

*Full Length Research Paper*

# Classification of genetic diversity and choice of parents for hybridization in cowpea *Vigna unguiculata* (L.) Walp for humid savanna ecology

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**Thirty-one accessions of cowpea of diverse eco-geographic origins were evaluated for genetic diversity using principal component analysis (PCA), single linkage cluster analysis (SLCA) and canonical techniques. The accessions were classified into six groups by PCA and SLCA while canonical technique identified five vector groups. There was no relationship between the clustering pattern and eco-geographic distribution. PCA and canonical techniques can be jointly used in multivariate analysis as both techniques performed complimentary role in identifying characters responsible for variation in cowpea. SLCA alone provided a clearer and more informative display of the group of accessions based on character performance. The three techniques revealed most distant accessions as having widest variation and possible choice of parent stocks in hybridization.**

**Key words:** Accessions, cowpea, genetic diversity, hybridization, parents, techniques.

## INTRODUCTION

The classification of a range of variability among accessions is pivotal to the maintenance and further acquisition of germ plasma resources even as accessions from diverse origins are needed as parent stocks for the development of improved varieties. Cowpea breeding in humid savanna requires parent stocks with specific adaptation to such humid ecology. Porter et al. (1974), reported that large morphological variability abound in the tropics suggesting adequate knowledge of the germ-plasm structure for the development of hybrids with specific ecology adaptation. The limitations resulting from normal pollination relating biparental heredity makes a critical choice of parents valuable in breeding programs, especially when polygenic characters are involved. Numerical taxonomic techniques have been successively used by many workers to classify variation patterns at both intra and interspecific levels (Sneath and Sokal, 1973; Chheda and Fatokun, 1982; Ariyo, 1991). Principal component analysis (PCA) is a descriptive technique which reveals the pattern of character variation among

individual accession. It further reduces a multivariate data into units or component thereby accounting for a meaningful amount of variation in a population (Atchley and Bryant, 1975). Unlike the PCA, the canonical analysis separates and forms two sets of varieties from which highly correlated variables are separated to form a new unit of within and between groups (Lawley and Maxwell, 1971). Single linkage cluster analysis (SLCA) decreases the number of individual variable units by classifying such variation into group which is translated into a dendrogram using the coefficient of similarity (Sneath and Sokal, 1973; Tatineni et al., 1996). The studies reported here aims at identifying the major characters responsible for variation among cowpea accessions with a view to grouping the accessions and identify potential parent stocks within the groups. The relative effectiveness of the techniques is also to be compared.

## MATERIALS AND METHODS

The 31 accessions of cowpea used in this study comprised 16 collected from diverse geographical region in Nigeria, 5 from Burkina-Faso, and 2 each from Cameroon, Niger republic, Benin republic, Senegal and Ghana (Table 1). Following land preparation, in the Teaching and Research farm of University of Agriculture

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**Table 1.** Origin/source of cowpea genotypes.

Number	Genotypes	Source/Origin	Morphological characteristics
1	LDPD	Kaduna North Western Nigeria	Erect
2	TVX-3236	Ogun State South Western Nigeria	Erect
3	Danilla	Kaduna North Western Region	Erect
4	Owode	Ogun State South Western Nigeria	Erect
5	Ife- brown	Oyo State South Western Nigeria	Erect
6	IT90K-277-2	Oyo State South Western Nigeria	Erect
7	IT95K-1091-3	Oyo State South Western Nigeria	Erect
8	IT97K-508-2	Oyo State South Western Nigeria	Erect
9	IT95K-1090-12	Oyo State South Western Nigeria	Erect
10	IT90K-76	Oyo State South Western Nigeria	Erect
11	IT97K-499-39	Oyo State South Western Nigeria	Erect
12	IT93K-686-2k	Oyo State South Western Nigeria	Erect
13	IT97K-1034-94	Oyo State South Western Nigeria	Erect
14	IT90K-59	Oyo State South Western Nigeria	Erect
15	AGRIBVI	Oyo State South Western Nigeria	Erect
16	KVX-745-119	Oyo State South Western Nigeria	Erect
17	KVX-745-11k	Burkina-Faso (Northern)	Erect
18	KVX-745-17k	Burkina-Faso (Southern)	Erect
19	KVX-414-22-72	Burkina-Faso (Southern)	Erect
20	KVX-795-17p	Burkina-Faso (Eastern)	Erect
21	IAR 48B	Kaduna, Zaria	Erect
22	TVU 14912	Niger republic	Prostrate
23	TVU 13096	Niger republic	Prostrate
24	TVU 10817	Cameroun, South West Province	Semi-Erect
25	TVU 10840	Cameroun, Eastern Province	Semi-Erect
26	TVU 14345	Senegal	Erect
27	TVU 14390	Senegal	Erect
28	TVU 13240	Benin Republic	Erect
29	TVU 13241	Benin Republic	Erect
30	TVU 12200	Ghana	Prostrate
31	TVU 12201	Ghana	Prostrate

Abeokuta, (humid savanna ecology), the seeds were grown using a randomized complete block design with three replications in single row plots to minimize environmental variations. Planting was done during the late season (August) of 2002 in 4 m long rows with 60 cm inter and 40 cm intra row spacing with two seeds were sown per hole. Blocks were separated from each other using 1 m spacing. Each row therefore contained 20 plants and the 16 inner competitive plants within each row were utilized for observation. Weeding was done 6 week after planting (WAP). Insect pests were controlled using karate at 50 ml per 20 litres of water.

Data were collected from the origin and growth habits of the 31 accessions as presented in Tables. Observations on the 27 characters were made according to the procedures outlined in Table 2. The genetic divergence among the genotypes was analyzed by standardizing the characters using a basic matrix of 31 x 27 and data subjected to the GENSTAT classificatory program at the biometrics unit of International Institute of Tropical Agriculture (IITA, 1982), Ibadan, Nigeria. The PCA matrix was prepared to calculate for each component, a latent vector and a latent root. The character loadings were used to calculate the accession component scores. The first three components were extracted for a three

dimensional ordination of accessions. Using the character variation, SLCA reduced the number of accessions into groups with the objective to minimize loss of information (Rhodes and Martins, 1972). Using the FASTCLUS procedure of SAS (2000), the group sorted the accessions into a dendrogram at intervals of 5% level of similarity starting from 100 to 80% level of similarity when all the 31 accessions occurred in a single cluster. Canonical vectors were used to describe the contributions obtained and separated to form two sets of variants from which highly correlated variables are separated to form a new unit of within and between groups of accessions. This analysis was also performed using the GENSTAT classificatory program.

## RESULTS

Using the procedure of Atchley and Bryant (1975), only six of the 31 Principal Component axes had eigen values greater than 1.00 and all together accounted for 63.62%

**Table 2.** Characters measured and methods of scoring/measurement.

Serial number	Characters	Method of Measurement	Rating scale	Nature of character
1	Flower colour	Visual estimation	White=0, purple=1, yellow=2	Qualitative
2	Stem pigmentation	Visual estimation	None=0, Node only=1, Hastate=3	Qualitative
3	Leaf shape	Visual estimation	Oblique=1, lanceolate=2, Hastate=3	Qualitative
4	Seed coat mixture	Visual estimation	Rough=1, smooth=2	Qualitative
5	Seed coat colour	Visual estimation	White=1, brown=2, milk=3, dark=5, red=6	Qualitative
6	Seed eye colour	Visual estimation	Black=0, brown=1, dark brown=2, white=3	Qualitative
7	Greenness of leaf	Visual estimation	Green=1, dark green=2	Qualitative
8	Leaf pubescence	Visual estimation	Glaberous=1, medium=2	Qualitative
9	Fresh pod colour	Visual estimation	Green=1, dark green=2	Qualitative
10	Dry pod colour	Visual estimation	Brown=0, purple=1, purple + patches=2	Qualitative
11	Pod shape	Visual estimation	Straight=1, curved=2	Qualitative
12	Days to 50% flower	Observed	-	Quantitative
13	Number of branch/plant	Counted	-	Quantitative
14	Length of branch	Measured (cm)	-	Quantitative
15	Number of peduncle per plant	Counted	-	Quantitative
16	Number of pods per peduncle	Counted	-	Quantitative
17	Number of pods per peduncle	Counted	-	Quantitative
18	Pod length	Measured (cm)	-	Quantitative
19	Peduncle length	Measured (cm)	-	Quantitative
20	Days to 95% maturity	Counted	-	Quantitative
21	Height at flowering	Measured (cm)	-	Quantitative
22	Number of seeds per pod	Counted	-	Quantitative
23	100-seed weight	Weighed (g)	-	Quantitative
24	Leaf width	Measured (cm)	-	Quantitative
25	Height at maturity	Measured (cm)	-	Quantitative
26	Pod width	Measured (cm)	-	Quantitative
27	Length of internodes	Measured (cm)	-	Quantitative

**Table 3.** Eigen value and percentage total variation accounted for by the first six principal component axes.

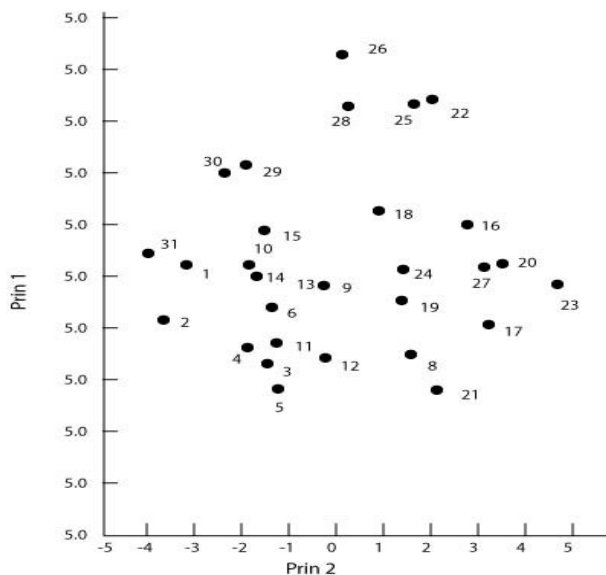
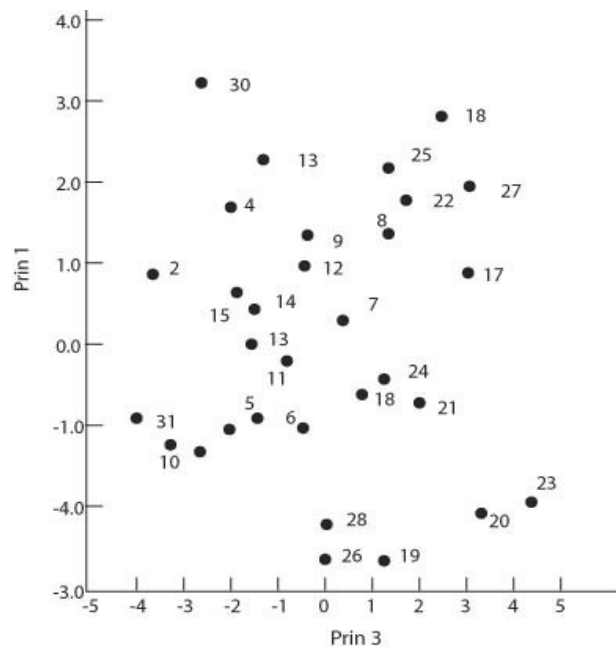
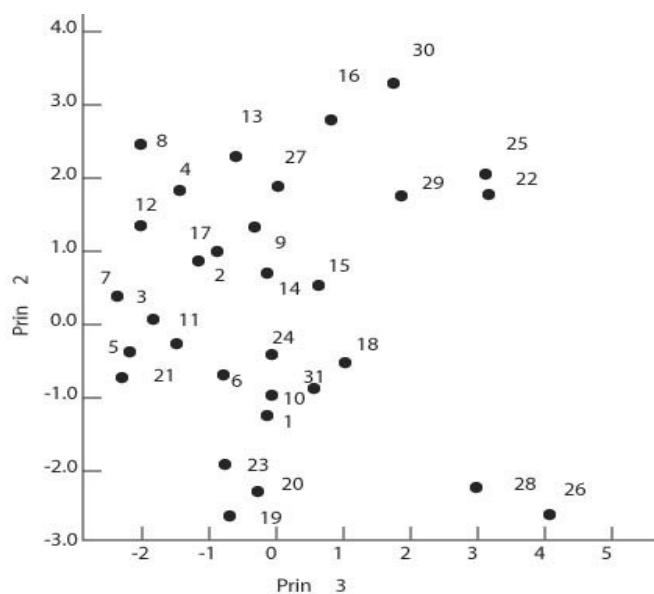
Principal component	Eigen value	Accounted total variation (%)	Cumulative (%)
1	4.73	18.17	18.17
2	3.10	11.93	30.10
3	2.78	10.68	40.78
4	2.28	8.62	49.39
5	2.05	7.89	57.28
6	1.64	6.33	63.62

of the total variations were retained (Table 3). The first three principal axes accounted for 40.78% of the variation among the 27 characters describing the accessions. The major characters describing the first three principal components and their respective scores are presented in Table 4. The first axis revealed only vegetative characters of flowering and heights, number of branches and peduncles as most important characters. The second axis included pod and seed characters while the third axis included reproductive phase of flowering and matu-

riety periods. From the plot of the accessions, principal axis 1 and 2 showed that TVU 13096 (26) from Senegal, (Figure 1) was most distant from the other accessions. Figure 2, graphing axes 2 and 3, illustrated TVU 14345 (22), TVU 13240 (28) and TVU 12220 (30) from Senegal and Benin republic to be most distinct. Only TVU 12220 (30) from Ghana was still distant from the other accessions (Figure 3). The accessions were classified into six distinct groups (Table 5), using the FASTCLUS procedure of SAS (2000). Groups I, II and V had three, two

**Table 4.** Scores of major traits of the first principal components used in ordination.

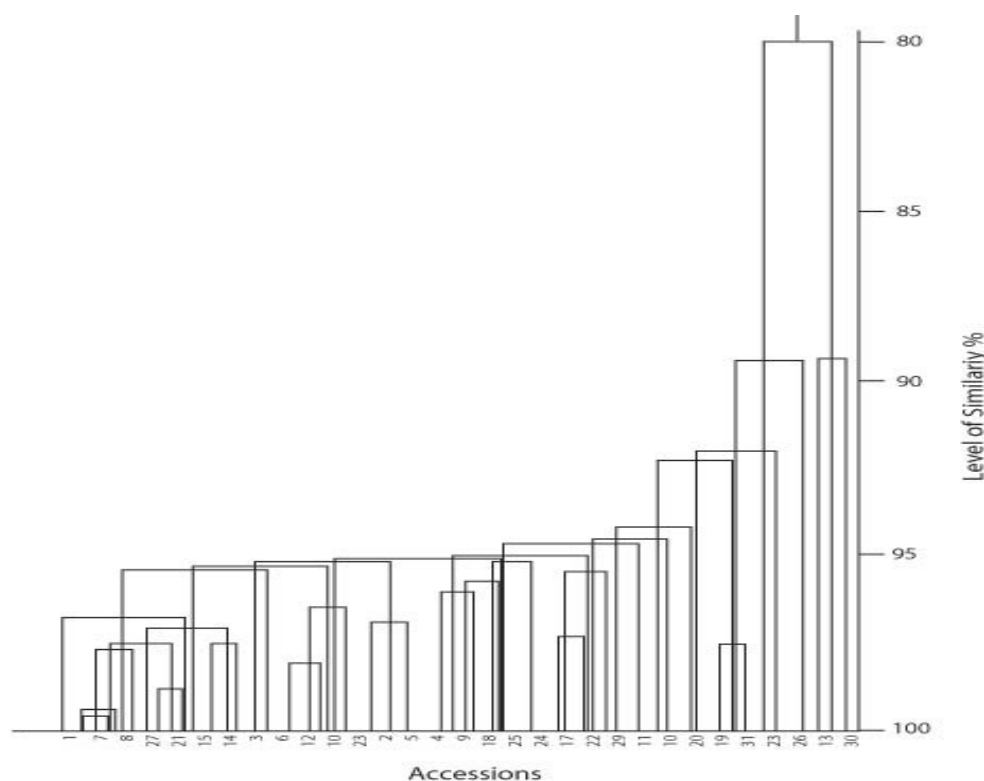
Axis 1		Axis 2		Axis 3	
Character	Score	Character	Score	Character	Score
No. of branches/plant	0.25	Seed Coat colour	0.30	Leaf Pubescence	-0.33
No. of peduncles/Plant	0.25	Number of Seed/pod	0.42	Days to 50% flower	-0.48
Length of Peduncle	0.26	Pod width	-0.26	Length of branch	-0.28
Height at flowering	0.35	Fresh pod colour	0.36	Days to maturity	-0.47
Height of maturity	0.34	Dry pod colour	0.34	Leaf width	0.31

**Figure 1.** Configuration of the cowpea genotypes under principal component axis 1 and 2.**Figure 3.** Configuration of the 31 cowpea genotypes under principal component axis 3 and 1.**Figure 2.** Configuration of the 31 cowpea genotypes under principal component axis 2 and 3.

and one accessions respectively while groups IV and VI had five and eight accessions. Group III had the highest number containing twelve accessions. Accessions in groups I, III, IV and V had reduced number of branches with more number of pods. Even as those in groups II and VI recorded highest number of branches. The number of peduncles carrying pods was same for accessions in groups II and V. Accessions in groups II and III flowered and matured earliest and followed by members in groups I and IV which matured later and same date. The tallest plants were found within the members in group II while the short plants with long branches were within the group I accessions. The dendrogram drawn from the SLCA illustrated the association between the 31 accessions (Figure 4). At the 100% level of similarity, all the 31 accessions were distinct from each other, while at 97.5% level almost half of the 31 accessions were similar to each other. At 90% level of similarity 9 of the accessions had joined to the 15 accessions leaving only

**Table 5.** Mean, Coefficient of Variation (CV) in parenthesis of six clusters with major characteristic patterns of 31 cowpea genotypes by FASTCLUS procedure clusters.

Character	I	II	III	IV	V	VI
Genotype	18,26,28	20,23	1,2,3,9,10,1 2,14,15, 25,29,30, 31	4,5,6,11,1 3	22	7,8,16,17, 19,21,24, 27
Length of branch	217.6 (27)	173 (34)	57 (10)	135 (43)	84 (7)	148 (39)
No of branch	4 (35)	6 (23)	4 (34)	4 (35)	4 (35)	6 (22)
Days 50% flower	44 (15)	42 (16)	47 (14)	45 (15)	53 (13)	52 (13)
Pod length	12 (20)	12 (19)	12 (19)	14 (17)	16 (15)	11 (22)
Weight at maturity	74 (75)	261 (21.4)	69 (80)	66 (83)	186 (30)	112 (49)
No of peduncles	2 (30)	2 (28)	1.9 (35)	1.8 (37)	2 (34)	0.8 (85)
No of pods/plant	48 (39)	29 (66)	47 (41)	23 (81)	35 (55)	21 (90)
Height at flower	4.5 (110)	222 (23)	56 (90)	59 (87)	180 (28)	110 (46)
Internodes length	5 (26)	4 (40)	6 (25)	3 (42)	7 (21)	5 (30)
Days to 95% maturity	65 (10)	62 (11)	67 (10)	65 (11)	68 (10)	72 (9)



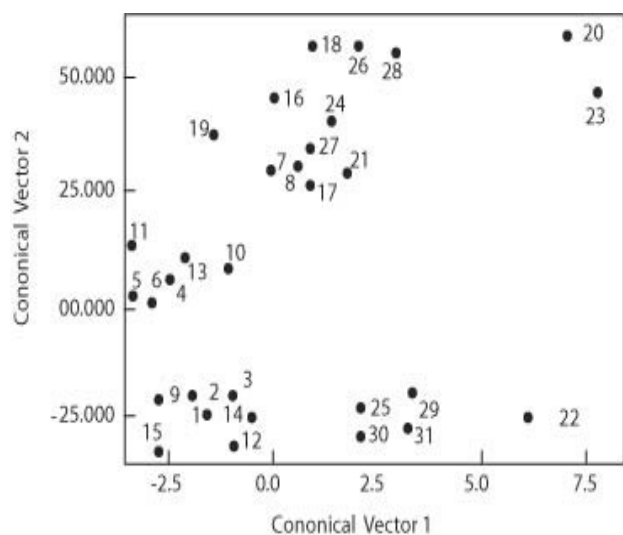
**Figure 4.** Denogram from single linkage cluster analysis (SLCA) of 31 cowpeas.

accessions TVU12201 (31), TVU13096 (23), TVU14345 (26), IT97K – 1034 - 94 (13) and TVU12200 (30) from Senegal, Niger Republic, Nigeria and Ghana respectively. By the 85% level of similarity, all the accessions had joined to form a single cluster, only TVU 12200 (30) could be distinguished from the rest of the population.

The first three canonical variables had eigen values greater than 2.0 and accounted for 58.97% of the total variation (Table 6). Number of seeds per pod was the most important variable in the first canonical axis even as branching trait and maturity period were most important in the second and third axes. Plant height was important

**Table 6.** Eigen values, total variance, cumulative variance and correlation between canonical variables in 27 characters measured on 31 cowpea genotypes.

Canonical variables	Eigen value	Total variance	Cumulative (%)	Correlation of canonical variable with				
				Seed coat texture (0.61)	Seed coat colour (0.54)	Greenness of leaf (0.46)	Length of branch (0.48)	Number of seeds per pod (0.51)
1	4.71	28.32	28.37	Seed eye colour (0.39)	Number branch (0.43)	Length of branch (0.61)	Pod length (-0.31)	Length of peduncle (-0.29)
2	3.12	20.41	48.78	Seed coat colour (-0.55)	Seed eye colour (0.45)	Number of branch (0.36)	Number of peduncles/plant (-0.39)	Number of pods/plant (-0.55)
3	2.07	11.19	58.97	Leaf Pubescence (0.34)	Length of branch (0.42)	Height of flower (-0.37)	Number of seeds (0.28)	Height at maturity (-0.37)
4	1.62	10.35	69.32	Days to 50% flower (0.39)	Pod length (-0.41)	Days to maturity (0.33)	Internode length (0.25)	Leaf width (0.27)
5	1.03	9.02	78.34					



**Figure 5.** Centroids of the 31 accessions along the first and second canonical vectors

in the fourth axis. However, no particular character was particularly important in the fifth canonical axis. The plot of the centroids of the 31 accessions along the first and second canonical vectors is detailed in Figure 5. The two axes captured 48.78% of the total variation. AGRIB VI (accessions 15) and K VX-795-AP (accession 20) each from Nigeria and Burkina Faso were most separated.

## DISCUSSION

The assumption in multivariate analysis is based on the use of genotypes with maximum genetic divergence

(Bhatt, 1970; Amurrio et al., 1995). The clustering pattern of the cowpea accessions did not indicate any relationship between genetic divergence and eco-geographic distribution. Clusters III and V1 contained the most geographically diverse genotypes from Nigerian, Cameroon, Benin Republic, Ghana and Senegal and Burkina Faso. This finding support the work of Dasputa and Das (1984), Ariyo (1991), Everett and Dun (1990) and Tatineni et al. (1996) on black gram, Okro, wheat and cotton, respectively. Growing the accessions in humid savanna revealed members of groups I, II and III to possess earliness to flower and maturity as well as having short branches and still retain high number of pods bearing seeds. This implied that members of these groups could be reliably used as parent stocks for hybridizations when breeding for reduced plant canopy and reduced date to flowering for humid savanna ecology. Cowpea is noted for high disease incidence especially in the humid tropics. However, reduced plant forms would allow enough sunshine penetration to every crop parts and automatically prevent mould growth synonymous with continuous wet crop parts. It may be understood that maturity period, plant height and a seed character on principal axes I, II and III really separated TVU 13096, TVU 13240 and TVU 12220 from other accessions. Furthermore, SLCA having identified accessions in groups I, II III to possess these same characters in addition to having increased number of branches and pods per plant, explains therefore, the suitability in using these characters in conjunction with pod color as criteria for grouping cowpea accessions. Padulosi et al. (1995), identified length and number of branches, date to flowering and maturity as important traits in multivariate studies in cowpea accessions.

By the PCA, maturity period, plant height and seed characters were important in accession grouping while the first three canonical variables identified branching, seed and pod characters as important. Since the canonical correlating values of these important characters are low, it becomes pertinent therefore, to use another technique. Shalini et al. (2003), complimented canonical technique with  $D^2$  statistics of Mahalanobis (1936). By this study, it is suggested that when numerous characters are considered simultaneously, PCA alone would not give an adequate character representation in terms of relative importance and hence the use of canonical or any other technique be complemented with it. Considering PCA and SLCA, it is noteworthy that SLCA is a better technique in multivariate analysis in that it sufficiently provides a clearer and more informative display of the relative positions of the accessions. Similar results have been reported earlier for yam and okra (Onyilagha, 1980; Ariyo, 1990).

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