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Genetic Diversity of Ethiopian Cowpea [*Vigna unguiculata* (L) Walp] Genotypes Using Multivariate Analyses

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ደገራ በድርቅ ተጋላጭ በሆኑ የኢትዮጵያ ክፍሎች የሚበቅልና ዝቅተኛ ገቢ ባላቸዉ አርሶ አደሮች የሚመረት ባለብዙ ጥቅም ጥራጥሬ ነዉ::በደገራ ዝርያወች ዉስጥ ያለዉ የዘረመል ልዩነት መረጃ ማወቅ ለሰብል ማሻሻል እና አሁን ያሉትን የዘረመል ሀብቶች በብቃት ለመጠቀም በጣም አስፈላጊ ነዉ::ስለዚህ ከጥናቱ ዓላማዎች መካከል፤ የስነቅርፅ ልዩነት እና መጠንን መገምገም እና የተተነተኑ ትንታኔዎችን በመጠቀም የስነ ባህሪ ብዝሀነትን ለመለየት የሚያስችሏቸዉን ባህሪያት መለየት ነዉ::የመስክ ሙከራዉ የተካሄደዉ በ2016 የመከር ወቅት በመልካሳ ግብርና ምርምር ማዕከል እና በመኢሶ ንዑስ ማእከል 324 የደገራ ዝርያዎችን በመጠቀም ነበር:: የመጀመሪያዎቹ ሰባት ዋና አካላት principal components) ከጠቅላላዉ ልዩነት ሰባ ሰባት በመቶ አብራርተዋል::ሁሉም በሚባል ደረጃ የተፈተኑ ማሳያዎች(ባህሪዎች) ለመጀመሪያዉ ፒሲ ዉስጥ ለተለዋዋጭነት ወሳኝ አስተወፅኦ ነበራቸዉ:: ቁጥራዊ ባህሪ ላይ የተመሰረተ ትንተና በዘጠና በመቶ ተመሳሳይነት ደረጃ ሰድስት የተለያዩ ቡድኖችን አሳይቷል::የዘረመል ቡድኖች (clustering of genotypes)የጂኦግራፊያዊ ስርጭትና መነኛ መካከል ምንም ግኑኝነት እንደሌለዉ አመላክቷ:: ከፍተኛዉ የሸግግር ቡድን በክላስተር 4 እና በክላስተር 5(D^2 = 41.62) መካከል ተመዝግቧ::የintra እና inter ቡድን ርቀት በቅደምተከተል ከ6.08 እስከ 22.72 እና 17.37 እስከ 41.61 ክፍሎች ነበር:: ስለሆነም ከፍተኛ የሆኑ የዘረመል ርቀት በቡድን ዉስጥ እና መካከል ታይቷል::የሚታየዉ ታይቷል::የሚታየዉ ከፍተኛ የዘር ልዩነት ለወደፊቱ ለደገራ ማዳቀል እና እርባታ መርሀ ግብር በጣም የተሳኡ ወላጆችን በመምረጥ በጥቅም ላይ መዋል አለበት::

Abstract

Cowpea is a multipurpose pulse crop grown by poor farmers in marginal and drought prone areas of Ethiopia. Information on the extent of genetic variation in cowpea genotypes is crucial to identify diverse genotypes for crop improvement and for efficient utilization of the existing genetic resources. Therefore, the objectives of the study were to assess the extent and pattern of morphological diversity among cowpea genotypes and to identify the traits contributing to the genetic diversity using multivariate analyses. The field experiment was conducted using 324 genotypes at Melkassa Agricultural Research Center and Miesso sub-center during the2016 cropping season. The first seven principal components explained 77% of the total variation. Almost all tested traits were important contributors to the variability in the first PC. The cluster analysis based on quantitative traits revealed six distinct groups at 90% similarity level. The clustering of genotypes did not follow patterns of geographical origin, indicating no relationship between genetic and geographic distribution. The highest inter cluster D^2 was recorded between cluster IV and cluster VI (D^2=41.62 units). The range of intra and inter cluster distance was 6.08 to 22.72 units and 17.37 to 41.62 units, respectively. Hence, the high genetic distance exhibited within and among clusters has to be exploited via crossing and selection of the most divergent parents for future cowpea breeding program.

Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.] is one of the most important native food and forage legumes, and it is grown in Sub-Saharan Africa and some temperate regions of the world (Srivastava *et al.,* 2016; Timko and Singh, 2008). It is predominantly cultivated by resource-limited smallholder farmers (Sivakumar *et al*., 2013). The grain and leaves are sources of proteins, minerals, and vitamins and have very high level of folic acid (Timko and Singh, 2008). Therefore, cowpea could play significant role in mitigating malnutrition such as micronutrient deficiencies for poor farmers of Sub-Saharan countries (Menssena *et al.,* 2017). Cowpea grain is highly nutritious and contains about 15.06 - 38.5% protein (Ravelombola *et al.,* 2016) and 50-60% carbohydrates (Diouf and Hilu, 2005). Cowpea plays a fundamental role in the human diet in many developing countriesand is being referred to as "poor-man's meat" (Ravelombola *et al.,* 2016). Ragab *et al*. (2004) fractionated cowpea protein into albumin (71.4%), globulin (11.1%), prolamin (2.2%) and glutelin (11.0%). Compared to cereals, the storage proteins of cowpea are rich in the amino acids lysine and tryptophan, but low in methionine and cysteine compared to animal protein.

Cowpea is a versatile crop and adapts to high temperature and drought (Baidoo and Monchiah, 2014; Santos *et al.,* 2015). The crop has a great ability to associate with nitrogen fixing bacteria (Ehlers and Hall, 1997). Hence, it is grown in marginal areas having low soil fertility because of its ability to fix atmospheric nitrogen (Ghalmi *et al.,* 2010). Cowpea can derive up to 99% of its N nutrition from symbiotic fixation and fix substantial amounts of symbiotic N (Nkaa *et al.,* 2014). In fact, cowpea has been shown to contribute about 240 kg N/ha, with N benefit of 60–70 kg/ha to succeeding crops in rotation in unfertile soils (Naab *et al.,* 2009).

Understanding the level of genetic diversity in germplasm is helpful to plant breeders as it supports their decision on the selection of parental genotypes and is important in widening the genetic base for cowpea breeding (Prasanthi *et al.,* 2012). Morphological traits are routinely used for estimating genetic diversity, but recently many molecular marker techniques have developed into powerful tools to analyze genetic relationships (Pandey, 2007). Collection, characterization and evaluation of available cowpea germplasm, quantification of the magnitude of diversity and classification into homogeneous groups to facilitate identification of genetic variability enable breeders to select traits of interest for an improvement program (Abe *et al.,* 2015). The analysis of genetic diversity in germplasm collections can facilitate the classification and identification of groups of accesses with superior characteristics to be used for breeding purposes (Tosti andNegri, 2005).

Several researchers applied multivariate statistical tools for genetic diversity studies in cowpea. Among these techniques, clustering large number of genotypes into homogenous groups, estimation of genetic distance among and within clusters and principal component analysis for identifying the most important contributing traits for the overall diversity are widely employed (Abe *et al.,* 2015; Molosiwa *et al.,* 2016; Udensi *et al.,* 2016).

However, in Ethiopia most of studies on cowpea were conducted using very limited number of genotypes, less number of quantitative traits, and single location experiments. Besides, there is no sufficient information on genetic diversity of Ethiopian cowpea genotypes by using multivariate analyses. Therefore, the objective of the present study were to determine the extent of and pattern of genetic diversity among 324 cowpea genotypes based on phenotypic traits and using multivariate analyses.

Materials and Methods

The study area

The field experiment was conducted at Melkassa Agricultural Research Center (MARC) and Miesso sub-center during the 2016 main cropping season from July to November. MARC is located at 39^0 12'E and 8^0 24'N with altitude of 1550 meters. It received five months mean rainfall of 140.96 mm (total 704.8mm) with minimum and maximum temperatures of 14.35°C and 28.22°C, respectively. The soil type of the test site was Andosol with the pH 7.6. The second testing site (Miesso) is located at 9° 14' N and 40° 45' E with an elevation of 1470 meters. It received five months mean rainfall of 84 mm (total 420mm). The minimum and maximum temperature was 15.2°C and 31.1°C, respectively. The soil type of the test site is Vertisol.

Plant materials

The experimental plant materials comprised 324 cowpea genotypes collected from different agro-ecological regions of Ethiopia. Of these, 72, 55, 52, 50, 14 genotypes were collected from Oromia, Amhara, Gambella, SNNP, Tigray regions, respectively, while 68 of the genotypes were of unknown origin lacking passport data. Out of the test genotypes, 311 were obtained from Melkassa Agricultural Research Center (MARC), five genotypes of Ethiopian origin were obtained from International Institute of Tropical Agriculture (IITA), while the rest eight are improved (released) varieties.

Experimental design and procedures

The experiment was laid out using 18 ×18 simple lattice design. The plot size was 2 m long, with spacing of 0.75 m between rows and 0.2 m between plants. It consisted of two rows accommodating 10 plants per row. The distance between plots, intra-blocks, and replications was 1m, 1.5m and 2m, respectively. Data were collected from the two rows.

Data collection

The descriptor of cowpea developed by the International Board for Plant Genetic Resources (IBPGR, 1983) was followed for data collection. The data collected on plot basis were days to flowering, days to maturity, grain filling period (days), hundred seed weight (g), seed length (mm), seed thickness (mm), seed width (mm), seed yield (g), biomass (g) and harvest index (%).In addition, the data collected on individual plant basis were number of pods per plant, number of seeds per pod, pod clearance (cm), pod length (cm), peduncle length (cm), number of pods per peduncle, terminal leaflet length (cm) and terminal leaflet width (cm). For single plant based traits, the average of data from the five random samples of plants per plot were used for analyses.

Data analysis

The mean data of the genotypes over all experimental sites for the eighteen traits were used for analyses. As per the method of Sneath and Sokal (1973,), the genotypes mean data were pre-standardized to a mean of zero and a variance unity to avoid biases due to difference in the scales of measurements. Multivariate analyses were performed using Minitab Statistical Software (Minitab, 2010) and SAS software (SAS, 2008). Principal component analysis was performed using correlation matrix to determine principal components, proportions of eigenvalues and the scores of the principal components. Hierarchical (Ward, 1963) cluster analysis was performed to group genotypes and construct a dendrogram by Ward's method by using Minitab software. The measure of dissimilarity was Euclidean distance. The average intra- and inter-cluster distances were calculated using the generalized Mahalanobis's D^2 statistics (Mahalanobis, 1936). The R^2 (RSQ), Cubic Clustering Criteria (CCC), pseudo-F statistics (PSF) and pseudo-T^2 statistics were considered for defining optimum cluster numbers (Milligan and Cooper, 1985). The contributions of each the traits to divergence were estimated as described as Sharma (1998) with the formula [CTIC=$\frac{SD}{\overline{\chi}}\chi 100$] where SD and $\overline{\chi}$ are the standard deviation and mean performance of each trait, respectively.

Results and Discussion

Principal component analysis

The patterns of variation and the relative importance of each 18 quantitative traits in explaining the observed variability were assessed through principal component analysis. As per Hair *et al.,* (2009), the loading effect of any traits greater than ±0.3 was regarded meaningful and significant while according to Chatfield and Collins (1980) principal components with an eigenvalues of less than one were eliminated from PCA because they were not significant (Chatfield and Collins,1980). According to the principle of Syafii *et al.* (2015), the first principal component accounts for maximum variability in the data with respect to succeeding components. In this study, the first seven principal components having eigenvalues greater than unity explained 77% of the total variation among the studied genotypes for all morphological traits (Table 1). This result is in line with that of Belul *et al.*(2014) regarding consideration of proportion of variation higher than 75% of the total variation as acceptable for characterization and evaluation of genetic collections for all legumes.

The first principal component had an eigenvalue of 4.07 and accounted for 22.6% of the total variation. Pod length, seed length, seed width and seed yield per plot showed high contribution to PC1. Different authors (Arora *et al.,* 2018; Rekha *et al.,* 2013) reported similar results. According to Chahal and Gosal (2002), the largest absolute value within the first PC is influencing the cluster more than those with lower absolute values closer to zero. Seed thickness, hundred seed weight, and number of pods per peduncle had negative contribution and which had an eigenvalues 2.7 and contributed 15% of variation in the second PC between the genotypes. Similarly, days to flowering, days to maturity and number of pods per plant were the higher contributor to the third PC, which accounted for

11.6% of the variation with an eigenvalue of 2.09. With respect to PC3 phenological traits had higher contribution for this component. The fourth PC explained 8.4% of the total variation, which was correlated with terminal leaflet length, terminal leaflet width, grain filling period, pod clearance, and number of pods per plant and peduncle length. In the fifth PC, days to flowering, number of seed per pod, pod length, and biomass were the major contributors, similarly, days to flowering, grain-filling period, number of pods per plant and harvest index contributed most to the six PC, while for the seventh PC the major contributor was grain-filling period. PC5, PC6, and PC7 accounted for about 7.4%, 6.2%, and 5.8% of the total variation, respectively (Table 1).

In general, the present study generally confirmed the existence of wide phenotypic variation in the Ethiopian cowpea genotypes in many of the traits assessed. This variation offers ample opportunities for the genetic improvement of the cowpea through simple selection based on the novel traits and crossing potential parents. Similarly, Molosiwa *et al.* (2016) used principal component analysis and identified the major traits for detecting phenotypic diversity in cowpea genotypes.

Table 1. Eigenvectors, eigenvalues, percent of variance explained by the first seven principal components (PCs) for 18 traits of cowpea genotypes tested over two locations

Trait	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Days to flowering	0.20	0.05	-0.48	0.14	-0.07	-0.36	-0.31
Grain filling period	-0.04	-0.15	-0.22	-0.26	0.40	0.18	0.63
Days to maturity	0.16	-0.05	-0.58	-0.04	0.19	-0.21	0.12
Pod clearance	0.05	0.01	0.08	0.04	0.51	-0.43	0.09
Number of pods per plant	0.18	0.12	0.30	-0.07	0.30	0.18	-0.30
Peduncle length	0.14	0.08	0.14	0.05	0.61	0.01	-0.32
Number of pods per peduncle	0.11	-0.50	0.15	0.14	0.03	-0.07	-0.06
Terminal leaflet length	0.22	-0.21	0.00	-0.54	-0.09	-0.01	-0.17
Terminal leaflet width	0.14	0.07	0.00	-0.69	-0.11	0.00	-0.11
Pod length	0.34	0.23	-0.15	0.09	0.01	0.26	-0.02
Number of seeds per pod	0.15	0.18	-0.07	0.28	-0.07	0.34	0.18
Seed length	0.31	0.11	-0.13	-0.01	0.07	0.30	-0.10
Seed width	0.41	0.04	-0.10	0.06	-0.07	0.18	0.03
Seed thickness	0.29	-0.46	0.05	0.12	-0.05	0.05	0.01
Hundred seed weight	0.25	-0.49	0.06	0.11	-0.06	0.01	0.01
Seed yield per plot	0.37	0.14	0.26	0.04	-0.14	-0.29	0.26
Biomass	0.22	0.24	0.11	0.01	-0.17	-0.42	0.11
Harvest index	0.26	0.16	0.33	-0.05	-0.01	-0.07	0.36
Eigenvalue	4.07	2.7	2.09	1.51	1.33	1.11	1.04
Proportion of variance explained (%)	22.6	15.0	11.6	8.4	7.4	6.2	5.8
Cumulative variance explained (%)	22.6	37.6	49.2	57.6	65	71.2	77.0

The extent of variance and relationship among different traits as explained by loading plot (Figure 1) showed that the magnitude of the relationship between the quantitative traits. Genotypes allocated in quadrant I were similar in pod clearance, number of pods per plant, pod length, peduncle length, terminal leaflet length, terminal leaflet width, number of seeds per pod, seed yield per plot, biomass and harvest index. The traits had relatively strong association and they have positive contribution to the discrimination of genotypes.

In the second and in the third quadrant, the genotypes were strongly associated with the single trait number of pods per peduncle and grain filling period, respectively. Genotypes found in the fourth quadrant were similar for days to flowering, days to maturity, seed length, seed width, seed thickness, and hundred seed weight (Figure 1). Moreover, the scattered plot showed that the genotypes were distributed in all the four quadrants. Breeders to identify the presence of genetic variability within the tested cowpea genotypes, and select donor parents for specific traits can use these. Genotypes overlapping in the two principal axes have similar phenotypic expression of the traits.

The loading plot indicates the similarity and differences between these 18 traits. In the biplot, the traits found near to the origin (x, y) such as number of pods per peduncle and pod clearance have smaller loading and the traits had little influence, whereas the traits far from the origin have higher loading and great influence in this classification. Hence, days to maturity, seed yield per plot and harvest index have higher loading effect among the traits. Furthermore, genotypes classified using quantitative traits were explained by the first two dimensions (PC1 and PC2 (Figures 1 and 2). Genotypes close and overlapping on loading plot showed that the genotypes had similar characteristics and they are found near to the origin, whereas, the genotypes far apart from each other and distantly far from the origin are genetically diverse (Figure 2).

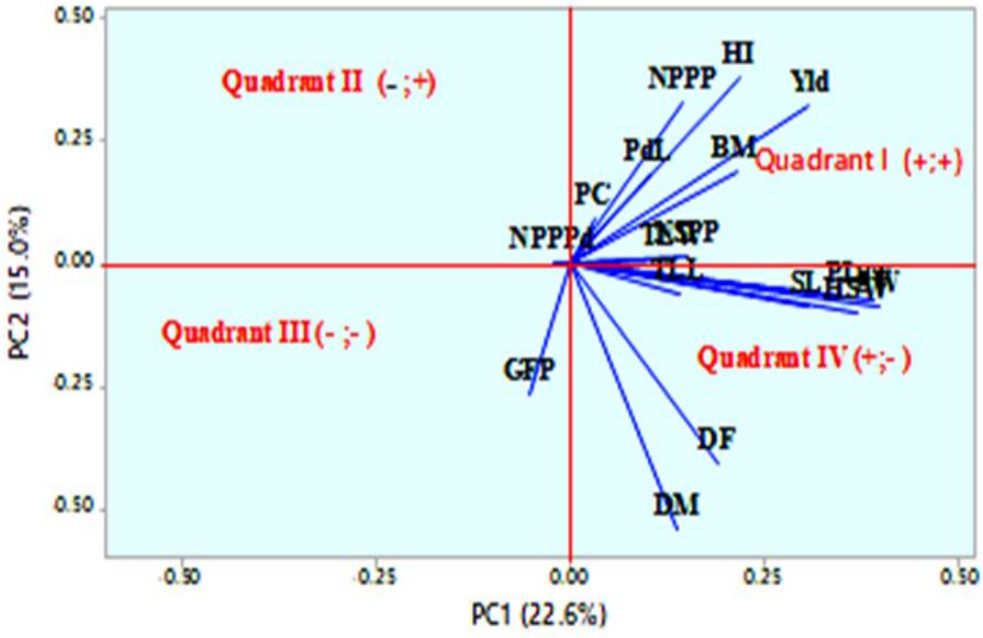

Figure 1. Loading plot showing association of 18 quantitative traits of 324 cowpea genotypes tested over two locations

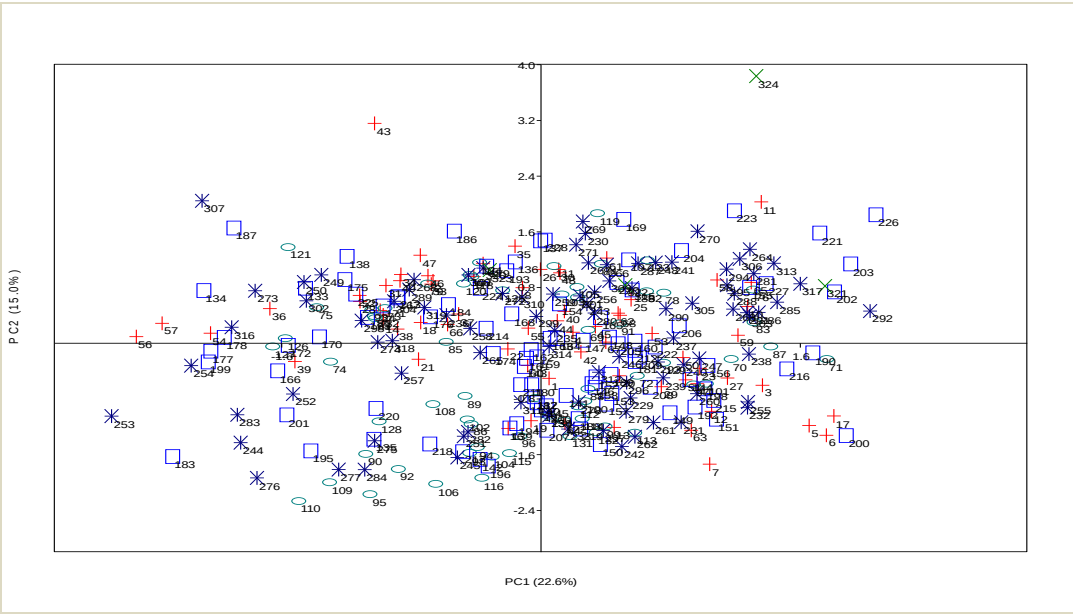

Figure 2. Score plot showing the overall variation among 324 cowpea genotypes by using 18 quantitative traits.

Clustering of genotypes

The tested genotypes were grouped in six different clusters with the number of genotypes per cluster varying from 35 to 77 (Table 2). Cluster IV was the largest cluster comprising 77 genotypes, followed by clusters I and II that contained 63 and 52 genotypes, respectively. Likewise, clusters III, V and VI contained 45, 35 and 52 genotypes, respectively. Of the 77 genotypes grouped in cluster IV, 25.98%, 18.18% and 16.68% of genotypes originated from Oromia, SNNP and Gambella regions, respectively. Similarly, in cluster I 25.4%, 22.22% and 17.46% of the total genotypes grouped in the second largest cluster (Cluster 1) had in that order origins that are unknown, and Gambella and SNNP regions (Table 4). The cowpea genotypes originating from the same regions entered into different clusters indicating the absence of relationships between genetic diversity and geographic origin. For instance, the genotypes from Oromia, Amhara, SNNP, Tigray Gambella and Unknown regions of origin grouped into the six distinct clusters (Tables 2 and 3). The main reasons for the grouping of genotypes of the same origin into different clusters could be the exchanges of germplasm by farmers among neighboring regions, natural and artificial selection, genetic enrichment, genetic drift and environmental variation. Jivani, *et al*. (2013) stated that genetic drift and selection in varied environments could cause greater diversity than geographic distance. Besides, free exchange of seed materials among the different regions causes characters constellations because of the human interference such that the material may lose its individuality. Furthermore, Dwevedi and Lal (2009) reported no parallelism between geographic distribution and genetic diversity.

Region	№ of genotypes per cluster with respective percentage						Total № of genotypes
	I	II	III	IV	V	VI	
Oromia	9 (14.29%)	10 (19.23%)	10 (22.22%)	20 (25.98)	4(11.43%)	19 (36.54%)	72
Amhara	9 (14.29%)	12 (23.08%)	11 (24.44%)	11 (14.29%)	6 (17.14%)	6 (11.54%)	55
Gambella	11(17.46%)	7 (13.46%)	11 (24.44%)	13 (16.88%)	2 (5.71%)	8 (15.38%)	52
SNNP	14 (22.22%)	5 (9.62%)	2 (4.44%)	14 (18.18%)	9 (24.71%)	6 (11.54%)	50
Tigray	4 (6.35%)	2 (3.85%)	0	1 (1.30%)	4 (11.43%)	3 (5.77%)	14
Unknown	16 (25.4%)	12 (23.08%)	11 (24.44%)	12 (15.58%)	8 (22.86%)	9 (17.31%)	68
IITA	2 (3.17%)	2 (3.85%)	0	1 (1.3%)	2 (5.71%)	0	5
Others	0	2 (3.85%)	0	5 (6.49%0	0	1 (1.92%)	8
Total	63	52	45	77	35	52	324
% of clusters	19.44	16.05	13.89	23.77	10.80	16.05	100
Origin of genotypes	1-7	1-8	1,2,3,4,5	1-8	1-7	1,2,3,4,5,6,8	

Table 2. Distribution of 324 cowpea genotypes in six different clusters

1=Oromia, 2= Amhara, 3= Gambella, 4= SNNP, 5= Tigray, 6= Unknown 7= IITA and8= others (Released variety)

Table 3. List of cowpea genotypes in each of the six different clusters

Cluster	Genotypes					
I	NLLP-CPC-07-25	NLLP-CPC-07-93-A	NLLP-CPC-07-92-A	NLLP-CPC-07-86	ACC-211-436-A	ACC-244-804-A
	NLLP-CPC-07-90	NLLP-CPC-07-93-B	NLLP-CPC-07-94-A	NLLP-CPC-07-135	ACC-211-436-B	ACC-211-446-B
	NLLP-CPC-07-98	NLLP-CPC-07-90-B	NLLP-CPC-07-28-B	NLLP-CPC-07-140	NLLP-CPC-0724	ACC-244-804-B
	NLLP-CPC-07-70	NLLP-CPC-07-88-A	NLLP-CPC-07-81-C	NLLP-CPC-07-163	NLLP-CPC-0769	ACC-244-804-C
	NLLP-CPC-07-81	NLLP-CPC-07-88-B	NLLP-CPC-07-37-D	NLLP-CPC-07-131	NLLP-CPC-0772	ACC-211-557
	NLLP-CPC-07-79	NLLP-CPC-07-93-21	NLLP-CPC-07-06-B	NLLP-CPC-07-108	NLLP-CPC-0775	ACC-228-624
	NLLP-CPC-07-87	NLLP-CPC-07-26-A	NLLP-CPC-07-15-A	NLLP-CPC-119-B	NLLP-CPC-0778	ACC-244-804
	NLLP-CPC-07-71	NLLP-CPC-07-38-C	NLLP-CPC-07-19-B	NLLP-CPC-07-100	NLLP-CPC-0785	ACC-216-748
	NLLP-CPC-07-93	NLLP-CPC-07-25-B	NLLP-CPC-07-32-B	NLLP-CPC-07-170	NLLP-CPC-0797	ACC-227-104
	NLLP-CPC-07101	NLLP-CPC-0714-B	NLLP-CPC-07-95-A-21	NLLP-CPC-07-06	ACC-235122-A	Jshartesfirbir
	NLLP-CPC-07-80	NLLP-CPC-119-A	NLLP-CPC-0707			
II	NLLP-CPC-07-50	NLLP-CPC-112-A	NLLP-CPC-0746-B	NLLP-CPC-0745	ACC-211-491-A	Dass 005
	NLLP-CPC-07-17	NLLP-CPC-07-128	NLLP-CPC-07-96-21	NLLP-CPC-0728	ACC-211441-B	MCP-23-D
	NLLP-CPC-07-17	NLLP-CPC-07-129	NLLP-CPC-07-43-B	ACC-214-147-A	ACC-211441-C	MCP-23-E
	NLLP-CPC-07-84	NLLP-CPC-07-130	NLLP-CPC-07-27-B-21	ACC-227-104-A	ACC-211-441	TVU-7149
	NLLP-CPC-106-A	NLLP-CPC-07-134	NLLP-CPC-07-67-B-21	ACC-227-104-C	ACC-211-383	TVU-15548
	ACC-214-147-B	NLLP-CPC-07-141	NLLP-CPC-07-81-B	ACC-241-761-A	ACC-211-447	Assebot
	NLLP-CPC-120	NLLP-CPC-07-158	NLLP-CPC-0746-A	ACC-211-446-A	ACC-220-575	Bekur
	ACC-222890	NLLP-CPC-07-44	NLLP-CPC-07-25-A	DOSS		
	NLLP-CPC-07-22	NLLP-CPC-118-D	NLLP-CPC-07-151	NLLP-CPC-0727	NLLP-CPC-07-172	ACC-216-747
	NLLP-CPC-07-65	NLLP-CPC-07-124	NLLP-CPC-07-152	NLLP-CPC-0749	NLLP-CPC-0716-A	ACC-208-778

III	NLLP-CPC-07-20	NLLP-CPC-07-67-A	NLLP-CPC-07-159	NLLP-CPC-0754	NLLP-CPC-0716-C	ACC-215-762
	NLLP-CPC-07-92	NLLP-CPC-07-27-B	NLLP-CPC-07-160	NLLP-CPC-0756	NLLP-CPC-07-19	TVU-14568
	NLLP-CPC-07-62	NLLP-CPC-07-59-A	NLLP-CPC-07-161	NLLP-CPC-0760	NLLP-CPC-07-36-21	NLLP-CPC-07-19
	NLLP-CPC-07-35	NLLP-CPC-07-67-B	NLLP-CPC-07-165	NLLP-CPC-0764	NLLP-CPC-07-37-A	NLLP-CPC-07-126
	NLLP-CPC-07-28	NLLP-CPC-07-53-B	NLLP-CPC-07-08	NLLP-CPC-110-B		
IV	NLLP-CPC-07-132	NLLP-CPC-07-20-B	NLLP-CPC-109-A	NLLP-CPC-07-10	NLLP-CPC-07-66-A	ACC-211-490
	NLLP-CPC-07-136	NLLP-CPC-07-92-B	NLLP-CPC-07-36	NLLP-CPC-106-B	NLLP-CPC-07-67-C-21	ACC-208-776
	NLLP-CPC-07-137	NLLP-CPC-07-87-B	NLLP-CPC-07-91	NLLP-CPC-105-A	NLLP-CPC-07-06-B-21	MCP-23-B
	NLLP-CPC-07-138	NLLP-CPC-07-53-A	NLLP-CPC-07-37	NLLP-CPC-07-54	NLLP-CPC-07-37-A-21	MCP-23-C
	NLLP-CPC-07-139	NLLP-CPC-07-66-B	NLLP-CPC-07-169	NLLP-CPC-07-48	NLLP-CPC-07-166	TVU-7146
	NLLP-CPC-07-142	NLLP-CPC-07-28-A	NLLP-CPC-07-171	NLLP-CPC-07-57	NLLP-CPC-07-03-A	Dass 002
	NLLP-CPC-07-143	NLLP-CPC-0748-A	NLLP-CPC-103-B	NLLP-CPC-115	ACC-235122-B	White wonder
	NLLP-CPC-07-146	NLLP-CPC-116-B-21	NLLP-CPC-122-A	NLLP-CPC-0751	ACC-211-440-A	Kanketi
	NLLP-CPC-07-147	NLLP-CPC-109-A-21	NLLP-CPC-0705	NLLP-CPC-0755	NLLP-CPC-113	Asrat
	NLLP-CPC-07-157	NLLP-CPC-07-167-21	NLLP-CPC-0709	NLLP-CPC-0782	NLLP-CPC-117	Bole
	NLLP-CPC-07-167	NLLP-CPC-07-145-21	NLLP-CPC-0729	CP-EXTERETIS	NLLP-CPC-114	IT
	NLLP-CPC-07-168	NLLP-CPC-07-99-21	NLLP-CPC-0742	ACC-211-491-C	NLLP-CPC-121	
V	NLLP-CPC-0701	NLLP-CPC-0714-A	NLLP-CPC-0783	NLLP-CPC-07-38-D	NLLP-CPC-07-74	ACC-223-402
	NLLP-CPC-0712	NLLP-CPC-07-43-A	NLLP-CPC-07-16	NLLP-CPC-07-21-E	NLLP-CPC-07-76	ACC-222-867
	NLLP-CPC-0723	NLLP-CPC-07-87-A	NLLP-CPC-07-11	NLLP-CPC-07-03-B	ACC-211-440-B	MCP-23-A
	NLLP-CPC-0747	NLLP-CPC-07-81-A	NLLP-CPC-07-21	NLLP-CPC-07-90-A	ACC-211-430	TVU-7144
	NLLP-CPC-07-144	NLLP-CPC-07-162	NLLP-CPC-07-59-B	NLLP-CPC-07-32-A	NLLP-CPC-116-B	ACC-216-749
	NLLP-CPC-07-145					
VI	NLLP-CPC-07-148	NLLP-CPC-07-61	NLLP-CPC-07-38-B	NLLP-CPC-07-48-21	NLLP-CPC-07-96	NLLP-CPC-123
	NLLP-CPC-07-149	NLLP-CPC-07-68	NLLP-CPC-07-21-A	NLLP-CPC-07-133-21	NLLP-CPC-07-99	ACC-215-821
	NLLP-CPC-07-150	NLLP-CPC-118-B	NLLP-CPC-07-30-C	NLLP-CPC-07-08-21	NLLP-CPC-07-41	MCP-23-B-21
	NLLP-CPC-07-153	NLLP-CPC-118-E	NLLP-CPC-07-67-C	NLLP-CPC-0739-A	NLLP-CPC-0753	Back Eye Bean
	NLLP-CPC-07-154	NLLP-CPC-07-125	NLLP-CPC-07-95-B	NLLP-CPC-0739-B	NLLP-CPC-0789	Dass 001
	NLLP-CPC-07-155	NLLP-CPC-07-127	ACC-244-804-B-21	NLLP-CPC-122-B	NLLP-CPC-0726	
	NLLP-CPC-07-156	NLLP-CPC-103-A	NLLP-CPC-117-21	NLLP-CPC-116-A	NLLP-CPC-104	
	NLLP-CPC-0777	NLLP-CPC-07-91-B	NLLP-CPC-07-33	NLLP-CPC-07-73	ACC-233-403	
	NLLP-CPC-0752	NLLP-CPC-07-91-A	NLLP-CPC-07-02	NLLP-CPC-07-88-C	ACC-221-727	TVU-7148

Cluster mean performance

The mean values of 18 quantitative traits per cluster are presented on Table 4. In this study, the mean values varied among clusters for all traits. Genotypes those took longer days to flowering and maturity were found in cluster VI, and extended grain filling period was recorded in cluster II. Cluster IV exhibited maximum mean values for number of pods per plant, peduncle length, seed yield per plot, biomass and harvest index. The highest mean values of seed length, seed width, seed thickness and hundred seed weight were recorded for cluster III. The highest mean values of terminal leaflet length, peduncle length and number of racemes per plant were recorded for cluster II. On the contrary, early flowering and maturity were recorded for cluster V and II, respectively.

On the basis of overall mean performance, cluster IV showed the best performance for most of the traits including seed yield per plot. Therefore, cluster IV would be preferable for selection of parents with high mean values for the improvement of genotypes. Conversely, cluster I had minimum values for yield and yield related traits. It showed the poorest performance of traits while the highest plant stature was recorded in this cluster. Therefore, this cluster is preferable for increasing number of pods per peduncle. In general, there was highly significant variation in mean performance among the clusters for most of the traits, and this offers a huge opportunity to select potential parents across the clusters for specific traits for future cowpea improvement. Overall, the variation observed among the 324 cowpea genotypes suggests that quantitative traits can reveal diversity existing among cowpea genotypes. Molosiwa *et al.* (2016) and Moolendra *et al.* (2018 had also reported similar results.

Seed yield per plot, biomass and harvest index were the major contributors for genetic divergence to the entire genotypes (Table 4). In contrast, days to maturity, terminal leaflet width and terminal leaflet length had small contribution towards genetic divergence. The levels of trait contribution for inter cluster divergence studies for cowpea were ≥15% as high contributor, ≥ 8% < 15% as medium contributor and <8% as little contributor for inter cluster divergence.

Table 5. Mean performance of cowpea genotypes grouped into six clusters based on 18 quantitative traits evaluated over two locations

Trait	Cluster						Mean	SD (±)	CTIC (%)
	I	II	III	IV	V	VI			
Days to flowering	52.02	46.00	57.11	53.54	51.64	57.43	52.96	4.21	8
Grain filling period	26.74	27.01	25.49	22.85	25.90	25.46	25.57	1.48	6
Days to maturity	78.77	73.15	82.56	76.40	77.53	82.81	78.54	3.72	5
Pod clearance	37.73	33.65	34.07	35.25	33.57	34.55	34.80	1.56	5
Number of pods per plant	27.57	28.66	25.47	30.36	27.36	27.54	27.83	1.62	6
Peduncle length	23.82	22.29	20.66	23.87	21.80	23.49	22.65	1.29	6
Number of pods per peduncle	3.20	2.96	2.84	2.85	2.92	2.85	2.94	0.14	5
Terminal leaflet length	11.06	11.36	12.24	11.43	12.32	11.04	11.57	0.57	5
Terminal leaflet width	18.79	18.74	20.27	19.31	20.59	18.33	19.34	0.91	5
Pod length	13.45	14.67	16.25	16.42	13.43	16.69	15.15	1.50	10
Number of seeds per pod	11.97	12.37	12.83	13.41	11.32	14.55	12.74	1.14	9
Seed length	7.01	7.55	8.37	7.99	7.31	8.34	7.76	0.56	7
Seed width,	5.04	5.66	6.22	5.98	5.18	6.09	5.69	0.49	9
Seed thickness	4.83	5.46	5.99	5.70	4.98	5.78	5.46	0.46	9
Hundred seed weight	12.57	15.67	20.28	18.40	13.44	18.47	16.47	3.07	9
Seed yield per plot	223.09	288.63	303.54	442.17	255.27	295.58	301.38	75.20	25
Biomass	485.39	581.37	612.14	751.40	518.73	609.40	593.07	92.69	16
Harvest index	0.49	0.54	0.49	0.63	0.52	0.51	0.53	0.05	10

CTIC = Contribution to inter-cluster divergence

Intra- and inter-cluster distance

The square distances (D^2) between all clusters showed highly significant variation ($P \leq 0.01$), indicating wide diversity among genotypes in the six different clusters. The range of intra- and inter-cluster distance was 6.08 to 22.72 units and 17.37 to 41.62 units, respectively (Table 5.5). The highest average intercluster D^2 was recorded between cluster IV and cluster VI (D^2=41.62 units) followed by cluster II and cluster VI (D^2=40.49 units) and cluster I and cluster VI (D^2= 39.10 units).This indicates that the inter-cluster distances were more genetically divergent from each other. As per the inter-cluster distance (from cluster I, IV and VI), selection of parents for hybridization program among genotypes from diverse clusters would give novel recombinants that increase efficiency for improvement of seed yield in cowpea. The nearest inter-cluster distance was found between cluster II and III (17.72 units) and followed by cluster I and III (23.38 units). Genotypes in these clusters (cluster II and III) were not genetically diverse. Thus, crossing parents from these three clusters might not give higher heterotic value in the future hybrid breeding program in the subsequent generations and will not give a wide range of variability in the segregating F2 population.

The maximum intra-cluster distance (D^2) was recorded in cluster II (22.72 units) followed by cluster III (12.80 units) and cluster V (12.47 units). The present investigation indicates that the genotypes in cluster III and V were more diverged than any one of the other clusters. Thus, the genotypes belonging to the distant clusters could be used for cowpea breeding program to getting a wider range of variability. In addition, genotypes from these two distinct clusters (III and V) could be utilized as parents for hybrid breeding program or recombinant breeding program owing to their wider within group distance. In contrast, the lowest D^2 was recorded in cluster I (6.08 units), which showed the presence of less genetic variability or diversity within this cluster. In general, intra-cluster distance was much less than inter-clusters one. Similarly, Ahamed *et al.* (2014) reported that the inter-cluster distances were higher than the intra-cluster distances.

Table 5. Average intra cluster (bolded diagonal) and inter cluster (off-diagonal) distance (D2) values

Cluster	I	II	III	IV	V	VI
I	**6.08**					
II	29.53*	**22.72**				
III	23.38	17.37	**12.80**			
IV	32.92**	29.22*	30.41*	**6.35**		
V	28.79*	26.73*	25.06	29.70*	**12.47**	
VI	39.10**	40.49**	38.74**	41.62**	35.53**	**8.71**

***,* indicates significance at 1% and 5% level of significace; χ^2_{18}=26.30 and 32.00 at5% and 1%, probability level, respectively*.

Conclusion

The first seven principal components accounted for 77% of total variation observed among the cowpea test genotypes. Days to maturity, grain-filling period, peduncle length, number of pods per plant, pod length, pod clearance, seed thickness, seed width, seed yield per plot and harvest index were the most important traits that contributed to the major principal components accounting for a large portion of the phenotypic diversity. The 324 cowpea test genotypes clustered into six distinct groups, and the highest inter-cluster D^2 was recorded between cluster IV and cluster VI followed by cluster II and cluster VI, and then cluster I and cluster VI. This indicates that the clusters distances were genetically divergent from each other. Intra-cluster distance was much lesser than inter-cluster distance showing the presence of high genetic divergence among the clusters. The clustering of the genotypes did not follow patterns of geographical distribution. Seed yield per plot, biomass and harvest index were the most discriminating traits for grouping the entire genotypes into the different clusters. In general, the present investigation indicated the existence of high genetic diversity and this offers many opportunities to exploit through breeding *via* crossing and selection of the most divergent parents from the clusters.

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