

# **Phenotypic marker-based diversity analysis of some African yam bean** *Sphenostylis stenocarpa*  **(Hochst. Ex. A. Rich. Harms) accessions**

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Received November 25, 2018 Accepted for publication June 21, 2019 Published July 7, 2019

#### **Abstract**

Phenotypic characterization is a preliminary germplasm assessment programme meant to provide primary information on the evaluated genetic resources. Detail from such study becomes a guide to gene banking system. This report on 96 accessions of African yam bean (AYB) is within the 2017 characterization programme for AYB germplasm at the Genetic Resources Centre (GRC), International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Twenty-three quantitative (vegetative and reproductive) descriptors were employed for a diversity study of the 96 accessions. Significant (P < 0.01) variation existed among the accessions for majority of the studied quantitative characters. Stepwise discriminant analysis identified 18 phenotypic traits to be most discriminatory for the 96 AYB accessions. The mean genetic similarity among the accessions was 0.80; the least (0.53) and the highest (0.95) was between TSs53 and TSs83 and TSs5 and TSs22 respectively. The first three principal component axes accounted for 53.4% of the total variation among the 96 accessions. Ward clustering system grouped the 96 accessions into three main clusters of 54, 31 and 11 accessions. Accessions in cluster I attained peduncle initiation and first flowering at 95±0.13 and 119±0.69 days after planting (DAP). Accessions in cluster III reached 50% primary branching at 24±0.13 DAP and had the least seed sizes. Significant intra-cluster variation existed, hence; meaningful selection of accession(s) for further work should be at the sub-cluster or lower similarity level within the main cluster. The present study unveils the phenotypic identity of the 96 accessions and provides a platform for trait-based parental stock selection for genetic improvement of the crop.

**Key words:** accessions, genebank, phenotypic markers, morphotypes, improvement.

## **Introduction**

Meeting the present and future global food security targets has remained a global challenge. As against agricultural intensification, a mechanism whose goal identifies few crops for large and intensive production, Waha *et al.* (2018) had remarked that agricultural system which engages in more diverse farming systems and hence production of many crops (diversification) can contribute to household food and nutritional security. Within the components of diversification, traditional and indigenous crops are usually integrated. A recent remark by Li and Siddique (2018) hinted the neglected and underutilized crop species to be characteristically nutritious, climate resilient, economically viable (when in the right setting) and adapts to local conditions, especially, marginal ecologies.

Grain legumes contribute significantly to total world food production (Snapp *et al.*, 2018) and represent a major source of protein in many developing countries, supplementing for the nutritional deficiencies in cereal and tuber crops. Examples of food legumes are: Soybean, Cowpea, Groundnut, Chickpea, *Faba* bean, Lentil, Pea, Bambara ground nut, Winged bean, Pigeon pea etc. Depending on the extent of global cultivation, production and awareness, the most notable legumes are: Soybean, Cowpea and Groundnut. African yam bean (AYB) which is not in the main class is an indigenous and tuberous leguminous species; which despite many reported potentials (Rachie, 1973; Nwokolo, 1987; Obizoba and Nnam, 1992) and positive contribution to nutrition in Africa has not received the favour of agricultural policies for increased awareness and promotion. The dual economic product from AYB (edible grain and tuber) have the potential to broaden man's food base in Subsahara Africa (Adewale, 2010).

By the regional demarcation of the centre of diversity of *Sphenostylis stenocarpa* Hochst. Ex. A. Rich (Harms) as presented by the Germplasm Resources Information Network [GRIN] (2009), the distribution of the area for the crop's domestication and production includes parts of the East, West, Central and South Africa. However, it is of reference to note that no documentation is available to reflect any other niche outside African for the existence and survival of the crop; this may suffice for the justification of the name "African yam bean". The multiple regions and cultures of Africa in which this crop survive might reflect a very wide adaptation of the crop (Gruneberg, 2016). Since the crop has had a long and significant feature in African culture (Potter, 1992), demographic influence of the various environments may have initiated both genetic and phenotypic variation in the crop which Moyib *et al*. (2008), Popoola *et al.* (2011), Adewale *et al.* (2012, 2015), Ojuederie *et al.* (2014), Shitta *et al.* (2016), etc. had significantly reported.

Intraspecific variations research provides an informative document for enhanced awareness of a species and its taxa, the same also encourages subsequent proposition of improvement programme of the crop. Moreover, because characterization information on accessions are very necessary for germplasm conservation; 96 of the newly explored AYB accessions by the Genetic Resources Centre (GRC), International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria were subjected to a routine phenotypic characterization during the 2017 genepool characterization program to understand their diversity.

## **Materials and Methods**

The experiment was carried out in a screen house (whose floor was not concreted) at IITA, Ibadan (7 $^0$ 29'53"N, 3<sup>0</sup>53'59"E), Nigeria. Ninety-six accessions African Yam Bean (See the list in Table 1) were planted for characterization assessment. Completely randomized design was employed for the study. Two seeds were planted per hill on the prepared seed beds at inter and intra row spacing of 1metre x 1metre in two replications. The seedlings were thinned to one/hill three weeks after seedling emergence. Staking was done at 3 weeks after planting (WAP). At 4WAP, NPK (15:15:15) fertilizer was applied at the rate of 60 kg ha<sup>-1</sup> by ring application as recommended by (Togun and Olatunde, 1988). Hoe weeding was carried out regularly at 3 weeks interval.

The morphological and agronomic characters were measured using the descriptor developed by Adewale and Dumet (2011). Twenty-three quantitative agronomic descriptors (including vegetative and reproductive parameters) were generated for each of the 96 accessions. The 23 quantitative data were subjected to analysis of variance (ANOVA) and the means of the 96 accessions were separated using Tukey's Honestly Significant Difference (HSD) at  $P = 0.05$ . The same data were subjected to the Stepwise discriminant analysis (PROC) STEPDISC in SAS) to be able to identify the individual discriminatory significance of the twenty-three characters for the 96 accessions. The process identified eighteen significance ( $P \le 0.05$ ) variables. Furthermore, means were generated across the two replications for the 96 accessions, giving rise to a 96 x 18 table matrix which was subjected to Gower genetic distance (Gower, 1971). The resultant paired products from the genetic distance were further subjected to principal component and clustering analysis of Ward (1963). All analysis was carried out in SAS (Version 9.4, 2011). Intra-cluster variability and/or similarities among the grouped accessions was accessed for the eighteen phenotypic characters using ANOVA.

## **Results and Discussion**

## *Results*

The descriptive and variance characteristics of the twenty-three quantitative traits employed for the characterization of the 96 African yam bean (AYB) accessions are presented in Table 1.

There was significant (P≤0.01) variation among the 96 accessions for all the characters except length of matured flower buds (Table 2). The range and means of each traits were equally presented in the Table. The coefficient of variation for the 23 traits seems moderate, the least (5.04%) and the highest (23.02%) was recorded for terminal leaves and petiole length at 8 weeks after planting (WAP) and pods per peduncle respectively (Table 2).

Table 3 present the stepwise discriminant analysis process for the selection of significant discriminatory variables. The procedure selected only 18 phenotypic characters whose significance probability was less than or equal to 0.05 (Table 3). Seed width was the last to be selected for significance, with  $P = 0.0166$ , the remaining five characters below seed width had probability  $> 0.05$  (Table 3).

Eighteen principal component (PC) axes explained the total (100%) variation among the 96 accessions (Table 4). The first five PC axes had eigenvalues  $> 1.0$ , the same explained 71% of the total variation among the 96 accessions (Table 4). Within the first PC axis, the number of primary branches measured at 4, 5 and the 6<sup>th</sup> WAP positively and significantly (eigenvector > 0.2) contributed to explaining the 23% variance in the axis (Table 4). However, within the same axis, the contribution of the floral traits (days to peduncle initiation, days to first, days to 50% and days to 100% flowering) were negative but significant (eigenvector > - 0.2). Pod and seed characters were positively and significantly prominent in their contribution to PC2 axis variance (Table 4). Leave characteristics and days to pod maturity had significant eigenvectors in PC3 (Table 4).

At the 0.075 similarity inflection point in Figure 1, three main clusters were feasible with the respective accession populations of: 54, 31 and 11. Five clusters were conspicuous at 0.05 similarity and eight were feasible at 0.025 (Figure 1). At 0.00 point of similarity in Figure 1, no duplicate accession(s) was observed; however, the cluster history revealed TSs22 and TSs5 as the most similar accessions.

From Gower genetic distance method, the genetic similarity within cluster I from Table 5 was 0.83. The 54 accessions in the cluster had superior performance for the three seed metrics, pod length and all traits relating to primary branching. The thirty-one accessions in cluster II (whose genetic distance was 0.82) had prominent performance for leave-related characters, peduncle initiation and pod maturity (Table 5). The 54 (cluster I) and 31 (cluster II) accessions showed significant ( $P < 0.01$ ) variation for the eighteen characters. Cluster III had a genetic similarity of 0.80, seven out of the eighteen variables had probability  $> 0.05$ . The remaining 11 characters significantly ( $P \le 0.01$ ) differentiated the eleven accessions in the cluster.

# *Discussion*

The Analysis of Variance revealed significant differences among the ninety- six accessions of African yam bean for the twenty- two phenotypic traits; this seem to depict that each of the 96 accessions exhibited unique genetic identity. In the present study, TSs 22A exhibited early maturity, being the first accession to attain flowering; moreover, the highest seed length and seed thickness was recorded in TSs 120. The significant and higher quantitative score for primary branching observed in some accessions in this study is a reflection of vigor; aggressive growth habit may have supported the production of many flowers that gave rise to many pods and seeds. There are some documented similar reports on the phenotypic performance of different accessions of African yam bean germplasm: twenty-five accessions (Popoola *et al.*, 2011), seventy-nine accessions (Adewale *et al.,* 2012), fifty accessions (Aremu and Ibirinde, 2012), twelve accessions (Abdukareem, *et al.*, 2015) etc. In this study, the cluster history could not identify duplicate accessions at the similarity point of 0.00 in the Ward dendogram and the highest point of similarity among the 96 accessions by Gower genetic distance was 0.95;

with respect to the studied variables, we therefore speculate that each of the 96 AYB accessions seem to have unique genetic composition. The significance of the finding is that each of the 96 accessions is a credible genotypic candidate, none is a duplicate of the other and the conservation of each in the gene bank as a unique taxon could be worthwhile. Traits with significant discriminatory properties among the 96 accessions included: vegetative/vigour (number of primary branches at 4, 5 and the  $6<sup>th</sup>$  weeks after planting), floral (days to peduncle initiation, days to first, 50% and 100% flowering) and pod and seed characters including days to pod maturity. However, further understanding of the genomic diversity of the same accessions used in the present study could be investigated for an approval or disapproval of our present claim.

Our result depicts substantial (about 20%) intraspecific variability among the 96 accessions. Furthermore, high intra-cluster variability existed within clusters I and II, the 18 quantitative traits differentiated the 54 and 31 accessions in clusters I and II. Seven phenotypic characters (number of leaves 3 WAP, terminal leave width, terminal leave length and petiole, pod length and the three seed metric measures) could not differentiated the eleven accessions in cluster III. The seven variables therefore provided the basis for similarity among the eleven accessions, while the remaining eleven differentiated them phenotypically. Our result concurs with the norm: while similarities exist among genotypes, cultivars and accessions of the same species for some phenotypic features, specific significant phenotypic variation(s) distinguishes the taxa in a species (Taia, 2005). Furthermore, we observed high and significant intra-cluster diversity within clusters containing large number of taxa. The high intra-cluster variability seems to mask the unifying feature/trait for such groupings. Therefore, for meaningful and reliable selection within a highly variable cluster, sub-clusters or sub-sub-clusters would be most appropriate group for selection because of low within-group diversity.

The use of phenotypic traits for characterization of germplasm is highly subjective because phenotypic expression is a function of the genotype, the environment and their interaction (Mohammadi and Prasanna, 2003). Meanwhile, phenotypic characterization is preliminarily very useful and primary to providing a guide to gene banking, especially for core collection identification. Reliable characterization of germplasm is possible with phenotypic data if "trash variables" according to Kaufman and Rousseeuw (1990) are disallowed. It is remarkable to state here that non-informative variables (redundant variables) yields lots of random terms in distances; thereby hiding the useful information provided by the other variables (Kaufman and Rousseeuw, 1990) and their effect could mask reliable classification, thus leading to misleading conclusion of a diversity assessment (Adebo *et al.*, 2016). Fowlkes *et al.* (1988) cited in Kaufman and Rousseeuw (1990) documented some trait selection processes. Stepwise discriminant analysis, a trait selection programme in SAS have been successfully employed to trim down the number of qualified traits for genotype classification by Gutierrez *et al*. (2003), Adewale *et al.* (2012) and Adebo *et al.* (2016).

The essence of the selection procedure before proceeding to diversity study is to identify variables which truly distinguishes the genotypes under assessment, such that variables whose distinguishing properties are weak or low (P>0.05) are rejected and disallowed to be used for diversity analysis. The incorporation of trait selection statistical procedure before phenotypic-based diversity analysis would lead to the generation of reliable genotype grouping and meaningful principal component estimates. Therefore, from our study, with the eighteen variables selected, we have reliably presented a worthwhile trait-based selection guide for the 96 African yam bean accessions. This documentation will support their reliable conservation in the germplasm at the Genetic ResourcesCentre, IITA, Ibadan, Nigeria.

# **Acknowledgements**

The authors wish to acknowledge the Genetic Resources Centre (GRC), International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria for providing the genetic resources materials and other resources for the conduct of this reported research.

## **Conflict of Interests**

none

#### **Tables, Figures and Charts**

Table1: List of the ninety-six African yam bean accessions obtained from the Genetic Resources Centre, International Institute of Tropical Agriculture, Ibadan, Nigeria.



Table 2: Descriptive and variance statistics of the twenty-three quantitative traits employed for diversity analysis of the ninety-six African yam bean accessions



† HPP3WK= Height at primary branching 3 weeks, NOPB4WAP= Number of primary branching 4 weeks after planting, NOPB5WAP= Number of primary branching 5 weeks after<br>planting, NOPB6WAP= Number of primary branching 6 weeks after height 3 weeks, PETLEN8WK= Petiole length 8 weeks, TMLL8WK= Terminal leaf length 8 weeks, TMLW8WK= Terminal leaf width 8 weeks, TMLAPL8WK= Terminal leaf length and petiole length 8 weeks, DTPEDINI= Days to peduncle initiation, DT1Flower= Days to first flowering, DT50Flower= Days to 50% flowering, DT100%Flower= Days to 100% flowering, LOMTB= Length of matured flower bud, PODL= Pod length, POD/PED= Pods per peduncle, DTPODM= Days to pod maturity, SEEDL= Seed length, SEEDW= Seed width, SEEDT= Seed thickness

# Table 3: Significant trait selection by Stepwise Discriminant Analysis procedure



**†** SEEDW= Seed width, SEEDT= Seed thickness, NOL3W= Number of leaves 3 weeks,DT50PB= Days to 50% primary branching, DT1, 50 and 100 FL= Days to first, 50% and 100% flowering, PETL8W= Petiole length 8 weeks, POD/P= Pods per peduncle, DTPEDIN= Days to peduncle initiation, DT50S= Days to 50% secondary branching, PODL= Pod length, NOPB 4, 5 and 6W= Number of primary branching4, 5 and 6 weeks, SEEDL=Seed length, HPB3W= Height at primary branching 3 weeks, TMLL8W= Terminal leaf length 8 weeks, LMATFB= Length of matured flower bud, TLW8W= Terminal leaf width 8 weeks, TLAPL8W= Terminal leaf length and petiole length 8 weeks, DTPM= Days to pod maturity.

Table 4: Eigenvalues, proportion of variance of the eighteen principal component axes and eigenvectors of the eighteen phenotypic traits.



**†**PC= Principal Components, NOL3WAP= Number of leaves 3 weeks after planting, NOPB4WAP= Number of primary branching 4 weeks after planting, NOPB5WAP= Number of primary branching 5 weeks after planting, NOPB6WAP= Number of primary branching 6 weeks after planting, DT50PB= Days to 50% primary branching, DT50SB= Days to 50% secondary branching, TMLW8WK= Terminal leaf width 8 weeks after planting, TMLAPL8WK= Terminal leaf length and petiole length 8 weeks after planting, DTPEDINI= Days to peduncle initiation, DT1FL= Days to first flowering, DT50FL= Days to 50% flowering, DT100FL= Days to 100% flowering, PODL= Pod length, PODSPED= Pods per peduncle, DTPODM= Days to pod maturity, SEEDL= Seed length, SEEDW= Seed width, SEEDT= Seed thickness



Figure 1: Dendrogram showing the grouping of ninety six accessions of African yam bean based on similarities for the eighteen phenotypic traits



Table 5: Intra-cluster descriptive information for the three clusters

**†** Pr>F= Measure of probability, CV= Coefficient of Variation, NOL3WAP= Number of leaves 3 weeks, PH3 WAP= Plant height 3 weeks, HPB3WAP=Height at primary branching 3 weeks, NOPB4, 5 and 6 WAP= Number of primary branching 4, 5and 6 weeks, DT50PB= Days to 50% primary branching, DT50SB= Days to 50% secondary branching, PETL8 WAP= Petiole length 8 weeks, TERMLL8 WAP= Terminal leaf length 8 weeks, TERMLW8 WAP= Terminal leaf width 8 weeks, TERMLAPL8 WAP= Terminal leaf length and petiole length8 weeks, DTPEDINI= Days to peduncle initiation, DT1,50 and100FLOWER= Days to first, 50% and 100% flowering, LOMATFBUD= Length of matured flower bud, PODS/PED= Pods per peduncle, DTPOMAT=days to pod maturity, SEEDTHICK= Seed thickness.

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