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Genetic diversity for immature pod traits in Ethiopian cowpea [*Vigna unguiculata* (L.) Walp.] landrace collections

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This study was undertaken to evaluate the extent and pattern of genetic diversity for immature pod traits in Ethiopia cowpea landrace collections. Eighty one landraces and improved cultivars were tested in a 9 x 9 simple lattice design. Analysis of variance revealed highly significant (P<0.01) or significant (p<0.05) differences among the genotypes for all traits. The first four principal components were able to explain 81% of variation for quantitative traits and 76% for qualitative traits. The genotypes were grouped into three distinct clusters, the first, second and third clusters with 60, 15 and 25% of the genotypes in that order. The landraces were distributed all over the clusters while the improved cultivars were absent in the second cluster. Shannon-Weaver diversity indices also showed existence of adequate genetic variability among the genotypes for qualitative traits. Shannon-Weaver diversity indices ranged from the lowest of 0.50 for pod curvature to the highest of 0.99 for pod shape. The study clearly showed that, even if the genotypes were classified into a few cluster, there was adequate divergence among the clusters showing existence of considerable genetic variability for immature pod traits for exploitation in future breeding for better green pod yield and quality in cowpea.

Key words: Cowpea, diversity, genotypes, immature pod, qualitative traits, quantitative trait.

INTRODUCTION

Cowpea (*Vigna unguiculata (L.)* Walp) is one of the most important grain legumes native to Africa (Timko and Singh, 2008). Cowpea is globally grown in tropical Africa, Asia, Latin America and Southern USA (Trinidad et al., 2010) under a wide range of soil pH including under low soil fertility, acidic soils and under drought-prone conditions (Badiane et al., 2012). The global area coverage of cowpea is estimated to around 11 million hectare from which 6 million tons of grain is produced with the share of Africa being 96% of world cowpea grain production (FAOSTAT, 2018). In Ethiopia, cowpea is grown almost in all lowland areas but the major

production complexes are located in the Southern and Western parts of the country (Bedru et al., 2019; Sisay et al., 2019).

Cowpea is grown by farmers for different purposes including the nutritious tender leaves, immature pods, fresh peas and dry grain (Chikwendu et al., 2014; Gerrano et al., 2017; Mamiro et al., 2011). In terms of nutritive value, cowpea is an excellent source of protein, particularly to the poor people who could not afford animal products, essential minerals, vitamins and folates (USDA, 2015). Cowpea hay is also an important feed for animals during the dry seasons (Timko and Singh, 2008). The crop has deep roots which help to stabilize the soil, protect the surface and preserve the moisture (NRC, 2006). Like other legumes, cowpea also restores soil fertility by fixing atmospheric nitrogen (Nielsen et al., 1997). Thus, this crop can contribute greatly towards meeting the food requirement of people in areas where food security and malnutrition are major challenges.

Despite the manifold merits of cowpea in Ethiopia, the national production and productivity is far below the potential partially due to the biophysical challenges constraining productivity in smallholder farms and the inadequate technological interventions. Information on the cowpea production and productivity is scantly but a few sources show that the average national yield of cowpea in Ethiopia is estimated to be 400 kg ha^{-1} (Bedru et al., 2019), while yields of 2200 to 3200 kg ha^{-1} have been commonly recorded from improved varieties with proper crop management and protection practices (MoA, 2012). It showed the comparative advantages of genetic and agronomic interventions. From the breeding efforts by the research system hitherto, however, only six improved varieties were released (MoA, 2018), indicating the potential for further research particularly in cowpea breeding efforts.

It is obvious that genetic diversity in plant genetic resources provides an opportunity for plant breeders to develop new and improved cultivars with desirable characteristics including for farmers' preferred traits (Govindaraj et al., 2015). The success of crop breeding programs by and large depends on the magnitude of genetic diversity present in the breeding source materials, heritability of the trait under consideration and the level of selection intensity applied (Falconer, 1989). Genetic diversity is a prerequisite for the identification of superior genotypes, which may be recommended for direct release as commercial cultivars for wider production, but also for the identification of desirable parents to use as source materials in breeding programs (Rajaravindran and Natarajan, 2011). Ethiopia is believed to have considerable genetic diversity for cowpea both at phenotypic and genotypic levels (Belayneh et al., 2016; Mulugeta et al., 2016; Sisay et al., 2019; Tesfaye et al., 2019). Apart from genetic diversity for the regular morpho-agronomic traits at phenotypic level and for molecular diversity at genotypic level, information on the magnitude and pattern of phenotypic diversity for immature pod traits in Ethiopian landrace collections is scanty. Thus, this study was proposed to determine the extent and pattern of genetic diversity among cowpea collections using immature pod related phenotypic traits.

MATERIALS AND METHODS

Plant material and experimental design

Eighty one cowpea genotypes including 77 Ethiopian landraces collections kindly provided by the Ethiopian Institute of Biodiversity and Melkassa Agricultural Research Center and four improved varieties were selected for the study (Table 1). The experiment was conducted in the experimental field of Melkassa Agricultural Research Center in Ethiopia under irrigated conditions. Complete block design like randomized complete block design become less efficient as the number of treatment increases, primarily block size increases proportionally with the number of treatments, and the homogeneity of experimental plot within a large black is difficult to maintain (Gomez and Gomez, 1984). To this effect, 9 x 9 simple lattice design was employed. The seeds were directly sown on a 2.25 m x 3.0 m plot of three rows with 75 cm inter row and 20 cm intra row spacing. All crop management and protection practices were kept constant as per the recommendation for the site.

Data collection

Quantitative characters including days to 50% flowering, days to first pod picking, terminal leaflet length, terminal leaflet width, number of primary branches, green pod length and width, average green pod weight and pod yield per plant were recorded. All data were taken on plant basis except days to 50% emergence and days to first pod picking which were recorded on plot basis. Qualitative variables including growth pattern, pod attachment to peduncle, pod color, immature pod pigmentation pattern, absence or presence of secondary color in the pod, hue of secondary color, pod shape, pod curvature, pod suture string and pod texture of the surface were also scored using the standard descriptors for cowpea germplasm characterization (IBPGR, 1983).

Statistical analysis

Analysis of variance was performed based on the model for simple lattice design following Gomez and Gomez (1984). SAS version 9.2 statistical package was used for the analysis (SAS Institute Inc., 2010). Records on the quantitative variables were pre-standardized to means of zero and variances of unity before clustering to avoid bias due to differences in measurement scales (Manly, 1986). The optimum number of clusters in data set were determined by using gap statistics (Tibshirani et al., 2001). Analysis were done using different packages of R in R environment (R Development Core Team, 2019). Hierarchical cluster analysis was used to group the genotypes based on their similarities. The distance was measured using Euclidean distance and the distance matrix was used to construct the dendrogram using ward D^2 linkage method. Intercluster distances were calculated based on the standardized Mahalanobis's D^2 statistics as:

$$D^2_{ij} = (xi - xj)' \, cov^{-1} \, (xi - xj)$$

Table 1. Genotypes, collection site and source of the genotypes.

No.	Genotype	Collection region	Source	No	Genotype	Collection region	Source
1	208776	Oromia	EBI	42	NLLP-CPC-07-27	Oromia	MARC
2	211441A	Gambela	EBI	43	NLLP-CPC-07-28	SNNPRS	MARC
3	211441B	Gambela	EBI	44	NLLP-CPC-07-29	Gambela	MARC
4	211441C	Gambela	EBI	45	NLLP-CPC-07-31	SNNPRS	MARC
5	211490	SNNPRS	EBI	46	NLLP-CPC-07-32	Gambela	MARC
6	211491B	SNNPRS	EBI	47	NLLP-CPC-07-33	Oromia	MARC
7	211557	Amhara	EBI	48	NLLP-CPC-07-39A	Gambela	MARC
8	216749A	Gambela	EBI	49	NLLP-CPC-07-39B	Gambela	MARC
9	216749B	Gambela	EBI	50	NLLP-CPC-07-42	Oromia	MARC
10	220575	Amhara	EBI	51	NLLP-CPC-07-45	SNNPRS	MARC
11	222867	Gambela	EBI	52	NLLP-CPC-07-46A	SNNPRS	MARC
12	222890	Gambela	EBI	53	Dass 007	Gambela	MARC
13	223402	Oromia	EBI	54	NLLP-CPC-07-46B	SNNPRS	MARC
14	228624	Amhara	EBI	55	NLLP-CPC-07-47	SNNPRS	MARC
15	233403	Amhara	EBI	56	NLLP-CPC-07-48B	SNNPRS	MARC
16	235122B	Tigray	EBI	57	NLLP-CPC-07-82	Tigray	MARC
17	235122A	Tigray	EBI	58	NLLP-CPC-07-83	Tigray	MARC
18	244804	SNNPRS	EBI	59	NLLP-CPC-07-49	SNNPRS	MARC
19	Dass 001	Gambela	MARC	60	NLLP-CPC-07-51	SNNPRS	MARC
20	NLLP-CPC-07-01	Amhara	MARC	61	NLLP-CPC-07-52	SNNPRS	MARC
21	NLLP-CPC-07-02	Tigray	MARC	62	NLLP-CPC-07-53	Oromia	MARC
22	NLLP-CPC-07-03	Tigray	MARC	63	NLLP-CPC-07-54	Oromia	MARC
23	NLLP-CPC-07-04	Amhara	MARC	64	NLLP-CPC-07-48A	SNNPRS	MARC
24	NLLP-CPC-07-05	Amhara	MARC	65	NLLP-CPC-07-55	Oromia	MARC
25	Dass 002	Gambela	MARC	66	NLLP-CPC-07-56	Oromia	MARC
26	NLLP-CPC-07-07	Amhara	MARC	67	NLLP-CPC-07-58	Oromia	MARC
27	NLLP-CPC-07-09	Amhara	MARC	68	NLLP-CPC-07-60	Oromia	MARC
28	NLLP-CPC-07-10	Amhara	MARC	69	NLLP-CPC-07-64	Oromia	MARC
29	NLLP-CPC-07-101	Tigray	MARC	70	NLLP-CPC-07-69	Amhara	MARC
30	NLLP-CPC-07-11	Amhara	MARC	71	NLLP-CPC-07-72	Amhara	MARC
31	NLLP-CPC-07-12	Amhara	MARC	72	NLLP-CPC-07-75	Amhara	MARC
32	NLLP-CPC-07-14A	Tigray	MARC	73	NLLP-CPC-07-77	Tigray	MARC
33	NLLP-CPC-07-14B	Tigray	MARC	74	NLLP-CPC-07-78	Tigray	MARC
34	NLLP-CPC-07-16A	Oromia	MARC	75	NLLP-CPC-07-85	Tigray	MARC
35	NLLP-CPC-07-16B	Oromia	MARC	76	NLLP-CPC-07-89	Tigray	MARC
36	Dass 005	Gambela	MARC	77	NLLP-CPC-07-97	Gambela	MARC
37	NLLP-CPC-07-16C	Oromia	MARC	78	Kenketi	Improved	MARC
38	NLLP-CPC-07-18	SNNPRS	MARC	79	Bole	Improved	MARC
39	NLLP-CPC-07-23	Tigray	MARC	80	Black eye bean	Improved	MARC
40	NLLP-CPC-07-24	Tigray	MARC	81	TVU	Improved	MARC
41	NLLP-CPC-07-26	Oromia	MARC				

EIB=Ethiopian Institute of Biodiversity, MARC= Melkassa Agricultural Research Center.

Where, D^2ij = the distance between cases i and j; xi and xj = vectors of the values of the variables for cases i and j; and cov^{-1} = the pooled within groups variance-covariance matrix. The significance of D^2 values was tested by comparing the D^2 values between any two clusters against tabulated chi-square (χ^2) values at p = -1 degrees of freedom where p refers to the number of quantitative characters considered.

The diversity index (H') of Shannon and Weaver (1949) was used to measure the level of genetic variation for qualitative traits. The index (H') was estimated as follows:

$$H' = 1 - \sum_{i=1}^{n} pi \log_e pi$$

Table 2. Range, genotypes showing the extreme values, mean value from analysis of variance and coefficient of variance of 10 quantitative traits in 81 cowpea genotypes.

Trait	Minimum		Maximum		MS	Grand mean	CV (%)
	Value	Genotype	Value	Genotypes			
1	42	222867, 216749B, NLLP-CPC-07-29	99	NLLP-CPC-07-56	286***	58	5.1
2	76	NLLP-CPC-07-82	236	NLLP-CPC-07-77	1373***	149	13.5
3	54	222867	110	NLLP-CPC-07-04, NLLP-CPC-07-56	280.2***	70	5.6
4	3.4	235122A	8.4	NLLP-CPC-07-48B	0.9***	5.9	10.2
5	7.2	NLLP-CPC-07-39A	13.1	NLLP-CPC-07-16B	1.6***	10.3	5.1
6	4.3	NLLP-CPC-07-39A	8.72	Black eye bean, NLLP-CPC-07-32	1.1***	7.0	7.2
7	9	NLLP-CPC-07-69	16.8	NLLP-CPC-07-97	2.7***	12	7.9
8	2.6	NLLP-CPC-07-69	6.8	TVU	0.4*	4.0	13.1
9	0.6	NLLP-CPC-07-69	4.5	NLLP-CPC-07-97	0.6***	2.0	25.0
10	30	NLLP-CPC-07-24	747	NLLP-CPC-07-54	32348***	305	32.0

1=Days to 50% flowering, 2= Plant height (cm), 3= Days to 1st pod picking, 4= Terminal leaflet length (cm), 5= Terminal leaflet width (cm), 6= Number of primary branches, 7= green pod length (cm), 8= green pod width (mm), 9=Av. weight of green pod (g), 10= Pod yield (g/plant).

Where: H' = Shannon diversity Index; pi = the proportion of accessions in the ith class of an n–class character; n = the number of phenotypic classes of traits. Each diversity index value was divided by its maximum value (log_en) and normalized, to keep the values between 0 and 1. Principal components based on correlation matrix were calculated using the same software as in clustering.

RESULTS AND DISCUSSION

Analysis of variance

The range, mean square and CV of 10 quantitative characters of 81 cowpea genotypes are presented in Table 2. The range in the period from sowing to 50% flowering and days to first pod set was almost equal to 57 and 56 days, respectively. Similarly, the difference between the maximum and minimum mean value of plant height, terminal leaflet length, terminal leaflet width, pod length, pod width, average pod weight and pod yield were 160 cm, 5 cm, 5.9 cm, 4.4, 7.8 cm, 4.2 mm, 3.9 g and 717 g, respectively. The mean number of days to 50% flowering ranged from 42 days for genotypes 222867, 216749 B and NLLP-CPC-07-29 to 99 days for genotype NLLP-CPC-07-56. For days to first pod picking, the mean number of days ranged from 54 days for genotype 222867 to 110 days for genotypes NLLP-CPC-07-04 and NLLP-CPC-07-56. These phenological traits, 37% of the genotypes had taken more number of days to flowering compared to the grand mean (58 days) while 28% of the genotypes had taken more number of days to first pod picking compared to the grand mean (70 days). A wide range of variation in immature pod yield was recorded, which ranged from 30 g per plant for genotype NLLP-CPC-07-24 to 747 g per plant for genotype NLLP-CPC-07-54. The wide range for most of the traits indicated the diversity among the cowpea genotypes and also suggests

the possibility of improving these traits through selection. The range and mean of days to 50% flowering and first green pod picking, plant height, number of primary branch, average pod weight and immature pod yield were generally larger than the previous reports (Khanpara et al., 2015; Shanko et al., 2014; Srinivas et al., 2017).

Most of the characters studied showed very highly significant (P<0.001) differences among genotypes except immature pod width which was significant at p<0.05. The analysis of variance revealed significant differences among the genotypes for the ten quantitative characters indicating the presence of adequate variability, which can be exploited through selection. Different reports indicated there is substantial phenotypic variability among cowpea varieties for different characters (Animasaun et al., 2015; Cobbinah et al., 2011; Gerrano et al., 2015; Lazaridi et al., 2017; Menssen et al., 2017; Molosiwa et al., 2016).

Diversity based on quantitative traits

Principal component analysis (PCA) reflects the importance of the largest contributor to the total variation at each axis of differentiation (Sharma, 1998). To decide how many components to maintain; different methods were recommended, an eigenvalue greater than one indicates that PCs account for more variance than accounted by one of the original variables in standardize data. This is commonly used as a cutoff point for which PCs are retained (Kaiser, 1960). An alternative method to determine the number of PCs is to look at a scree plot, which is the plot of eigenvalues ordered from the largest to the smallest. The number of component is determined by the point beyond which the remaining eigen values are

Table 3. Eigenvectors and eigenvalues of the first four principal components (PCs) for 10 quantitative pod characters of 81 cowpea genotypes.

Trait	Principal components			
	PC1	PC2	PC3	PC4
Eigenvalue	2.79	2.38	1.59	1.33
Proportion	27.9	23.8	15.9	13.3
Cumulative	27.9	51.7	67.6	80.9
Eigenvectors				
Days to 50% flowering	0.44	0.69	-0.45	0.32
Plant height (cm)	-0.19	0.62	-0.10	-0.32
Days to 1st pod picking	0.36	0.73	-0.46	0.30
Number of primary branches	0.32	-0.45	-0.14	0.54
Terminal LL length(cm)	0.04	0.44	0.77	0.29
Terminal LL width(cm)	-0.16	0.62	0.65	0.10
Pod length (cm)	0.86	0.02	0.28	-0.06
Pod width (mm)	0.74	-0.10	0.06	-0.55
Av. weight of green pod (g)	0.91	0.02	0.10	-0.26
Pod yield (g/plant)	0.42	-0.46	0.22	0.52
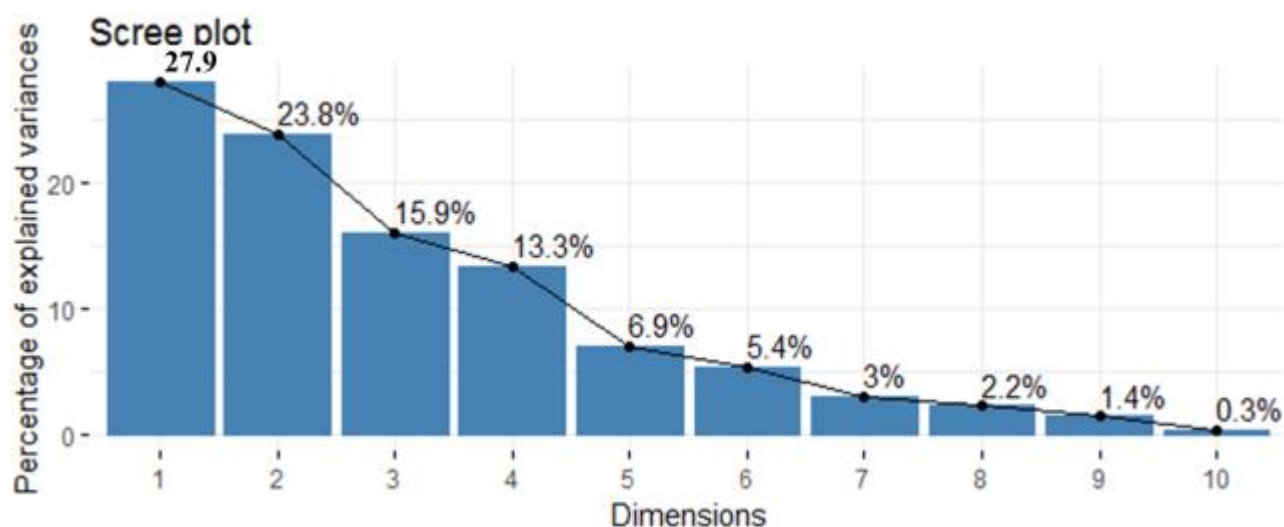
Figure 1. Scree plot of principal components (PCs) for 10 quantitative traits in 81 cowpea genotypes.

all relatively small and of comparable size (Jolliffe, 2002; Peres-neto et al., 2005). Accordingly, based on the scree plot (Figure 1) and eigenvalues (Table 3) four principal components (PCs) having eigenvalues between 1.33 and 2.79, extracted a cumulative of about 81% of the total phenotypic diversity maintained (Table 3). Manggoel and Uguru (2011) reported the first three components contributed 78.11% of the variability among the 10 cowpea accessions using 10 quantitative traits evaluated.

According to Chahal and Gosal (2002) characters with the largest absolute values closer to unity within the principal components influence the clustering more than those with lower absolute values closer to zero. The first principal component explains up to 27.9% of the total variance, and this was due mainly to variations in pod characters (average pod weight, pod length and width (Table 3). High contribution of pod character reported by Tesfaye et al. (2019) and in their report, about 77% of the total variance in 18 quantitative traits of 324 Ethiopian cowpea accessions could be explained on the basis of seven principal components and the first 22.6% of the total variance was due to mainly to pod length, seed
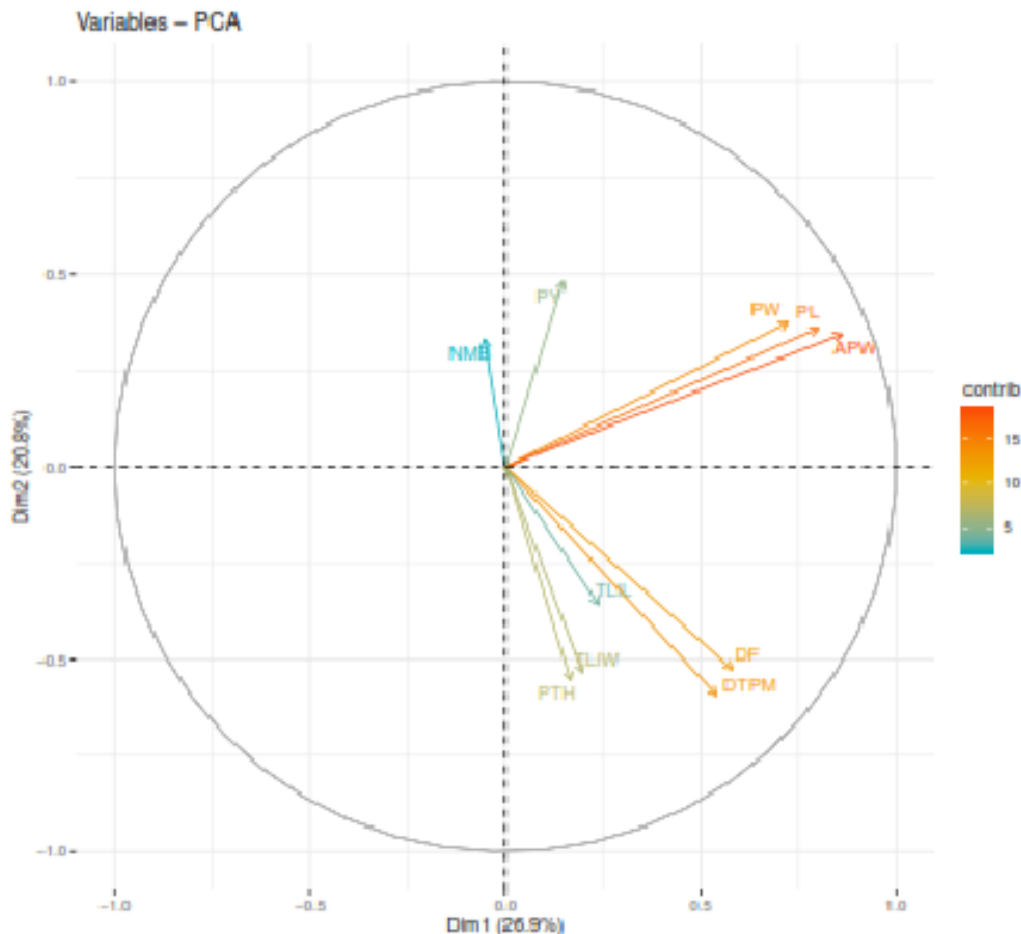
Figure 2. Variable correlation plot showing the relationships between 10 quantitative traits in 81 cowpea genotypes collected from different region of Ethiopia. DF=Days to 50% flowering, PH= Plant height, DTPM= Days to 1st pod picking, TLLL= Terminal leaflet length, TLLW= Terminal leaflet width, NMB= Number of primary branches, PL= immature pod length, PW= immature pod width, APW=Average weight of immature pod, PY= Pod yield.

length, seed width and seed yield per plot. Days to 50% flowering, days to first pod picking, plant height, and terminal leaf width were the main contributor for the variation in the second PC, which contribute 23.8% from the total variations. Similarly, the proportion of the total phenotypic variance of the genotypes accounted for by the third and fourth PCs were about 15.9 and 13.3%, respectively. The major contributor character for the third PC were variations mainly in the terminal leaflet length and width; and by less extent the phenological traits while variations in pod width, number of primary branches and pod yield contribute to the fourth PC. The PCA confirmed that the collected Ethiopian cowpea landraces have high diversity and all of the traits considered appeared to have high contributions towards the total phenotypic variability.

In the variable correlation plot (Figure 2), variables that are closed to the center of the plot are less important for the first components. Accordingly, a number of primary branches (NMB) and terminal leaflet length (TLLL) contribute less for principal components 1 and 2. Pod characters such as pod length (PL), pod width (PW), and average pod weight (APW), and crop maturity parameters (days to 50% flowering (DF) and first pod picking (DTPM)) were the major variables contributed for the variance of the first two components.

To observe the overall distribution pattern and correlations among the data attribute cluster analysis was done based on ten morphological traits related to the immature pod character of cowpea. The optimum number of clusters as determined by gap statistics was 3 (Figure 3a). The average linkage clustering method classified the 81 cowpea genotypes into three distinct clusters (Figure 3b). The number and name of genotypes in each cluster with their collection regions are presented in Table 4.

Cluster I was the largest group having 49 (60%) genotypes comprised from all collection regions, improved
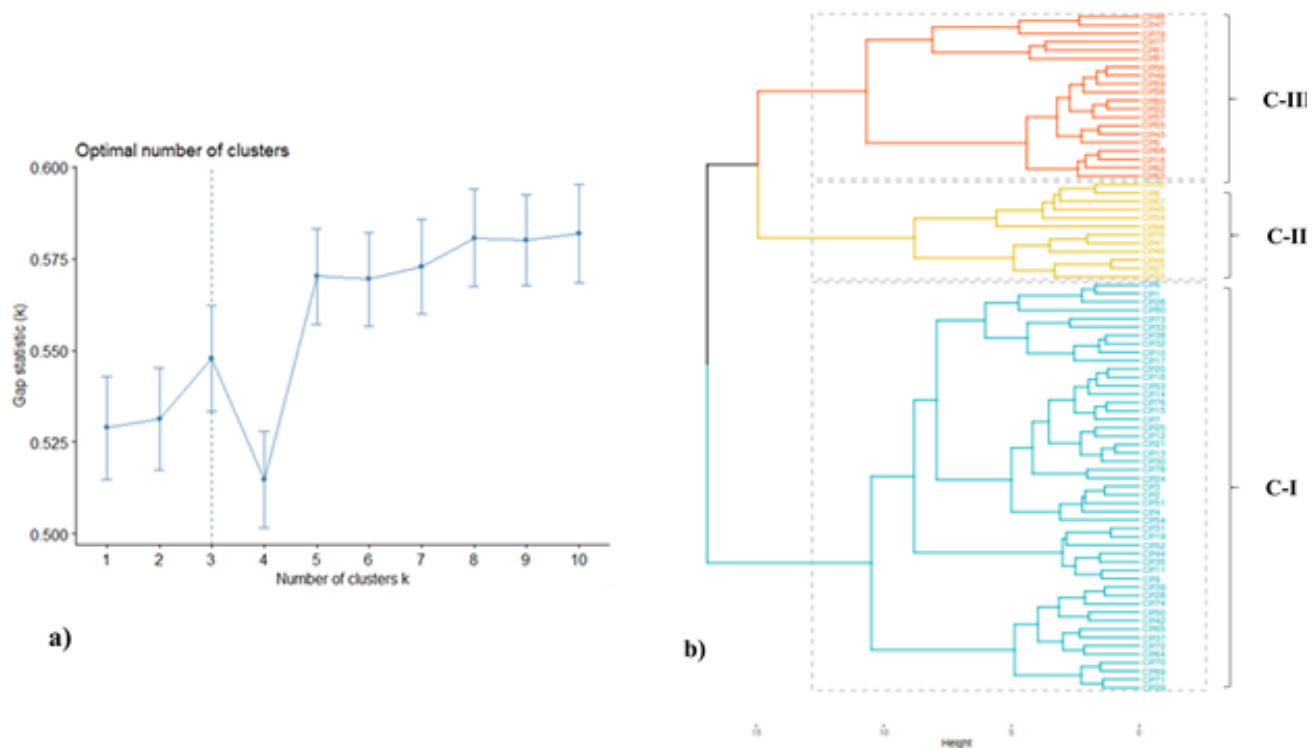
Figure 3. Cluster analysis: (a) Gap statistics to determine the number of clusters (K). (b) Dendrogram showing the relationships among the 81 cowpea genotypes evaluated for 10 quantitative traits. (Blue, yellow and red color represent cluster I, II and III, respectively).

cultivars and are characterized by early maturing genotypes, with the mean value of 51 days and 64 days for 50% flowering and first pod picking, respectively. The pod length, width and weight of this group were smaller. The second cluster consisted of about 15% of the genotype but all are landraces from all collection regions. This group was characterized by high yield and yield component traits (pod length, pod width, average pod weight and a number of the primary branch). The third group had 20 (25%) genotypes with large leaf areas (leaflet length and width), highest plant height and late maturing genotypes. The third cluster with high yield and genotypes in this cluster is a good source of pod yield per plant. The mean value of the 10 quantitative characters in each cluster is indicated in Table 5.

The pairwise generalized squared distance (D^2) among the three clusters based on Mahalanobis's D^2 statistics revealed the maximum and highly significant genetic distance was recorded between cluster I and III (D^2= 34.73) followed by cluster II and cluster III (D^2= 22.15). Non-significant (P>0.05) inter-cluster distance was observed between clusters I and II (D^2=9.96), indicating that genotypes in these two clusters had relatively little genetic divergence (Table 6). Thus, the crossing of genotypes from these two least distanced clusters produces little heterotic expression in the F1 population

and low range of variability in subsequent segregating populations.

Diversity based on qualitative traits

In the principal component analysis (PCA) of 10 qualitative traits in 81 cowpea genotypes, four principal components were retained based on eigenvalues and scree plot (Table 7 and Figure 4). The qualitative traits showed that the first four PCs explained 35, 19, 12 and 10%, respectively of the total variance. The correlation between the variables and components for PC1 was mainly due to pod secondary color, the hue of secondary color, immature pod pigmentation, and pod attachment to peduncle and pod suture string. The texture of the pod surface and pod shape contributes to the variance in the PC2. The third component was related to the Intensity of green color in the immature pod and pod degree of curvature while PC4 was mainly due to the growth pattern of the genotypes.

Frequency distribution patterns, percent of proportion and Shannon-Weaver Diversity Index (H') were estimated for 81 cowpea genotypes from 10 qualitative traits (Table 8). The dominant (81%) growth pattern among the genotypes was indeterminate growth habit and 68% of
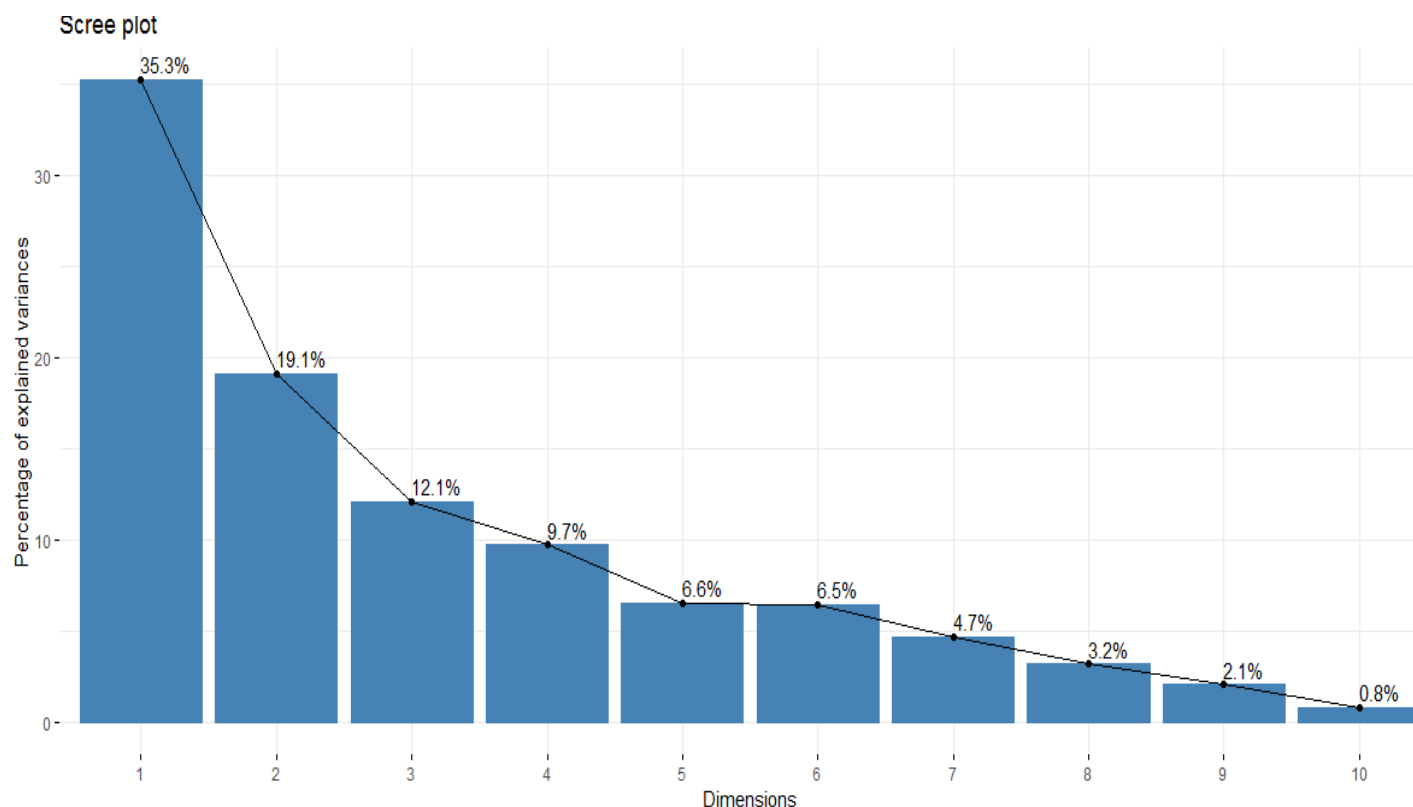
Figure 5. Scree plot of principal Components (PCs) for 10 qualitative traits in 81 cowpea genotypes.

Table 4. Clustering of 81 cowpea genotypes into four clusters using mean of 10 agro-morphological characters.

Cluster	Number of accessions	Accessions included	Collection region
I	49	211441A, 211491B, 220575, 222867, 223402, 228624, 233403, 211441B, 211441C, 222890, 235122A, 208776, 211557, 235122 B, 216749, Dass 001, Dass 002, Dass 007, Kenketi, Black eye Bean, NLLP-CPC-07-01, NLLP-CPC-07-02, NLLP-CPC-07-05, NLLP-CPC-07-07, NLLP-CPC-07-10, NLLP-CPC-07-101, NLLP-CPC-07-11, NLLP-CPC-07-12, NLLP-CPC-07-14A, NLLP-CPC-07-14B, NLLP-CPC-07-16B, NLLP-CPC-07-16C, NLLP-CPC-07-18, NLLP-CPC-07-23, NLLP-CPC-07-27, NLLP-CPC-07-29, NLLP-CPC-07-42, NLLP-CPC-07-45, NLLP-CPC-07-46A, NLLP-CPC-07-46B, NLLP-CPC-07-48A, NLLP-CPC-07-55, NLLP-CPC-07-64, NLLP-CPC-07-69, NLLP-CPC-07-72, NLLP-CPC-07-75, NLLP-CPC-07-77, NLLP-CPC-07-78, NLLP-CPC-07-89	Amhara, Gambella, Oromia, SNNPRS, Tigray, Improved
II	12	216749A, Dass 005, NLLP-CPC-07-04, NLLP-CPC-07-09, NLLP-CPC-07-16A, NLLP-CPC-07-24, NLLP-CPC-07-26, NLLP-CPC-07-31, NLLP-CPC-07-32, NLLP-CPC-07-56, NLLP-CPC-07-58, NLLP-CPC-07-85	Amhara, Gambella, Oromia, SNNPRS, Tigray,
III	20	244804, 211490, NLLP-CPC-07-03, NLLP-CPC-07-28, NLLP-CPC-07-33, NLLP-CPC-07-39A, NLLP-CPC-07-39B, NLLP-CPC-07-47, NLLP-CPC-07-48B, NLLP-CPC-07-82, NLLP-CPC-07-83, NLLP-CPC-07-49, NLLP-CPC-07-51, NLLP-CPC-07-52, Bole, TVU, NLLP-CPC-07-53, NLLP-CPC-07-54, NLLP-CPC-07-60, NLLP-CPC-07-97	Gambella, Oromia, SNNPRS, Tigray, Improved

Table 5. Mean value of immature pod yield and yield components of 81 cowpea genotypes in each cluster.

Character	Clusters		
	I	II	III
Days to 50% flowering	50.98	61.65	79.79
Plant height (cm)	150.44	135.12	164.78
Days to 1st mature pod	63.63	72.53	91.42
Number of primary branch	5.70	6.40	5.64
Terminal LL length(cm)	10.28	9.89	10.98
Terminal LL width(cm)	7.05	6.45	7.80
Pod length (cm)	11.61	13.02	12.58
Pod width (mm)	3.90	4.31	4.00
Av. weight of green pod (g)	1.74	2.45	2.17
Pod yield (g/plant)	286.29	394.80	232.48

Table 6. Average inter-cluster distance among clusters.

Clusters	I	II
I		
II	9.96^{ns}	
III	34.73**	22.15**

χ^2=16.92.0 and 21.67 at 5 and 1% probability level, respectively; **highly significant, at p<0.01. ns=not significant.

Table 7. Eigenvectors and eigenvalues of the first four principal components (PCs) for 10 qualitative pod characters of 81 cowpea genotypes.

Variable	Principal components			
	PC1	PC2	PC3	PC4
Eigenvalue	3.5	1.9	1.2	1.0
Proportion	35.3	19.1	12.1	9.7
Cumulative	35.3	54.4	66.5	76.2
Eigenvectors				
Growth Pattern	-0.39	-0.18	-0.26	0.75
Pod attachment to peduncle	-0.70	0.42	-0.02	0.21
Immature pod pigmentation	0.75	0.47	-0.17	-0.16
Intensity of green color in immature pod	-0.19	0.53	0.55	0.32
Pod secondary color	0.81	0.47	-0.16	0.16
Hue of secondary color	0.79	0.49	0.07	0.16
Pod shape	-0.52	0.55	0.11	-0.01
Pod degree of curvature	0.13	-0.29	0.84	-0.09
Pod seture string	0.67	-0.25	0.26	0.38
Texture of pod surface	0.55	-0.55	-0.06	0.22

the genotypes did not have pigment in its immature pod. The proportion of genotypes with preferred immature pod characters like medium to dark green pod color, with the absence of secondary color in its pod, round shape cross-section, straight pod and fibreless were 27, 72, 51, 89 and 52%, respectively. Immature pod with very

Table 8. Estimate of frequency, proportion and Shannon-Weaver diversity index (H') of qualitative traits of 81 Ethiopian cowpea genotypes.

Trait	Description	Code	Frequency	Proportion (%)	H'
Growth pattern	Determinate	1	15	19	0.80
	Indeterminate	2	66	81	
Pod attachment to peduncle	Pendent	3	38	47	0.96
	30°-90° down from erect	5	23	28	
	Erect	7	20	25	
Immature pod pigmentation	None	0	55	68	0.57
	Pigmented tip	1	8	10	
	Pigmented sutures	2	7	9	
	Pigmented valves, green sutures	3	3	4	
	Splashes of pigment	4	8	10	
Intensity of green color in immature pod	Very light green	1	19	23	0.79
	Light green	3	40	49	
	Medium green	5	19	23	
	Dark green	7	3	4	
Pod secondary color	Absent	0	58	72	0.91
	Present	1	23	28	
Hue of secondary color	Pink	1	19	83	0.79
	Red	2	4	17	
Pod shape	Round	1	41	51	0.99
	Flat	3	40	49	
Pod degree of curvature	Straight	0	72	89	0.50
	Slightly curved	3	6	7	
	Curved	5	3	4	
Pod seture string	Absent	0	42	52	0.73
	Moderately stringy	1	37	46	
	Very stringy	3	2	2	
Texture of pod surface	Very smooth	1	8	10	0.78
	Smooth	3	27	33	
	Moderately rough	5	29	36	
	Rough	7	15	19	
	Very rough	9	2	2	

smooth and smooth surfaces were 10 and 33%, respectively. Proulx et al. (2010) indicated that for fresh pod quality the acceptable pod quality is pods with bright green color with no secondary color, tender, and firm, snap very easily, no sign of shriveling and abnormality. Thus, this result indicated the existence of considerable diversity to develop varieties for green pod purposes. For most genotypes, the pod attached to the peduncle was pendent (47%). Those genotypes with secondary color in their pods, the hue of secondary color were dominantly pink (83%) and only 17% of the genotypes were red colored.

Shannon-Weaver diversity index (H') was used to compare diversity among qualitative traits. The value of the Shannon diversity index ranged from 0.50 to 0.99. Higher value of H' observed for pod shape (0.99) followed by pod attachement to peduncle (0.96) and pod secondary color (0.91). The frequency class of pod curvature was unbalanced, 72, 6 and 3 for straight, slightly curved and curved pods, respectively and due to

this the H' was low (0.50). A low H' indicates extremely unbalanced frequency classes for an individual trait and a lack of genetic diversity (Perry and McIntosh, 1991).

Conclusion

Ethiopian cowpea landraces were found to be divers for immature pod characters based on the quantitative and qualitative traits tested. Thus, there is an opportunity to develop cowpea varieties for immature pod purposes from available local germplasm collections either through selection or hybridization. Genotypes with good quality for immature pod purposes can be used as a source of new cultivars with improved features or as a gene pool of useful traits in breeding programs.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Animasaun DA, Oyedeji S, Azeez YK, Mustapha OT, Azeez MA (2015). Genetic variability study among ten cultivars of cowpea (Vigna unguiculata L . Walp) using morpho-agronomic traits and nutritional composition. The Journal of Agricultural Sciences 10(2):119-130.

Badiane FA, Gowda BS, Cissé N, Diouf D, Sadio O, Timko MP (2012). Genetic relationship of cowpea (Vigna unguiculata) varieties from Senegal based on SSR markers. Genetics and Molecular Research 11(1):292-304. https://doi.org/10.4238/2012.February.8.4

Bedru B, Berhanu A, Mulugeta T, Dagmawit T, Bezawit Y, Selamawit K (2019). Cowpea: Production, marketing and utilization in Ethiopia. Research Report 121.

Belayneh AD, Mohammed S, Dagne K, Timko MP (2016). Assessment of genetic diversity in Ethiopian cowpea [Vigna unguiculata (L.) Walp.] germplasm using simple sequence repeat markers. Plant Molecular Biology Reporter 34(5):978-992 https://doi.org/10.1007/s11105-016-0979-x

Chahal GS, Gosal SS (2002). Principles and procedures of plant breeding: Biotechnological and conventional approaches. Alpha Science International Ltd

Chikwendu JN, Igbatim C, Obizoba IC (2014). Chemical Composition of Processed Cowpea Tender Leaves and Husks. International Journal of Scientific and Research Publications 4(5):1-5.

Cobbinah F, Addo-Quaye A, Asante IK (2011). Characterization, evaluation and selection of cowpea (Vigna Unguiculata (L.) Walp) accessions with desirable traits from eight regions of Ghana. ARPN Journal of Agricultural and Biological Science 6(7):21-32.

Falconer DS (1989). Introduction to Quantitative Genetics (3rd ed.). Longman Scientific and Technical.

FAOSTAT (2018). FAOSTAT. Food and Agriculture Organization of the United Nations. Statistics Division.

Gerrano AS, Adebola PO, Jansen van Rensburg WS, Laurie SM (2015). Genetic variability in cowpea (Vigna unguiculata (L.) Walp.) genotypes. South African Journal of Plant and Soil 32(3):165-174. https://doi.org/10.1080/02571862.2015.1014435

Gerrano AS, Sternberg W, Rensburg JV, Adebola PO (2017). Nutritional composition of immature pods in selected cowpea [Vigna unguiculata (L.) Walp.] genotypes in South Africa. Australian Journal of Crop Science 11(02):134-141. https://doi.org/10.21475/ajcs.17.11.02.p72

Gomez KA, Gomez AA (1984). Statistical procedures for agricultural research (2nd ed.). John Wiley, and Sons.

Govindaraj M, Vetriventhan M, Srinivasan M (2015). Importance of genetic diversity assessment in crop plants and its recent advances : An overview of its analytical perspectives. Genetics Research International http://dx.doi.org/10.1155/2015/431487%0A

IBPGR (1983). Descriptors for Cowpea. Internationa Board for Plant Genetic Resource.

Jolliffe IT (2002). Principal Component Analysis (2nd editio). Springer.

Kaiser HF (1960). The Application of electronic computers to factor analysis. Educational and Psychological Measurement 20:141-151. https://doi.org/http://dx.doi.org/10.1177/001316446002000116

Khanpara SV, Jivani LL, Vachhani JH, Kachhadia VH (2015). Genetic variability, heritability and genetic advance studies in vegetable cowpea [Vigna unguiculata (L.) Walp.]. Electronic Journal of Plant Breeding 7(2):408-413. https://doi.org/10.5958/0975-928X.2016.00050.8

Lazaridi E, Ntatsi G, Savvas D, Bebeli PJ (2017). Diversity in cowpea (Vigna unguiculata (L.) Walp .) local populations from Greece. Genetic Resources and Crop Evolution 64:1529-1551. https://doi.org/10.1007/s10722-016-0452-6

Mamiro P, Mbwaga A, Mamiro D, Mwanri A, Kinabo J (2011). Nutritional quality and utilization of local and improved cowpea varieties in some regions in Tanzania. African Journal of Food, Agriculture and Development 11(1):4490-4506.

Manggoel W, Uguru MI (2011). Comparative study on the phenology and yield components of two photoperiodic groups of cowpea (Vigna unguiculata (L.) Walp.) in two cropping seasons. African Journal of Agricultural Research 6(23):5232-5241. https://doi.org/10.5897/AJAR11.156

Menssen M, Linde M, Otunga E, Abukutsa-onyango M, Fufa F, Winkelmann T (2017). Genetic and morphological diversity of cowpea (Vigna unguiculata (L.) Walp.) entries from East Africa. Scientia Horticulturae 226:268-276.

MoA (2018). Ministry of Agriculture: Plant Variety Release, Protection and Seed Quality Control Directorate. Crop Variety Register Issue No.21.

MoA, (Ministry of Agriculture) (2012). Animal and Plant Health Regulatory Directorate. Crop Variety Register. Issue No. 15.

Molosiwa OO, Gwafila C, Makore J, Chite SM (2016). Phenotypic variation in cowpea (Vigna unguiculata [L.] Walp.) germplasm collection from Botswana. International Journal of Biodiversity and Conservation 8(7):153-163. https://doi.org/10.5897/IJBC2016.0949

Mulugeta A, Asfaw Z, Woldu Z, Fenta BA, Medvecky B (2016). Cowpea (Vigna unguiculata (L.) Walp.) (Fabaceae) landrace diversity in Northern Ethiopia. International Journal of Biodiversity and Conservation 8(11):297-309. https://doi.org/10.5897/IJBC2016.0946

Nielsen SS, Ohler TA, Mitchell C (1997). Cowpea leaves for human consumption: production, utilization, and nutrient composition. In LEN Dashiell, BB Jackai, DR Singh, R Mohan (Eds.), Advances in Cowpea Research 326-332. Co publication of International Institute of Tropical Agriculture (UTA) and Japan International Research Center for Agricultural Sciences (JIRCAS).UTA.

NRC (2006). Lost Crops of Africa: volume II Vegetables. In National Academies Press: Vol. II. The National Academies Press. http://www.nap.edu/catalog/11763.html

Peres-neto PR, Jackson DA, Somers KM (2005). How Many Principal Components ? Stopping Rules for Determining the Number of Non-Trivial Axes Revisited. Computational Statistics and Data Analysis 49:974-997. https://doi.org/10.1016/j.csda.2004.06.015

Perry MC, McIntosh MS (1991). Geographical patterns of variation in the USDA soybean germplasm collection: I. Morphological traits. Crop Science 31:1350-13. https://doi.org/10.2135/cropsci1991.0011183X003100050054x

Proulx E, Yagiz Y, Nunes MCN, Emond EP (2010). Quality attributes limiting snap bean (Phaseolus vulgaris L.) postharvest life at chilling and non-chilling temperatures. HortScience 45(8):1238-1249.

R Development Core Team (2019). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing.

Rajaravindran M, Natarajan S (2011). Genetic distance and diversity among some Cowpea (Vigna unguiculata L. Walp) genotypes. International Journal of Research in Plant Science 2(1):9-14.

SAS Institute Inc. (2010). SAS/STAT® 9.22 User's Guide. Cary, NC: SAS Institute Inc.

Shanko D, Andargie M, Zelleke H (2014). Genetic variability and heritability of yield and related characters in cowpea (*Vigna unguiculata* L . Walp .). Research in Plant Biology 4(2):21-26.

Sharma JR (1998). Statistical and Biometrical Techniques in Plant Breeding. New Age International (P) Limited Publishers.

Singh SR, Jackai LE (1985). Insect pests of cowpeas in Africa, their life cycle, Economic importance and potentials for control. In S Singh, K

Sisay A, Alemu M, Asfaw Z, Woldu Z, Berhanu FA (2019). Cowpea (*Vigna unguiculata* (L .) Walp ., Fabaceae) landrace (local farmers ' varieties) diversity and ethnobotany in Southwestern and Eastern parts of Ethiopia. African Journal of Agricultural Research 14(24):1029-1041. https://doi.org/10.5897/AJAR2018.13641

Srinivas J, Kale VS, Nagre PK (2017). Study of genetic variability, heritability and genetic advance in cowpea [Vigna unguiculata (L.) Walp]. *I*nternational Journal of Current Microbiology and Applied Sciences 6(6):3314-3318.

Tesfaye W, Mekbib F, Amsalu B, Gedil M (2019). Genetic diversity of Ethiopian cowpea [*Vigna unguiculata* (L.) Walp] genotypes using multivariate analyses. Ethiopian Journal of Agricultural Sciences 29(3):89-104.

Tibshirani R, Walther G, Hastie T (2001). Estimating the number of clusters in a data set via the gap statistic. Journal of the Royal Statistical Society 63:411-423.

Timko MP, Singh BB (2008). Cowpea, a Multifunctional Legume. In PH Moore, R Ming (Eds.), Genomics of Tropical Crop Plants33(2):227-258 https://doi.org/10.1007/s13398-014-0173-7.2

Trinidad TP, Mallillin AC, Loyola AS, Sagum RS, Encabo RR (2010). The potential health benefits of legumes as a good source of dietary fibre. British Journal of Nutrition 103:569-574. https://doi.org/10.1017/S0007114509992157

USDA (2015). Nutrient data for: 11202, Cowpeas, leafy tips, cooked, boiled, drained, without salt. USDA National Nutrient Database for Standard Reference 27.