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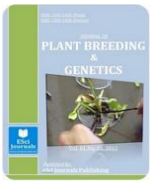

DESCRIPTIVE AND DISCRIMINATORY SIGNIFICANCE OF POD PHENOTYPIC TRAITS FOR DIVERSITY ANALYSIS OF COCOA GENOTYPES

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ABSTRACT

Intra-specific genetic diversity analysis precedes crop breeding proposal for species improvement. Sixteen and twenty-four parental and hybrid cocoa genotypes were respectively laid out in a randomized complete block design of six replications at Ibadan, Nigeria. A sampling unit of fifteen uniformly ripe pods was collected for assessment from each plot. Six quantitative data from the pods were subjected to statistical analysis. Highly significant ($P < 0.0001$) variability existed among the 40 genotypes. Range of performance of the genotypes were: pod weight (0.43 – 0.86kg), pod length (15.9 – 27.96cm), pod girth (21.51 – 34.07cm), pod thickness (1.26 – 5.71cm), number of beans per pod (20 - 51) and bean weight per pod (0.017 - 0.41kg). Positive and significant ($P < 0.001$) correlation existed between pod weight and length, pod girth and bean number/pod. The mean Gower genetic distance among the 40 genotypes was 0.228; the least (0.023) existed between G25 and G30 while the highest (0.529) was between G17 and G35. The first three principal component axes explained 73% of the total variation. Three distinct groups emerged from the Ward clustering technique. Significant ($P<0.05$) intra and inter cluster variability existed in the study. High genetic diversity lies within the studied population. Pod traits were important descriptors for cocoa genotypes classification.

Keywords: pod value, bean value, genetic distance, eigenvector, eigenvalue, clustering.

INTRODUCTION

Breeding for sustainable yield is dependent on availability and accessibility of intra-specific diversity of species. Since maintenance of sustainable and productive yield is the utmost challenge in crop production, adequate variability within a species would provide genetic buffer for their survival in the face of inclement conditions. Gotsch (1997) had remarked that the production capacity of cocoa could be greatly boosted by generating high quality hybrids for wider cultivation. The use of superior cocoa hybrids made a significant contribution to cocoa productivity in Brazil (Dias *et al*., 2003). High productivity per hectare of cocoa has been a long production target in Nigeria (Oyedokun *et al.*, 2011). Nigeria cocoa production would receive a boost in tonnage when high quality and superior hybrids replace the traditional cultivars in the farmers' plot.

The most recent challenge in agriculture is the global climatic and environmental changes. The change presently threatens and endangers the sustainable continuity and productivity of species with narrow genetic diversity. For cocoa germplasm in Nigeria, Aikpokpodion (2010) had observed significant genetic diversity in the cocoa genetic resources maintained in the field genebanks and farmers' fields. A good utilization of these available genetic resources in Nigeria could provide insurance against any present or future abiotic and biotic threat to cocoa cultivation and production.

Periodic quantification and evaluation of the diversity status of a germplasm is a necessary exercise. The essence is to update the breeders on the genetic resources of the germplasm and form a basis for the proposition of further and timely breeding programme. Diversity analysis based on the genomic profile of genotypes had been remarked to be more reliable in the characterization of cocoa (Sounigo *et al.*, 2005 and

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Aikpokpodion *et al.*, 2009) and other crops (Mohammad and Prasana, 2003). However, phenotypic traits with high heritability are still useful for germplasm characterization (Klug and Cummings, 1994; Sounigo *et al.* 1997). Lachenaud and Oliver (2005) and Bekele *et al.* (2006) approved of the use of such traits for classification of cocoa genotypes.

The Cocoa Research Institute of Nigeria (CRIN) had previously generated hybrids of cocoa from some selected parents in a breeding programme. The evaluation of the parents and progenies for some pod and bean values is necessary to understand variation among the two populations and to ascertain the level of success of the hybridization programme.

The present study utilized some pod and bean phenotypic descriptors for a multivariate characterization of two populations (parents and hybrids) to understand diversity within and between them. While the study produced an update on the diversity status of the forty cocoa genotypes studied (parent and hybrid inclusive), it additionally informs cocoa breeders of the inherent genetic potentials in the forty genotypes for subsequent selection and further improvement.

MATERIALS AND METHODS

This study was hinged on the need to understand the pattern of diversity within population and between different populations of cocoa genotypes for guided selection for subsequent improvement. Fifteen uniformly matured and ripe cocoa pods were selected and harvested from each genotype in the parental and hybrid plots. The parental and hybrid plots were experimentally laid out in randomized complete block design (RCBD) of six replications as Local Clone Trial (LCT) and Hybrid Trial (HT) respectively at CRIN, Ibadan. There were sixteen and twenty-four genotypes in the LCT and HT plots respectively. Table 1 has the list of the forty cocoa genotypes considered in the study.

Table 1: List of the genotypes.

S/N	Code	Pedigree
	Hybrids	
1	G1	T65/7 x T22/28
2	G2	T12/11 x N38
3	G3	T65/7 x T9/15
4	G4	PA150 x T60/887
5	G5	P7 x T60/887
6	G6	P7 x PA150
7	G7	T65/7 x T57/22
8	G8	T53/5 x N38
9	G9	T65/7 x N38
10	G10	T53/5 x T12/11
11	G11	T65/35 x T30/13
12	G12	T86/2 x T9/15
13	G13	T9/15 x T57/22
14	G14	F3 Amazon (Check)
15	G15	T86/2 x T22/28
16	G16	T82/27 x T12/11
17	G17	T86/2 x T16/17
18	G18	T65/7 x T53/8
19	G19	T65/7 x T101/15
20	G20	T86/2 x T53/8
21	G21	T86/2 x T65/35
22	G22	T101/15 x N38
23	G23	T82/27 x T16/17
24	G24	T86/2 x T57/22
	Local Clones	
25	G25	T12/5
26	G26	T57/22
27	G27	T82/27
28	G28	T22/28
29	G29	C77
30	G30	T30/13
31	G31	T12/11
32	G32	T53/8
33	G33	T65/35
34	G34	T16/17
35	G35	T9/15
36	G36	T65/7
37	G37	T53/5
38	G38	T101/15
39	G39	T86/2
40	G40	N38

The harvested pods were weighed and metric measures of the length and girth were taken. The pods were carefully broken and pod thickness was estimated as the difference between the outer (ridge to ridge) and the inner diameter of the fresh pod husk using the venier caliper. The number of beans per pod was counted and the weight of the beans was recorded per pod. Data were analysed using the statistical analysis system, SAS V. 9.2 (SAS Institute Inc., 2007). The data of the six parameters were subjected to analysis of variance (ANOVA), using PROC GLM in SAS. Phenotypic and genotypic coefficients of variation (PCV and GCV respectively) were calculated following the method of Singh and Chaudhary (1985). The contribution of the genetic component in the phenotypic coefficient of variation was calculated as the ratio of GCV to PCV.

The interrelationship among the six parameters was verified using the Pearson correlation coefficient method in SAS Analyst. Means were generated for each genotypes across the six replication for the six parameters; giving a 40 x 6 multivariate data matrix. The data was standardized (mean = 0; standard deviation = 1) to harmonize the various units of measurements among the parameters following the approach of Ofori *et al.* (2006). The data matrix was then subjected to Gower genetic distance (Gower, 1971) analysis. The resulting product was further subjected to Principal Component Analysis (PCA) and WARD clustering analysis. ANOVA was conducted to understand variability between and within the generated cluster groups.

RESULTS AND DISCUSSION

A wide range of differences existed among the forty cocoa genotypes for the six studied variables. For instance, minimum pod weight and the number of beans were 0.43Kg and 20 as against the highest 0.86Kg and 51 respectively (Table 2).

Table 2: Descriptive and variability statistics of the six phenotypic traits of the 40 cocoa genotypes.

Traits	Min.	Max.	Mean	Variance	GCV (%)	PCV (%)	GCV/PCV
Pod Weight (Kg)	0.43	0.86	0.50	0.118***	18.18	26.53	0.69
Pod Length (cm)	15.90	27.96	20.43	15.35***	33.39	44.03	0.76
Pod Girth (cm)	21.51	34.07	27.69	17.05***	30.40	39.19	0.78
Pod Thickness (cm)	1.26	5.71	3.52	3.15***	37.26	44.98	0.83
Bean wt./Pod (Kg)	0.02	0.42	0.12	0.007***	9.54	13.05	0.73
Beans/Pod	20.17	51.17	36.55	67.91***	47.31	85.96	0.55

NB: *** - Significance at $P < 0.001$, PCV – Phenotypic Coefficient of variation, GCV - Genotypic Coefficient of variation.

The proportion of the husk to the pod by weight ranged between 51 – 95%. The husk component of the pod for most of the genotypes was very huge. While a breeding programme could be proposed to lower the husk:pod ratio for higher economic yield, an effective utilization of the pod husk for local soap, organic fertilizer (through composting) etc. will save its wastage in farms. A plough back of the pod husk as fertilizer in the cocoa farms as suggested by Agbeniyi *et al*. (2011) would ameliorate the soil, improve subsequent yield and enhance farmer's net profit. G20, G19, G8, G10, G6, G23, G3 and G11 were the first eight genotypes with significant ($P<0.001$) higher bean weight per pod. All the other genotypes came behind G6, G1, G23, G16 and G12 with less than 40 beans per pod. Specifically, the ratio of the bean weight to the pod weight was above 41% in G20. The significant performance of the hybrids in this study re-emphasizes the outstanding role of crop breeding technique in generating variants (genotypes) with better performances than the parents in addition to increasing the plant genetic diversity and resources. National cocoa productivity in Nigeria would receive improvement, just like Brazil (Dias *et al*., 2003) if some of these hybrids are given wider adoption and utilization. Since the bean is the most economic part of cocoa (Oyedokun *et al*., 2011) selection focus on genotypes with higher bean weight will enhance greater production of cocoa.

Moreover, the six quantitative variables significantly ($P<0.001$) differentiated the forty cocoa genotypes. The six variables proved very important in discriminating among the forty genotypes; they would be very important phenotypic descriptors for classifying genotypes within the *Theobroma cacao* species. The PCV was generally higher than the GCV. The highest GCV (47.3%) and PCV (85.9%) occurred in the number of beans per pod while the least (9.5% and 13.1%) occurred in bean weight per pod. The ratio of the GCV to PCV ranged between 0.55 (number of beans per pod) to 0.83 (pod thickness). The percentage of the genetic component in the total phenotypic variation among the forty studied genotypes for the six traits was higher than 50%. The role of the environment in the phenotypic expression of the six traits was lower than the genetic counterpart. Selection of genotypes based on these traits may be reliable.

Table 3 presents the relationship by correlation between the six phenotypic traits. The weight of the pod exhibited highly significant ($P<0.001$) and positive correlation with pod length ($r = 0.509$), pod girth ($r = 0.536$) and bean weight per pod ($r = 0.403$). Positive and highly significant ($P<0.001$) correlation existed between pod length and girth (Table 3). The above relationships seem to stress that metric length and circumference of the pod are good determinants of pod weight. The positive relationship of the pod weight with its length, girth and the bean weight could facilitate simultaneous selection of traits; thus the selection of genotypes with heavier pods equally means a selection for genotypes with higher bean weight.

Table 3: Pearson Correlation coefficient of the six pod and bean values.

Traits	Pod Weight	Pod Length	Pod Girth	Pod Thickness	Bean wt./Pod
Pod Length	0.509***	-	-	-	-
Pod Girth	0.536***	0.574***	-	-	-
Pod Thickness	0.250	0.230	0.048	-	-
Bean wt./Pod	0.403**	0.243	0.254	0.044	-
Beans/Pod	0.195	0.259	0.188	0.122	0.175

NB: ** and *** - Significance at P ≤ 0.01 and < 0.001.

Table 4: Summary of the minimum, mean and maximum Gower genetic distances among the sixteen parents, twenty-four hybrids and the forty genotypes combined.

	Gower distances	Linked genotypes
Twenty-four hybrids		
Mean	0.345	
Minimum	0.032	G9 and G14
Maximum	0.450	G6 and G17
Sixteen parental genotypes		
Mean	0.210	
Minimum	0.023	G25 and G30
Maximum	0.527	G34 and G35
Forty genotypes		
Mean	0.228	
Minimum	0.023	G25 and G30
Maximum	0.529	G17 and G35

The summary of the genetic distances among the 40 combined genotypes are presented in Table 4. The hybrids had the highest mean genetic distance of 0.345 but the sixteen parental clones had a mean Gower genetic distance of 0.210. The genetic distance among the parents was narrower compared to that of the hybrids. The justification for wider genetic distance among the hybrids could be due to the heterozygous nature of hybrids. Bartley (2005) noted that populations that are products of recombination usually have greater genetic diversity. The cocoa hybrids are new genetic variants; therefore, they add to the quantity of the genetic resources of the Nigerian cocoa germplasm. However, G9 (T65/7 x N38) and G14 (F3 Amazon) had the highest similarity (1-d; where d = genetic distance) of 0.968. The genetic base of G9 is Amazon/Amelonado. Both genotypes had Amazonian genetic base (though in varied proportion). However, cytoplasmic inheritance could be implicated for the high genetic similarity between them. G6 (P7 x PA150) and G17 (T86/2 x T16/17) were farthest apart among the hybrids; this is reasonable because none of the two progenies had a common parent. Among the parental clones, G25 (T12/5) and G30 (T30/13) had the highest similarity; this could be explained by the fact that both were from the Amazonian primary genetic resource. In the whole study the highest genetic distance was between T86/2 x T16/17 (a hybrid) and T9/15 (a parental clone).

From Table 5, the total genetic variation among the 40 cocoa genotypes was accounted for by the six PC-axes, with variance proportions ranging from 41.3% (PC1) to 6.2% (PC6). The eigenvalues for each axes followed the descending trend as the variance proportion. Each of the six traits had higher (> 0.30) but varied magnitude of vector loading in the first three PC-axes which accounted for about 73% of the total genetic variation among the forty cocoa genotypes. By the magnitude of the eigenvector loadings, pod weight (0.52), length (0.50) and girth (0.49) were most prominent in PC1. Pod thickness and number of beans per pod had the highest loading in PC2 and PC3 respectively. Bean weight per pod had the highest vector loading (0.77) in PC4 (Table 5). The six phenotypic traits were very valuable in describing and distinguishing the forty cocoa genotypes.

The WARD clustering technique grouped the forty cocoa genotypes as revealed in Figure 1. Three main groups or clusters evolved at the 0.1 inflection point or similarity coefficient. The number of genotypes grouped within the three clusters was 22, 7 and 11 respectively. The grouping which was irrespective of the population types indicated that uniformity within each of the population is independent of any of the six studied variables. The six pod and bean traits could not separate/demarcate the parental clones from the hybrids; this seem to justify that both population are components within the single *Theobroma cacao* species entity. It further informs that each genotype (parent or hybrid) have different inherent genetic potentials for the six studied parameters. Our observation in this study is strongly supported by report of Cacaonet (2012) which stated that cacao genetic resources comprise wide range of genetic variability. The three clusters differ significantly (P<0.001) from each other for the six quantitative characters (Table 6).

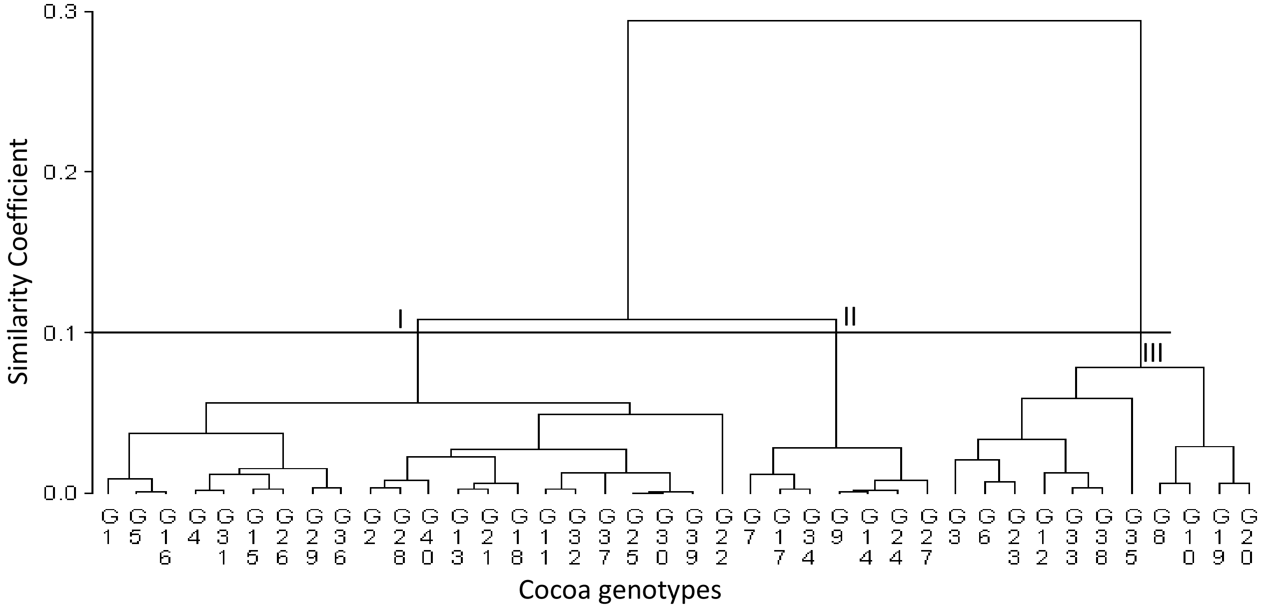

Figure 1: Dendrogram showing the grouping of the forty cocoa genotypes.

Table 5: Eigenvalues, variance proportions and Eigenvectors showing the prominence of each trait to each PC axes.

PC-Axes	Eigenvalue	Variance Proportion	Cumulative	Eigenvectors of the six phenotypic traits					
				PW	PL	PG	PT	BW	BP
PC1	2.48	41.31	41.31	0.52	0.5	0.49	0.21	0.35	0.28
PC2	1.00	16.63	57.94	-0.04	0.04	-0.28	0.87	-0.33	0.22
PC3	0.89	14.76	72.71	-0.19	-0.17	-0.23	-0.19	0.27	0.88
PC4	0.82	13.68	86.39	0.17	-0.31	-0.36	0.27	0.77	-0.29
PC5	0.45	7.42	93.81	-0.68	0.65	-0.12	0.05	0.3	-0.13
PC6	0.37	6.19	100	-	-	-	-	-	-

NB: PC – Principal Components, PW - Pod Weight, PL - Pod Length, PG - Pod Girth, PT - Pod Thickness, BW - Bean wt./Pod and BP - Beans/Pod.

Pod girth and number of beans per pod clearly and significantly (P<0.001) separated the forty cocoa genotypes to three clusters. Pod weight, its length and bean weight per pod could not differentiate the 29 genotypes in clusters I and II; meaning that the two clusters are similar with respect to the three phenotypic traits. Moreover, any variation in the thickness of the pod among the 18 genotypes in clusters I and III could be due to chance since it did not significantly (P<0.05) differentiates the genotypes in the two clusters. Therefore, pod thickness cannot be used to partition genotypes within the two clusters (Table 6). The very highly significant (P<0.001) variation observed among the forty genotypes especially for the number of beans per pod (an economic trait) depicts a unique inherent genetic potential within the tested population for enhanced higher productivity in cocoa.

Table 7 presents the measures of variability within each of the three clusters. The mean values for the six variables was least in cluster I and highest in cluster III. Selection of individual genotypes from these two extreme clusters (i.e. I and III) for a hybridization programme could lead to raising progenies with different genetic constitution. Such hybridization success will lead to increase in the genetic diversity and resources in cocoa.

The selection of genotypes within cluster III for wide cultivation could be a short term breeding programme for higher cocoa productivity in Nigeria, since the genotypes in the cluster had heavier pod and bean weight. Highly significant (P≤0.01) variation existed among the 22 and 11 genotypes in clusters I and III for the six variables (Table 7). The six variables characteristically differentiated the grouped genotypes in the two clusters. The non-significant variation exhibited by the seven genotypes in cluster II for pod weight, pod thickness and number of beans per pod depicted that the three traits are good indices for ascertaining uniformity within the cluster and similarity among the genotypes. However, pod length girth and bean weight per pod differentiated among the seven genotypes in the cluster.

Table 6: Inter-Cluster variability as revealed by paired cluster comparison across the six phenotypic traits.

Phenotypic Traits		Cluster 1	Cluster 2
Pod Weight (Kg)	Cluster 2	Ns	
	Cluster 3	***	***
		Cluster 1	Cluster 2
Pod Length (cm)	Cluster 2	Ns	
	Cluster 3	***	***
		Cluster 1	Cluster 2
Pod Girth (cm)	Cluster 2	***	
	Cluster 3	***	***
		Cluster 1	Cluster 2
Pod Thickness (cm)	Cluster 2	***	
	Cluster 3	Ns	***
		Cluster 1	Cluster 2
Bean wt./Pod (Kg)	Cluster 2	Ns	
	Cluster 3	***	***
		Cluster 1	Cluster 2
Beans/Pod	Cluster 2	***	
	Cluster 3	***	***

NB: *** - Significance comparison at P < 0.001, ns – Non significant comparison.

Table 7: Intra-cluster variability of the six phenotypic traits.

Phenotypic Traits	**Cluster I**		**Cluster II**		**Cluster III**	
	Mean	Variance	Mean	Variance	Mean	Variance
Pod Weight (Kg)	0.45	0.09***	0.49	0.03	0.62	0.13***
Pod Length (cm)	19.73	6.37***	19.82	4.64**	22.19	15.35***
Pod Girth (cm)	26.81	9.93***	27.86	15.76***	29.40	6.97**
Pod Thickness (cm)	3.75	1.19***	2.35	0.57	3.76	2.79***
Bean wt./Pod (Kg)	0.097	0.003***	0.11	0.0006**	0.15	0.01**
Beans/Pod	36.18	45.70**	33.91	25.07	39.97	83.48***

NB: ** and *** - Significance at P ≤ 0.01 and < 0.001

The forty genotypes have unique genetic constitution; they differ from each other in the phenotypic expression of the six traits. The general rule is: cluster formation depends on similarities among sets of genotypes for specific phenotypic trait. However in this study, diversity existed within the same group of genotypes. Therefore, genotypic uniformity may be dependent on the number of genotypes forming a cluster. Our study noted that the cluster with smaller sample size of genotypes displayed higher uniformity while those with larger sample size exhibited significant (P≤0.01) intra-cluster variability. Inherent genetic potential is feasible within the three clusters for subsequent breeding programme. Wider genetic variability provides raw material for breeding new and improved varieties to achieve a more economically sustainable cocoa production system (Cacaonet, 2012). The observed diversity in this study informs of the possibility for selection of genetic materials for further breeding programme. As a follow up to the earlier remark of Aikpokpodion (2010), there lies a very high diversity within the genetic resources of Nigerian cocoa.

REFERENCES

Agbeniyi, S.O., K.A. Oluyole and M.O. Ogunlade 2011. Impact of cocoa pod husk fertilizer on cocoa production in Nigeria. World J. Agric. Sci. 7:113–116.

Aikpokpodion, P.O., J.C. Motamayor, V.O. Adetimirin, Y. Adu-Ampomah, I. Ingelbrecht, A.B. Eskes, R.J. Schnell and M. Kolesniova-Allen. 2009. Genetic diversity assessment of sub-samples of cacao, *Theobroma cacao L*. collections in West Africa using simple sequence repeats marker. Tree Genet. Geno. 5:699 – 711.

Aikpokpodion, P.O. 2010. Variation in agro-morphological characteristics of cacao, *Theobroma cacao* L., in farmers' fields in Nigeria. New Zeal. J. Crop Horti. Sci. 38:157-170.

Bartley, B.G.D. 2005. The Genetic diversity of Cacao and its utilization. CABI Publishing, Wallingford, UK.

Bekele, F.L., I. Bekele, D.R. Butler and G.G. Bidaisee. 2006. Patterns of morphological variation in a sample of cacao (*Theobroma cacao* L.) germplasm from the International Cocoa Genebank, Trinidad. Genet. Res. Crop Evol. 53:933 – 948.

CacaoNet. 2012. A Global Strategy for the Conservation and Use of Cacao Genetic Resources, as the Foundation for a Sustainable Cocoa Economy. Bioversity International, Rome.

Dias, L.A.S., J. Marita, C.D. Cruz, E.G. Barros and T.M.F. Salomao. 2003. Genetic distances and its association with heterosis in Cocoa. Braz. Arch. Bio. Tech., 46:339-347.

Gotsch, N. 1997. Cocoa biotechnology: status, constraints and future prospects. Biotech. Adv. 5:333-352.

Gower, J.C. 1971. A general coefficient of similarity and some of its properties. Biomet. 27:623–637.

Klug, W.S. and M.R. Cummings. 1994. Concepts of Genetics. Macmillan College Publishing Company, USA.

Lacheaud, P.H. and G. Oliver. 2005. Variability and selection for morphological bean traits in wild cacao trees (*Theobroma cacao* L.) from French Guiana. Genet. Res. Crop Evol. 52:225 -231.

Mohammadi, S.A. and B.M. Prasanna. 2003. Analysis of genetic diversity in crop plants-salient statistical tools and considerations. Crop Sci. 43:1235-1248.

Ofori, K., F.K. Kumaga and A. Tonyigah. 2006. Morphological characterization and agronomic evaluation of Bambara groundnut (*Vigna subterranean* (L.) verdc.) germplasm in Ghana. Plant Genet. Res. Newsl. 145:23-28.

Oyedokun, A.V., A. A. Omoloye, B.D. Adewale, O. O. Adeigbe, O. O. Adenuga and P. O. Aikpokpodion. 2011. Phenotypic variability and diversity analysis of bean traits of some Cocoa hybrids in Nigeria. Asian J. Agric. Sci. 3:127-131.

SAS Institute. 2007. The SAS system for Windows. Release 9.2. SAS. Inst., Gary, NC, USA.

Singh, R.K. and S.D. Chaudhary. 1985. Biometrical methods in quantitative genetic analysis. Kalyan Publishers, New Delhi.

Sounigo, O., F. Bekele, G. Bidaisee, Y. Christopher and R. Umaharan. 1997. Comparison between genetic diversity data obtained from morphological, biochemical and molecular studies. In: Cocoa Research Unit Report for 1997. St Augustine, Trinidad, University of West Indies.

Sounigo, O., R. Umaharan, Y. Christopher, A. Sankar and S. Ramdahin. 2005. Assessing the genetic diversity in the International Cocoa Genebank, Trinidad (ICGT) using isozyme electrophoresis and RAPD. Genet. Res. Crop Evol. 52:1111–1120.