

# Evaluation of the Genetic Variation of Cowpea Landraces (*Vigna unguiculata*) from Western Cameroon Using Qualitative Traits

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## Abstract

Characterization of the genetic diversity and analysis of the genetic relationship between accessions of a crop species is a key step in breeding superior cultivars. The main objective of the hereby study was to determine the genetic variation between 30 cowpea accessions collected throughout the eight divisions of the Western Region of Cameroon using qualitative traits. Phenotypic variation of these accessions was evaluated using diversity indices and cluster analyses. A total of twenty qualitative traits were used for the study. Fifteen of them (75%) were polymorphic, displaying each at least two phenotypic classes. The monomorphic characters were growth pattern, leaf color, leaf hairiness, plant hairiness and pod hairiness, each with only one phenotypic class. Results showed a relatively significant level of genetic diversity among the studied cowpea accessions. Overall, the average of the observed and effective number of phenotypic classes per qualitative trait were  $N_a = 2.350$  and  $N_e = 1.828$  respectively. The Nei's genetic diversity and the Shannon weaver diversity index were  $H_e = 0.369$ , ranging from zero (monomorphic trait) to 0.655 (growth habit) and  $H' = 0.609$ , ranging from zero (monomorphic trait) to 0.996 (seed crowding), respectively. The dendrogram constructed from the twenty qualitative traits revealed 05 accessions clusters with the number of accessions in each cluster varying from one to eleven. Information obtained from this study is likely be useful for future cowpea breeding program.

**Keywords:** genetic variation, phenotypic class, qualitative trait, *Vigna unguiculata*

## Introduction

Cowpea, *Vigna unguiculata* (L.) Walps is an important food legume in developing countries, mostly in sub-Saharan Africa, Asia, Central and South America (Singh *et al.*, 1997). Cowpea has been referred to as "poor man's meat" because of its high protein content (20-25%) (Diouf and Hilu, 2005; Kereen and Taiwo, 2007; Sharmar *et al.*, 2013). Cowpea young leaves, pods and peas contain vitamins and minerals useful for human consumption and animal feeding (Rachie, 1985; Nielsen *et al.*, 1997). This crop tolerates low soil fertility due to its high rate of nitrogen fixation (Eloward and Hall, 1987). Because of its high capacity to cover the ground, this legume is also used to control soil erosion (Kariaga, 2004). Sub-Saharan Africa accounts for the majority (more than 64%) of the world's cowpea production, which is estimated to about 12.5 million tons (Langyintuo *et al.*, 2003).

In Cameroun, the Far North Region is the largest contributor to the national production of cowpea and the

crop ranks second after groundnuts in the category of leguminous crop (Dudje *et al.*, 2009). The production in this region ranges from 300 to 500 kg/ha in farmers' fields and 1,200 to 2,000 kg/ha in research stations (Dudje *et al.*, 2009). According to Bidima (2012), the national annual production in Cameroon is estimated to about 110,000 tons from a planted area of 105,000 hectares. The three northern regions of Cameroon (Adamaoua, North and Far North) account for about 90,000 tons of the national production (Bidima, 2012).

Despite the low contribution of the West Region to the national production, cowpea is a commodity of high market, cultural and economic value due to its high local consumption and cultural considerations (Mbiendou, 2012). The market price of cowpea in the West Region is almost the double of that of Northern Cameroon (Mbiendou, 2012). Despite its importance, the principal constraints to the production of this legume is the lack of the high yielding improved varieties, resistant to the abiotic and biotic stresses among which diseases and insects pests are the most prevalent (Nkamleu *et al.*, 2000).

The loss of genetic diversity, due to conventional breeding modern agricultural practices that promote monoculture has been dramatic for many cultivated species (Wilkes, 1983). As a better performing crop varieties are released by research institutions and agencies, farmers are encouraged to adopt the latter by extension services because of their higher profitability. This shift will result to the abandon of landraces and loss of genetic diversity. A consequence of this is the increase vulnerability to biotic and abiotic stress of the elite germplasm due to a narrowing genetic base of the crop. The knowledge, the access and the use of the available diversity in domesticated and wild relatives of a plant are essential for sustainable crop improvement (Singh, 2001). Assessing and maintaining genetic diversity are therefore central to ensure more prospects in breeding of cowpea and other plant species (St.Clair and Howe, 2011).

Because of their direct observation on plants during the vegetative and reproductive stages, phenotypic qualitative traits have been widely used to assess genetic variability between accessions in cowpea and other plant species (Bennet-Lartey and Ofori, 1999; Geleta and Labuuschagne, 2005; Ofori *et al.*, 2006). In Cameroon, the lack of detailed information on the genetic diversity of cowpea limits the understanding of its genetic structure and evolution. This lack also hampers the achievements in breeding of this legume plant. The present investigation was therefore undertaken to assess the extent of the genetic variation available in cowpea accessions examined in order to assist in setting up a breeding program for cowpea improvement.

## Materials and Methods

### Plant materials and study site

Cowpea landraces were collected from all the eight divisions of the Western Region of Cameroon. Accessions were collected from market places, farmers' fields and at the Institute of Agricultural Research for Development. A total of 30 accessions were used for the study. The study was carried out at the Research and Teaching farm of the Faculty of Agronomy and Agricultural Sciences of the University of Dschang, located in the West Region of Cameroon at latitude of 5°20' North and longitude of 10°05' East, and 1407 m above the sea level. The annual rainfall of the study site ranges from 1,800 to 2,000 mm.

The average annual temperature and relative humidity are closed to 20.50 °C and 76.8%, respectively.

### Experimental design

The thirty accessions were sown in the field under rainfed conditions from August to December 2016 in a randomized complete block design (RCBD) with three replications, on a total area of 2000 cm × 1980 cm. The blocks of 600 cm × 2000 cm were outdistanced of 90 cm from each other. Each block consisted of 30 experimental units of 200 cm × 200 cm, for each of the 30 accessions to evaluate. Each experimental unit comprised 16 plants distant to each other of 50 cm within the lane and the row in order to avoid competition among neighboring plants and to ensure a sound establishment of accessions. Four plants were sampled at the center of each experimental unit populated with sixteen individuals for qualitative scoring. A total of 12 plants per accessions were analyzed. Agronomic and plant protection practices were applied to provide plants with adequate growing conditions. Plants were evaluated for twenty qualitative traits at various stages of development.

### Evaluated qualitative traits

In order to categorize each accession morphologically, data on twenty traits were recorded according to International Board for Plant Genetic Resources descriptors for cowpea (IBPGR, 1983). Table 1 presents the 20 qualitative traits, their codes and their description classes used in the analysis. Each accession was scored for the most frequent character shown in each qualitative trait.

### Data analysis

Phenotypic frequency distributions of the characters were worked out for all the sample germplasm and were established using GrahPad Prism 6.0. To assess the overall phenotypic diversity of each character for all accessions, the observed ( $N_a$ ) and the expected ( $N_e$ ) number of phenotypic classes were calculated. Phenotypic classes' frequencies were used to compute Nei's (1987) gene diversity ( $H_e$ ) and Shannon-Weaver diversity index ( $H'$ ). The Nei's gene diversity was calculated as follow:

$$H_e = 1 - \sum_{i=1}^n x_i^2$$

Table 1. List of qualitative traits used in this study and observed description classes

No	Qualitative trait name	Code	Description classes
1	Flower colour	Fcol	Violet (2), Mauve-pink (3)
2	Growth habit	GH	Erect (2), Semi-erect (3), Semi-prostrate (5), Prostrate (6), Climbing (7)
3	Growth pattern	GP	Indeterminate (2)
4	Immature pod pigmentation	IPP	None (0), Pigmented tip (1), Uniformly pigmented (5)
5	Leaf "V" marking	Mark"V"	Present (1), Absent (0)
6	Leaf colour	LCol	Dark green (7)
7	Leaf texture	LT	Intermediate (2), Membranous (3)
8	Leaf hairiness	LH	Glabrescent (5)
9	Plant hairiness	PH	Glabrescent (3)
10	Plant pigmentation	PP	Very slight (1), Moderate (3), Extensive (7)
11	Pod attachment to peduncle	PAP	30° - 90° down from erect (5), Erect (7)
12	Pod curvature	PC	Straight (0), Slightly curved (3), Curved (5)
13	Pod hairiness	PodH	Glabrescent (3)
14	Raceme position	RP	Mostly above canopy (1), In upper canopy (2), Throughout canopy (3)
15	Seed colour	SCol	White pale (1), Cream (2), Cream grey spotted (3), Cream brown spotted (4)
16	Seed crowding	SCrow	Semi-crowded (3), Crowded (5)
17	Seed shape	SS	Kidney (1), Ovoid (2), Rhomboid (5)
18	Terminal leaflet shape	TLS	Sub-globose (2), Sub-hastate (3), Hastate (4)
19	Testa texture	TT	Smooth (1), Smooth to rough (3)
20	Twinning tendency	TwT	None (0), Slight (3), Pronounced (7)

The Shannon-Weaver diversity index was estimated as suggested by Hennink and Zeven (1991) using the formula:

$$H' = -\sum_{i=1}^n x_i \ln(x_i) / \ln(n)$$

Where ' $x_i$ ' represents the relative frequency of the ' $i^{\text{th}}$ ' class of a trait and ' $n$ ' represents the number of phenotypic classes of the trait.

Minimum and maximum values for the diversity indices ( $H_e$  and  $H'$ ) are 0 and 1 respectively. Value of zero indicates a monomorphic character with all the individuals belonging to the same category and having the same phenotypic class.  $H_e$  or  $H'$  equal to one specifies maximum diversity with individuals equally dispersed among the different phenotypic classes of the character. The coefficient of dissimilarity was computed for all the pairwise comparisons of accessions using XLSTAT, version 2014.5.0. and a dendrogram was created by cluster analysis with Agglomerative Hierarchical Clustering (AHC) using the same XLSTAT computer program.

## Results

### *Genetic diversity*

Of the twenty qualitative traits presented in Table 1, five traits (25%) were monomorphic (growth pattern, leaf colour, leaf hairiness, plant hairiness and pod hairiness) whereas fifteen traits (75%) were polymorphic.

Among the fifteen polymorphic traits, six traits were polymorphic with two phenotypic classes (leaf texture, "V" leaf marking, flower colour, testa texture, seed crowding and pod attachment to peduncle). Concerning the leaf texture (Fig. 1A), membranous texture was the dominant leaf texture observed (80%) over all the studied accessions as opposed to intermediate (20%). With regard to "V" leaf marking distribution it was observed that 63% of the accessions studied presented the "V" mark at the upper face of their leaves and 37% of the accessions do not present any "V" mark (Fig. 1B). Flower colour of cowpea accessions analyzed was either violet or violet-pink. Most accessions had violet flowers (80%), while violet-pink flowers were on 10% of accessions (Fig. 1C). The testa texture of the 67% of the accessions studied was smooth, while 33% of these accessions had smooth to rough testa texture (Fig. 1D). It was observed that 53% of the studied accessions produced crowded seed and 47% of accessions were semi-crowded (Fig. 1E). In the study, two classes of pod attachment to peduncle were observed (Fig. 1F), whereas 87% of the accessions studied were 30-90° down from erect and 13% showed erect pod attachment to peduncle.

Seven characters were polymorphic with three phenotypic classes (plant pigmentation, pod curvature, terminal leaflet shape, seed shape, immature pod pigmentation, raceme position and twinning tendency). Out of the accessions studied, 57% showed very slight plant pigmentation, 23% showed moderate plant pigmentation and 20% of the accessions showed extensive plant pigmentation (Fig. 1G). The general trend of pod curvature is that 57% of the accessions had pods slightly curved, while 23% of the accessions had curved pods and 20% had straight pod curvature (Fig. 1H). It was observed that 53% of the accessions studied produced a globose terminal leaflet shape and 43% were sub-hastate terminal leaflet shape. Only 3% of the accessions studied showed hastate terminal leaflet shape (Fig. 1I). Among the five main seed shape descriptor

described by the International Board for Plant Genetic Resources IBPGR (1983) for cowpea, three seed shape were observed (Fig. 1J) as follow: 60% of the studied accessions showed kidney seed shape, while 37% had rhomboid seed shape. Only 3% of the accessions studied had an ovoid seed shape. The distribution of pigmentation in immature pods of cowpea accessions studied was as follow: 63% of accessions had no pigmentation (green pods), 20% had pigmented tips, and 17% had splashes of pigment left throughout the surface of the immature pods (Fig. 1K).

The distribution of raceme position of the studied cowpea accessions showed that 43% of the accessions had their raceme positions at the same level as the canopy, while 40% of the accessions produced racemes within the canopy and only 17% had their raceme positions above the canopy (Fig. 1L). Three categories of twining tendency in the cowpea accessions studied were observed: 47% of accessions studied showed pronounced twining tendency, while 43% had slight twining tendency. 10% of the accessions studied had no twining tendency (Fig. 1M).

Concerning the distribution of seed color in the cowpea accessions studied, four categories were observed (Fig. 1N). 50% had cream seed color, while 27% produced white pale seed color. The two others categories of seed color, less represented were cream grey spotted observed on 13% of accessions and cream brown spotted observed on 10% of the accessions analyzed. It was observed that 50% of the studied accessions displayed secondary branches which form acute angles with main stem, producing an erect growth habit. A ratio of 13.33% of accessions was semi-erect, 27% of accessions had semi-prostrate growth habit and 3% showed prostrate growth habit. Seven percent of accessions showed climbing growth habit, needed stalk to twin on (Fig. 1O).

The observed number of phenotypic classes ranges from 1 (monomorphic trait) to 5 (growth habit) with a mean of 2.530 for all the twenty traits (Table 2). The mean effective number of phenotypic classes was  $N_e = 1.828$ , with values ranging from 1 (monomorphic trait) to 2.901 (growth habit). The mean Nei's gene diversity and Shannon Weaver diversity index were 0.369 and 0.609 respectively (Table 2). Nei's gene diversity ranged from zero (monomorphic trait) to 0.655 (growth habit) and Shannon Weaver diversity index ranged from zero (monomorphic trait) to 0.996 (seed crowding) (Table 2)

### *Cluster analysis*

The Cluster analysis with UPGMA using dissimilarity coefficient resulted in the identification of five groups of accessions as shown by the dendrogram (Fig. 2). The number of accession in each cluster varied from one (Cluster 5) to eleven (Cluster 4) (Table 3). Cluster 4 was the largest and consisted of eleven accessions. In this group, accessions have predominantly erect pod attachment to peduncle; kidney and ovoid seed shape; sub-hastate and hastate terminal leaflet shape (Table 4). The second largest group is the cluster 1 made of ten accessions. This cluster is characterized by an erect and semi-erect growth habit; a raceme located mostly above and in upper the canopy; they have rhomboid seed shape. The cluster 2 consists of five accessions and is singularized by extensive plant pigmentation; erect pod attachment to peduncle and have a pronounced twinning tendency (Table 4). Cluster 3 is made of tree accessions having predominantly "V" leaf marking and a sub-hastate terminal leaflet shape. Only one accession (KEB-CP039)

Table 2. Diversity parameters of phenotypic qualitative traits in cultivated *Vigna unguiculata*

Qualitative trait name	Na	Nc	He	H'
Flower colour	2	1.219	0.180	0.469
Growth habit	5	2.901	0.655	0.784
Growth pattern	1	1.000	0.000	0.000
Immature pod pigmentation	3	2.134	0.532	0.828
Leaf "V" marking	2	1.873	0.466	0.951
Leaf colour	1	1.000	0.000	0.000
Leaf texture	2	1.471	0.320	0.722
Leaf hairiness	1	1.000	0.000	0.000
Plant hairiness	1	1.000	0.000	0.000
Plant pigmentation	3	2.393	0.582	0.892
Pod attachment to peduncle	2	1.301	0.231	0.567
Pod curvature	3	2.406	0.584	0.895
Pod hairiness	1	1.000	0.000	0.000
Raceme position	3	2.662	0.624	0.935
Seed colour	4	2.866	0.651	0.864
Seed crowding	2	1.991	0.498	0.996
Seed shape	3	2.018	0.504	0.717
Terminal leaflet shape	3	2.113	0.527	0.738
Testa texture	2	1.799	0.444	0.918
Twinning tendency	3	2.406	0.585	0.895
<b>Mean</b>	<b>2.350</b>	<b>1.828</b>	<b>0.369</b>	<b>0.609</b>
<b>Std. Dev.</b>	<b>1.089</b>	<b>0.663</b>	<b>0.252</b>	<b>0.382</b>
<b>Std. Error of means</b>	<b>0.244</b>	<b>0.148</b>	<b>0.059</b>	<b>0.086</b>

Na = Number of observed phenotypic classes; Nc = Number of effective phenotypic classes. He = Nei's genetic diversity; H' = Shannon-Weaver diversity index

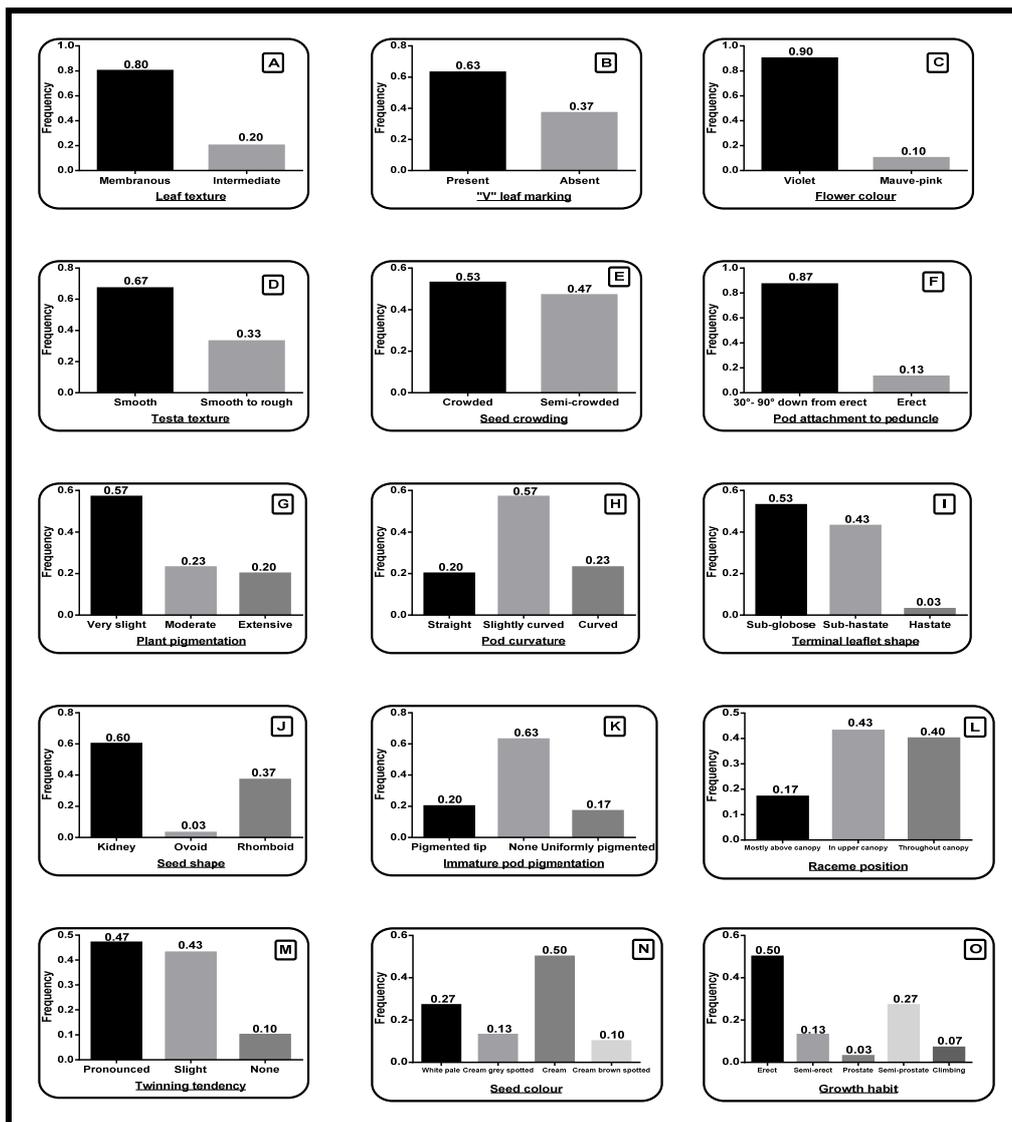


Fig. 1. Phenotypic classes' frequencies of fifteen polymorphic qualitative traits in cultivated *Vigna unguiculata* from the Western Region of Cameroon

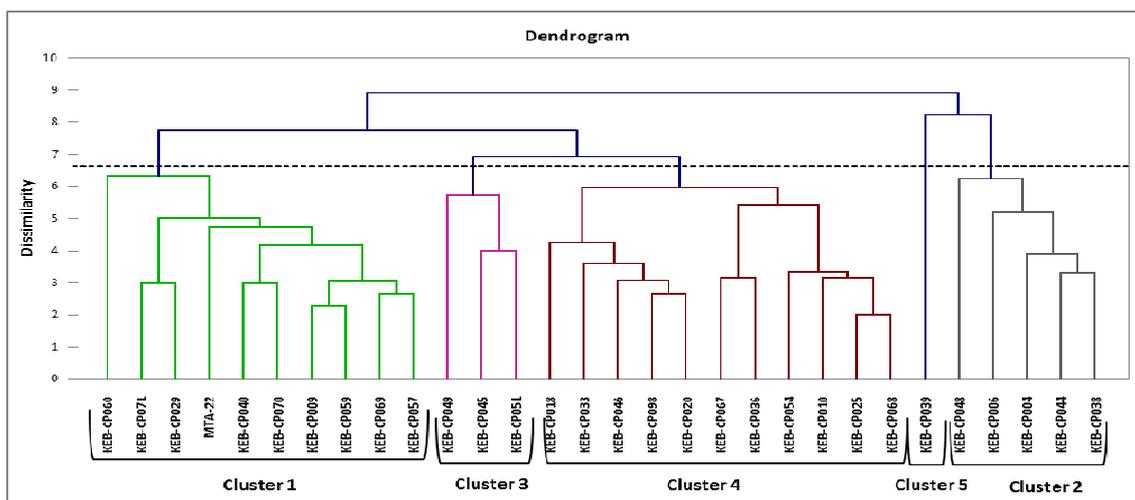


Fig. 2. Phylogenetic relationship between the thirty accessions of cultivated *Vigna unguiculata* from the Western Region of Cameroon

Table 3. Accessions distribution between clusters based on 19 qualitative traits at cultivated *Vigna unguiculata*

Cluster	Accessions	Number of accession
Cluster 1	KEB-CP057, KEB-CP009, KEB-CP040, KEB-CP071, KEB-CP069, KEB-CP060, KEB-CP059, KEB-CP070, MTA22, KEB-CP029	10
Cluster 2	KEB-CP006, KEB-CP004, KEB-CP044, KEB-CP038, KEB-CP048	5
Cluster 3	KEB-CP045, KEB-CP049, KEB-CP051	3
Cluster 4	KEB-CP018, KEB-CP067, KEB-CP010, KEB-CP025, KEB-CP036, KEB-CP054, KEB-CP046, KEB-CP098, KEB-CP020, KEB-CP068, KEB-CP033	11
Cluster 5	KEB-CP039	1

Table 4. Qualitative characteristics of the different clusters of *Vigna unguiculata*

Qualitative trait name*	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
Flower colour	2	2,3	2	2,3	2
Growth habit	2,3	3,5,7	3,5	2,3,5,7	6
Growth pattern	2	2	2	2	2
Immature pod pigmentation	0,1,5	0,1,5	5	0,1	0
Leaf "V" marking	0,1	0,1	1	0,1	1
Leaf colour	7	7	7	7	7
Leaf texture	2,3	2,3	3	2,3	3
Leaf hairiness	5	5	5	5	5
Plant hairiness	3	3	3	3	3
Plant pigmentation	1,3	7	1,3	1,3	7
Pod attachment to peduncle	5,7	5	3	5	5
Pod curvature	0,3	3,5	3,5	0,3,5	3
Seed colour	1,2,4	2,3	3,4	1,2,4	2
Seed crowding	3,5	3,5	3,5	3,5	3
Pod hairiness	3	3	3	3	3
Raceme position	1,2	2,3	1,3	1,2,3	2
Seed shape	5	1,5	1	1,2	1
Terminal leaflet shape	2,3,4	2,3	3	2,3	2
Testa texture	1,3	1,3	1,3	1,3	1
Twinning tendency	0,3	7	3,7	3,7	0

\*: Description of each qualitative trait and its classes is given in Table 1

constitutes cluster 5, the only prostrate growth habit accession. It has no twinning tendency.

**Discussion**

Scoring qualitative traits appears as an important alternative to molecular and biochemical techniques in assessing genetic variation on plant germplasm and

information on genetic relationship needed in breeding programs. Cowpea in the Western region of Cameroon has a lot of economic and cultural consideration and there was an imperative need for genetic characterization and preservation. For the description and classification of the genetic resources of plant species, morphological characterization appears as the first step (Smith and Smith, 1989). In the present study, divergence among cowpea

accessions was expressed in term of differences in phenotypic classes. The study indicated a wide distribution of phenotypic classes for the traits considered and showed the presence of numerous genetically distinct accessions in the local collections. Because of the direct observation on plants, qualitative traits are considered as the most significant characters to identify a particular plant accession or variety and can help plant breeders with strategies for the conservation of the genetic diversity. These traits are mostly genetically controlled and are less influenced by environmental factors compared to quantitative traits.

Polymorphism was found in 15 out of 20 qualitative traits studied hereby. Therefore, clear morphological differences exist between the analyzed accessions for 75% of the studied traits (Table 2). For the overall phenotypic variation in the study, 75% of phenotypic traits showed significant variation in the cowpea collection. A total of 47 phenotypic classes were detected by the 20 phenotypic traits in our study, which is significantly lower compared to cowpea germplasm in Ghana that found 31 phenotypic classes with only 7 qualitative traits (Cobbinah *et al.*, 2011). However, the results obtained were closed with those of Gonné *et al.* (2013) who found 9 phenotypic classes from only four qualitative traits in Northern Cameroon. The growth habit of a crop is of high significance to the cropping system. The growth habit of cowpea greatly affects the harvest of the crop (Fery, 1985; Bennett-Lartey and Ofori, 1999). The prostrate types are used by farmers in mixed cropping (Doku, 1970; Rachie and Rawal, 1976). It was reported that the prostrate and climbing types induce extended periods of pod maturation, resulting in an uneven periods of harvesting and specially required bending very low to pick the pods. Aryeetey (1971) and Cobbinah *et al.* (2011) reported similar observations. Erect and sub-erect plants do not need stalks to keep pods from touching the ground. This prevents rotting of pods, which usually happen when pods come into contact with the soil. This is also in agreement with Cobbinah *et al.* (2011). All phenotypic classes of the twinning tendency documented in the IBPGR (1983) descriptor list for cowpea was observed for the accessions studied. The results indicated that most of the accessions need staking since majority of them show trailing tendency. The pigmentation of the cowpea accession ranged from green (no pigmentation) to purple, with different intensities of the purple coloration. This pigmentation is dependent on the concentration of anthocyanin in the plant part as previously highlighted (Fery, 1985; Harland, 1919).

According to Bennet-Lartey and Ofori (1999), it is more desirable to have racemes above the canopy than either at the same level of the canopy or within the canopy. This position facilitates pod visibility and harvesting. In this study, that character and observation were confirmed when harvesting 17% of accessions which presented their raceme mostly above the canopy. The results of the flowers color are consistent with those found by Cobbinah *et al.* (2011), Nkouannessi (2005) and Bennet-Lartey and Ofori (1999) where the violet flowers were largely represented. According to Purselglove (1968) flower colors that are mostly encountered on the field are pink, white and violet. So, in spite of the fact that the results of this study showed only two colors of flowers documented by IBPGR (1983), Gibbon and Pain (1985) reported additional flower colors

such as pale blue and yellow, which were not observed in the current study. This may be attributed to the relatively large number of accessions studied as compared to the smaller number used in the present study. In addition to the three shapes observed in this study, Nkouannessi (2005) observed two other shapes: crowder and globose. He also found two other testa textures (rough and wrinkled). This should be attributed to the large number of accessions used. Three types of pod curvature was reported in this study and was similar to those reported by Nkouannessi (2005) who also found some accession with "V" leaf marking as reported in this study. The maximum Nei's genetic diversity value of 0.655 was contributed by growth habit, where 5 different phenotypic classes were identified. This is higher than the reports of Gonné *et al.* (2013) who reported 3 types of growth habits in 18 cowpea accessions. The hereby results are however lower as compared to 6 phenotypic classes of growth habits reported by Cobbinah *et al.* (2011) when studying 134 cowpea accessions. The Nei's average genetic diversity value recorded was  $H_e = 0.359$ . This result was comparatively higher than that reported in cultivated cowpea using Allozyme markers ( $H_e = 0.061$ ; Pasquet, 2000), AFLP markers ( $H_e = 0.108$ ; Coulibaly *et al.*, 2002) and RAPD Markers ( $H_e = 0.123$ ; Ba *et al.*, 2004). The Shannon-Weaver diversity index found in this study was  $H' = 0.609$ . Similar high values were reported in others cultivated inbred species like rice (Roy *et al.*, 2016) and wheat (Hailu *et al.*, 2010).

## Conclusions

Traditional cowpea landraces from western Cameroon that are highly preferred by consumers because of their taste and cultural considerations needed to be characterized prior to their conservation. The genetic characterization of this crop was based on qualitative phenotypic descriptors. For the trait growth habit, five different phenotypic classes were detected, followed by seed colour with four different phenotypic classes. Growth habit, seed colour, plant pigmentation, pod curvature, raceme position and twinning tendency were the primary determinants for explaining the existing genetic variation in this collection of cowpea genotype. The thirty accessions were grouped in five distinct clusters with varied number of accessions.

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