Evaluation and Identification of Genetic Variation Pattern in Cowpea [*Vigna unguiculata* (L.) Walp] Accessions Using Multivariate Analyses

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Abstract: Thirty accessions of cowpea from Ebonyi, Enugu and Kogi States, Nigeria were used for the study. Seeds of the accessions were randomized in a plot measuring 50x50 meters for three growing seasons. Our results on yield and yield-related traits showed that three principal components were extracted, which contributed 82.23% of the total variability. It revealed that number of seeds pod^{-1}, 100-seed weight, pod length, days to 50% maturity, seed yield, number of leaves plant^{-1} contributed significantly to the total genetic variability while for proximate composition, four principal components accounted for 93.75% of the total genetic divergence. Cluster analysis revealed that accessions were grouped not necessarily based on geographical location but genetics. Selection for high yielding accessions should be done on cluster 2 as we recommend selection and hybridization of accessions from cluster 1, 2, and 3 for optimal benefit.

Keywords: Germplasm collection, cowpea, selection, hybridization, multivariate analysis.

INTRODUCTION

As farmers adopt the best yielding crop varieties, the tendency of shifting attention to crops that will give better financial returns becomes inevitable. This practice might cause diversity lost. It should be emphasized that elite germplasm has narrow genetic variability. This narrow genetic base might increase their vulnerability to biotic and abiotic stresses. According to [1], this could be broadened through the knowledge, access and use of the available diversity in domesticated and wild relatives. Selection of genetically distant parents for hybridization is a basic need for generation of genotypes with desirable traits.

Landraces of cowpea (*Vigna unguiculata* L. Walp) present high variability, adaptability and superb nutritive profile [2, 3]. Strikingly, the challenging, precarious and worsening ecological conditions in the world demands crops with intrinsic capacity to withstand these conditions and still give good yield [4]. Evaluation of available genetic stock to assess the genetic variation for economically important characteristics is a *sine-quo-non* for introgressing desirable genes in a single accession [5].

According to [6], variation is a highly heritable characteristic. While qualitative traits provide an estimate of diversity, quantitative traits are exploited to determine the magnitude of genetic variation within collection or germplasm [6]. Various numerical

taxonomic techniques have been successfully used to classify and measure the pattern of phenotypic variation in the relationship of germplasm collections in a variety of crops by many researchers such as in black gram [7], soybean [8], lentil [9], pea [10] and *Nigella sativa* [11].

Multivariate analysis provided a method of evaluation and identification of land races that could be further characterized by DNA molecular markers [12. 13] considered multivariate analysis appropriate for choosing parents for hybridization. Determination of germplasm diversity and genetic relationships among breeding materials is valuable aid in crop improvement strategies [11]. A benefit of the cluster analysis on the basis of quantitative traits for selection of phenotypically distinct germplasm with more breeding values has been reported previously by [14]. Consequently, principle component analysis also provided help for identification of superior accessions based on multiple traits performance. Studies on genetic divergence using multivariate analysis has been reported by [15] in mungbean (*Vigna radiata*), [16-24] in cowpea (*Vigna uniguiculata*).

For conservation and exploitation of genetic resources for crop improvement, it becomes imperative that the knowledge about genetic structure and relationships among genotypes/accessions be known, especially in cowpea landraces in Nigeria. This is the pivot of the present research.

MATERIALS AND METHODS

Thirty accessions of cowpea [*Vigna unguiculata* (L.) Walp] were obtained from cowpea cultivating areas in

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Ebonyi, Enugu, and Kogi States, Nigeria, respectively (Table **1**). A plot of land measuring 40x40 meters was manually cleared in the University of Calabar Experimental Farm, Calabar. Twenty beds were made with a spacing of 2 meters between beds. Three seeds were sown in a hole of 4cm deep per variety according to the [25]. The 30 accessions were randomized on each bed with 2 replications per bed per variety using randomized complete block design (RCBD). A spacing of 50 x 75cm was maintained. After seedling emergence, each stand of individual variety was thinned down to 2. Weeding was done manually as the need arose while staking was done 4 weeks after planting. This research was conducted during the 2010, 2011, 2012 planting seasons while data obtained were pooled together.

Data Collection and Analysis

After two weeks of planting, percentage germination and days to seedling emergence were estimated. Other morphological traits such as plant height, number of branches plant^{-1}, number of leaves plant^{-1}, leaf area

Table 1: Cowpea [*Vigna uniguiculata* (L.) Walp] Accessions Collected from Different Sites in Nigeria with their Altitude During 2011/2012 Growing Season

Accession	Collecting site	State	Altitude (m.a.s.l.)
AFB$_1$	Afikpo	Ebonyi	57.69
AFB$_2$	Afikpo	Ebonyi	57.69
OHB$_1$	Okposi	Ebonyi	56.00
OHB$_2$	Okposi	Ebonyi	56.00
OHB$_3$	Okposi	Ebonyi	56.00
UDE$_1$	Udi	Enugu	326.00
UDE$_2$	Udi	Enugu	326.00
NSE$_1$	Nsukka	Enugu	422.94
NSE$_2$	Nsukka	Enugu	422.94
NSE$_3$	Nsukka	Enugu	422.94
NSE$_4$	Nsukka	Enugu	422.94
NSE$_5$	Nsukka	Enugu	422.94
OBC$_1$	Obanliku	Cross River	1,575.76
ABK$_1$	Abocho	Kogi	412.42
ABK$_2$	Abocho	Kogi	412.42
ABK$_3$	Abocho	Kogi	412.42
OCK$_1$	Ochadamu	Kogi	412.42
OCK$_2$	Ochadamu	Kogi	412.42
OCK$_3$	Ochadamu	Kogi	412.42
OCK$_4$	Ochadamu	Kogi	412.42
OFK$_1$	Oforachi	Kogi	128.00
OFK$_2$	Oforachi	Kogi	128.00
OFK$_3$	Oforachi	Kogi	128.00
ODK$_1$	Odolu	Kogi	41.00
ODK$_2$	Odolu	Kogi	41.00
ODK$_3$	Odolu	Kogi	41.00
IGK$_1$	Igalamela	Kogi	41.00
IGK$_2$	Igalamela	Kogi	41.00
IGK$_3$	Igalamela	Kogi	41.00
IGK$_4$	Igalamela	Kogi	41.00

*The acronyms were arrived at by using the first 2 alphabets of the collecting site and the first alphabet of the state while the subscript denotes the number of accessions collected per site.
M.a.s.l. = meters above sea level.

plant^{-1}, internode length plant^{-1}, petiole length plant^{-1}, days to 50% flowering, number of flowers plant^{-1}, number of pods plant^{-1}, pod length, number of seeds pod^{-1}, days to 50% maturity, seed yield plant^{-1}, and 100-seed weight were also recorded at 10 weeks. For the estimation of the leaf area, the leaves were laid on a 1-cm grid (graph paper) and their outlines traced. The number of square centimeters were calculated, including the partial squares and multiplied by 0.1cm^2. However, all partial squares that were less than half covered were excluded. The seed yield per plant was estimated by multiplying the average number of seeds per pod per plant and the average number of pod per plant [26].

Preparation of Samples for Proximate and Anti-Nutritive Contents Analyses

Extraneous matter from the seeds of the 30 accessions obtained such as unhealthy seed, infected seed, sand and chaff were removed from the samples. The seeds were dehulled with water at room temperature (27ºC), sundried for 2 days and then dried at 70-80ºC for 2 hours in an oven (Astell Hearson type). Finally, the dried dehulled samples were pulverized using an electric blender (Model 4250 Braun, Germany) and sieved to a particle size of 1mm and stored in dissector for analysis.

Determination of Proximate Composition

The proximate analysis of samples for moisture, crude fat, fibre, protein and ash were determined using the methods described by [27]. The protein content was determined using micro kjeldhal method (N x 6.25) and the carbohydrate content was determined by the difference obtained after subtracting the total organic nitrogen, protein, lipid, ash, fibre, from the total dry matter and expressing as percentage. The gross food energy (calorific value) was estimated by multiplying the crude protein, crude fat and total carbohydrate by factors 4, 9 [28].

Toxicant Analysis

Hydrocyanic acid (HCN) was estimated by the alkaline titration method. For HCN determination, alkaline sample solution was titrated with standard 0.02N AgNO$_3$ to a permanent turbid KI indicator end point (1 ml of 0.02N AgNO$_3$ = 1.08 mg HCN). Phytic acid was determined as iron precipitate with the assumption that iron: phosphorus molecular ratio is 4:6 according to [29] (The molecular formula of phytic acid is $C_6H_{18}O_{24}P_6$ with molecular mass of 660g/mol), while

oxalate was determined according to [30]. Oxalate determination involved three steps- digestion, oxalate precipitation and permanganate titration.

Determination of Mineral Composition

The ash of each sample was digested with 5mL of 2M HNO$_3$ and heated to dryness on a heating mantle. Five (5) mL of 2M HNO$_3$ was added again, heated to boil and filtered through a Whatman No 1 filter paper into a 100ml volumetric flask. The filtrate was made up with distilled water. Calcium was determined using Jenway Digital Flame Photometer (PFP7 model) while other minerals apart from phosphorus were determined using Buck Scientific Atomic Absorption Spectro-photometer (BUCK 210VGP model). The phosphorus in the sample filtrate was determined by using Vanadomolybdate reagent at 400nm using colorimetric method (Colorimeter SP 20, Bausch and Lamb). Vitamins A and C were also estimated [27].

Data Analyses

Principal component and cluster analyses were performed by computer software (Predictive Analytics SoftWare "PASW") version 18.0. Estimation of genetic distance was according to the method of [31]Mohammadi and Prasanna (2003). $GD_{(I,j)} = [(x_1-y_1)^2 + (x_2 - y_2)^2 + . . . (x_p-y_p)^2]$; where GD = genetic distance between two accessions; I and j = different accessions; p = quantitative traits denoted by x_1, x_2,. . . x_p and y_1, y_2, . . . y_p. Note: Representative accession(s) from each cluster was used in the estimation of genetic distance.

RESULTS

Principal Component Analysis for Yield and Yield Related Traits

Three principal components with Eigen value > 1 contributed 82.23% of the total variability among 30 accessions evaluated for 13 traits. The following traits contributed positively to PC$_1$ that had 46.8% variation proportion to the total variability: inter-node length plant^{-1} (0.944), number of leaves plant^{-1} (0.912), days to 50% maturity (0.881), number of flowers plant^{-1} (0.599), while leaf area plant^{-1} (-0.931), vein length plant^{-1} (-0.847) and seed yield (-0.714) contributed negatively to the variability. Pod length plant^{-1} (0.904), 100-seed weight (0.861), number of seeds pod^{-1} (0.824) and number of flowers plant^{-1} (0.767) contributed high variability in PC$_2$ accounting for 22.67% of the total variation. In PC$_3$ accounting for 12.74% of the total variability, number of leaves plant^{-1}

(0.490) and days to 50% flowering (0.765) contributed positively to the total variation while vein length plant[-1] (-0.617) contributed negatively to the variation (Table **2**).

Principal Component Analysis for Proximate, Anti-Nutritive and Mineral Compositions

Four principal components were extracted which accounted for 93.75% variability among the 30 cowpea accessions evaluated. PC_1 accounted for 46.07% of the total variation and was correlated positively with the ash content (0.722), crude fibre (0.902), carbohydrate (0.987), caloric value (0.946), calcium (0.962), phosphorus (0.984), iron (0.899), vitamin C (0.668) while vitamin A (-0.664) contributed negatively. PC_2 accounted for 26.36% and mainly correlated with oxalate (0.925) and negatively with the moisture content (-0.720), protein (-0.861), and magnesium (-0.543). However, PC_3 had 11.34% of the total variation. Fat content contributed 0.762, HCN (0.570), magnesium (0.734) while PC_4 accounted for 7.64% of the variation and correlated with HCN (0.725) only (Table **3**).

Cluster Analysis

Single linkage cluster analysis was adopted with the view to classifying the 30 accessions based on similarity indices of yield and yield related traits, proximate, anti-nutritive and mineral components. For yield and yield-related traits, two clusters were observed with cluster –1 having 23 accessions and cluster – 2 with only 7 accessions (Figure **1**). Accessions from cluster 2 produced high seed yield (933.68±3.64), broad leaf surface (70.56±0.34cm^2), long vein (192.77±0.82cm), increased pod number (46.91±0.19), reduced days to 50% maturity (57.10±0.40 days) while accessions in cluster 1 produced more number of leaves (147.30±2.57) (Table **4**).

Additionally, 4 clusters were observed for proximate, anti-nutritive and mineral components where clusters 1, 2, 3 and 4 had 11, 8, 10 and 1 accessions, respectively (Figure **2**). Accessions from cluster 3 had higher protein content (26.25±0.51%), followed by cluster 4 accessions (24.25±0.45%). The only accession in cluster 4 gave higher fat content

Table 2:   Principal Components (PCs) for 16 Yield and Yield Related Traits in 30 Accessions of Cowpea Landraces [*Vigna uniguiculata* (L.) Walp]

		Component matrix		
Yield and yield related traits		**PC_1**	**PC_2**	**PC_3**
Eigen value		7.488	3.628	2.039
Proportion of variation		46.800	22.672	12.743
Cumulative variance		46.800	69.472	82.215
Communality	Eigen factors			
Inter-node length plant[-1]	0.947	0.944	-0.174	-0.157
Leaf area plant[-1] @ 5 weeks	0.945	-0.931	0.267	0.088
Number of leaves plant[-1] @10 weeks	0.866	0.912	-0.088	-0.164
Days to 50% maturity	0.979	0.881	0.202	-0.401
Leaf area plant[-1] @ 10 weeks	0.983	-0.867	0.399	0.268
Vein length @ 10 weeks	0.719	-0.847	-0.044	-0.020
Number of leaves plant[-1] @5 weeks	0.830	0.767	0.032	0.490
Seed yield	0.966	-0.714	0.474	0.480
Vein length @ 5 weeks	0.821	-0.659	-0.080	-0.617
Days to seedling emergence	0.385	-0.369	0.363	-0.343
Number of pods plant[-1]	0.171	-0.328	0.243	0.068
Pod length plant[-1]	0.936	0.314	0.904	-0.142
100-seed weight	0.833	0.177	0.861	-0.247
Number of seeds pod[-1]	0.943	0.399	0.824	0.325
Number of flowers plant[-1]	0.947	0.599	0.767	-0.004
Days to 50% flowering	0.882	0.478	-0.262	0.765

Table 3: Principal Components (PCs) for Proximate and Anti-Nutritive Compositions in 30 accessions of cowpea landraces [*Vigna uniguiculata* (L.) Walp]

Proximate and anti-nutritive compositions	Component matrix			
	PC_1	PC_2	PC_3	PC_4
Eigen value	7.371	4.217	1.815	1.222
Proportion of variation	46.066	26.355	11.341	7.635
Cumulative variance	46.066	72.421	83.761	93.747
Communality	Eigen factors			
Moisture (%)	0.796	-0.464	-0.720	0.041	0.248
Protein (%)	0.936	0.194	-0.861	-0.271	0.287
Fat (%)	0.841	-0.341	-0.365	0.762	0.105
Ash (%)	0.813	0.792	0.309	0.300	0.021
Crude fibre (%)	0.870	0.902	-0.194	-0.087	-0.103
Carbohydrate (%)	0.982	0.987	-0.012	-0.044	0.071
Calorific value (kcal/100g)	0.955	0.946	-0.078	-0.077	0.218
Phytate (mg100g^{-1})	0.898	0.261	0.818	-0.069	0.395
Hydrocyanide (mg100g^{-1})	0.893	-0.006	0.208	0.570	0.725
Oxalate (mg100g^{-1})	0.941	-0.017	0.925	0.280	-0.087
Calcium (mg100g^{-1})	0.970	0.962	-0.169	-0.010	0.123
Magnesium (mg100g^{-1})	0.958	0.179	-0.543	0.734	-0.302
Phosphorus (mg100g^{-1})	0.971	0.984	0.046	-0.035	-0.011
Iron (mg100g^{-1})	0.897	0.899	-0.242	-0.074	0.154
Vitamin A (mg100g^{-1})	0.943	-0.664	0.635	-0.195	0.247
Vitamin C (mg100g^{-1})	0.958	0.668	0.574	0.248	-0.349

while accessions in cluster 1 had the least crude fibre content and carbohydrate and the accessions with the highest carbohydrate content was found in cluster 2, resulting to increased caloric value of 210.85±1.64 Kcal/100g. Anti-nutritive components were found to be much reduced in cluster 4 accessions; phytate (0.61±0.02mg/100g), hydrocyanide (3.43±0.42mg/100g) and oxalate (6.10±0.28mg/100g). These components were highest in cluster 2 accessions (Table **5**). Calcium, phosphorus and vitamin C levels in cluster 2 accessions were higher when compared with accessions in other clusters. However, magnesium level was high in the cluster 4 accession (19.20±0.46mg/100g) while vitamin A level was high in accessions in cluster 1 (78.10±1.75mg/100g) (Table **5**).

Genetic Distance

The genetic distance between UDE_1 (cluster -1) and ABK_3 (cluster -2) was 341.73, NSE_2 (cluster-1) and IGK_2 (cluster-2) (283.41) while OFK_1 (cluster-1) and OCK_4 (cluster-2) was 274.92. Additionally, for proximate, anti-nutritive and mineral compositions, the genetic distances between representative accessions are as follows; ODK_2 (cluster-1) and NSE_5 (cluster-2) was 61.17; ABK_3 (cluster-1) and OHB_1 (cluster- 3) was 36.73 and OHB_2 (cluster-1) and NSE_1 (cluster-4) was 69.25, respectively. OFK_1 (cluster-2) and NSE_2 (cluster 3) was 40.66; IGK_2 (cluster-2) and NSE_1 (cluster-4) was 51.17 while NSE_2 and NSE_1 was 51.37.

DISCUSSION

Analysis of genetic relationships in crop species is obviously an important component of crop improvement programmes. This is because (a) it provides information about genetic diversity as well as a platform for stratifying samples for breeding populations [31], (b) aids in the identification of diverse parental combinations that will create segregating progenies [32], (c) provides a method of evaluation and identification of landraces that would be further characterized by DNA molecular markers [12].

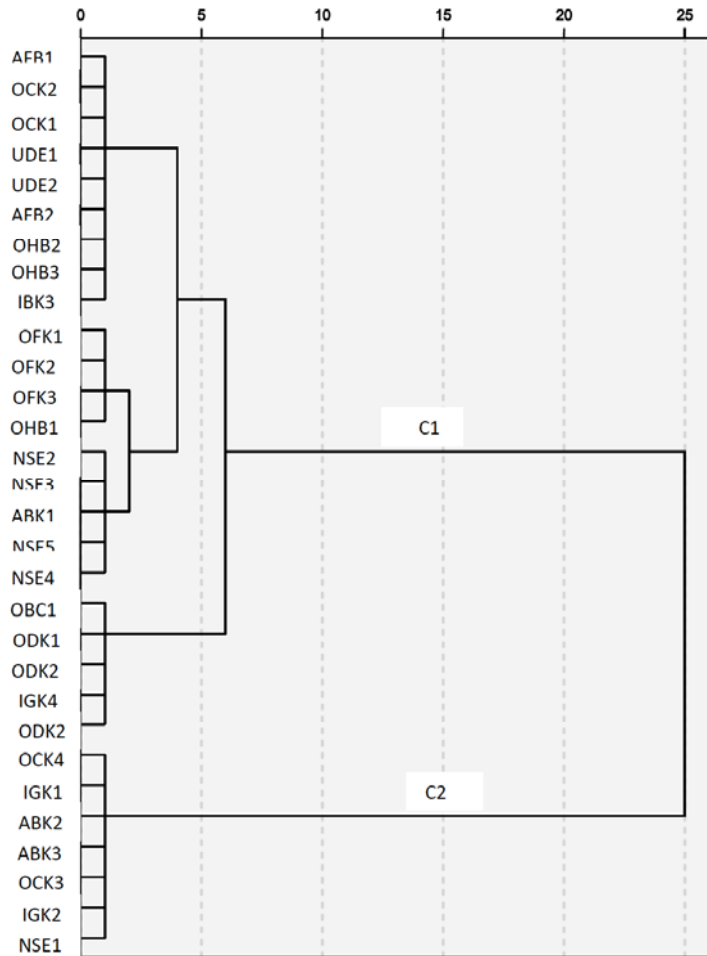According to [33], cultivated cowpea and indeed legumes [34] has lower genetic variability than many

Figure 1: Cluster pattern for yield and yield-related traits constructed by nearest neighbour in 30 cowpea [*Vigna uniguiculata* (L.) Walp] accessions.

Table 4:   Variation within Clusters for Yield and Yield- Related Traits in 30 Accessions of Cowpea [*Vigna unguiculata* (L.) Walp]

| Parameters	Cluster-1	Cluster-2
	23	7
	Mean±SE	Mean±SE
Days to seedling emergence (days)	4.05±0.03	3.96±0.06
Vein length @5 wks (cm)	58.70±1.46	62.29±0.34
Vein length @10wks (cm)	162.83±3.66	192.77±0.82
Number of leaves @5 wks	51.43±3.03	41.93±0.48
Number of leaves @ 10 wks	147.30±2.57	112.13±0.69
Leaf area @ 5 months (cm^2)	30.61±0.63	40.27±0.32
Leaf area @ 100 wks (cm^2)	39.15±1.08	70.56±0.34
Inter-node length (cm)	7.78±0.18	5.34±0.06
Days to 50% flowering (days)	49.04±0.58	48.06±0.32
Number of flowers	62.17±2.61	63.78±0.23
Days to 50% maturity (days)	63.88±0.84	57.10±0.40
Number of pods	33.99±1.34	46.91±0.19
Number of seeds	18.50±0.65	20.88±0.42
Pod length (cm)	15.02±0.35	16.05±0.18
Seed yield (g/variety)	618.47±1.10	933.68±3.64
100-seed weight (g)	9.53±0.26	10.09±0.17
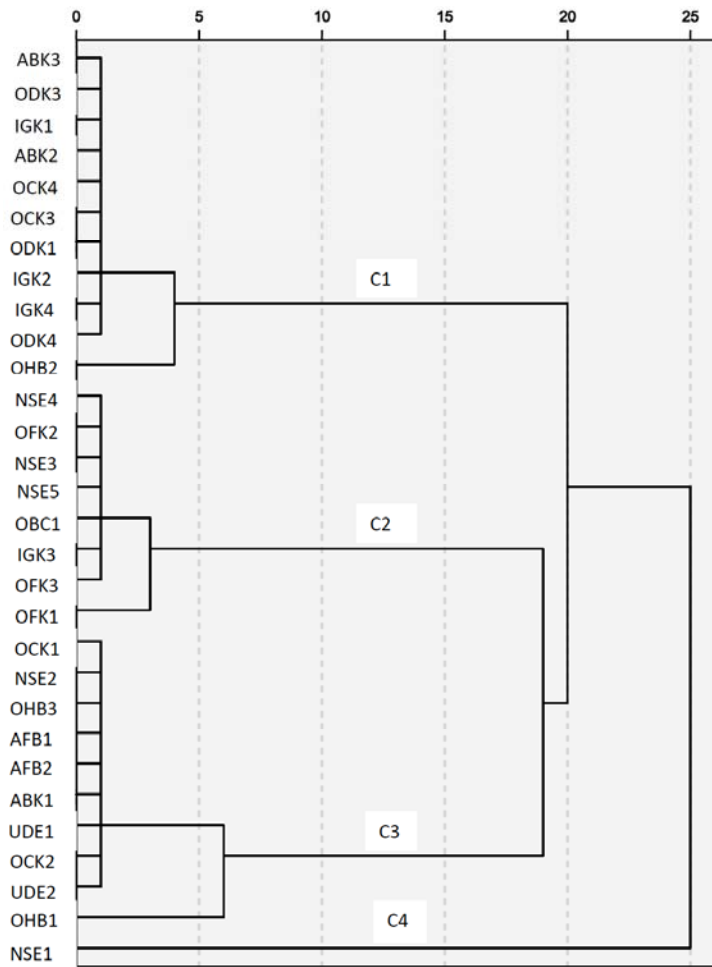
Figure 2: Cluster pattern for proximate and anti-nutritive compositions constructed by nearest neighbor in 30 cowpea [*Vigna uniguiculata* (L.) Walp] accessions.

Table 5:   Variation within Clusters for Proximate, Antinutritive and Mineral Compositions in 30 Accessions of Cowpea [*Vigna unguiculata* (L.) Walp]

Parameters	Cluster-1	Cluster-2	Cluster-3	Cluster-4
	11	8	10	1
	Mean±SE	Mean±SE	Mean±SE	Mean±SE
Moisture	50.05±0.67	45.49±0.41	50.26±0.73	51.80±0.12
Protein	22.52±0.23	21.85±0.21	26.25±0.51	24.25±0.45
Fat	3.78±0.10	3.29±0.11	3.95±0.19	4.60±0.21
Ash	5.72±0.24	7.17±0.17	6.25±0.08	5.90±0.23
Crude fibre	1.41±0.05	1.90±0.02	1.82±0.07	1.90±0.08
Carbohydrate	12.15±0.12	18.33±0.15	16.93±0.33	13.11±0.56
Caloric value (kcal/100g)	179.57±0.28	210.85±1.64	208.60±1.60	172.50±1.13
Phytate	1.26±0.01	1.45±0.02	1.14±0.05	0.61±0.02
Hydrocyanide	4.19±0.06	4.21±0.08	4.23±0.16	3.43±0.42
Oxalate	7.14±0.06	7.72±0.12	6.16±0.16	6.10±0.28
Calcium	12.23±0.11	27.10±0.11	26.88±0.49	14.40±0.78
Magnesium	8.24±0.11	9.42±0.15	11.23±0.78	19.20±.46
Phosphorus	0.19±0.01	1.26±0.02	0.93±0.05	0.40±0.02
Iron	0.02±0.003	0.11±0.01	0.12±0.01	0.05±0.001
Vitamin A	78.10±1.75	59.22±0.27	47.35±0.27	25.12±0.59
Vitamin C	40.03±0.28	74.70±1.00	41.26±0.34	59.20±1.10

other crops. Our results on yield and yield-related traits, only three principal components were extracted, which contributed 82.23% of the total variability among 30 accessions evaluated. It revealed that number of seeds pod^{-1}, 100-seed weight, pod length, days to 50% maturity, seed yield, number of leaves plant^{-1} contributed significantly to the total genetic variability and divergence. These results corroborate those of earlier reports by [35-37].

For proximate, anti-nutritive and mineral compositions, four principal components accounted for 93.75% of the total genetic divergence where phosphorus, iron, ash, crude fibre, protein, vitamins A and C, carbohydrate, magnesium, fat, oxalate and HCN contributed maximally to the PCs. This variation according to [38] is attributable to environmental and genetic factors. [39] reported that when the communalities are high, it indicates that the similarities among the ecotypes or accessions are high. Our present results revealed high communalities except for number of pods plant^{-1} (0.171) and days to seedling emergence (0.385). This suggests high similarities among the accessions evaluated.

The aspiration of breeders and farmers is to produce crop species that will have high yielding capacity, resistance to ecological conditions such as pest/diseases, drought, salinity, notwithstanding. From our cluster analysis, it does appear that selection for high yielding accessions should be done in cluster 2. For emphasis, all the accessions in cluster 2 were obtained from Kogi State, Nigeria, except NSE$_1$ that was obtained from Nsukka, Enugu State. This implies that the accessions found in cluster 2 (Figure **1**) may have superior genes as they were raised in the same ecological conditions with those in cluster 1. This could inform importantly that the chances of environmental influences were reduced drastically with genetic factor playing an active role.

Cluster analysis for proximate, anti-nutritive and mineral compositions revealed that accessions in cluster 3 (Figure **2**) possess high protein content, comparable carbohydrate, calcium and iron contents with accessions in cluster 2. However, accessions in cluster 1 contain high vitamin A while those in 2 possess high vitamin C. Regrettably, accessions that exhibited high yield capacity did not contain high protein, which is the major proximate component in cowpea cultivation. What this suggests is that depending on the aim of the breeder, the gene(s) to use for introgression is basically accession-specific.

[40] observed that introgressing genes from diverse germplasm into the available genetic base are striking in plant biotechnology. It does imply that accessions from different clusters could be selected and hybridized to obtain a variety fortified with important economic traits.

According to [41], genetic distance is any quantitative measure of genetic differences, be it at the sequence level or the allele frequency level that is calculated between individuals, populations or species. Our results showed that the genetic distances between representative accessions in different clusters was generally not wide. Importantly, the low divergence among accessions studied indicates the possibility of the accessions originating from the same genetic background. [42] reported high level of resemblance among cowpea varieties, which was attributed to their self-pollinating nature.

Though cluster analysis grouped together accessions with greater morphological, proximate, anti-nutritive and mineral similarities together in the present work, the cluster did not necessarily include all accessions from the same geographical sites. It is probable that the lack of differentiation among regions is an indication of both high level of gene flow between regions as well as lack of sufficient time for significant genetic differentiation along geographical lines. [37] demonstrated that genetic drift and selection pressure under different environments would have caused greater divergence rather than geographical distance.

CONCLUSION

Succinctly, the yield performance and chemical compositions of accessions were more genetic-based than environmentally influenced. Additionally, selection for high yielding accessions should be done on cluster 2 in Figure **1** as we recommend selection and hybridization of accessions from clusters 1, 2, and 3 in Figure **2** for optimal benefit.

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