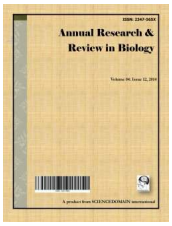

Annual Research & Review in Biology

14(4): 1-10, 2017; Article no.ARRB.34264
ISSN: 2347-565X, NLM ID: 101632869

Fruit Morphometric and RAPD Evaluation of Intraspecific Variability in Some Accessions of African Yam Bean (*Sphenostylis stenocarpa* Hochst. ex. A. Rich. Harms)

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Authors' contributions

This work was carried out in collaboration between all authors. Author JOP designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ACO and BDA managed the analyses of the study. Author BMA managed the literature searches and RAPD protocol. Author AEA review the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2017/34264
Editor(s):
(1) Jin-Zhi Zhang, Key Laboratory of Horticultural Plant Biology (Ministry of Education), College of Horticulture and Forestry Science, Huazhong Agricultural University, China.
(2) George Perry, University of Texas at San Antonio, USA.
Reviewers:
(1) Marcos Vinicius Bohrer Monteiro Siqueira, Universidade do Sagrado Coração, Brazil.
(2) Zeynel Dalkiliç, Adnan Menderes University, Turkey.
Complete Peer review History: http://www.sciencedomain.org/review-history/20004

Original Research Article

Received 22nd May 2017
Accepted 9th June 2017
Published 12th July 2017

ABSTRACT

African Yam Bean (AYB) (*Sphenostylis stenocarp*a, Hochst. ex A. Rich, Harms) is an indigenous underutilized legume mainly grown in Sub-saharan African as a source of protein. Intraspecific variability studies were carried out on 10 accessions of AYB obtained from the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria. Fourteen (14) fruit morphometric characters and nine

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(9) arbitrary RAPD primers were employed for evaluation of genetic intra-specific variability among the accessions. A total of 410 bands were generated with 261 (63.66%) polymorphic bands. There is significant correlation among some reproductive characters; days to 50% flowering, pods per peduncle, number of locules per pod, number of seeds per pod, pod length and seed set percentage. These characters represent good markers of the taxon suitable for breeding and genetic improvement purposes. Morphometric and RAPD cluster analysis using UPGMA resulted in a dendrogram each; with membership similarity ranging from 72% to 93%. Two accessions (TSs56 and TSs94) recorded higher level of similarity index of 93% based on RAPD profiling. The morpho-metric evidences shows inherent stability of AYB across varied eco-geographical settings, which demands further investigation and exploitation. However, the RAPD evidences show that the species have evolved and adapted to distinct geographical setting with a clear Nigeria and Ghana demarcation. This fact can be engaged to guide future studies, germplasm collection, characterization, documentation, utilization and conservation of AYB to boost knowledge and awareness on the genetic diversity and utility of the species.

Keywords: AYB; Sphenostylis stenocarpa; genetic intra-specific variability; RAPD characterization.

1. INTRODUCTION

African yam bean (AYB) (*Sphenostylis stenocarpa* Hochst ex A. Rich) is a grain legume cultivated throughout Africa for its edible seeds and tubers [1,2]. It is considered rich in plant protein (21% by wt) with lysine and methionine contents comparable to that of soybean (*Glycine max*) [3,4]. In addition, AYB has high metabolic energy, low true protein digestibility, moderate mineral content, and amino acid content that compares favourably with most pulses [5]. However, with all the rich nutritional and chemical composition, AYB is listed among the neglected and underutilized species (NUS) [6,7]. NUS are traditional crops / landraces cultivated by farmers with little or no research attention and usually not traded as agricultural commodity [7]. NUS are highly adapted to marginal, complex, and difficult environments and contribute significantly to diversification and resilience of agro-ecosystems in order to withstand the impacts of climate change scenarios. The use of these species, whether wild, managed or cultivated, can have immediate consequences on the food security and well-being of the poor [8,9]. The greater use of NUS will also reduce pressures on other crops; reduce nutritional, environmental and financial vulnerability in times of change [6,8].

Several authors have reported that the pods and seeds of AYB contain galactose-specific lectins which confer resistance to cowpea pests such as *Clavigralla tomentosicollis* and legume pod borer *Maruca vitrata* [10,11,12].

Inspite of agronomic and economic advantage of AYB, its utilization, conservation and management have not been properly harnessed [13,14]. The crop has not received a well-coordinated systematic research attention to exploit its agronomic and genetic qualities to advance its utilization and genetic improvement [15]. Presently, there are no established varieties of AYB while the available landraces are generally low yielding and requires extra agronomic practices like staking. Previous studies on phenotypic and genetic diversity of AYB did not combine phenotypic characters with molecular markers (RAPD) to assess intraspecific variability of the species [16,17,18, 19]. The present study therefore exploits nine (9) RAPD primers and (14) fruit morpho-metric traits to assess the intraspecific genetic variation existing among ten (10) accessions of AYB obtained from the Genetic Resources Centre (GRC) of IITA Ibadan. The ten accessions were selected from earlier morphological characterization, phenotypic and agronomic similarities [12,18]. The combination of morpho-metric and RAPD markers will enhance better and reliable analysis of the genetic relationships existing among the studied accessions of AYB.

2. MATERIALS AND METHODS

2.1 Sources of Materials

Ten (10) accessions of AYB were collected from the Genebank of International Institute of Tropical Agriculture (IITA), Ibadan. Table 1 shows passport data of the accessions studied.

2.2 Field Cultivation and Design

The cultivation was carried out on an experimental field at IITA, Ibadan. Field design was carried out as described by Popoola et al. [18].

Table 1. Passport data of the ten accessions used for this study

S/N	Accessions	Origin/Locality	Seed coat colour	Seed shape
1.	TSS 11	Nigeria	Creamy white	Globose
2.	TSS 40	Nigeria	Brown	Ovoid
3.	TSS 56	Nigeria	Brown	Globose
4.	TSS 104[B]	Unknown	Greenish white	Globose
5.	TSS 116[B]	Nigeria	Brown	Globose
6.	TSS 125	Unknown	Brown	Ovoid
7.	TSS 139	Unknown	Creamy white	Ovoid
8.	TSS 90	Nigeria: Ikot-Ekpene	Brown black (Speckled)	Ovoid
9.	TSS 94	Nigeria: Ikot-Ekpene	Brown	Ovoid
10.	TSS 118	Ghana	Creamy white	Globose

2.2.1 Fruit morpho-metric characterization

Fourteen (14) quantitative characters were evaluated: Days to 50% flowering (DF), Days from flowering to maturity (DFF), Days to pod maturity (DPM), Number of flower per peduncle (NFP), Number of pods per peduncle (NPP), Total pods per plant (PPP), Pod length (PL), Number of locules per pod (NLP), Number of seeds per pod (NSPP), Seed set percentage (SS%), Seed length (SL), Seed width (SW), Seed thickness (ST) and 100 seed weight (WT). Qualitative characters were scored based on visual evaluation while quantitative characters were measured and recorded as SI units following the descriptor for African yam bean developed by Adewale and Dumet [20].

2.3 Characterization

Young leaves were harvested at four weeks after seedling emergence and lyophilized for 48 h and stored at -20℃ at the Bioscience laboratory of the International Institute of Tropical Agriculture (IITA), Ibadan.

2.3.1 AYB DNA extraction and quantification

Total genomic DNA was extracted using the mini-preparation protocol described by Dellaporta et al. [21]. Fifteen (15) RAPD primers were screened, nine (9) were selected and employed for further analysis. The concentration and purity of the extracted DNA was monitored using spectrophotometer wavelength 260:280 nm using the NanoDrop ND-1000 Spectrophotometer. Extracted DNAs were stored at -40℃ until used for RAPD-PCR.

2.3.2 RAPD-PCR

The PCR reactions were performed with a total volume of 10 µl containing: 2.0 µl of template DNA (10 ng/ µl), 0.5 µl of primer (5 µM/ µl), 0.4 µl of $MgCl_2$ (50 mM), 0.5 µl of DNTPs (2.5 mM), 0.5 µl of DMSO, 0.1 µl of Taq DNA polymerase (5 µ/µl), 1.0 µl of 10 X Buffer and 5.0 µl of Ultra-Pure Water (UPW). The amplification process was performed using the Applied Biosystem Thermal Cycler for an initial denaturation of 94℃ for 3 minutes (1 cycle), denaturation at 94℃ for 20 seconds, annealing at 38℃ for 40 seconds and final extension at 72℃ for 5 minutes. The PCR product were analyzed on 1.5% agarose with 1x TBE. The molecular fragments were estimated using 50 bp PCR marker and the gel was observed on a UV-transilluminator.

2.4 Data Analysis

Descriptive statistics, correlation coefficients and hierarchical clustering (dendrogram) were carried out on the 14 morpho-metric characters considered in this study. Data matrix from RAPD profiles were scored as present (1) or absent (0). The data obtained from scoring the RAPD bands were subjected to genetic similarity matrix using Jaccard's similarity coefficient. The cluster analysis of phylogenetic relatedness of the accessions was determined by using UGPMA (unweighted pair-group method with arithmetic averages) with the NTSYS-pc software [22]. The clusters membership segregation pattern generated from the mean values of morpho-metric characters and RAPD bands were compared.

3. RESULTS AND DISCUSSION

3.1 Descriptive Statistics of Morpho-metric Characters

The mean, standard deviation, range and variance showed variability among the 10

accessions analyzed for all except three of the 14 quantitative morpho-metric characters evaluated (Table 2 and Fig. 1a). The accessions revealed similarity in flowering and maturity periods. Two accessions TSs94 and TSs116B recorded longer flowering period (101 days: 3 months 11 days) compared to TSs118 which had a maximum 82 day flowering period and closely followed by TSs111 with 87 days. Accession TSs56 recorded shorter period of days from flowering to maturity (48 days) while TSs116B again had longer period with 77 days. Accessions TSs40 and TSs125 recorded earlier days to pod maturity (137 and 139 days), described as early maturing accessions while TSs94 recorded 165 days indicating late maturity days. TSs11 recorded lower number of flowers per peduncle (9) while TSs116B recorded higher value (14.56). Average pods per peduncle ranged from 30.14 in TSs90 to 78.11 in TSs94 indicating potential good yield. Fig. 1b shows the pod formation in *S. stenocarpa* studied. In terms of size, TSs90 recorded lower values in pod length (12.58 cm), number of locules per pod (8.03) and number of seeds per pod (6.72) while TSs139 recorded higher values in pod length (28.5 cm), number of locules per pod and seeds per pod which resulted in higher seed set percentage (95.8%). Seed characters; seed length, seed width, seed thickness and 100-seed weight also reflected significant variations amongst the ten accessions. Seed length ranged from 8.19 mm in TSs11 to 9.15 mm in TSs118 while 100 seed weight ranged from 25.56 g in TSs116B to 33.97 g in TSs118.

3.2 Pearson Correlation of the Characters

Table 3 shows the Pearson correlation coefficients for the 10 accessions using the 14 morpho-metric characters. Days from flowering was significantly correlated with days to 50% flowering (DF) r = 0.70, while days to pod maturity was fairly correlated with days from flowering to maturity with r = 0.54. There was no correlation between number of flowers per plant and other characters. Number of locules per pod showed higher correlation with pod length r = 0.97, number of locules per pod r = 0.99 and negatively correlated with days from flowering to maturity with r = - 0.66. Seed weight was observed to be correlated with seed length and seed width. There was no correlation among seed characters like seed length, seed width, seed thickness with pod characters. Seed weight was observed to be fairly correlated with seed length and seed width.

3.3 Clustering Pattern of *Sphenostylis stenocarpa* based on Fruit Morphometric Characters

Cluster analysis produced three cluster groups (Fig. 2). Group I clustered with six (6) accessions; TSs40, TSs56, TSs11, TSs104B, TSs125 and TSs118. Group II consist of three (3) accessions; TSs94, TSs116, TSs139 while group III had a single accession, TSs90. Accessions TSs40, TSs56 and TSs11 appeared to be the most closely related accessions in group I, and accession TSs90, most distant amongst the ten accessions.

Table 2. Descriptive statistics of reproductive characters of *S. stenocarpa* accessions studied

S/N	Character	Mean	SD	Range	Variance
1	Days to 50% Flowering	93.40	6.45	82 (TSs118) - 101 (TSs116B)	41.60
2	Days from Flowering to Maturity	60.50	9.35	48 (TSs56) - 77 (TSs116B)	87.39
3	Days to Pod Maturity	149.60	9.09	137 (TSs40) - 165 (TSs94)	82.71
4	Number of Flower Per Peduncle	11.36	2.06	9 (TSs11) - 15 (TSs104B)	4.24
5	Number of Pods Per Peduncle	4.52	0.72	3. 67 (TSs56) - 6 (TSs118)	0.51
6	Pods Per Plant	52.84	13.64	30.14 (TSs90) - 78.11 (TSs94)	186.01
7	Pod Length (cm)	23.67	4.72	12.58 (TSs90) - 28.54 (TSs139)	22.29
8	Number of Locules Per Pod	17.88	3.87	8.03 (TSs90) - 21.44 (TSs139)	14.99
9	Number of Seed Per Pod	16.06	3.70	6.72 (TSs90) - 20.10 (TSs139)	13.67
10	Seed Set Percentage	88.63	4.46	80.0 (TSs116B) - 95.8 TSs139)	19.90
11	Seed Length (mm)	8.49	0.31	8.19 (TSs11) - 9.15 (TSs118)	0.10
12	Seed Width (mm)	6.74	0.23	6.4 (TSs139) - 7.13 (TSs94)	0.05
13	Seed Thickness (mm)	6.94	0.18	6.69 (TSs11) - 7.2 (TSs56)	0.03
14	100 Seed Weight (g)	29.88	2.57	25.56 (TSs116B)-33.97(TSs118)	6.62

SD- Standard deviation (an index of the disparity between the 14 characters)

a)

b)

Fig. 1a. Variation in pod shape and size of the *S. Stenocarpa* accessions studied. b. Pod formation in *S. stenocarpa*

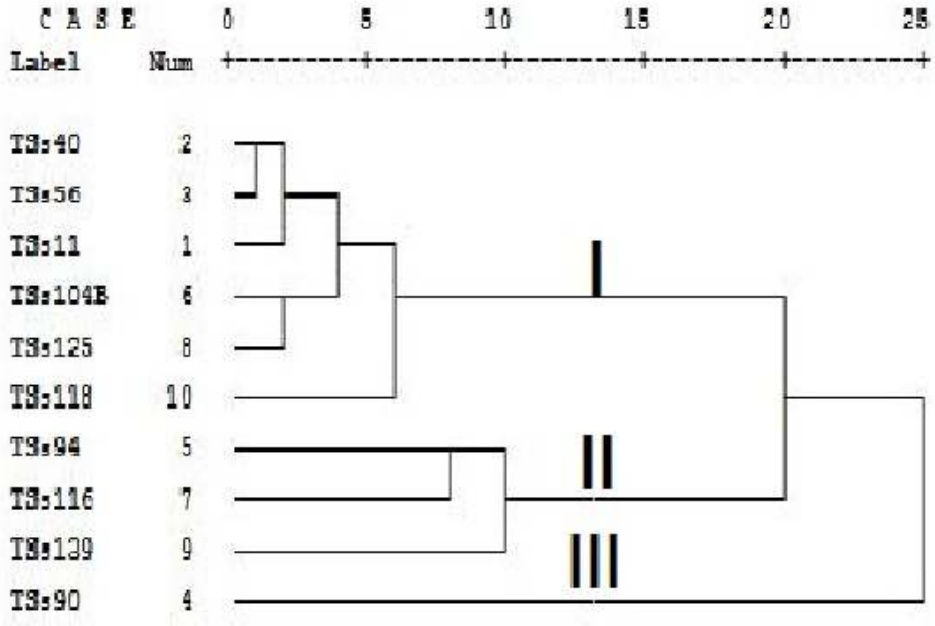

Fig. 2. Clustering pattern of *Sphenostylis stenocarpa* accessions using fruit morphometric characters

Table 3. Pearson correlation coefficient of the character among the accession of *S. stenocarpa* studied

	DF	DFM	DPM	NFP	NPP	PPP	LP	NLP	NSPP	SS	SL	SW	ST	WT
DF	1													
DFM	0.70**	1												
DPM	0.40*	0.54*	1											
NFP	0.30	0.19	-0.10	1										
NPP	-0.47	-0.26	0.04	0.01	1									
PPP	0.46*	0.18	0.46*	-0.06	-0.29	1								
PL	-0.13	-0.66**	-0.27	0.13	0.18	0.35	1							
NLP	-0.24	-0.70**	-0.26	0.12	0.34	0.36	0.95**	1						
NSPP	-0.16	-0.66**	-0.22	0.13	0.28	0.40*	0.97**	0.99**	1					
SS	-0.09	-0.61	-0.12	-0.23	0.20	0.11	0.80**	0.69**	0.73**	1				
SL	-0.41	-0.13	0.22	-0.17	0.46*	-0.12	-0.01	0.09	0.06	-0.08	1			
SW	-0.08	-0.06	0.06	-0.56	-0.31	0.22	-0.27	-0.26	-0.27	-0.10	-0.34	1		
ST	0.07	-0.51	-0.17	0.23	-0.19	0.23	0.52*	0.48	0.49	0.42*	-0.22	0.29	1	
WT	-0.29	-0.35	0.30	-0.55	0.23	0.07	0.02	0.09	0.07	0.21	0.59*	0.40*	0.33	1

*** Correlation is significant at the 0.05 level.*

DF = Days to 50% flowering, DFM = Days from Flowering to Maturity, DPM = Days to Pod Maturity, NFP = Number of Flower Per Peduncle, NPP = Number of Pods Per Peduncle, PPP = Pods Per Plant, PL =Pod length, NLP = Number of locules per pod, NSPP = Number of Seeds per pod, SS = Seed set percentage, SL = Seed length, SW = Seed Width, ST = Seed thickness, WT = 100 seed weight

3.4 Cluster Analysis of RAPD Markers of *Sphenostylis stenocarpa*

Number of amplified fragments per primer ranged from 30 (OPN-13) to 60 (OPT-16). The primer OPN-13 generated 17 polymorphic bands, primer OPB07 generated 40 polymorphic bands while OPT-16 generated 43 polymorphic bands. The primer sequences, the number of polymorphic bands generated and percentage polymorphism are shown in Table 4. Dendrogram of the cluster analysis of the ten accessions from the sequence polymorphism profile is shown in Fig. 3. Three clusters were identified at 76% similarity level; groups I and II and III. Group I comprised seven accessions; TSs11, TSs40, TSs104B, TSs90, TSs116B, TSs56 and TSs94 while group II comprised two accessions; TSs125, TSs139 and group III composed of only TSs118. Group I cluster segregated into 3 subgroups which were similar at 77% similarity index; subgroup Ia (TSs11) which was independent at 77%, subgroup Ib (TSs40, TSs104B, TSs90 and TSs116B) at 82% and Ic (TSs56 and TSs94) with 99% genetic similarity. Group II on the other hand had two subgroups IIa and IIb which were similar by 74%. The shortest genetic distance was recorded for accessions TSs56 and TSs94, accounting for 99% similarity.

Table 4. Primers, sequences, number of bands and percentage polymorphic

Primers	Sequences	NB	PB	%P
CPU17	5^1 – ACC TGG GGA G – 3^1	50	22	44%
ACO09	5^1 – AGA GCG TAC C – 3^1	40	20	50%
ACO03	5^1 – CAC CCT AGT C – 3^1	50	40	80%
OPN13	5^1 – AGC GTC ACT C – 3^1	30	17	57%
OPH09	-5^1 – TGT AGC TGG G – 3^1	50	26	52%
OPT15	5^1 – GGA TGC CAC T – 3^1	40	31	78%
OPT16	5^1 – GGT GAA CGC T – 3^1	60	43	72%
OPB07	5^1 – GGT GAC GCA G – 3^1	40	40	100%
OPB04	5^1 – GGA CTG GAG T – 3^1	50	22	44%
Total		**410**	**261**	**63.66%**

NB = Number of Bands, PB = Polymorphic Bands, %P = Percentage Polymorphism

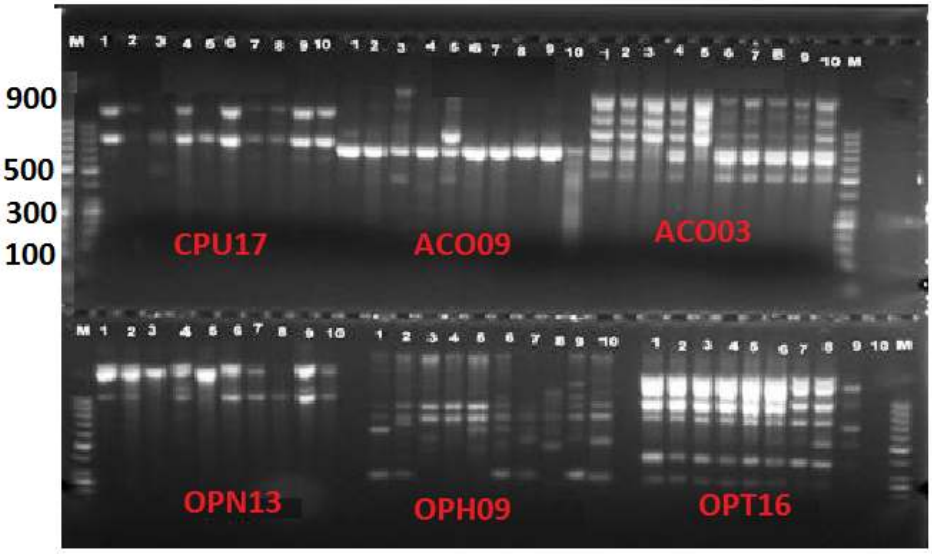

Fig. 3. RAPD DNA banding profile 1% gel electrophoresis of primers CPU17, ACO09, ACO03, OPN13, OPH09 and OPT16

(M = Marker; 1 – 10 = Accession numbers TSs11, TSs40, TSs56, TSs90, TSs94, TSs104B, TSs116B, TSs125, TSs139, TSs118). (M = Marker; 1 – 10 = Accession numbers TSs11, TSs40, TSs56, TSs90, TSs94, TSs104B, TSs116B, TSs125, TSs139, TSs118)

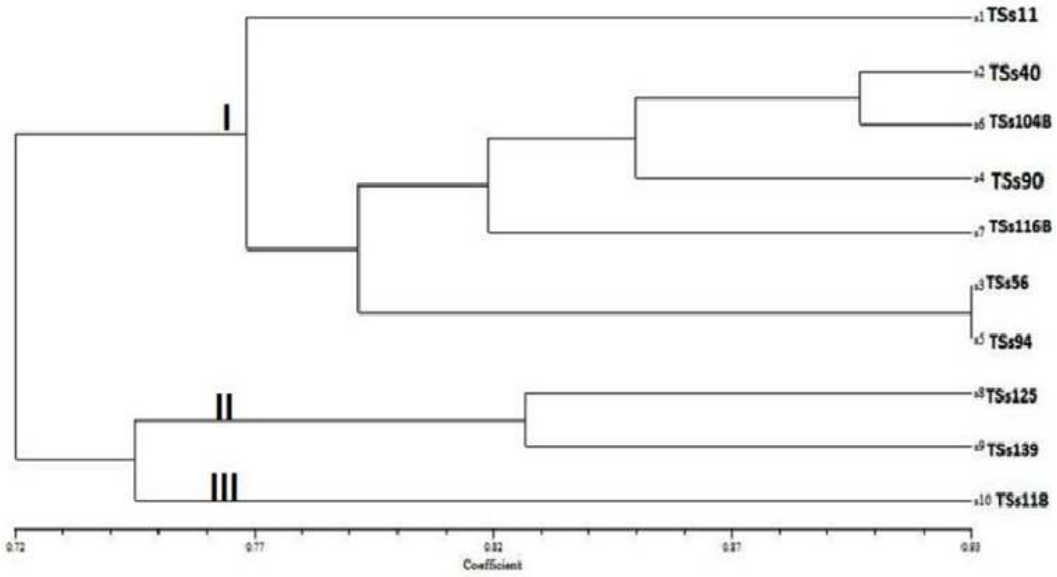

Fig. 4. Cluster pattern based on RAPD profile of the 10 accessions of *Sphenostylis stenocarpa*

3.5 Discussion

Fruit morpho-metric characters and Random Amplified Polymorphic DNA were employed to evaluate the genetic intra-specific variation among ten accessions of African yam bean (*Sphenostylis stenocarpa*). The results revealed considerable variation among the accessions in both fruit morphometric characters and DNA data.

In underutilized crops, longer flowering and fruiting periods, extensive branching and twinning habit have been connected with higher/increased yield which were observed in some of the accessions; although other factors such as genetic composition and environmental differences may interplay [18,23]. Number of pods per peduncle and number of locules per pod are directly linked to seed set percentage which is generally high in all the accessions, which indicates potential for high yield and their usefulness for cultivation and commercial purposes. In addition, the correlation analysis indicates that any selection based on pod length, number of seeds per pod, seed weight, seed length and width will enhance the genetic improvement of AYB. The application of phenotypic characters to unravel intra-specific variations in AYB has been well reported by [16,17,18,19,20]. The genetic distances observed are moderate particularly between TSs139 and TSs56. The fruit morpho-metric cluster using the reproductive characters did not reflect eco-geographic delimitation of the accessions indicating that the accessions are either from similar eco-geographical setting, or share common source of propagation material or the taxon show strong resistance to eco-geographical influences. The phenotypic grouping indicates high degree of morphological similarity, nevertheless, the separation of the clusters showed a considerable degree of morphological variation within the species. The accession; TSs139 showed better agronomic qualities like higher pod and seed numbers than others. The accession can be a target candidate as parental line in the effort to improve the AYB.

3.6 RAPD Variability

The percentage polymorphisms expressed by the primers indicate that only four primers (ACO03, OPT15, OPT16 and OPB07) can be said to be effective, efficient and highly polymorphic to assess the genetic variability in AYB. The primer, OPB07 recorded 100% polymorphic across the AYB accessions. The RAPD clusters were congruent with results of fruit morpho-metric dendrogram. This may point to the greater strength of molecular techniques at detecting variability and offering a better picture of the variation present within the taxon. The level of polymorphisms is relatively high and

comparable to earlier studies where RAPD was employed for genetic diversity of AYB [24]. The dendrogram from the RAPD analysis generated three major groups with four subgroups with possible connections with geographical demarcation. This grouping and thus RAPD evidences eliminates the ambiguity connected with morphological evidences. Accessions that were segregated in fruit morpho-metric characters dendrogram were grouped together. Group I clustered seven accessions (TSs11, TSs40, TSs104B, TSs90, TSs116B, TSs56 and TSs94) most of which were collected from Nigeria and though a few were recorded as of unknown origin, they are clearly of close origin with the others and may thus be classified as of Nigerian origin. Group II clustered two accessions (TSs125 and TSs139). The third cluster housed a single accession (TSs118), classified by the RAPD analysis as the most distant accession in the lot, a clear departure from the morphometric grouping. This distant accession also delimited on geographical of unknown origin. From morpho-metric and sequence data, accession TSs56 is the most basal of all the accessions and may well constitute the closest to the ancestral form of the species. Its origin should thus be an area of interest, with possible finds of most native genetic constituent for the species and an area for probably extensive collection of accessions and germplasm for conservation and improvement efforts on the species. In the present study, the RAPD analysis delimited the 10 accessions into three groups, with Nigeria and possibly Ghana being key origins of spread of the accessions.

4. CONCLUSION

The combination of morpho-metric traits and RAPD has provided a better understanding of the relationships existing among the ten accessions of AYB studied and eliminated possible duplication of germplasm. The distantly related accessions such as TSs11, TSs118, TSs139 and the morphometrically and RAPD constantly delimited accession TSs56 can be employed as parent lines in heterosis breeding. The morpho-metric evidences shows inherent stability of AYB across varied eco-geographical settings, which demands further investigation and exploitation. However, the RAPD evidences show that the species have evolved and adapted to distinct geographical setting with a clear Nigeria, Ghana demarcation in the accessions studied. This fact can be engaged to guide future studies, germplasm collection, characterization, documentation, utilization and conservation of AYB to boost knowledge and awareness on the genetic diversity and utility of the species.

ACKNOWLEDGEMENT

The authors acknowledge the management of Covenant University and Centre for Research, Innovation and Discovery (CUCRID) for Publication Support. We also extend thanks to the Genetic Resources Centre of the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria for providing the seeds used for this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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