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EXPRESSION OF TRAITS IN COWPEA (*VIGNA UNGUICULATA* L. WALP) AS REVEALED BY CLUSTER ANALYSIS

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Abstract

In plant improvement programs, knowledge of germplasm diversity and genetic relationships among breeding materials cannot be over emphasized. Cluster analysis has been an important multivariate statistical tool used by many researchers for diversity studies; it has been found effective, irrespective of the data set involved in the study. The genetic variability among 10 genotypes of cowpea was assessed based on the expression of the quantitative and qualitative traits. Quantitative traits revealed a dendrogram which grouped genotypes into two major clusters, A and B, with cluster A having two (2) sub clusters (1 and 2). Cluster B had no sub-cluster. Sub-cluster 1 of A had one (1) genotype while sub-cluster 2 of A had only five (5) genotypes. Cluster B had four (4) genotypes. Qualitative traits revealed a dendrogram which also grouped genotypes into two major clusters (A and B). Cluster A had two sub-clusters (1 and 2); with sub-cluster 1 having two (2) genotypes, sub-cluster 2 having five (5) genotypes. Cluster B had only three (3) genotypes. All quantitative traits had very high level of contribution to the total variability observed among the genotypes, making these characters important for discriminating among genotypes of cowpea. Moreover, for any breeding work to be successful in cowpea, these characters must be considered. The level of variations showed by qualitative traits was very high, as ten traits out of thirteen studied were able to discriminate effectively among the genotypes studied, while the level of similarity for phenotypic expressions among the rest of the traits was very high. This study identified genotypes TVu3, TVu5, TVu4 and TVu7 for further genetic studies based on high number of seeds per plant. Furthermore, the choice of cluster analysis as a tool for this work proved to be a wise decision, as dendrograms were able to reveal similarities and differences among the tested genotypes.

Keywords: *Vigna unguiculata*, dendrogram, cluster analysis, genotypes, germplasm, diversity

Introduction

Cowpea (*Vigna unguiculata* L. Walp) is an important grain legume widely distributed in the tropical and subtropical regions of the world (Ajayi and Adesoye, 2013); where it serves as a source of protein for both urban and rural populations (Uarrotta, 2010). It is widely cultivated in Nigeria and also regarded as being very affordable source of protein. It has protein content of approximately 29% and has potential to fix atmospheric nitrogen for soil improvement (Adetiloye et al., 2013). Extensive improvements have been done on this crop by International Institute of Tropical Agriculture (IITA) in collaboration with other research stations which has led to distribution of varieties expressing combination of desired traits in more than 65 countries in the world (Fatokun et al., 2002; Ajayi and Adesoye, 2013). Still, the production of the crop is hindered by low grain yields, grain quality and susceptibility to pests and diseases, and lack of improved cultivars (Magloire, 2005). Inadequate knowledge of the genetic diversity of the existing germplasm has made improvement of the crop to be very slow. The fact that the crop is being required on varietal basis in terms of many traits, which are region specific, has made breeding program for cowpea improvement difficult, even though it is a single crop species (Magloire, 2005).

Before any crop improvement program can be taken up, in any cowpea; in order to obtain maximum benefits from selection procedures, plant breeders must be able to identify and manipulate a combination of traits that positively enhance grain yield in cowpea (Oladejo et al., 2011). A thorough knowledge concerning the level of genetic diversity existing within the species for various traits is very important. Information on the nature and magnitude of diversity in the existing plant materials and association of among various traits is a pre-requisite for yield improvement (Ajayi and Adesoye, 2013). Germplasm collection and diversity records are therefore highly desirable for a successful plant breeding program (Knotova, 2010).

Diversity is traditionally estimated by measuring variation in phenotypic or qualitative traits such as flower colour, growth habit, or quantitative agronomic traits such as yield potential, stress tolerance (Kameswara, 2004). Diversity has been used as a powerful tool in the classification of cultivars and also to study taxonomic status based on morphological traits in cowpea. However, morphological traits are greatly influenced by environmental factors, which may result in varying relationship patterns (Selvi et al., 2003), in spite of this, they are still effective for selection (Magloire, 2005).

9	TVU9252	TVu6
10	TVU11979	TVu7

The 10 cowpea genotypes were evaluated in a field laid out in a Randomized Complete Block Design with three (3) replications. Each replicate consisted of 10 row plots of 10 genotypes, each of which was regarded as a treatment, with 1m distance between treatments. Each treatment consisted of 30 plants, and the total number of plants on the field was 900. Each treatment was sown at a spacing of 30cm between plants and 50cm between rows with planting depth being approximately 2.5 cm. Weeding of the experimental field was done as when required to keep the weed pressure low. Sniper and Cypermethrin (cyperform) were used at different growth stages to control insect pests at appropriate time.

Data were collected from 10 randomly selected plants and their means were recorded for all observations. Ten quantitative traits and thirteen qualitative traits were measured according to International Board for Plant Genetic Resources (IBPGR), cowpea descriptors. The qualitative traits (collected at 8 weeks after planting) are as shown in table 2. The quantitative traits measured are: Plant height (cm), number of main branches, and number of nodes on main stem, number of leaves per plant (at 8 weeks after planting), number of days to flowering, number of pods per peduncle, number of pods per plant, seeds per pod, seeds per plant and 100-seed weight (g) (at maturity).

Diversity study is a process which involves the use of specific a method or combinations of methods for analysis of variation among individuals or groups of individuals or populations. Generated data often involves numerical measurements and sometimes, combinations of different types of variables. Morphological data, pedigree data, biochemical data, storage protein data and more recently, DNA data have been used by researchers to analyze genetic diversity in crop plants; with each of these data giving different types of information. Still, choice of data set to be used and analytical methods must depend on the objectives of the study, the resources and technological infrastructure available as well as the time constraints (Mohammadi and Prasanna, 2003). With advances in crop improvement programs, as a result of increases in the sample sizes of breeding materials and germplasm accessions; methods to classify and order genetic variations are assuming considerable importance. The use of multivariate statistical tool is a desirable strategy for germplasm classification and for analyzing variability among large breeding materials. Among the multivariate analytical tools, with ability to analyze multiple measurements on each individual involved, irrespective of the available data set; and widely used is the cluster analysis technique (Mohammadi and Prasanna 2003). Cluster analysis groups individuals based on traits they possess; and hence, members mathematically combined in the same cluster are individuals with the same or similar descriptions (Hair et al. 1995). The resulting clusters of individuals reflect high internal homogeneity and high external heterogeneity (Hair et al 1995).

Cluster analysis has been extensively used as a tool for diversity studies in crop species and has now become popular among researchers (Vural and Karasu, 2007). It has been used in breeding programs and has been found to be effective at grouping accessions based on morphological traits even though it would be very important if morphological traits with high discriminatory capacities are strictly adhered to in a diversity program (Ajayi and Adesoye, 2013). The objective of this study was to identify ideal genotypes of cowpea based on quantitative and qualitative traits that are suitable for selection for improved grain yield; and also to classify 10 genotypes of cowpea into groups based on their quantitative and qualitative traits with the aid of cluster analysis.

Materials and Methods

The experiment was conducted at the Research Field of the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko (latitude 7.2° N, longitude 5.44° E, Altitude 423M above sea level), Ondo State Nigeria, between August and December, 2013. 10 cowpea genotypes used in this study were collected from International Institute of Tropical Agriculture (IITA) Ibadan. The collected genotypes are presented in Table 1.

Table 1: List of Tested Genotypes

S/N	GENOTYPE	CODE
1	IT98K-205-8	IT1
2	IT98K-555-1	IT2
3	TVU4886	TVu1
4	TVU4886	TVu2
5	IT89KD-288	IT3
6	TV U9253	TVu3
7	TVU11986	TVu4
8	TVU9256	TVu5

9	TVU9252	TVu6
10	TVU11979	TVu7

The 10 cowpea genotypes were evaluated in a field laid out in a Randomized Complete Block Design with three (3) replications. Each replicate consisted of 10 row plots of 10 genotypes, each of which was regarded as a treatment, with 1m distance between treatments. Each treatment consisted of 30 plants, and the total number of plants on the field was 900. Each treatment was sown at a spacing of 30cm between plants and 50cm between rows with planting depth being approximately 2.5 cm. Weeding of the experimental field was done as when required to keep the weed pressure low. Sniper and Cypermethrin (cyperforce) were used at different growth stages to control insect pests at appropriate time.

Data were collected from 10 randomly selected plants and their means were recorded for all observations. Ten quantitative traits and thirteen qualitative traits were measured according to International Board for Plant Genetic Resources (IBPGR), cowpea descriptors. The qualitative traits (collected at 8 weeks after planting) are as shown in table 2. The quantitative traits measured are: Plant height (cm), number of main branches, and number of nodes on main stem, number of leaves per plant (at 8 weeks after planting), number of days to flowering, number of pods per peduncle, number of pods per plant, seeds per pod, seeds per plant and 100-seed weight (g) (at maturity).

Table 2: International Board for Plant Genetic Resources (IBPGR) Cowpea Descriptors

S/N	Traits	Score and Trait form
1	Growth Habit	1, Acute erect; 2, Erect; 3, Semi-erect; 4, Intermediate; 5, Semi-prostrate; 6, Prostrate; 7, Climbing
2	Growth Pattern	1, Determinate; 2, Indeterminate
3	Terminal Leaflet Shape	1, Globose; 2, Sub-globose; 3, Sub-hastate; 4, Hastate
4	Terminal Leaflet Colour	3, Pale green; 5, Intermediate green; 7, Dark green
5	Twinning Tendency	0, None; 3, Slight; 5, Intermediate; 7, Pronounced
6	Plant Pigmentation	0, None; 1, Very slight; 3, Moderate; 5, Intermediate; 7, Extensive; 9, Solid
7	Plant Hairiness	3, Glabrescent; 5, Short appressed hairs; 7, Pubescent to hirsute
8	Flower Colour	1, White; 2, Violet; 3, Mauve-pink; 4, Other
9	Raceme Position	1, Mostly above canopy; 2, In upper canopy; 3, Throughout canopy
10	Pod Attachment to Peduncle	3, Pendant; 5, 30-90 degree down from erect; 7, Erect
11	Immature Pod Pigmentation	0, None; 1, Pigmented tip; 2, Pigmented sutures; 3, Pigmented valves; 4, Splashes of Pigment; 5, Uniformly pigmented; 6, Other
12	Pod Curvature	0, Straight; 3, Slightly curved; 5, Curved; 7, Coiled
13	Seed Shape	1, Kidney; 2, Ovoid; 3, Crowder; 4, Globose; 5, Rhomboid

Data on quantitative traits were subjected to analysis of variance using general linear model (GLM) procedure for randomized complete blocks design in SPSS (version 20); while the data on both quantitative and qualitative traits were subjected to cluster analysis using paleontological statistics software package for education and data analysis (PAST).

Results and Discussion

Analysis of variance showed that the mean squares for all traits (Table 3) indicated the existence of high significant differences among the genotypes. Wide range significant variations were observed in plant height (11.70cm to 27.19cm), number of nodes on main stem (4.33 to 8.55), number of leaves per plant (12 to 37.44), number of days to flowering (44days to 87 days), number of pods per peduncle (0 to 4.44), number of pods per plant (0 to 71.11), number of seeds per pod (0 to 19.67), number of seeds per plant (0 to 1260) and 100-seed weight (0.00g to 16.53g). The range of variations among the genotypes for other traits was low (Table 4).

Table 3: Analysis of Variance for all the Traits of the Tested Ten Genotypes of Cowpea

TRAIT	REPLICATION	TREATMENT	ERROR	CV (%)
Plant Height (cm)	6.54	65.58*	15.63	19.47
Number of Main Branches	0.63*	0.94**	0.18	9.07
Number of Nodes on Main Stem	0.41	7.68**	0.84	14.63
Number of Leaves	35.98	297.23**	35.17	24.32
Number of Days to Flowering	4.91	561.07**	5.31	4.48
Number of Pods per Peduncle	0.07	6.03**	0.05	6.87
Number of Pods per Plant	1.66	1567.49**	41.35	4.41
Seeds per Pod	0.10	93.08**	1.29	8.59
Seeds per Plant	1349.95	518033.59**	5074.05	9.97
100-Seed Weight (g)	1.52	59.24**	1.45	10.58

** , Highly significant; *: Significant; CV, Coefficient of Variation

For the 13 qualitative traits, 5 out of the 10 genotypes had acute erect growth habit (IT1, IT2, IT3, TVu5 and TVu7), one (1) had erect growth habit (TVu4), one (1) was semi erect (TVu2), one (1) was prostrate (TVu1) and the remaining one was climbing (TVu6). Five (5) accessions had determinate growth pattern (IT1, IT2, TVu2, TVu3 and TVu5) while the remaining five (5) were indeterminate (TVu1, IT3, TVu4, TVu6 and TVu7). Two (2) genotypes had globose terminal leaflet shape (TVu4 and TVu5), 2 had sub-globose (TVu3 and IT1) while the rest had hastate terminal leaflet shape (IT2, TVu1, TVu2, IT3, TVu6 and TVu7). Leaflet colour was pale green for two genotypes (IT1 and IT2), intermediate for 5 genotypes (TVu2, IT3, TVu3, TVu4 and TVu7) while the rest had dark green colour (TVu1, TVu5 and TVu6). Twinning tendency was pronounced for three (3) genotypes (TVu1, TVu2 and TVu6), intermediate for three (3) genotypes (TVu3, TVu4 and TVu7), and slight for three (3) genotypes (IT1, IT2 and TVu5) while the remaining one (IT3) did not any twinning tendency. Two (2) genotypes were moderately pigmented (IT1 and IT2), three (3) intermediately pigmented (TVu3, TVu5 and TVu6), three solidly pigmented while the remaining one (TVu7) had no pigment. Six (6) genotypes were glabrescent for plant hairiness (IT1, TVu1, TVu2, IT3, TVu4 and TVu6), three (3) had short appressed hairs (IT2, TVu3 and TVu5), while the remaining one (TVu7) had pubescent hairs. Flower colour was white for only genotype IT1; it was violet for genotypes IT2 and TVu4, mauve-pink for genotypes TVu3, TVu5 and TVu7, while the remaining four (4) genotypes had other kinds of flowers. Four (4) genotypes (IT1, TVu3, TVu5 and TVu7) had their racemes mostly above canopy; two genotypes (IT2 and TVu4) had it in upper canopy while the remaining four (4) genotypes had their racemes throughout canopy. Pod attachment to peduncle was 30° to 90° in seven (7) of the genotypes (IT2, TVu1, TVu2, IT3, TVu3, TVu4 and TVu5), with two (2) of the genotypes (IT1 and TVu7) having pod attachments that are pendants. All genotypes had only one variant for immature pod pigmentation, while pod curvature was the same for all genotypes (slightly curved); seed shape was kidney for six (6) genotypes (IT1, TVu1, TVu2, IT3, TVu3 and TVu5), rhomboid for two (2) genotypes (TVu4 and TVu7) and ovoid for one (IT2). Overall, there were wide variations among the genotypes for most of the qualitative traits except for immature pod pigmentation and pod curvature (Table 5).

Data on quantitative traits produced a dendrogram that grouped the 10 genotypes into two major clusters, A and B, with cluster A having two (2) sub clusters (1 and 2). Cluster B had no sub-cluster. Sub-cluster 1 of A had one (1) genotype (TVu6) while sub-cluster 2 of A had only five (5) genotypes (TVu1, TVu2, IT3, IT2 and IT1). Cluster B had four (4) genotypes (TVu3, TVu5, TVu4

The dendrogram constructed based on the data from qualitative traits revealed two major clusters (A and B) for the 10 genotypes. Cluster A had two sub-clusters (1 and 2); with sub-cluster 1 having two (2) genotypes (TVu4 and TVu7), sub-cluster 2 having five (5) genotypes (IT2, TVu3, TVu5, IT3 and IT1). Cluster B had only three (3) genotypes (TVu1, TVu2 and TVu6) without sub-clusters (Figure 2). The genotypes with the highest number of seeds are grouped in the same cluster by data from quantitative traits (cluster B), while the ones with the highest seed weights are grouped in same sub-cluster by quantitative data (Cluster A; sub-cluster 2). Genotype TVu6, occupied sub-cluster 1 of cluster A, making it totally different from all other genotypes tested. TVu1, TVu2 and TVu6 are grouped together in same cluster by qualitative traits just as in quantitative traits; this shows they are very similar in most of the qualitative features. Also, qualitative data grouped IT1, IT2, and IT3 in the same cluster just as in data from quantitative traits. All quantitative traits had very high level of contribution to the total variability observed among the genotypes,

making these characters important for discriminating among genotypes of cowpea. Moreover, for any breeding work to be successful in cowpea, these characters must be considered. This agrees with some of the findings of Vural and Karasu (2007), Stoilova and Pereira (2013), Ajayi and Adesoye (2013) on cowpea and Olanrewaju and Cyril (2012) on *Corchorus olitorius*. The level of variations showed by qualitative traits was very high, as ten traits out of thirteen studied were able to discriminate effectively among the genotypes studied, while the level of similarity for phenotypic expressions among the rest of the traits (pod attachment to peduncle, immature pod pigmentation and pod curvature) was very high.

The variability, as observed among the accessions was generally high, and the improvement of this can be done through the conventional breeding techniques (selection and hybridization). The morphological traits also had different discriminatory capacity as observed among the genotypes, as some efficiently discriminated than others, making those better traits of choice in plant diversity programs. The results from this research have shown that enough variability exist in the studied traits among the tested cowpea genotypes. High mean values for yield and its component traits, warrant effective selection for further improvement. This study identified genotypes TVu3, TVu5, TVu4 and TVu7 for further genetic studies based on number of days to flowering and high number of seeds per plant. Furthermore, the choice of cluster analysis as a tool for this work proved to be a wise decision, as dendrograms were able to reveal similarities and differences among the tested genotypes. To consider any statistical package, user-friendliness must be one of the primary targets of choosing such a tool, and this was observed with the tools used during this study.

Table 4: Mean Values of the quantitative Traits

Genotypes	PH (cm)	NB	NN	NL	NDFL	NPPED	NPP	SDP	SDPL	100-SW (g)
IT1	11.70a	3.33a	4.78ab	13.33a	44.44ab	3.89d	46.78c	16.11d	731.77d	13.40cd
IT2	16.59ab	4.99bc	5.00ab	19.44ab	47.89b	3.89d	66.33de	11.33b	741.55d	14.77de
TVu1	19.65bc	5.00bc	8.00cd	36.11c	45.78ab	2.22b	35.11b	11.00b	386.33bc	13.20cd
TVu2	23.10bcd	5.11bc	8.33d	37.22c	47.22b	2.33b	31.56b	10.33b	310.45b	11.50bc
IT3	17.33ab	4.55bc	4.55a	14.22a	62.44c	3.00c	35.33b	13.66c	476.78c	16.53e
TVu3	18.19ab	4.55bc	6.22b	25.33b	46.67c	4.00c	74.33e	16.00d	1192.67f	10.47b
TVu4	22.04bcd	4.33b	4.33a	12.00a	45.56b	4.44ef	56.22cd	17.78d	997.56e	10.33b
TVu5	20.83bcd	4.66bc	6.44bc	27.11bc	42.22a	4.11de	71.11e	16.22d	1260.44f	11.27bc
TVu6	26.36cd	5.33c	8.55d	37.44c	87.11c	0.00a	0.00a	0.00a	0.00a	0.00a
TVu7	27.19d	4.89bc	6.44bc	21.67ab	45.44ab	4.67f	53.11c	19.67e	1043.56e	12.30bc

Means followed by the same alphabet within a column are not significantly different from one another at $P \leq 0.01$ Duncan Multiple Range Test (DMRT).

PH, Plant height; NB, Number of main branches; NN, Number of nodes on main stem; NDFL, Number of days to first flowering; NPPED, Number of pods per peduncle; NPP, Number of pods per plant; SDP, Seeds per pod; SDPL, Seeds per plant; 100-SW, 100-Seed weight

Table 5: Mean values of the qualitative traits

Genotypes	GH	GP	TMS	TLC	TWT	PG	PHR	FC	RP	PAP	IPP	PC	SS
IT1	1	1	2	3	3	3	3	1	1	3	4	3	1
IT2	1	1	3	3	3	3	5	2	2	5	0	3	2
TVu1	6	2	3	7	7	7	3	4	3	5	0	3	1
TVu2	3	1	3	5	7	7	3	4	3	5	0	3	1
IT3	1	2	3	5	0	7	3	4	3	5	0	3	1
TVu3	4	1	2	5	5	5	5	3	1	5	0	3	1
TVu4	2	2	1	5	5	1	3	2	2	5	0	3	5
TVu5	1	1	1	7	3	5	5	3	1	5	0	3	1
TVu6	7	2	3	7	7	5	3	4	3	0	0	3	0
TVu7	1	2	3	5	5	0	7	3	1	3	0	3	5

GH, Growth habit; GP, Growth pattern; TMS, Terminal leaflet shape; TLC, Terminal leaflet colour; TWT, Twining tendency; PG, Plant pigmentation; PHR, Plant hairiness; FC, Flower colour; RP, Raceme position; PAP, Pod attachment to peduncle; IPP, Immature pod pigmentation; PC, Pod curvature; SS, Seed shape

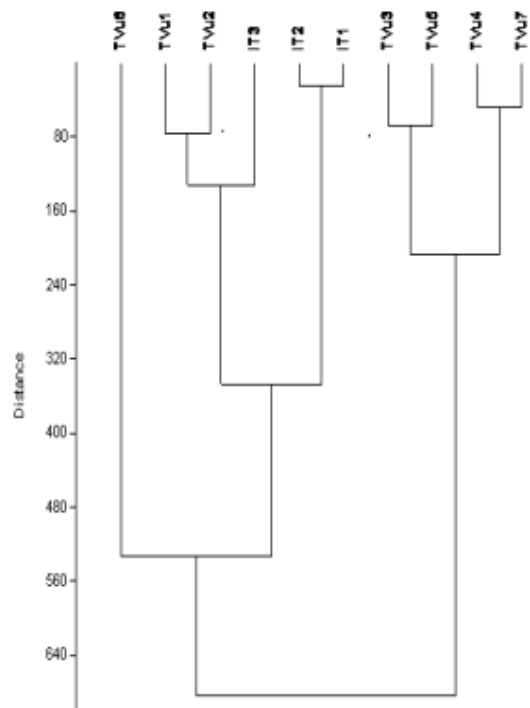


Figure 1: Dendrogram based on quantitative traits of the tested 10 genotypes of cowpea

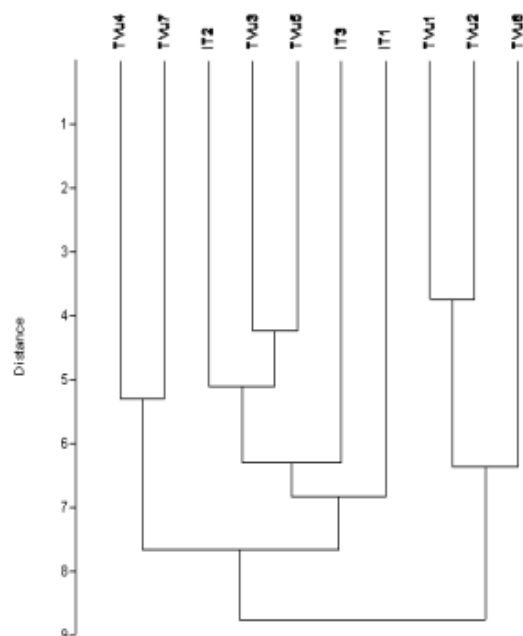


Figure 2: Dendrogram based on qualitative traits of the tested 10 genotypes of cowpea

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