

Research Article

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Pre-breeding highlights of intraspecific polymorphism and genetic estimates of seed traits of African yam bean (*Sphenostylis stenocarpa* Hochst Ex. A. Rich.) Harms breeding lines

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Abstract

Discriminatory morpho-metric features are obvious on legume seeds. This study utilized seven quantitative and 11 qualitative seed traits to characterize 139 African yam bean (AYB) breeding lines which were developed through single seed descent procedure. The seven quantitative data were subjected to analysis of variance, their means were combined with qualitative scores for genetic distance, principal component (PC) and clustering analyses. Significant ($P \leq 0.001$) variation existed among the breeding lines for the seven traits. Mean ranges of seed length (SL), width (SW), thickness (ST) and a single seed weight (SSW) among the 139 breeding lines were respectively: 6.77–10.22 mm, 5.70–7.86 mm, 4.96–7.45 mm and 0.15–0.42 g. Positive and significant ($P \leq 0.05$) genotypic correlation existed among SSW, SL, SW and ST. Seed colours, pattern, shapes, sizes, surface texture, brilliance varied among the breeding lines. Ranges of phenotypic and genotypic coefficient of variation and broad-sense heritability were: 5.49–23.84%, 2.95–19.88% and 28.91–69.54% respectively. Fourteen (quantitative and qualitative) traits contributed higher (≥ 0.30) eigenvector loadings to the first three PC axes which explained 57.9% of the total variation among the breeding lines. Similarity among the lines was 0.75. Four clusters ensued in the dendrograph and each group had genetic similarities of: 0.85 (I), 0.82 (II), 0.78 (III) and 0.80 (IV). This research unveiled significant variation among AYB breeding lines with promising reliability for breeding opportunities of the qualitative and quantitative seed traits, which could contribute to higher grain yield and acceptability.

Introduction

Food, feed and nutrition security is only attainable by increase production of different crop types at optimal quality and quantity. However, legumes pulses are ahead of other plants part as the major source of dietary protein (Balan and Predeep, 2021). The present study is part of the move to promote acceptability of less known crops, especially legumes in human and livestock meals because they differ in profile, digestibility, bioavailability, consumers' acceptability etc. (Henchion *et al.*, 2017). African yam bean (AYB) (*Sphenostylis stenocarpa* Hochst A. Rich.) Harms, a tuberous legume evidentially hosts lots of food and nutritional values in the seed and the tuber for human and livestock. Its significance among major legumes and tuberous crop is very poor and its global production statistics is unknown till date, however, Nigeria obviously leads in research attention, cultivation and production (Adewale and Nnamani, 2022; Adewale, 2023).

Genetic resources for most previous AYB characterization had been mostly accessions and sometimes landraces. Accessions and landraces are 'raw' plant genetic resources with unknown genetic profile but are usually the basic stocks for the development of breeding lines. Accessions are recovered genetic resources from domesticated and explored landraces; they hold significant characteristics which are discovered during characterization and evaluation programmes. While accessions and landraces have not been selected, breeding lines have undergone some cycles of breeding/selection. Genetic resources for the present study are breeding lines. Moreover, phenotypic profiling of breeding lines leads to identification of individuals with potential to provide platform for selection of genotypes for cultivar development programmes. Breeding lines have traceable link to landrace(s) or accession(s), however, with some peculiar characteristic needing to be assayed and explored.



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Pre-breeding profiling or characterization (genotypic or phenotypic) describes, classifies and unveils genetic resources potentials within a gene pool for guided trait-based selection. The same process equally identifies superior genotype(s) as possible parents for subsequent hybridization, linking superiority to specific trait(s), and direct listing and recommendation of genetic materials for advancement. Genetic estimates of quantitative traits provide a predictive measure of the responses of traits to genetic improvement. According to Muluaem and Michael (2013) higher values for most genetic estimates suggests the occurrence of additive gene action with low environmental influence on the trait.

It is expected that germplasm collectors and the primary user of genetic resources must be thoroughly versed and familiar with intra-specific variation of their target taxa (Hanelt and Hammer, 2011). The present investigation therefore employed seed characteristics of the developed 139 breeding lines of AYB diversity assessment. Seed is central to human and livestock livelihood on earth (N'Danikou *et al.*, 2022), seed characteristics of legumes reveal significant intra-specific variabilities (Moles *et al.*, 2005; Bareke, 2019), most of which are highly heritable (Blair *et al.*, 2010). Seed traits therefore are very important for phenotypic diversity assessment, providing reliable leverages for genetic improvement and presenting reliable determinants for commercial acceptability of varieties (Adewale *et al.*, 2010; Rana *et al.*, 2015). The present study employed diversity assessment among 139 AYB breeding lines using some phenotypic traits whose genetic estimates were equally discovered to reliably guide direct or indirect selection of genotypes for subsequent cultivar improvement programme for the crop.

Materials and methods

Source of basal genetic materials for the experiment

In 2019, seeds of 30 AYB accessions were collected from the Genetic Resources Centre (GRC), International Institute of Tropical Agriculture, Ibadan Nigeria. Some quantities of seed were purchased from Pagede village, Owo, Ondo state, Nigeria same year and seven landraces were sorted from the seed mixtures, using size, colour and colour patterns as descriptors.

Experimental layout and breeding system

The 37 genetic materials were grown out in row plots of five plants in three replicates at the Teaching and Research farms, Federal University Oye-Ekiti, Ikole-Ekiti Campus, Nigeria. The single seed descent breeding method was employed. Each plant within a row was isolated by bagging with a fine mesh plastic net. This was done according to selfing technique for AYB previously reported by Adewale and Adegbite (2018). At harvest of the isolated plants, 59 genetic resources (S1) emerged based on testa colours and colour patterns. In 2021, five uniform seeds, each of 51 S1 were sown in a single row of unreplicated trial. One plant in each row plot was isolated. Harvest from the isolated 51 S1 plant gave rise to 72 different S2 seed lots which were planted out in 2022 for further selfing to generate S3 progenies. Only 16 variants (with significant features) different from lines in S1 and S2 were recorded for S3. From the three cycles of selfing plants from single seeds, (i.e. S1 (51), S2 (72) and S3 (16)), a total of 139 genetic materials or breeding lines were considered for this study.

Data collection and analysis

Four quantitative data (individual seed weight, seed length, width and thickness) were generated from 10 random seed samples in the seed lots. Furthermore, three ratios between seed length, width and thickness were estimated and 11 qualitative data were also recorded.

Individual seed weight, seed length, width and thickness were assessed for normal distribution. To assess the reliability of the measured traits for quantitative description, means of seed length, width, thickness and their ratios were all compared in pairs using paired *t*-test statistics. For the seven traits, minimum and the maximum data were utilized to generate the coefficient of range (CoR) as:

$$\frac{\text{Largest value} - \text{Smallest value}}{\text{Largest value} + \text{Smallest value}} \quad (1)$$

Analysis of variance (ANOVA) was conducted as completely randomized design for the seven quantitative data, using PROC GLM and the treatment means (genotypes) were separated using Tukey honestly significant differences in SAS (version 9.4, 2011). The relationship among the seven quantitative traits was verified using analysis of covariance (ANCOVA). Phenotypic, genotypic coefficient of variations and broadsense heritability were estimated from variance components according to Singh and Chaudhury (1985). From the variance components in the ANCOVA, phenotypic and genotypic correlation coefficients were estimated following Miller *et al.* (1958) and their respective significance were tested following Singh and Chaudhury (1985) to generate the calculated *r*-values. The significance of each correlation coefficients was detected by the comparison of the calculated with the tabulated *r*-values at *g*-2 degrees of freedom, where *g* is the number of genotypes. Coheritability was estimated as: $(GCOV_{X_1X_2}/PCOV_{X_1X_2})$ following Sahu (2013) and Farshadfar and Estehghari (2014); where: $GCOV_{X_1X_2}$ and $PCOV_{X_1X_2}$ were the respective genotypic and phenotypic covariances for paired traits. Mean of the seven quantitative traits and the descriptive scores for the 11 qualitative traits for each genotype were prepared as 139×18 mean matrix table for multivariate analysis. From the data matrix, genetic distance, principal component (PC) and clustering analysis were carried out in SAS. Mean performances, similarity and variability among members within each cluster were equally estimated for the 18 phenotypic variables.

Results

From Fig. 1, the mean ranges of the different metric dimensions and individual seed weight of the 139 AYB breeding lines were: 6.77–10.22 mm (seed length), 5.70–7.86 mm (seed width), 4.96–7.45 mm (seed thickness) and 0.15–0.42 g (individual seed weight). ANOVA revealed significance ($P \leq 0.001$) among the 139 AYB breeding lines for: individual seed weight, seed length, width, thickness and their ratios; furthermore, the coefficient of variation for the seven traits was less than 10% (Table 1). The length, width, thickness of AYB seeds and their ratios differed significantly ($P \leq 0.001$) from each other (online Supplementary Table S1). From this study, mean individual seed weight, seed length, width and thickness of AYB were respectively: 0.28 g, 8.61, 6.69 and 6.23 mm. However, in some breeding lines, a single seed could weigh 0.42 g, be as long as 10.5 mm with possible width and thickness

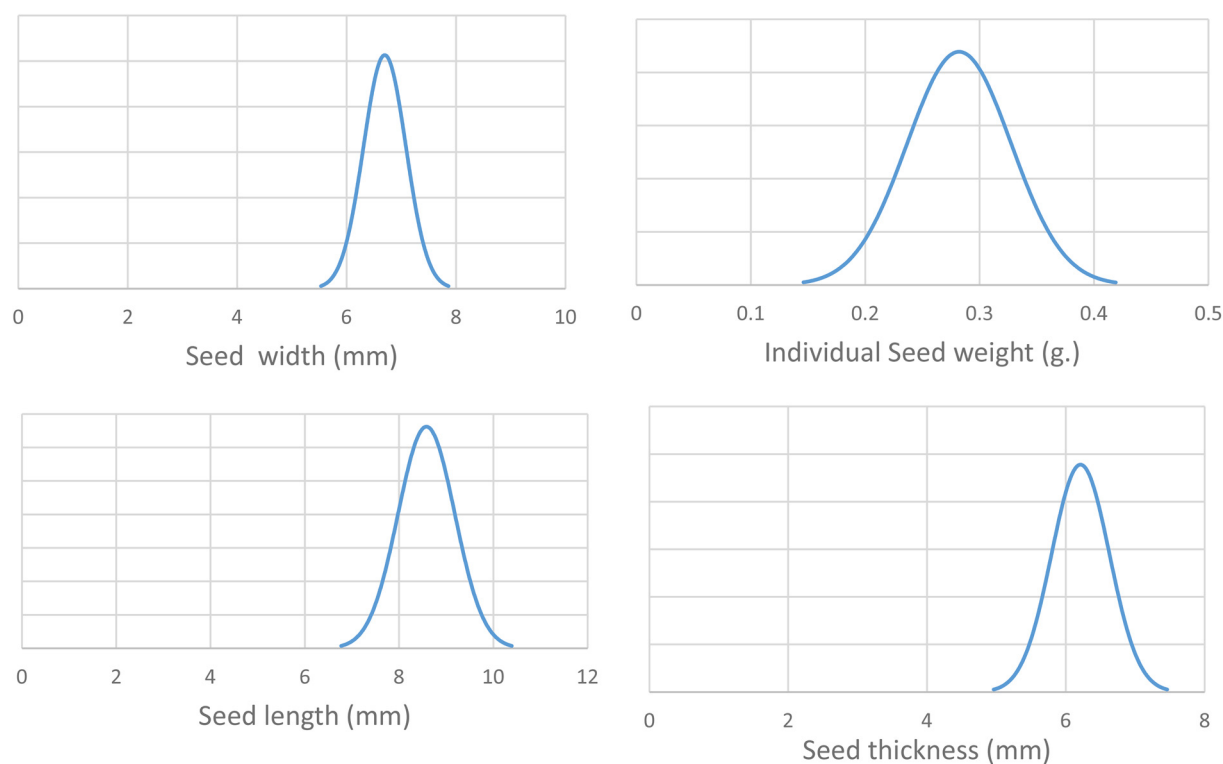


Figure 1. Normal distribution pattern of four seed metrics of the 139 African yam bean accessions.

of: 7.88 and 7.57 mm (Table 1). CoR was highest (38.10%) in individual seed weight and least (12.25%) in width to thickness ratio. The seed width to thickness ratio had the least values for HB, PCV and GCV, but the highest values for the same occurred in seed length (Table 1). The highest and the least genetic advance respectively occurred in individual seed weight and width to thickness ratio (Table 1). Generally, by magnitude, PCV were higher than GCV. Six of the quantitative traits had $\geq 50\%$ broad sense heritability, the seed length had the highest (Table 1). Table 2 presents the phenotypic and genotypic correlation coefficients among the seven quantitative traits. Among the 42 correlation coefficients, six were negative, five of which were phenotypic correlations. Only four were positively significant ($P \leq 0.05$), three of which were genotypic correlations among pairs of individual seed weight, seed length, width and thickness. Only the correlation between individual seed weight and seed length had positive and significant phenotypic and genotypic correlation (Table 2). Among the 21 coheritability estimates, only four were negative; all occurring between seed thickness and the three seed metrics ratios (LW, LT and WT) and between seed width with seed length: thickness. The highest positive coheritability was between seed length and width, and the least was between LW and WT (Table 2). Only six PC axes had eigenvalue ≥ 1.0 , the six explained 79% of the total variation among the 139 AYB breeding lines and eigenvalues, and variance proportion declined from PC1 to PC6 (Table 3). Contribution to variability among the 139 breeding lines as revealed by eigenvectors loadings identified SCC, SCCP, TBCOVs and PTestaV to be most prominent in PC1 with loadings > 0.30 . Prominently in PC2 and PC3, the seven quantitative variables, seed shape and size were most significant, with eigenvectors loadings well above 0.30 (Table 3).

The 139 AYB breeding lines grouped into four distinct clusters at the inflection point of 0.05 in Fig. 2. Memberships in each of the

four cluster were: 29, 46, 19 and 45 respectively. At the inflection point of 0.1 in Fig. 2, clusters I, II and III had merged into a group, furthermore at the similarity coefficient point of 0.185, the 139 breeding lines could no more be distinguished. In Table 4, common features within each cluster were presented. Cluster I had the highest mean for all the seven quantitative traits. Genotypes in cluster I were monocoloured, 62% (white) and 38% (brown). The mostly shining and smooth seeds fell within the medium and large seeds, majority (55%) of which were oblong in shape (Table 4). Least values for individual seed weight, seed length, width and thickness were obtained in cluster II. Within this group, the different forms of possible testa colours in AYB existed, the four possible seed shapes (round, oval, oblong and rhomboid) also existed in different proportions in the cluster. Furthermore, sizes of the 46 breeding lines were within small and medium, more of them were smooth with shining brilliance (Table 4). The 19 genotypes in cluster III had the least values for the three seed metric ratios, 84% were monocoloured. Most of them were round, and few genotypes had oval and oblong shapes. Among the mostly (42%) medium sized-seed breeding lines, 68 and 63% of the breeding lines respectively had matt brilliance and wrinkle testa (Table 4). Cluster IV had the highest proportion (84%) of the breeding lines with mosaic seeds with different patterns in the study. Among the 45 lines in the cluster, percentage shining brilliance was 89 and 71% of the genotypes had medium size (Table 4). Online Supplementary Fig. S1 present arrays of seed sizes, shapes, brilliance, testa colours and colour patterns in AYB.

Discussion

Breeding lines are plant breeders' 'working-germplasm' during cultivar development programmes. The present study is the first report on characterization of AYB breeding lines; genetic

Table 1. Analysis of variance showing components, descriptive statistics and genetic estimates of the seven quantitative traits employed in the diversity assessment of the 139 African yam bean breeding lines

Traits	ANOVA			Descriptive statistics				Genetic estimates				
	Mean square (MS)	Error MS	CV (%)	Means	Min.	Max.	CoR (%)	HB (%)	PCV (%)	GCV (%)	GCV/PCV (%)	GA (%)
Wgt. (g.)	0.022***	0.001	9.57	0.28	0.19	0.42	38.1	68.64	10.65	8.82	82.82	28.46
SL(mm)	3.721***	0.138	4.31	8.61	6.96	10.54	20.46	69.54	23.84	19.88	83.39	11.64
SW(mm)	1.574***	0.069	3.92	6.69	5.57	7.88	17.17	63.57	18.45	14.71	79.73	9.34
ST(mm)	0.078***	1.795	4.47	6.23	5.17	7.57	18.84	58.34	19.66	15.02	76.4	9.47
LW	0.075***	0.005	5.27	1.28	1.07	1.53	17.66	53.58	9.26	6.78	73.22	9.03
LT	0.151***	0.007	6.16	1.39	1.09	1.91	27.62	49.9	10.88	7.69	70.68	9.49
WT	0.028***	0.002	4.25	1.08	0.99	1.28	12.25	28.91	5.49	2.95	53.73	3.14

ANOVA, Analysis of variance; Wgt, Individual seed weight; SL, Seed length; SW, Seed width; ST, Seed thickness; LW, Length to width ratio; LT, Length to thickness ratio; WT, Width to thickness ratio; CoR, Coefficient of range; HB, Broad-sense heritability; PCV, Phenotypic coefficient of variation; GCV, Genotypic Coefficient of variation; GA, Genetic advance.

*** – Significance at the probability level of $P \leq 0.001$.

Sample size (n) = 139.

Table 2. Phenotypic (p) Genotypic (g) correlation coefficients and coheritability (CoHb) for the seven quantitative traits

	SL	SW	ST	LW	LT	WT
Wgt						
p	0.2841*	0.1317	0.1308	0.0003	0.0005	0.0001
g	0.3459**	0.2448*	0.2596*	0.0633	0.0797	0.0427
CoHb	0.4646	0.3831	0.3659	0.0062	0.0081	0.0015
SL						
p		0.0842	0.0752	0.0036	0.0007	0.0002
g		0.0178	0.0189	0.0046	0.0058	0.0031
CoHb		0.8374	0.8115	0.5048	0.5452	0.0869
SW						
p			0.1339	-0.0021	-0.0024	0.0002
g			0.0268	0.0065	0.0082	0.0044
CoHb			0.8326	0.6008	-0.4495	0.0684
ST						
p				-0.0018	-0.0042	-0.0005
g				0.0062	0.0077	0.0042
CoHb				-0.5414	-1.3449	-0.1771
LW						
p					0.0089	0.0001
g					0.0318	0.017
CoHb					0.1984	0.0002
LT						
p						0.0005
g						0.0135
CoHb						0.0461

Wgt., Individual seed weight; SL, Seed length; SW, Seed width; ST, Seed thickness; LW, Length to width ratio; LT, Length to thickness ratio; WT, Width to thickness ratio.

*, ** – Significance at the probability level of $P \leq 0.05$ and 0.01 respectively.

Table 3. Proportions of variance, eigenvalues and eigenvector loadings of the 18 traits in each of the principal component axes

Principal components	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	4.67	3.72	3.20	1.76	1.38	1.07
Variance proportion (%)	23.34	18.61	15.98	8.80	6.88	5.37
Cumulative variance (%)	23.34	41.95	57.93	66.73	73.61	78.98
Traits	Eigenvectors					
Individual seed weight	0.24	0.01	0.44	-0.03	0.13	0.04
Seed length	0.22	0.32	0.28	0.07	0.18	0.06
Seed width	0.22	-0.10	0.44	0.04	-0.18	-0.09
Seed thickness	0.16	-0.30	0.37	-0.10	0.19	0.04
Seed length to width ratio	0.04	0.42	-0.10	0.04	0.34	0.14
Seed length to thickness ratio	0.05	0.49	-0.05	0.14	-0.02	0.02
Seed width to thickness ratio	0.05	0.31	0.03	0.20	-0.51	-0.16
SCC	0.37	-0.13	-0.17	-0.01	-0.04	0.13
SCCP	0.38	-0.08	-0.24	0.10	0.12	-0.10
Sshape	0.03	0.37	0.05	-0.08	0.22	0.19
Punching	0.12	0.10	0.06	0.14	-0.54	0.09
SEBRI	-0.15	-0.07	-0.05	0.57	0.07	0.25
SPLtesta	-0.04	-0.23	0.06	0.17	0.00	0.38
TestaTex	-0.12	-0.10	0.09	0.52	0.11	0.35
TBCOVS	0.37	-0.04	-0.20	0.16	-0.06	-0.07
PTestaV	0.34	-0.06	-0.27	0.10	0.10	-0.09
ECOWS	-0.02	-0.10	0.10	0.45	0.12	-0.58
Sesize	0.20	0.15	0.34	0.08	0.06	-0.08

SCC, Seed Coat colour; SCCP, Seed Coat Colour Pattern; TestaTex, Testa Texture; SP Testa, Splitting/Cracking of Testa; Sshape, Seed shape; SEBRI, Seed Brilliance; TB COVS, Testa Basal Colour on Variegated Seed; P Testa V, Pattern of Testa Variegation; TB COVS, Testa Basal Colour on Variegated Seed; ECOWS, Eye Colour of White Seed; sesize, Sizes of Seed.

resources for earlier diversity reports had either been accessions and/or landraces. Since they are the genetic materials for future advancement to new cultivars, harnessing the morpho-metric diversity of their seeds was a priority, hence the present investigation. The present study wishes to stage a platform for parental seed stock selection for future cross breeding and hybrid development based on identification of the individual potentials among the lines and the genetic estimate of the studied traits.

The length, width, thickness and their ratios were distinct variables, paired comparison by *t*-test statistics informed significant differences among them, thereby ascertaining their uniqueness as phenotypic traits capable of employment in descriptors and characterization of AYB. Above information is an update and in consonance with earlier remarks from: Adewale *et al.* (2010), Aina *et al.* (2020) and Shitta *et al.* (2022). The parametric information from the range (minimum to maximum), coefficients of range and variation hinted on the presence of wide variability among the 139 AYB breeding lines for the studied traits, this informs of available diversity among the 139 genotypes. Moreover, higher magnitude for phenotypic and genotypic coefficient of variation informs of inherent wider variability among the genetic resources under study for the specific trait. The present study identified, seed coat colour, seed coat colour pattern, testa basal colour on variegated seed, pattern of testa variegation, seed shape and sizes (single seed weight, dimensional metric

measures and their ratios) as very important distinguishing characteristics for AYB seeds. Notable testa colours in AYB include grey, white, light to dark brown, dark purple/black etc. and different forms of variegations or marbling patterns. Qualitative variabilities in AYB ranges in various sizes and intensities. Variations on testa are prominent features in legumes (Cervantes *et al.*, 2016; Bareke, 2019; Bria *et al.*, 2019; Balan and Predeep, 2021). Seed size, colour and shape have very high taxonomic values to distinctively reveal divergence among genetic resources compared to the vegetative variables (Rana *et al.*, 2015; Cervantes *et al.*, 2016).

The quantitative traits showed wide range of variability among the 139 breeding lines of AYB. This is in consonance with report of Rana *et al.* (2015) on 4274 accessions of *Phaseolus vulgaris* and Ruelle *et al.* (2019) who worked on 1296 genetic resources of: *Phaseolus vulgaris* L., *Pisum sativum* L., *Vicia faba* L., *Arachis hypogaea* L. and *Trigonella foenum-graecum* L. Nnamani *et al.* (2021) observed outstanding performances of large-seeded AYB accessions, attributing this to the possession of higher food reserve. This seems to infer that larger seeds hold better promises for higher protein content, larger or heavier seeds could positively correlate with high protein content. Major proteins in pulses are contained in the cotyledon and the embryonic axis of the seed, however, fairly good quantities are present in the seed coat of various legumes (Henchion *et al.*, 2017). Therefore, selection of

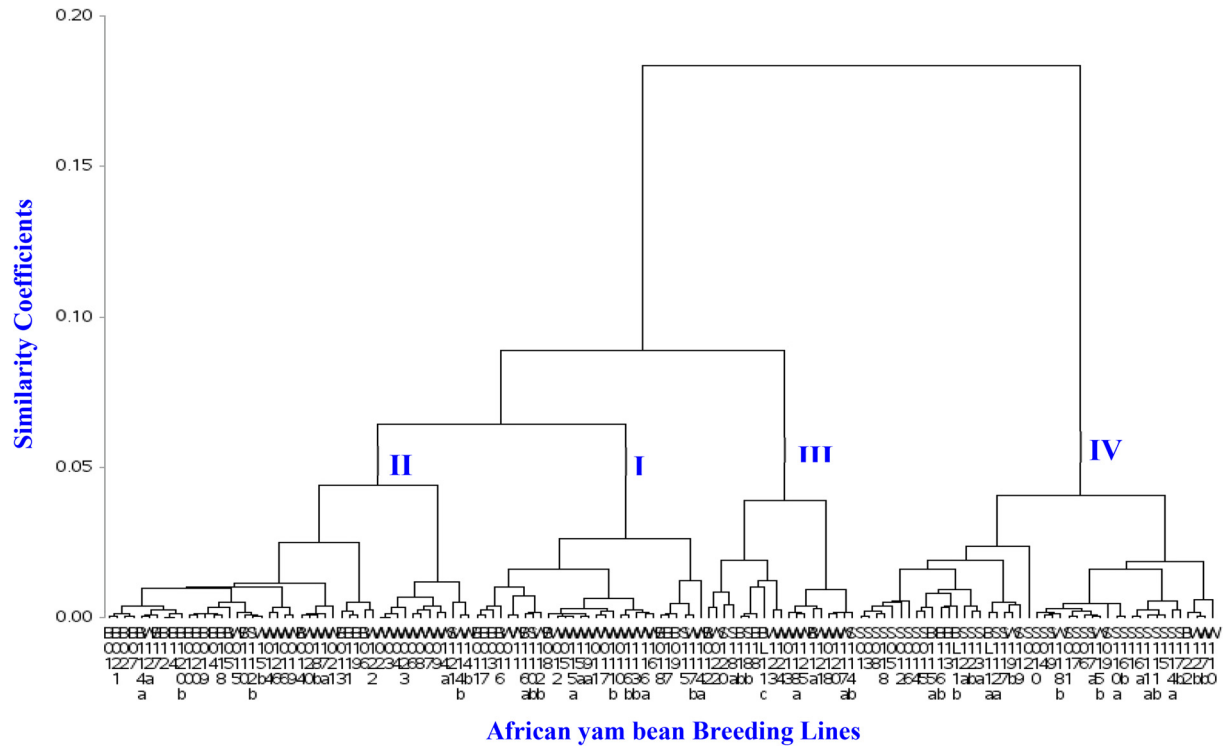


Figure 2. Dendrogram showing aggregation by similarity and partitioning in to groups of 139 African yam bean breeding line.

W01 with mean individual seed weight of 0.42 g and its recommendation for continual production may be promising for increased protein supply. Moles *et al.* (2005) added that large-seeded species or genotypes have seedlings that are better able to tolerate many of the stresses encountered during seedling establishment. Furthermore, seed weight determines suitability for end-use, heavier seeds commands better market price (N'Danikou *et al.*, 2022).

Proportion of the genetic component in the phenotypic measurements highlights the level of reliability for such traits. For instance, traits whose expression is less dependent on the environment offer significant promises for genetic advance in the breeding programme. High broad sense heritability, genetic advance and very high genotypic:phenotypic coefficient of variability ratio are vital genetic estimates to guide direct selection of the best individuals for successful genetic improvement. The additive component in the broad sense heritabilities of the various traits in this study, was not available, further work is needed to ascertain this. However, the broadsense heritability and genetic gain obtained in this study was like that of Adjei *et al.* (2023). Broad-sense heritabilities recorded for seed length (70%) and width (64%) in our study was lower compared to what Rana *et al.* (2015) obtained in the characterization of 4274 *Phaseolus vulgaris* accessions. Among all the quantitative traits in the study of Bareke (2019) on some *Phaseolus vulgaris* genotypes, the highest (0.97) broad-sense heritability was obtained in seed length; the same trait had the leading value in the present study. Selection for traits with broad-sense heritability $\geq 70\%$ according to Idahosa *et al.* (2010) is reliable as such magnitude reveals high genetic contribution to the phenotype. However, trait-based selection of genotypes is more reliable if it is dependent on high heritability and genetic advance (Muluaem and Michael, 2013).

Bareke (2019) noted that seed length had a strong and direct relationship with seed width and thickness, our study discovered strong genetic correlation between: individual seed weight, seed length, width and thickness. Selection process in breeding programme is eased when positive correlation (especially genotypic correlation) exists among traits, such situation enhances selection of genotype with numerous associated traits. In this study, heavier seeds of AYB were proportionally longer, wider and thicker. These four traits are therefore well associated such that selection of one had positive, simultaneous and possibly direct influence on the others. The association further inform of the possibility of prediction of values of one trait from another. The significant positive correlation and medium (0.36–0.47) co-heritability of seed weight with seed length, width and thickness showed that selection for any of these traits will proportionally favour simultaneous transmissibility or joint inheritance and improvement of other traits. The reports of Akhtar *et al.* (2007) and Farshadfar and Estehghari (2014) corroborated this assertion. The strong genotypic correlation between seed weight and metric measures affirms the possibility of significant improvement of these seed trait simultaneously in AYB breeding.

From the clustering analysis, every genotype was initially distinct at the 0.0 point of similarity coefficient. Variability among the genotypes began to disappear as the inflection point rose beyond 0.0, leading to the assembly of many genotypes into different clusters each with unique similarity for some traits. Seed coat colour of genotypes in each cluster was not homogenous, thus creating within-group variations among genotypes in the same cluster based on testa colours. This seems to inform that testa colour is not the primary delineating variable for the cluster. Rana *et al.* (2015) who had similar observation in *Phaseolus vulgaris* germplasm suspected random mating among the different coloured groups. Selection of different genotypes has been made

Table 4. Description of the four clusters generated from the dendrogram based on the quantitative and qualitative traits

	Cluster I (<i>n</i> = 29, Genetic similarity = 0.85)	Cluster II (<i>n</i> = 46, Genetic similarity = 0.82)	Cluster III (<i>n</i> = 19, Genetic similarity = 0.78)	Cluster IV (<i>n</i> = 45, Genetic similarity = 0.80)
Quantitative				
Traits	Mean ± SE			
Wt.(g.)	0.317 ± 0.007	0.253 ± 0.005	0.282 ± 0.013	0.292 ± 0.006
SL(mm)	9.157 ± 0.091	8.276 ± 0.059	8.247 ± 0.129	8.729 ± 0.086
SW(mm)	6.918 ± 0.045	6.450 ± 0.064	6.805 ± 0.104	6.774 ± 0.042
ST(mm)	6.276 ± 0.076	6.025 ± 0.065	6.509 ± 0.097	6.301 ± 0.052
LW	1.325 ± 0.011	1.289 ± 0.015	1.214 ± 0.012	1.291 ± 0.012
LT	1.468 ± 0.023	1.383 ± 0.018	1.269 ± 0.014	1.391 ± 0.016
WT	1.107 ± 0.012	1.072 ± 0.005	1.046 ± 0.006	1.078 ± 0.008
Qualitative				
Traits	Proportion (%)			
SCC	White (62.1%), Brown (37.9%)	White (34.8%), Grey (15.2%), Cream (2.2%), Brown (41.3%), Creamy brown (4.4%), Milky brown (2.2%), Mosaic (2.2%)	White (47.4%), Brown (21.1%), Creamy brown (10.5%), Grey dark brown (5.3%), Mosaic (15.8%)	White (6.7%), Brown (6.7%), Black (2.22%), Mosaic (84.4%)
SCCP	Non-variegated (100%)	Non variegated (93.5%), Variegated (6.5%)	Non-variegated (84.2%), Variegated (15.8%)	Variegated (100%)
Sshape	Oval (13.8%), Oblong (55.2%), Rhomboid (31%)	Round (28.3%), Ova l (34.8%), Oblong (32.6%), Rhomboid (4.3%)	Round (63.2%), Oval (31.6%), Oblong (5.3%)	Round (31.1%), Oval (33.3%), Oblong (31.1%), Rhomboid (4.4%)
Punching	Non-punched (69%), Punched (31%)	Non punched (93.5%), Punched (6.5%)	Not punched (100%)	Not punched (75.6%), Punched (24.4%)
SEBRI	Shining (96.6%), Matt (3.4%)	Shining (78.3%), Matt (21.7%)	Shinning (31.6%), Matt (68.4%)	Shinning (88.9%), Matt (11.1%)
SPLtesta	Testa not splitted (100%)	Non splitted (100%)	Testa not splitted (47.4%), Testa splitted (52.6%)	Testa not splitted (75.6%), Testa splitted (24.4%)
TestaTex	Smooth (86.2%), Rough (6.9%), Wrinkle (6.9%)	Smooth (95.7%), Rough (4.3%)	Smooth (26.3%), Rough (10.2%), Wrinkle (63.2%)	Smooth (88.9%), Rough (8.9%), Wrinkle (2.2%)
TBCOV5	White (82.8%), Grey (17.2%)	Non-variegated (63%), White (34.8%), Grey (2.2%)	Non-variegated (68.4%), White (15.8%), Cream (5.3%), Light brown (5.3%), Reddish brown (5.3%)	White (11.1%), Cream (17.8%), Light brown (40%), Reddish brown (11.1%), Dark brown (4.4%), Purple (4.4%), 8(11.1%)
PTestaV	No variegation pattern (100%)	Variegated (93.5%), Sparse black uneven stripes (4.3%), Black patches with uneven stripes (2.2%)	Variegated (84.2%), Sparse black uneven stripe (15.2%)	Dense black uneven stripes (44.4%), Sparse black uneven stripe (55.5%), Black patch uneven stripe (40%)
ECOWS	Non-variegated (17.2%), Clean (44.2%), Brown (20.7%), Dark brown (10.3%), Black (6.9%)	Non-variegated (26.1%), Clean-eyes (54.3%), Brown (10.9%), Dark brown (8.7%)	Non-variegated (5.3%), Clean-eye (26.3%), Brown (42.1%), Dark brown (15.8%), Black (10.5%)	Non-variegated (31.1%), Clean-eye (22.2%), Brown (28.9), Dark brown (2.2%), Black (13.3%), Purple (2.2%)
Sesize	Medium (56.6%), Large (41.4%)	Small (23.6%), Medium (71.7%)	Small (36.8%), Medium (42.1%), Large (21.1%)	Small (6.7%), Medium (71.1%), Large (22.2%)

Wgt, Individual seed weight; SL, Seed length; SW, Seed width; ST, Seed thickness; LW, Length to width ratio; LT, Length to thickness ratio; SCC, Seed Coat colour; SSCP, Seed Coat Colour Pattern; TestaTex, Testa Texture; SPLtesta, Splitting/Cracking of Testa; Sshape, Seed shape; SEBRI, Seed Brilliance; TBCOV5, Testa Basal Colour on Variegated Seed; TBCOV5, Testa Basal Colour on Variegated Seed; ECOWS, Eye Colour of White Seed; sesize, Sizes of Seed.

possible by the groupings leading to unveiling the potentials within each cluster. For example, genotypes with high seed yield potential appeared in cluster I and cluster III contained genotypes with matt brilliance and wrinkle testa surfaces; significant inferential features for low (shortened) cooking time.

The potentials of the 139 AYB breeding lines for the 18 seed traits, their genetic estimates and diversity among them for the traits is unveiled this study. Possibilities for selection of parents in different clusters for crossbreeding programmes that could enhance the production of heterotic hybrids and discovery of gene actions for different quantitative and qualitative traits is equally presented. This primarily offers a reliable platform for selection. Subsequent genotyping of the same 139 genotypes with single nucleotide polymorphism (SNP) technique would be necessary to identify the level of homozygosity/heterozygosity and similarities among genotypes developed through S1, S2 and S3 cycles. The availability of precise genetic profiles through SNP markers for the population can be mapped and correlated with existing phenotypic data, thereby reducing breeding duration and accelerating the crop breeding programme.

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