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RESEARCH ARTICLE

Intraspecific Variability in Agro-Morphological Traits of African Yam Bean *Sphenostylis stenocarpa* (Hochst ex. A. Rich) Harms

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Abstract

Intraspecific variabilities in 40 accessions of African yam bean (AYB) were assessed through characterization of 48 agro-morphological traits for two cropping seasons between June and December 2011 and 2012. Data were analyzed using descriptive statistics, analysis of variance (ANOVA), correlation analysis, principal component analysis, and cluster analysis (Semi - partial R squared method). The accessions showed significant differences ($P \leq 0.05$, $P \leq 0.01$, $P \leq 0.0001$) in 16 reproductive traits. Accessions TSs 66 (144.50 days), TSs 51 (144.67 days) and TSs 154 (144.67 days) were identified as early maturing accessions. The first five principal component axes explained 69.7% of the total variation with PC1 and PC2 contributing 38.9% to the total variation. Correlation coefficients were high and significant for yield traits. A highly significant correlation ($r = 0.99^{***}$) was observed between seed yield (kg ha^{-1}) and weight of total pods per plant. Tubers were produced from 42.5% of the accessions. The accessions of AYB were meaningfully grouped into five clusters at the R-squared distance of 0.04 similarity index. Phenotypically, AYB 57 and TSs 123 were the most similar accessions with the closest distance of 0.0071. Four seed shapes were identified; oval (82.5%), globular/round (5%), oblong (10%), and rhomboid (2.5%). In order to improve the yield of AYB, the number of seeds per pod, number of pods per plant, weight of total seeds per pod, and weight of total seed per plant are important determinant factors. The genetic variabilities observed in the traits studied could be utilised for improvement of AYB.

Key words: African yam bean, characterization, genetic variability, stepwise discriminant analysis, underutilised crops
Abbreviations: AYB: African yam bean, NUS: Neglected and underutilised species, PCA: Principal component analysis

Introduction

Across the world, many of the plant species that are cultivated for food are neglected and underutilised while they play a crucial role in the food security, nutrition, and income generation of the rural poor (Magbagbeola et al. 2010). Most of these neglected and underutilised crop species (NUCS) are nutritionally rich, therefore their erosion can have immediate consequences on the nutritional status and food security of the poor and their enhanced use can bring about better nutrition and fight hidden hunger (Dansi et al. 2012). The concentration on a few major staple crops has resulted in an alarming reduction not only on crop diversity but also the variability within them, especially the neglected and underutilised species (NUS) (Adewale et al. 2013). NUS are indigenous,

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relatively common, available, accessible, well-adapted, easy and cheap-to-produce crops. Moreover, they are culturally linked to the people who use them traditionally (Jaenicke and Pasiecznik 2009; Okigbo 1973; Oniang'o et al. 2006; Padulosi et al. 2003).

One of such underutilised crops is the African yam bean (AYB) *Sphenostylis stenocarpa*, a dual food crop in tropical Africa. It is the most economically important among the seven species of *Sphenostylis* (Potter 1992). The economic importance of AYB as a food security crop for the alleviation of poverty in Sub-Saharan African cannot be over-emphasized. It has high nutritive value producing yields of edible seeds and tubers that contain more than twice the protein in sweet potatoes or Irish potatoes and more than 10 times the amount in cassava roots (NRC 2006). Both the seed and tubers are high in protein content between 15 to 29% and the amino acid values are higher than those in other common legumes; pigeon pea, cowpea and bambara groundnut (Adewale and Dumet 2010; Uguru and Madukaife 2001). AYB can be used to replace cowpea in most food preparation, especially during the lean period when food is scarce among the rural farmers. This exceptionally nutritious pulse has a very significant link with African socio-cultural life. There is a widespread use of AYB in the Nigerian diet, especially in southern states (Obizoba and Nnam 1992). A special meal from it features during the marriage ceremony among the Ekiti's in Nigeria. The rich nutritional content makes AYB of immense importance as a potential food security crop for Africans.

Despite the nutritional benefits of AYB, it is faced with several production constraints which make it remain neglected and underutilised. Increasing the use of underutilised crops is one of the better buffers to reduce nutritional, environmental and financial vulnerability in times of change (Jaenicke and Pasiecznik 2009). This crop requires further research and commercial exploitation. Most landraces remain uncharacterized and are in the hands of resource-poor farmers. Hence, assessing the genetic diversity in the available landraces will enhance breeding for improved varieties. Some reports have been made in literature on the morphological characterization of a few to more accessions of AYB (Akande 2009; Popoola et al. 2011; Adewale et al. 2012; Aremu and Ibirinde 2012). Agro-morphological characterization is the first step for the assessment of genetic variability and identification of desirable traits of interest in AYB. The objective of this research therefore, was to assess the genetic diversity present in 40 accessions of African yam bean accessions using AYB phenotypic descriptors.

Table 1. Descriptive statistics of 48 agro-morphological traits of 40 African yam bean accessions

Characters	Means	Mean Square	CV%
Hypocotyl length	6.45	2.24***	17.01
Number of main branches	2.77	1.49***	27.80
Days to 50% emergence	9.77	1.63	25.09
Days to peduncle initiation	75.20	105.27***	11.94
Days to 50% bud initiation	81.55	84.33**	10.88
Days to 1st flowering	96.58	159.44*	11.36
Days to 50% flowering	106.58	142.46***	9.32
Days to physiological maturity	156.65	234.54**	8.44
Petiole length	9.40	13.90**	55.61
Peduncle length	17.02	16.02	42.20
Number of flowersper peduncle	7.33	9.88	62.49
Number of leaves per meter	29.17	32.02**	13.62
Number of peduncle per plant	24.24	72.27***	36.35
Number of total pods per plant	15.83	205.29	84.88
Number of locules per pod	13.89	7.79	16.96
Number of seeds per pod	11.54	9.05**	24.58
Stem diameter	6.32	1.99	23.92
Internode distance	11.45	7.39	29.13
Terminal leaf length	10.16	3.29*	14.61
Terminal leaf width	4.02	0.39	16.84
Length of pod	20.73	58.49*	33.08
Weight of total pods per plant	79.45	4706.03***	63.28
Weight of total seeds per plant	29.55	718.33***	56.46
Weight of total seeds per pod	3.73	4.13***	28.21
Number of tubers	0.05	0.03*	331.84
Tuber length	0.10	0.13	317.99
Tuber width	0.08	0.08	327.29
Tuber population	0.04	0.02	311.58
Tuber shape	0.06	0.04	308.70
Tuber skin colour	0.05	0.02	307.80
Tuber branching	0.01	0.00*	507.68
Extent of tuber branching	0.02	0.01	497.93
Tuber yield (kgha-1)	21.23	21094.49	679.98
Seed length	8.57	1.54***	7.98
Seed width	6.77	0.59***	7.53
Seed thickness	6.66	0.63***	7.31
100g seed weight	28.80	43.13***	13.19
Seed set percentage	82.71	316.82**	16.92
Shelling percentage	42.99	880.37***	49.07
Seed yield (kgha-1)	291.71	75568.97***	57.68
Seed shape	0.49	0.85***	14.22
Testa colour variegation	0.11	0.72***	129.37
Presence of seed cavity ridges	0.27	0.15***	33.40
Testa basal colour	0.78	15.52***	24.90
Basal colour of variegated seeds	0.20	2.91***	117.36
Eye colour pattern	0.52	10.31***	43.84
Pigmentation of plant parts	0.09	0.65***	153.07
Pattern of testa variegation	0.17	0.33***	137.02

* Significant at $P \leq 0.05$, ** Significant at $P \leq 0.01$, *** Significant at $P \leq 0.0001$

Materials and Methods

Location of study

The field work was carried out at the experimental field of the Institute of Agricultural Research and Training (IAR&T), Obafemi Awolowo University; Moor Plantation Ibadan (latitude 7° 22' 37.5" and longitude 3° 50' 38.46") in the 2011 and 2012 cropping seasons. The altitude is 192 m above sea level. The location received a total of 1,246.15 mm rainfall (June to December 2011) and 914.5 mm rainfall (June to December 2012) during the cropping seasons. The minimum and maximum recorded temperature ranged from 19.70 to 33.50°C with an average temperature of 21.78 to 30.27°C during the 2011 cropping season and the minimum and maximum recorded temperature ranged from 21.70 to 33.10°C with an average of 21.97 to 29.87°C during the 2012 cropping season.

Table 2. Descriptive statistics of reproductive traits of forty accessions of African yam bean

Accessions	DPI	DF	DPM	NPPP	NTPP	PDL	LP	WTP	WTST	WTSD	HSWT	SL	SW	ST	NSPP	NLP	SSP	SP	TY	SY
AYB1	77.67	110.83	163.67	25.11	12.33	14.12	17.27	75.95	15345	2.67	24.81	8.02	6.47	6.35	11.08	12.05	91.47	20.55	3.22	154.47
AYB4	76.00	107.83	157.17	24.09	18.67	16.40	23.10	94.68	21.29	3.45	27.45	8.48	6.75	6.13	13.31	14.93	89.23	30.16	3.21	212.92
AYB9	70.83	102.33	147.33	21.38	10.17	20.41	21.89	75.01	26.52	3.04	29.28	8.56	6.96	7.00	11.58	13.36	83.73	34.63	0.00	265.17
AYB23	80.33	110.67	161.17	27.53	11.17	19.24	17.71	56.51	21.22	3.70	24.83	7.89	6.56	6.65	11.14	14.94	72.65	37.65	13.55	212.17
AYB26	72.33	108.67	156.00	23.83	18.00	16.95	23.57	54.12	28.11	3.42	25.80	7.75	6.53	6.91	11.92	13.75	86.03	56.30	0.00	281.07
AYB34	81.67	109.00	155.33	24.51	16.67	18.40	23.19	64.39	31.72	3.50	25.80	8.72	6.46	6.32	12.28	14.61	83.74	49.67	2.95	317.15
AYB45	76.00	110.50	164.50	23.58	15.67	20.19	20.05	71.61	31.39	4.60	29.06	8.77	6.95	6.50	10.64	12.03	87.39	60.16	100.00	313.88
AYB50	80.67	114.67	161.67	23.33	13.50	16.90	22.47	66.84	45.10	3.65	26.80	8.64	6.29	6.31	10.98	13.44	81.58	69.16	0.00	451.00
AYB56	75.50	106.50	154.33	25.34	18.17	15.26	22.33	68.31	28.59	4.24	28.71	7.95	7.13	7.34	13.07	14.97	85.47	42.79	0.59	285.88
AYB57	74.33	105.67	155.67	25.03	14.50	17.61	20.15	100.30	27.58	4.66	32.93	8.65	6.86	6.63	10.72	12.38	85.83	41.52	59.25	162.88
AYB61	74.83	107.00	153.50	26.10	10.33	15.71	21.65	93.18	20.59	4.02	28.01	7.83	6.72	6.82	10.28	13.90	74.36	23.77	11.96	205.88
AYB70B	73.67	104.50	157.17	24.19	12.83	18.19	20.21	76.05	21.22	3.80	29.67	8.47	6.70	6.74	12.29	14.78	83.07	38.42	24.44	212.17
AYBIFE	77.17	107.50	160.67	27.19	8.33	17.56	18.31	43.99	19.12	2.98	25.25	8.28	5.70	5.79	12.48	14.28	87.10	44.09	3.21	191.17
Tss5	70.33	101.00	155.00	20.56	35.67	15.88	20.61	119.67	39.11	3.39	30.72	8.01	6.73	6.78	12.47	13.61	91.78	33.05	14.62	391.10
Tss19	68.83	98.83	149.50	29.59	9.67	20.13	18.39	54.40	23.62	3.10	27.17	9.13	6.71	6.60	10.88	13.50	73.61	46.20	0.00	236.18
Tss26	67.50	98.17	144.83	18.85	14.67	18.39	23.65	85.90	25.34	4.20	29.57	8.19	7.36	6.45	11.03	14.70	75.40	32.69	0.00	253.38
Tss41	72.00	103.50	157.50	15.85	12.67	17.12	20.84	57.12	19.67	2.38	29.20	9.56	7.42	7.26	8.59	13.18	65.18	36.20	0.00	196.67
Tss42	82.00	113.67	162.50	18.40	16.67	16.37	24.05	80.17	30.53	3.25	30.47	8.82	6.68	6.49	11.26	14.07	79.35	40.77	0.00	305.25
Tss45	73.83	107.17	165.83	22.68	23.17	17.56	22.29	75.52	21.58	3.56	29.39	8.62	6.74	6.60	10.20	14.68	69.06	30.50	0.00	215.83
Tss51	66.67	97.50	144.67	23.10	22.33	16.83	23.70	76.34	35.21	3.51	28.70	8.26	6.68	6.52	11.87	14.95	79.26	49.09	0.00	352.07
Tss52	68.67	100.33	147.33	20.95	22.83	15.73	22.03	114.37	40.99	3.38	28.84	7.82	6.46	6.47	10.20	12.67	78.72	47.13	0.00	409.87
Tss66	68.50	98.83	144.50	25.37	12.50	16.20	25.33	51.92	33.48	3.96	29.20	8.57	6.78	6.56	12.27	14.65	84.47	66.86	0.00	334.75
Tss68	69.33	99.17	159.67	25.05	18.33	16.63	24.03	108.44	53.43	4.47	27.51	9.29	6.43	6.51	12.90	14.58	87.98	58.23	0.00	534.32
Tss78	78.50	114.00	160.50	23.27	9.67	19.68	17.84	44.33	15.40	3.49	25.32	8.92	7.00	6.51	12.25	14.45	85.45	43.75	0.00	154.00
Tss88	78.83	111.00	159.67	27.15	3.83	14.79	19.28	34.77	10.03	1.72	22.32	8.70	6.67	6.55	10.38	15.27	69.12	34.39	0.00	100.33
Tss107	74.50	103.33	159.50	27.88	8.83	15.78	11.78	47.16	24.12	5.80	33.00	8.84	6.99	7.09	10.63	13.60	79.28	54.34	0.00	241.17
Tss123	75.17	103.33	153.50	22.71	19.00	16.76	25.07	93.89	21.68	4.26	32.18	8.87	6.72	6.46	11.37	14.37	80.29	30.43	220.73	177.83
Tss133	75.00	106.33	150.50	24.39	21.00	16.64	20.15	123.46	33.82	6.04	33.71	9.18	7.34	6.91	12.58	15.27	80.30	28.46	0.00	338.15
Tss134	73.50	105.17	150.50	24.29	12.17	16.32	19.28	146.74	52.61	4.66	31.04	8.96	7.10	6.82	13.87	14.62	95.34	36.53	9.20	526.13
Tss137	81.83	112.00	167.17	22.19	17.67	18.21	20.22	52.81	20.53	3.19	29.10	8.37	6.84	6.77	9.93	10.95	90.20	41.03	0.00	205.28
Tss138	77.50	109.00	161.50	22.37	11.00	18.77	19.64	55.84	23.86	3.29	30.07	8.56	6.89	6.76	12.02	14.55	82.47	44.51	0.00	238.62
Tss139	79.50	112.00	164.17	21.49	12.33	14.05	13.23	67.18	23.42	3.06	32.13	10.23	7.14	7.14	13.02	14.03	93.17	41.84	0.00	234.22
Tss140	80.17	113.00	162.83	25.47	21.33	14.18	25.06	111.71	34.37	5.24	33.49	9.29	6.77	6.68	11.78	13.10	89.44	35.68	64.90	343.70
Tss148	74.33	104.50	158.17	35.07	19.50	19.05	18.35	81.56	34.03	3.15	28.72	8.47	6.77	6.67	12.15	14.30	85.10	48.86	22.39	340.25
Tss150	76.00	108.83	157.83	31.82	16.83	17.53	20.13	73.08	23.92	3.68	30.30	8.56	6.85	6.83	9.94	12.70	78.86	40.49	0.00	239.22
Tss152	72.67	107.00	159.00	19.79	10.33	16.24	20.87	64.84	45.28	4.04	32.43	8.46	7.09	7.36	10.47	14.22	73.67	72.65	0.00	452.75
Tss153	77.83	104.83	156.67	24.62	13.83	15.90	15.33	59.94	18.14	3.38	24.93	8.29	6.49	6.40	13.22	13.93	94.79	31.91	0.00	181.35
Tss154	76.50	102.83	144.67	24.00	19.50	16.17	25.72	109.63	41.26	4.11	28.98	8.46	6.56	6.18	14.20	15.38	91.43	42.22	0.00	412.60
Tss156	80.50	115.83	163.50	25.01	25.33	15.86	18.53	156.98	50.48	3.56	26.96	8.09	6.64	6.84	8.69	10.49	80.78	40.97	0.00	504.78
Tss157	77.00	106.17	157.00	26.47	22.00	17.15	21.89	89.45	48.30	3.50	28.20	8.47	6.78	6.65	12.01	14.50	82.19	61.93	294.83	482.98
LSD	7.55	8.75	13.13	6.12	13.92	4.29	6.79	50.11	15.49	0.77	3.36	0.21	0.42	0.22	1.98	2.68	13.97	20.31	162.77	152.91
Std. error	3.82	4.43	6.65	3.10	7.05	2.17	3.44	25.37	7.84	0.39	1.70	0.11	0.21	0.11	1.42	1.36	7.07	10.28	82.40	77.41
Pr. Value	***	***	**	***	Ns	Ns	*	***	***	***	***	***	***	***	*	Ns	**	***	Ns	***

Ns-Not significant Pr-Probability Std. error-Standard error*- P $\leq$ 0.05 **- P $\leq$ 0.01 ***- P $\leq$ 0.001

DPI-Days to peduncle initiation, DF-Days to 50% flowering, DPM-Days to pod maturity, NPPP-Number of peduncle per plant, NTPP-Number of total pods per plant, PDL-Peduncle length, LP-Length of pod, WTP-Weight of total pods, WTST-Weight of total seeds per plant, WTSD-Weight of total seeds per pod, SL-Seed length, SW-Seed width, ST-Seed thickness, NSPP-Number of seeds per pod, NLP-Number of locules per pod, SSP-Seed set percentage, SP-Shelling percentage HSWT-100g seed weight, TY-Tuber yield(g), SY-seed yield (kg h^{-1})

Source of plant materials

Twenty-seven accessions of AYB were obtained from the Genetic Resources Center of the International Institute of Tropical Agriculture (IITA) Ibadan. They consisted of two accessions from Ghana, one from Bangladesh and 24 from Nigeria, six of which were from Enugu State and two from Imo State in South East Nigeria. The 13 other accessions were from the Institute of Agricultural Research and Training (IAR&T) Obafemi Awolowo University, Moor Plantation Ibadan, all of Nigerian origin (Table S1). Most of the accessions from IAR&T were collected from southwest Nigeria except AYB 34 which was from Kaduna State in northwest Nigeria (Fig. 1).

Morphological characterization

The accessions were sown on 5 × 40 m^2 plots with inter and intra row spacing of 1 × 1 m^2 in the experimental plot of IAR&T Ibadan for two planting seasons (June to December 2011 and June to December 2012). Seeds were dusted with macozeb fungicide prior to planting. Three seeds were sown per hole and later thinned to one plant stand 2 weeks after planting (WAP), to give a total of five representative plant stands per accession. At three WAP, the seedlings were staked. Manual weeding was done regularly to keep the field free of weeds. Insects were controlled with Cyperdiforce applied at the rate of 35 - 60 mL in 20 L of water at 2-week-intervals during the flowering period and subsequently for 2

Table 3. Stepwise discriminant analysis for continuous, log transformed ordinal, and binary variables

Steps	Variables	Partial R^2	F-Value (Pr > f)
1	Days to 50% flowering	0.474	6.97 (0.0004)
2	Length of pod (cm)	0.383	4.50 (0.0060)
3	Number of total pods per plant	0.358	3.90 (0.0121)
4	Number of tubers	0.377	3.02 (0.0431)
	Ordinal variable		
1	Basal colour of variegated seeds	0.464	6.50 (0.0007)
	Binary variables		
1	Testa color variegation	0.902	76.01 (<0.0001)
2	Pigmentation of plant parts	0.555	9.97 (<0.0001)
3	Presence of seed cavity ridges	0.307	2.98 (0.0367)

weeks for a total of three applications prior to harvesting. A total of 48 agro-morphological variables were recorded, using IITA phenotypic descriptors for AYB (Adewale and Dumet 2011). Ten readings were taken for each of the quantitative characters. Data on leaves, stems, branches, flowers and pods were taken on three representative plants (sampling units) for all the accessions on a plot basis. The dried matured pods were harvested at maturity and later on the tubers if present, were harvested. The vegetative and reproductive traits assessed are presented (Table S2). The experimental design used was a randomized complete block design with three replications. The testa basal colors were determined using the Methuen Handbook of Color (Kornerup and Wanscher 1978).

Data Analysis

The Gower (1971) distances between pair of accessions using the mixture of variables (continuous, ordinal and binary) were calculated and a cluster analysis of the accessions using the Ward minimum variance method (Ward 1963) was applied on the distances matrix. The mixed model analysis of variance (ANOVA) was used on entry basis for the individual traits combined across both years. The 40 accessions of AYB were the fixed factors while the blocks were the random factors. The Statistical Analysis System (SAS) procedure used for the ANOVA was the general linear model (GLM). The linear additive models used for individual year and for the 2 years combined are presented below.

Individual year, the linear statistical model used was:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \lambda_k + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

Where:

Y_{ijk} = the observation made in the i^{th} accessions on the j^{th} blocks in the k^{th} year,

μ= the overall mean of the traits,

α_i = the fixed effect of the i^{th} accessions,

β_j = the random effect of the j^{th} block,

λ_k = the effect of the k^{th} year,

$(\alpha\beta)_{ij}$ = the interaction effect of the i^{th} accession in the j^{th} block and

ε_{ijk}= the residual effects.

Cluster analysis, ANOVA and stepwise discriminant analysis, were made using the procedures CLUSTER, GLM

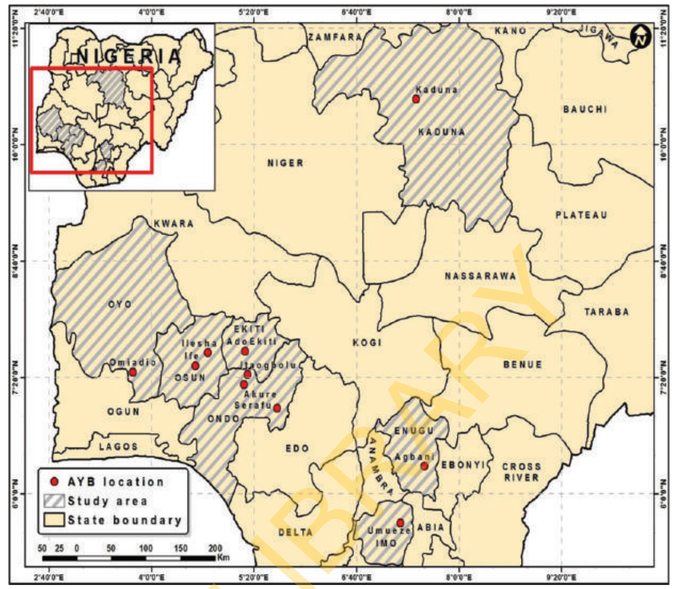

Fig. 1. African yam bean accession collection sites in Nigeria

and STEPDISC from the Statistical Analysis System, SAS-V9.3 (SAS / STAT user's guide 2010). The data matrix of the 40 AYB accessions and all the characters measured were standardized using the standard deviation procedure (mean = 0, standard deviation = 1) according to Chadran and Pandya (2000) and Ofori et al. (2006) to eliminate the differences in measurement scales and to equalize the effects of each variable (Hoft et al. 1999). Analysis of variance (ANOVA) and stepwise discriminant analysis approach were applied on the continuous binary and the log-transformed ordinal variables according to the method of Gutierrez et al. (2003) to identify the most important continuous, binary and ordinal variables on the clustering groups. A maximum *P*-value of 0.15 was used to accept a new variable in the set of significant variables as recommended by Gutierrez et al. (2003). The standardized data was used for Principal Component Analysis (PCA) to examine the interrelationship among the set of variables for the agro-morphological characters of the accessions. This was carried out using the PRINCOMP procedure SAS-V9.3 (SAS / STAT user's guide 2010). Those principal components with eigen values > 1.0 were selected. Pearson correlation coefficient (Pearson 1976) was calculated to understand the relationship between pairs of morphological traits.

Results

Analysis of variance of morphological characters

The 48 agro-morphological variables differed in their strength in distinguishing the 40 AYB accessions. There were significant differences in the performances of the 40 accessions of AYB for some of the traits studied over a period of 2 years as shown by the high mean square values (Table 1). Days to physiological maturity had a significant mean square value of 234.54 ($P \leq 0.01$), weight of total seed per plant 718.33 ($P \leq 0.0001$), seed set percentage 316.82 (P

Table 4. Frequencies of the three binary and five ordinal variables per cluster.

Variables	Clusters (No.of accessions)	I(5)	II(12)	III(9)	IV(8)	V(6)
	Variations			Percentage(%)		
Pigmentation of plant part	Non-pigmented	100.00	16.67	11.11	37.50	66.67
	Pigmented	-	83.33	88.89	62.50	33.33
Testa colour variegation	Non-variegated	100.00	16.67	100.00	25.00	-
	Variegated	-	83.33	-	75.00	100.00
Presence of seed cavity	Present	100.00	100.00	77.78	100.00	100.00
	Absent	-	-	22.22	-	-
Eye colour pattern	ECP1	-	33.33	-	100.00	83.33
	ECP 2	20.00	8.33	-	-	-
	ECP 4	60.00	41.67	88.89	-	16.67
	ECP 5	-	-	-	-	-
	ECP 6	-	8.33	-	-	-
	ECP 7	20.00	8.33			
Testa basal color	Yellowish grey	-	8.33	-	-	-
	Pale green	20.00	-	-	-	-
	Greyish green	-	-	-		
	Brown	80.00	66.67	88.89	25.00	-
	Purple	-	-	11.11	-	-
	Mosaic	-	16.67	-	75.00	100.00
Seed shapes	Oval	60.00	100.00	77.78	87.50	83.33
	Oblong	20.00	-	-	12.50	16.67
	Rhomboid	20.00	-	-	-	-
	Globular	-	-	22.22	-	-
Basal color of variegated seeds	Non-variegated	100.00	83.33	100.00		
	Cream	-	-	-	-	-
	Brown	-	16.67	-	100.00	100.00
Pattern of testa variegation	PTV0	100.00	83.33	100.00	-	-
	PTV2	-	16.67	-	100.00	83.33
	PTV3	-	-	-	11.11	16.67
Tuber Production	Tuberous	80.00	8.33	33.33	12.50	100.00
	Non-tuberous	20.00	91.67	66.67	87.50	-

NB = Cluster population in parenthesis. Num = Number. PTV0 = No variegation. PTV2 = Sparse black dots on creamy brown background with concentration around the hilum. PTV3 = Patchy light brown dots on dark brown background ECP1 = Brown testa with continuous narrow black stripe around the hilum. ECP2 = Brown testa with dark brown fork-like eye pattern. ECP4 = Brown testa with dark brown incision-like pattern below and parallel to the hilum. ECP5 = White testa with reddish brown vase-like eye. ECP6= White testa with black vase-like eye pattern. ECP7 = Light green testa with continuous narrow reddish brown stripe around the hilium

≤ 0.01), shelling percentage 880.37 ($P \leq 0.0001$), and weight of total pods per plant 4,706.03 ($P \leq 0.0001$) while seed yield (kg ha^{-1}) had the highest mean square of 75,568.97 ($P \leq 0.0001$). Four seed shapes were identified. A total of 82.5% had oval shape, 5% globular, 10% oblong and 2.5% rhomboid. The phenotypic variability in the testa basal colour of some AYB accessions is presented (Fig. 2). Out of 20 reproductive traits studied, there was no significant difference among the accessions for number of total pods per plant (NTPP), peduncle length (PDL), number of locules per pod (NLP) and tuber yield (TY). Other reproductive traits had varying degrees of significance. Days to peduncle initiation (DPI), days to 50% flowering (DF), number of peduncles per plant (NPPP), weight of total pods (WTP), weight of total seeds per plant (WTST), weight of total seeds per pod (WTSD), 100 g seed weight (HSWT), seed length (SL), seed width (SW), seed thickness (ST), shelling percentage (SP) and seed yield (SY) were highly significant ($P \leq 0.0001$). Days to physiological maturity (DPM) and seed set percentage (SSP) were significant at $P \leq 0.01$ while pod length (LP) and number of seeds per pod were significant (NSPP) at $P \leq 0.05$ (11). TSs 66 (144.50 days), TSs 51 (144.67 days) and TSs 154 (144.67 days) are early maturing accessions while TSs 137 is a late maturing accession (167.17 days). TSs 154 had the longest (25.72 cm) pod length, the highest (15.38) number of locules per pod and still the highest (14.20) number of seeds per pod. TSs 156 had the highest (156.98 g) weight of total pods while TSs 78 had the lowest (17.84 g). TSs 68 had the highest weight of total seeds per pod (4.47 g) and weight of total seeds per plant (53.43 g) while AYB 1 had the lowest 2.67 g and 15.45 g, respectively. TSs 88 had very low (100.33 kg ha^{-1}) seed production (Table 2). Seed set percentages ranged from 65.18% (TSs 41) to 94.79% (TSs 153). The accessions studied had significant differences which show the degree of diversity amongst them.

Cluster Analysis

The WARD technique of clustering grouped the 40 accessions of AYB meaningfully into five clusters at the R-squared distance of 0.04 similarity index (Fig. 3). The distances between the accessions spanned 0.0071 to 0.1132 with a mean distance of 0.0256. Phenotypically, AYB 57 and TSs 123 were the most similar accessions with the closest distance of 0.0071. Cluster I had five accessions: TSs 123, AYB 57, TSs 148, AYB 34 and AYB 23. The twelve accessions in cluster II were: TSs 78, TSs 139, TSs 88, AYB 50, TSs 156,

Fig. 2. Phenotypic variability in the testa basal color of some African yam bean accessions AYB 70B & TSs 45 (mottled), TSs 5 (agate brown), TSs 154 (hazel brown), TSs 150 (brown), TSs139 (yellowish gray), TSs 66 (purplish gray), AYB 1 (grayish green), and AYB 23 (pale green)

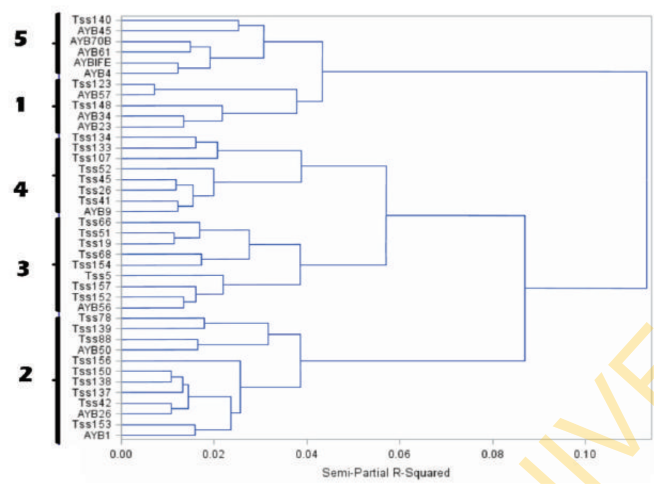

Fig. 3. Dendrogram produced from Ward's minimum variance cluster analysis showing genetic relationship among 40 African yam bean accessions. NB = Semi partial R squared is the increase in sum of squares within a new cluster formed by joining the two clusters below it

TSs 150, TSs 138, TSs 137, TSs 42, AYB 26, TSs 153 and AYB 1. The nine accessions in cluster III were: TSs 66, TSs 51, TSs 19, TSs 68, TSs 154, TSs 5, TSs 157, TSs 152 and AYB 56. The eight accessions placed in cluster IV were: TSs 133, TSs 134, TSs 107, TSs 52, TSs 45, TSs 26, TSs 41 and AYB 9. TSs 140, AYB 45, AYB 70B, AYB 61, AYB IFE and AYB 4 were grouped in cluster V (Fig. 3). Of the 48 agro-morphological traits analyzed, testa color variegation was the most discriminatory trait, classifying the accessions in the proportion of 35% of variegated and 65% of non-variegated seeds.

Considering the continuous, binary, and log-transformed ordinal variables in Table 4, the most significant discriminative continuous variables were days to 50% flowering ($P \leq 0.01$) followed by length of pod ($P \leq 0.01$), number of total pods per plant ($P \leq 0.01$) and number of tubers ($P \leq 0.05$). The most significant discriminative binary variables were testa color variegation ($P < 0.0001$), pigmentation of plant parts ($P < 0.0001$), and presence of seed cavity ridges ($P \leq 0.05$) while the most significant discriminative ordinal variable was basal color of variegated seeds ($P < 0.01$) (Table 3). The frequencies of the three binary and five ordinal variables per cluster is presented (Table 4). Accessions in cluster I where non-pigmented (100%), 80% produced tubers and 100% were seed cavity ridges on the pods (100%). TSs 123, AYB 57 and TSs 148 had oval seed shape while AYB 34 and AYB 23 had rhomboid and oblong seed shapes, respectively. Eighty percent of the accessions (Cluster I) had brown testa color while 20% had pale green testa color (AYB 23). In cluster II, only one accession had grayish green oblong seeds (AYB 1), others (91.67%) had oval seeds. Vines of 33.33% of accessions in this cluster were pigmented while 66.67% were non-pigmented. Only AYB 1 in cluster II was tuberous (8.33%). In terms of testa basal colour, TSs 139 had yellowish grey testa color, TSs 88 and AYB 50 had mosaic testa basal color while the other accessions in the clusters had different shades of brown testa color. Accessions in cluster III had 77.78% oval and 22.22% globular seed shapes. 33.33% of accessions in cluster III produced tubers while 66.67% were non-tuberous. All the accessions in cluster III were non-variegated. TSs 66 had purplish grey testa basal color while 88.88% had brown seeds (hazel brown-TSs 19, TSs 51, AYB 56, and TSs 154). Cluster IV consists of TSs 133, TSs 134, TSs 52 and TSs 26 which were non-pigmented (50%) while TSs 107, TSs 45, TSs 41 and AYB 9 were pigmented (50%). Only TSs 134 (12.5%) in cluster IV produced tubers, other accessions were non-tuberous. 87.5% of accessions in cluster IV had oval seed shape except TSs 45 which had oblong seed shape (12.5%). In terms of testa basal color, 75% were mosaic while 25% had hazel brown testa color. All the accessions in cluster V had mosaic testa basal color and produced tubers (100%). AYB 4 and AYB 61 had pigmentation on plant parts (33.33%) while the other accessions were non-pigmented (66.67%). 100% of accessions in cluster V had oblong seeds (AYB IFE) while 83.33% of the seeds were oval. 42.5% of accessions produced tubers. Most of these accessions were from States in southwest Nigeria.

Pearson's correlation coefficient of morphological variables

The Pearson correlation coefficients among 20 reproductive traits of AYB are presented (Table 5). Days to peduncle initiation was strongly and positively correlated with days to bud initiation ($r = 0.96^{***}$), days to 50% flowering ($r = 0.89^{***}$) and days to physiological maturity ($r = 0.69^{***}$). Days to 50% flowering had a strong correlation with days to pod maturity ($r = 0.77^{***}$). There was also a strong and significant correlation between weight of total pods per plant and number of total pods per plant ($r = 0.63^{***}$) as well as weight of total seeds per plant ($r = 0.66^{***}$). 100 g seed weight was strongly correlated with weight of total seeds per pod (0.65) but weakly correlated with weight of total seeds per pod ($r = 0.38^{***}$). Seed thickness was weakly correlated with 100 g seed weight ($r = 0.45^{***}$). Number of seeds per pod was significantly correlated with number of locules per pod ($r = 0.62^{***}$). These are

Table 5. Pearson's correlation coefficient of some agro-morphological variables evaluated in 2011 and 2012

	DPI	DBI	DF	DPM	WTP	WTST	WTSD	HSWT	SL	SW	ST	NSPP	NLP	SSP	NT	TL	TW	TY	SY
DPI	1.00																		
DBI	0.96***																		
DF	0.89***	0.88***																	
DPM	0.69***	0.67***	0.77***																
WTP	-0.12	-0.16	-0.09	-0.22															
WTST	-0.19	-0.18	-0.15	-0.21	0.66***														
WTSD	-0.11	-0.13	-0.14	-0.16	0.41**	0.36*													
HSWT	-0.20	-0.24	-0.19	-0.12	0.39**	0.28	0.65***												
SL	0.11	0.04	0.07	0.18	-0.04	0.01	0.14	0.39**											
SW	-0.21	-0.28	-0.12	-0.16	0.10	-0.03	0.29	0.55**	0.37*										
ST	-0.14	-0.14	0.00	0.06	0.00	0.09	0.16	0.45**	0.18	0.70***									
NSPP	-0.03	-0.01	-0.21	-0.30	0.12	0.17	0.21	0.01	0.10	-0.12	-0.27								
NLP	-0.20	-0.17	-0.33	-0.39	-0.17	-0.05	0.08	-0.05	0.10	0.05	-0.12	0.62***							
SSP	0.19	0.20	0.10	0.05	0.27	0.22	0.18	0.07	0.03	-0.20	-0.24	0.68***	-0.13						
NT	0.17	0.15	0.09	0.12	0.24	-0.07	0.29	0.29	0.07	-0.10	-0.13	0.09	-0.03	0.17					
TL	0.14	0.08	0.04	0.10	0.17	-0.08	0.20	0.12	-0.16	-0.14	-0.13	0.12	0.04	0.14	0.79***				
TW	0.18	0.10	0.06	0.12	0.07	-0.06	0.18	0.04	-0.10	-0.13	-0.19	0.06	0.02	0.08	0.63***	0.91***			
TY	0.10	0.05	0.12	0.19	0.19	0.06	0.35	0.30	0.12	0.06	-0.09	-0.06	-0.25	0.18	0.69***	0.66***	0.73***		
SY	-0.18	-0.15	-0.14	-0.19	0.62***	0.99***	0.32*	0.22	0.00	-0.04	0.09	0.19	-0.02	0.21	-0.14	-0.15	-0.12	-0.02	1.00

DPI-Days to peduncle initiation, DBI-Days to bud initiation, DF-Days to 50% flowering, DPM-Days to physiological maturity, WTP-Weight of total pods, WTST-Weight of total seeds per plant, WTSD-Weight of total seeds per pod, HSWT-100g Seed weight, SL-Seed length, SW-Seed width, ST-Seed thickness NSPP-Number of seeds per pod, NLP-Number of locules per pod, SSP-Seed set percentage, NT-Number of tuber, TL-Tuber length, TW-Tuber width, TY-Tuber yield, SY-Seed yield. Levels of significance *- $P \leq 0.05$, **- $P \leq 0.01$ ***- $P \leq 0.0001$

Table 6. Eigen values and contribution to the first five principal component axes to variationin African yam bean accessions evaluated on 14 agro-morphological traits.

Variables	PC1	PC2	PC3	PC4	PC5
Days to 50% flowering	-	-0.236	-	0.505	0.416
Petiole length (cm)	-0.201	0.336	-	0.354	-
Number of peduncle per plant	-	-0.265	0.260	-	-0.582
Number of total pods per plant	-0.205	0.381	0.223	-	-
Pod Length (cm)	-	0.461	-	-0.296	-
Seed length (cm)	-	-0.243	-	-0.399	0.503
Shelling percentage	-	-	-0.337	-	-0.318
Number of tubers	-	-	0.623	-	0.209
Tuber branching	-	-0.272	0.518	-0.264	-
Pigmentation of plant parts	0.343	-0.284	-0.221	-	-
Presence of seed cavity ridges	0.213	-	-	0.490	-
Testa colour variegation	0.509	-	-	-	-
Testa basal colour	0.459	0.275	-	-	-
Basal colour of variegated seeds	0.476	0.239	-	-	-
Eigen values	3.220	2.236	1.823	1.371	1.106
Percent variation	0.230	0.159	0.130	0.098	0.079
Cumulative proportion	0.230	0.389	0.520	0.618	0.697

PC = Principal Component
Only eigenvectors with values ≥ 0.20 which largely controlled each PC axes are presented

important attributes for seed yield. A strong and highly significant correlation was observed between seed set percentage and number of seeds per pod ($r = 0.68^{***}$). Tuber length had a positive and highly significant correlation with number of tubers ($r = 0.79^{***}$) and tuber width ($r = 0.91^{***}$). Tuber width also had a positive and highly significant correlation with number of tubers ($r = 0.63^{***}$). Tuber yield was strongly correlated with number of tubers ($r = 0.69^{***}$), tuber length ($r = 0.63^{***}$) and tuber width ($r = 0.73^{***}$), respectively. A highly significant correlation was also observed between seed yield (kg ha^{-1}) and weight of total seeds per plant ($r = 0.99^{***}$) as well as weight of total pods per plant ($r = 0.62^{***}$) but weakly correlated with weight of total seeds per pod ($r = 0.32^{*}$).

Principal Component Analysis of morphological traits of AYB

In order to assess the patterns of variation, principal component analysis (PCA) was carried out by considering 14 agro-morphological traits simultaneously based on the results obtained from the stepwise discriminant analysis. The principal component analysis and percentage contribution of each component to the total variation in the 2 years combined (Table 6) showed that the first five principal components explained 69.7% of the total variation, the principal component axis one (PC1) with eigen value 3.220 contributed 23% of the total variability while PC2 with eigen value of 2.236 contributed 15.9% of total variability observed among the 40 accessions of AYB. PC3 had an eigen value of 1.823 and contributed 13% of the total variability (Table 6). The eigen values ranged from 3.220 (PC1) to 1.106 (PC5). Eigen values equal to or greater than 0.2 were identified as the logical cut-off points where each selected trait made an important contribution to the PC axis. The first principal component was mostly correlated with qualitative traits; presence of seed cavity ridges (0.213), pigmentation of plant parts (0.343) with testa basal color (0.459) and testa color variegation (0.509) having higher loadings to PC1. Also contributing to the loading negatively on PC1 are petiole length (-0.201) and number of total pods per plant (-0.205). Testa basal color (0.275), petiole length (0.336), number of total pods per plant (0.381) and pod length (0.461) contributed positively to the variation in the second principal component. The remaining variables contributed negatively to the variation in PC2. Number of peduncle per plant (0.260), number of total pods per plant (0.223), and tuber-related traits; tuber branching (0.518) and number of tubers (0.623) were the major characters that contributed positively to the third principal component while shelling percentage (-0337) and pigmentation of plant parts (-0.221) contributed negatively to the variation. The fourth principal component showed 9.8% of the total variation in the population. Petiole length (0.354), presence of seed cavity ridges (0.490) and days to 50% flowering (0.505) contributed positively to the variation in PC4 while the other traits tuber branching (-0.264), pod length (-0.296) and seed length (-0.399) contributed negatively to the variation. The major contributors to the loading on the fifth principal component were number of tubers (0.209), days to 50% flowering (0.416), and seed length (0.503) while shelling percentage (-0.318) and number of peduncle per plant (-0.582) contributed negatively to the variation.

Discussion

The result of the means square analysis revealed highly significant differences among the 48 qualitative and quantitative traits investigated. This explains the variation existing among the accessions studied which can be exploited for use in future breeding programs. Genetic variability studies enable selection of parents for hybridization (Chaudhury and Singh 1982) as sound crop improvement depends upon the magnitude of variability in the base population (Adebisi et al. 2001). Accessions with promising agronomic traits were identified. Early flowering accessions such as TSs 26, TSs 19, TSs 52, TSs 51, TSs 68, TSs 66, AYB 9 and TSs 154 identified in this study could be selected as parents for early maturity. Similar results for early maturity accessions were reported by Popoola et al. (2011). The variation of the days to flowering among accessions could be due to genotypic factor as the earliness of the accessions was consistent for both years. Accessions with higher values of number of leaves per meter, number of branches, internode distance, and number of flowers per peduncle were observed to produce a higher number of pods per plant and consequently a higher number of seeds per plant, Popoola et al. (2011) observed such in their report. TSs 154 had the longest pod length (25.72 cm), an average of 14 seeds per pod and seed set percentage of 91.43%. It may be a good candidate for high seed yield. TSs 66 could also be considered for high seed yield as the pod length was 25.33 cm, seed set percentage 84.47%, and number of seeds per pod 12.27. The high seed yield recorded for some of the accessions could be due to the long vine length and luxuriant growth habit of the accessions, which invariably encouraged production of large number of flowers that matured into pods and seeds. Such observations have been reported for cowpea and pigeon pea (Popoola et al. 2011; Wien and Summerfield 1984).

TSs 88 showed poor performance for some agronomic traits recording the lowest number of total pods per plant, weight of total pods per plant, and invariably the lowest seed yield (100 kg ha^{-1}). Pigmentation was found to be more intense in TSs 78 than in any other accession. Other pattern of pigmentation showed that TSs 139 had slight pigmentation on the stem but it also had poor seed yield. Pigmentation of plant parts as well as variegation of seeds played a vital role in characterizing the accessions of AYB studied.

TSs 156 had the highest total weight of pods (156.98 g) while TSs 78 had the least (17.84 g). Four seed shapes were identified in this study: oval (82.5%), globular/round (5%), oblong (10%), and rhomboid (2.5%). This is in agreement with the report of Adewale et al. (2010) that the oval seed shape is the most prominent shape in AYB while rhomboid is the least. Significant differences ($P < 0.0001$) were observed in the some traits of the 40 accessions indicating considerable phenotypic and genetic differences among them.

Hence, these genetic variations could be utilised as raw materials for genetic improvement of the species. High number of flowers per peduncle indicates high floral productivity and probably high pod and seed yields. Most of the flowers produced by some accessions aborted at early stages of development leading to poor yield. The correlation analysis indicated that terminal leaflet length, peduncle length, pod length, number of locules per pod, and number of seeds per pod were characters that contributed significantly to the high seed set percentages in all the accessions; Popoola et al. (2011) observed the same in their work. Therefore, selections based on these characters could contribute immensely to higher seed productivity in AYB. The Ward's (1963) minimum variance cluster analysis grouped the 40 accessions of AYB into five clusters based on similarity for the quantitative and qualitative characters.

Phenotypically, AYB 57 and TSs 123 were the most similar accessions in this study. The stepwise discriminant analysis approach applied on the continuous, the log-transformed ordinal and binary variables identified the most important variables on the clustering groups. The importance of each variable is defined by the stepwise discriminant approach in a sequential manner beginning with the highest F value from an ANOVA considering the possible relation between the descriptive variables (Adewale 2011). The stepwise discriminant analysis revealed that days to 50% flowering, pod length, number of total pods per plant, number of peduncles per plant, number of tubers, seed length, petiole length, shelling percentage, tuber branching, and (continuous variable), basal color of variegated seeds (ordinal variable), testa color variegation, pigmentation of plant parts, and presence of seed cavity ridges (binary variables) were the most discriminative variables used for grouping the 40 AYB accessions into clusters.

Principal component analysis (PCA) identifies plant traits that characterize the distinctness among selected genotypes (Chakravorty et al. 2013). These are often extended to the classification of a population into groups of distinct orders based on similarities for one or more characters, and thus guide in the choice of parents for subsequent hybridization (Ariyo 1987; Nair et al. 1998). Hair et al. (1998) had earlier remarked that eigen values greater than one are considered significant and component loadings greater than or equal to ± 0.2 were considered to be meaningful. In this study, 69.7% of the total variation was explained by the first five principal components with PC1 to PC3 accounting for 51.9% of the total variation with eigen values ranging from of 3.220 to 1.823, respectively. The 14 agro-morphological traits had a significant contribution to detecting variation among the 40 accessions of AYB.

The Pearson's (1967) correlation coefficients were high and significant for yield and yield related traits which are important for improving the yield of AYB. Similar observations have been reported by Ibirinde and Aremu (2013) and Togun and Olatunde (1998) on vegetative and reproductive traits and their effects on yield.

Conclusion

The assessment of 40 African yam bean accessions revealed the extent of genetic variability in the 48 agro-morphological traits. Terminal leaf length, peduncle length, pod length, number of locules per pod, and number of seeds per pod are characters that contributed significantly to the high seed set percentages in all the accessions. Selections based on these characters could contribute immensely to higher seed productivity. In order to improve the yield of AYB, the number of seeds per pod, number of pods per plant, weight of total seeds per pod and weight of total seed per plant are important determinant factors.

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