

Morphological Characterization of Selected Cowpea [*Vigna unguiculata* (L.) Walp.] Accessions from International Institute of Tropical Agriculture, Ibadan, Nigeria

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# ABSTRACT

Characterization of available germplasm is a necessary step to facilitate breeding programs worldwide. Therefore, this study aimed to characterize twenty accessions of Cowpea from the International Institute of Tropical Agriculture, Nigeria. The accessions were planted in a randomized complete block design with three replicates. The morphological characters of seedlings were assessed following the International Board for Plant Genetic Resource cowpea descriptor list (IBPGR) and twenty-nine descriptors were used. Eighteen accessions showed semi-erect habits while two showed erect plant growth habits. The number of Days to first flowering among the selected accessions was 35-45 while the number of days to first pod was 37-47. Peduncle length was observed to be 12-35 cm, Pod length was 10.61-22.81 cm while seed length was 6.10-9.93 cm among sampled accessions respectively. One accession TVu-12501 formed an outgroup owing to the uniqueness of its terminal petiole length. The cluster analysis substantiated the existence of diversity among the twenty accessions for the morphological traits studied and posits TVu-12501 accession as a morphologically superior accession to be studied further for selection in breeding programmes in the West African region.

Keywords: Characters, Cowpea, Diversity, IITA, Poaceae, Phenotype

# INTRODUCTION

Cowpea (Vigna unguiculata (L.) Walp), is one of the ancient and most important human food source and forage legume crops in the world (Zimba et al., 2022). It is a self-pollinating dicotyledonous crop plant belonging to the family Fabaceae and native to Central Africa. It is an annual herbaceous legume cultivated for its edible seeds or fodder. Cultivated cowpeas are either erect, prostrate, or climbing annuals with a tap root and virtually all are glabrous. They are mostly grown for grain but a small proportion (about 10%) are grown as green leafy vegetables and fodder in Africa or as fresh pods in eastern Asia (Boukar et al., 2015). Cowpeas are of vital importance to the livelihood of several millions of people in West and Central Africa. Rural families that make up the larger part of the population of these regions derive food, animal feed and income from the cultivation of the crop (Gerrano et al., 2015). According to Nameirakpam and Khanna (2018), its chromosome is diploid (2n=2x=2). The understanding of its genetic diversity and the relationship that exists among its accessions is of utmost importance. This helps to improve the available genetic resources in any hybridization program (Mafakheri et al., 2017), eliminate duplications within the germplasm and select representative samples for utilization and conservation in gene banks (Karapanos et al., 2017).

Genetic diversity studies help in crop breeding by analyzing the genetic variability in cultivars, identifying diverse parental combinations to create segregating progenies with maximum genetic variability for further selection as desirable genes are introgressed from diverse germplasm into the available genetic base (Xiong *et al.*, 2016). Hence it is a very important step in breeding for agronomic and

economically desired traits (Nameirakpam and Khanna, 2018). Variation studies have long been initiated by researchers to determine viability status for breeding programs due to their importance in guantifying trait expression. The degree of genetic variety present in the offered crop species determines the potential enhancement of crop plants (Arya et al., 2021). Hence characterization and cataloging of genetic resources is a vital condition for an effective improvement program. Researchers have the chance to create new, enhanced varieties with desired qualities that satisfy the preferences of both growers and breeders, owing to plant genetic variety (Kumar et al., 2015). Plant morphological variation typically provides feedback for the evolving climatic circumstances and indicates adaptive evolution, even though genetic variation is more precise in assessing variety. This helps breeders in taking into account both the innate genetic makeup and environmental influences involved in crop improvement (Karuwal et al., 2021). Researches then study genetic variations and diversity by observing phenotypic expressions of the plant through the use of morphological characters.

Gerrano *et al.* (2015), examined the genetic variability in cowpea genotypes and reported 79.30% variability among the genotypes with cluster analysis showing five distinct groups of genotypes. Also in 2015, Animasaun *et al.* (2015) studied genetic variability among ten cultivars of cowpea using morpho-agronomic traits and nutritional composition. Their results showed that the cultivars were divided into two broad genetic groups (A&B). Group A consist of two clusters of low performing cultivars in terms of economic traits and Group B comprised two clusters of superior

cultivars for most of the traits studied. Mafakheri *et al.* (2017), studied the genetic diversity in cowpea germplasm using morphological and molecular characters. They found that there was genetic similarity across all the genotypes. Similar morphological characterization studies from Menssen *et al.* (2017), Lazaridi *et al.* (2017), Ajayi *et al.* (2017) and El-Nahrawy, (2018) all showed high variation among accessions although cluster analysis showed no more than three groups in any of the studies. These observed variations have tremendous implications for breeding programs as they showcase the broad range of traits and genotypes that breeders have to work with in breeding for desirable traits (Mofokeng *et al.*, 2020). The practical applications of these variations are also tied to their phenotypic expressions.

More recently, Karuwal et al. (2021), examined the seed of regional cowpea cultivars from Southwest Maluku. Indonesia for their morphological and nutritional characters. Their findings demonstrated that the samples varied significantly. Similarly, Gomes et al. (2021), investigated the diversity of Cowpea in Mozambique with the goal of breeding for trait expression. Their findings showed that local landraces maintain a high degree of morphological variety without following a particular geographic pattern, which suggests that there has been gene movement. However, accessions from a single landrace. Maringué, appeared to be the most viable in terms of productivity and nutrition-related characteristics. Thus, might be incorporated into the current preservation and breeding efforts in the area towards the generation of elite cowpea varieties. Variations in Cowpea have tremendous implications for breeding programs as they showcase the broad range of traits and genotypes that breeders have to work with in breeding for desirable traits (Mofokeng et al., 2020). In this study, our aim was to assess the genetic variation existing among selected cowpea accessions for potential use in cowpea breeding and improvement programmes in the West African sub-region.

#### MATERIALS AND METHODS Sample Collection

All the cowpea seeds that were used for the purpose of this research were obtained from International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State Nigeria (Table 1).

# Seed Planting and Germination

The research was carried out in a screen house which was built in the Botanical Garden of the University of Lagos, Lagos, Nigeria. The screen house was constructed to protect the plants from insects and other forms of pest attacks. The structural framework of the screen house was made of wood which was painted in other to prevent insects from feeding on it and it was covered with mosquito nets. A white transparent nylon was used in the roofing of the screen house. Seeds of each accession were planted in 5 replicates using planting bags and properly labeled using tags made from cardboard. Adequate spacing of samples was ensured within the screen house in other to give equal chances of survival and avoid overcrowding. The plants were watered between 7 am and 8 am every other day, throughout the duration of the research. The entire screen house and the surroundings were kept clean and weeding was done by handpicking. The germination period was recorded for all the samples.

#### Morphological Characterization

Twenty accessions of Cowpea were planted and characterized using IPGRI (1987) descriptors. The experiment was carried out at the Botanical Garden of the Department of Botany, Faculty of Science University of Lagos, Akoka using randomized complete block design [RCBD]. The layout was done in 3 replicates with a plot size of  $3 \text{ m}^2$ , spacing of 0.75 - 1 m inter-row spacing, and 0.5 m for intra row spacing on the field. Measurements were taken weekly early in the morning using a meter rule.

#### Data Analysis

All the measurements were performed in replicates. Descriptive statistics of mean of the replicate values was calculated using Microsoft Excel package. Individual and group mean  $\pm$  standard deviations (SD) values were calculated for the data. Pair-wise analysis of the morphological characters recorded was carried out by computing distance matrices for the characters assessed following Onuminya *et al.* (2021). Hierarchical cluster analysis (HCA) was performed using Ward's clustering method and squared Euclidean distance while a dendogram was generated following Quesada *et al.* (2009).

# RESULTS

All the seeds of the selected cowpea accessions planted in the screen house germinated and were pruned to five plant stands per accession. Some of the seeds germinated within four days while others germinated between 5-7 days. Table 2 shows the qualitative morphological characteristics of the cowpea accessions studied. Eighteen of the accessions showed semi-erect growth habits while two showed erect habits. The majority of the accession exhibited intermediate twinning (n=17) while few (n=3) exhibited slightly twinning character. Extreme (n=1), moderate= (n=1), slight = (n=16) and no (n=2) plant pigmentation was recorded. Raceme position observed include above the canopy (n=6), throughout the canopy (n=3) and upper canopy (n=11). The flower color is either pink (n=13), white (n=4) or violet (n=3)among the accessions studied. All the accessions showed flower pigmentation except TVu-12501. The pod shape was straight (n=2) and slightly curved (n=18) among the evaluated samples. On the pod, uniform, splashes, and tip pigmentation were observed in TVu-12100, TVu-15096, and TVu-10826, respectively.

 Table 1: List of cowpea samples used in the study

S/N	ACCESSION NAME	COUNTRY OF ORIGIN	CULTIVAR NAME	DIGITAL OBJECT IDENTIFIER
1	TVu-4595	Tanzania	S.V.S.46	10.18730/ZMPZ
2	TVu-10826	Cameroon	EX MOGODE	10.18730/NM3N
3	TVu-11472	Philippines	UPLB-113	10.18730/P85\$
4	TVu-11477	Philippines	UPLB-118	10.18730/P8A2
5	TVu-11502	Philippines	UPLB-913	10.18730/P93V
6	TVu-11503	Philippines	UPLB-915	10.18730/P94W
7	TVu-11979	Sudan	TB79-914	10.18730/PQPD
8	TVu-12084	India	APC 451	10.18730/PTZ7
9	TVu-12100	India	APC 537	10.18730/PVFQ
10	TVu-12482	Zambia	ZM 2091	10.18730/N0R1Z
11	TVu-12485	Zambia	ZM 2136	10.18730/N0R4\$
12	TVu-12501	Zambia	ZM 2803	10.18730/N0RMD
13	TVu-13196	India	APC 131B	10.18730/N1DYX
14	TVu-13659	Nigeria	AO83-24	10.18730/QD3*
15	TVu-15096	Malawi	AG87M-196	10.18730/HT76
16	TVu-16015	Botswana	B-442	10.18730/JNCR
17	TVu-16071	Somalia	AFGOI	10.18730/JQ35
18	TVu-16123	Zimbabwe	PS87Z-308	10.18730/JRQM
19	TVu-16666	Uganda	UCC - 17	10.18730/K7MG
20	TVu-16805	Cameroon	OU 16 B	10.18730/KB9P

There were variations in the quantitative traits measured (Table 3). Days of first flowering among the samples ranges between 35-45 days while days of first pod appearance range between 37-47 days. Peduncle length is 12 -35 cm while pod length is 10.61-22.81 cm and pod width are 6.22-9.88 cm among sampled accessions. Seed length is 6.10-9.93 cm and seed thickness is 3.35-5-75 cm. The cluster analysis based on data matrices generated from the morphological characters of the selected accessions resolved the twenty sampled accession at about 0.24 similar co-efficient while the accessions were grouped as one at a similarity level of 0.04 (Fig. 1). There are various levels between these two extremes which gave rise to different clusters and sub-cluster, with the accession survey as an operational taxonomic unit (OTU).

The OG is a unique accession with diverse traits different from others, but at 0.09 it has a similarity with cluster A. This is the only accession that has terminal petiole length, terminal rachis length, and peduncle length distinct from the others. Cluster B consists of 9 accessions (TVu-11472, TVu-12100, TVu-16193, TVu-13659, TVu-16015, TVu-12482, TVu-13196, TVu-16071 and TVu-16805) with two sub cluster (B1 and B2). The first sub-cluster (B1) consists of five accession (TVu-11472, TVu-12100, TVu-16193, TVu-13659 and TVu-16015,) which were closely related at about 0.137 similarity coefficient. The second sub-cluster comprises four clusters (TVu-12482, TVu-13196, TVu-16071., TVu-16805). Cluster C consists of four accessions (TVu-11477, TVu-11979, TVu-11502 and TVu-15096) with two sub-cluster (C1 and C2) having two accessions each (TVu-11477, TVu-11979) and (TVu-11502, TVu-15096), respectively

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ACCN NO	PPP	PGH	TERMINAL LEAFLET SHAPE	TWINNING TENDENCY	DETERMINACY	RP	FLOWER COLOR	FPP	PS	POPP	EC	TESTA TEXTURE
TVu-4595	Very slight	SE	Globose	Intermediate	Determinate	AC	Pink	WP	SC	NP	BS	Intermediate
TVu-10826	Very slight	SE	Globose	Intermediate	Indeterminate	ТС	Pink	WP	SC	TP	BS	Intermediate
TVu-11472	Very slight	SE	Sub-hastate	Intermediate	Indeterminate	UC	Pink	WP	SC	NP	BS	Intermediate
TVu-11477	Very slight	SE	Sub-hastate	Intermediate	Indeterminate	UC	Violet	WP	SC	NP	BS	Intermediate
TVu-11502	Very slight	SE	Sub-globose	Intermediate	Determinate	AC	Pink	WP	SC	NP	Green	Intermediate
TVu-11503	Very slight	SE	Globose	Intermediate	Indeterminate	UC	White	WP	SC	NP	BS	Intermediate
TVu-11979	Very slight	SE	Sub-globose	Intermediate	Indeterminate	UC	Violet	WP	SC	NP	BS	Intermediate
TVu-12084	Very slight	SE	Sub-hastate	Intermediate	Determinate	UC	Pink	WP	SC	NP	BS	Intermediate
TVu-12100	Extensive	SE	Hastate	Intermediate	Determinate	AC	Pink	WP	SC	UP	BS	Intermediate
TVu-12482	Moderate	SE	Sub-globose	Intermediate	Indeterminate	UC	Pink	WP	SC	NP	BS	Smooth
TVu-12485	Very slight	SE	Sub-globose	Intermediate	Determinate	ТС	Pink	WP	SC	NP	BIS	Intermediate
TVu-12501	Very slight	SE	Globose	Intermediate	Determinate	UC	Pink	NP	ST	NP	BS	Smooth
TVu-13196	Very slight	SE	Sub-hastate	Intermediate	Determinate	UC	Violet	WP	SC	NP	BS	Intermediate
TVu-13659	None	SE	Sub-hastate	Slight	Determinate	UC	White	WP	SC	NP	Ab	Intermediate
TVu-15096	None	SE	Sub-hastate	Intermediate	Indeterminate	ТС	Pink	WP	SC	SP	BIS	Intermediate
TVu-16015	Very slight	Е	Sub-hastate	Slight	Indeterminate	UC	Pink	WP	ST	NP	BIS	Intermediate
TVu-16071	Very slight	SE	Globose	Slight	Indeterminate	AC	White	WP	SC	NP	BS	Intermediate
TVu-16193 TVu-16666	Very slight Very slight	E SE	Sub-hastate Sub-hastate	Intermediate Intermediate	Determinate Indeterminate	AC UC	Pink White	WP WP	SC SC	NP NP	BS BS	Smooth Intermediate
TVu-16805	Very slight	SE	Hastate	Intermediate	Indeterminate	AC	Pink	WP	SC	NP	BS	Intermediate

 Table 2: Qualitative morphological evaluation of the selected cowpea accessions

\*Values represents mean of data obtained.

Key: Accn no: Accession number PPP: Plant Pigmentation Pattern PGH: Plant Growth Habit - SE: Semi-erect; E: Erect; FPP: Flower pigmentation Pattern - WP: Wing Pigmented; RP: Raceme Position – AC: Above Canopy; TC: Throughout Canopy; UC: Upper Canopy PS: Pod Shape – SC: Slightly curved; ST: Straight; PoPP: Pod Pigmentation Pattern - NP: None; UP: Uniform; TP: Tip; SP: Splash EC: Eye Color; Splash; BIS: Black Spot; BS: Brown Ab: Absent Nigerian Journal of Basic and Applied Sciences (June, 2023), 31(1): 65-72

ACCN_NO	DFF	DFP	NMB	NMSN	PL	TLL	TLW	FW	CL	CLL	TPL	LRL	POL	NL/P	PW	SL	ST
TVu-4595	40	42	7	19	28.3	10.4	8.7	2.6	0.54	0.30	12.9	16.0	15.7	17.2	7.6	6.4	4.3
TVu-10826	40	42	2	19	29.7	8.7	6.0	2.9	0.52	0.30	7.9	10.2	17.6	14.0	9.1	8.7	5.1
TVu-11472	39	41	4	15	27.3	9.0	6.4	2.6	0.56	0.32	8.8	11.2	18.3	16.8	8.9	8.5	4.6
TVu-11477	41	43	5	18	29.6	11.5	8.8	3.0	0.54	0.34	10.4	13.3	17.3	19.8	7.3	7.1	4.6
TVu-11502	38	40	5	14	26.8	11.1	6.6	2.6	0.50	0.32	11.1	13.6	15.2	17.2	6.3	6.8	3.8
TVu-11503	35	37	4	14	28.0	9.1	5.8	3.0	0.52	0.34	8.0	10.6	14.3	14.4	6.3	6.5	3.5
TVu-11979	40	42	5	18	31.4	12.3	7.6	3.1	0.54	0.32	7.3	10.6	20.1	20.0	8.0	6.5	3.7
TVu-12084	37	39	2	12	30.5	12.5	7.5	2.7	0.52	0.30	9.2	11.9	22.8	12.8	7.7	8.8	4.2
TVu-12100	39	41	4	12	29.4	10.6	5.9	2.5	0.54	0.32	7.5	9.5	15.2	13.6	6.3	7.5	3.9
TVu-12482	42	44	4	16	12.3	10.5	6.9	2.9	0.54	0.34	9.6	12.3	15.4	14.2	9.9	9.7	5.2
TVu-12485	40	42	4	12	16.8	9.8	6.4	2.8	0.52	0.34	6.4	8.5	22.0	18.4	7.8	9.3	5.8
TVu-12501	44	46	7	18	30.4	10.7	7.5	2.7	0.52	0.32	15.1	18.8	16.0	20.8	6.9	6.7	3.9
TVu-13196	38	40	4	13	29.2	7.7	4.6	2.8	0.54	0.34	6.7	8.9	17.1	15.0	6.9	7.6	4.6
TVu-13659	35	38	4	15	22.6	7.7	5.9	2.7	0.54	0.30	6.5	8.5	13.7	11.0	7.3	9.9	5.0
TVu-15096	38	40	3	10	22.0	11.9	8.7	2.6	0.54	0.36	11.6	14.7	17.6	17.4	8.1	8.2	4.6
TVu-16015	36	38	4	13	25.5	7.4	6.0	2.6	0.54	0.32	9.5	11.7	10.6	11.0	7.0	6.4	4.6
TVu-16071	42	45	4	17	34.6	9.7	8.2	3.0	0.54	0.36	8.9	11.7	16.6	16.0	6.2	6.1	4.5
TVu-16193	37	39	4	11	23.3	9.8	7.8	2.8	0.54	0.32	9.7	12.3	20.2	14.0	7.7	7.3	3.8
TVu-16666	40	42	5	20	22.2	11.5	9.1	2.9	0.52	0.34	11.0	14.4	16.8	17.0	6.8	6.8	3.7
TVu-16805	45	47	7	17	16.4	11.7	7.1	2.5	0.54	0.34	11.5	14.1	13.8	15.2	6.4	6.9	3.7

Table 3: Quantitative morphological evaluation of the selected cowpea accessions

\*Values represents mean of data obtained. All measurements of length, width and thickness are in centimeters (cm)

Key: Accn no – Accession number, DFF - Days to first flowering, DFP - Days to first pod, NMB - Number of Main Branches, NMSN - Number of Main Stem Nodes, PL - Peduncle Length, TLL - Terminal leaflet Length, TLW - Terminal leaflet Width, FW - Flower Width, CL - Calyx Length, CLL - Calyx Lobe Length, TPL - Terminal Petiole Length, LRL - Leaf rachis Length, POL - Pod Length, NL/P - Number of Locules/pod, PW - Pod Width, SL - Seed Length, ST - Seed thickness.

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**Figure 1:** Similarities among the selected cowpea accessions studied based on morphological characters. **Key:** TVu represents the assigned accession code for the cowpea studied with corresponding identifier numbers. **Note:** Accessions were resolved into three main clusters (A, B and C) with two sub-clusters each (A1, A2; B1, B2; and C1, C2 respectively) and a distinct operational unit (OG) at similarity coefficient 0.09.

### DISCUSSION

Many researchers have used morphological traits to characterize cowpea such as plant pigmentation, plant height, pod traits, seed traits, etc. These traits were all found to be of great importance to assess the level of genetic variability and have led to a better classification of cowpea species. Our study showed great variations in morphological traits such as in the growth forms with only two being erect forms while the rest are semi-erect. This variation is suspected be as a result of adjustment to different environmental factors overtime and as a result of breeding and hybridization efforts over many years. This is consistent with the findings of Ajavi et al. (2017) and El-Nahrawy (2018). Differences in phenotypic characters such as growth style (twinning or non-twinning), flower colour, pod shape and germination period were also reported by Gerrano et al. (2015), Animasaun et al. (2015) and Mafakheri et al. (2017). The work agrees that different accessions with different genotypes will have differences in growth form, growth shape and growth time. Our study observed differences in phenology and petiole length which are important traits in morphological characterization. This is similar to the reports of Lazaridi et al. (2017), Ajavi et al. (2017) and El-Nahrawy (2018) on cowpea morphology assessment.

Morphological variation is the first step utilized by traditional farmers during selective breeding for creating hybrids with desired traits. While the accessions showed variations in phenotypic expression after germination and during growth phases, it is hard to determine if such variations were due to environmental conditions (soil, humidity, water etc.) or inherent genetic composition. Previous researchers have reported usage of such variation in their cluster analysis to provide clusters and aid in their overall analysis. This is always done to provide insight into the possible evolutionary relationship between the accessions under examination (Horn and Shimels, 2020). The constructed dendogram revealed that the accessions were grouped into three clusters with observable variation hinting at common ancestry for all three clusters due to similarities and presence of common dominant features in all. The dendrogram revealed that one accession (TVu-12501) had a markedly different petiole length compared to the others with its petiole length being terminal. It also had terminal rachis length, and peduncle length distinct from the other accessions. These attributes separated it as an out-group from the other accessions in the dendogram. Outliers in variations of this nature are usually as a result of previous selective breeding or a result of extreme environmental adaptation (Iseki et al., 2020). The resultant accession showing this marked difference in genotype will confer advantages over the other accessions due its evolving trait adaptation. Inculcation of TVu-12501 accession into future breeding programs for further examination could result in transmission of its superior genotypic traits down to future progenies.

This study is significant for having revealed this particular variation among the selected accessions as previous studies had no such high variation between clusters or any outlier accessions. The study recommends that further studies be carried out on accession TVu-12501 to determine its desired traits that may be transferred into other accessions for breeding and expression.

# CONCLUSION

The study found significant genotype variation, which may be a result of unique environmental factors or responses. The variations observed in the accessions verify the work of breeders and taxonomists as to the grouping of the accessions and their attempts to make them better. The outgroup accession (TVu-12501) might prove valuable in breeding practices hence a need for closer inspection.

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