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Multivariate analysis of seed yield and morphological characters of Okra (*Abelmoschus esculentus* L. Moench) accessions

M.A. Murtadha*, S. Adetoro, K.A. Shittu

Department of Agronomy, Faculty of Agricultural Production and Management, College of Agriculture, Osun State University, Ejigbo, Osun State, Nigeria.

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Abstract. *Enhancement of okra fruit production requires identification of genotypes with promising seed yield attributes, thus multivariate techniques were employed to reveal genetic and morphological attributes of sixteen diverse okra lines during wet and dry seasons in 2019 at Teaching and Research Farm, College of Agriculture, Osun State University, Ejigbo Campus. Seeds were sown in single-row plots of 5 m long, spaced 0.70 m apart and 0.50 m within on sandy loam soil in a randomized complete block design. The dry season crop was supported by the application of 12 mm water weekly. Data collected on growth and seed traits were subjected to the General Linear Model (GLM), principal component analysis (PCA), and cluster analysis using the Statistical Analysis System (SAS, 2018). The petiole color was scored according to IPGRI (1991) square root transformed prior to the analysis. Results showed highly significant lines, season, and their interactions for almost all traits. Four PCAs accounted for 85.77% and the first two PCA captured 51% of the total variations. Both PCA and cluster analysis grouped the lines into four and revealed the potentials of SAHARI F1, NGB01197, and LD-88 for high seed yield. It is concluded that these lines can be incorporated into okra yield improvement program.*

Keywords: Okra fruits, cluster analysis, dendrogram, principal components, vegetable

Introduction

The global competitiveness of the okra crop among prominent fruit vegetables relies on the ability of farmers to grow the crop all year round using high yielding seeds. Such farming practice will enhance the financial contribution of okra (*Abelmoschus esculentus*) fresh fruit to the livelihood of the farming community in rural areas of almost all developing countries. Farmers, particularly in the tropics, respond to prevailing rainfall patterns and intensities, by extensive production of the fruit during wet seasons purposely to maximise its financial return (Farinde et al., 2009). However, in the dry season, obtainable fruit yield is limited to a few cultivable areas where the soil water level is relatively high or irrigation facility is adequate. Low income farmers are handicapped and usually under the mercy of the erratic nature of dry season raindrops. During the dry season, water evaporates heavily from the soil surface thus turning the once productive land into less productive. The absence of irrigation facilities in farmers' farms hinders the successful production of okra fruits in dry periods consequently leading to low supply, scarcity and expensive fruits (Katung, 2007; Agbugba, 2013).

Despite this obstacle, incessant production of okra throughout the year emanates from farmers‘ awareness of high nutritive value of its raw or cooked fruits (dos Santos et al., 2013), medicinal and economic values of all plant organs (Al-Kanani et al., 2019), wide adaptability, export

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*e-mail: mosobalaje.murtadha@uniosun.edu.ng potential and bountiful return (Thirupathi et al., 2012). The statistics showed that in 2018 (Faostat, 2020), Nigeria, with over two million tons of fresh pod, came after India, as the second-largest producer of okra in the world. Despite this second position of the nation in okra fruit production, the gap between the leading countries and Nigeria is wide due to lack of improved seeds among other factors which if properly addressed can support okra fruit yield across all seasons (Ibitoye and Kolawole, 2022).

A series of efforts were made to improve okra fruit yield for dry season (Sugri et al., 2015; Alake, 2019; Maldonado-Peralta et al., 2021). It was agreed that okra fruit yield fluctuates seasonally due to the influence of genotype × season interaction limiting the potential for improvement. Seed improvement carried out in a number of ways, is highly dependent on the amount of genetic variability available in the gene pool of a seed. To improve okra seed successfully, it is essential to assemble, characterize and evaluate genetic resources of okra in order to maximize their utilization in the improvement program. Since the development of improved cultivars depends on genetic diversity among the available materials, such preliminary efforts will enhance rapid utilization of heterosis phenomenon or identification of high yielding cultivars through genetic recombination (Koundinya et al*.,* 2013). Phenotypic characterization of Okra cultivars to aid the selection strategies that could be used for its improvement had been employed (dos Santos et al., 2013; Mihretu et al., 2014; Eshiet and Brisibe, 2015; Maciel et al., 2018; Mohammed et al., 2022).

Principal Component Analysis (PCA) is a prominent multivariate tool (Alake, 2019) followed by Discriminant Canonical Analysis (DCA) and Cluster Analysis (CA) methods. PCA, among others as prescribed by Hair et al. (2009) and reviewed by Jolliffe and Cadima (2016), gained importance due to its robustness in determining the multivariate response of treatments by transforming a set of interrelated variables into a set of uncorrelated variables while the newly formed variables (PCs) are linear combinations of the original variables. This tool can specify the most influential variables on system variability and identify plant characters that contribute most to the variation within a group of entries. Usually, among the formed PCs, only the first three PCs are retained particularly when the subsequent eigenvalues were less than one (Ahiakpa et al., 2014; Mihretu et al., 2014; Amoatey et al., 2015; Jolliffe and Cadima 2016;). In a separate investigation, Kyriakopoulou et al. (2014) and Alake (2019) concluded that diversity among okra landraces as provided by these tools can be used as sources of genetic variation to improve agro-morpho-nutritional traits and broaden the gene pool.

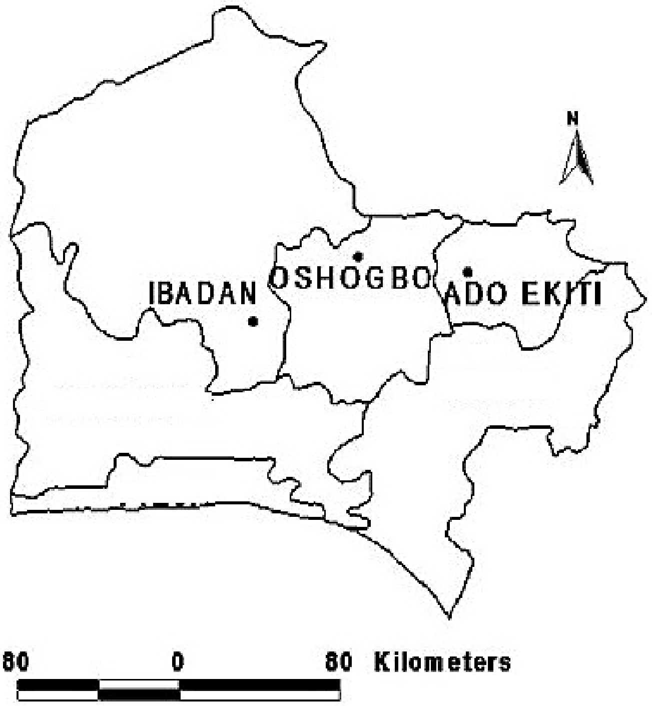
Nevertheless, PCA carries information about the patterns of variations in individual variables and the relationships between the variables (Qi and Luo, 2014), but does not adequately display the importance of each character relative to each other in a simultaneous analysis (Shalini et al*.,* 2003). PCA generally plays a complementary role with cluster analysis in genetic diversity analysis among genetic resource collections in the identification and classification of accessions for specific breeding purposes of okra (Kyriakopoulou et al., 2014). Cluster analysis often extends to genotype grouping to cluster entries that show similarity in one or more characters and thus guide the choice of genotypes for crop improvement (Singh et al., 2018). The cluster technique is therefore employed to reveal the pattern of relationship between genotypes within a population and also classify the genotypes. Genotypes within a cluster are homogenous and differ from genotypes in other clusters. In other words, homogeneity and heterogeneity are maximized between them, thus within-cluster genotypes have the same genetic constitution, i.e. homogeneous are less divergent than those that occurred in a different cluster. Hybridization is usually performed among genotypes belonging to inter clusters to obtain transgressive segregants with a broad spectrum of genetic variability for traits of interest to a researcher. Alternatively, Labroo et al. (2021) proposed the use of genomic selection to choose founders in heterotic pools when costs are limited. These heterotic pools can further be used in hybrid crop improvement.

Several methods to measure distance among clusters are reportedly available and vary according to how closest is defined at each stage of merging groups. Andersson et al. (2007) stressed that for any two or more accessions to be considered genetically identical, their genetic similarity index (GS) should be equal to or greater than 95%. The broad genetic similarity indices and clustering patterns of lines suggest useful variability within the collection for future genetic improvement of the crop through a direct selection of accessions with the desired characteristics or hybridization using genetically divergent ones as parents (Torkpor et al., 2006; Ahiakpa et al., 2014). In this context, this study was designed to estimate the genetic diversity, relationship and yield potential of the selected unclassified okra lines for possible inclusion into okra seed yield improvement program.

Material and methods

Plant material and Experimental site description

Sixteen (16) okra lines sourced from National Genetic Resources and Biotechnology (NAGRAB), seed markets, and farmers' own seeds were evaluated from April to July, and October to December 2019 wet and dry farming seasons, respectively, at the Teaching and Research farm of Osun State University, Ejigbo, Osun State, Nigeria (Latitude 7°, 52'28.37" N and Longitude 4°, 18'13.76" E 426 m altitude) 29ºC. Names and sources of the materials were listed in Table 1, while the geographical map of the site is presented on Figure 1.



Key: • State capitals

Figure 1. Location of farmers' own seeds (Aderogba et al., 2012)

Table 1. Name and source of okra lines used in the study

S/N	Line Codes	Sources
1.	NGB 01197	NACGRAB, Ibadan, Oyo State
2.	SAHRI_F1	Seed shop, Ibadan, Oyo State
3.	U/EJ/AO2	Farmers own seed, Ejigbo, Osun State
4.	U/EJ/AO3	Farmers own seed, Orisunbare, Osogbo, Osun State
5.	U/EJ/AO4	Farmers own seed, Ejigbo, Osun State
6.	U/EJ/AO5	Farmers own seed, Oko, Oyo State
7.	U/EJ/AO6	Farmers own seed, Ibadan, Oyo State
8.	U/EJ/AO7	Farmers own seed, Ikole-Ekiti, Ekiti State
9.	C/AM/OO2	Farmers own seed, Eleyele, Ibadan, Oyo State
10.	C/AM/OO3	Farmers own seed, Ido- Ekiti, Ekiti State
11.	C/AM/OO4	Farmers own seed, Ado- Ekiti, Ekiti State
12.	C/AM/OO5	Farmers own seed, Oja-Oba, Osogbo, Osun State
13.	C/AM/OO6	Farmers own seed, Oye- Ekiti, Ekiti State
14.	47-4	NIHORT, Ibadan, Oyo State
15.	Clemson Spineless (C/Spine)	Exotic seed shop, Ibadan, Oyo State
16.	LD_88	NIHORT, Ibadan, Oyo State

The soil had the physical and chemical properties listed. The sandy loam soil of the experiment station had chemical properties pH (H_2O) = 6.40, Org.Carbon %= 0.52, total N %= 0.041, available P (mg/kg)= 0.11, Na^+(C mol/kg)= 0.18, K^+(C mol/kg)= 1.22, Ca^{2+} (C mol/kg)= 2.00 and Mg_2+(C mol/kg)= 0.09. It also had as physical components, bulk density (cm^3) = 1.20, Silt %= 25.50, Clay %= 15.00 and Sand %= 60.00. The climatological data are presented in Table 2. Three seeds of each okra line were dibbled into the soil using single-row plots of 5 m long, spaced at 0.70 m apart with 0.50 m within the row. The experiment was conducted using a Randomized Complete Block design with three blocks.

Table 2. Climatological data of the area from January to December 2019

Month	Average Rainfall (mm)	Average Temperature ºC	Relative Humidity %
January	0.98	33.39	51.23
February	2.50	32.71	59.21
March	1.77	31.95	71.29
April	3.60	31.87	75.47
May	8.86	29.58	83.29
June	13.11	26.83	90.30
July	10.58	25.43	89.19
August	8.63	25.33	92.23
September	8.92	25.55	92.33
October	13.88	25.90	91.37
November	4.22	29.28	82.40
December	0.15	32.32	60.84

C = Celsius degree

Source: https://tcktcktck.org/nigeria/osun/ejigbo/january-2019

Crop management and Studied traits

Two weeks after emergence, the okra plants were thinned to one stand. Weeds were removed regularly by hand. The dry season crop was irrigated throughout the growing season with a watering can, which applied 12 mm of water per plant per week. A total of 150 kg.ha^{-1} of NPK (15-15-15) was applied in three doses. Insect pest was controlled as recommended by the Teaching and Research farm. Data were collected on vegetative and reproductive characters such as emergence (number of days from sowing to 50% seedling emergence), flowering (number of days from sowing to 50% full bloom flowering), petiole color (scored immediately before flower opened on 50% of the plants (scored according to IPGRI, 1991), plant height (measured in centimeter from the soil level to the tip of the plant at maturity), branch plant^{-1} number, weight of 100 seeds (g) and yield (seed t.ha^{-1}).

Statistical analyses

Data were analyzed using the General Linear Model (GLM) of Statistical Analysis Software (SAS, 2018) with a two-way analysis of variance to assess the interactions. The two seasons data collected on seven morphological traits were combined to obtain analysis of variance (ANOVA) and revealed the effects of blocks, lines, seasons and interaction of lines and seasons. Means of all lines were compared using least significant difference (LSD). The petiole color scores were subjected to square root transformation before the analysis. PRINCOMP (SAS, 2018) was employed to identify the contribution and importance of each group of traits to the observed variance and was further used in determining the correlation between each trait and PC scores, thus traits that did not significantly associate with PC scores were unimportant. The cluster analysis was performed by the Cluster procedure of SAS. The standardized data for the variables were analyzed by the Unweighted Paired Group Method using Arithmetic averages (UPGMA) (Panchen, 1992). In this method, each line was initially considered as a separate cluster, and new clusters were formed using the smallest distance between a pair of lines thereby leading to the formation of a dendrogram.

Results and discussion

Highly significant lines, seasons and their interaction mean sum of squares was obtained for almost all traits (Table 3). However, branch plant^{-1} and seed yield t.ha^{-1} had insignificant lines x season interaction. The lines characterized with highly significant seed yield (t plant^{-1}) and early maturity: SAHRI_F1, 47-4, and U/EJ/A07 could be utilized for a breeding program aiming at increasing seed yield and early maturity. The lines x season interaction revealed a significant increase in the performance of okra in dry season over wet season in all morphological traits except seed yield which was insensitive to changes in growth season (Figure 2).

Table 3. Growth and yield performance of okra (*Abelmoschus esculentus* (L.) Moench) lines grown in wet and dry seasons

Lines	DTE	DM	PH (cm)	NB plant^{-1}	100-SW (gm)	PC	SY (t ha^{-1})
47-4	6.31^{a}	95.92^{bc}	37.35^{de}	1.69^{c}	6.88^{a}	1.42^{bc}	27.15^{ab}
C/Spine	6.00^{ab}	99.50^{abc}	33.07^{e}	4.00^{a}	5.30^{d}	1.00^{c}	19.46^{ab}
C/AM/002	6.00^{ab}	104.00^{a}	46.45^{cde}	2.71^{bc}	6.75^{ab}	1.67^{b}	20.32^{ab}
C/AM/003	5.83^{ab}	103.00^{ab}	41.85^{cde}	2.50^{bc}	6.55^{abc}	1.33^{bc}	28.87^{ab}
C/AM/004	5.50^{b}	98.67^{abc}	46.8^{cd}	2.79^{bc}	6.66^{ab}	1.00^{c}	20.52^{ab}
C/AM/005	6.33^{a}	98.17^{abc}	49.67^{cd}	2.63^{bc}	7.27^{a}	1.50^{bc}	17.71^{b}
C/AM/006	5.50^{b}	99.33^{abc}	46.21^{cde}	2.83^{bc}	6.25^{abcd}	1.00^{c}	21.36^{ab}
LD_88	6.00^{ab}	97.33^{abc}	34.7^{de}	2.67^{bc}	5.40^{cd}	1.67^{b}	24.83^{ab}
NG_01197	5.67^{b}	101.00^{abc}	110.15^{a}	2.33^{bc}	6.78^{ab}	2.17^{a}	20.68^{ab}
SAHRI_F1	5.50^{b}	96.50^{bc}	87.51^{b}	2.17^{bc}	5.61^{bcd}	1.50^{bc}	32.49^{a}
U/EJ/A02	5.50^{b}	95.17^{c}	52.75^{c}	1.67^{c}	6.45^{abcd}	1.17^{bc}	21.88^{ab}
U/EJ/A03	5.83^{ab}	96.17^{bc}	55.09^{c}	3.00^{ab}	6.21^{abcd}	1.17^{bc}	20.31^{ab}
U/EJ/A04	5.50^{b}	95.50^{c}	48.56^{cde}	2.79^{bc}	7.11^{a}	1.50^{bc}	22.32^{ab}
U/EJ/A05	5.67^{b}	95.00^{c}	50.4^{cd}	2.58^{bc}	6.54^{abc}	1.00^{c}	18.57^{b}
U/EJ/A06	5.50^{b}	95.50^{c}	43.7^{cde}	2.00^{bc}	7.03^{a}	1.00^{c}	19.22^{ab}
U/EJ/A07	5.83^{ab}	94.50^{c}	56.76^{c}	2.54^{bc}	6.72^{ab}	1.33^{bc}	24.38^{ab}
Means	5.78	97.83	52.56	2.56	6.47	1.34	22.50
S.E	0.06	0.56	03.32	0.11	0.10	0.05	0.93
Lines	**	**	**	**	**	**	*
Seasons	**	**	**	**	**	**	*
Lines x season	**	*	**	NS	*	**	NS

NS, Not significant; *,**, significant at 0.05 and 0.01 levels of probability, respectively.
DTE= Days to emergence, DM= Days to 50% maturity, PH= Plant height in centimeter, NBr= Number of branch per plant, SW= 100-seed weight in gram, PtC= Petiole color, and Seedtha= Seed yield ton ha^{-1}. S.E= Standard error of means. Means within columns followed by the same letter are not significantly different, $p < 0.05$.

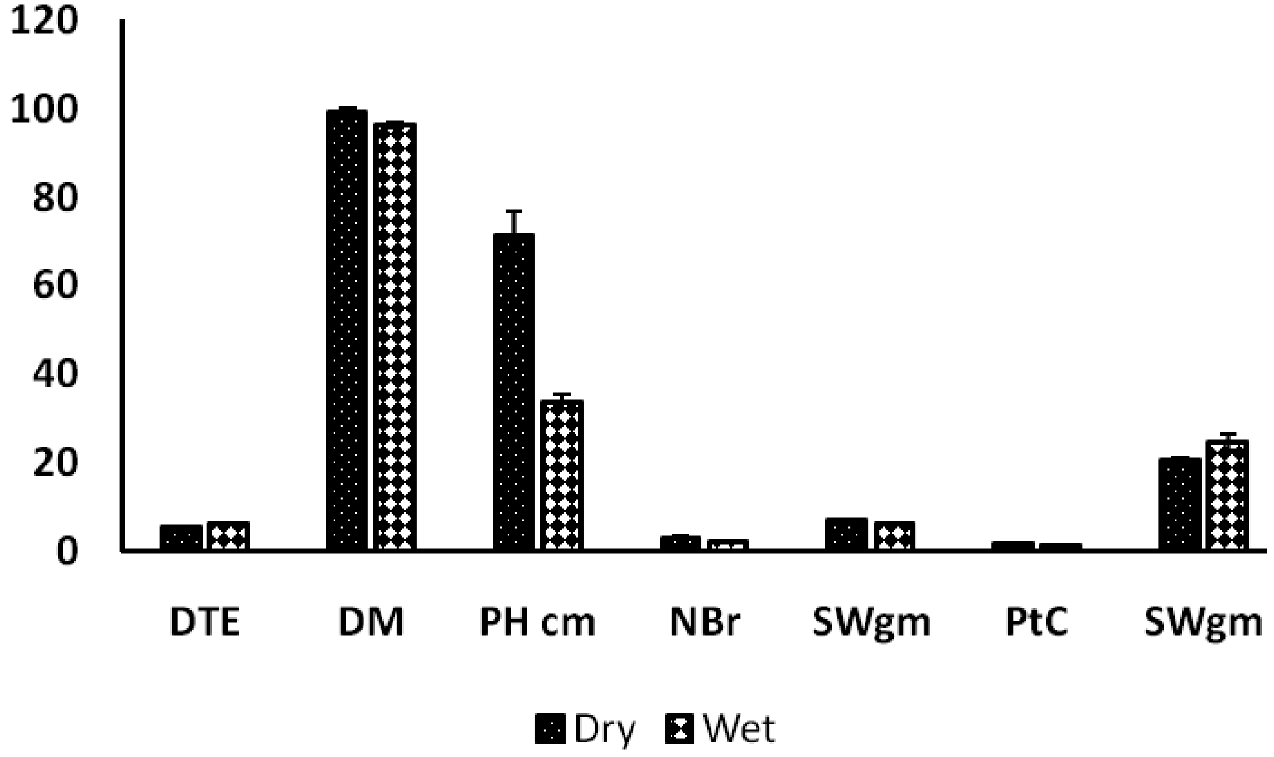


Figure 2. Seasonal variability in the growth and seed yield of sixteen okra lines
Key: DTE= Days to emergence, DM= Days to 50% maturity, PH= Plant height in centimeter, NBr= Number of branch per plant, SW= 100-seed weight in gram, PtC= Petiole color, and Seed ttha= Seed yield t.ha^{-1}. S.E= Standard error of means.

Presence of large genotypic variation among the okra lines employed as revealed by the observed significant mean sum of squares for all traits indicated a great diversity among the materials. Such diversity which is the first requirement for studying and grouping the lines can be considered satisfactory. Morphological variation among okra was reported (Alake, 2019; Massucato et al., 2020; Mohammed et al., 2022) as a prerequisite for selection among parents for hybridization for okra improvement.

In the PCs from correlation matrix, the cut-off value used in identifying traits that significantly contributed to the PC axis had eigenvectors equal to or greater than 1 (Table 4). The first four PCs displayed values above the cut-off and accounted for 85.77%: (PC 1 (28.30%), PC 2 (22.81%), PC 3 (19.22%) and PC 4 (15.44%)) of all phenotypic variations among the lines. These PCs were thus employed in matching these lines by comparing the spatial distribution of the traits on the PCs. On PCI, okra plant height (0.561) and petiole color (0.531) had positive loadings, while the variation on PC2, PC3 and PC4 was mainly attributed to days to maturity (0.632), 100-seed weight (0.740) and seed emergence (0.601), respectively.

The lines and traits were plotted on a biplot to display their associations and differences using the first two PCAs which explained 51% of multivariate variations among the lines (Figure 3).

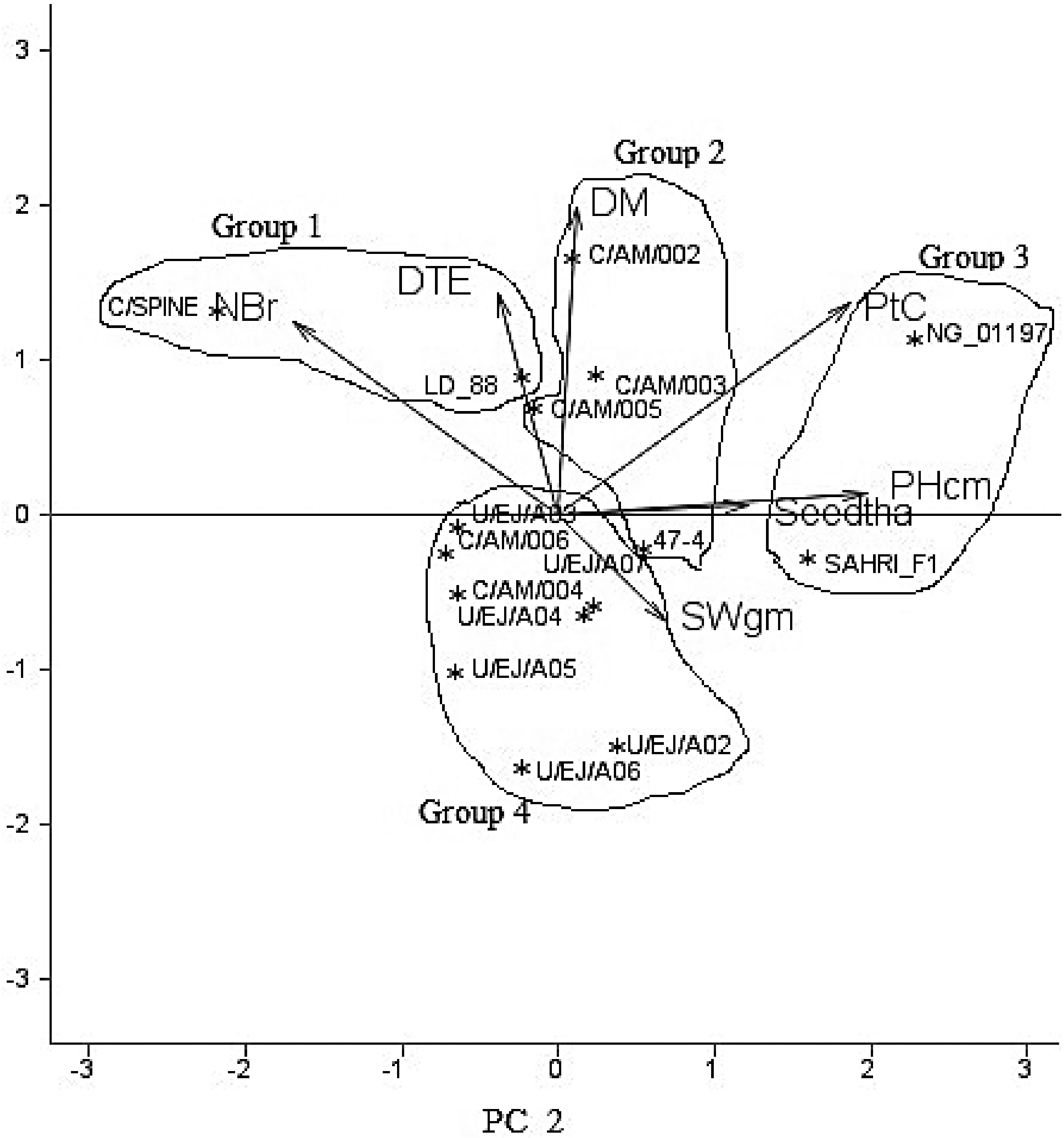


Figure 3. Bi-plot of PC 1 and PC 2 scores of okra lines evaluated for growth and seed yield in wet and dry seasons DTE= Days to emergence, DM= Days to 50% maturity, PH= Plant height in centimeter, NBr= Number of branch per plant, SW= 100-seed weight in gram, PtC= Petiole color, and Seed ttha= Seed yield t.ha^{-1}. S.E= Standard error of means.

The traits loaded on the two axes were also used to group the lines into closely relative members. These characters: days to emergence, days to maturity, petiole color and plant height are the most discriminating traits (Figure 3). The scores of the two PCs divided the lines into four distinct groups. The first group is made up of two lines, namely: LD_88 and Clemson spineless both were sourced from Ibadan and characterized by long days to germination and shared a high number of branch plant^{-1} in common though not a discriminating character. The second group consisted of 47-4 and C/AM/002, both from Ibadan, C/AM/003 (Ado Ekiti), C/AM/005 (Osogbo), and characterized by the longest period of maturity. The third group contained two lines, which are NG_01197 and SAHRI_F1 sourced from Ibadan and had conspicuous pigmentation and associated with high seed yield (t.ha^{-1}) which was not a discriminating character, either. The fourth group consisted of eight lines, namely: C/AM/004, C/AM/006, U/EJ/A02, U/EJ/A03, U/EJ/A04, U/EJ/A05, U/EJ/A06, and U/EJ/A07. These lines shared, in common, short days to germination, maturity, relatively dwarf plant and stem green color.

Clustering the lines based on their similarity for the seven quantitative characters produced four major clusters at a normalized distance of 0.4 (Figure 4). The four clusters derived from Ward's minimum variance cluster analysis showed remarkable correspondence with the PCA. The deep focus on the clusters showed that Cluster 1 had four lines corresponding to the members of Group 2 in the PCA, namely: 47-4, C/AM/005, C/AM/002 and C/AM/003. The members of Cluster 2 were similar to the members contained in group 1 of PCA which were Clemson spineless and LD_88. Cluster 3, further, consisted of members that have characteristics of group 4 in the PCA. The fourth cluster had two lines which are also members of group 3 in the PCA.

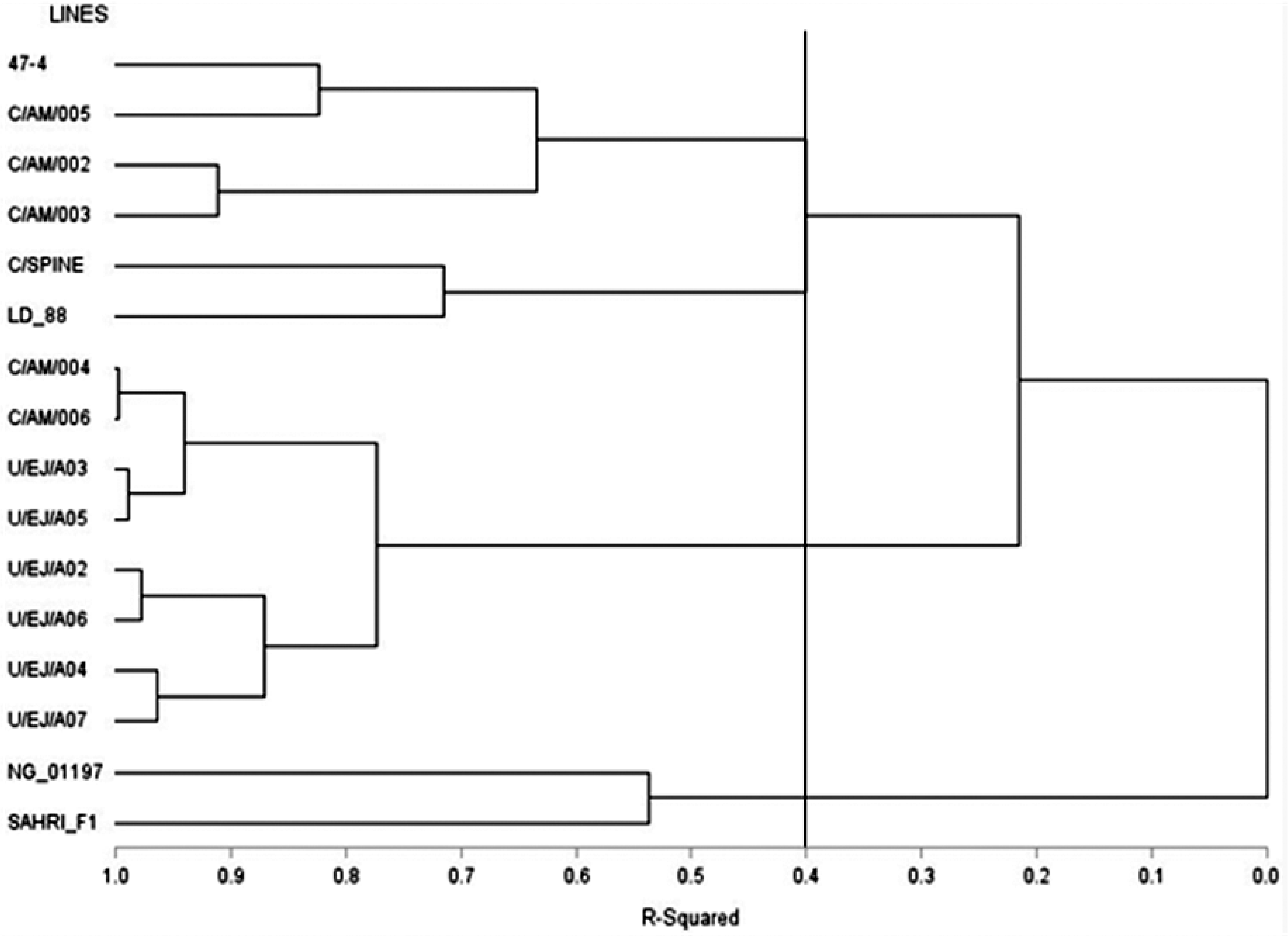


Figure 4. Dendrogram for okra lines evaluated for growth and seed yield in wet and dry seasons

The multivariate clustering method assumes that members of the same cluster belong to the same heterotic group and are highly homogeneous, suggesting that hybridization should be performed between clusters, not within clusters, meaning that members with good performance “sourced” from different clusters can be used for seed yield improvement. It is also understood that the two techniques employed here gave a clearer understanding of similarities and dissimilarities in the accessions. The lines considered in this study are useful for a further breeding program to develop high seed yielding varieties. The classification of genotypes into different clusters aids in their selection to design future breeding programs. Diverse varieties may be useful for the transfer of the desired gene for seed yield improvement.

Given the fact that genetic variation among inbred or pure lines is additive variance (Hallauer et al., 1988), the phenotypic value should closely approximate the genetic worth of the lines. In this instance, evaluation of okra via lines per se based on morphological traits is appropriate in placing them in heterotic groups and further steps can be taken towards seed yield improvement. A particular interest is that the grouping of the lines was independently far from the genetic background of the lines implying that they have diverse genetic backgrounds and could be a good source of useful genetic components. The obtained results suggested that superior offspring (hybrid combinations) could be obtained at early generations by crossing between, rather than within clusters. Secondary traits are very valuable in selection for improved okra seed yield under diverse environments. In this study, the weak association observed between seed yield and other related characters suggested the masking effects of genetic by environment interaction on the expression of these characters.

In general, a high correlation suggests that selection directed to one character directly affects the other. Conversely, low or non-correlation suggests independence of association that would be possible to select independently for the two characters for diverse directions. The grouping of lines into clusters usually enhances the selection of genotypes needed for a breeding program. Genetically diverse lines are potentially useful for gene transfer for yield and other attributes. This study identified promising lines that possessed high seed yield and short stems which could be used as sources for seed yield development. The promising lines: SAHRI_F1, NG_01197, 47-4 and LD_88 can be utilized in a future breeding program.

Conclusion

Genetic variation exists among the studied okra lines for almost all characters. The evaluated genotypes SAHRI_F1, NG_01197, 47-4 and LD_88 showed more potential in terms of yield as they outperformed other lines, indicating their utility as promising lines. These results are of practical orientation and contribution to the breeding programs of *Abelmoschus esculentus* L. Moench.

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