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Full Length Research Paper

Morphological classification of genetic diversity in cultivated okra, *Abelmoschus esculentus* (L) Moench using principal component analysis (PCA) and single linkage cluster analysis (SLCA)

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29 okra accessions sourced from different agro-ecological regions in Nigeria and grown during the rainy season of 2007 at Abeokuta (derived savannah) were evaluated for genetic diversity using principal component analysis (PCA) and single linkage cluster analysis (SLCA). The experiment was laid out in a randomized complete block design (RCBD) with five replications. The accessions were classified into six and five cluster groups by PCA and SLCA respectively. The mean contributions of plant height, days to flowering, branches per plant, fresh pod width, mature pod width, fresh pod length, pod weight per plant, pod per plant, seeds per pod, and seed weight per plant were relatively high in the principal axes confirming the major contributions of these traits to seed yield in okra. The first four principal axes accounted for over 60% of the total variation among the 18 characters describing the accessions. Accessions 29, 9 and 14, which appears to be the most diverse may be useful as source for variable characters in okra improvement among the accessions studied been the most distant.

Key words: Derived savannah, principal axes, accessions, variation, cluster analysis, okra.

INTRODUCTION

Okra, *Abelmoschus esculentus* [L.] Moench is a dicotyledonae, belonging to the order Malvales, family Malvaceae and genus *Abelmoschus* (syn. *Hibiscus*) (Schippers, 2000). It is an important vegetable crop widely grown in the tropical and subtropical regions of the world (Tindall, 1983; Siemonsma and Kouame, 2004). The plant is a robust, erect, annual herb, ranging between 1 to 2 m in height, with simple leaves, which are alternate and palmately veined. The flowers are regular and solitary, with superior ovaries and numerous stamens. The fruit is a pod, variable in color (when fresh), pubescent or glabrous, 10 to 30 cm long, 2 to 3 cm wide with ridges ranging between 7 and 9.

In West Africa, the plant is cultivated as a vegetable crop and the leaves, buds and flowers are often eaten (Siemonsma and Kouame, 2004). The leaves and fruit produce mucilaginous substance, which makes most African delicacies especially soup, slimy and thick, thereby making consumption of bulky food such as eba, pounded yam, (fufu) etc. easy. The leaves are sometimes used as cattle feed. Fresh okra fruit contains 2.1 g protein, 0.2 g fat, 8 g carbohydrate, 36 calories, 1.7 g fiber, 175.2 mg minerals, 232.7 mg vitamin and 88 ml of water per 100 g of edible portion (Berry et al., 1988). Its edible leaf per 100 g contains about 81 ml water, 56 calories, 11 g carbohydrate and 4.4 g protein. Mature seed of 100 g okra contains 20% edible oil and 20.23% crude protein due to high lysine content and it is a good source of vitamin C (Berry et al., 1988; Siemonsma and Kouame, 2004).

In addition to its usefulness as a vegetable crop, okra

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Abbreviations: NIHORT, National Horticultural Research Institute, Ibadan; **UNAAB,** University of Agriculture, Abeokuta; **BU,** Babcock University Ilishan-Remo; **NACGRAB,** National Centre for Genetic Resources and Biotechnology; **IAR&T,** Institute of Agricultural Research and Training, Ibadan.

fruit is useful medicinally, in curing ulcer and suppressing the pains and effects of haemorrhoid. The mucilage has been used as a plasma replacement or blood volume expander (Siemonsma and Kouame, 2004). Reports from research in China revealed that an alcohol extract from *Abelmoschus* leaves can eliminate oxygen free radicals, alleviate renal tubular-interstitial diseases, improve renal function and reduce proteinuria (Siemonsma and Kouame, 2004). Compared to other fleshy fruit-vegetables (eggplant, and tomato), okra is particularly rich in calcium and ascorbic acid (Siemonsma and Kouame, 2004). *A. esculentus* is a high yielding crop under a good cropping system, with yield varying from 4480 to 5500 kg/ha of green pods (Ayodele, 1993).

Crop improvement through breeding depends on the availability of genetic variability in the crop species and how easily this variability could be fixed in genotypes (Ariyo, 1990a; Kiran Patro and Ravisankar, 2004). Genetic diversity has been reported in the West African and South Asian accessions of okra (Ariyo, 1993; Bisht et al., 1995).

A good understanding of genetic variability in the different characters of okra is a useful tool in the genetic improvement of the crop. This will enhance the identification of useful genes and their behaviour as an aid in hybridization program. There exists a wide genetic diversity among cultivated species of the genus *Abelmoschus* (Bisht et al., 1995, Omohinmin and Osawaru, 2005). These variations are highest amongst species found in countries such as Turkey and India (Bisht et al., 1995). Porter et al. (1974), reported that large morphological variability abound in the tropics suggesting adequate knowledge of the germplasm structure for development of hybrids with specific ecological adaptation. Variability is more prominent in days to flowering, plant height and various fruit characteristics among okra germplasm. Hence, these traits could be important in differentiating varieties of *A. esculentus* (Ariyo and Odulaja, 1991).

The classification of a range of genetic variability among genotypes is pivotal to the maintenance and further acquisition of germplasm resources even as accessions from diverse origins are needed as parents stocks for the development of improved varieties (Aremu et al., 2007). Numerous approaches have been employed to estimate genetic variability in germplasm collections. The coefficient of racial likeness (CRL) proposed by Pearson (1926) and principal component analysis (PCA) proposed by Gower (1966) have been employed to measure or estimate genetic divergence among genotypes. The CRL technique standardizes the difference between pairs of observations by dividing them with the within-variety standard error before combining in Euclidean numerical fashion (Ariyo, 1990b). The canonical technique separates and forms two sets of variables from which highly correlated variables are separated to form a new unit of within and between groups (Lawley and Maxwell,1971). Numerical taxonomic techniques have also been successively used by many workers to classify variation patterns at both intra and interspecific levels (Sneath and Sokal, 1973, Ariyo and Odulaja, 1991). Principal component analysis (PCA) is a descriptive method which shows the pattern of covariation of characters among the individuals (Rhodes and Martins, 1972). It tends to reduce the dimension of multivariate data by removing inter-correlation among variables and allows a multi-dimensional relationship to be plotted on two or three principal axes (Hayman, 1967). The relative discriminating power of the axes and their associated characters are measured by eigen-values and factor scores, respectively. However, PCA alone would not give an adequate character representation in terms of relative importance when numerous characters are considered simultaneously (Shalini et al., 2003). To complement the results of such multivariate analysis, metroglyph analysis and single linkage cluster analysis (SLCA) are often employed to classify the variation. SLCA is an agglomerative technique which shows the pattern of relationship between individuals of a population (Ariyo and Odulaja, 1991). SLCA is generally employed to summarize the position of accessions by sorting them into distinct groups. It is often used to illustrate patterns of covariation of characters among individuals. Thus, this study is aimed at identifying the major characters responsible for variation among okra accessions with a view to grouping accessions and identify potential parental stocks within groups employing the combined technique of PCA and SLCA.

MATERIALS AND METHODS

The 29 okra genotypes that were used for this study were sourced from the exploratory collections deposited in the gene banks of five research institutions in Nigeria, namely University of Agriculture Abeokuta (five genotypes), NIHORT(16 genotypes), Babcock University (four genotypes) NACGRAB (four genotypes) and IAR&T (one genotype) (Table 1). Land preparation was done by ploughing as practiced at the University of Agriculture Abeokuta (Humid Savanna ecology) and seeds were sown using a randomized complete block design with five replications in single row plots. Each row was 8 m long with intra-row spacing of 30 cm and inter-row spacing of 60 cm. Each row contained 25 plants. Planting was done during the early season (June 2006). Three seeds were sown per hole at 1 cm depth and were later thinned down to one plant per stand on establishment. Weeding was done manually at three weeks after planting, while a compound fertilizer NPK 16:16:16 was applied at three weeks interval after sowing to enhance vegetative growth at the recommended rate of 60 kg/ha. Insect pest control was done by spraying Cyperfits (synthetic pyrethrum) at the rate of 80 gai/ha. Harvesting was done when the fruits were fully dried. Data were collected on eight randomly selected plants per accession in each block by visual observation for qualitative traits while data on quantitative traits were scored according to the descriptors for okra (Charrier, 1984) (Table 2).

Data analysis

Data collected on the quantitative characters were analyzed using

Table 1. Accessions and their sources.

S/N	Accession name	Source
1	Lady's Finger	UNAAB
2	OLA KA-1-6-05	NIHORT
3	OLA V1	NIHORT
4	OLA K2005	NIHORT
5	NIHORT Ila-gidi	UNAAB
6	LD88/1-8-11-1	NIHORT
7	LD88/1-8-5-2	NIHORT
8	Short Mouth Ibarapa	UNAAB
9	Clemson spineless	NACGRAB
10	V45-2	NIHORT
11	NH99/DA	NIHORT
12	LD88/1-8-16-2	NIHORT
13	OLA 99/13	NIHORT
14	OSADEP Purple Tall	UNAAB
15	47-4-5	NIHORT
16	ENUGU-1	NACGRAB
17	47-4	NIHORT
18	V2-OYO	UNAAB
19	V-35	IAR&T
20	OLA 3 LOCAL	NIHORT
21	OK 20	NIHORT
22	NH99/28	NIHORT
23	Dajofolowo 1	BU
24	CCN2005/2	BU
25	NH88/1-8-16-2	NIHORT
26	NH88/82	NIHORT
27	NH99/9	NIHORT
28	Jokoso 2	BU
29	CCN2005/1	BU

NIHORT, National Horticultural Research Institute, Ibadan; UNAAB, University of Agriculture, Abeokuta; BU, Babcock University Ilishan-Remo; NACGRAB, National Centre for Genetic Resources and Biotechnology; IAR&T, Institute of Agricultural Research and Training, Ibadan.

SAS Microsoft windows 8.0 (SAS Institute, 1999), employing the method outlined by Steel and Torrie (1980). PCA and SLCA were used for the determination of genetic variation and percentage similarity within accessions. The PCA produced eigen-vectors and factor scores that were used respectively to measure the relative discriminative power of the axes and their associated characters. The FASTCLUS procedure was used to produce distinct groups of the 29 genotypes on the basis of the genetic relationship while using the character variation. SLCA summarized the position of accessions into a dendrogram at intervals of 5% level of similarity, starting from 100 to 65% level of similarity, when all the 29 accessions occurred in a single cluster.

RESULTS

The result of the PCA in early rainy season at Abeokuta showed that four of the 14 principal component axes had eigen values greater than two and altogether accounted for 64.32% of the total variation. The first three accounted 56.11% with PCA 1 accounting for 25.56%, PCA 2 accounting for 17.66% and PCA 3 accounting for 12.90%. The PCA 1 and 2 was loaded with fresh pod width (0.27), mature pod width (0.33) and seed weight per plant (-0.76), whereas PCA 3 was loaded by days to flowering (0.34), ridge per pod (0.30), pod weight per plant (0.36), and seed weight per pod (-0.30). The relative discriminating power of the PCA as revealed by the eigen values was high in PCA 1 (7.67) and lower in PCA 4 (2.46) (Table 3).

The genotypes were classified into six distinct groups (Table 4), using the FASTCLUS procedure. Cluster I had the highest number of accessions which was ten accessions followed by Cluster V with six accessions. Clusters III and VI had two accessions each, while Clusters II and IV had one and three accessions, respectively. Clusters II, III and VI contain the earliest flowering accessions, while the latest in flowering were accessions found in Clusters IV and I. The tallest accessions were found in Cluster IV, followed by Clusters II and I. However, the shortest accessions were found in Clusters III and VI. The highest in number of branches per plant were found in Clusters V and II, whereas cluster VI contain accessions with the least number of branches. The highest mature pod length was found in accessions in Cluster VI, followed by accessions in Cluster IV. The least pod length was found in accessions in Cluster II. The plot of genotypes in axes 1 and 2 (Figure 1) in the early rainy season, also revealed that CCN2005/1 (29), Jokoso (28), V-35 (19), Lady's finger (1), Dajofolowo (23) and Clemson spineless (9), were the most distant and distinct of the other accessions. CCN2005/1 (29) and Clemson spineless (9) were most described by characters in principal axes 1 while Jokoso (28) and V-35 (19) were most described by characters in axes 2.

The dendrogram drawn from the SLCA shows the relationship between the 29 accessions in the early rainy season (Figure 2). All the accessions were distinct from each other at 100% level of similarity and had formed one single cluster at 28% level of similarity. At about 66% level of similarity, Lady's Finger (one) OLA V1 (three), NH88/82 and 47-4-5 (15) had joined with V_2-OYO (18) to form one single cluster. At 60% level of similarity, 19 accessions had already joined to form a cluster. At 26% level of similarity, all the accessions had formed a single cluster except OSADEP Purple tall (14), Clemson spineless (nine) and CCN2005/1(29), which were still distinct from all other accession.

DISCUSSION

The output of the PCA revealed that different characters contributed differently to the total variation. The mean contributions of plant height, days to flowering, branches per plant, fresh pod width, mature pod width, fresh pod

Table 2. List of morphological characters considered.

Character	Estimation
Stem texture	'1'-spiny and '2'- spineless
Stem colour	1'-green and '2'- pigmented
Petiole colour	'1'-green and '2'- pigmented
Fresh pod colour	1'- light green, '2'-green, '3'- dark green, '4'-green and red and '5'-red
Fruit orientation	'3'-horizontal, '5'-slightly upright and '7'- Upright
Pod pubescence	'3'-downy, '5'- slightly rough and '7'- prickly
Leaf vein pigmentation	1'-green and '2'- pigmented
Calyx pigmentation	'1'- green and '2'-pigmented
Peduncle colour	'1'- green and '2'- pigmented
Peduncle texture	'2'- *pubescence and* '1'- glabrous
Seed texture	'1'- smooth and '2'- rough
Seed shape	'1'- round and '2'- reniform
Petal colour	'1'- light yellow and '2'- yellow
Fresh pod length (cm)	Measured from the tip of pod to pedicel attachment seven days after flowering
Fresh pod width (cm)	Measure as circumference of the pod using callipers seven days after flowering
Days to flowering (days)	Determined as average of the number of days to flowering of selected plants
Mature pod length (cm)	Measured from the tip of pod to the pedicel attachment when pods are dry
Mature pod width (cm)	Measure as circumference of mature pod
Number of seeds per ridge (cm)	Determined at maturity by counting seeds per ridge in ten randomly selected pods
Number of seeds per pod	Determined at maturity by counting the total number of seeds in ten randomly selected pods
Number of ridges per pod	Determined by counting the number of ridges in ten randomly selected pods
100-seed weight (g)	Determined by weighing 100 dry seeds as sample from the bulk of each accession
Plant height at maturity(cm)	Measured from the soil level to the tip of the plant at maturity
Plant height at flowering(cm	Measured from the soil level to the tip of the plant at flowering
Plant height at bud initiation (cm)	Measured from the soil level to the tip of the plant at bud initiation
Number of pods per plant	Average number of pods from eight randomly selected competitive plants
Length of peduncle (cm)	Measured from the pedicel to the point of attachment to the axis
Number of fruits per main stem	Determined by counting the average number of fruits on the main stem excluding the branches
Number of branches per plant	3'- weak, '5'- medium, and '7'- strong
Pod weight per plant	Average value of the summation of the weighed mature harvested pods from eight inner plants
Seed weight per plant	Determined by bulking and weighing of the dry seeds as sample from the bulk of each accessions

length, pod weight per plant, pod per plant, seeds per pod, 100 seed weight and seed weight per plant were relatively high in the principal axes. This observation confirmed the individual contributions of these traits to the variations observed in the 29 okra accessions as well as confirm the contributions of these traits to seed yield in the 29 accessions. This agrees with the report of Ariyo and Odulaja (1991) and Ogunbodede (1997). It further suggests that, selections from any cluster group for seed yield must take into consideration these traits. This corroborates the report of Aremu et al. (2007) working with cowpea and Olika et al. (2011) working with coffee.

The first four principal axes accounted for over 60% of the total variation among the 18 characters describing the accessions. Thus, the characters associated with these should be used in differentiating okra accessions (Clifford and Stephenson, 1975). Accessions in Clusters II, with high potentials for high pod weight per plant and seed weight per plant and accessions in Clusters III with high potential for early flowering could be reliably used as parent stocks in breeding for high seed and pod yield, as well as useful when selection favors earliness in flowering especially since earliness is an advantage, where environmental conditions may be unfavorable. The clustering scores among principal components axes suggest that some relationships exist among individuals within a cluster. But SLCA is a better technique in that it sufficiently provides a clearer and more informative display of relative positions of the genotypes (Aliyu and Fawole, 2001; Aremu et al., 2007). It also provides information on the minimum percentage similarity among the accessions.

Furthermore, the dendrogram generated from similarity or genetic distance matrices has provided an overall pattern of variations as well as degree of relatedness among accessions. Genotypes 29, 9 and 14, which appears to be the most diverse may be useful as source for variable characters in okra improvement among the

Table 3. Principal component analysis of the 29 okra accessions at Abeokuta early rainy season showing the factor scores, eigen values and percentage total variance accounted for by the first four principal component axes.

Character	PC axis 1	PC axis 2	PC axis 3	PC axis 4
Days to flowering	0.14	0.02	0.34	-0.14
Plant height at bud initiation	0.27	0.02	0.12	0.30
Plant height at maturity	0.27	-0.01	0.03	0.28
Plant height at flowering	0.28	-0.03	0.10	0.27
Branches per plant	0.26	0.03	-0.08	-0.19
Fresh pod length	-0.30	0.03	-0.05	0.10
Mature pod length	-0.31	0.03	-0.02	0.06
Fresh pod width	0.24	0.27	-0.04	-0.02
Mature pod width	0.19	0.33	-0.05	-0.04
Ridge per pod	0.14	0.21	0.30	-0.22
100 seed weight	0.04	0.24	-0.19	-0.12
Peduncle length	-0.07	-0.14	0.05	0.13
Pod per main stem	0.17	-0.13	-0.23	0.31
Seeds per ridge	0.11	-0.05	-0.21	-0.19
Seeds per pod	0.19	0.05	0.04	-0.39
Pod per plant	0.23	-0.15	-0.28	0.09
Pod weight per plant	0.21	-0.02	-0.36	0.04
Seed weight per plant	0.25	-0.76	-0.30	0.01
Eigen-value	7.67	5.30	3.87	2.46
% Variance	25.56	17.66	12.90	8.20
Cumulative percentage variance	25.56	43.21	56.11	64.32

Table 4. Mean, coefficient of variation (cv) (in parenthesis) of the six clusters with major characteristic patterns of the 29 accessions of okra by FASTCLUS cluster procedure at Abeokuta rainy season.

Character	I (11, 13, 17, 20, 23, 25, 28, 6, 7)	II (14)	III (10, 16)	IV (2, 22, 4)	V (1, 15, 18, 26, 3, 5)	VI (29, 9)
Days to flowering	30.92(3.4)	25.20(0)	24.80(4.8)	31.00(4.9)	29.13(3.3)	25.60(0.0)
Plant height at bud initiation	48.08(6.2)	55.76(0)	23.59(1.7)	53.79(5.3)	34.53(1.8)	21.28(5.6)
Plant height at maturity	136.49(11.9)	154.63(0)	61.54(4.3)	170.53(9.3)	104.19(10.0)	50.83(20.5)
Plant height at flowering	75.18(6.8)	91.97(0)	39.38(4.0)	89.57(4.1)	57.48(4.0)	33.21(12.8)
Branches per plant	3.04(0.12)	3.40(0)	3.20(0.3)	2.73(0.6)	3.63(0.5)	0.90(1.3)
Fresh pod length	7.05(0.4)	7.41(0)	8.44(0.8)	8.62(1.7)	7.71(1.0)	12.84(4.9)
Mature pod length	8.71(0.5)	8.63(0)	10.68(0.6)	11.18(2.1)	9.98(1.0)	16.98(6.7)
Fresh pod width	2.98(0.2)	2.97(0)	2.67(0.2)	2.92(0.01)	3.01(0.2)	2.27(0.02)
Mature pod width	3.70(0.31)	3.90(0)	3.51(0.3)	3.65(0.3)	3.82(0.2)	2.98(0.08)
Ridge per pod	8.04(0.31)	6.80(0)	7.50(0.4)	8.07(0.12)	7.93(0.24)	7.60(0.28)
100 seed weight	5.59(0.41)	5.91(0)	5.85(0.54)	5.75(0.20)	6.24(0.3)	5.06(1.07)
Peduncle length	3.00(0.39)	2.65(0)	3.07(0.32)	3.52(0.18)	3.59(0.81)	3.37(0.75)
Pod per main stem	4.78(1.0)	6.40(0)	4.20(0.28)	4.60(0.69)	4.50(0.91)	2.80(1.13)
Seeds per ridge	13.98(0.9)	14.40(0)	14.60(0.85)	13.07(0.81)	13.57(0.9)	12.60(0.6)
Seeds per pod	107.18(5.1)	81.60(0)	104.50(2.12)	102.47(4.2)	102.03(9.0)	87.30(5.2)
Pod per plant	6.52(1.19)	9.00(0)	6.70(0.4)	5.33(1.21)	6.20(0.6)	2.90(1.3)
Pod weight per plant	59.54(8.9)	87.22(0)	67.77(3.28)	50.52(10.4)	63.35(4.75)	22.34(1.4)
Seed weight per plant	35.50(5.89)	48.10(0)	36.97(1.54)	28.07(5.9)	33.70(4.8)	10.91(2.28)

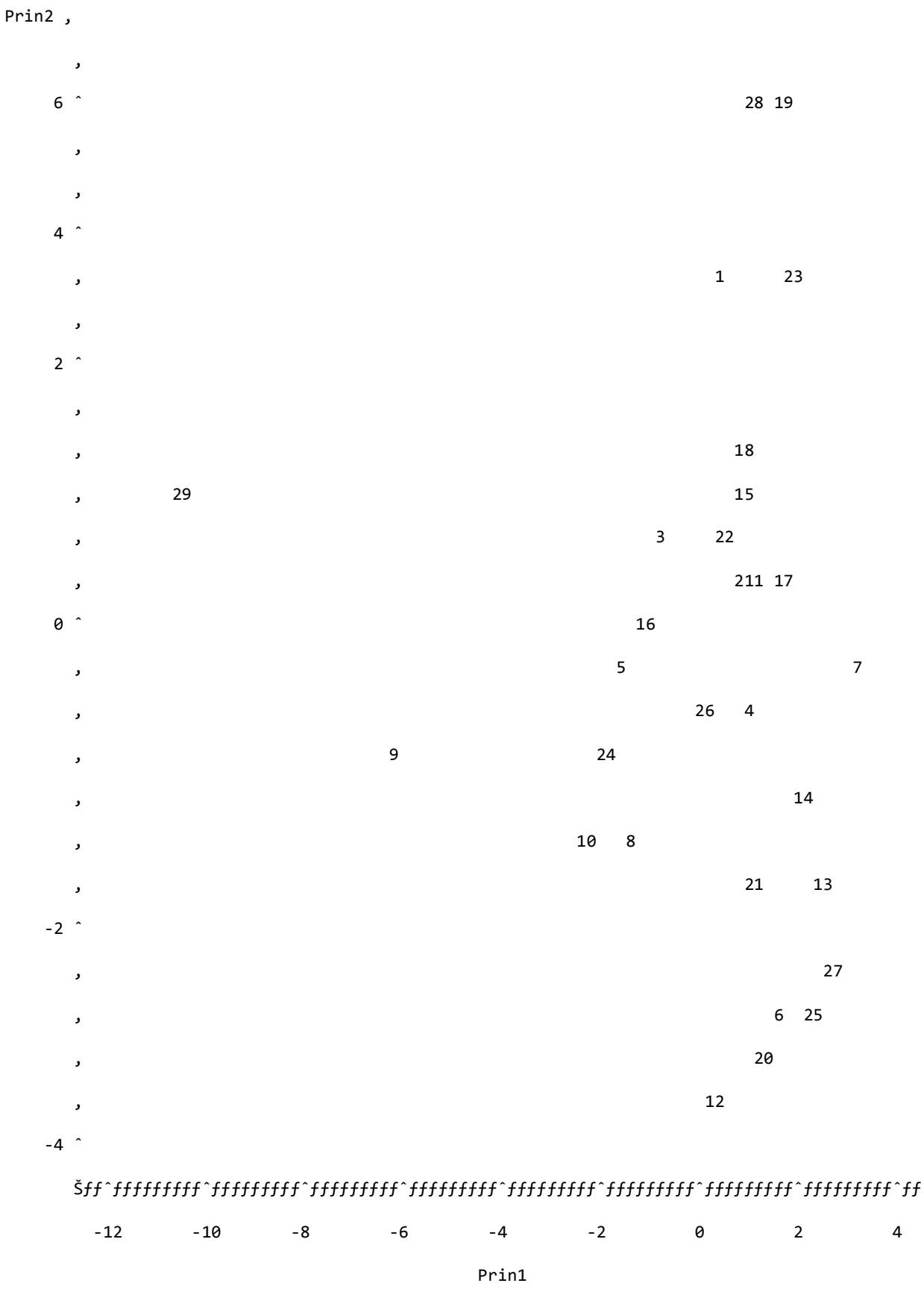

Figure 1. Configuration of the 29 okra genotypes under principal component axes 1.

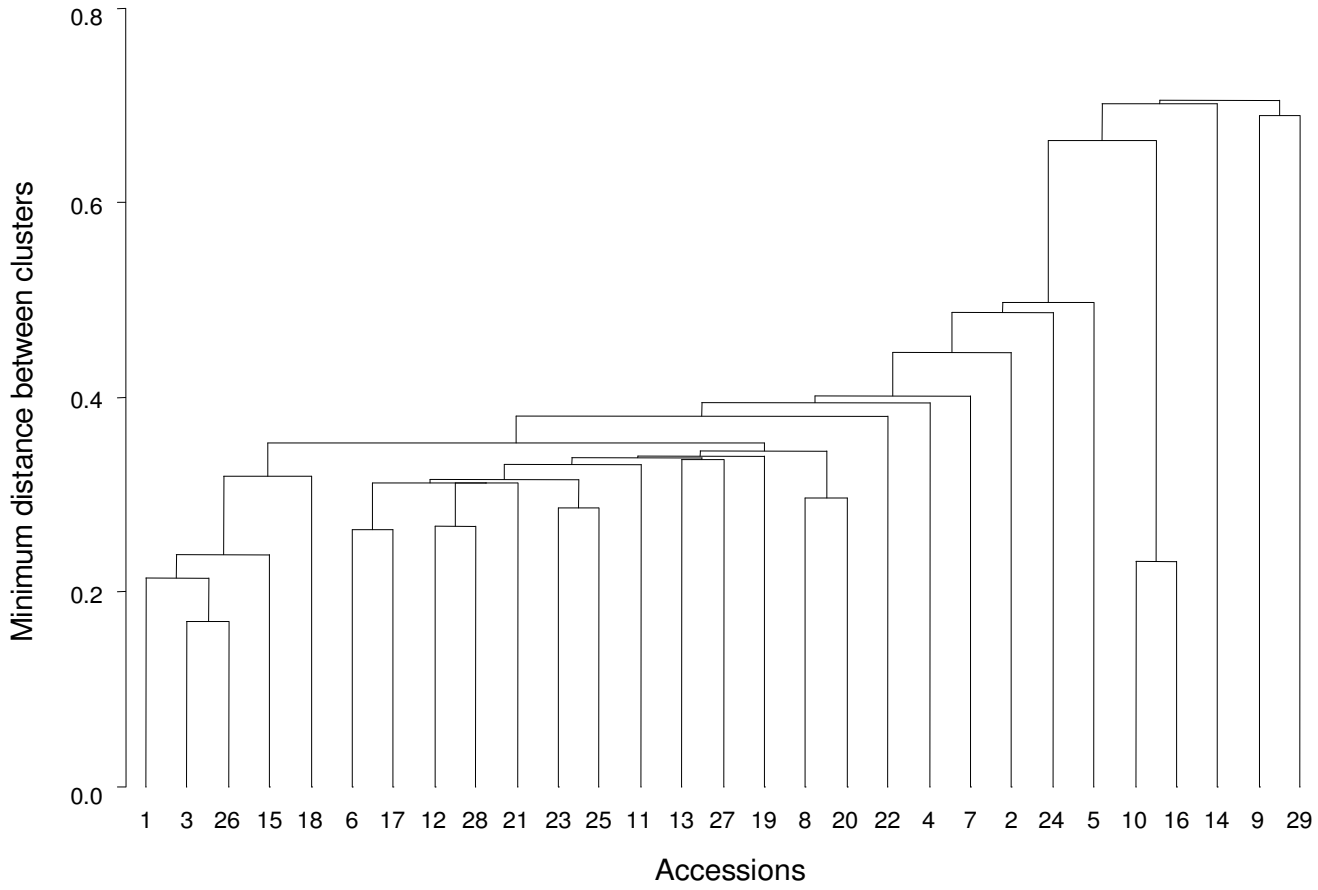

Figure 2. Dendrogram SLCA of the 29 okra accessions in the early rainy season Abeokuta.

genotypes studied been the most distant. From this study, a combination of PCA and SCLA will produce better results when considering genetic variability among okra accession. This agrees with the finding of Aremu et al. (2007). However, SCLA proved to be a better tool in multivariate analysis since it provided much clearing information concerning the extent of relationship among the genotypes. This also agrees with earlier reports for cowpea and yam (Onyilagha, 1980; Aremu et al., 2007).

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