Original Research Article

Genetic variability among cowpea (*Vigna unguiculata* L. Walp) mini core collection

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ABSTRACT

Aims: To evaluate cowpea genotypes selected from mini core collection for variability that can be exploited in breeding programme and also to provide information on high yielding genotypes to aid further use of the genotypes in cowpea improvement programmes.
Study design: A completely randomized design in two replications was used for the study.
Place and Duration of Study: International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria between March and August, 2017.
Methodology: Two hundred cowpea genotypes were grown in the glass house at IITA. Data were collected on twelve agronomic characters including plant height, chlorophyll content, days to first flower initiation, days to first pod harvest, hundred-seed weight, pod length, number of pods per plant, mean number of seeds per pod, grain yield per plant, seed length, seed width and seed thickness. The data were subjected to analysis of variance, cluster and principal component analyses.
Results: Significant genetic variability was observed among the cowpea genotypes for all the agronomic characters investigated in this study. The genotypes were grouped into five clusters with TVu1059 and TVu43 identified as the most distinct. The first four principal components (PC) with eigen values > 1 accounted for 72.5% of the total variation among the cowpea genotypes with loadings of hundred-seed weight, seed length, seed width, seed thickness, days to first pod harvest, days to first flower, pod length, mean number of seeds per pod, pods per plant and grain yield per plant.
Conclusion: The broad genetic variation observed among the cowpea genotypes evaluated in this study for yield and yield-related characters can be explored to improve cowpea.

Keywords: Cowpea mini core collection; principal component analysis; cluster analysis; agronomic characters of cowpea.

1. INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) is an essential food legume in the tropics and sub-tropics, especially in sub-Saharan Africa [1]. It is used as a grain crop, fodder for livestock, or as a vegetable. Cowpea grain is an important source of protein (23.4%), carbohydrate (60.3%) and fat (1.8%) [2]. Cowpea also has the ability to fix atmospheric nitrogen through symbiosis with nodule bacteria to improve soil fertility [3]. According to estimates by FAO, approximately 5.5 million tonnes of cowpea grain are produced annually on about 10.5 million hectares worldwide [4]. In spite of the importance of cowpea to millions of people in the world, especially Africa, its production does not meet the need of consumers [5] due to biotic and abiotic stresses [6]. This therefore calls for improvement of yield and yield-related characters of cowpea to meet the global requirement of cowpea and increase profit of cowpea farmers.

The most important factor and raw material for plant breeding is the genetic variation available in the breeding population for the desired characteristics. Genetic variability present in breeding materials plays an essential role in the success of a breeding programme [7] and the availability of this diversity is an important tool useful in selection process [8]. The yield potential of underutilised and recently composed cowpea mini core collection derived from the entire collection of over 15,000 accessions at the genetic resource centre of IITA which also captures most of the available diversity of species [9] has not yet been fully exploited. It is therefore important to collect, characterise, and evaluate genotypes in order to select superior ones for desirable traits. Research studies have been done on insect and multiple disease resistances, drought, early-maturing, medium-maturing and late-maturing grain types, content of protein and mineral and general morphological characteristics [10,11,6]. There is little information on the genetic similarities and differences among the genotypes of the mini core collection. Better knowledge of the genetic similarities and differences of breeding materials could help to conserve genetic diversity which provides the basis of the genetic variation and genetic relationships among cowpea genotypes and also sustain long term selection gain for crop improvement. Information on the nature and degree of genetic diversity would assist plant breeders in selecting parental materials for hybridisation [12] and also help in widening the genetic base of the crop [5]. Collection, characterisation, maintenance and evaluation of available cowpea germplasm, quantifying the magnitude of diversity and also classifying them into groups based on similarities are necessary to ease the identification of genetic variability that enables selection of desirable traits for an improvement in breeding programme [13].

This study aimed to evaluate cowpea genotypes selected from mini core collection for variability that can be exploited in breeding programme and also to provide information on high yielding genotypes to aid further use of the genotypes in cowpea improvement programmes.

2. MATERIAL AND METHODS

2.2 Glass house experiment

This study was carried out at International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria between March and August, 2017. The 200 genotypes used for the study were selected from the mini core collection of the Genetic Resources Centre of IITA (Table 1). The genotypes were planted in plastic pots arranged in a completely randomized design with two replicates. Thoroughly mixed top soil sample was collected and taken to microbiology laboratory for soil analysis to determine the nutrients composition. Dried soil sample (at 65^{o}C for 48 hours) was grounded and passed through 0.5 mm sieve. 0.5 ml of distilled water was added and the mixture was stirred vigorously and allow to stand for 30 minutes. The solution was then used for determination of nutrients composition of the soil following the standard procedures (Table 2). The plastic pots were each filled with 5 kg top soil and then watered to field capacity before planting the seeds. All the seeds were treated with apron star (active ingredients: imidacloprid (10%), metalaxyl (10%) and carbendazim (10%)) at a dosage of 10g per 4kg of seeds to prevent insect attack. Five seeds of each of the two hundred genotypes were planted per pot. After two weeks, the plants were thinned to two plants per pot to achieve required plant population. White flies were controlled using a mixture of Courage (imidacloprid (17.8% SL)) and Karate (50 g/l lambda-cyhalothrin) at dosage of 25ml and 75ml per 20 litres of water, respectively. Leaf thrips were also controlled by spraying plants with Dynamic (abamectin (1.84% w/w) and cyclohexanol) at dosage of 20ml per 20 litres of water. Pod borers were controlled with Termex (chlorpyriphos) at dosage of 80ml per

Table 1. List of genotypes evaluated for genetic variability at Ibadan in 2017

No	Genotype	No	Genotype	No	Genotype	No	Genotype	No	Genotype
1	TVu6439	41	TVu2449	81	TVu6477	121	TVu13950	161	TVu15450
2	TVu21	42	TVu2500	82	TVu6493	122	TVu13958	162	TVu15487
3	TVu43	43	TVu2548	83	TVu6641	123	TVu13965	163	TVu15500
4	TVu53	44	TVu2606	84	TVu6663	124	TVu13968	164	TVu15610
5	TVu109	45	TVu2680	85	TVu6837	125	TVu13979	165	TVu15636
6	TVu113	46	TVu2723	86	TVu6966	126	TVu14172	166	TVu4943
7	TVu132	47	TVu2736	87	TVu6968	127	TVu14173	167	TVu15653
8	TVu201	48	TVu2769	88	TVu7798	128	TVu14190	168	TVu15661
9	TVu13573	49	TVu2845	89	TVu8262	129	TVu14195	169	TVu15687
10	TVu393	50	TVu2933	90	TVu8612	130	TVu14224	170	TVu15742
11	TVu408	51	TVu2968	91	TVu8622	131	TVu14248	171	TVu15775
12	TVu415	52	TVu2971	92	TVu8631	132	TVu14253	172	TVu15860
13	TVu456	53	TVu3043	93	TVu8656	133	TVu14272	173	TVu15861
14	TVu467	54	TVu1124	94	TVu8671	134	TVu14290	174	TVu15878
15	TVu945	55	TVu3107	95	TVu8673	135	TVu14336	175	TVu15913
16	TVu969	56	TVu3156	96	TVu8713	136	TVu14345	176	TVu15926
17	TVu972	57	TVu3282	97	TVu8775	137	TVu14346	177	TVu15945
18	TVu1004	58	TVu3310	98	TVu8877	138	TVu14393	178	TVu5500
19	TVu1016	59	TVu3346	99	TVu8883	139	TVu14401	179	TVu15976
20	TVu1036	60	TVu3360	100	TVu9073	140	TVu14406	180	TVu15982
21	TVu1037	61	TVu3552	101	TVu9486	141	TVu14533	181	TVu16220
22	TVu1059	62	TVu3565	102	TVu9256	142	TVu14539	182	TVu16237
23	TVu1280	63	TVu3652	103	TVu9259	143	TVu14558	183	TVu16253
24	TVu1330	64	TVu3657	104	TVu9265	144	TVu14621	184	TVu16269
25	TVu1477	65	TVu3830	105	TVu9357	145	TVu14632	185	TVu16278
26	TVu1556	66	TVu3947	106	TVu9391	146	TVu14633	186	TVu16304
27	TVu8072	67	TVu9508	107	TVu9393	147	TVu14683	187	TVu16368
28	TVu1637	68	TVu4545	108	TVu9468	148	TVu14691	188	TVu16403
29	TVu1656	69	TVu4557	109	TVu9761	149	TVu14759	189	TVu16408
30	TVu1727	70	TVu4622	110	TVu9820	150	TVu14788	190	TVu16430
31	TVu1811	71	TVu4632	111	TVu9848	151	TVu14862	191	TVu16449
32	TVu1886	72	TVu4669	112	TVu10179	152	TVu14875	192	TVu16461
33	TVu1916	73	TVu4760	113	TVu10366	153	TVu14970	193	TVu16465
34	TVu2168	74	TVu4761	114	TVu10394	154	TVu15037	194	TVu16486
35	TVu2185	75	TVu4783	115	TVu10513	155	TVu15114	195	TVu16504
36	TVu2252	76	TVu4808	116	TVu10559	156	TVu15299	196	TVu16505
37	TVu2280	77	TVu4823	117	TVu11610	157	TVu15400	197	TVu16514
38	TVu2322	78	TVu5443	118	TVu12873	158	TVu15411	198	TVu16521
39	TVu2398	79	TVu6365	119	TVu13778	159	TVu15426	199	TVu16528
40	TVu2418	80	TVu6464	120	TVu13939	160	TVu15445	200	TVu16574

Table 2. Physico-chemical properties of the soil used for evaluation of cowpea genotypes at Ibadan in 2017

Sample	pH: H_2O (1:1)	OC (%)	N (%)	Bray P (ppm)	Particle size (%)			Ca	Mg	K	Na	Zn	Cu	Mn	Fe
					Sand	Silt	Clay	(cmol$_c$/Kg)				(ppm)			
1	6.94	1.01	0.15	10.97	90	8	2	7.19	1.63	0.88	4.88	7.78	1.10	88.04	55.76

20 litres of water. Harvesting was done when the pods had dried.

2.2 Data collection and analysis

Data were collected on agronomic traits such as plant height (cm), days to first flower initiation, days to first pod harvest, hundred-seed weight (g), pod length (cm), number of pods per plant, mean number of seeds per pod, grain yield per plant (g) from which yield per hectare (kg/ha) was estimated, seed length (mm), seed width (mm) and seed thickness (mm).

Chlorophyll content (chlorophyll b) present in the leaves of the cowpea plants were determined with the use of SPAD-502 chlorophyll meter (Konica-Minolta, Japan), that measures the absorbance of leaf in the red (650 nm) and infrared (940 nm) regions of the electromagnetic spectrum [14].

Data were subjected to analysis of variance (ANOVA) to determine if there are significant differences among the genotypes using SAS 9.4 version software. The means for each trait were subjected to Cluster Analysis and Principal Component Analysis (PCA) to determine the pattern of variability and relationships among evaluated genotypes using SAS 9.4 version software. Rank summation index (RSI) [15] was used to rank genotypes and selection was done based on their performances across the traits considered. Means were compared using LSD at 5% probability level.

3. RESULTS AND DISCUSSION

The 200 cowpea genotypes were evaluated based on their phenological, yield and yield related traits. The cluster pattern showed two main clusters with the first cluster having two members and the other containing the remaining 198 genotypes (Fig. 1). The dendrogram further divided the cluster into sub-clusters using the average distance between cluster procedure. At average distance of 250, the dendrogram showed five sub-clusters containing 2, 18, 8, 76 and 96 genotypes, respectively which indicates the existence of genetic diversity among the genotypes used (Table 3). Conservation of cowpea genetic variability is essential for future use and the actual utilization of the accessions is equally important [16]. The genotypes joining different clusters at different average distances showed the relationship among the genotypes and also indicates that they are genetically distant thereby creating a chance for improvement. TVu1059 and TVu43 in cluster V belong to the same cluster and also have distant relationship with other genotypes because they had the highest quantity of yield. Selection of highly divergent clusters is expected to express wide variability, therefore the variability between groups could be useful in hybridization while within group variability could be useful for selection process.

There were highly significant ($P \leq .0001$) differences among the genotypes for all the traits evaluated (Table 4). From the mean performance of 20 selected cowpea genotypes for phenological, yield and yield related traits based on rank summation index method, TVu12873 (61 days) had the shortest days to first pod harvest while TVu9259 (185 days) took the longest days to first pod harvest (Table 2). TVu8262 (17.85 g) had the highest value for hundred-seed weight while TVu2968 (4.80 g) had the lowest value. The highest value for pods per plant was observed in Tvu12873 (28) while TVu15913 had the lowest value of 2.75. TVu3310 (16.10) had the highest mean number of seeds per pod while TVu14253 (6.20) had the lowest value. The highest grain yield value was observed in TVu8631 (1,470 kg/ha) while the lowest value was observed in TVu15861 (131.67 kg/ha). For seed length, TVu12873 (10.44 mm) had the highest value while the lowest was recorded in TVu14970 (5.70 mm). The highest seed thickness was observed in TVu109 (5.52 mm) while the lowest value was observed in TVu8262 (3.16 mm). Genotype TVu8631, followed closely by TVu109 had the best performance across the traits considered. The cowpea genotypes are genetically diverse for the traits considered for the study thereby suggesting the existence of variability among the genotypes which makes them amenable to selection pressure. This agrees with the reports of Yadav et al. [17] and Ali et al. [18] who worked on chickpea and soybean, respectively.

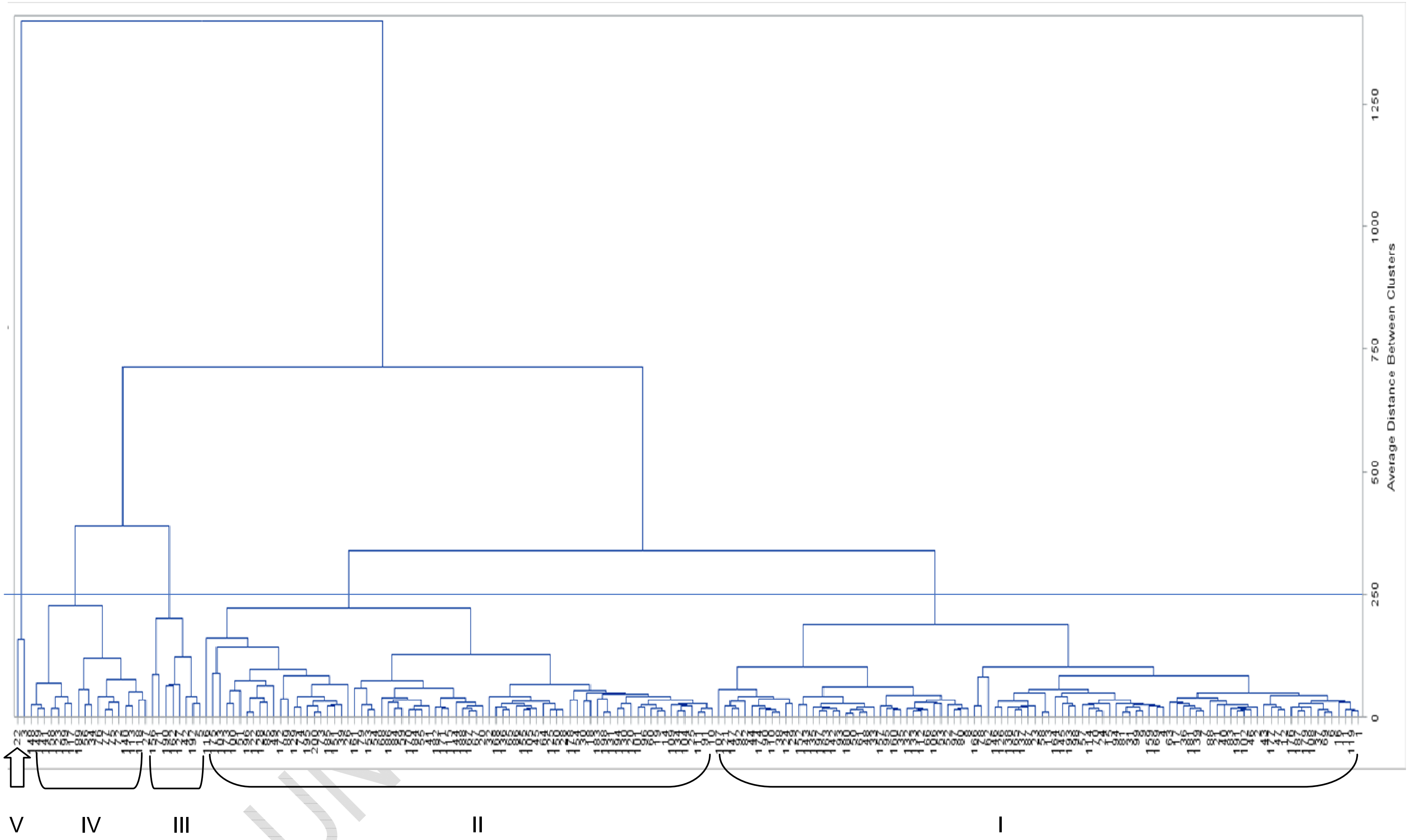

Figure 1. Dendrogram demonstrating the relationships among 200 cowpea genotypes based on phenological, yield and yield-related traits

Table 3. List of genotypes in the different clusters

Cluster I				Cluster II				Cluster III	Cluster IV	Cluster V
TVu9393	TVu15400	TVu3310	TVu132	TVu10559	TVu14970	TVu9357	TVu1477	TVu15299	TVu4669	TVu1059
TVu13950	TVu8673	TVu456	TVu7798	TVu15913	TVu1124	TVu2680	TVu9848	TVu4632	TVu14406	TVu43
TVu14683	TVu15445	TVu15610	TVu1037	TVu9259	TVu4545	TVu3657	TVu8622	TVu16430	TVu10366	
TVu8631	TVu2185	TVu14632	TVu2418	TVu15861	TVu16304	TVu13979	TVu393	TVu15487	TVu12873	
TVu6493	TVu14253	TVu16465	TVu6641	TVu9073	TVu6663	TVu14788		TVu14173	TVu8072	
TVu2606	TVu14272	TVu8877	TVu16449	TVu2968	TVu3346	TVu1637		TVu2500		
TVu15878	TVu10179	TVu3282	TVu9256	TVu16505	TVu15742	TVu5500		TVu16461		
TVu8612	TVu3947	TVu10394	TVu2723	TVu13958	TVu16269	TVu15037		TVu109		
TVu9820	TVu9391	TVu4622	TVu21	TVu14190	TVu3107	TVu1727				
TVu2322	TVu3043	TVu1330	TVu2548	TVu6837	TVu2449	TVu1004				
TVu13968	TVu2971	TVu945	TVu15945	TVu2845	TVu16237	TVu16253				
TVu1656	TVu8775	TVu8671	TVu2736	TVu15860	TVu15775	TVu16521				
TVu14875	TVu6464	TVu6477	TVu415	TVu8262	TVu10513	TVu14248				
TVu14558	TVu201	TVu1811	TVu15926	TVu14195	TVu14621	TVu16486				
TVu14336	TVu4943	TVu8883	TVu16368	TVu4761	TVu16403	TVu14224				
TVu16514	TVu5443	TVu13573	TVu15976	TVu16504	TVu15653	TVu13939				
TVu15500	TVu3565	TVu15426	TVu9468	TVu16574	TVu9508	TVu9486				
TVu14539	TVu14633	TVu15687	TVu2280	TVu4760	TVu1036	TVu8713				
TVu8656	TVu14172	TVu53	TVu4557	TVu16278	TVu1886	TVu3360				
TVu15982	TVu14393	TVu3652	TVu113	TVu14862	TVu15661	TVu1016				
TVu2933	TVu15636	TVu972	TVu969	TVu2398	TVu14345	TVu467				
TVu3552	TVu14346	TVu2252	TVu408	TVu1556	TVu3830	TVu9761				
TVu2769	TVu6968	TVu16220	TVu13778	TVu15450	TVu6966	TVu14290				
TVu1916	TVu1280	TVu14401	TVu6439	TVu6365	TVu15114	TVu9265				

Table 4: Mean performance for phenological, yield and yield-related traits in the best 10, 5 intermediate and 5 lowest genotypes evaluated at Ibadan in 2017

Genotype No.	**Genotype name**	**DTH (days)**	**100SWT (g)**	**Number of pods/plant**	**Number of seeds/pod**	**Yield (kg/ha)**	**Seed length (mm)**	**Seed thickness (mm)**	**Rank summation index**
92	TVu8631	89.50	12.95	11.75	12.00	1470.00	10.38	5.37	316
5	TVu109	73.00	11.00	20.00	12.10	586.67	8.73	5.52	352
22	TVu1059	67.50	19.95	18.50	10.20	870.00	7.13	4.09	372
72	TVu4669	81.00	16.25	14.75	8.70	776.67	8.72	4.90	385
189	TVu16408	75.50	12.25	10.00	14.70	731.67	7.57	5.03	400
118	TVu12873	60.50	8.80	28.00	7.80	1383.33	10.44	4.36	402
75	TVu4783	79.00	13.30	18.75	10.40	531.67	9.42	4.68	412
132	TVu14253	76.50	12.40	15.25	6.20	1466.67	9.94	4.47	420
27	TVu8072	77.00	8.95	16.00	11.40	668.33	9.78	4.63	428
2	TVu21	75.00	13.75	10.25	8.50	938.33	8.38	4.84	429
58	TVu3310	116.00	10.00	7.25	16.10	616.65	8.16	4.24	688
175	TVu15913	166.50	11.05	2.75	15.90	593.33	8.76	4.48	690
24	TVu1330	90.50	12.40	7.00	14.40	656.67	7.02	3.91	692
95	TVu8673	112.00	11.25	7.75	13.60	346.67	8.01	4.91	693
176	TVu15926	81.50	5.90	23.00	11.80	581.67	7.02	3.90	694
153	TVu14970	113.00	13.80	6.75	7.00	575.00	5.70	3.40	1031
51	TVu2968	134.50	4.80	8.50	8.20	516.67	6.76	3.98	1036
89	TVu8262	129.00	17.85	4.75	7.00	611.67	5.99	3.16	1036
173	TVu15861	108.00	8.70	4.00	9.50	131.67	6.92	3.91	1102
103	TVu9259	185.00	11.10	5.50	7.20	228.33	5.91	3.56	1193
Overall mean		87.50	11.90	10.65	10.03	659.49	7.45	4.12	
Mean squares		760.66	32.70	61.61	12.36	205199.76	3.24	0.96	
Error		324.66	0.82	2.79	3.83	10112.9	0.25	0.39	
CV (%)		20.60	8.03	15.68	19.50	15.25	6.70	15.09	
LSD (0.05)		30.91	1.78	2.99	3.72	192.60	0.93	1.22	

All mean square values are significant at $P \leq .0001$

DTH- Days to first pod harvest; 100SWT- Hundred-seed weight

The principal component analysis revealed that the first four principal components (PC) each with eigen values >1 accounted for 72.5% of the total variation in the data set (Table 5). The first principal component which captured 28.7% of the total variation had the highest eigen value of 3.45. The second principal component with eigen value of 2.27 accounted for 18.9% while the third and fourth principal components with eigen values of 1.61 and 1.37 contributed 13.4% and 11.4% of the total variation in the population, respectively. The hundred-seed weight (0.515), seed length (0.472), seed width (0.497) and seed thickness (0.372) were strongly correlated and loaded on the first principal component (PC). The second PC was loaded with days to first pod harvest (0.588) and days to first flower initiation (0.581). Pod length (0.646) and mean number of seed per pod (0.588) were loaded on the third PC while the fourth PC was loaded with number of pods per plant (0.396) and grain yield (0.629). The loadings of hundred-seed weight, seed length, seed width and seed thickness on the first principal component indicates that the four traits are the characters that chiefly accounted for most of the variation among the genotypes and that maximum variability occurred in the first PC. Days to first pod harvest, days to first flower initiation, pods per plant, grain yield, pod length and mean number of seeds per pod also contributed highly to the variation observed in the population. This suggests that the ten traits should be considered for selection in cowpea improvement programme. It also suggests that these traits are very important to maintain variability within the breeding population. Similar observations were made by Doumbia et al. [19] in an evaluation of 94 cowpea genotypes from Mali and Ghana. The study also identified seed weight, seed length, seed width and seed thickness as important traits in diversity analysis. Bozokalfa et al. [20] observed that wide variation found in the quantitative characters can be useful in the development of variety description and identification.

Table 5. Principal component analysis of agronomic traits of 200 cowpea genotypes evaluated at Ibadan in 2017

Character	Prin1	Prin2	Prin3	Prin4
Days to first pod harvest (days)	0.04	**0.59**	-0.05	0.31
Days to first flower (days)	0.01	**0.58**	-0.02	0.34
Hundred-seed weight (g)	**0.52**	-0.03	-0.10	0.08
Pod length (cm)	0.21	-0.07	**0.65**	-0.14
Number of pods/plant	-0.23	-0.39	-0.24	**0.40**
Mean number of seeds/pod	-0.13	0.06	**0.59**	0.33
Grain yield (kg/ha)	0.09	-0.37	0.07	**0.63**
Seed length (mm)	**0.47**	-0.08	0.16	-0.12
Seed width (mm)	**0.50**	0.01	-0.06	0.08
Seed thickness (mm)	**0.37**	-0.04	-0.28	0.16
Plant height (cm)	0.04	0.08	-0.21	-0.11
Chlorophyll content	-0.07	0.06	-0.09	-0.22
Eigen value	3.45	2.27	1.61	1.37
Proportion (%)	28.74	18.92	13.43	11.43
Cumulative (%)	28.74	47.65	61.08	72.51

Values in bold within each column indicate most important variables that contributed most to the variation of a particular factor

4. CONCLUSION

TVu1059 and TVu43 formed separate cluster from the rest of the genotypes with respect to yield potential. This shows their distinctness from the population of cowpea used in this study and can therefore be used as parents to cross with genotypes in other clusters for cowpea improvement.

Significant differences were observed among the 200 cowpea genotypes for phenological, yield and related traits. TVu8631 had the highest worth based on RSI and the highest grain yield. This genotype can therefore be directly selected for further evaluation at advanced breeding stages before nomination for release to farmers. Also the ten top ranking genotypes identified in this study can be selected for further improvement in cowpea breeding programmes at IITA and other research institutes.

Considerable genetic variability existed among the cowpea genotypes used in this study. Most variations were attributed to hundred-seed weight, seed length, seed width, seed thickness, days to first pod harvest, days to first flower, number of pods per plant, grain yield, pod length and mean number of seeds per pod. These characters can be used as selection criteria in future breeding programme.

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