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Assessment of Genetic Diversity in *Sphenostylis stenocarpa* (Hochst Ex. A Rich Harms) Using Morphological Markers

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

African Yam Bean [*Sphenostylis stenocarpa* (L.)] as an unexploited, neglected and underutilized crop, calls for urgent attention for its breeding and improvement to avoid extinction. Fifty AYB seed samples from Idomi, Obubra, Ekoli-Edda, Abakaliki, Jos as well as IITA, Ibadan, Nigeria was assessed for genetic diversity using morphological and *rbc*L gene markers. Morphological characterization of AYB was carried out on twenty yield-related and yield traits. Analysis of Variance (ANOVA), Principal Component Analysis and Clustering Analysis were done using Genstat discovery Edition 4 and PASW version 20.0 software. The results revealed low genetic variations in morphological traits like pod weight, pod length, number of leaves, days to 50 % maturity, seed length and width and days to 50% flowering in different accessions investigated. Principal Component analysis generated eight principal components accounting for 57.47 % of the total variation while the hierarchical cluster analysis revealed two major clusters without traceable

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pattern of clustering. The relatively low genetic variation observed in the AYB accessions indicates inter-national germplasm exchange of AYB which has been a common practice among countries in Africa and Asia. Accessions from Ekoli-Edda in cluster 2 distantly clustered away from those from Abakaliki even when they were from same state. The Principal component analysis lend credence to the analysis of variance results and stems from the low degree of morphological diversity possibly due to an interaction of genetic and environmental factors. The traits which had large loading values (pod weight and length) in the first two principal component should be considered for selection in *S. sternocarpa* breeding programme. We conclude that there was very narrow genetic diversity in the AYB collections investigated.

Keywords: Sphenostylis stenocarpa; clustering analysis; food production; nutritional benefits.

1. INRODUCTION

The alarming increase in the world's population has its direct effect on food security and sustainability which has increased the demands for food production. Although science has made enormous strides in improving the world's ability to feed itself over the past decades, a large proportion on world population are still suffering from hunger and malnutrition [1]. Nearly eight hundred million people in the developing world do not have enough to eat. Many important crop plants native to Africa with potentials to alleviate and reduce food insecurity in the continent are mostly neglected, unimproved and under-utilized. One of such crops with unexploited potentials and quality nutritional value is *Sphenostylis stenocarpa* (Hochst ex A. Rich Harms) popularly called African yam bean [2].

African yam bean (AYB) is a leguminous crop of tropical African origin belonging to the family Fabaceae which is the second biggest and one of the most economically important families among the dicotyledons [1,3]. African yam bean (*Sphenostylis stenocarpa*) originates from Ethiopia and both wild and cultivated species are found in tropical Africa from northern Ethiopia (Eritrea) to Mozambique including Tanzania and Zanzibar and as far south as Zimbabwe in East Africa [1]. It is also cultivated throughout West African countries particularly, Nigeria, Cameroun, Ghana, Togo and Cote d'Ivoire. The crop is an annual grain legume and has a pattern of growth similar to those of other grain legumes [4].

African Yam Bean has edible tubers and most culturally and economically important of the seven species in the genus Sphenostylis [5]. It is a minor grain legume and under-exploited (Saka et al., 2004), usually cultivated in association with yam, cassava, maize and sorghum and other crops [6]. The legume has long been used as a traditional dual seed grain and tuber food crop in Africa and it has other greater potentials [7]. African yam bean has been reported as a good source of essential amino acids including cystein, lysine, methionine, phenylalanine and proline [8]. The amino acid contact in African yam bean seeds is higher than those in cowpea, pigeon pea [9]. Despite the nutritional benefits of African yam bean, it is faced with several production constraints. African yam bean source of protein and rich in minerals such as phosphorous, potassium and iron minerals [10]. The value of the protein in both seeds and tubers is comparatively higher than what could be obtained from other legume crops [11].

The utilization of African yam bean has links with the socio-cultural values of some ethnic groups within the African society. For instance, the ‘Avatimes,’ in Ghana prepare a special meal from it during the celebration of puberty rites of adolescent girls. Likewise, a special meal from it features during the marriage ceremony among the Ekiti people in Nigeria, pointing that the different forms of local recipes are prepared from the crop to meet the dietary needs of the people [12] Unfortunately, there is a decrease in agro-biodiversity [13] of these species in many parts of Nigeria probably due to lack of awareness of the potential of this neglected crop, poor methods of propagation, processing, marketing and consumption of the crop [8]. Others factors include the presence of anti-nutritional factors such as oxalate, phytate, lectin, saponin, tannin, trypsin inhibitors and hydrogen cynanide [14] low seed yield [15], hardness of the seed coat which makes high demand on the cost and time of cooking, agronomic demand for stakes and the long maturation period, presence of secondary metabolites [5], [16] as well as biotic factor like insect infestation. The crop has also undergone little or no genetic improvement to boost its agronomic and nutritional qualities [17].

The role of physiological traits in grouping plant accession is important and cannot be overemphasized [18]. [19], revealed that the relatively high variations observed in pods per peduncle, pod per plant, number of filled pods, number of seeds per pod and days to pod maturity were indicative of varietal differences of African yam bean accessions. With the use of morphological markers, estimates of genetic parameters across accessions will reveal the relative various characters of importance as it affects the total variation in the crop for its improvement [20]. *Sphenostylis stenocarpa* is a crop with great potentials in agricultural productivity and food security, especially is sub-Saharan Africa. Also, its continued existence is threatened with genetic erosion and extinction of its landraces and wild relatives due to lack of proper conservation and breeding strategies. Up until now, the genetic diversity of *S. stenocarpa* remains poorly understood. Consequent upon this, an understanding of the genetic diversity and its relationship will provide the needed baseline data for efficient breeding scheme.

This research is therefore designed to provide information on the genetic diversity of 50 accessions of S. stenocarpa using morphological markers. This will enable proper characterisation as well as the development of proper conservation and breeding strategies for this crop.

2. MATERIALS AND METHODS

2.1 Sample Collection and Establishment

Fifty seeds of African Yam Bean (*Sphenostylis stenocarpa*) accessions were obtained from Cross River, Ebonyi and Plateau States, as well as International Institute of Tropical Agriculture (IITA), respectively for the study (Table 1). The experimental plot was ploughed, harrowed and ridged before planting. Three seeds of each accession were planted per stand; which was thinned to one plant per stand after seedling emergence and establishment. The sowing was in the spacing of 1 × 1 m. The experiment was laid out in a Randomized Complete Block Design (RCBD) with five (5) replicates. After establishment, sticks of about 3 m length were pro-vided to support the plants as stakes at three weeks after planting. The field was kept clean by regular hand weeding with hoes (Plate 1).

2.2 Data Collection

Data was collected monthly to maturity on quantitative traits. Quantitative traits were counted, measured using metric rulers or vernier caliper and weighed using weighing balance. On the field, data were recorded on the five middle plants (sampling units). Five readings were made for each of the quantitative characters per accession. All the characterizations were based on Genetic Resources Center, International Institute of Tropical Agriculture (ITTA) descriptors for African Yam Bean. Data was collected on the following parameters;

i) Days to 50 % Seedling emergence
ii) Days to 50% flowering
iii) Days to 50% maturity
iv) Number of Leaves per plant
v) Vine length
vi) Peduncle length
vii) Petiole length
viii) Terminal Leaf length
ix) Terminal Leaf width
x) Number of pods per Plants
xi) Pod Length
xii) Pod Weight
xiii) Number of Seeds per pod
xiv) Seed Weight per pod
xv) Seed length
xvi) Seed width
xvii) Internode length
xviii) Leaf Area
xix) Vine diameter
xx) Number of Branches per plant

2.3 Data Analysis

Morphological data obtained were calculated by using analysis of variance (ANOVA) and means were separated using the Least Significant Difference (LSD) test. Cluster patterns for quantitative trait in 50 accessions of AYB were generated using Ward’s method. The quantitative data was subjected to principal component analysis (PCA). All analyses were done using Predictive Analytic Software (PASW) version 20.0.

Table 1. Accessions of *S. stenocarpa* used in the study, their origin and ecological zone

S/N	Accessions	Origin	Ecological zone
1	Idomi	Cross River	Forest
2	Obubra	Cross River	Forest
3	JOS31	Plateau	Savanna
4	JOS30	Plateau	Savanna
5	Abakaliki	Ebonyi	Forest
6	Ekoli-Eda	Ebonyi	Forest
7	TSs-602	Nigeria	Savanna
8	TSs-625	Nigeria	Savanna
9	TSs-561	Nigeria	Savanna
10	TSs-224	Unknown	Unknown
11	TSs-592	Nigeria	Forest
12	TSs-168	Unknown	Unknown
13	TSs-593	Nigeria	Forest
14	TSs-438	Nigeria	Savanna wood land
15	TSs-69	Nigeria	Forest
16	TSs-42	Nigeria	Forest
17	TSs-591	Nigeria	Forest
18	TSs-111	Nigeria	Forest
19	TSs-581	Nigeria	Forest
20	TSs-84	Nigeria	Forest
21	TSs-571	Nigeria	Forest
22	TSs-128	Nigeria	Swampy with trees and grasses
23	TSs-5	Nigeria	Savanna
24	TSs-138	Nigeria	Savanna
25	TSs-46	Nigeria	Savanna
26	TSs-68	Ghana	Forest
27	TSs-55	Nigeria	Savanna wood land
28	TSs-155	Nigeria	Forest
29	TSs-65	Zaire	Forest
30	TSs-66	Bangladesh	Forest
31	TSs-93	Nigeria	Forest
32	TSs-120	Nigeria	Forest
33	TSs-430	Nigeria	Forest
34	TSs-98	Nigeria	Forest
35	TSs-87	Nigeria	Swampy area
36	TSs-67	Bangladesh	Savanna
37	TSs-445	Nigeria	Savanna
38	TSs-8	Nigeria	Savanna
39	TSs-38	Nigeria	Forest
40	TSs-130	Nigeria	Forest
41	TSs-61	Nigeria	Savanna
42	TSs-77	Ghana	Savanna
43	TSs-45	Nigeria	Savanna
44	TSs-116	Nigeria	Savanna
45	TSs-12	Nigeria	Savanna
46	TSs-153	Nigeria	Savanna
47	TSs-19	Nigeria	Savanna
48	TSs-133	Nigeria	Savanna
49	TSs-30	Nigeria	Forest
50	TSs-150	Nigeria	Forest

Plate 1. The experiment was laid out in a Randomized Complete Block Design (RCBD) with five (5) replicates

3. RESULTS

Analysis of variance of yield related and yield traits of AYB obtained from IITA germplasm and AYB growing regions.

Fifty accessions of AYB were characterized based on 20 morphological quantitative traits. The results show significant differences ($P<0.05$) in all the traits among the accessions studied except for seed width and the branch number of the plant per parameters.

The mean days to 50% seedling emergence in S. sternocarpa accessions evaluated ranged from 9 to 14 days. TSs591 produced the highest number of leaves (132.67) followed by TSs168 (108.33), TSs105 (105.33) while TSs65 produced the least (88.00). However, for plant height, AYB accession obtained from Idomi produced the tallest plant(91.33cm) while the shortest was observed for T593. The final leaf length showed differences that were so significant and width in most accessions investigated. There was however no significant difference ($P<0.05$) in terminal leaf width for accessions obtained from Obubra and Idomi as they produced same loading values for terminal leaf width. Accessions TSs84 showed widest internode length, followed by TSs168, TSs571 and TSs42 while accessions obtained from Jos showed the least internode length, followed by idomi, Obubra and Jos respectively. There was no significant difference at $P<0.05$ among accessions obtained from AYB growing regions (Jos, Idomi, Obubra, Abakaliki and Ekoli-Edda) based on internode length.

The widest leaf area was observed in accessions obtained from Jos (57.70 cm^2). TSs130(47.00 cm^2) showed the narrowest leaf area. The widest vine diameter was observed in TSs68(1.50cm), followed by Obubra, Idomi, and Ekoli-Edda (1.33 cm), and TSs438(1.23cm) respectively. The narrowest value for vine diameter was observed in TSs38(0.70cm) and TSs5(0.70cm), followed by TSs593(8.33cm) and TSs42, respectively.

Accessions obtained from Jos (16.33) showed the highest number of branches followed Idomi (16.00) while the least number of branches was observed in TSs581, TSs84, TSs130 and TSs30 (8.00), followed by TSs593(8.33) and TSs42(8.35). The result obtained shows that accession TSs161had the highest number for50% days to flowering while TSs591 had the lowest. Accessions obtained from Ekoli-Edda produced the longest peduncle length followed by Jos 31(14.2) and TSs602(13.5) while accessions obtained from TSs592 had the shortest peduncle length followed by TSs65 and TSs68.Petiole length revealed that accessions obtained from Idomi and Obubra produced the highest petiole length (4.43cm) and (4.44cm) followed by TSs67(4.2cm) while TSs42showed the lowest petiole length (3.0cm), followed Ekoli-Eda and TSs592(3.1cm) and T111(3.2cm).

Table 2. Principal Component Analysis (PCA) in morphological traits of African yam bean

	Community	**PC_1**	**PC_2**	**PC_3**	**PC_4**	**PC_5**	**PC_6**	**PC_7**	**PC_8**
Eigen value	-	2.85	1.76	1.48	1.34	1.26	1.18	1.45	1.06
Proportion of variance (%)		13.55	8.36	7.50	6.37	5.99	5.61	5.47	5.05
Cumulative variance (%)		13.55	21.91	28.96	35.33	41.33	46.95	52.42	57.47
days 50% to seedling emergence.	0.517	-0.079	0.543	0.330	0.179	0.057	0.189	-0.178	0.062
days to 50% flowering	0.786	-0.072	0.161	-0.073	0.217	0.436	-0.061	0.572	0.426
days to 50% maturity.	0.671	-0.048	-0.188	0.029	0.706	0.123	0.148	-0.311	0.013
No of leaves	0.670	0.198	0.090	0.552	0.071	-0360	0.097	0.029	0.416
Plant height(cm)	0.438	0.217	0.354	0.296	0.185	-0.143	-0.077	0.300	-0.044
Peduncle length(cm)	0.455	0.118	-0360	-0.243	0.216	0.102	-0.380	0.133	-0.80
Petiole length(cm)	0.608	0.309	0.431	-0.142	0.232	0.412	0.074	0.010	-0278
Terminal leaf length(cm)	0.652	0.228	0.158	0.359	-0.148	0.046	-0.446	-0.447	-0.153
Terminal leaf width(cm)	0.560	0.456	0.208	-0.398	-0.253	-0.131	0.024	-0.103	0.239
No. of pods per plant.	0.380	0.472	0.119	-0.087	-0.235	0.097	-0.241	-0.101	-0.057
Pod length(cm)	0.465	0.108	0.610	-0.120	-0.021	-0.037	0.236	-0.067	0.072
No. of seeds/pod	0.555	0.210	0.360	0.190	0.520	-0.020	-0.039	-0.021	0.262
Seed weight/pod(g)	0.523	0.592	-0.137	0.120	-0.091	-0.331	0.143	-0.016	0.021
Seed length(mm)	0.705	0.089	-0.009	0.440	-0.158	0.198	0.601	0.087	-0.268
Seed width (mm)	0.650	-0.126	-0.178	0.290	-0.218	0.611	0.144	-0.193	0.198
Pod Weight(g)	0.540	0.673	0.143	-0.534	0.171	0.107	-0.165	0.061	0.227
Internode length(cm)	0.642	-0.643	0.296	0.013	-0.133	-0.328	-0.039	0.096	-0.073
Leaf area(cm2)	0.629	0.502	-0.294	0.021	-0.034	-0.038	0.181	0.342	-0.372
Vine diameter(cm)	0.582	0.569	0.040	0.230	-0.089	0.120	-0.219	0.069	0.358
No. of branches	0.563	0.274	-0.351	-0.417	-0.038	-0.181	0.362	-0.064	0.149

Accession TSs68 showed earliest days to 50% Pod maturity while Abakaliki had the latest days. Accession TSs153 produce the highest number of pods, followed by Idomi and Jos while TSs12 produced the lowest number of pods, followed by TSs591, TSs571 and Abakaliki, TSs504, TSs593, TSs66, and TSs87respectively. For Pod length, the longest pod length was observed in Obubra and TSs161 (23 cm), followed by Idomi (22.33 cm) and TSs591(21cm) while the shortest pod lengths were observed in Abakaliki (9.7cm), followed by Jos (10cm) and Ekoli-Eda (10.7 cm) respectively.

Accessions obtained from Obubra showed the heaviest pod weight followed by Idomi and TSs69. The lightest pod weights were observed in TSs571(4.0g), followed by TSs84(4.1g) and TSs591(4.5g). The greatest number of seeds per pod was observed in TSs153 (17.7) followed by Idomi and TSs161(16.7), and Jos (16.33) whileTSs168 showed the smallest number of seeds per plant(6.7) followed by TSs438, TSs128(7.7) and TSs93(8.0) respectively. Accessions obtained from Idomi showed the heaviest seed weight followed by Jos and Obubra while TSs42 showed the lightest weight, followed by TSs38 and TSs571for seed weight.

Principal Component Analysis (PCA) in morphological traits of African yam bean from IITA germplasm and AYB growing regions.

The PCA of 20 quantitative traits in AYB accessions is presented in Table 2. It revealed that the total cumulative variation observed in the AYB was 57.47 % where PC1 to PC8 contributed 13.55 %, 8.36 %, 7.5 %, 6.3 %, 5.99 %, 5.61 %, 5.47 % and 5.05 %, respectively. Among Parameters studied, Days to 50% flowering, days to 50% maturity, leaf number. Length of seed as well as its width, had communalities of 0.786,0.671,0.670,0.705 and 0.650 respectively thus accounting for Variation of 70 % and above. (Table 2) Cluster analysis of morphological characters of 50 African yam bean accessions

The dendrogram for Cluster analysis using ward's method for the fifty AYB accessions based on morphological trait revealed two major clusters. The dendrogram shows that accessions were not grouped based on location as there were inter-mixing of accessions between the different locations within the sub-clusters. The Dendrogram further revealed that cluster I contained only accessions obtained from Abakaliki while cluster 2 contained 49 accessions. Cluster 2 had two sub-clusters, denoted as cluster 2A and 2B. cluster 2A contained only one accession (Tss602) while 2B contained the remaining 48 accessions. Cluster 2Bwas further grouped into two sub-clusters, $2B_1$ and $2B_2$. $2B_1$ consist of 15 accessions while cluster $2B_2$ consists of accessions. Revealing a phonetic analysis or phenotypic relationship within the accessions studied is shown in the Dendrogram below (Fig. 1).

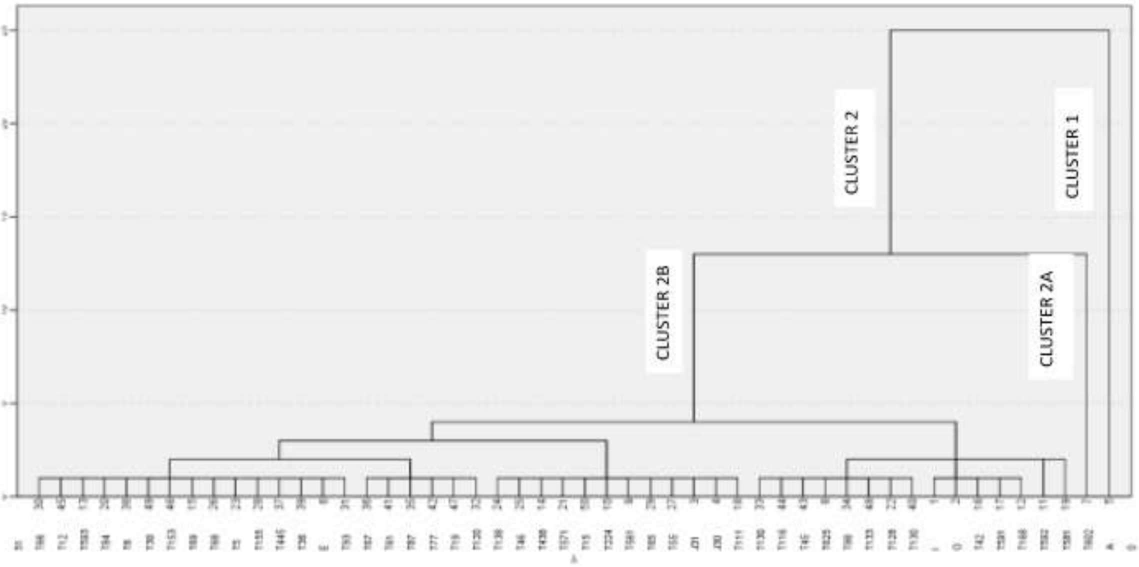


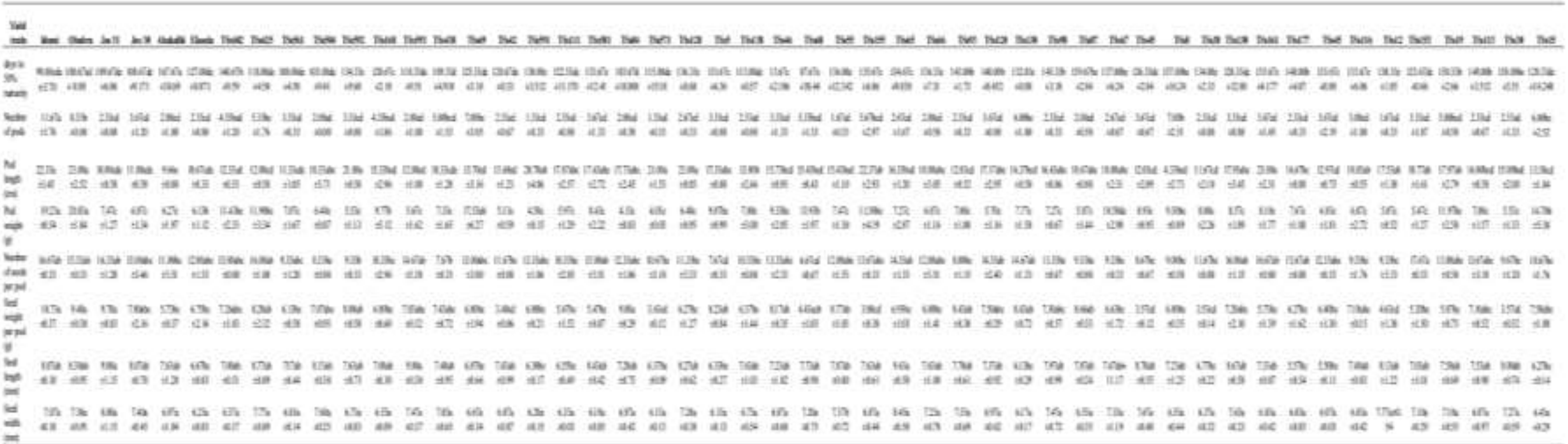
Fig. 1. Genetic relationship of 50 AYB accessions from morphological traits data

Table 3. Yield related traits of 50 AYB accessions obtained from AYB growing regions and IITA germplasm

Results are presented in mean ± standard error
Mean values with different superscript along the same horizontal axis are significantly different (P<0.05)

https://journalajob.com/files/contexts/45/library/2023_AJOB_108813_Table_3.pdf

Table 4. Yield traits of 50 AYB accessions obtained from AYB growing regions and IITA germplasm



Results are presented in mean ± standard error
Mean values with different superscript along the same horizontal axis are significantly different (P<0.05)

https://journalajob.com/files/contexts/45/library/2023_AJOB_108813_Table_4.pdf

4. DISCUSSION

Analysis of variance (ANOVA) results of the twenty quantitative morphological trait studied in 50 S.stenocapa as summarized in Tables 3 and 4 showed significant morphological variation in all parameters evaluated except for number of branches and seed width. The variation in morphological trait among accessions may be attributed to the fact that the crop is mainly vegetative propagated in nature and perhaps genotype environment interactions. The result indicated a low level of morphological variation among the accessions, pointing to difficult possibilities of obtaining desirable trait combinations of specific accession. The accessions with a greater number of leaves in each plant, vine in each plant, branch number of each plant, plant height as well as least internode showed that the longer the plant the greater the chance of having many numbers of seeds. The great seed harvest observed in most of the accessions is as a result of the vine being very tall and the healthy way that the plant grows that encourage the making of many flowers production of large number of flowers that mature to become pods and finally seeds. This research work agrees with the report of [21] who revealed that accession of AYB with a greater branch number length at internode had in return a greater number of seed in each plant.

In delimitating the genetic and taxonomic relationship in crops, characters that are known to seed alone has been noted to be very essential [22], [23]; [21], [24]. For AYB seeds, different parameters were seen and recorded as revealed by [25] and [5] in the AYB to be an important physical form traits that the AYB germplasm to stand out and be different in the population. With this research work, we took into consideration the seed width, length and weight as morphological characters to distinguishing AYB. The result revealed that accessions from Jos had the largest seeds while accessions from Idomi had the heaviest seeds. The differences in seed traits in accessions may be attributed to variations in genetic architecture of different accessions and prevailing environmental conditions. [26] revealed that accessions with wanted scientific agriculture characters like how long the pod is, how many the number of seeds or pods is, not too long it takes to bring flowers and a greater percentage of seed were the distinguishing characters in AYB accession.

Generally, some local accessions performed better in all parameters studied except for days to 50% seedlings emergence, leaf number and branch number compared to other accessions from IITA, Accessions from Idomi had better performances in seed weight, plant height, actual leaf length and length of the pod, stem diameter etc. It is apparent that Idomi accession had adequately adapted to the climatic and agro-geological conditions of Cross River State.

The principal analysis component as seen in Table 2 lend credence to the analysis of variance results and stems from the low degree of morphological diversity possibly due to an interaction of genetic and environmental factors. The traits which had large loading values (pod weight and length) in the first two principal component should be considered for selection in S. sternocarpa breeding programme. This result is consistent with the work of [27], who reported eight principal components (PC1 to PC8) for principal component analysis in germplasm collection analysis of African Yam Bean. He further stated that PCI to PC8 accounted for 68.68% of the phenotypic variability observed in the accessions.

Cluster analysis for morphological characters revealed two major clusters for 20 quantitative morphological traits in 50 accessions (Fig 1). The morphological clustering in this research is an indication that the accessions at the genetic level are morphologically related since they were not grouped based on locations. The dendrogram further revealed that the accessions obtained from Idomi, Obubra, Jos, and Ekoli-Edda were grouped closely with some germplasm collections of IITA. It is probable that the relatively low genetic variation observed in the AYB accessions may indicates inter-national germplasm exchange of AYB which has been a common practice among countries in Africa and Asia. Accessions from Ekoli-Edda in cluster 2 distantly clustered away from those from Abakaliki even when they were from same state.

5. CONCLUSION

Fifty accessions AYB were characterized based on 20 yield related and yield morphological traits. ANOVA results revealed minimal variations in morphological traits like Pod weight, Pod length, number of leaves, days to 50 % maturity, Seed length and width and days to 50 % flowering in different accessions investigated. Principal Component Analysis of twenty morphological traits revealed eight principal component and expressed 57.47 % of the total variation observed with Pod weight and length as major contributors. Cluster analysis revealed two major clusters. Therefore, it is recommended that the use of morphological and molecular (DNA) markers to determine the extent of variability and the contributing trait among accessions will provide the needed baseline for effective characterization of this crop.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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