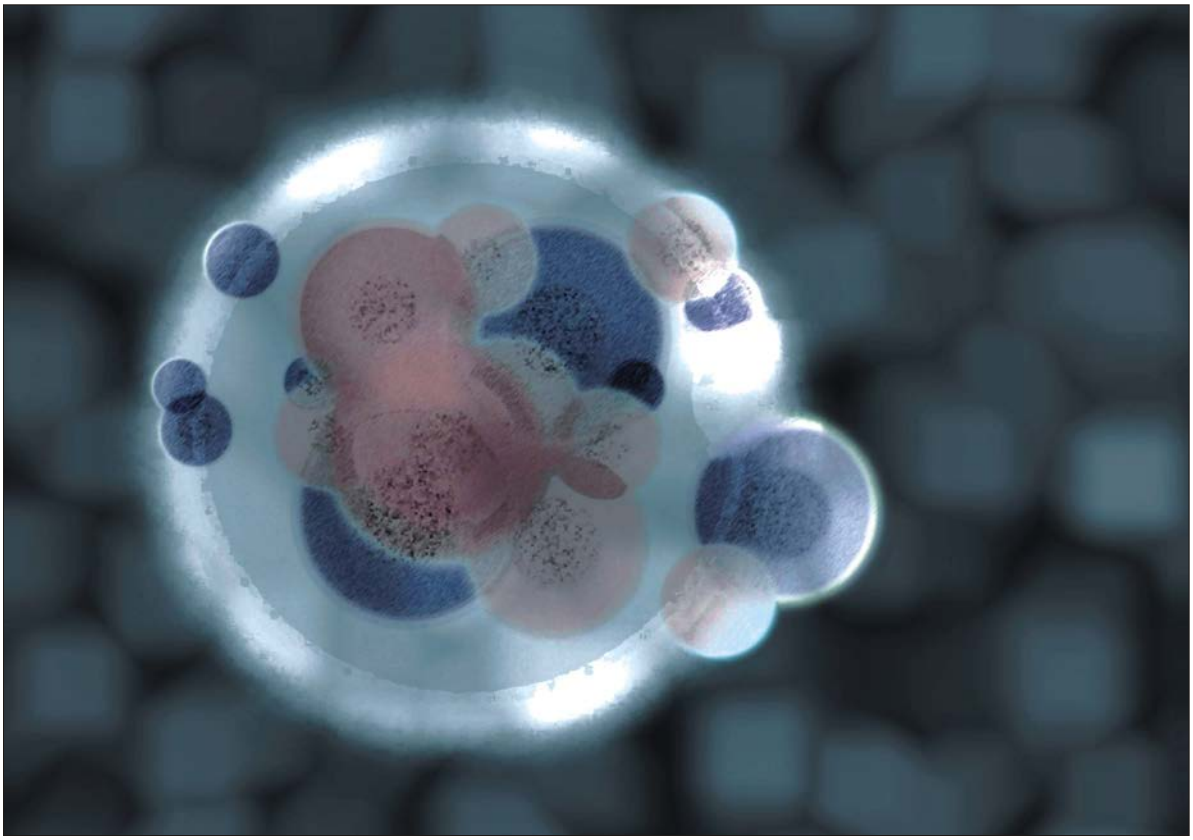

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Morphometric Analysis of some Species in the Genus *Vigna* (L.) Walp: Implication for Utilization for Genetic Improvement

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ABSTRACT

The genus *Vigna* (L.) Walp is a large cosmopolitan leguminous genus comprising both cultivated and wild species. *Vigna unguiculata* (L.) Walp. (Cowpea) is an important food legume that is widely cultivated in the tropics, but its production and storage are hampered by insect pests. The present study evaluated the intra-and inter-specific morphological variabilities among 20 accessions of six *Vigna* species, comprising wild and cultivated species. The species are *Vigna unguiculata* (L.) Walp, *V. vexillata* (A. Richard), *V. oblongifolia* (A. Richard), *V. ambacensis* (Baker), *V. luteola* (Jacq.) (Bentham) and *V. racemosa* (G. Don) (Hutch and Dalziel). Thirty-one morphological characters involving qualitative and quantitative vegetative and reproductive traits were used for the evaluation. Multivariate analysis such as Pearson Correlation Coefficient (PCC), Principal Component Analysis (PCA) and Cluster Analysis (CA) were employed to evaluate the intra and inter specific variabilities. The paired-sample T test indicated significant differences among the 20 accessions ($p \leq 0.000$, df = 19) in their morphometric traits. The relative significant correlation observed among some traits such as pod length, number of locules per pod and number of seeds per pod indicated their closeness and potential for genetic improvement of cowpea. The PCA showed that reproductive traits such as days to 50% flowering (0.984), days to 50% ripe pod (0.993), number of pods per peduncle (0.340) and 100-seed weight (0.132) are the major traits that accounted for the variations among the species. The UPGMA using the average (between groups) segregated the 20 accessions into two main clusters, cluster I (comprising mainly wild *Vigna* species) and cluster II (comprising both wild and cultivated species). The taxonomic affinities and genetic diversity among the species are of great importance in the utilization of the species for food and nutrition, fodder for ruminant animals, cover crop for rotational farming and more importantly genetic improvement of cowpea.

Key words: Genus *Vigna*, wild *Vigna* species, morphometric analysis, cluster analysis

INTRODUCTION

The genus *Vigna* (L.) Walp consists of more than 80 different species widely distributed in tropical and subtropical regions (Pasquet, 2001; Andargie *et al*., 2013). The species *V. unguiculata* commonly referred to as beans, black-eye bean, Kaffir pea, Southern pea, marble pea or cowpea and its wild relatives constitute the section Catiang (Padulosi, 1997; Andargie *et al*., 2013). The taxonomy of the genus *Vigna* is somehow controversial, due to the composition of species of different origin characterized by varied ecological and morphological diversity (Fatokun *et al*., 1993; Padulosi, 1997; Delgado-Salinas *et al*., 2011).

The economic importance of the genus *Vigna* cannot be overemphasized. Cowpea plays an important role as source of plant protein and as fodder crop in the cropping systems of tropical, sub-tropical, arid and semi-arid regions (Pule-Meulenberg *et al*., 2010; Sprent *et al*., 2010). The use of cowpea as green manure, animal fodder and for medicinal purposes is gaining unprecedented attention in the sub-Sahara Africa (Sprent *et al*., 2010). In the traditional agricultural practices, cowpea is usually intercropped with a number of crops such as cereals, root crops, cotton and several plantation crops. Some of the wild *Vigna* species are variously utilized as supplements to native diets, fodder feeds and as medicine. *Vigna vexillata* produces edible tuber that is consumed due to its medicinal values in Sudan and Ethiopia (Moray *et al*., 2014). Insect pest attack remains the most serious constraints to the cultivation and storage of cowpea and efforts made so far to solve this problem have not yielded the expected results.

Wild *Vigna* species particularly *V. vexillata*, are known to contain pest resistance genes that could be transferred to the cultivated cowpea through interspecific hybridization for genetic improvement of cowpea. The wild *Vigna* species such as *V. vexillata*, *V. oblongifolia*, *V. luteola*, *V. racemosa* and *V. ambacensis* which are found in many African countries could be exploited for food, medicine, agriculture (as cover crops and fodder) and more importantly for genetic improvement of cowpea (Kouam *et al*., 2012; Vijaykumar *et al*., 2012). The present study is aimed at evaluating the taxonomic affinities and genetic variabilities among 20 accessions of six *Vigna* species using morphometric analysis and to identify *Vigna* species that can serve as a bridge species between *V. unguiculata* and *V. vexillata* for inter-specific hybridization. The six species under study are *Vigna unguiculata*, *V. vexillata*, *V. oblongifolia*, *V. luteola*, *V. racemosa* and *V. ambacensis*.

MATERIALS AND METHODS

Collection and acquisition of seed samples: This study was conducted in Covenant University Farm, Ota, Nigeria in 2013 during the early dry season (October-December) and late dry season (January-March). Seeds of thirteen accessions of five wild *Vigna* species and two accessions of cultivated cowpea were obtained from the Genetic Resources Centre of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Five local varieties of cowpea (landraces) that are usually cultivated within Ogbomoso agricultural zone were collected from farmers in Ogbomoso North Local Government area, Oyo state, Nigeria. The sources and seed characters of the accessions used for this study are shown in Table 1. The seed samples comprised five accessions of *V. vexillata*, two accessions each of *V. oblongifolia*, *V. luteola*, *V. ambacensis*, *V. racemosa* and seven accessions of *V. unguiculata*. Information on plant habit and local names were obtained from the passport data of the IITA accessions and from the local farmers for the landraces of cowpea.

Table 1: Passport data and source of seeds used for this study

Species	Accession number	Source	Status	Local name	Plant habit	Seed coat colour	Seed texture	Seed size
V. vexillata	TV*nu*84	IITA	Wild	Unknown	Climbing	Blackish brown	Smooth	Very small
V. vexillata	TV*nu*72	IITA	Wild	Unknown	Prostrate	Greenish brown	Smooth	Small
V. vexillata	TV*nu*635	IITA	Wild	Unknown	Climbing	Darkish green	Smooth	Small
V. vexillata	TV*nu*327	IITA	Wild	Unknown	Climbing	Darkish green	Smooth	Very small
V. vexillata	TV*nu*719	IITA	Wild	Unknown	Semi-erect	Greenish brown	Smooth	Small
V. oblongifolia	TV*nu*144	IITA	Wild	Unknown	Climbing	Darkish brown	Smooth	Very small
V. oblongifolia	TV*nu*42	IITA	Wild	Unknown	Climbing	Black	Smooth	Small
V. luteola	TV*nu*28	IITA	Wild	Unknown	Spreading and twining	Blackish brown	Smooth	Small
V. luteola	TV*nu*1023	IITA	Wild	Unknown	Spreading and twining	Blackish brown	Smooth	Small
V. ambacensis	TV*nu*1682	IITA	Wild	Unknown	Climbing	Darkish brown	Smooth	Small
V. ambacensis	TV*nu*104	IITA	Wild	Unknown	Climbing	Darkish brown	Smooth	Small
V. racemosa	TV*nu*126	IITA	Wild	Unknown	Creeper and twining	Darkish brown	Smooth	Very small
V. racemosa	TV*nu*45	IITA	Wild	Unknown	Creeper and twining	Darkish brown	Smooth	Very small
V. unguiculata	IT90K-277-2	IITA	Breeding line	Ewa funfun	Erect	White	Rough	Medium
V. unguiculata	TV*u*2027	IITA	Breeding line	Ewa funfun	Semi-erect	White	Rough	Big
V. unguiculata	PJ2K-01N	Orile-igbon	Landrace	Abewehe	Spreading and twining	White	Rough	Medium
V. unguiculata	PJ2K-02N	Egbeda	Landrace	Ewa pupa	Erect	Brown	Rough	Medium
V. unguiculata	PJ2K-03N	Gambari	Landrace	Tede local	Semi-erect	White	Rough	Medium
V. unguiculata	PJ2K-04N	Igbon	Landrace	Beweja	Spreading and twining	White	Rough	Medium
V. unguiculata	PJ2K-05N	Idi-eni	Landrace	Ewa pupa	Erect	Brown	Rough	Medium

Table 2: Qualitative and quantitative traits used for characterization

Qualitative traits	Quantitative traits
Plant habit	Terminal leaflet length
Terminal leaflet shape	Terminal leaflet width
Leaf texture	Petiole length
Stem texture	Rachis length
Flower colour	Stipule length
Raceme position	Stipule width
Pod colour	Days to 50% flowering
Pod attachment to peduncle	Days to 50% ripe pod
Pod shattering	Standard petal length
Seed colour	Standard petal width
Seed texture	Calyx lobe length
Seed size	Peduncle length
	Pod per peduncle
	Pod length
	Pod width
	Locules per pod
	Seeds per pod
	Seed set percentage
	100 seed weight

Cultivation of the accessions: Four seeds of each accession were planted in each of four plastic buckets filled with top soil for all the accessions. The four plastic buckets for each accession were arranged in twenty straight lines. Some of the wild *Vigna* seeds were scarified before planting to enhance germination. The plants were thinned to two per bucket after establishment. The plants were regularly watered and hand weeded throughout the period of study. Data were collected from the four plants in the middle plastic buckets for each accession, totaling 80 plants out of a grand total of 160 plants raised for the study.

Morphometric characterization: A total of thirty-one morphological traits, comprising 12 qualitative and 19 quantitative characters were used for this study (Table 2). Descriptors for cowpea *Vigna unguiculata* was used for the characterization (IBPGR., 1983). Qualitative characters were scored based on visual evaluation while quantitative characters were measured and recorded in SI units.

Scoring of qualitative traits: Twelve qualitative traits were scored based on visual evaluation. The data obtained were standardized on a numerical scale using the IPGRI (2006) system of coding of qualitative data to allow for statistical analysis (Table 2).

Measurement of quantitative traits (vegetative and reproductive characters): Ten measurements were taken for each of the 19 quantitative characters studied using metric ruler, weighing balance and actual counting. All harvested pods and seeds were sun dried to reach constant weight before recording their weights. Seed set percentage was estimated as follows:

$$\text{Seed set percentage} = \frac{\text{No. of seeds per pod}}{\text{No. of locules per pod}} \times 100$$

Data analysis: The means of the measurements for each character were computed for all the accessions. The mean values were used to design a similarity data matrix. The differences among the studied species were tested by paired-sample T-test. The resultant data matrix of 20×31 was standardized (mean = 0 and standard deviation = 1) and subjected to multivariate analysis. Inter-relations and correlation coefficient (r) among the quantitative traits were evaluated using SPSS version 20 (SPSS., 2011). Phenetic relationships among the species were evaluated by Principal Component Analysis (PCA) and Cluster Analysis (CA) from the matrix of average values of the morphometric characters.

RESULTS

Species description

***Vigna vexillata* (A. Richard):** *Vigna vexillata* is a wild relative of cowpea characterized by heavy pubescence of the leaves, stem and pods, a trait that protects the species against insect pest attack. It is a twining, climbing or prostrate herb that is widely distributed in tropical Africa, Asia and Australia. The species showed tremendous variation in vegetative, floral, pod and seed characters. Variabilities observed in plant hairiness in the accessions studied include scabrous in T*Vnu*84, puberulous in *Tvnu*72, glabrecent in T*Vnu*635 and pubescent in T*Vnu*719. The leaf shape/forms observed in the studied accessions include; subglobose in T*Vnu*84, hastate in T*Vnu*719, lanceolate in T*Vnu*327 and linear in T*Vnu*635. The flower color also varied which includes; purple in T*Vnu*84, iolet in T*Vnu*72, T*Vnu*635 and deep purple in T*Vnu*327 and T*Vnu*719, respectively. Variations in growth habit and seed characters are shown in Fig. 1 and 2.

***Vigna oblongifolia* (A. Richard):** *Vigna oblongifolia* is characterized by twining/climbing growth habit, highly vegetative with linear to lanceolate, scabrous leaves, scabrous stem and yellow flowers. The pods are straight and very tiny, held in erect position on the peduncle, black in colour and shatter after drying. The seeds are dark brown, smooth and small (Fig. 2).

***Vigna racemosa* (G. Don) (Hutch and Dalziel):** *Vigna racemosa* is found in many African countries and characterized by deep blue flowers, puberulous leaf texture, pubescent stem and abundant pod production. The black pods shatter at maturity to release the seeds. The seeds are smooth, darkish brown and small in size (Fig. 2).

***Vigna luteola* (Jacq.) (Bentham):** *Vigna luteola* is characterized by yellow flowers, ovate and glabrous leaves. The raceme position is within the canopy. The pods are erect, black in colour containing darkish brown smooth seeds (Fig. 2).

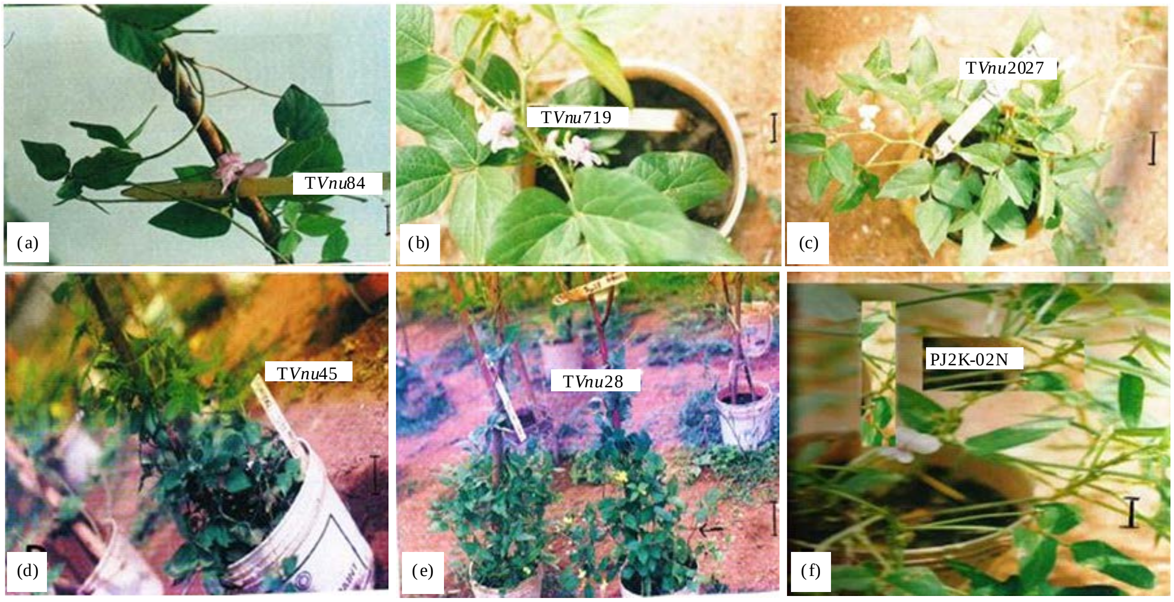

Fig. 1(a-f): Growth habit in some *Vigna* species accessions studied, (a) *Vigna vexillata* (TV*nu*84) showing twinning growth habit, (b) *Vigna vexillata* (TV*nu*719) showing semi-erect growth habit, (c) *Vigna unguiculata* (TV*nu*2027) showing semi-erect growth habit, (d) *Vigna racemosa* (TV*nu*45) showing twining growth habit, (e) *Vigna luteola* (TV*nu*28) showing twining growth habit and (f) *Vigna unguiculata* (PJ2K-02N) showing erect growth habit, scale line represent 3.50 cm

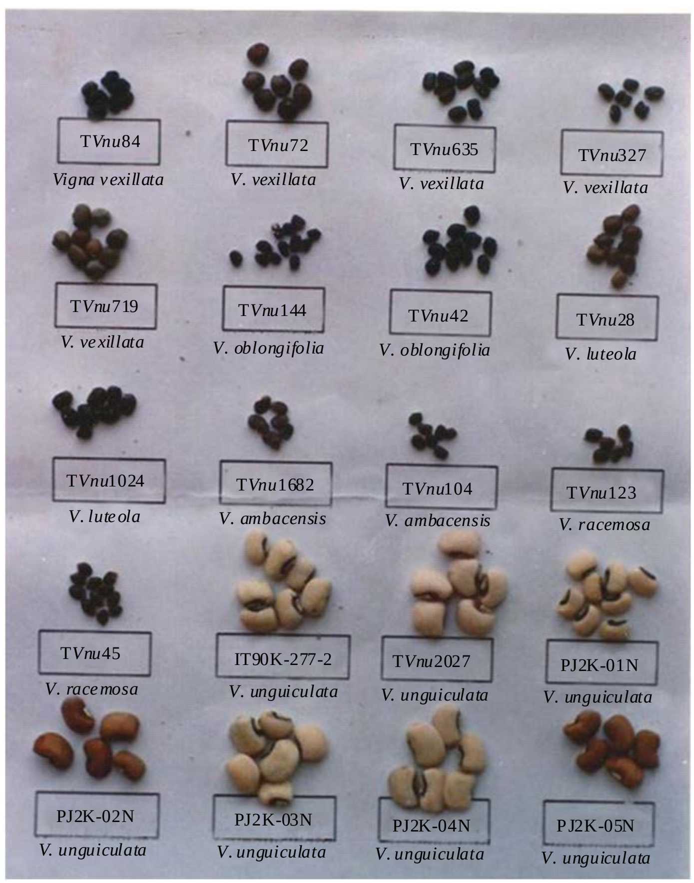

Fig. 2: Seed samples of the 20 accessions of six *Vigna* species studied showing variations in size, shape and colour

***Vigna ambacensis* (Baker):** This species is characterized by deep yellow flowers, puberulous ovate leaves, scabrous stem and abundant pod production. The pods are tiny and black in colour, while the seeds are small, smooth and darkish brown in colour (Fig. 2).

***Vigna unguiculata* (L.) Walp:** *Vigna unguiculata* is an annual herb with variable growth habits ranging from erect, sub-erect and prostrate to climbing habit (Fig. 1). The stem is usually stout, smooth or sub glabrous which could be determinate or indeterminate. Pods are coiled, round, crescent or straight. The seeds are relatively big and rough in texture (Fig. 2).

Qualitative and quantitative evaluation of morphological characters: All the 20 accessions of the six species of *Vigna* considered in this study varied considerably in their morphometric traits. The variabilities expressed in their qualitative traits are shown in Table 3 and Fig. 1 and 2. The *Vigna* species showed determinate and indeterminate growth habits. All the wild *Vigna* species were observed to be perennial and exhibited twining or prostrate growth habit with indeterminate main stem, while the *Vigna unguiculata* accessions are annuals that are either erect or semi-erect, except accession (PJ2K-04N) that showed spreading and twining habits (Table 3). The variabilities expressed in growth habit include twining or climbing, prostrate, semi-erect and erect (Fig. 1). Terminal leaflet shape observed in the *Vigna* species studied include ovate, lanceolate, subhastate and linear (Table 3). The *Vigna* species also vary in flower, pod and seed colours and in flowering and fruiting periods. The flower colours observed in the *Vigna* species include purple, deep purple, violet, yellow/deep yellow, blue and white. Raceme position observed in the species studied are; within canopy in *V. vexillata* (T*Vnu*84, T*Vnu*327), *V. oblonlongifolia*, *V. luteola*, *V. ambacensis* and all the *V. unguiculata* accessions, under canopy in *V. racemosa* and above canopy in *V. vexillata* (T*Vnu*72, T*Vnu*635 and T*Vnu*719). The pods of the *Vigna* species also varied in colour, size, shape and texture (Table 3). All the 20 accessions showed different patterns of pigmentation on stem, petiole and rachis, variable leaf shape and pubescence on leaves, stems and pods. Though the leaves are petiolate and stipulate in all the accessions, these characters varied quantitatively among the accessions.

The Terminal Leaflet Length (TLL) ranged from 5.15 cm in *V. luteola* (T*Vnu*1024) to 11.57 cm in *V. unguiculata* (PJ2K-05N). The Terminal Leaflet Width (TLW) ranged from 0.88 cm in *V. oblongifolia* (T*Vnu*144) to 7.15 cm in *V. unguiculata* (PJ2K-05N). Terminal leaflet length and width among the *V. vexillata* accessions ranged between 8.32 cm (length), 2.37 cm (width) in T*Vnu*84 and 10.52 cm (length), 4.52 cm (width) in T*Vnu*72. Petiole length ranged from 1.12 cm in *V. vexillata* (T*Vnu*327) to 10.30 cm in *V. unguiculata* (T*Vu*2027). Flowering and pod maturity time vary widely from 42 days in *V. oblongifolia* (T*Vnu*42) to 95 in *V. racemosa* (T*Vnu*45) and 56 days in *V. oblongifolia* (T*Vnu*42) to 107 days in *V. ambacensis* (T*Vnu*1682, TVnu104, respectively), as do standard petal length and width (from 1.02 and 1.82 cm in *V. racemosa* (T*Vnu*45) to 2.66 cm and 3.77 cm in *V. vexillata* (T*Vnu*84). Pod length varied (from 3.30 cm in *V. racemosa* (T*Vnu*45) to 15.25 cm in *V. unguiculata* (IT90K-277-2) and 100-seed weight (from 1.07 g in *V. oblongifolia* (T*Vnu*144) to 25.32 g in *V. unguiculata* (T*Vnu*2027). Seed set percentage was generally high in all the accessions ranging between 80.43% in *V. luteola* (T*Vnu*28) and 100% in *V. ambacensis* (T*Vnu*1682). Minimum and maximum values, the mean and the variance values of the nineteen quantitative morphometric characters considered in this study are recorded in Table 4. The paired-sample T test indicated significant differences among the 20 accessions of the *Vigna* species evaluated ($p \leq 0.000$, df = 19). Vegetative characters recorded low variance values while reproductive traits such as floral, pod and seed traits recorded high variance values as reflected in days to 50% flowering (294.766), days to 50% ripe pod (371.22), pod length (18.18), number of locules per pod (28.49), number of seeds per pod (27.04), seed set percentage (28.20) and 100-seed weight (62.61) (Table 4).

Correlation coefficient (r) of the 19 quantitative traits of the 20 accessions of six *Vigna* species studied: All the vegetative traits studied were significantly correlated at 0.01 level, but recorded negative correlations with reproductive traits such as days to 50% flowering and days to 50% ripe pod. Standard petal length and width were significantly correlated with terminal leaflet length (r = 0.75, 0.72), respectively. Pod length was also significantly correlated with vegetative traits such as terminal leaflet length and width (r = 0.81 and 0.72), stipule length and width (r = 0.80 and 0.85) while it was negatively correlated with days to 50% flowering (r = -0.48) and days to 50% ripe pod (r = -0.57). Number of locules per pod was significantly correlated with standard petal length and width (r = 0.86 and 0.82), calyx lobe length (r = 0.87) and pod length (r = 0.71). Similarly, number of seeds per pod was significantly correlated with terminal leaflet length, standard petal length and width, calyx lobe length and pod length (Table 5).

Principal component analysis of the 20 accessions of six *Vigna* species studied: The first five PC axes were observed to have influenced the variation with 98.60% of the total variation (Table 6). Eigen values ranged from 10.44 in PC5 to 679.72 in PC1. The PC1 alone accounted for 78.87% of the total variation. Reproductive traits such as days to 50% flowering (0.984), days to 50% ripe pod (0.993), number of pods per peduncle (0.340) and 100-seed weight (0.132) were the most important traits that contributed to the variation in PC1. The PC2 accounted for 9.10% of the variation which was mostly influenced by reproductive traits which recorded higher

Table 3: Qualitative traits of the accessions of *Vigna* species studied

Species	Accession number	Growth habit	Terminal leaflet shape	Leaf texture	Stem texture	Flower colour	Raceme position	Pod colour	Pod attachment to peduncle	Pod shattering	Seed colour	Seed texture	Seed size
V. vexillata	TV*nu*84	Twining	Ovate	Scabrous	Scabrous	Purple	Within canopy	Black	Horizontal	Present	Black brown	Smooth	Very small
V. vexillata	TV*nu*72	Erect	Ovate	Puberulous	Pubescent	Violet	Above canopy	Dark brown	Horizontal	Present	Greenish brown	Smooth	Small
V. vexillata	TV*nu*635	Twining	Linear	Glabrescent	Pubescent	Violet	Above canopy	Brown	Erect	Present	Dark green	Smooth	Small
V. vexillata	TV*nu*327	Twining	Lanceolate	Pubescent	Pubescent	Deep purple	Within canopy	Brown	Pendant	Present	Dark green	Smooth	Very small
V. vexillata	TV*nu*719	Semi-erect	Hastate	Pubescent	Pubescent	Deep purple/violet	Above canopy	Black	Erect	Present	Greenish brown	Smooth	Small
V. oblongifolia	TV*nu*144	Twining	Linear	Scabrous	Scabrous	Yellow	Within canopy	Black	Pendant	Present	Dark brown	Smooth	Very small
V. oblongifolia	TV*nu*42	Twining	Lanceolate	Scabrous	Scabrous	Yellow	Within canopy	Black	Pendant	Present	Black	Smooth	Small
V. luteola	TV*nu*28	Twining	Ovate	Glabrous	Glabrous	Yellow	Within canopy	Brown	Erect	Present	Blackish brown	Smooth	Small
V. luteola	TV*nu*1023	Twining	Ovate	Glabrous	Glabrous	Yellow	Within canopy	Brown	Erect	Present	Blackish brown	Smooth	Small
V. ambacensis	TV*nu*1682	Twining	Ovate	Puberulous	Scabrous	Deep yellow	Within canopy	Black	Erect	Present	Darkish brown	Smooth	Small
V. ambacensis	TV*nu*104	Twining	Ovate	Puberulous	Scabrous	Deep yellow	Within canopy	Black	Erect	Present	Darkish brown	Smooth	Small
V. racemosa	TV*nu*126	Twining	Ovate	Puberulous	Pubescent	Deep blue	Under canopy	Black	Horizontal	Present	Darkish brown	Smooth	Very small
V. racemosa	TV*nu*45	Twining	Ovate	Puberulous	Pubescent	Deep blue	Under canopy	Brown	Horizontal	Present	Darkish brown	Smooth	Very small
V. unguiculata	IT90K-277-2	Erect	Ovate	Glabrous	Glabrous	White	Within canopy	Brown	Erect	Absent	Whte	Rough	Medium
V. unguiculata	TV*u*2027	Semi-erect	Ovate	Glabrous	Glabrous	White	Within canopy	Brown	Horizontal	Absent	Whte	Rough	Big
V. unguiculata	PJ2K-01N	Twining	Ovate	Glabrous	Glabrous	White	Within canopy	Brown	Erect	Absent	Whte	Rough	Medium
V. unguiculata	PJ2K-02N	Erect	Subhastate	Glabrous	Glabrous	White	Within canopy	Brown	Erect	Absent	Brown	Rough	Medium
V. unguiculata	PJ2K-03N	Semi-erect	Ovate	Glabrous	Glabrous	White	Within canopy	Brown	Erect	Absent	Whte	Rough	Medium
V. unguiculata	PJ2K-04N	Twining	Ovate	Glabrous	Glabrous	White	Within canopy	Brown	Erect	Absent	Whte	Rough	Medium
V. unguiculata	PJ2K-05N	Erect	Subhastate	Glabrous	Glabrous	White	Within canopy	Brown	Erect	Absent	Brown	Rough	Medium

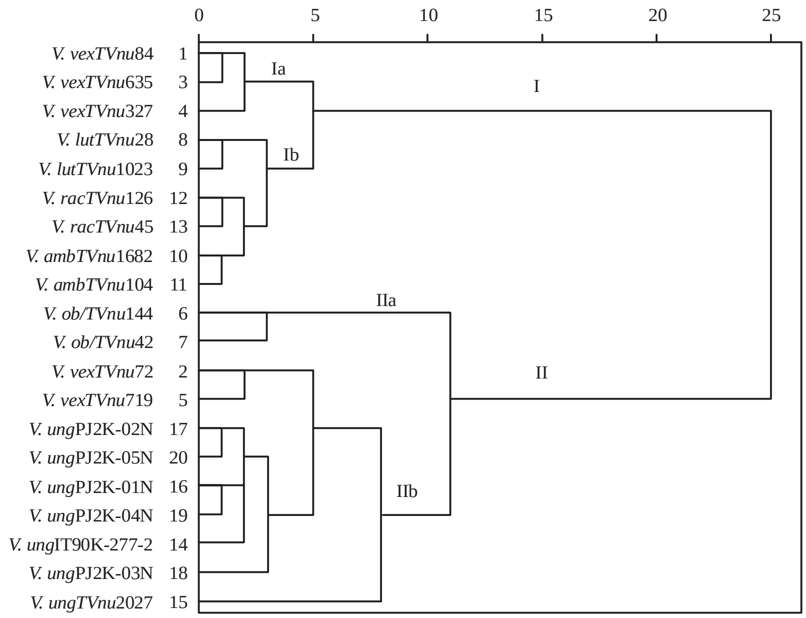

Fig. 3: Dendrogram generated from the mean values of the 19 quantitative traits of the 20 accessions of six *Vigna* species studied. *V. vex*: *Vigna vexillata*, *V. lut*: *Vigna luteola*, *V. rac*: *Vigna racemosa*, *V. amb*: *Vigna ambacensis*, *V. obl*: *Vigna oblongifolia* and *V. ung*: *Vigna unguiculata*. 1-20 represent the accession numbers

Table 4: Descriptive statistics and variance of traits of the six *Vigna* species studied

Traits	Minimum	Maximum	Mean	SE	Variance
Terminal leaflet length (cm)	5.15	11.57	8.5395	0.39339	3.095
Terminal leaflet width (cm)	0.88	7.15	3.6735	0.45707	4.178
Petiole length (cm)	1.12	10.30	5.5720	0.56634	6.415
Rachis length (cm)	0.55	3.25	1.7410	0.17368	0.603
Stipule length (cm)	0.32	1.44	0.8040	0.09130	0.167
Stipule width (cm)	0.14	0.51	0.2880	0.02593	0.013
Days to 50% flowering	42.00	95.00	69.8500	3.83905	294.766
Days to 50% ripe pod	56.00	107.00	84.2000	4.30825	371.221
Standard petal length (cm)	1.02	2.66	1.8300	0.12617	0.318
Standard petal width (cm)	1.82	3.77	2.7785	0.14458	0.418
Calyx lobe length (cm)	1.04	2.54	1.6340	0.10831	0.235
Peduncle length (cm)	5.40	16.84	10.2040	0.84577	14.307
Pod per peduncle	2.00	6.00	4.0500	0.26631	1.418
Pod length (cm)	3.30	15.25	9.2125	0.95347	18.182
Pod width (cm)	0.20	1.35	0.6995	0.09535	0.182
Locules per pod	6.00	21.80	12.6170	1.19364	28.495
Seeds per pod	6.00	20.80	11.7200	1.16277	27.041
Seed set percentage	80.43	100.00	92.4680	1.18736	28.196
100 seed weight (g)	1.07	25.32	8.0030	1.76927	62.606

T-test $p \leq 0.000$, df = 19, SE: Standard error

values, pod length (0.756), locules per pod (0.729) and seed per pod (0.719) compared to vegetative traits with lower values such as terminal leaflet length and width (0.652 and 0.517), rachis length (0.382) among others. The PC3 was largely influenced by both vegetative and pod and seed traits which accounted for 6.40% of the total variation while PC4 and PC5 accounted for 3.02 and 1.21%, respectively (Table 6).

Cluster analysis of the 20 accessions of some *Vigna* species studied: A dendrogram was generated from the cluster analysis using average linkage (between groups) methods (Fig. 3). Two cluster groups were generated at 25 coefficient similarity. Cluster I comprised 9 accessions of wild *Vigna* species which segregated into two sub clusters; Ia and Ib, respectively. Sub cluster Ia comprised three *V. vexillata* accessions (T*Vnu*84, T*Vnu*635 and T*Vnu*327) while sub cluster Ib comprised six accessions of three wild *Vigna* species viz; *V. luteola* (T*Vnu*28 and TVnu1023), *V. racemosa* (T*Vnu*124 and T*Vnu*45) and *V. ambacensis* (T*Vnu*1682 and T*Vnu*104). Cluster II on the other hand comprised 11 accessions of both wild and cultivated *Vigna* species. This cluster group also segregated into two main sub cluster groups, IIa and IIb. Sub

Table 5: Pearson correlation coefficient among the quantitative traits of the 20 accessions of some *Vigna* species studied

Traits	TLL	TLW	PL	RL	STL	STW	DF	DRP	SPL	SPW	CLL	PDL	PPP	PODL	PODW	LPP	SPP	SS	SW
TLL	1																		
TLW	0.602**	1																	
PL	0.574**	0.869**	1																
RL	0.653**	0.915**	0.843**	1															
STL	0.745**	0.807**	0.672**	0.838**	1														
STW	0.753**	0.812**	0.780**	0.807**	0.722**	1													
DF	-0.499*	-0.374	-0.388	-0.586**	-0.440	-0.567**	1												
DRP	-0.589**	-0.408	-0.386	-0.608**	-0.537*	-0.598**	0.965**	1											
SPL	0.745**	0.371	0.233	0.328	0.555*	0.513*	-0.109	-0.238	1										
SPW	0.720**	0.362	0.246	0.332	0.547*	0.501*	-0.081	-0.198	0.989**	1									
CLL	0.583**	0.112	0.008	0.068	0.407	0.294	0.037	-0.093	0.937**	0.933**	1								
PDL	0.347	0.406	0.441	0.342	0.155	0.589**	-0.213	-0.183	0.352	0.318	0.244	1							
PPP	-0.142	0.169	0.138	0.047	-0.092	-0.060	0.394	0.305	-0.087	-0.074	-0.141	0.077	1						
PODL	0.810**	0.716**	0.657**	0.722**	0.800**	0.845**	-0.488*	-0.571**	0.739**	0.731**	0.583**	0.530*	-0.148	1					
PODW	0.471*	0.790**	0.687**	0.763**	0.671**	0.745**	-0.452*	-0.455*	0.322	0.331	0.097	0.474*	0.019	0.753**	1				
LPP	0.686**	0.259	0.157	0.189	0.504*	0.415	-0.170	-0.308	0.869**	0.820**	0.871**	0.349	-0.208	0.714**	0.280	1			
SPP	0.680**	0.210	0.116	0.150	0.475*	0.364	-0.142	-0.285	0.870**	0.826**	0.886**	0.299	-0.213	0.678**	0.213	0.995**	1		
SS	0.227	-0.217	-0.183	-0.169	-0.012	-0.267	0.168	0.097	0.248	0.284	0.338	-0.316	-0.057	-0.061	-0.441	0.198	0.293	1	
SW	0.622**	0.728**	0.768**	0.760**	0.630**	0.803**	-0.378	-0.406	0.424	0.458*	0.202	0.429	-0.011	0.736**	0.748**	0.211	0.178	-0.112	1

**Correlation is significant at the 0.01 level, *Correlation is significant at the 0.05 level, TLL: Terminal leaflet length, TLW: Terminal leaflet width, PL: Petiole length, RL: Rachis length, STL: Stipule length, STW: Stipule width, DF: Days to 50% flowering, DRP: Days to 50% ripe pod, SPL: Standard petal length, SPW: Standard petal width, CLL: Calyx lobe length, PDL: Peduncle length, PPP: Pod per peduncle, PODL: Pod length, PODW: Pod width, LPP: Locules per pod, SPP: Seeds per pod, SS: Seed set percentage, SW: 100 seed weight

Table 6: Eigen values, proportion of variation and contribution of the 19 quantitative traits to the total variation in the first five PC axes of the 20 accessions of six *Vigna* species studied

Traits	PC1	PC2	PC3	PC4	PC5	Total (%)
TLL	-0.594	0.652	0.130	0.148	0.049	
TLW	-0.440	0.517	-0.425	-0.027	-0.089	
PL	-0.432	0.474	-0.520	0.054	0.055	
RL	-0.638	0.382	-0.420	0.094	-0.073	
STL	-0.537	0.558	-0.071	0.074	-0.275	
STW	-0.634	0.561	-0.351	-0.136	0.027	
DF	0.984	0.132	0.041	0.012	-0.095	
DRP	0.993	0.009	-0.067	-0.017	0.075	
SPL	-0.229	0.821	0.335	-0.045	-0.002	
SPW	-0.196	0.827	0.285	0.026	0.017	
CLL	-0.073	0.740	0.512	-0.060	0.022	
PDL	-0.234	0.471	-0.255	-0.478	0.532	
PPP	0.340	0.079	-0.210	0.008	-0.264	
PODL	-0.590	0.756	-0.048	-0.117	-0.004	
PODW	-0.500	0.474	-0.476	-0.239	-0.090	
LPP	-0.286	0.729	0.568	-0.238	-0.003	
SPP	-0.259	0.719	0.619	-0.159	0.015	
SS	0.132	0.176	0.530	0.785	0.222	
SW	-0.449	0.632	-0.589	0.205	-0.036	
Eigenvalue	679.719	78.400	55.190	26.064	10.437	
Variance (%)	78.867	9.097	6.404	3.024	1.211	98.597

PC: Principal component

cluster group IIa contained two accessions of *V. oblongifolia* (T*Vnu*144 and T*Vnu*42) while sub cluster group IIb further segregated into two, the first containing two accessions of *V. vexillata* (T*Vnu*72 and T*Vnu*719) and the second group containing 7 accessions of *V. unguiculata* which are PJ2K-02N, PJ2K-05N, PJ2K-01N, PJ2K-04N, IT90K-277-2, PJ2K-03N and T*Vu*2027 with T*Vnu*2027 being isolated from the other *V. unguiculata* accessions.

DISCUSSION

In this study, a total of thirty-one morphometric traits comprising 12 qualitative and 19 quantitative characters, which included vegetative, floral, pod and seed traits were used to assess intra and inter-specific variations among 20 accessions of six species of *Vigna*. The plants comprised of thirteen accessions of five species of wild *Vigna* (wild relatives of cowpea) and seven accessions of cultivated cowpea, *V. unguiculata*. The accessions showed considerable variabilities in their growth habits, vegetative traits, flowering and reproductive attributes. Accessions with short flowering time exhibited early pod maturity and showed better potentials for pod production and consequently higher seed production potentials. These observations corroborated the findings of Amao (2005) on *V. vexillata*, *V. racemosa*, *V. ambacensis*, *V. luteola* and *V. unguiculata* and Adegbite (2006) on *V. unguiculata* and *V. vexillata*. Similar results for early maturity and variabilities among *Vigna* species were reported by Cobbinah *et al.* (2011) and Garba and Pasquet (1998a, b).

Vigna oblongifolia flowered within 42 days of planting and matured within 56 days, making it a good potential parent for genetic improvement for early maturity. Also, the accessions with longer pods possessed higher number of locules per pod, number of seeds per pod and consequently higher seed set percentage are potential raw materials to boost seed production on *Vigna* species, especially cowpea. The significant correlation among some variable characters such as days to 50% flowering, days to 50% ripe pod, pod length, number of locules per pod and seeds per pod could be exploited in breeding efforts and genetic improvement of the cultivated cowpea (*V. unguiculata*).

The morphometric analysis highlighted a high degree of taxonomic affinities of the different species and diversity within the genus *Vigna*. Morphological similarities such as growth habit, leaf type (trifoliolate compound), flower type (Papilionaceous) and fruit type (pod) justified the grouping of the six species into the same genus *Vigna*. Though, the taxonomic affinities of the species in the genus *Vigna* have been variously reported (Marechal *et al*., 1978; Padulosi, 1993; Pasquet, 2001), its complexity had left many taxonomic issues in the genus unraveled. The genus *Vigna* has been traditionally divided into seven (7) subgenera containing 81 species (Marechal, 1976; Pasquet, 2001; Moray *et al*., 2014). Four subgenera viz; *Vigna*, *Haydonia*, *Plectrotropis* and *Macrorhyncha* which are distributed in Africa and show similarity in their morphological characters contain 54 species (Padulosi, 1993; Pasquet, 2001). The subgenus *Vigna* is divided into nine sections that contain the largest number of species (Pasquet, 2001). The section Catiang has two recognized species; *V. unguiculata* and *V. nervosa* Markotter. The species *V. unguiculata* according to Padulosi (1993) includes the cowpea and its wild relatives. In the present study, the groupings (I and II) in the dendrogram from the average linkage (between groups) revealed taxonomic affinities and phenetic variabilities of the species studied. Two major cluster groups formed (cluster I wild *Vigna* and cluster II both wild and cultivated cowpea) reinforced their grouping together as cultivated and wild species *V. unguiculata* (L.) Walp which constitute the section Catiang. As expected, all the wild *Vigna* species were grouped together in the cluster group I except two accessions of *V. vexillata* (T*Vnu*72 and T*Vnu*719) and *V. oblongifolia* which clustered with seven accessions of *V. unguiculata*. This is a clear indication that the species studied shared common morphological traits that justified their groupings. The wild *Vigna* species were clustered based on their growth habit, leaf texture, flower colour, seed texture and days to flowering. The segregation of two wild *Vigna* species *V. vexillata* (T*Vnu*72 and T*Vnu*719) and *V. oblongifolia* (T*Vnu*144 and T*Vnu*42) from the wild *Vigna* cluster group I can be attributed to their wide character differentiation and closeness to the cultivated cowpea *V. unguiculata*, especially in leaf size and shape, pod length, number of locules and seeds per pod, seed size and seed set percentage. This observation reflects considerable alignment with the classification of Pasquet (2001) and Marechal *et al*. (1978).

By raising the accessions in the same location and subjected to similar environments, the effects of the environment on their phenotypic expressions would have been removed or reduced, hence the variabilities observed are largely due to genetic factors and phenotypic similarities will be an indication of genetic relationships. Thus, the phenotypic groupings arising from the analysis reflects the closeness of the species and accessions in each group/cluster which suggest the potential crossability of the members of the same group. Thus, the closeness observed among the accessions of *V. vexillata* (T*Vnu*84, T*Vnu*635, T*Vnu*327), *V. luteola* (T*Vnu*28 and T*Vnu*1023), *V. racemosa* (T*Vnu*126 and T*Vnu*45) and *V. ambacensis* (T*Vnu*104) on one hand and the phenotypic closeness of *V. vexillata* (T*Vnu*72 and T*Vnu*719), *V. oblongifolia* and *V. unguiculata* on the other hand is indicative of their genetic closeness and possible crossability. With respect to the above observation, *V. racemosa*, *V. luteola* and *V. oblongifolia* could be used as potential bridging species in the hybridization between *V. vexillata* and *V. unguiculata*. Adegbite (2006) and Olatunde *et al*. (2007) separately reported the closeness and possibility of crossing wild *Vigna* species especially *V. vexillata* with *V. unguiculata* to produce a viable offspring. The highest phenetic distance observed was in *V. oblongifolia* with coefficient of 62.685 while the minimum phenetic distance observed was among the following species; *V. vexillata*, *V. luteola*, *V. racemosa* and *V. unguiculata* (PJ2K-0N2 and PJ2K-0N5). The result of the principal component analysis indicated the consistency and stability of reproductive characters in the taxonomic delimitation of the species in the genus *Vigna*. The PCA also confirmed a high phenetic relationship among the species. High phenotypic/genetic diversity among related plant groups or species is a good factor for genetic improvement for species in the group, such that some of the species/accessions with superior or better characteristics or traits can be used as parents for breeding purposes. This principle can be adopted for the *Vigna* species studied for genetic improvement of cowpea i.e., *V. unguiculata*.

Some of the desirable characters found in wild *Vigna* species that can be explored for genetic improvement of cowpea are hairiness, abundant pod production, high number of locules and seeds per pod, longer life span and extensive branching habit which could lead to higher seed yield. Such observations have been reported for *Vigna unguiculata* (cowpea), *Cajanus cajan* (pigeon pea) and *Sphenostylis stenocarpa* (African Yam Bean) (Popoola *et al*., 2011). The variability observed in the hairiness (glabrescent to pubescent among the wild *Vigna* species and smooth for the cultivated species) on plant parts, flower colour and size and shape of leaves (rhombic, subhastate, ovate) are quite remarkable. All the wild *Vigna* species were observed to be prolific in pod production. Some of the attributes of the wild *Vigna* species especially, *V. vexillata* are also highly desirable. The characteristic hairiness of leaves and pods is a desirable character which could be exploited in breeding for host plant resistance to insect pests. This trait is not prominent among the cultivated cowpea. In addition to using the *Vigna* species for breeding purposes, some of the species can be utilized as animal feed/fodder and for medicinal purposes. This knowledge can be expanded through cultivation and utilization of the *Vigna* species. The phytochemicals present in the wild species that are utilized as medicine in some parts of Africa need to be analyzed. Further studies on this group of plants could be directed towards agro-morphological and molecular characterization of the species in the genus *Vigna* for genetic evaluation of diversity and similarity to unravel the taxonomic complications in the genus and to channel the researches for genetic improvement of the cultivated species in the genus.

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