Original Article

Genetic distance and Diversity among some Cowpea (*Vigna unguiculata* L. Walp) genotypes

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Abstract

Diversity analysis has been a fundamental protocol for crop improvement; it unravels the genetic potentials of genotypes. Nine breeding lines of cowpea and a common cowpea cultivar were evaluated in a randomized complete block design. The phenotypic traits measured includes: plant height at 4 weeks, number of pods per plant, pod length, number of days to first flower, number of days to first ripe pod, number of peduncles per plant, peduncle length and number of branches per plant. The ten cowpea differed significantly ($P \leq 0.001$) from each other for the eight phenotypic traits. 24881-2 significantly ($P \leq 0.05$) produced more pods than Ife-brown. However, days to first flowering and pod ripening were significantly ($P \leq 0.05$) lower in Ife-brown than 24881-2. The mean Euclidean distance between the genotypes was 3.7479, the least (0.9494) was between 25016-2 and 24901-1 and the highest (6.4590) was between 24884-1 and 24881-2. The coefficient of variation for the phenotypic traits ranged between 5.51(days to first pod ripening) and 105. 63 (number of branches per pod). The first three principal component axes explained 80% of the total variation. Two groups of genotypes distinctly evolved by the principal component analysis. Group I had genotypes with high pod productivity but late flowering and ripening. The genotypes in group II flowered and produced ripen pods much earlier. Selection of genotypes within the groups could facilitate a breeding programme for highly productive and early maturing cowpea genotypes.

Key words: Euclidean distance, genetic divergence, principal component analysis, genotypes, breeding

Introduction

Successful conservation of germplasm largely depends on the understanding of the diversity within the species (Karuri *et al*., 2010). Germplasm with wider genetic base provides buffer and resilience against climatic and other environmental changes and ensures sustainable food security. The presence of genetic variability among crop genotypes depicts the richness of the genepool and assures plant breeders of the possibilities of combating subsequent food security crisis for the crop. Moreover, understanding the nearness and diverseness of genotypes within the same species is an important primary knowledge to guide selection and improvement in a breeding programme. In essence, diversity leads to the identification of superior genotypes which may be recommended as cultivar and identification of genotypes with desirable phenotypic traits for selection as parents for eventual breeding and improvement programmes.

Cowpea comes first ahead of other arable food legumes in the sub-Sahara Africa. The recent annual global production of cowpea approximates 3.3 million tons; Central and West Africa region are the major areas of its cultivation (CGIAR, 2011). While it is chiefly a vegetable and grain crop for human who values it as a nutritional supplement to cereals and an extender of animal proteins, it provides a very safe fodder for livestock animals. Cowpea has vast utility in the food culture of both man and animal (Tarawali *et al*., 2002; Diouf and Hilu, 2005; Fang *et al.*, 20
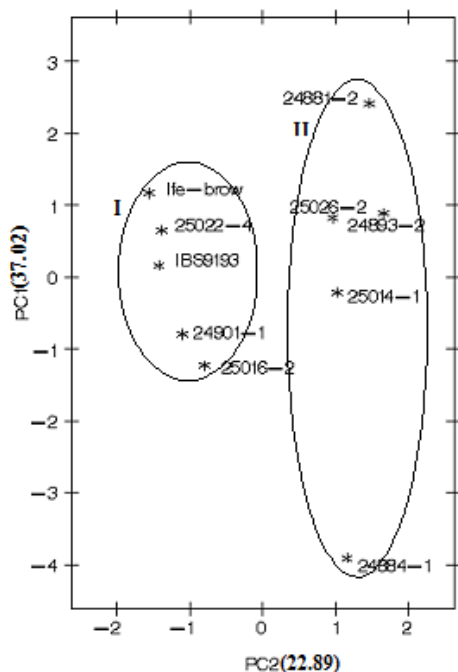
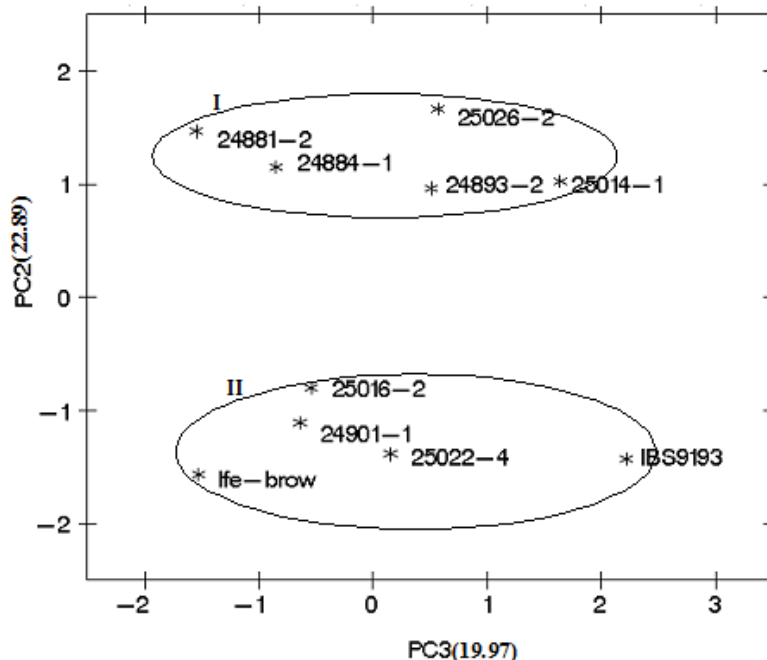Figure 1: The spatial figuration of the ten cowpea genotypes by the principal component axes 1 and 2.

Figure 2: The spatial figuration of the ten cowpea genotypes by the principal component axes 2 and 3.

Porter *et al*. (1974) reported that morphological variability in cowpeas (*Vigna unguiculata* L. Walp) abounds in the tropics. Poor concern for available but unutilized cowpea variants may promote the losses of diversity in the species. Consequently, narrow genetic base of germplasm may increase the potential for vulnerability to biotic and abiotic stress. Genetic distance between pair of genotypes offers the basis for understanding the structure of the diversity of any intra-species population. It constructs an all-to-all matrix to describe the distance between each sequence pair of genotypes; thereby guiding plant breeder in their selection procedure. Euclidean distance was remarked by Hoque and Rahman (2007) as a suitable method of measuring distances between would be parents.

Hybridization is a unique way of increasing variation within a species; resultant offspring adds to the genetic diversity within the germplasm. Phenotypic evaluation of genotypes for morphological classification remains the only tool for discovering genetic variability within a population, where higher-precision diversity analysis techniques are not within the reach of the scientist.

The objective of the present study is to understanding the genetic identity of the ten cowpea genotypes via morphological characterization method with the aim of using their breeding values for subsequent breeding programme. Furthermore, the study aimed at understanding the relatedness and diverseness among the genotypes through genetic distance.

Materials and Methods

Nine breeding lines of cowpea from the University of Ibadan, Nigeria and one improved variety from the Obafemi Awolowo University, Ile Ife, Nigeria were evaluated for some phenotypic characteristics. Some passport and descriptive information of the ten cowpea genotypes is presented in Table 1.

Two seeds of the ten cowpea genotypes were planted per hill in a spacing of 20cm by 60cm. The experiment contained a row plot of 15 stands per genotype laid out in a randomized complete block design of three replications. Weeding was done as at when due and Monocrotophos (Nuvacron) was used to control insect pests at an interval of two weeks after planting to harvest.

Data on eight quantitative traits were collected from the five plants within the row. The measured traits includes: plant height at four weeks after planting, number of pods per plant, pod length, number of days from planting to first flowering, number of days from planting to first ripe pod, number of peduncles per plant, peduncle length and number of branches per plant. Multivariate Analysis of Variance (MANOVA) by PROC GLM was performed on the eight quantitative variables to understand the variability among the genotypes using Statistical Analysis System, SAS-V9.2 (SAS/STAT user's guide 2007). The mean performance of each genotype for each variable was estimated to produce

Table 1: Basic passport and some quantitative descriptive information on the ten Cowpea genotypes

Genotypes	Passport Information			Some quantitative descriptors							
	Testa colour	Testa texture	Sources	PH4W	PodPP	PL	DFF	DPRP	PDPP	PDL	BPP
24881-2	Brown	Smooth	UI	21.33	17.33	13.20	49.33	64.22	10.11	24.83	2.44
24884-1	Tan brown	Rough	UI	29.33	2.67	16.17	56.00	72.44	6.67	14.17	0.78
24893-2	Tan brown	Rough	UI	25.78	9.56	17.33	44.56	64.44	9.89	23.19	1.67
24901-1	Light brown with dark brown patches	Rough	UI	26.56	8.11	14.66	46.78	63.33	7.56	15.07	0.44
25014-1	Brown	Rough	UI	36.11	11.56	17.56	47.78	64.56	11.22	16.70	0.44
25016-2	Tan brown	Rough	UI	23.22	8.00	15.56	47.11	64.78	7.78	14.01	0.11
25022-4	Tan brown	Rough	UI	23.00	10.33	15.93	43.44	60.00	8.11	20.32	0.33
25026-2	Light brown	Rough	UI	27.67	13.33	17.22	47.22	66.44	9.89	24.02	1.67
IBS9193	Brown	Rough	UI	47.56	11.00	15.21	46.89	62.00	7.00	28.26	0.00
Ife-brown	Light brown	Rough	OAU	20.44	10.78	13.37	44.33	59.56	7.22	19.64	1.33

* UI – University of Ibadan, Ibadan, Nigeria; OAU – Obafemi Awolowo University, Ile-Ife, Nigeria
* PH4W - Plant height at 4 weeks, PodPP - Number of pods per plant, PL - Pod length, DFF - Number of days from planting to first flower, DPRP - Number of days from planting to first ripe pod, PDPP - Number of peduncles per plant, PDL - Peduncle length and BPP - Number of branches per plant

Table 2: Measures of variability among the eight quantitative characters

Phenotypic variables	ANOVA			Eigenvectors in each PC-axis		
	Mean	CV (%)	P-values	PC1	PC2	PC3
PH4W	28.10	18.05	<0.0001	-0.1237	-0.0477	0.6683
PodPP	10.27	40.58	<0.0001	0.5331	0.1532	0.0451
PL	15.62	13.08	<0.0001	-0.1751	0.3191	0.5141
DFF	47.34	7.58	<0.0001	-0.3935	0.3792	-0.1524
DPRP	64.18	5.51	<0.0001	-0.3997	0.5116	-0.0621
PDPP	8.54	28.85	<0.0001	0.2958	0.4970	0.2004
PDL	20.02	18.46	0.0010	0.4058	0.0642	0.2974
BPP	0.92	105.63	<0.0001	0.3207	0.4643	-0.3623

* PH4W - Plant height at 4 weeks, PodPP - Number of pods per plant, PL - Pod length, DFF - Number of days from planting to first flower, DPRP - Number of days from planting to first ripe pod, PDPP - Number of peduncles per plant, PDL - Peduncle length and BPP - Number of branches per plant
ANOVA - Analysis of Variance, CV – Coefficient of variation, PC-axis - Principal Component axes, PC1, 2 and 3 – Principal Component axes 1, 2 and 3,
*Eigen vectors ≤ 0.2 are significant

Table 3: Proportion of the total variation explained by the different Principal Component axes

PC-Axes	Eigenvalue	Variance (%)	Cumulative variance (%)
1	2.96	37.02	37.02
2	1.83	22.89	59.91
3	1.59	19.97	79.87
4	1.10	13.80	93.67
5	0.40	5.02	98.69
6	0.07	0.94	99.64
7	0.01	0.23	99.87
8	0.01	0.13	100

Table 4: GD –Euclidian distance between the ten cowpea genotypes

Genotypes	24881-2	24884-1	24893-2	24901-1	25014-1	25016-2	25022-4	25026-2	IBS9193
24884-1	6.4590								
24893-2	3.7990	5.2972							
24901-1	4.5018	4.0992	3.2742						
25014-1	4.7826	5.0799	2.7231	3.3622					
25016-2	4.8928	3.8320	3.3106	0.9494	3.1953				
25022-4	4.4554	5.6423	2.6244	2.0477	3.3546	2.2318			
25026-2	3.1780	5.1546	1.3950	3.6149	2.6029	3.6224	3.2378		
IBS9193	5.3731	5.8380	4.3044	3.8493	4.1941	4.3018	3.6954	4.2084	
Ife-brown	3.5977	5.8634	3.5184	2.3231	4.6644	3.0001	2.1825	3.9303	4.3590

a 10 x 8 multivariate data matrix. Genetic divergence among the genotypes was measured by the Euclidian distance method of Cruz and Regazzi (1994) as cited by Oliveira and Valls (2003). The Euclidian distance between two genotypes x and y was calculated as:

$$dxy = \Sigma((xk-yk)^2)^{0.5}$$

where;

dxy – Euclidean distance

xk – performance of x parent for kth character

yk - performance of y parent for kth character

The distance was further subjected to principal component analysis using PRINCOMP in SAS. Coordinates were obtained for the first three PC axes and two-dimensional figures were therefore produced with two PC-axes in each case by the % *plotit* procedure in SAS.

RESULTS

Among the ten studied genotypes of cowpea, days to first flowering and ripening of pods occurred latest in 24884-1 (Table 1). However, 25022-4 reached first flowering at 43 days after planting while 25022-4 and Ife-brown produced the first ripened pod at 60 days after planting. 24881-2 had the highest number of branches and the highest number of pods per plant. 25014-1 had the longest pod and the highest number of peduncles per plant. The highest height of plant at 4weeks after planting and the longest peduncle length was obtained in IBS9193. 24884-1 had the least number of peduncles and pods per plant. IBS9193 which had the highest plant height at four weeks did not produce any branch (Table 1). Across the ten genotypes the least plant height at four weeks (20.44cm) was obtained in Ife-brown.

Considering the eight variables in this study, the ten genotypes differs significantly (P≤0.001) from each other (Table 2). The mean height of plant at four weeks after planting was 28.1cm and mean pod length for the ten genotypes was 15.6cm. Mean number of pods per plant was about 10 and the mean number of days to first flowering and pod ripening were 47 and 64 days respectively. From this study, first pod ripened within seventeen day from first flowering (Table 2). The average number of peduncles per plant was about 9 with an average length of 20.02 cm. In this experiment, the least coefficient of variation (5.51) was from days to first pod ripening, and highest (105. 63) from number of branches per pod (Table 2).

The proportion of the contribution of each variable to determining the total variation within each Principal Component (PC) axis was also shown in Table 2. PDPP and BPP among other variables had significant (Eigenvector ≥ 0.2) contribution to determining variation within each of the three PC-axes. Six of the quantitative characteristics were significant (Eigenvector ≥ 0.2) in their contribution to the total variation in PC1. The dimension of the contribution of DFF and DPRP to the total variation in PC1 was negative. The five phenotypic trait of significance (Eigenvector ≥ 0.2) in PC2 contributed to the total variation positively. Within PC3, five phenotypic traits were significant (Eigenvector ≥ 0.2), among others BPP's contribution to the total variation was negative (Table 2).

Eight PC-axes with varied proportions contributed to explaining the total variation among the ten cowpea genotypes. The cumulative proportion of variation explained by the first three PC-axes was 80%, the remaining five axes explained just about 20% of the total variation (Table 3).

Table 4 presents the Euclidian distance among the ten cowpea genotypes. The range of distance was between 0.9494 and 6.4590 with the mean of 3.7479. The most similar genotypes in this study were 24901-1 and 25016-2. The widest Euclidean distance in this study was between 24881-2 and 24884-1.

The relationship between the ten cowpea genotypes was revealed by Fig. 1. PC1 and PC2 which formed the component of the figure explained 59.91% of the total variation. The zero (0) on PC axis 2 separated the ten genotypes to two groups of equal population. Genotypes in group I included Ife-brown, 25022-4, IBS9193, 24901-1 and 25016-2, while 24881-2, 25026-2, 24893-2, 25014-1 and 24884-1 which lies westward formed group II. 24884-1 was farther apart from other genotypes in group II (Fig. 1).

24881-2 and 24884-1 were the most diverse genotypes within the group; each had the extreme value for number of pods per plant (Fig. 1; Table 1). The description of the ten cowpea genotypes by PC 2 and PC 3 (Fig. 2) was based on 43% explanation of the total variation. The separation pattern of the ten genotypes into two major groups of equal population in Fig. 2 was same as Fig. 1. However, while the grouping was eastward and westward in Fig. 1, the separation was northward and southward in Fig. 2. The description of the relationship and diversity of the ten genotypes by PC1 and PC3 (Figure not shown) was similar to the description by PC1 and PC2.

Diversity among genotypes within group I (Fig. 1) was narrower compared to group II (Fig. 1). Euclidean distance within the two groups in Fig. 1 was 3.1920 and 3.5984 respectively. PH4W and PDL were the discriminatory traits for genotypes within group I (Fig. 1). Genotypes with the extreme values for the two traits existed in the group. The phenotypic variables determining the degree of diversity in group II (Fig. 1) were: PodPP, PL and PDPP; their respective CV were: 40.58, 13.08 and 28.85 (Table 2). Moreover, genotypes with the extreme values for the three traits existed within the group.

The zero (0) coordinate on PC 2 (Figures 1 and 2) separated genotypes with earlier days to flowering and pod ripening (group II; Fig. 1) from those which produces flowers later and whose pod ripens much later (group I; Fig. 1).

DISCUSSION

Phenotypic variation was evident among the ten cowpea genotypes. All the eight quantitative variables had relative importance in determining their variations but none of them could identify any genotype as a duplicate of the other. High variability among cowpea genotypes based on phenotypic characters has been reported by some authors (Aremu *et al.*, 2007; Adewale *et al.*, 2010). Specific variation which differentiates genotypes with respect to some phenotypic characters may have ensued from natural and environmental mutations of the phenotypic traits. Morphological plasticity as reported by Austin (1997), cited by Karuri *et al*. (2010) stated that the variation in the morphological characters of genotypes which are phenotypically similar is due to genetic differentiation especially in the presence of varying conditions and parallel evolution.

The significant performance of some of the cowpea lines (for some phenotypic traits e.g. number of pods per plant and pod length) over Ife-brown in this study indicates that cowpea breeding and improvement programme is advancing in Nigeria. However, the trait for earliness in Ife-brown is of high significance; further breeding programme could aim at incorporating this characteristic in high yielding cowpea genotypes.

Number of pods per plant is one of the phenotypic traits with high contributions to the genetic variability in legumes e.g. grass pea (*Lathyrus sativus* L.) (Turk et al., 2007), pigeon pea (*Cajanus cajan* L.) (Upadhyaya et al., 2007) black gram (*Vigna mungo* L.) (Singh et al., 2010). The same trait was implicated in this study along with two vegetative traits: height of the plant at 4 weeks and number of branches per plant. However, days to first flowering and ripening of pod were most significant in unifying the ten genotypes. 24901-1 and 25016-2, the most similar genotypes had an Euclidean distance of 0.9494; their similarity was further supported by the same source of collection and testa texture. Interestingly, the presence of the dark brown patches on the seeds of 24901-1 significantly and morphologically differentiated it from 25016-2. Assessment of the diversity of the two genotypes by molecular techniques may present a different pattern of variation.

Genetic distance and proximity of genotypes within a population for different characters of any crop are very important to determine their phylogenetic relationship and their evolutionary pattern (Hoque and Rahman, 2007). The mean Euclidean distance of 3.7479 in the present study indicates that very high genetic divergence exist among the ten cowpea genotypes. This wide differentiation signifies the existence of high heterogeneity within the population.

Genotypes in group II had higher number of branches and produced more pods; this seems to indicate a link between the vegetative and the productive stage in cowpea (Vidya, 2002). The ten cowpea genotypes in this study could therefore be presented as parents for concerted breeding and improvement programme on cowpea for some of the studied traits. For instance, 24881-2 with high pod productivity in group II and 25022-4 and or Ife-brown with earlier days to reaching first flowering and pod ripening in group I could be selected for hybridization programme. Resultant progenies from such breeding programme could combine higher pod productivity with early maturity.

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