and Swaray *et al.* [12]. Eight experimental units each for Pendembu and Kpuwabu planted at 3 m × 3 m were randomly selected while four experimental units were selected from Njala lower nursery. Plant population per plot was 1111. Therefore, a total of twenty genotypes were randomly selected and labelled systematically for this study.

The management practices at these locations were uniform. Cleaning of the farms at was usually done twice in a year while the Njala Lower Nursery site. This activity was followed by excessive pruning of unproductive branches, chupons and epiphytes, etc. Incidence of pests of cocoa such as *Distantiella theobroma*, *Sahlbergella singularis*, etc. was minimal during the study periods, while the black pod disease (*Phytophthora megakarya*) is becoming a major threat to the cocoa sector in the country. The control measures usually adopted by smallholder farmers and private sector organizations is proper farm sanitation, allowing aeration and ease of movement for pest and/or disease surveillance. However, one of the exporters of cocoa from Sierra Leone (Tradin Organic) has started experimenting the use of *Trichoderma asperellum* PRR11 as soil treatment against *P. megakarya.* Organic fertilizers such as compost are used for cocoa production, enabling Sierra Leonean cocoa to have premium price in the world market.

2.3. Data Collection

A total of 15 agro-morphological traits were evaluated following the standard cocoa descriptor according to Aikpokpodion *et al.* [13] and Bekele *et al.* [14]. The sampled cocoa trees were evaluated for their yield performance and corresponding yield contributing factors. Data collected included pod length, pod width, pod thickness, pod diameter, number of pods per tree, number of flowers per tree, dry bean weight per pod, estimated dry bean yield per tree, single dry bean weight, pod set percentage, pod length and breadth, stem diameter, leaf length, leaf width and leaf area. Pods were harvested 4 to 5 months after flower set (*i.e.* when they had attained maturity). The data collected, acronyms, method of evaluation and sampling periods are presented in **Table 1**.

2.4. Statistical Analysis

Data collected were subjected to analysis of variance (ANOVA) while significant means (at probability of 0.05%) were separated with Duncan's Multiple Range Test (DMRT) using SAS, version 9.3 software. Also, mean standard deviation, and coefficient of variation (CV) were recorded for each of the traits measured. The relationships among the traits were determined using Pearson's correlation analysis. Genetic variance data generated were analyzed based on Euclidian distance method, Dice's and Jaccard's similarity coefficient. Genetic relationships among cocoa genotypes were determined using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm and SAHN methods. For the genetic analysis, the data collected were subjected to a linear mixed model using residual maximum likelihood (REML) procedure [15] for estimation of the variance parameters and Best Linear Unbiased Predictions (BLUPs) for random effects [16].

The pod set percentage per genotype was calculated following the methodology of Vithya *et al.* [2].

$$\text{Pod set}(\%) = \frac{\begin{array}{c}\text{Number of pods harvested} + \text{cherelle wilted pods}\\ + \text{ rodents and pod rot affected pods}\end{array}}{\text{total number of flowers per tree}} \times 100 \quad (1)$$

Table 1. List of quantitative traits recorded in cocoa genotypes for morphological characterization.

Growth/yield trait	Acronym	Unit	Method of evaluation	Sampling period
Pod width	PW	cm	Measure the wideness of the pod using Vernier caliper	October, 2023
Pod length	PL	cm	Measure from the apex to the basal constriction	October, 2023
Pod size (pod area)	PS	cm^2	Product of the pod width to pod length	October, 2023
Pod thickness	PT	cm	Measure from the inner core (between the bean set and the first layer of the pod) to the end	October, 2023
Pod diameter	PDIA	cm	Measure from one point of the transverse cut to the other	October, 2023
Number of flowers per tree	NFT	-	Count flowers from the base to the branches at the jorquette	July, 2023
Number of pods per tree	NPT	-	Count total number of pods per tree	September, 2023
Number of beans per pod	NBP	-	Count number of beans per pod	October, 2023
Dry bean weight per pod	DBWP	g	Weigh dry bean (of 7% moisture content) per pod	December, 2023
Estimated dry yield per tree	EDYT	g	Total dry weight of beans from all pods per tree	December, 2023
Pod set percent	PSP	-	Divide number of pods harvested plus cherelle wilted pods plus rodents and pod rot affected pods by the number of flowers per tree and multiply by 100	September, 2023
Stem diameter	SD	cm	Measure the diameter using Vernier caliper	January, 2024
Leaf length	LL	cm	Measure from the apex to the tip	January, 2024
Leaf width	LW	cm	Measure from the widest portion of the leaf (end to end)	January, 2024
Leaf area	LA	cm^2	Measure using millimeter graph paper method by tracing the contour of the leaf with a pencil and counting the number of squares within the contour	January, 2024

Note: cm = centimeter, g = gram. Leaf area (LA) measurement was obtained using the equation, leaf area (cm^2) = x/y, where x is the weight (g) of the area covered by the leaf outline on a millimeter graph paper, and y is the weight of one cm^2 of the same graph paper [18]. The estimated dry yield per tree (EDYT) was calculated by multiplying the number of pods per tree with the number of beans per pods and the average individual bean weight (1.5 g/bean) [18].

The means of each genotype were analyzed using the Statistical Analysis System (SAS) Version 9.4 (SAS Institute, Cary, NC, USA). The general linear model used for estimation of the expected mean squares is presented in **Table 2**. The Duncan's new multiple range test (DNMRT) at 5% level of probability was used for comparison of means. The relationships among the traits were determined using correlation coefficient analysis. The performance of each of the genotypes was compared with the trial means for each trait.

2.5. Phenotypic and Genotypic Variability

Variation among genotypes was estimated using the range, mean, standard error, phenotypic and genotypic variance, and coefficient of variation, and the resulting variance components were used to calculate phenotypic and genotypic variation as well as genetic advance as follows:

$$\sigma_g^2 \left(\text{Genetic variance}\right) = \frac{\sigma_t^2 - \sigma_e^2}{r} \quad (2)$$

where σ_g^2 = genotypic variance, σ_t^2 = mean square of treatment and σ_e^2 = error mean square, r = number of replications.

$$\sigma_p^2 = \sigma_g^2 + \sigma_e^2$$

where σ_p^2 = phenotypic variance. The Phenotypic Coefficient of Variance (PCV) and Genotypic Coefficient of Variance (GCV) were calculated following the method of Singh and Chaudhary [19] as presented in the formulae below:

$$\text{PCV}(\%) = \frac{\sqrt{\sigma_p^2}}{\bar{X}} \times 100 \quad (3)$$

$$\text{GCV}(\%) = \frac{\sqrt{\sigma_g^2}}{\bar{X}} \times 100 \quad (4)$$

The technique for the estimation of PCV and GCV, described by Singh and Chaudhary [19] was followed where population mean of the traits = X, PCV and GCV were categorized as low (0% - 10%), moderate (10% - 20%) and high (20% and above) following Subramanian and Madhava-Menon [20].

Table 2. Analysis of Variance (ANOVA) and mean squares for flower, pod and bean traits in cocoa genotypes.

Source of Variation	Df	MS	EMS
Replications (R)	r^{-1}	RMS	σ^2 e + n'σ^2 g + n'gσ^2 r
Genotypes (G)	g^{-1}	GMS	σ^2 e + n'σ^2 r + n'rσ^2 g
Error	$(r^{-1})(g^{-1})$	EMS	σ^2 e

Note: n' denotes harmonic mean destined for cocoa genotypes/block or replicate; g denotes genotypes' harmonic mean; r, denotes number of blocks or replications; MS, mean squares for the respective sources of variations; EMS, expected mean squares based on the sum of squares of type III.

2.6. Heritability, Genetic Advance (GA) and Genetic Advance as Percent of Mean

The broad-sense heritability (%) estimate was calculated following the procedures of Johnson *et al.* [21], and was categorized as either being low (h^2B < 30%), moderate (h^2B = 30% - 60%), or high (h^2B > 60%).

The expected genetic advance (GA) under selection as a percentage (%) of mean was estimated using the formula developed by Assefa *et al.* [22]. The selection intensity (K) as a constant is 2.06 when K is presumed at 5%, with the

$$\text{Phenotypic standard deviation} = \frac{\sqrt{\sigma_p^2}}{\bar{X}} \quad (5)$$

Genetic advance (GA): $$\text{GA} = K \times \sqrt{\sigma_p^2} \times h^2\text{B} \quad (6)$$

and Genetic advance as percentage of the mean (GA%)

$$\text{GA}(\%) = K \times \frac{\sqrt{\sigma_p^2}}{\bar{X}} \times h^2\text{B} \times 100 \quad (7)$$

Genetic advance was categorized as low (0% - 10%), moderate (10% - 20%) and high (>20%) based on the methods described by Johnson *et al.* [21].

3. Results and Discussion

3.1. Analysis of Variance for Yield and Agronomic Traits

Data collected on yield and vegetative parameters from the 20 clones across two agro-ecological zones showed a highly significant variation ($p < 0.01$) particularly for stem diameter, leaf length, leaf width and leaf area, while a corresponding significant difference was observed in pod width, pod length, pod thickness, pod diameter, number of beans per pod, dry bean weight per pod and estimated dry bean yield per tree. This result corroborates with the findings of Kunikullaya *et al.* [22] where significant difference was recorded for the aforementioned traits. However, pod size, number of flowers per tree and number of pods per tree were not significantly different (**Table 3**).

Table 3. Statistical and genetic parameter estimates of selected morpho-agronomic traits of cocoa.

Trait	Statistical estimates		Genetic parameter estimates			
	Mean	SD	HB (%)	PCV (%)	GCV (%)	GA
LL	34.05	4.77	87.97	14.09	13.21	25.53
LW	12.62	1.77	96.34	14.17	13.91	28.12
LA	437.7	121.02	95.85	27.89	27.31	55.08
SD	8.349	1.59	94.98	19.28	18.79	37.73
PDIA	7.357	0.73	81.05	10.02	9.02	16.73
NFT	37.54	4.45	0.00	11.70	0.00	0.00
PSP	45.63	11.86	68.68	26.08	21.61	36.90
NPT	16.23	3.72	99.82	23.25	23.23	47.80
PL	14.07	0.50	71.34	3.58	3.02	5.26
PW	7.375	0.46	58.65	6.36	4.87	7.69
PSA	103.8	8.50	68.88	8.29	6.88	11.76
PT	1.27	0.10	54.48	7.88	5.82	8.85
NBP	40.97	5.16	72.91	12.67	10.82	19.03
DBWP	17.08	4.95	88.27	29.38	27.60	53.42
EDYT	292.5	145.82	95.04	50.50	49.24	98.88

The high genetic variability present within genetic resources of crops is mainly used as a source material and these provide the basis for selecting suitable parents for use in breeding programs [24]. The coefficient of variation is mainly used as a measure to observe the range of variations available in genotypes [25]. High value of coefficient of variation in this study signified the existence of greater degree of variability among the clones across the two agro-ecological zones.

Analysis of variance showed high level of variabilities in traits such as leaf area, pod set per cent, number of beans per pod, dry bean weight per pod, number of flowers per tree and leaf length ($p < 0.05$) (**Table 3**). The analysis further revealed low variability in pod width, pod length, pod thickness, pod diameter, stem diameter and leaf width. Despite the low variabilities, there was however, a high level of significant difference in these traits ($p < 0.05$) (**Table 4**). These variabilities observed were the resultant effect of a combination of genotypic and environmental factors.

Table 4. Mean performance of cocoa genotypes for selected growth and flower traits.

Trait	LL	LW	LA	SD	NFT
KPU010	41.6	16.0	663.9	9.0	42.8
KPU011	35.7	13.7	494.2	9.9	39.5
KPU012	33.7	11.7	398.3	7.0	40.1
KPU013	37.9	12.5	472.6	6.0	37.4
KPU014	34.9	11.6	405.2	8.0	41.1
KPU015	33.4	13.6	452.2	6.8	35.6
KPU016	43.9	17.2	755.9	8.4	37.6
KPU09	39.1	13.3	524.6	7.9	39.9
NJA017	27.4	11.1	304.0	8.1	41.4
NJA018	37.5	13.9	521.0	10.8	39.8
NJA019	28.1	10.8	303.1	8.4	38.0
NJA020	39.1	13.3	520.9	9.2	32.1
PEN01	30.4	10.3	314.6	8.3	36.1
PEN02	32.1	11.2	360.8	9.2	35.9
PEN03	28.3	11.1	316.9	7.4	36.8
PEN04	30.0	10.4	313.9	7.7	37.5
PEN05	31.0	12.8	403.9	10.2	32.6
PEN06	29.5	11.8	348.4	12.0	33.6
PEN07	33.9	12.8	431.7	7.6	39.2
PEN08	33.7	13.4	448.3	5.4	33.8
SED	1.66	0.34	24.86	0.36	4.53
Fpr	<0.001	<0.001	<0.001	<0.001	0.604

Data collected on yield and vegetative parameters from the 20 clones across two agro-ecological zones showed a highly significant variation ($p < 0.001$) particularly growth and flower traits (**Table 4**) and pod traits (**Table 5**).

Table 5. Mean performance of cocoa genotypes for selected pod traits.

Trait	PSP	NPT	PDIA	PL	PW	PSA	PT
KPU010	46.4	19.0	6.6	13.9	6.6	91.9	1.2
KPU011	58.0	22.0	8.6	14.0	7.6	106.1	1.3
KPU012	37.8	15.0	7.4	13.9	7.0	96.9	1.3
KPU013	27.8	10.0	7.9	13.9	7.2	99.9	1.3
KPU014	44.1	18.0	7.4	14.0	7.2	100.9	1.4
KPU015	34.7	12.0	8.2	13.9	7.2	100.2	1.2
KPU016	44.3	16.0	6.8	13.9	7.4	102.0	1.2
KPU09	43.8	17.0	6.8	13.9	7.3	101.7	1.2
NJA017	39.7	16.0	7.2	14.0	7.6	106.0	1.2
NJA018	65.8	25.0	7.9	13.8	8.0	111.0	1.4
NJA019	35.2	13.0	7.7	13.6	7.9	107.5	1.4
NJA020	62.9	19.0	8.5	15.2	8.1	122.7	1.5
PEN01	45.0	15.0	7.2	15.6	7.7	119.6	1.2
PEN02	53.2	17.0	6.5	14.2	7.8	110.3	1.3
PEN03	37.4	12.0	6.1	13.9	7.2	99.9	1.2
PEN04	40.9	15.0	6.3	13.9	7.2	99.3	1.2
PEN05	51.9	15.5	7.9	14.0	6.6	92.1	1.2
PEN06	66.4	21.0	7.4	13.9	7.0	97.2	1.3
PEN07	43.5	16.0	7.5	13.9	7.5	103.5	1.3
PEN08	33.9	11.0	7.3	14.0	7.7	107.4	1.2
SED	6.66	0.16	0.31	0.27	0.30	4.69	0.07
Fpr	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Separation of means from the pooled data of the three zones using the Duncan's Multiple Range Test (DMRT) showed that the clones PEN 05, PEN 01, KPU 010, PEN 03, KPU 011, NJA 020, NJA 019, KPU 012, KPU 015, PEN 02, PEN 06, PEN 08, NJA 017, KPU 016 and NJA 018 were significantly different for pod width, pod length, pod size, pod thickness, pod diameter, number of flowers per tree, number of pods per tree, number of beans per pod, dry bean weight per pod, estimated dry yield per tree, pod set per cent, stem diameter, leaf length, leaf width and leaf area, respectively (**Table 5**, **Figure 1**). The presence of significant variations among the genotypes indicates their importance in breeding programs [15]. Furthermore, the differences in phenotypic values between genotypes in different environments gives a clue in the measure of genotype by environment (G × E) interactions [26].

Figure 1. Morphological variations in pod set, size, shape and color among cocoa genotypes.

3.2. Heritability, Selection Gain and Coefficient of Variation

Heritability is a good indicator of the transmission of traits from parents to their offspring. Estimates of heritability helps breeders select genotypes from genetically diverse populations. Therefore, high heritability contributes to effective selection for a particular trait. More than 60% of the traits in this study showed high heritability (68.68% - 99.82%) while about 13% showed moderate heritability (54.48% - 58.65%). Number of flowers per tree was the only trait that showed low heritability. The highest broad sense heritability was manifested by number of pods per tree (99.82%) followed by estimated dry yield per tree and stem diameter (95.04% and 94.98%), respectively (Table 3).

The analysis of PCV and GCV of the different traits are presented in Table 3 with results indicating that the PCV is slightly greater than the GCV. This is an indication that the variabilities were less influenced by the environment, suggesting that the variations were mostly due to the genetic constituents of the genotypes. The highest PCV and GCV were found in pod set per cent (26.08% and 21.61%), dry bean weight per pod (29.38% and 27.06%), leaf area (27.89% and 27.31%), number of pods per tree (23.25% and 23.23%) and estimated dry yield per tree (50.50% and 49.24%), respectively. As moderate PCV and GCV were recorded for leaf width (14.17% and 13.91%), leaf length (14.09% and 13.21%) and number of beans per pod (12.67% and 10.82%), low PCV and GCV was recorded for pod length (3.58% and 3.02%) respectively. An inverse proportionality of PCV and GCV was however recorded for number of flowers per tree (11.70% and 0.00%), pod diameter (10.02% and 9.02%), pod thickness (12.47% and 6.02%) and pod size (7.88% and 5.82%) respectively.

This study recorded moderate genetic advances for pod diameter (14.91%), number of beans per pod (16.73%) and leaf length (25.53%) while high genetic advances were recorded for number of pods per tree (47.80%), dry bean weight per pod (53.42%), leaf area (55.08%), stem diameter (37.73%), pod set per cent

(36.90%) and leaf width (28.12%). Low genetic advances ranged from 0% for number of flowers per tree to 7.88% for pod size (Table 3).

Analysis of variance showed high level of variabilities in traits such as number of beans per pod, dry bean weight per pod and estimated yield per tree ($p < 0.001$). Despite these variabilities, there was a corresponding high level of significant differences in these traits ($p < 0.05$) (Table 6). The top 10 genotypes with dry bean weight per pod ranging from 17.3 (KPU 014) to 28.8 g (NJA 018) are presented in Table 6. Of the 20 genotypes studied, NJA 018 exhibited the heaviest dry bean weight per pod (28.75 g), whereas PEN08 had the lowest of 7.7 g. These variabilities observed were the resultant effect of a combination of genotypic and environmental factors.

Table 6. Mean performance of cocoa genotypes for selected bean yield and related traits.

Trait	NBP	DBWP	EDYTR
KPU010	44.1	20.7	393.5
KPU011	41.2	18.9	416.2
KPU012	32.4	13.7	204.7
KPU013	36.1	9.7	97.0
KPU014	47.9	17.3	311.0
KPU015	44.5	12.8	154.1
KPU016	43.0	23.4	373.8
KPU09	41.0	19.4	329.5
NJA017	42.1	17.8	284.2
NJA018	26.4	28.8	718.7
NJA019	40.5	13.9	180.8
NJA020	41.4	20.5	389.9
PEN01	40.1	16.5	247.7
PEN02	43.0	19.7	335.3
PEN03	42.4	13.7	164.2
PEN04	44.5	16.7	249.7
PEN05	37.7	13.4	211.0
PEN06	43.8	21.8	458.6
PEN07	42.0	15.4	245.8
PEN08	45.2	7.7	84.7
SED	2.70	1.68	32.08
Fpr	<0.001	<0.001	<0.001

3.3. Correlation between Yield Parameters

The result of Pearson's correlation coefficient (Table 7) showed that pod width recorded highly significant and positive correlation with pod length ($r = 0.25^{**}$) and pod size ($r = 0.91^{**}$). Pod length recorded highly significant and positive correlation with pod size ($r = 0.63^{*}$). Records of pod thickness showed highly significant and positive correlation with pod diameter (r = 0.24*), number of pods per tree (r = 0.24*) and stem diameter (r = 0.21*). Number of pods per tree had highly significant ($p < 0.0001$) and positive correlation with dry bean weight, estimated dry yield per tree, pod set percent, stem diameter, leaf length, leaf width and leaf area (0.74**, 0.87**, 0.60**, 0.75**, 0.21**, 0.21** and 0.23**) respectively (Table 7). Dry bean weight per pod recorded highly significant and positive correlation with estimated dry yield per tree, dry bean weight per pod, pod set per cent, stem diameter, leaf length, leaf width and leaf area. Estimated dry yield per tree equally was highly significant and positively correlated with dry bean weight per pod, pod set per cent, stem diameter, leaf length, leaf width and leaf area. Dry bean weight per pod recorded highly significant and positive correlation with estimated dry yield per tree, dry bean weight per pod, pod set per cent, stem diameter, leaf length, leaf width and leaf area. Estimated dry yield per tree equally was highly significant and positively correlated with dry bean weight per pod, pod set per cent, stem diameter, leaf length, leaf width and leaf area. Pod set per cent was highly significant and positively correlated with stem diameter (r = 0.56**). Leaf length was highly significant and positively correlated with leaf width (r = 0.61**), while leaf width was highly significant and positively correlated with leaf area (r = 0.90**). Highly significant ($p < 0.001$) and negative correlations were observed between pod thickness and number of beans per pod (r = −0.21**), pod diameter and number of beans per pod (r = −0.20**), number of pods per tree and number of beans per pod (r = −0.19**), number of flowers per tree and pod set per cent (r = −0.70**) and between number of beans per pod and dry bean weight per pod (r = −0.20**) (Table 7).

Table 7. Pearson genotypic and phenotypic path coefficient analysis in cocoa genotypes for morphological and yield traits.

	PW	PL	PS	PT	PDIA	NFT	NPT	NBP	DBWP	EDYT	PSP	SD	LL	LW	LA
PW	1														
PL	0.25**	1													
PS	0.91**	0.63**	1												
PT	0.05^{ns}	−0.052^{ns}	0.02^{ns}	1											
PDIA	0.08^{ns}	0.13*	0.12^{ns}	0.24**	1										
NFT	0.01^{ns}	−0.11^{ns}	−0.04^{ns}	0.03^{ns}	−0.10^{ns}	1									
NPT	0.09^{ns}	0.03^{ns}	0.09^{ns}	0.24**	0.17*	0.09^{ns}	1								
NBP	−0.05^{ns}	0.06^{ns}	−0.02^{ns}	−0.21**	−0.20**	−0.07^{ns}	−0.19**	1							
DBWP	0.13*	0.02^{ns}	0.11^{ns}	0.14*	−0.01^{ns}	0.05^{ns}	0.73**	−0.20**	1						
EDYT	0.12^{ns}	0.04^{ns}	0.12^{ns}	0.17*	−0.03^{ns}	0.09^{ns}	0.87**	−0.16*	0.84**	1					
PSP	0.06^{ns}	0.13^{ns}	0.11^{ns}	0.14*	0.17*	−0.70**	0.60**	−0.10^{ns}	0.44**	0.52**	1				
SD	−0.05^{ns}	−0.01^{ns}	−0.04^{ns}	0.21**	0.11^{ns}	−0.05^{ns}	0.75**	−0.16*	0.62**	0.68**	0.56**	1			
LL	−0.80^{ns}	−0.02^{ns}	−0.06^{ns}	−0.01^{ns}	0.09^{ns}	0.01^{ns}	0.21**	−0.04^{ns}	0.24**	0.28**	0.11^{ns}	0.01^{ns}	1		
LW	−0.04^{ns}	−0.13^{ns}	−0.09^{ns}	−0.03^{ns}	0.08^{ns}	−0.01^{ns}	0.21**	−0.03^{ns}	0.23**	0.28**	0.10^{ns}	0.06^{ns}	0.61**	1	

Continued

LA	-0.07^{ns}	-0.80^{ns}	-0.09^{ns}	-0.04^{ns}	0.07^{ns}	0.01^{ns}	0.23**	-0.03^{ns}	0.27**	0.33**	0.11^{ns}	0.05^{ns}	0.90**	0.89**	1

Prob > |r| under H_0: Rho = 0; ** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed) PW = Pod width; PL = Pod length; PS = Pod size; PT = Pod thickness; PDIA = Pod diameter; NFT = Number of flowers per tree; NPT = Number of pods per tree; NBP = Number of beans per pod; DBWP = Dry bean weight per pod; EDYT = Estimated dry bean yield per tree; PSP = Pod set percentage; SD = Stem Diameter; LL = Leaf Length; LW = Leaf Width and LA = Leaf Area.

3.4. Principal Component and Cluster Analysis of Cocoa Genotypes for Yield and Yield Contributing Traits

Broadly, the UPGMA clustered the cocoa genotypes into four main groups at dissimilarity coefficient of 0.44 (Figure 2) implying the existence of a high level of genetic diversity in the cocoa genotypes. The use of UPGMA to clearly differentiate the genotypes into distinctive groups validates the effectiveness of morphological or quantitative traits in grouping cocoa genotypes. This grouping further indicates that although the genotypes have central point of origin, however, the further the genotypes are from the centre of origin, the better they would be in terms of selection for the development of improved cultivars (Figure 3).

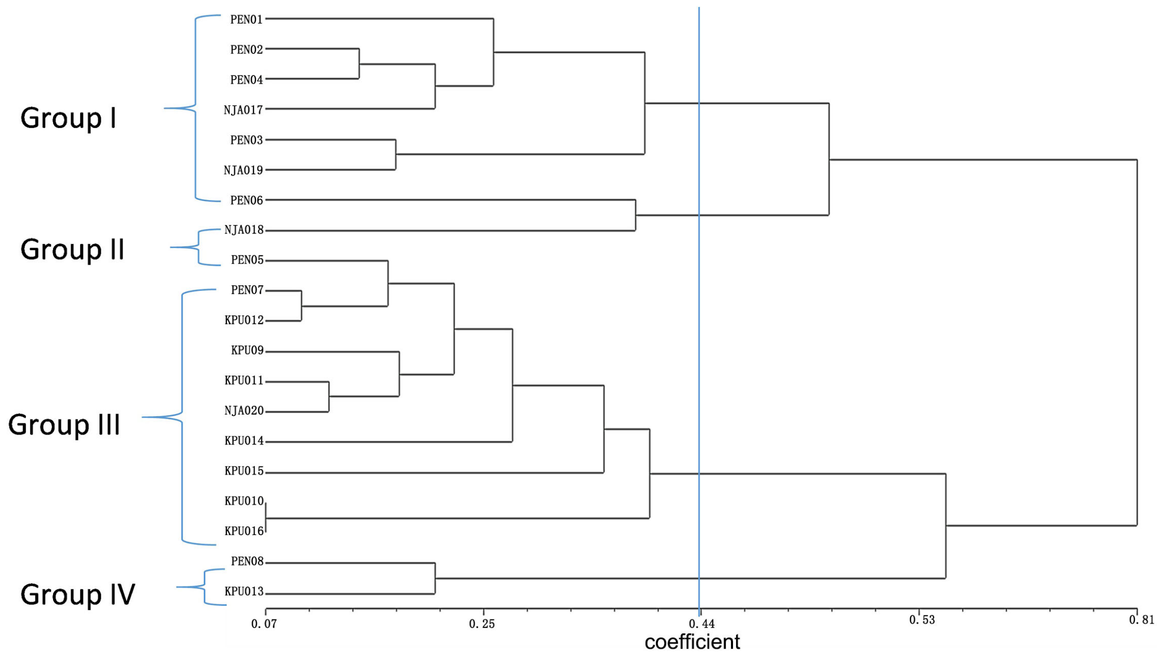

Figure 2. Cluster dendrogram for 20 cocoa genotypes based on UPGMA analysis.

Knowledge of the genetic stability of a quantitative trait in different environments is important for the selection of genotypes for subsequent breeding programs [27]. The result of research and experience in crop breeding underscores that the success of plant breeding programs depends largely on the presence of genetic variations and inheritance of traits of interest. Analysis of genetic variation within a base population helps breeders decide on the appropriate strategies and selection criteria to use to improve target traits. Analysis of data from this study showed that traits assessed had highly significant variations across the two

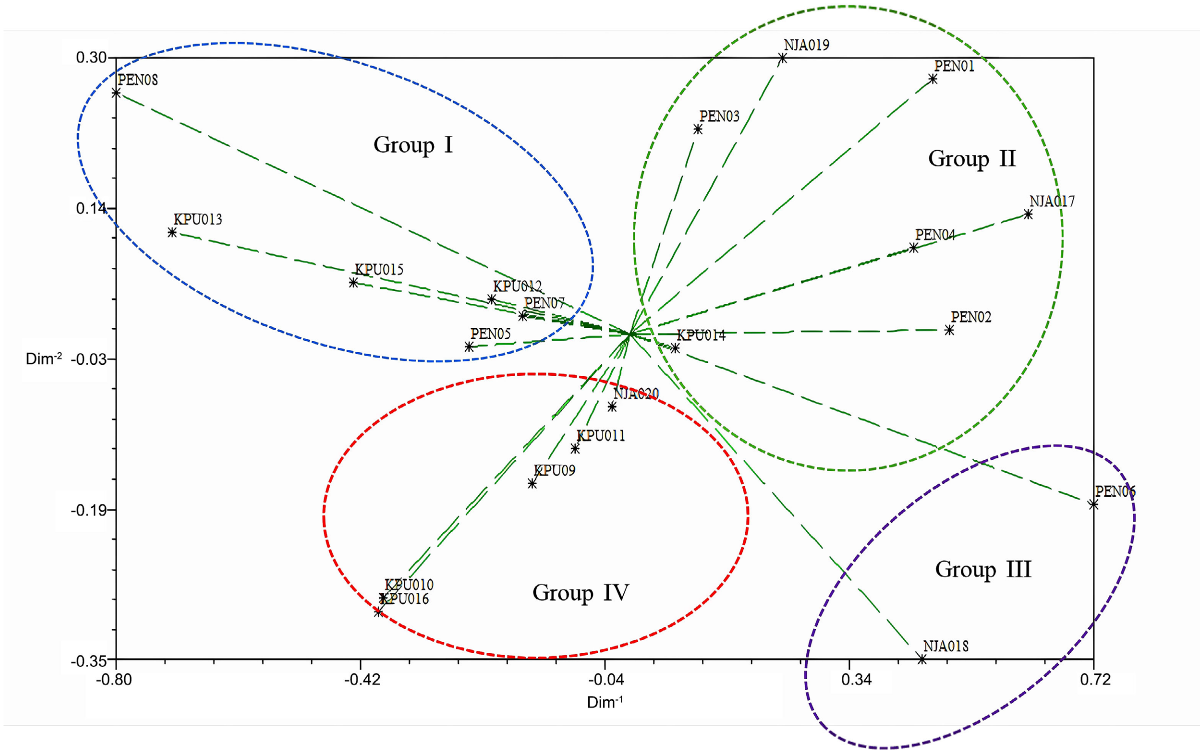

Figure 3. Grouping of 20 cocoa genotypes based on cluster analysis.

agro-ecological zones supporting the findings of Vashistha *et al.* [28] that the existence of variability is essential for resistance to abiotic and biotic stress as well as for wide adaptability of genotypes. This means that the genotypes exhibit different levels of variations in the relationship between observed traits in terms of heritability, selection gains and coefficient of variations (PCV and GCV) among the populations. These findings are in conformity to the result of Terfa and Gurmu [29]. These differences could be due to different origins of the genotypes and are therefore subject to genetic confirmation through molecular characterization. Traits showing the highest values of PCV and GCV gives an indication of the presence of high variability, while traits showing low PCV and GCV values indicates low genetic variability. The presence of high variability in traits indicates the possibility of effective selection during improvement of cocoa genotypes for that particular trait. Traits showing lowest GCV (pod length) and highest PCV (dry bean weight per pod) indicate that the variability is largely influenced by the environment because PCV was higher than GCV. The moderate PCV and GCV values for dry bean weight per pod and number of beans per pod with a corresponding GA of 47.46% and 47.65%, respectively, revealed consistently high variability in these traits, suggesting the reliability of these traits for yield improvement in cocoa. Significant genetic variation of any breeding material is a prerequisite as it does not only provide basis for selection but also provides valuable information regarding the choice of parents' diversity for use in breeding programs [30] [31]. Generally, the higher differences observed between phenotypic and genotypic variances for number of flowers per tree, pod thickness, pod size and pod width suggest that these characters were greatly influenced by environmental factors.

Heritability is the proportion of overall trait variation due to genetic causes, and it is the index of transmission of traits from parents to their offspring [32]. Traits that exhibit high heritability with high level of genetic advance as a percentage of the mean and higher genotypic coefficient of variation provides better clue than individual parameter and indicates that bulk of the variabilities is as a result of additive genetic factors for those traits, whereas traits with moderate heritability and with low genetic advance as a percentage of the mean is mainly driven by non-additive traits and direct genetic factors [23]. In this case, therefore, selection may not be possible because much of the variation is influenced by environmental factors. Only traits with high genotypic coefficient of variation, heritability and genetic advance should be selected. Reddy *et al.* [33] reported that these environmental factors may be the resultant effect of heterogeneity in soil fertility status and other unpredictable factors. Different scholars have therefore suggested that in order to improve the performance of such traits, much attention should be given to management practices rather than selection [28] [34]. Selection from amongst these populations should consider genetic advance because it serves as an important indicator for the expected result [35]. In this study, we observed heritability indicating that the observed traits were less influenced by the environment in their expression. Therefore, the plant breeder can safely conduct selection based on the phenotypic expression of these traits in individual plants by applying simple breeding methods like conventional breeding method. The genetic advance as a percentage of the mean that coincides with high heritability is more useful than heritability alone for predicting performance gains when selecting best individual genotype, as was obtained in this study for leaf area, dry bean weight per pod and number of pods per tree. However, highly heritable traits associated with moderate genetic advance as a percentage of the mean are given consideration for improvement of the trait through selection, because the trait can be improved more easily than other traits. This corroborates with the findings of Singh *et al.* [30]. Since the development of cocoa hybrids takes length of time, it is therefore, imperative that breeders develop suitable methodology for future breeding programs.

The existence of positive correlation coefficients between vegetative traits and yield parameters in this study is an indication that the quantitative traits measured are suitable for predicting and selecting yield for breeding programs aimed at developing progenies with better heterosis. Conclusively, correlation analysis revealed the association of different traits, indicating characters that must be considered during selection for improvement in genetic yield.

In the present study, 20 genotypes were grouped into four major clusters on the basis of morphological diversity. The distinct variation in the cocoa germplasm suggests that the genotypes might possess genes, in high frequencies, for adaptation in the studied area, whereas the high genetic variation is indicative of a high amount of additive genetic variance, needed for genetic progress in cocoa breeding. The high genetic variability also represents a heterotic pool that provides an opportunity for the systematic exploitation of hybrid vigor in cocoa. These findings agree with the view that analysis of genetic diversity helps to identify unique phenotypes and genotypes for breeding and conservation [36]-[38]. Clustering of genotypes with considerable variabilities has been achieved by Guimarães *et al.* [39] who analyzed the agronomical performance of eight yield components and resistance traits of 145 cocoa accessions. These authors found that accession POUND 12 had the best performance regarding weight of wet seeds from healthy fruits, average weight of wet seeds per fruit, and number of fruits with witches' broom symptoms per plant. In rice, Mazid *et al.* [40] clustered 41 rice genotypes into 6 main groups on the basis of 13 morphological traits.

4. Conclusions

Broadly, the UPGMA clustered the cocoa genotypes into four main dissimilar groups.

The 20 cocoa genotypes showed considerable variations in average performance in terms of vegetative, pod and bean traits. High PCV and GCV values for most of the tested traits indicate the good breeding value of the germplasm material. Traits with high heritability combined with high average genetic advance can be used as selection criteria since they are least affected by environmental factors. Correlation analyses also revealed associations between different traits and determined component traits on which selection could be relied upon for genetic improvement of yield.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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