

## Phenotypic Variability and Diversity Analysis of Bean Traits of Some Cocoa Hybrids in Nigeria

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**Abstract:** It is essential to understand the economic potential and superiority among cocoa hybrids. Therefore, the present study aims at detecting variability among cocoa hybrids for bean index in Nigeria. Dried bean of fourteen genotypes of cocoa were evaluated for their bean values. Analysis of variance (ANOVA) was used to understand the variability among the fourteen genotypes and Principal Component Analysis (PCA) was employed to identify distinguishing traits and the grouping of the genotypes based on similarities. The fourteen cocoa genotypes were significantly ( $p \leq 0.05$ ) different from each other with respect to weight of one bean, bean length, width, thickness, 100 bean weight, bean length to width, length to thickness and width to thickness ratio. All the studied morphometric characters exhibited high ( $>70\%$ ) broad sense heritability. G8, the hybrid between T53/5 and N38 was the most superior genotype for bean weight and some other bean characteristics. The mass of seventy-four dried cocoa bean of G8 approximated 100 g. The first three Principal Component axes explained 91% of the total variation and the PCA grouped the fourteen genotypes into four distinct clusters. Genotypes could be selected for specific traits and improvement of traits seemed to be genetically reliable.

**Key words:** Bean value, diversity, heritability, hybrids, seed index

### INTRODUCTION

Cocoa (*Theobroma cacao* L.) is an important commodity crop of Neo-tropical origin. It is principally grown in West Africa, Central and South America and Asia. In the order of annual production size, the eight largest cocoa producing countries at present are Côte d'Ivoire, Ghana, Indonesia, Nigeria, Cameroon, Brazil, Ecuador and Malaysia. These countries represent 90% of the total world Cocoa production (UNCTAD, 2010).

The most interesting economic part of the crop is the duly fermented and dried seed otherwise called the beans. The bean size is one of the most important components of yield in Cocoa (Soria, 1978). The protein content in dried cocoa bean ranged between 15 to 20% and the fat content is nearly 50% (Biehl *et al.*, 1982; Spencer and Hodge, 1992). The highest percentage (52%) of the total seed storage proteins in cocoa bean is the albumin fraction. The content of the globulin (a vicilin-class) fraction of storage protein in cocoa bean is about 43% (Voigt and Biehl, 1993).

Morphological and structural characteristics on seeds often exhibit large and high discriminatory variations within species (Adewale *et al.*, 2010a). Physical

properties of the seeds are important in the determination of their shapes (Balkaya and Odabas, 2002; Adewale *et al.*, 2010a); they are required for the development of post harvest equipment (Dash *et al.*, 2008), they are very important as inferential factors or components in determining some polygenic traits e.g., yield (Omokhafa and Alika, 2004), protein and oil content (Kaushik *et al.*, 2007). Some characters which are physical-quantitative in nature (e.g., seed size, seed shell, fat content) are related to the quality of the cocoa beans (Monteiro *et al.*, 2009). While the morphometric values of cocoa beans could act as an important characteristics in breeding for yield, the indices could equally act as an indicator for storage pests' resistance or susceptibility.

Production capacity of Cocoa promises to receive a great boost in the next decade if hybridization method of breeding could be applied (Gotsch, 1997). The development of superior hybrids has made a significant contribution to cocoa productivity, particularly in Brazil (Dias *et al.*, 2003). Hybrids of Cocoa was reported by Dias *et al.* (1998) to show wide adaptability, low interaction with years and an outstanding performance for yield and its components, when compared to unimproved traditional local cultivars.

Variation in colour, format, weight, and size of the beans is according to race and cultivar group (Souza and Dais, 2004). Direct and significant variation of different hybrids of cocoa to traits relating to dried bean may provide trustworthy criteria for selection. The present study looked at the phenotypic variations of some quantitative traits on cocoa beans and the diversity of fourteen cocoa hybrids based on the studied traits.

**MATERIALS AND METHODS**

Twenty matured cocoa pods of uniform sizes and ripening were harvested per genotype in 2010 from a hybrid trial plot established in 1999 at Cocoa Research Institute of Nigeria (CRIN), Headquarters, Ibadan, Nigeria. The field consisted of 14 hybrids laid out in Randomized Complete Block Design. A total of 840 pods which resulted from fourteen genotypes in three replications constituted an experimental unit. The fourteen cocoa genotypes included thirteen cocoa hybrids and F3 Amazon (a common cocoa genotype in most farmers' field in Nigeria). List of the genotypes and their pedigree is shown in Table 1. Seeds from the pods of each genotype were removed and bulk fermentation was done in trays per genotype in three replicates. The fermented seeds were sun-dried naturally and stored.

The weight of 100 beans was recorded per genotype. Other quantitative measurements were made on thirty uniformly selected dry beans per genotype. Individual weight of each of the thirty selected bean was recorded per genotype. The length, width and thickness of beans were measured in millimeters using the vernier caliper according to Kaushik *et al.* (2007). The ratios between the bean length, width and thickness were estimated from

Table 1: List of the fourteen Cocoa hybrids and their pedigree

Genotypes	Pedigree
G1	T65/7 X T22/28
G2	T12/11 X N38
G3	T65/7 X T9/15
G4	Pa 150 X T60/887
G5	P7 X T60/887
G6	P7 X Pa 150
G7	T65/7 X T57/22
G8	T53/5 X N38
G9	T65/7 X N38
G14	F3 Amazon
G15	T86/2 X T30/13
G17	T86/2 X T16/17
G19	T65/7 X T101/15
G21	T86/2 X T53/8

the individual values of the length, width and thickness of the beans following Omokhafa and Alika (2004).

The means from the thirty sampling unit per replicate for each genotype were generated for eight variables. These were subjected to analysis of variance (ANOVA) to understand variability among the genotypes. Broad sense heritability for each trait was calculated as the ratio of the genotypic variance to the phenotypic variance. A data matrix of fourteen genotypes by eight quantitative variables was generated for multivariate analysis. The data set generated was standardized (mean = 0 and standard deviation = 1) according to Chandran and Pandya (2000) and Ofori *et al.* (2006) to eliminate the effects of different scales of measurements. Pearson correlation coefficient was calculated to understand the relationship between pairs of morphological traits. ANOVA, Principal Component (PC) Analysis and tri-dimensional graph were made using the procedures of GLM, PRINCOMP and g3d, respectively in the Statistical Analysis System, SAS-V9.2 (SAS Institute Inc., 2007).

Table 2: Means, ranges and estimates of some seed traits of Cocoa beans

Morphological variables	Means±S.E.	Min.	Max.	CV (%)	Heritability (%)	Genotypic F-values
Weight of one bean (g)	1.09±0.02	0.84	1.47	5.51	91.74	11.11***
Bean length(cm)	2.16±0.02	1.91	2.51	2.49	93.35	14.03***
Bean width(cm)	1.20±0.01	1.07	1.35	3.63	70.42	2.38*
Bean thickness(cm)	0.73±0.01	0.61	0.89	7.87	71.66	2.53*
100 bean weight (g)	125.26±0.99	114.11	146.63	3.34	78.11	3.57**
Bean length to width ratio	1.83±0.03	1.60	2.50	7.56	70.80	2.43*
Bean length to thickness ratio	3.07±0.05	2.46	3.69	7.04	76.27	3.21**
Bean width to thickness ratio	1.71±0.03	1.29	2.08	7.80	74.38	2.90*

CV-Coefficient of variation in percentage; \*, \*\* and \*\*\*: Level of significance at p≤0.05, 0.01 and 0.001, respectively

Table 3: Pearson correlation coefficients of eight morphological traits on Cocoa beans

	LT	WDT	TKN	S100	LtoW	LtoT	WtoT
WT	0.8518***	0.6470*	0.9516***	0.6074*	0.6462*	0.2372 <sup>ns</sup>	- 0.0798 <sup>ns</sup>
LT		0.6142*	0.9508***	0.5209 <sup>ns</sup>	0.7338**	0.3594 <sup>ns</sup>	- 0.0785 <sup>ns</sup>
WDT			0.6945*	0.3661 <sup>ns</sup>	0.0175 <sup>ns</sup>	0.4061 <sup>ns</sup>	0.3885 <sup>ns</sup>
TKN				0.5586*	0.7029*	0.2065 <sup>ns</sup>	- 0.1530 <sup>ns</sup>
S100					0.3223 <sup>ns</sup>	0.3294 <sup>ns</sup>	0.0909 <sup>ns</sup>
LtoW						- 0.1135 <sup>ns</sup>	- 0.5766*
LtoT							0.8487***

WT: Weight of one seed; LT: Bean length; WDT: Bean width; TKN: Bean thickness; S100: 100 bean weights; LtoW: Bean length to width ratio; LtoT: Bean length to thickness ratio; WtoT: Bean width to thickness ratio; \*, \*\* and \*\*\*: Level of significance at p≤0.05, 0.01 and 0.001, respectively

**RESULTS**

The fourteen genotypes of cocoa differed significantly ( $p \leq 0.05$ ) with respect to the eight quantitative variables. The weight and length of individual beans most significantly ( $p \leq 0.001$ ) differentiated the fourteen genotypes (Table 2). From Table 2, the coefficient of variation (CV) of the eight variables ranged between 2.49 (bean length) and 7.87 (bean thickness). The overall means of the fourteen genotypes for each of the eight variables were presented in Table 2. The minimum weight of an individual (0.84 g) and a hundred (114.11 g) beans were obtained in G2. The least length (1.91 cm), width (1.07 cm) and thickness (0.61 cm) of cocoa bean was obtained in G5, G4 and G19 respectively. The ratio of the bean length and width to thickness was lowest in G1 while the least ratio for bean length and width was obtained in G5. The highest (146.63 g) 100 bean weight among the fourteen genotypes was obtained in G7, while G8 had the highest mass and length (1.47 g and 2.51 cm, respectively) for an individual bean. The genotype (G1) with the highest thickness (0.89 cm) equally had the highest ratio of 2.5 for bean length to width of 2.5. The highest ratio of length and width to thickness (3.7 and 2.1, respectively) was obtained in G19 (Table 2). The broad sense heritability for the eight variables ranged between 70.42 and 93.35% for bean width and length, respectively.

From Table 3, the weight of an individual bean positively and significantly ( $p \leq 0.05$ ) correlated with the length, width, thickness of the bean, the 100 bean weight

and the ratio of the bean length to its width. The bean length had a positive and significant ( $p \leq 0.05$ ) correlation with the bean width, thickness and the ratio of the bean length and width ( $r = 0.61, 0.95$  and  $0.73$ , respectively; Table 3). The correlation between bean width and its thickness was positive and significant ( $p \leq 0.05$ ). The relationship between bean thickness with 100 bean weight and the ratio of the length to its width was positive and significant (Table 3). The ratio of the bean length to width and bean width to thickness was significant and negatively correlated, however, the correlation between the ratio of bean length to thickness and width to thickness was significant and positive (Table 3).

The Eigen values which represents the proportion of variations explained by each PC axis and the eigenvectors of each morphological variable are presented in Table 4. The first three PC axes explained 91% of the total variation among the fourteen genotypes of cocoa. The contribution of the first three PC-axes to the total variance were in the proportion of 59, 32 and 9%, respectively (Table 4). Six morphological traits (weight of one bean, bean length, width, thickness, 100 bean weight and bean length to width ratio) significantly (eigenvector  $\geq 0.2$ ) distinguished the 14 genotypes in PC1. The three ratios from the metric measurements on the beans distinguished the 14 genotypes in PC2. However, five of the eight variables (i.e., bean width, thickness, 100 bean weight, bean length to width and bean length to thickness) were found to significantly (eigenvector  $\geq 0.2$ ) discriminated the 14 genotypes in PC3 (Table 4).

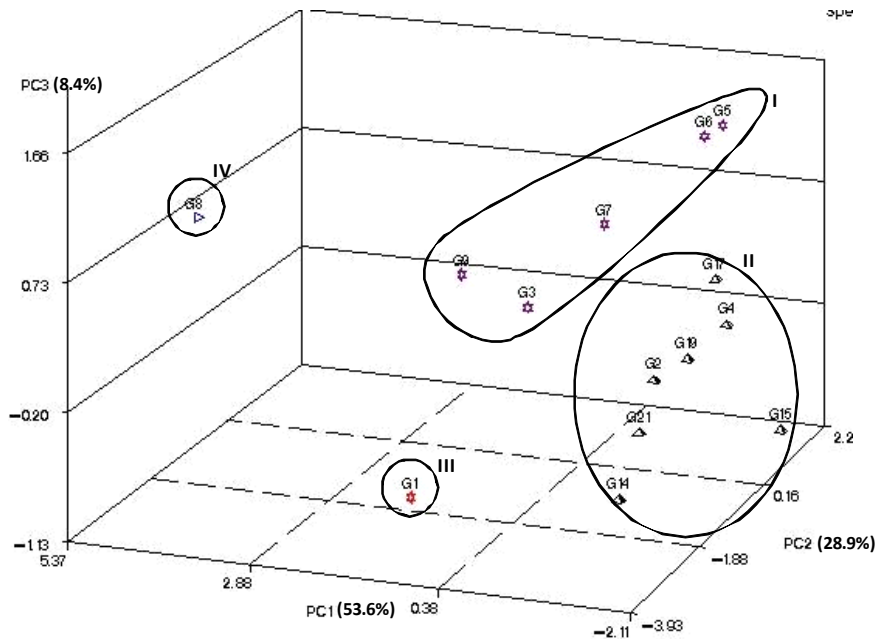


Fig.1: Tri-dimensional graph showing the grouping of the fourteen cocoa genotypes

Table 4: The Principal components showing the eigenvalues, proportions of variation and the eigenvectors

	PC1	PC2	PC3	PC4	PC5
	<b>PC-axes</b>				
Eigenvalues of each axis	4.2845	2.3098	0.6730	0.5620	0.1353
Variation explained in each axis	0.5356	0.2887	0.0841	0.0703	0.0169
Total variation explained across axes	0.5356	0.8243	0.9084	0.9787	0.9956
	<b>Eigenvectors</b>				
Morphological variables	<b>Eigenvectors</b>				
Weight of one bean	0.4578	-0.0545	-0.0702	-0.0917	0.7953
Bean length	0.4600	-0.0448	-0.0535	0.3072	-0.4782
Bean width	0.3406	0.3010	-0.5875	-0.2962	-0.2266
Bean thickness	0.4715	-0.0861	-0.2023	-0.0101	-0.0358
100 bean weight	0.3255	0.0866	0.6610	-0.6399	-0.1819
Bean length to width ratio	0.3238	-0.4102	0.2527	0.4289	0.0533
Bean length to thickness ratio	0.1724	0.5460	0.3225	0.4459	-0.0354
Bean width to thickness ratio	-0.0090	0.6505	0.0363	0.1306	0.2213

Table 5: The within-cluster means and the coefficient of variation of the eight quantitative traits

Variables	Clusters					
	I		II		III	IV
	Mean ± SE	CV	Mean ± SE	CV	Mean	Mean
WT	1.07±0.03	6.95	1.02±0.01	3.57	1.12	1.30
LT	2.17±0.05	5.03	2.11±0.02	3.12	2.22	2.44
WDT	1.20±0.02	4.60	1.20±0.01	2.38	1.18	1.25
TKN	1.31±0.03	4.76	1.28±0.01	2.01	1.36	1.47
S100	128.40±1.60	2.79	122.12±0.47	1.03	122.40	134.40
LtoW	1.82±0.02	2.94	1.77±0.02	3.21	2.09	2.11
LtoT	3.20±0.09	6.25	3.01±0.05	4.75	2.59	3.23
WtoT	1.78±0.04	5.10	1.72±0.04	6.22	1.40	1.65

NB: WT - Weight of one seed, LT - Bean length, WDT - Bean width, TKN - Bean thickness, S100 - 100 bean weights, LtoW - Bean length to width ratio, LtoT - Bean length to thickness ratio, WtoT - Bean width to thickness ratio

Figure 1 presented the tri-dimensional spatial figuration of the fourteen genotypes. Four basic clustering groups were visible in Fig. 1; clusters I and II had a membership of five (G3, G5, G6, G7 and G9) and seven (G2, G4, G14, G15, G17, G19 and G21) genotypes respectively. Clusters III and IV had G1 and G8 as single member. The mean bean weight of the five genotypes in cluster I was 1.07 g, while the seven genotypes in cluster II had 1.02 g. G1 and G8 had outstanding individual bean weights of 1.12 and 1.30 g, respectively (Table 5). Cluster I had the highest bean width to thickness ratio, this characteristic feature was important for the grouping of the five genotypes into cluster I. Genotypes in cluster II had the least performances for the following characters: bean weight, length, thickness, 100 bean weight and length to width ratio (Table 5). G1 outstandingly had the least value for: bean width, length to thickness and width to thickness ratios but G8 was most outstanding for the highest value for seven of the eight morphological variables except width to thickness ratio (Table 5, Fig. 1). The CV within the clusters ranged between 1.03 to 6.95% (Table 5).

## DISCUSSION

From this study, bean length was about twice the width and about thrice the thickness; moreover, the bean width is about twice the thickness. The generally very low CV observed for the eight traits indicated a high level of

uniformity in the sample from which data were obtained. Bean size is often expressed by the seed index (average weight of the dried bean). The very high significant correlation of the weight of the bean to its thickness and length seem to depict their importance and significance in determining the bean weight.

Cocoa varieties with a large seed index are of higher economic values to chocolate industries (Ruinard, 1961). The general mean of an individual bean weight for the 14 genotypes was 1.09 g. As remarked by Monteiro *et al.* (2009), cocoa genotypes with bean weight higher than 1g are superior, hence, most of the genotypes involved in this study may boost cocoa production in Nigeria. High broad sense heritability is a good indicator of reliability for genetic improvement of phenotypic traits (Adewale *et al.*, 2010b). The moderately high (>70%) broad sense heritability obtained in this study depicted that the contribution of the environmental factor to the phenotypic variances of the eight quantitative traits is low.

Genotypes in cluster II had lower than the expected and economic weight of individual bean of 1.07 g as recommended by Toxopeus (1985). F3 Amazon (G14) coincidentally fell in this cluster. The need for improved cocoa varieties to replace poorly productive cocoa varieties in most farmers' plots in Nigeria is overdue. However, the identified superior Cocoa hybrids from this study included: G1, G8 and the five genotypes in cluster I.

## CONCLUSION

High productivity per hectare of cocoa is a long awaited production target in Nigeria. Since tonnage yield is directly proportional to bean size, the study therefore revealed an outstanding contribution of breeding to Nigerian cocoa economy. Seven of the fourteen genotypes in this study had larger bean size compared to the most incumbent cocoa variety (i.e., F3 Amazon). These hybrids promise to heave cocoa production in Nigeria when they are released to farmers.

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