Ethnobotany and Genetic Diversity of South Chadian Cowpea Landraces as a Novel Source of Early Grain Production

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Abstract: - Cowpea is the main source of food crop in many sub-Saharan Africa countries, including Chad. However, very little is known on the genetic diversity of South Chadian cowpea, particularly in a context of global warming and climate change. This study was undertaken to determine cowpea genotypes, ethnobotanical nomenclature and to assess morphological and agronomical variability for future breeding program to create a new varieties and build the core collection. Prospecting missions in 14 villages were conducted in rural areas and allowed to collect sixty cowpea landraces. Farmers differentiated landraces using characteristics such as, seed taste, cooking duration, seed color and seed size. The qualitative traits assessed revealed the presence of variability, including plant pigmentation, seed color, seed shapes dominated by rhomboid shape (87.5%). Analysis of variance indicated significant difference ($p<0.01$) between accessions for assessed quantitative traits. Pearson correlation between grain yield and number of pod per plant was positive ($r=0.91$; $p<0.01$). Moreover, grain yield was negatively correlated with plant height ($r=-0.55$; $p<0.01$) and days to flowering ($r=-0.53$; $p<0.01$). Additionally, cluster analysis performed with non-highly correlated traits allowed clustering the accessions in 4 groups and cluster 2 found to be better with highest mean value of grain yield (2.034 $t.ha^{-1}$). The highest inter cluster divergence D2 was found between cluster 2 and 3, indicating divergence between accessions belonging to these clusters. In summary, addition of these findings in the national breeding program will help develop new varieties of cowpea with attributes that will enhance quality and productivity.

Key-Words: – Cowpea – diversity – ethnobotanical – improvement – Barh-Kôh – Chad.

1. Introduction

Cowpea (Vigna unguiculata (L.) Walp.) is a major staple food crop in many West and Central African countries. It is mainly grown in tropical Africa, Asia, North and South America. It is used as grain but also as a vegetable and fodder crop. Global production of dry seed is estimated at 12,577,845 tons worldwide of which over 95.9% are produced in Africa [1]. Cowpea contains high protein level (~30%), carbohydrate (50–60%), low fat content (1.5%), as well as vitamins and minerals (Ca, P, Fe). Cowpea is one of the main source of food in many developing countries, therefore it is being referred to as "poor man's meat" [2]. Cowpea can accommodate various types of soil and intercropping systems. It is also resistant to drought, and has the ability to improve soil fertility and reduce the loss of field's topsoil, thus preventing soil erosion [3].

In Chad, cowpea is a second most economically important leguminous behind peanut [4]. It is primarily grown for its fresh leaves and fresh pods, with the fresh and dry seeds used as a vegetable for human consumption; additionally cowpea haulms are used to feed livestock. In 2000, the overall production of cowpea was 71,621 tons and in 2018 was 151,737 tons which represent an increase of 85% [5]. The yield is very low (0.68 $t.ha^{-1}$) across the country, this is partly due to the fact that local varieties with poor production practices were used. However, the lack of reports on the genetic diversity of cowpea in the country, make it difficult to develop a strong breeding program. The importance to evaluate the agronomical and phenotypic diversity of cowpea accession in Chad is essential to know the whole potentiality we had in our area and make it available for future plant breeding. It could be help to access to current variability, essential to build the core collection.

It is well known that climate change as led to significant loss of genetic diversity of cowpea, thereby several strategies were implemented to minimize genetic erosion and safeguard genetic diversity around the world. In Chad, only a small part of the country were covered by these strategies [4] and [6] which showed a high level of cowpea genetic diversity. In addition, no studies on Barh-kôh cowpea have been carried out, although the area is known as a most cowpea production. Therefore there is a need to establish a new collection as novel source of early grain maturity, in breeding program. Meanwhile introducing the new accessions from germplasm collection into breeding program is also the best way to increase genetic diversity in cultivated crops [7]. An enhanced exploitation of genetic diversity through novel pre-breeding strategies and a fundamental reinforcement of the entire plant breeding chain is a vital part of a sustainable system for global food security [8].

Genetic diversity is a broad concept, which can be described by many aspects. Genetic diversity research provides the basis of the genetic variation and genetic relationships among cowpea genotypes, thus providing information for the preservation and utilization of germplasm resources and improvement of cultivars [7]. The genetic diversity and relationship research of cowpea is a challenging topic for geneticists and breeders [10]. Genetic diversity is important for a successful breeding program [9]. The success of good breeding and selection program usually depends on the genetic variability present in the breeding materials and the variation in the population. Genetic diversity in cowpea has been assessed by numerous studies using agronomical, morphological and molecular markers [10], [11], [12], [13] and [14]. Thereby agronomical and morphological variability of cowpea landraces could provide useful information on the genetic potential of germplasm.

In previous work [4] and [6], assessment of agro-morphological diversity of Chadian cowpea showed high difference among accessions through traits such as the number of days to first flowering (37–97 days), number of pod per plant (14–83), number of seeds per pod (5–20), seed weight per pod (2.11–4.89 g). This previous study allowed to see positive correlation between flowering and maturity ($r = 0.61$) and the negatives correlation between maturity and pod length ($r = -0.55$) and maturity and number of seeds per pod ($r = -0.54$). According to various studies, some morphological traits are mainly used as markers including pod per plant, seed per pod and seed size which affect on potential yield of cowpea [15].

The genotype by environment (GE) interactions plays a major role in the performance of any genotype and in identification of adaptable genotypes to varying environments [16]. The use of the novel source of genetic diversity through prospecting (varieties from farmer fields), agronomical and morphological assessment could help Chadian breeding program to create new varieties that are more adapted to the Soudanese arid area. The important part of genetic diversity and population structure research is to study the similarity and difference within populations under multiple spaces and times [7].

Therefore, the primary objective of this research was to determine the whole farmer varieties of cowpea and assess the agronomical and morphological variability of cowpea in the Barh-Kôh department in South Chad, which is the first step in the creation of new varieties, particularly when facing challenges of global climate change.

2. Material and methods

2.1. Site selection and survey for ethnobotanical assessment

The survey mission was carried out from 2017 to 2018 in Barh-Kôh department, South of Chad in the North of Moyen Chari Province, between latitudes 8.67° and 10.47 N and longitude 17.42° and 18.61° E. The department is located in South East of Chad and extends over 200 km long and 132 km wide with an area of 17,258 km^2 and has 435 villages distributed in five sub-prefectures, three of which were concerned by this current research. Previous prospecting of cowpea in Chad was out of this current prospecting area and was realized in Logone Oriental and Mayo Kebbi Est and Mayo Kebbi Ouest and allowed collecting 45 accessions of cowpea [4]. Farmers' knowledge was collected during a survey, in several villages known for their high cowpea productivity, population (number), ethnicity and distances between villages. We conducted individual interviews and occasional conversations as previously described [17].

The interviews were conducted with ten local peasants nominated by local village leaders according to their age, the size of their cowpea fields, depending on the accessions they cultivate (rare accessions for example) and their availability.

2.2. Study site, experiment design, and crop cultivation

Field experiments were performed from July to November 2018 at the Bébédjia Research Station (8°40’ N, 16°54’ E), of Chadian Institute of Agricultural Research for Development, in Sudanese zone. Annual minimal and maximal temperature averages ranged from 26 to 30°C and rainfall was 858.2 mm.

Sixty cowpea landraces collected in Barh-Kôh Department during this current study were used. The experiment was conducted in Randomized Completely Block Design (RCBD) with three replications. Seed were planted in 0.80 m rows having a within-row spacing of 0.50 m and planted at approximately a 2.5 cm depth. Each block consisted of tree plots and each plot contained 11 seed holes. Seeds were pretreated with Momtaz 45WS, an insecticide. Seeds were hand planted at a rate of two seeds per hole and weeds were controlled with hand hoeing. Recommended amount of fertilizer ($N_{15}P_{15}K_{15}$) was applied at the rate of 50 $kg.ha^{-1}$ and disease management was undertaken by using Cypercal 12EC/720EC.

2.3. Data collection

Totally 34 agronomical and phenotypic traits consisted of 17 qualitative traits and 17 quantitative traits were investigated and recorded based on the criteria of [18]. The 17 qualitative traits were growth habit; terminal leaflet shape; flower color; flowering pigment pattern; pod shape; dehiscence of pod; pod color; seed color; size of seed; seed shape; grain texture; particular features of seeds; eye pattern; eye color; plant pigmentation; attachment of testa and leaf marking [18]. For quantitative traits recorded, day to germination and days to flowering were sampled from sowing to stage when 50% of plants respectively have been germinated or have begun to flower. At the end of vegetative growth, the following quantitative traits were determined on nine randomly selected plants: plant height; plant width; number of main branches; number of nodes on main stem; peduncle length and number of pods per plant. Number of locules per pod, number of seeds per plant and pod length and width were the mean value of nine longest mature pods from the same nine randomly selected plants. Seed weight per pod was obtained on the nine longest mature pods after gathering. Seed length and width were recorded on 10 mature seeds excluding those from the extremities of pods. Grain yield per hectare was calculated using the formula of [19] and hundred seeds weight was measured on 100 seeds moisture content 12%.

2.4. Data analysis

The ethnobotanical data were used to calculate frequencies and construction of the graphs. The frequency distribution of the qualitative traits was determined and descriptive analysis of the quantitative traits was performed. Quantitative traits data were subjected to the analysis of variance (ANOVA) using GenStat 12th edition. Broad sense heritability (H^2) was estimated as the ratio of genotypic variance to the phenotypic variance and expressed in percentage [20]. The following analyses were done by using XLSTAT pro version 2016. Pearson correlation coefficients were calculated for every pair of quantitative traits and reported were the correlation coefficient (r) with respective probability (P). The principal component analysis (PCA) was conducted to identify the patterns of morphological variation. Hierarchical agglomerative clustering was performed and dendrogram was constructed based on the quantitative data with the Unweighted Pair-Group Method with Arithmetic Average (UPGMA) to provide overall phenotypic similarities among genotypes. Discriminant Factor Analysis (DFA) was done to characterize the cluster obtained. The average inter-cluster distances were calculated using the generalized Mahalanobis's D^2 statistics [21].

3. Results and discussion

3.1. Villages prospected and accessions collected

The survey was carried out in 14 villages and 60 cowpea landraces were inventoried and collected representing an average of 4.29 accessions collected

per village (table 1). This rate was high than 1.8 rate obtained in Benin cowpea [22].
This rate was different between cantons. In Koumogo 26 accessions were collected in six villages representing 4.3 accessions per village. In Moussafoyo, 19 accessions were collected in 5 villages with the proportion of 3.8 accessions per village. In Kokaga, 15 accessions were collected for 3 villages prospected with the rate of 5 accessions per villages. The number of varieties varied in a similar pattern across villages and canton. Similar results were found in some study [23], [22]. Regarding in the important of accessions collected per village, most accessions was in Molguidi where nine accessions were found, followed by Ferme with eight accessions and Goro I allowed to collect seven accessions. Some villages showed low amount of diversity with very low number of accessions collected: two from Sakogongo, Kaynodjo and Goro II; three from Maïkolo II, Moussafoyo, Gentille and Kokaga.

Table 1: Number of accessions collected per village

Cantons	Villages number	Number of accessions	Rate per village
Koumogo	6	26	4.3
Moussafoyo	5	19	3.8
Kokaga	3	15	5
Totally	14	60	4.29

3.2. Folk nomenclature

During the survey, three vernacular main names, *"Moudjo"* (56.43%), *"Mindji"* (25.71%) and *"Lair"* (17.86%) have been identified within the 9 ethnical groups (tableau 2). It has been found a diversity of vernacular terms that would be related to the ethnical diversity existing in Barh-Kôh Department. The success of vernacular name «*Moudjo*» was justified by the fact that it is part of the dialect of the majority "Sara" and some of its subgroups ("Sara kaba", "Mbaye", "Ngaman").
This current study showed that nomenclature of cowpea accession generally derive from their seed color («*Moudjo ndàh»* and *«Lair bil»* both mean white cowpea); their seed size («*Moudjo bôh»* means big cowpea); plant habit linked to animal name («*Moudjo Kass dog»* and *«Mindje li»* respectively mean cowpea buffalo feed and cowpea snake); their seed taste («*Moudjo sucre», «Moudjo tolba»* and *«Mindje maïnmané»* respectively means cowpea sugar, cowpea that make host happy and cowpea with sweet jus); their tutor («*Moudjo kague*» means cowpea with wood); their cultivation place («*Mindje guidekey*» means cowpea cultivated back of home); their pod shape («*Moudjo kou*, «*Mindje kouloulou*», «*Moudjo nengabille*», «*Mindje Kla*» respectively means cowpea curved, cowpea millipede, cowpea earring and cowpea rope) and their pod color («*Lai gue iri»* means black cowpea). A study [24] showed that local cowpea populations in Tanzania are named cowpea according to their growth habit, seed color, pod shape and coloration of the plant. Same results on cowpea were found in Greece [25], Portugal [26], Serbia [27] and Burkina Faso [23].
The ethnic group as "Gore" and "Mbaye" named their accession with some character like animal name. These nomenclatures would certainly result from similarity existing between the pod shape of accessions and the shape of these animals mentioned above. Otherwise, farmers named accession regarding on the person who brought the accession first in their village.

3.3. Insect pests control and seed conservation

In villages inspected, survey revealed that insect pests or their larvae and nymph (*"Kour moudjo"*, means cowpea pest in local language *"Sar"*) cause lot of damage in cultivated cowpea through all growth stages. In the study area, the most well-known pest of cowpea by the farmer, were locusts, *Helicoverpa armigera*, *Aphis croccivora* or aphids, *Mylabris sp, Anoplocnemis curvipes.* According to [22], among the factors influencing cowpea diversity, the most important are attack of pests, diseases and poverty of soil.
The survey showed that to protect their cowpea in field from all kind of pest attack, farmers often used chemical product: Cypercal 720EC and Conquest. They also used extract of fresh neem leaf (Azadirachta indica) or dry tobacco leaves and powdered soaps. The use of these essences or detergents would be made in consideration of their repellent effect for

insects. Most of farmers (85%) used chemicals to preserve their crop products and others 15% kept it, without or with natural products. According to some authors, 42% farmers use the chemical method [28].

Table 2: nomenclature of accessions collected, means and ethnical group

Local name	Means (literal translation)	Ethnic groups
"*Lai gue agne*"	Red cowpea	"*Tounian*"
"*Mindje osmoudejéni*"	Unknown	"*Gore*"
"*Lai gue bouhou*"	White cowpea	"*Tounian*"
"*Lai gue brai*"	Unknown	"*Tounian*"
"*Lai gue iri*"	Black cowpea	"*Tounian*"
"*Lai gue tega*"	Cowpea tree	"*Tounian*"
"*Lair bil*"	White cowpea	"*Boua*"
"*Lair gangoiri*"	Cowpea curve	"*Boua*"
"*Lair gnan/gnanl*"	Red cowpea	"*Boua*"
"*Loubia al abiate*"	White cowpea	"*Haoussa*", "*Misserié*", "*Dakara*"
"*Loubia al amar*"	Red cowpea	"*Haoussa*", "*Misserié*", "*Dakara*"
"*Loubia al argate*"	Variegate cowpea	"*Haoussa*", "*Misserié*", "*Dakara*"
"*Mindje maïmanè*"	Cowpea with sweet water	*«Gore»»*
"*Mindje kadje*"	Cowpea life	"*Gore*"
"*Mindje kague ngeum*"	Cowpea stalk of Grewia sp	"*Gore*"
"*Mindje guidekey*"	Cowpea of home	"*Gore*"
"*Mindje kla*"	Cowpea rope	"*Gore*"
"*Mindje kouloulou*"	Cowpea millipede	"*Gore*" *et autres*
"*Mindje li*"	Cowpea snake	"*Gore*"
"*Mindje mbodjibey*"	Cowpea for Mbodjibey	"*Gore*", "*Ngaman*", *etc.*
"*Moudjo bôh*"	Big size of cowpea	"*Sar*"
"*Moudjo ndàh*"	White cowpea	"*Sar*", "*Mbaye*"
"*Mindjo ngandenan*"	Sweet cowpea	"*Sara kaba*"
"*Mindjo gouri*"	Cowpea curve	"*Sara kaba*"
"*Mindjo jâha*"	White cowpea	"*Sara kaba*"
"*Moudjo kague*"	Cowpea tree	"*Sar*"
"*moudjo kaga*"	Cowpea tree	"*Sara kaba*" *de Banda/CST*
"*Moudjo kass*"	Cowpea feed	"*Sar*"
"*Moudjo kass dog*"	Cowpea feed of buffalo	"*Sar*"
"*Moudjo kou*"	Cowpea curve	"*Sar*"
"*Moudjo kré*"	Red cowpea	"*Sar*"
"*Moudjo mananbille*"	Cowpea earring	"*Sara kaba*" *de Banda/CST*
"*Moudjo nengabille*"	Cowpea earring	"*Mbaye*"
"*Mindje sucre*"	Sweet cowpea	"*Gore*"
"*Moudjo tolba*"	Cowpea makes host happy	"*Sar*"
"*Mindjo tohou*"	Unknown	"*Sara kaba*" *de Kyabé*
"*Moudjo tore*"	Unknown	"*Sara kaba*" *de Banda/CST*
"*Mindje dobal*"	Unknown	"*Gore*"

3.4. Agro-morphological assessment of accession collected

This study enabled the evaluation of 16 accessions. The other forty-four (44) accessions including very rare climbing accessions did not finish their reproductive development stage and have not been characterized. This failure would be due to the pocket of drought occurred earlier before the normal end of rainfall season which coincided with flowering stage. It would be explained by the late maturing for these accessions too. It would be wise to reach them in farmer area, for genetic improvement.

3.4.1. Analysis of qualitative traits assessed

The qualitative traits assessed showed significant variability between accessions. Most accessions were semi-erect (81.25%) and 75% of them had purple flowers (Table 3). Observation of the plants pigmentation (Figure 1) revealed that 12.5% of plants were not pigmented, 68.75% were low pigmented, 12.5% were medium pigmented and 6.25% were high pigmented. A study in Benin indicated three types of plant pigmentation, low, moderate and high [29].

Table 3: Plant description of cowpea accessions

Traits	Modality	Number of accessions	Frequency (%)
Growth habit	Semi-erect	13	81.25
	Climbing	3	18.75
Flower color	Purple	12	75
	Yellow	4	25
Plant pigmentation	None	2	12.5
	Very slight	11	68.75
	Medium	2	12.5
	High	1	6.25
Leaf marking	Smooth	16	100

Plant none pigmented *low pigmented* *medium pigmented* *high pigmented*

Fig. 1. Variation of plant pigmentation of cowpea accessions

The seeds of assessed accessions (Table 4 and figure 2) were most rhomboids (87.5%) and had black color (18.75%) or light orange and ocher brown color on same accession (18.75%). Many seeds were smooth (75%) with no eye (37.5%) or absent and elongated on same accession (37.5%) and with a testa firmly attached (100%). The accessions of Barh-Kôh showed regarding in seed coloration, a high variability compared to the Chadian cowpeas studied [4]. This difference could be explained by climatic differences between the two study areas. According to [30], the geographical distribution of cowpea cultivars is linked to ecological factors and human factors. Isolated populations and species accumulate genetic differences [31].

All pods were slightly curved (100%), generally had light yellow color (93.75%) and were not dehiscent (100%). This last observation is similar to those of [23] and [4]. According to [32] relative dehiscence of the pod is absent in advanced cultivars but present in some primitive cultivars.

Table 4: Seed description of 16 accessions based on eight qualitative traits

Traits	Modality	Frequency (%)
Seed shape	Rhomboid	87.5
	Kidney	12.5
Seed color	White	12.5
	Black, light orange, brown, ochre-brown	6.25
	Brown	12.5
	Black and light orange	6.25
	Ochre-brown and light orange	6.25
	Black	18.75
	Black, light orange ochre-brown	18.75
	Black, red and light orange	6.25
	Light orange	12.5
Seed size	Medium	18.75
	Small	81.25
Seed texture	Smooth	75
	wrinkly	25
Particular seed feature	Absents	100
Eye pattern	Absent	37.5
	Absent and elongated	37.5
	Elongated	25
Eye color	Brown dark	12.5
	Red	12.5
	Eye absent	37.5
	Eye absent and black	37.5
Attachment of testa	Testa firmly attached to seed	100
Curvature of pod	Slight curved	100
Dehiscence of pod	No	100
Color of pod	Light yellow	93.75
	Light yellow and dark purple	6.25

Qualitative traits observed on cowpea accessions revealed existence of diversity intra-accession (Figure 3). Same observation was made on western Burkina Faso white Caya [33]. This diversity within accession could be explained by the cowpea reproduction mode. In fact, Cowpea is indeed a self-pollinating species with a very low rate of allogamy (1%), except in wet areas where there is the high activity of pollinating insects this rate can reach 10%.

Finally, the seeds brought from farmer area and used for this study were the open-pollinated populations. An open-pollinated population or open-pollinated cultivars are developed for species that are naturally cross-pollinated [33]. During survey in this current study, farmer of Barh-Koh department recognized sowing a mixture of two or more accessions. This same observation has been made by many authors in cowpea [23], in Bambara groundnuts [28], in pearl millet [35] and in sorghum [36].

Fig. 2. Some accessions to show inter-accession diversity of cowpea (A: Moudjo boh; B: Moujo kou; C: Lai gue iri; D: Lair gnanl; E: Lai gue tega; F: Moudjo kag; G: Moudjo kass dog)

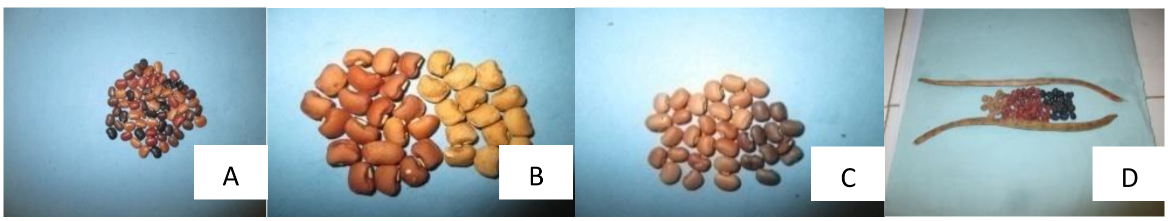

Fig. 3. Some accessions to show diversity within cowpea accessions (A: Moudjo kass; B: Lair gnanl; C: Lai brai; D: Moudjo kass)

3.4.2. Performances of accessions

The analysis of variance indicated that there were high significant difference ($p<0.001$) among accessions for most assessed traits (table 5). Hundred seed weight and number of pod per plant showed significant difference ($p<0.01$) among accessions. Only one trait plant height indicated no significant difference among accessions ($p = 0.136$). According to [7] the cowpea population of Africa had the most diversity in maturity, plant habit, and seed shape.

Coefficient of variation ranged from 7.49% (days to flowering) to 54.06% (grain yield per hectare). The high coefficient of variation was for grain yield per hectare (54.06%); followed by number of pod per plant (53.02%), plant height (34.7%), number of main branch (31.45%) and number of pod per peduncle (30.13%). According to [37], high coefficient of variation presents in the experimental material indicates the possibility of improving characters through phenotypic selection.

The heritability in broad sense (H^2) estimate varied from 28.45% to 96.93%, respectively for plant height and days to flowering. In this investigation many assessed traits except grain yield per hectare (58.57%), peduncle length (58.39%), number of pod per plant (58.01%), number of pod per peduncle (54.99%) and plant height (28.45%) had high heritability indicating that these traits could be governed by additive genes [38]. Heritability is a useful quantitative parameter, which considers the role of heredity and environment determining the expression of a trait [19]. This result indicates that these traits with high heritability could be easily improved by selection.

Analysis of variability of each trait was estimated based on means, minimum and maximum observed value and allowed to see the high difference between accessions for grain yield per hectare trait, which ranged at 0 to 5.41 $t.ha^{-1}$ with an average of 1.57 ± 0.55 $t.ha^{-1}$. The days to flowering ranged from 40 to 55 days with an average of 43.31 ± 3.87 days; however the short rainfall period did not allow some accessions to achieve their reproductive stage. Accession of this current study showed early days to flowering. Some authors found days to flowering ranged from 91 to 114 days [15], 52 to 71 days [12] and 64 to 82 days [13] very long than those of this current study. According to [39], most of farmers (83.7%) preferred cowpea varieties with early maturing.

There was high variability in cowpea grain size, with a length varied from 6 to 9 mm and a means value of 7.35±0.73 cm. Grains weight in this current study ranged from 9.48 to 12.64 compared to those in Senegal, Nigeria and Niger [40], grains in Chad were smaller.

Table 5: Agronomical performances of cowpea accessions evaluated

Traits	Min.	Max.	Mean ± SD	CV (%)	H^2 (%)
PHG (cm)	25	342	162.47 ± 29.94^{ns}	**34.79**	28.45
NPP	1	5	$2.70 \pm 0.38^{**}$	**30.13**	**54.99**
PWD (cm)	0.6	1.7	$1.27 \pm 0.16^{***}$	18.04	**75.99**
NLPO	9	20	$16.22 \pm 2.05^{***}$	17.36	**85.93**
NMB	1	10	$5.30 \pm 1.2^{***}$	**31.45**	**87.46**
NNMS	6	17	$11.39 \pm 1.28^{***}$	**21.45**	**62.66**
PLG (mm)	85	635	$447.68 \pm 50.73^{***}$	**23.22**	**58.39**
POLG (cm)	12.9	22.1	$18.42 \pm 2.37^{***}$	14.34	**94.59**
POWD (cm)	0.6	1	$0.76 \pm 0.07^{***}$	11.76	**72.83**
NPOP	0	128	$38.46 \pm 12.57^{***}$	**53.02**	**58.01**
SLG (mm)	6	9	$7.35 \pm 0.73^{***}$	11.99	**93.69**
SWD (mm)	5	6	$5.53 \pm 0.4^{***}$	10.68	**83.63**
NGPO	7	20	$15.29 \pm 2.54^{***}$	**20.12**	**88.15**
SWTP (g)	0.68	2.32	$1.50 \pm 0.24^{***}$	**24.74**	**86.14**
GYDH ($t.ha^{-1}$)	0	5.41	$1.57 \pm 0.55^{***}$	**54.06**	**58.57**
HSWT (g)	9.48	12.64	$10.98 \pm 1.86^{**}$	7.49	**81.52**
DFW (days)	40	55	$43.31 \pm 3.87^{***}$	11.25	**96.93**

Min: minimum observed value; Max.: maximum observed value; SD: standard deviation; CV: coefficient of variation; H^2: heritability; PHG: plant height; NPP: number of pod per peduncle; PWD: plant width; NLPO: number of locules per pod; NMB: number of main branch; NNMS: number of nodes on main stem; PLG: peduncle length; POLG: pod length; POWD: pod width; NPOP: number of pod per plant; SLG: seed length; SWD: seed width; NGPO: number of grains per pod; SWTP: seed weight per pod; GYDH: grain yield per hectare; HSWT: hundred seed weight; DFW: days to flowering; ns : no significant; ** p<o.o1; *** p<o.oo1.

3.4.3. Relationships among the traits

Pearson's correlations among the traits were observed during phenotypic assessment (table 6). Correlation studies help the plant breeder during selection and provide the understanding of yield components. Grain yield was positively and meaningfully correlated with number of pod per plant ($r = 0.91$, $p<0.01$) and number of locus per pod ($r = 0.6$, $p<0.01$), however, was negatively correlated with plant height ($r = -0.55$, $p<0.01$), days to flowering ($r = -0.53$, $p<0.01$), plant width ($r = -0.49$, $p<0.05$) and pod length ($r = -0.77$, $p<0.01$). Number of seeds per pod was negatively correlated with plant height ($r = -0.69$, $p<0.01$) and days to flowering ($r = -0.85$, $p<0.01$). Hundred seeds weight was positively correlated with plant height ($r = 0.53$, $p<0.01$) and days to flowering ($r = 0.61$, $p<0.01$). Accessions with late flowering time and a high vegetative development, produced heavier seed, but had less number of seed per pod and less grain yield per hectare.

The correlation between plant height and days to flowering was positive ($r = 0.89$, $p<0.01$). However, plant height was negatively correlated with the number of locules per pod ($r = -0.67$, $p<0.01$), pod length ($r = -0.71$, $p<0.001$), number of grain per pod ($r = -0.69$, $p<0.001$) and grain yield per hectare ($r = -0.55$, $p<0.01$). As well as plant height, days to flowering was negatively correlated with number of locules per pod ($r = -0.78$, $p<0.01$), pod length ($r = -0.76$, $p<0.001$), number of grain per pod ($r = -0.82$, $p<0.001$) and grain yield per hectare ($r = -0.53$, $p<0.01$). In opposition with this current study, a study has found a negative correlation between days to flowering time plant height [12]. Nonet less, another study found positively correlated between days to flowering and pod length [13].

The number of seed per pod was positively correlated to pod length ($r = 0.85$, $p<0.01$) and negatively to pod width ($r = -0.76$, $p<0.01$), indicated that accessions with long pods produced many seeds per pod and accessions with large pods produced few seeds per pod. Although the pod width was negatively correlated to hundred seed height ($r = -0.57$, $p<0.001$), it was able to produce long ($r = 0.6$, $p<0.01$) and large ($r = 0.63$, $p<0.001$) seed.

The quantitative data were subjected to principal component analysis (PCA), which revealed that the cumulative contribution rate of the first 4 principal components was 91.174% (Table 7), indicating that these principal components contain the most genetic information of the phenotypic traits and can be used for comprehensive evaluation of core collections. These finding are similar to that of [4] and [15], with 95.76% and 88.2%, respectively. However, there were significantly different from the results of [29] and [12] with 60.16% and 45.28 %, respectively.

According to the principle of some authors, the first principal component accounts for maximum variability in the data with respect to succeeding components [41]. In this current study the first PC I which concentrated 55.159% of the total variance of the agronomic data, was positively associated to number of locules per pod, number of nodes on main stem, peduncle length, pod length, number of grains per pod and grain yield per hectare; however, was negatively associated to plant height, plant width, pod width, seed length, seed width, hundred seed weight and day to first maturity pods.

PC II, with 15.156% contribution rate, was positively associated to number of pod per peduncle number of main branch, number of pod per plant and was negatively associated to number of locules per pod, peduncle length, pod length and seed weight per pod. PC III, with 14.205% of total variance, was negatively associated to number of main branch and positively associated to seed length and width and hundred seed weight. PC IV, with 6.654% contribution rate, was positively correlated to seed weight per pod and pod width and negatively to number of nodes on main stem.

Table 6: Pearson correlation coefficients between traits among cowpea accessions

Traits	PHG	DFW	PWD	NLPO	NMB	NNMS	PLG	POLG	POWD	NPOP	SLG	SWD	NGPO	HSWT	SWTP	GYDH
DFW	0.89**															
PWD	0.63	0.73**														
NLPO	-0.67**	-0.78**	-0.78**													
NMB	0.74**	0.74**	0.51**	-0.5**												
NNMS	-0.48	-0.72**	-0.53**	0.61**	-0.42											
PLG	-0.71**	-0.76**	-0.64**	0.73**	-0.76**	0.63**										
POLG	-0.71**	-0.81**	-0.78**	0.84**	-0.77**	0.68**	0.8**									
POWD	0.79**	0.79**	0.75**	-0.67**	0.42	-0.52**	-0.49*	-0.61**								
NPOP	-0.36	-0.3	-0.31	0.17	0.05	0.02	0.03	0.06	-0.69**							
SLG	0.24	0.25	0.60**	-0.58**	-0.1	-0.17	-0.26	-0.33	0.46*	-0.31						
SWD	0.13	0.29	0.63**	-0.62**	-0.16	-0.39	-0.25	-0.36	0.38	-0.15	0.89					
NGPO	-0.69**	-0.82**	-0.92**	0.91**	-0.55**	0.6**	0.74**	0.85**	-0.76**	0.36	-0.59**	-0.62**				
HSWT	0.53**	0.61**	0.73**	-0.65**	0.07	-0.48*	-0.34	-0.51**	0.78**	-0.62**	0.72**	0.73**	-0.72**			
SWTP	-0.19	-0.39	-0.57**	0.63**	-0.52**	0.27	0.55**	0.62**	-0.14	-0.25	-0.27	-0.36	0.65**	-0.06		
GYDH	-0.55**	-0.53**	-0.49**	0.39	-0.28	0.17	0.32	0.32	-0.77**	0.91**	-0.28	-0.13	0.6**	-0.56	0.08	
DFMP	0.73**	0.83**	0.90**	-0.88**	0.66**	-0.71**	-0.85**	-0.89**	0.76**	-0.26	0.49*	0.51**	-0.94**	0.64**	-0.57**	-0.50*

Min: minimum observed value; Max.: maximum observed value; SD: standard deviation; CV: coefficient of variation; H^2: heritability; PHG: plant height; PWD: plant width; NLPO: number of locules per pod; NMB: number of main branch; NNMS: number of nodes on main stem; PLG: peduncle length; POLG: pod length; POWD: pod width; NPOP: number of pod per plant; SLG: seed length; SWD: seed width; NGPO: number of grains per pod; SWTP: seed weight per pod; GYDH: grain yield per hectare; HSWT: hundred seed weight; DFW: days to flowering; * p<o.o5; ** p<o.oo1.

Table 7: Principle component analysis of quantitative traits recorded in cowpea

Principle component	PC I	PC II	PC III	PC IV
Eigen value	9.929	2.728	2.557	1.198
% total variance	55.159	15.156	14.205	6.654
Cumulative variance %	55.159	70.315	84.520	91.174
Plant height	**-0.807**	-0.140	-0.348	0.232
Days to flowering	**-0.901**	0.006	-0.259	0.220
Number of pod per peduncle	0.107	**0.638**	**0.577**	0.447
Plant width	**-0.909**	0.035	0.147	-0.151
Number of locules per pod	**0.906**	**-0.207**	-0.124	0.083
Number of main branch	-0.632	**0.225**	**-0.706**	-0.054
Number of nodes on main stem	**0.676**	-0.337	-0.033	**-0.513**
Peduncle length	**0.795**	**-0.309**	0.285	-0.025
Pod length	**0.881**	**-0.307**	0.173	-0.012
Pod width	**-0.844**	-0.411	0.018	**0.200**
Number of pod per plant	0.385	**0.899**	-0.089	-0.038
Seed length	**-0.551**	-0.091	**0.699**	-0.266
Seed width	**-0.552**	0.126	**0.786**	-0.147
Number of grains per pod	**0.971**	-0.029	-0.076	0.168
Hundred seed weight	**-0.749**	-0.322	**0.485**	0.185
Seed weight per pod	0.532	**-0.491**	0.109	**0.626**
Grain yield per hectare	**0.601**	0.732	0.145	0.108
Day to first mature pods	**-0.964**	0.143	-0.028	-0.002

3.4.4. Cluster analysis

The cluster analysis performed with non-highly correlated eight quantitative traits using agglomerative hierarchical clustering to construct a dendrogram (Figure 4) with amount of mean values of each in tableau 8. Truncation to level 10.236 allowed classifying the accessions into four meaningful independent clusters. The first cluster was found to be the largest cluster with eight landraces with reduces days to flowering (40.958 days), shortest plant height (151.903 cm) and highest seed weight per pod (1.583 g).

This cluster was classified into three sub cluster, of which the first had one accession, the second had three and the third had four accessions. In this cluster the accessions 27 and 38 were more closely related, it is case for accessions 3 and 9, whereas accession 45 was distantly related to other accessions in the same cluster.

Cluster 2 contained tree accessions showed higher values for the desirable agronomic traits, with highest mean values of grain yield per hectare (2.034 $t.ha^{-1}$) and highest number of pod per plant (52.778). In this cluster the accession 51 was slightly distanced than the accessions 14 and 48, which were closely related, and accession 51 had a highest mean value of grain yield per hectare and a highest mean value of number of pod per plant.

Cluster 3 was composed of four accessions presented means values of all assessed traits. It was divided in three sub clusters which the accessions 34, 15 and 33 were closely related among them and distantly related to accession 17 in same cluster.

Cluster 4 with only one accession was found to be unworthy in terms of grain yield per plant (19 $kg.ha^{-1}$) and has longest days to flowering (54 days) and highest plant height (244.778 cm).

Current study allowed seeing inter-cluster divergences through high significant differences ($p \leq 0.009$) between pair of clusters (Table 9). D^2 value ranged from 55.335 to 388.776, maximum inter-cluster distance ($D^2 = 388.776$) occurred between clusters II and III indicating greater diversity between genotypes belonging to these cluster. The minimum inter-cluster distance (D^2 = 55.335) was observed between cluster I and cluster III (p = 0.009) indicating a close relationship between accessions involved, while there was high significant difference (p≤0.009) between these cluster. According to [42], parents within a cluster can be used for hybridization.

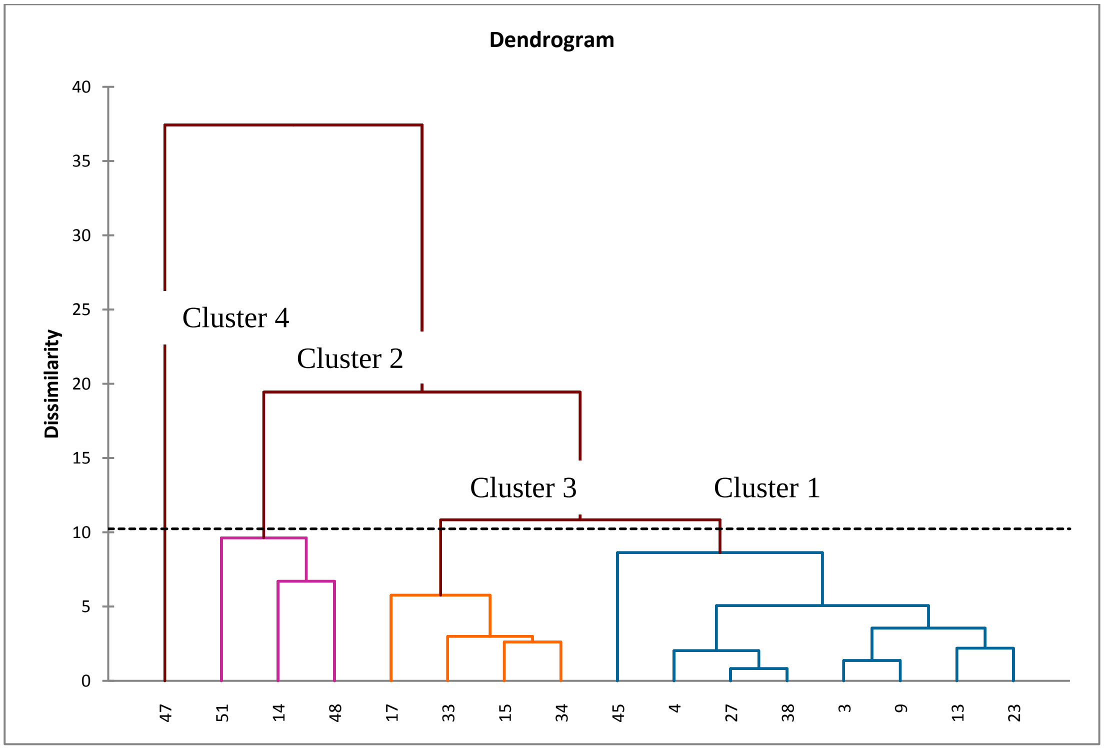

Figure 4: Clustering pattern of 16 cowpea accessions wards minimum variance dendrogram

Table 8: means values of four clusters derived from Ward's hierarchical clustering

Traits	Cluster 1	Cluster 2	Cluster 3	Cluster 4		
Frequency	8	3	4	1	F	Pr > F
Days to flowering	**40.958**	45.667	41.25	**54**	**34.564**	**< 0.0001*****
Number of pod per plant	38.042	**52.778**	27.639	3.556	**86.885**	**< 0.0001*****
Seed length	**7.417**	7.630	8.056	8.222	1.142	0.369 ns
Seed width	**5.625**	5.778	5.694	6	0.473	0.706 ns
Hundred seed weight	11.144	11.482	11.826	16.88	**10.515**	**0.001****
Seed weight per pod	**1.583**	1.233	1.443	1.518	1.991	0.165ns
Grain yield per hectare	1.669	**2.034**	1.140	**0.019**	**32.515**	**< 0.0001*****
Plant height	**151.903**	182.148	160.813	**244.778**	**13.276**	**0.0003*****

Table 9: Average of inter cluster distance, Fisher distance and P-value

Cluster (From/To)	D^2 value	F-value	P-value
Cluster I – Cluster II	165.224	20.797	0.001
Cluster I – Cluster III	55.335	8.513	0.009
Cluster I – Cluster IV	183.745	16.961	0.001
Cluster II – Cluster III	388.776	38.450	<0.001
Cluster II – Cluster IV	300.932	20.834	0.001
Cluster III – Cluster IV	213.722	16.440	0.002

4. Conclusion

Prospection mission allowed collected 60 cowpea landraces in Barh-Kôh department, with high amount of diversity within and among landraces. Farmers mostly named their accessions according to their growth habit, seed color, pod shape, the origin of this specific accession and the name of person you brought the accession of village. The negative and meaningful correlation between grain yield and days to flowering demonstrated the importance to develop early flowering varieties in order to increase productivity of cowpea in South of Chad. The study allowed finding out one accession which reached a highest potential for grain yield per hectare (5.41 $t.ha^{-1}$). There are accessions in this study which showed the highest number of pod per plant (128), the highest number of grains per pod (20), the heaviest seeds weight per pod (2.32 g) and longest plant height (342 cm). Assessment of agronomical and morphological variability clustered 16 assessed landraces in four clusters with high amount of inter-cluster divergence through high significant difference ($p \leq 0.009$) between clusters. The cluster 2 contained tree productive landraces seems interesting to get novel source of productivity and the cluster 1, put together shortest flowering landraces could be provided the novel source of early grain maturing, short plant height and high seed weight per pod. The use of landraces of these two clusters (I and II) in breeding program could provide a hope for creation of new early and productive varieties. This agronomical and phenotypic information will be used in our breeding program to develop the new early grain maturing accession with high grain yield and produced high biomass.

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