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RESEARCH ARTICLE

Characterization, evaluation and diversity studies of cowpea germplasm (*Vigna unguiculata* L.)

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Abstract

Characterization of the genetic diversity among cowpea (*Vigna unguiculata* L.) accessions is essential for breeding improved cultivars. This evaluated 50 cowpea accessions using 13 qualitative morphological traits. Twelve traits (92.3%) were polymorphic, each displaying at least two phenotypic classes, while leaf marking was monomorphic. The Shannon-Weaver diversity index ranged from 0 (monomorphic trait) to 0.38 (growth habit), indicating moderate diversity. The principal components analysis revealed that the first three components accounted for 47.97% of total variation, with eigenvalues of 2.46, 1.88 and 1.41, respectively. Cluster analysis showed clear differentiation among accessions, though some formed closely related groups. The results suggest the existence of useful genetic variability among the accessions, with those from distant clusters offering potential for use in breeding programs to broaden the genetic base of cowpea.

Keywords: Cowpea, variability, traits, diversity, characterization

Introduction

Cowpea (*Vigna unguiculata* L.) is a highly valuable legume crop, serving as a major source of dietary protein, vitamins, and minerals for millions of people in tropical and subtropical regions (Singh *et al.,* 2002; Timko and Singh, 2008). In addition to its nutritional value, cowpea plays an important role in improving soil fertility through biological nitrogen fixation and is widely used as animal feed and a cover crop (Fatokun *et al.,* 2002). Despite its significance in food security and sustainable agriculture, cowpea production faces numerous challenges, including limited genetic diversity, susceptibility to pests and diseases, and environmental stresses such as drought and poor soil conditions (Ehlers and Hall, 1997). Effective crop improvement programs require the identification and utilization of diverse genetic resources. In this regard, morphological characterization, particularly through qualitative traits, is a critical first step (Mendes *et al.,* 2020). Qualitative traits are distinct, visually observable characteristics controlled by a small number of genes and are less influenced by environmental factors (NARO, 2000; IBPGR, 1983). Traits such as growth habit, leaf shape, flower colour, pod form, and seed coat colour are key indicators of genetic variation and serve as reliable tools for distinguishing between genotypes (Kouam *et al.,* 2012;). The systematic breeding programme involves the steps like creating genetic variability, practicing selection and utilization of selected genotypes to evolve promising varieties (Katora *et al.*, 2018).

The study of qualitative morphological traits offers several advantages for cowpea improvement. It enables breeders to classify and cluster accessions, facilitating the identification of genetically diverse lines for use in hybridization (Omoigui *et al.,* 2006). Diverse parental lines, selected based on qualitative trait variation, increase the likelihood of obtaining superior progeny with desirable attributes such as improved yield, resistance to pests and diseases, and better adaptability to environmental conditions (Gerrano *et al.,* 2015). Additionally, morphological characterization aids in the conservation and management of cowpea germplasm by identifying unique accessions that can broaden the genetic base of breeding programs (Adebisi *et al.*, 2001). Although molecular characterization techniques are increasingly used in diversity studies, qualitative morphological trait analysis remains a simple, rapid, and cost-effective approach for the preliminary evaluation of large germplasm collections (Fatokun *et al.*, 1997). It provides valuable baseline information that can guide further detailed genetic analysis and breeding efforts. Therefore, this study was conducted to characterize fifty cowpea accessions collected from diverse locations using qualitative morphological traits. The aim was to assess the extent of genetic diversity among these accessions, identify distinct groups through cluster and principal component analysis, and ultimately provide essential information that can support cowpea breeding programs aimed at developing improved cultivars with a broader genetic base.

Materials and methods

The study was conducted at Chuka University Kairini farm (00°23'51.4"S, 037°46'24.0"E) at an elevation of 1400 m above sea level. The site receives approximately 300-700 mm annual rainfall with an average temperature of 28°C. Fifty cowpea accessions were selected from a preliminary evaluation of 208 accessions based on earliness, drought tolerance, pest resistance, and leaf production potential. These included 21 accessions from the International Institute of Tropical Agriculture (IITA, Nigeria), 27 from the National Gene Bank of Kenya (KALRO-Muguga), and 2 farmer-preferred varieties from western and eastern Kenya. The experiment employed a Randomized Complete Block Design (RCBD) Each experimental unit measured 4.2 m × 1.5 m, with replicates ridged 1.0 m apart. Accessions were planted in single rows (1.0 m long) with spacing of 60 cm within rows and 30 cm between rows. KK06 was used as the check in the experiment. Data was collected using the International Board for Plant Genetic Resources (IBPGR) cowpea descriptor. The qualitative data included: growth pattern, twining tendency, plant pigmentation, terminal leaflet shape, raceme position, pod attachment to peduncle, immature pod pigmentation, leaf colour intensity, leaf marking, mature pod curvature growth habit, plant hairiness, seed colour and flower colour.

Qualitative data was analyzed using goodness of fit Chi-square test. The Shannon-Weaver index (H) was computed using phenotypic frequencies to assess the diversity for each character for the 50 accessions and the entire set of F1s and their parent populations according to Hutchenson (1970) as follows:

$$H = -\sum_{i=1}^{n} P_i log_e P_i,$$

where p1 is the proportion of accession in the ith phenotype and n is the number of classes for given character. The standardized H´ ranging from 0 to 1 was obtained by dividing H by the loge of the total number of phenotypic classes as follows:

$$H^{`} = \frac{H}{log_e n}$$

Euclidean distances were used for cluster analysis.

Results and discussion

The Shannon-Weaver diversity index (H′) was used to evaluate diversity among 13 qualitative traits (Table 1). Growth habit showed the highest diversity (H′ = 0.38), followed by mature pod curvature (H′ = 0.36). These traits, which influence plant architecture and agronomic performance, are commonly reported as variable in cowpea germplasm and are considered important for yield improvement and adaptation (Ghalmi *et al.*, 2010). In contrast, leaf marking showed no diversity (H′ = 0), indicating a monomorphic trait across all accessions. This uniformity suggests either low genetic variation or stabilizing selection, possibly due to farmer preferences or adaptive significance under specific environments (Khady *et al.,* 2023). The observed regional variation in polymorphism levels for most traits (except leaf marking) highlights the role of geographic origin and farmer selection in shaping diversity. These findings underline the importance of considering regional sources when selecting parents for breeding programs aimed at maximizing genetic gain and enhancing resilience. Distinctive differentiation of characters among the genotypes was observed in leaf shape, pod curvature, seed colour, leaf colour and pod colour. The results are in agreement with those of Onuminya *et al.*, (2023) who found existence of wide diversity for the morphological traits in cowpea accessions. Six growth habits were observed ranging from climbing (32%), semi-erect (32%), intermediate (22%), acute erect (4%), prostrate (6%), and semi-prostrate (4%) (Table 1), suggesting a wide phenotypic plasticity within the germplasm. Growth habit significantly agronomic trait as it influences plant density, weed control and harvesting efficiency. Specifically, erect and semi-erect are preferred in mechanized or high-density planting systems due to their reduced lodging and improved harvestability (Karikari *et al.*, 2023; Cobbinah *et al.*, 2011). Wide range of phenotypic variability, were observed in all the characters indicated large amount of variability among the genotypes for these traits. The results were in agreement with those of (Katore *et al.*, 2018). The variation in twining tendency further supports the genetic diversity among the accessions. A majority (54%) exhibited pronounced twining, while 32% had intermediate and 12% slight twining (Table 1). This distribution suggests that twining is a polygenic trait, likely influenced by environmental interactions and multiple genetic factors. These findings are consistent with Barik *et al.*, (2023), who identified three twining tendency groups in cowpea. The presence of both non-twining and twining forms among the accessions makes this germplasm highly valuable for breeding programs targeting dual-purpose varieties as well as for optimizing performance under diverse cultivation systems. The findings of this study showed that hat indeterminate growth (74%) is predominant within the germplasm studied (Table 1). This is significant from a breeding perspective, as determinate types are often preferred for mechanized agriculture and uniform maturity, making them suitable for commercial grain production (Kumar *et al.*, 2015).

Table 1: Phenotypic frequency and diversity of qualitative traits

Variable	**Description**	**Frequency**	**Percentage**	**Shannon waver index (H)**	**Standardised Shannon-Weaver index (H')**
Immature pod pigmentation	None	30	60.0	1.27	0.32
	Pigmented tip	7	14.0		
	pigmented sutures	2	4.0		
	Pigmented valves	2	4.0		
	Splashes of pigment	3	6.0		
	Uniformly pigmented	6	12.0		
	Total	50	100.0		
Plant hairiness	Glabrescent	13	26.0	0.94	0.24
	short appressed	30	60.0		
	Pubescent	7	14.0		
	Total	50	100.0		
Terminal leaflet shape	Globose	17	34.0	0.74	0.19
	Sub globose	32	64.0		
	Sub hastate	1	2.0		
Growth pattern	Determinate	13	26.0	0.58	0.15
	Indeterminate	37	74.0		
Leaf marking	present	50	100	0	0
	Absent	0	0		
Leaf color intensity	Pale green	9	18.0	1.00	0.26
	Intermediate green	27	54.0		
	Dark green	14	28.0		
	Total	50	100.0		
Growth Habit	Acute erect	2	4.0	1.50	0.38
	Semi-erect	16	32.0		
	Intermediate	11	22.0		
	Semi prostrate	2	4.0		
	Prostrate	3	6.0		
	Climbing	16	32.0		
	Total	50	100.0		

Table 1(Continued): Phenotypic frequency and diversity of qualitative traits

Variable	Description	Frequency	Percentage	Shannon waver index(H)	Standardised Shannon-Weaver index(H’)
Twinning tendency	None	2	4.0	1.10	0.28
	Slight	6	12.0		
	Intermediate	16	32.0		
	Pronounced	26	52.0		
	Total	50	100.0		
Flower colour	violet	41	82	0.54	0.14
	white	8	16		
	Pink	1	2		
Raceme position	Mostly above canopy	0	0	0.77	0.20
	In upper canopy	3	6		
	Throughout	47	94		
Mature pod curvature	Straight	27	54	1.41	0.357
	Slightly curved	22	44		
	Curved	1	2		
	Coiled	0	0		
Seed colour	Brown	10	20	0.71	0.18
	Cream	18	36		
	Black	8	16		
	Gray	10	20		
	White	2	4		
	Maroon	2	4		

In contrast, indeterminate accessions (24%) offer advantages in subsistence or dual-purpose systems, where prolonged pod production or biomass is beneficial (Singh *et al.*, 2003). The observed ratio implies that growth habit may be controlled by a major gene or few loci. The observed plant pigmentation varied considerably from no pigment (8%) to extreme (2%) pigmentation (Table 1), demonstrates a broad phenotypic spectrum among the cowpea accessions. The majority of accessions exhibited moderate pigmentation (54%) with only a small proportion showing extreme pigmentation. This distribution suggests that pigmentation intensity is quantitatively inherited, likely controlled by multiple alleles with additive or cumulative effects. The variation in expression levels points to polygenic inheritance or the action of epistatic gene interactions, rather than a simple Mendelian pattern.

These findings are consistent with the genetic model proposed by Ishiyaku and Singh (2004), who reported that two genes, interacting through dominant and recessive epistasis, condition vegetative pigmentation in cowpea. Understanding the genetic basis of pigmentation is useful not only for taxonomic classification but also for marker-assisted selection, especially when pigmentation is linked to traits like stress tolerance or pest resistance. The observed seed colors diversity, which included cream (36%), brown (20%), grey (20%), black (16%), white (4%), and maroon (4%) (Table 1), indicates substantial phenotypic variation among the cowpea accessions. The presence of clearly distinguishable color categories suggests that seed color is qualitatively inherited, most likely under the control of a few genes with multiple alleles. This is consistent with recent genome-wide association studies, which identified four major genes involved in control of seed color in cowpea (Xiong *et al.,* 2024). These genes may act independently or interact epistatically to produce the diverse pigmentation patterns observed. Given that seed color can influence consumer preference and marketability, this trait holds importance for both breeding and commercialization efforts. Similarly, flower color was dominated by violet (82%), followed by white (16%), suggesting qualitative inheritance. The limited number of color classes and their clear segregation strongly support monogenic inheritance, as previously reported by Rawal *et al.,* (1976). Together, the variation in seed and flower color highlights the genetic diversity within the studied accessions and reinforces the value of morphological traits for selection, classification, and understanding inheritance patterns. The findings if the studied revealed that the majority of the accessions (94%) had racemes distributed throughout the plant canopy, while 6% had racemes above the canopy. Accessions with racemes above the canopy are agronomically advantageous as they facilitate easier harvesting and reduce pod loss, particularly in mechanical or large-scale production systems. These findings are consistent with those of Pandey and Ngarm (1985) and Bennett-Lartey and Ofori (1999), who emphasized the breeding potential of such traits for improving harvesting efficiency. In case of pod curvature, the majority of accessions had straight pods (54%), while others were slightly (44%) slightly curved or curved (2%). It suggests that pod shape is under the control of multiple alleles, which is consistent with genetics studies that have implicated 3 to 4 genes controlling pod shape in cowpea (Nwofia, 2014). The predominance of straight pods is notable, as they are often preferred in the market due to better packing, visual appeal and ease of processing (Salk *et al.,* 2008; Yanmaz, 2010). This makes pod curvature an important trait in breeding programs targeting both yield and market quality. Immature pod pigmentation showed great variation, which included no pigmentation (60%), pigmented tip (14%), uniformly pigmented (12%), pigmented splashes (6%), pigmented sutures (4%), and pigmented valves (4%). This distribution indicates a complex inheritance pattern. This broad spectrum of pigmentation types supports earlier findings by Porter et al. (1974) and Mustapha and Singh (2008), who reported multiple gene interactions governing pod pigmentation. Such variability not only contributes to the visual distinctiveness of accessions but may also be linked to physiological traits such as anthocyanin accumulation and stress response, making it relevant for both aesthetic and adaptive breeding goals. The observed variation in terminal leaf shapes (sub-globose (64%), globose (34%), and sub-hastate (2%) reflects a moderate level of morphological diversity among the studied cowpea accessions. The predominance of sub-globose and globose leaf types is consistent with patterns reported in other studies and may be influenced by both selection pressures and genetic factors.

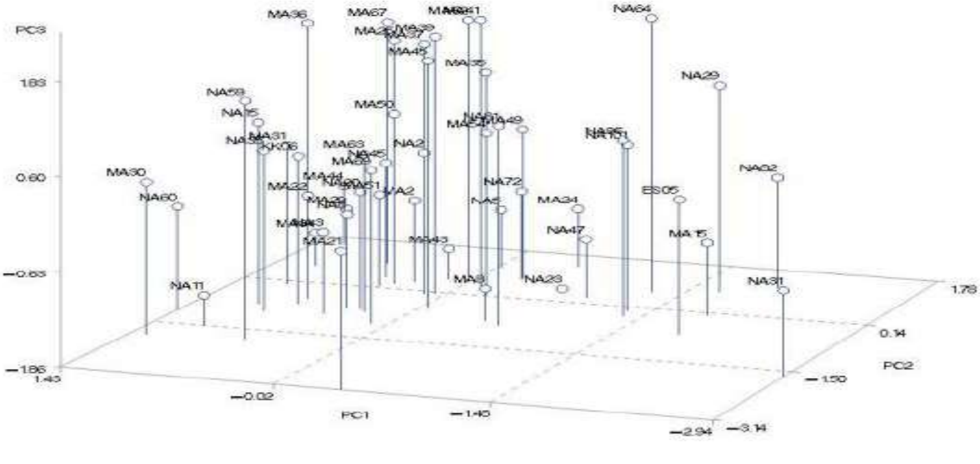
Globose leaf shapes are considered desirable for vegetable cowpea production, as they are associated with broader leaf surfaces that enhance photosynthetic efficiency and leaf biomass, both of which are critical for vegetable yield (Hutchinson *et al.*, 2017). The presence of a small percentage (2%) of accessions with sub-hastate leaves adds to the morphological diversity and could serve as a useful trait in breeding programs targeting novel ideotypes. From a genetic standpoint, Pottorff *et al.*, (2012) identified a major quantitative trait locus (QTL) associated with the control of hastate and sub-globose leaf shapes in cowpea, supporting the view that this trait is under relatively simple genetic control. This makes terminal leaf shape a reliable morphological marker for early selection and classification of cowpea germplasm. The principal component analysis (PCA) based on 13 morphological traits revealed substantial phenotypic diversity among the cowpea accessions studied. The first three principal components (PCs) accounted for 47.97% of total phenotypic variation, with eigenvalues of 2.46, 1.88, and 1.41 for PC1, PC2, and PC3, respectively (Table 2). This corresponds to explained variance of 42.70%, 32.73% and 24.57%. These results are consistent with those of Rekha *et al.*, (2013) and Arora et al. (2018). These findings are also in agreement with the principle established by Syafii *et al.*, (2015) that the first principal component captures the maximum variation in multivariate datasets.

Table 2: Principal component analysis of 50 cowpea accessions for 13 morphological traits

Principle component analysis	**Principle component Axis**		
	PC1	PC2	PC3
Eigen Value	2.458	1.884	1.414
Variation explained	2.458	1.884	1.414
Cumulative % of variation	20.49	36.19	47.97
Variables	**Factor loading**		
Terminal leaf shape	-0.40725	-0.43175	0.24520
Growth habit	0.56664	-0.22428	0.43494
Growth pattern	0.56086	0.57159	0.08687
Twinning tendency	0.74006	0.40932	0.17918
Plant pigmentation	0.31238	-0.20176	0.55269
Plant hairiness	-0.23093	0.61255	-0.29724
Leaf colour intensity	0.59290	-0.50281	-0.17927
Leaf marking	0.00000	0.00000	0.00000
Immature pod pigmentation	0.34909	0.12903	-0.58039
Flower colour	0.13757	-0.15022	0.06092
Raceme position	0.54896	-0.05416	-0.27390
Seed colour	-0.03129	0.55730	0.46656
Mature pod curvature	0.40465	-0.36291	-0.25640

The PCA provided meaningful insights into the multidimensional structure of phenotypic variation. Following the criteria of Hair *et al*., (2009), trait loadings above ±0.3 were considered meaningful. PC1 was predominantly defined by twining tendency, growth habit, and growth pattern, all showing high and positive loadings, indicating strong contributions to phenotypic variability. Traits such as terminal leaf shape, plant hairiness, and seed colour had negative loadings in PC1, suggesting an inverse relationship. PC2 was mainly influenced by plant hairiness, twinning tendency, growth pattern, and seed colour, all with high positive loadings, making them effective in distinguishing variation captured by this component. In contrast, terminal leaf shape, leaf colour intensity, mature pod curvature, and plant pigmentation exhibited negative loadings in PC2. PC3 showed strong positive associations with seed colour, terminal leaf shape, growth habit, plant pigmentation, and twining tendency, while traits such as immature pod pigmentation, raceme position, mature pod curvature, and plant hairiness had high negative loadings. Our results confirm with Jain *et al.*, (2022), Dairo (2024) and Chaudhary *et al*., (2025) who also identified plant hairiness and seed-related traits as major contributors to cowpea phenotypic diversity through PCA. Notably, twining tendency, plant hairiness, and plant pigmentation emerged as the most influential variables in PC1, PC2, and PC3, respectively. The trait leaf marking had a loading of zero across all three components, indicating it contributed negligibly to the variation and is of limited utility in discriminating among accessions. These results demonstrate that different traits contributed uniquely to each principal component, reflecting the multidimensional nature of morphological diversity. The PCA thus provides a robust framework for identifying key descriptors that can be prioritized in breeding programs and genetic conservation strategies. Cluster analysis grouped the 50 cowpea accessions into three major clusters (Fig. 1), highlighting a significant level of phenotypic diversity among them. Accessions such as NA31, NA82, NA11, NA60, NA64, ES5, NA29, and MA30 were the most divergent, suggesting their potential utility as parental lines in breeding programs aimed at introducing new trait combinations. In contrast, the majority of accessions are closely grouped, reflecting shared morphological characteristics, perhaps due to self-pollination and narrow genetic bases in domesticated cowpea lines. This pattern is consistent with the predominantly self-pollinated nature of cowpea, which tends to reduce genetic variability within populations (Padulosi, 1993; Kouam *et al.*, 2012). This pattern supports the use of PCA as an efficient method for summarizing multidimensional trait variation and identifying genetically distinct accessions for inclusion in breeding programs.

Fig 1: Cluster analysis of accessions based on thirteen qualitative traits



In conclusion this study revealed substantial morphological diversity among 50 cowpea accessions using 13 qualitative traits. Growth habit and mature pod curvature showed the highest polymorphism, while leaf marking was monomorphic. PCA and cluster analyses confirmed the variability, identifying twining tendency, plant pigmentation, and plant hairiness as key traits. Divergent accessions such as NA11, NA60, NA31, and MA30 offer valuable resources for breeding. These findings provide a basis for genetic improvement and highlight the need for complementary molecular analyses in future studies. The genotype KK6, which was used a check, is positioned near the center of the PCA space, closely surrounded by a dense cluster of other accessions. This indicates that KK6 shares similar morphological characteristics with the majority of the studied cowpea lines. Its central placement suggests low divergence, reaffirming its role as a reference or baseline genotype in the study. In contrast, accessions located at the periphery (such as NA11, NA60, NA64, NA82, and MA30) exhibit greater morphological diversity and may serve as potential candidates for broadening the genetic base in breeding programs. Future breeding strategies should focus on germplasm enhancement by developing core collections that capture the full spectrum of identified morphological diversity, with priority given to accessions exhibiting unique trait combinations and adaptive potential to diverse environmental conditions. Also it is evident that divergent accessions such as NA11, NA60, NA31, and MA30 should be used as parents in breeding programs. Traits such as twining tendency and plant pigmentation are key for selection. Future studies should integrate molecular markers to complement morphological data and guide effective conservation and utilization of cowpea genetic resources.

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