



INTRA-SPECIFIC VARIABILITY AND DIVERSITY ANALYSIS OF AFRICAN YAM BEAN BY SEED SIZE PARAMETERS

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ABSTRACT

Understanding the intra-specific variability within a species is preliminary to any genetic improvement of the crop. Thirty (30) African yam bean (AYB) accessions were tried in four locations in Nigeria. Resultant seed lots from harvest per location and per replication were evaluated in the study. The genetic variability, distance and the clustering of the 30 accessions were based on twelve seed size estimates. Accession and location significantly ($P \leq 0.05$) differed with respect to the twelve characters. Among other characters however, the interaction: Accession (Acc.) x Location (Loc.) was not significant for circumference and geometric mean diameter of the seed. The variance proportion due to location alone was significantly ($P < 0.0001$) the highest; ranging from 41.6% to 78.9%. The variance due to accession (11.2 - 33.2%) was significantly ($P < 0.001$) higher than that of Acc. x Loc. (4.8 - 15.4%). The contribution of Acc. x Loc. and the experimental error to the total variation did not differ significantly. The mean Gower genetic distance between the 30 accessions was 0.232. The least (0.036) was between TSs33 and TSs84 and the highest (0.672) existed between TSs9 and TSs86. The 30 accessions grouped in five clusters. TSs104B and TSs69 existed independently in clusters III and IV, respectively. Despite intra-cluster uniformity, some characters significantly ($P < 0.05$) differentiated the four, twenty and four accessions within clusters I, II and V, respectively. The present study on revealing the genetic variability among the 30 accessions, equally presented the platform for selection criteria for seed sizes parameters for AYB.

Keywords: African yam bean, seed size estimates, sources of variation, Gower genetic distance.

INTRODUCTION

The evolving campaign for food diversification is enhancing the realization of the place of the underutilized crop species in the global food system for food security. As remarked by Jaenicke and Pasiecznik (2009), increasing the use of underutilized crops is one of the ways to reduce nutritional, environmental and financial vulnerability in times of change. The importance of the less-used crop species is uncontroversially inevitable in the sustenance of the growing global population. The results of poor attention to underutilized species were critical erosion and extinction of the genetic resources of some crop species. Therefore, a continued research attention on these species may salvage them from imminent loss, enhance their awareness and restore their place in the food system of the populace.

African yam bean (*Sphenostylis stenocarpa* (Hochst Ex. A. Rich) Harms) is in the long list of the highly nutritious underutilized species (Evans and Boulter, 1974) in Africa. The record on the extent of the losses and rescue of its genetic resources in Africa is yet unknown. However, the research attention on the crop in the recent time is encouraging. Among the outstanding remarks on the importance of African yam bean (AYB) includes: cultural link to Africa (Potter and Doyle, 1992), nutritional potentials and status (Nwokolo, 1996; Uguru and Madukaife, 2001; Ekpo, 2006), suitability for wide climatic conditions (Anochili, 1984; Betche *et al.*, 2005), grain yield potentials (Okigbo, 1973; National Academy of Science, 1979), etc.

Genetic variation of a quantitative trait is assumed to be controlled by the collective effects of quantitative trait loci (QTLs), epistasis (interaction between QTLs), the environment, and interaction between QTL and environment (Semagn *et al.*, 2010). The contribution of each of these sources of variation is significant to the understanding of the variability of species. Breeding to improve AYB, (like every other crops) depends on understanding the intra-specific variability and pattern of classification of the species for all agronomic characters of importance.

Basically, multivariate analysis of a set of genotypes leads to the understanding of the genetic identity, characteristics and potential of such genotypes. Depending on the set of descriptors involved, the outcome of multivariate analysis launches the plant breeders to further improvement strategies on the crop, such as: identification and release of superior cultivars on a short term programme (Chheda and Fatokun, 1982; Ariyo, 1991), selection of parents for various characters for breeding and improvement programmes (Bhatt, 1970; Nassir and Ariyo, 2007), selection of parents for mean generation analysis to understand the gene action for specific characters etc.

Metric measurements on the seeds such as length, width, thickness, their ratios and mass of seeds are very important characteristic for seed characterization and evaluation (Omokhafe and Alika, 2004; Tetsuka and Uchino, 2005; Adewale *et al.*, 2010). The existence of wide variability in colour, shape and sizes of AYB seeds



have been observed by Oshodi *et al.* (1995) and Adewale *et al.* (2010). However, the efficacy of the seed-related characters, especially the quantitative, on the physical properties of the seed in distinguishing among accessions of AYB has not been investigated. As much as the knowledge of the physical properties of the seed will be useful parameters in designing handling and processing equipment (Asoiro and Ani, 2011), they are very important determinant of the proximate and nutritive values of the seeds. The present research addresses the significance of twelve seed size characteristics with the aim of understanding the variability within the AYB species. Moreover, the study equally aimed at understanding the genetic identity of each of the 30 AYB accessions and classifies them based on similarity for the twelve physical seed parameters.

MATERIALS AND METHODS

Seeds of thirty accessions of AYB were obtained from the Genetic Resources Centre of the International Institute of Tropical Agriculture (IITA), Ibadan. They were planted for evaluation in a randomized complete block design of three replications in a multi-locational trial at Ibadan, Mokwa, Ikenne and Ubiaja, Nigeria in 2007. At harvest in each location, seeds of each accession were bulked per replication. Thirty seeds of uniform sizes were selected per replication and the metric measurements on the seeds were done as described by Adewale *et al.* (2010) to generate seed length, width and thickness. Detail of the twelve descriptors used in the study is presented in Table-1.

Table-1. The descriptions of the seed parameters for the diversity study of the 30 AYB accessions.

#	Parameters	Descriptions/mode of estimation	References
1	Single seed weight (g)	Mass of a single seed	-
2	Seed length (mm)	Distance measured between the two ends of the seed, parallel to the hilum	Adewale and Dumet (2011)
3	Seed width (mm)	Distance on the seed measured from hilum to the keel	Adewale and Dumet (2011)
4	Seed thickness (mm)	Distance on the seed measured perpendicular to the seed length	Adewale and Dumet (2011)
5	Surface area (mm ²)	$S = D_g^2$	Asoiro and Ani (2011)
6	Seed volume (cm ³)	Volume of 100 randomly selected seeds in 94% ethanol	Adewale and Dumet (2011)
7	Seed density	Ratio of bulk weight to bulk volume	Asoiro and Ani (2011)
8	Surface area/mass ratio	Ratio of the seed surface area to the mass of single seeds	Asoiro and Ani (2011)
9	Seed circumference (cm)	The circumferential length round the seed perpendicular to the seed length; estimated as: $2\pi r$, where $r = \frac{1}{4}(W+T)$	-
10	Geometric mean diameter (mm)	$D_g = (abc)^{1/3}$	Asoiro and Ani (2011)
11	Seed sphericity	$\emptyset = D_g/a$	Asoiro and Ani (2011)
12	100 seed weight (g)	Mass of 100 randomly selected seeds taken from total seed yield in replicates	Adewale and Dumet (2011)

NB: a = seed length, b = seed width, c = seed thickness, $\pi = 22/7$, r = radius, S = surface area of the seed, D_g = geometric mean diameter, \emptyset = Sphericity, T = seed thickness, W = seed width

Analysis of variance (ANOVA) was conducted in SAS (2007), version 9.2 using the PROC GLM procedure, to partition all the sources of variation. The percentage contribution of each sources of variation was calculated as the proportion of each to the total variance. In the PAST software (Hammer *et al.*, 2001), T-test statistics was used to compare and test for significance between each pair of the sources of variation. The phenotypic and genotypic coefficient of variation was calculated following the procedure of Singh and Chaudhary (1985).

Furthermore, mean values for each parameter across the four locations were generated for each

replication. The multivariate data was subjected to another ANOVA to understand variation among the accessions alone. For genetic distance calculation and the cluster grouping techniques, a 30 x 12 data matrix was produced the means of each accession across the three replications for the 12 characters. The data was standardized (mean = 0; standard deviation = 1) to harmonize the various units among the parameters following the approach of Ofori *et al.* (2006).

The genetic distances between pairs of the 30 AYB accessions was produce from the continuous data by the PAST statistical software (Hammer *et al.*, 2001) using



the Gower distance option. The Gower distances were then subjected to multivariate analysis by Ward clustering method, using the PROC TREE procedure to generate a dendrogram in SAS version 9.2. The number of clusters that most reliably describe the grouping of the 30 accessions in the dendrogram was assessed by the K-means clustering method. To understand variability among member accession within each cluster, ANOVA (PROC GLM in SAS, version 9.2) was done.

RESULTS

For all the studied parameters, there existed significant ($P \leq 0.05$) variation among the 30 accessions and the four locations (Table-2). The 120 accession by location combinations equally exhibited significant ($P \leq 0.05$) variation for all the parameters except SC and GMD. Generally across all the parameters, the proportion of the contribution of location to the total variance was highest, followed by the contribution of the accession then the interaction between accession and location (Table-2). Among the twelve parameters, the least proportion of variance for accession, GGE and Acc. x Loc. was

observed in S100; however, the same parameter gave the highest variance contribution (76.85%) to location. From the ANOVA, the contribution of each sources of variation to the total variance varied significantly. Moreover, the proportions of each sources of variation differed with the various parameters studied.

Therefore, the mean contribution of accession, location and the interaction between both were 19%, 56% and 11.2%, respectively (Table-2). In a decreasing order, the variance proportion due to location was significantly ($P \leq 0.01$) higher than the variation contributed by accession (19%), the interaction of accession with location (11.19%) and the experimental error (13.69%). The mean contribution of the cumulative proportion of accession and Acc. x Loc. interaction was about one-third of the total variation. In all cases (Table-2), phenotypic coefficient of variation (PCV) was higher than the genotypic coefficient of variation (GCV). Both GCV and PCV were relatively low (less than 20%), with GMD having the least for both while the highest PCV (19.27%) occurred in SV (Table-2).

Table-2. Measures of variability for the seed parameters of African yam bean.

Seed parameters	Mean squares			Percentage contribution to total variance				GCV (%)	PCV (%)
	Accessions	Location	Acc. X Loc.	Accessions	Locations	Acc. X Loc.	GGE		
SSW	3928.53***	17483.9***	2861.40***	14.68	65.35	10.69	25.37	11.09	18.14
SL	1.18***	4.41***	0.42*	16.65	62.27	5.92	22.57	6.42	9.05
SW	0.42***	1.26***	0.29**	19.28	57.51	13.18	32.46	4.48	8.04
ST	0.73***	2.48***	0.50***	17.72	60.49	12.29	30.01	6.39	10.71
SA	97.30***	385.73***	53.08***	16.10	63.81	8.78	24.88	8.55	13.69
SV	6540.67***	19227.44***	5056.36***	18.31	53.84	14.16	32.47	10.73	19.27
SD	0.008*	1.014***	0.24***	17.81	55.56	13.19	31	4.10	9.66
SAMR	0.0007*	0.0104***	0.0028***	33.20	42.23	11.66	44.86	4.53	11.66
SC	0.042***	0.015*	0.003ns	22.92	41.60	9.42	32.34	4.32	8.17
GMD	0.33***	0.0019*	0.0006ns	18.46	45.72	15.42	33.88	3.71	6.41
SS	0.008***	0.095**	0.029*	21.69	48.19	14.82	36.51	5.73	7.64
S100	43.94***	302.16***	19.014***	11.18	76.85	4.84	16.02	14.46	19.21
Mean				19	56.12	11.19	30.19		

*- $P \leq 0.05$, ** - $P \leq 0.01$, *** - $P \leq 0.001$

GGE = cumulative variance of accession and accession by location interaction, PCV = Phenotypic coefficient of variation, GCV = genotypic coefficient of variation, SSW = single seed weight(mg), SL = seed length (mm), SW = seed width (mm), ST = seed thickness(mm), SA = surface area (mm²), SV = seed volume (cm³), SD = seed density, SAMR = surface area/mass ratio, SC = seed circumference (cm), GMD = geometric mean diameter (mm), SS = seed sphericity, S100-100 seed weight (g)

Only ten comparisons were possible between the five sources of variations identified in the ANOVA. The significance of a paired comparison between the sources of variations is presented in Table-3. But for the comparison between Error and Acc. x Loc. which was not significant, all the other comparisons (Table-3) were very and highly significant ($P \leq 0.01$).

The grouping of the 30 accessions of AYB by the WARD clustering system is presented in Figure-1. By similarity for the twelve seed size parameters, five distinct clusters were evident at the inflection point of 0.80

(Figure-1). Clusters I and V had four accessions each as members. Cluster II had the highest membership of 20 AYB accessions. Only one accession featured in Cluster III (TSs 104B) and IV (TSs 69). With reference to the tested seed size parameters; the most similar accessions with the closest distance (0.0009) were TSs33 and TSs84 (Figure-1). The most diverse clusters were I and V with the distance of 0.2877 (Figure-1); specifically, the widest distance was between TSs9 (cluster I) and TSs86 (cluster V).



Table-3. The probabilities associated with the student's paired T-test with two-tailed distribution for the comparison and test for significance among the sources of variation

	Accessions	Locations	Acc. x Loc.	GGE
Locations	0.000000395			
Acc. x Loc.	0.000315	0.0000000108		
GGE	0.000000019	0.0003067	0.0000001009	
Error	0.0104	0.0000000837	0.153	0.000000076

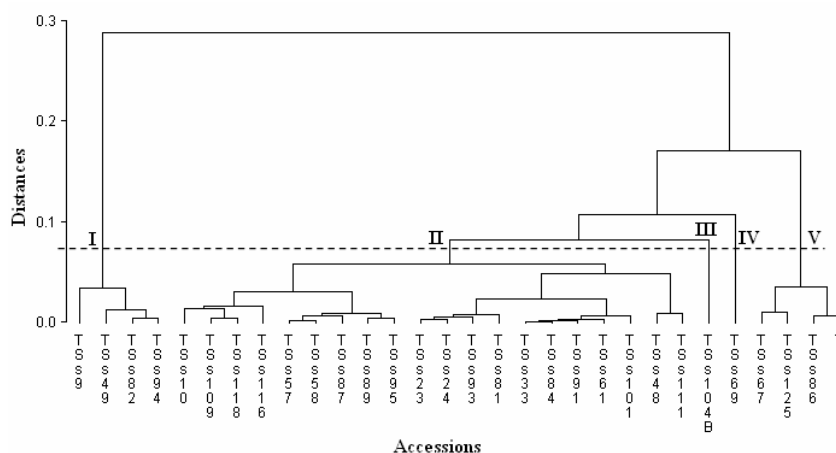


Figure -1. A WARD dendrogram showing the grouping of the thirty accession of African yam bean.

The Gower genetic distance in this study ranged between 0.036 and 0.672, with the mean of 0.232. The least distance (0.036) was between TSs33 and TSs84 and the highest (0.672) existed between TSs9 and TSs86 (Table not shown). The accessions in cluster I consists TSs9, TSs49, TSs82 and TSs94. Within the cluster, TSs49 and TSs94 were most similar (genetic distance = 0.059), however TSs9 and TSs82 were most diverse with the distance of 0.293. The mean distance within the cluster I was 0.136 (Table-4).

Table-4. Gower genetic distances between the four AYB accessions in cluster I.

	TSs9	TSs49	TSs82
TSs49	0.171		
TSs82	0.293	0.141	
TSs94	0.208	0.059	0.085

The genetic distances among the 20 AYB accessions which clustered in group III is presented in Table-5. The genetic distances ranged between 0.036 (TSs33 and TSs84) and 0.294 (TSs10 and TSs111). The mean genetic distance within this group was 0.145. TSs67, TSs86, TSs96 and TSs125 grouped in cluster V. Table-6 showed the genetic distance between the four accessions. The mean distance among them was 0.177. Least distance (0.106) existed between TSs86 and TSs96 while the

widest genetic distance within the cluster (0.236) was between TSs86 and TSs125 (Table-6).

From Table-7, ten of the twelve seed sizes parameters differentiated the 30 AYB accessions very significantly ($P < 0.01$). There were different unifying seed parameters for the accession members in each of the five clusters. Among the twelve characters, the seed length and the sphericity of the seed significantly ($P < 0.05$) differentiated the four AYB accessions in cluster I. The seed circumference is the only character which significantly ($P < 0.05$) differentiated the 20 AYB accessions in clusters II. Significant ($P < 0.05$) variation within cluster V was determined by seed thickness, seed density and seed sphericity (Table-7). The minimum value and the lowest mean for SSW, SW, ST, SA, SV, SC, GMD and S100 occurred in cluster V while the maximum value and the highest mean for SSW, SV, GMD and S100 occurred in cluster I. For SW, SD, SAMR, SC and SS the highest value occurred in cluster II. Moreover, the means for all the characters in cluster II were relatively similar to the general means of the 30 AYB materials for all the traits (Table-7). The significance of the singly appearance of TSs69 and TSs104B in clusters III and IV is evident from Table-7. TSs69 had the highest overall means for SL (9.40mm), SA (59.59mm²) and SAMR (0.24) and the lowest overall means for SW (6.32mm), ST (5.67mm) and SS (0.74). On the other hand, the uniqueness of TSs104B was evident with lowest overall mean of 7.95mm for seed length and the highest overall means of 6.54mm, 6.88mm and 0.89 for SW, ST and SS, respectively (Table-7).

**Table-5.** Gower genetic Distances between the 20 accessions in Cluster III.

	TSs10	TSs23	TSs24	TSs33	TSs48	TSs57	TSs58	TSs61	TSs81	TSs84	TSs87	TSs89	TSs91	TSs93	TSs95	TSs101	TSs109	TSs111	TSs116
TSs23	0.198																		
TSs24	0.148	0.066																	
TSs33	0.223	0.096	0.142																
TSs48	0.166	0.181	0.206	0.142															
TSs57	0.172	0.065	0.095	0.095	0.169														
TSs58	0.174	0.108	0.122	0.140	0.199	0.064													
TSs61	0.250	0.110	0.164	0.056	0.135	0.097	0.140												
TSs81	0.217	0.099	0.113	0.121	0.132	0.132	0.178	0.121											
TSs84	0.221	0.105	0.144	0.036	0.113	0.090	0.132	0.038	0.105										
TSs87	0.103	0.133	0.134	0.156	0.157	0.099	0.084	0.171	0.214	0.154									
TSs89	0.130	0.148	0.116	0.189	0.234	0.109	0.090	0.206	0.212	0.187	0.083								
TSs91	0.192	0.083	0.100	0.052	0.153	0.063	0.103	0.069	0.120	0.045	0.121	0.142							
TSs93	0.243	0.063	0.102	0.081	0.190	0.097	0.129	0.093	0.115	0.088	0.167	0.179	0.069						
TSs95	0.164	0.085	0.078	0.125	0.186	0.072	0.111	0.148	0.132	0.128	0.140	0.093	0.088	0.128					
TSs101	0.165	0.133	0.142	0.087	0.106	0.112	0.155	0.101	0.140	0.075	0.128	0.186	0.068	0.125	0.120				
TSs109	0.182	0.136	0.152	0.130	0.111	0.130	0.163	0.138	0.115	0.119	0.166	0.196	0.112	0.165	0.130	0.122			
TSs111	0.294	0.253	0.281	0.211	0.145	0.247	0.276	0.199	0.175	0.192	0.282	0.312	0.231	0.268	0.260	0.223	0.146		
TSs116	0.170	0.146	0.132	0.175	0.176	0.134	0.166	0.178	0.167	0.174	0.155	0.157	0.156	0.198	0.110	0.174	0.119	0.234	
TSs118	0.097	0.125	0.102	0.153	0.135	0.113	0.150	0.173	0.142	0.156	0.111	0.129	0.135	0.179	0.101	0.142	0.089	0.204	0.086

Table-6. Gower genetic distances between the four accessions in cluster V.

	TSs67	TSs86	TSs96
TSs86	0.167		
TSs96	0.167	0.106	
TSs125	0.136	0.236	0.203

**Table-7.** ANOVA summary and descriptive statistics of the twelve quantitative traits for the characterization of 30 African yam bean accessions.

Variability among the 30 accessions of AYB												
Items	SSW	SL	SW	ST	SA	SV	SD	SAMR	SC	GMD	SS	S100
Df	29	29	29	29	29	29	29	29	29	29	29	29
Minimum	209.82	7.59	5.83	5.49	46.89	260.07	0.68	0.18	1.86	6.36	0.69	14.99
Mean	261.22	8.48	6.35	6.07	54.03	329.65	0.79	0.21	2.02	6.88	0.82	23.62
Maximum	353.22	10.46	7.60	7.82	68.19	434.09	0.92	0.25	2.40	7.55	0.93	29.73
Pr > F	0.0004	<0.0001	0.0003	0.0015	0.0004	0.005	0.0808	0.0735	<0.0001	0.0022	<0.0001	<0.0001
Variability among the four accessions of AYB in cluster I												
Df	3	3	3	3	3	3	3	3	3	3	3	3
Minimum	262.62	8.45	6.32	5.86	55.01	332.73	0.78	0.18	1.99	6.92	0.76	22.45
Mean	294.40	8.93	6.53	6.20	58.43	363.79	0.81	0.20	2.08	7.12	0.79	26.61
Maximum	353.22	9.50	6.91	6.94	62.22	434.10	0.84	0.22	2.23	7.55	0.84	29.73
Pr > F	0.9256	0.0099	0.5779	0.3780	0.4056	0.8723	0.1026	0.6538	0.7878	0.9025	0.0126	0.0789
Variability among the four accessions of AYB in cluster II												
Df	19	19	19	19	19	19	19	19	19	19	19	19
Minimum	218.02	7.59	5.94	5.63	47.31	272.36	0.69	0.18	1.89	6.47	0.78	20.58
Mean	261.78	8.44	6.38	6.08	53.97	329.60	0.80	0.21	2.03	6.88	0.82	23.71
Maximum	300.87	9.25	7.59	6.77	62.26	388.43	0.91	0.25	2.41	7.28	0.92	28.48
Pr > F	0.9320	0.7178	0.2479	0.5869	0.7729	0.9040	0.4647	0.7612	0.0129	0.9306	0.1294	0.3101
Variability among the four accessions of AYB in cluster V												
Df	3	3	3	3	3	3	3	3	3	3	3	3
Minimum	209.82	7.81	5.83	5.49	46.89	260.07	0.76	0.19	1.86	6.36	0.77	14.99
Mean	230.64	8.15	6.01	5.81	49.04	286.61	0.81	0.22	1.93	6.57	0.81	20.39
Maximum	259.09	8.50	6.36	6.37	51.07	324.09	0.86	0.23	2.08	6.86	0.86	22.56
Pr > F	0.0872	0.0745	0.1071	0.0174	0.1593	0.0538	0.0276	0.0609	0.1471	0.0729	0.0165	0.1481
Means for each characters for clusters III and IV with a single accession												
TSs69	250.67	9.40	6.32	5.67	59.59	337.61	0.76	0.24	1.93	6.95	0.74	23.60
TSs104B	250.07	7.95	6.54	6.88	51.99	358.26	0.72	0.21	2.06	7.06	0.89	22.96

*SSW = single seed weight(mg), SL = seed length (mm), SW = seed width (mm), ST = seed thickness(mm), SA = surface area (mm²), SV = seed volume (cm³), SD = seed density, SAMR = surface area/mass ratio, SC = seed circumference (cm), GMD = geometric mean diameter (mm), SS = seed sphericity, S100 = 100 seed weight (g) and Df = degree of freedom

DISCUSSIONS

The contribution to total variance by the different sources of variation varied significantly. The mean contribution of location to the total variance was highest and above half (56%) of the total variation. As reported by Yan and Kang (2003), 59% and 80%, were respectively observed in a multi-environment trial of soybean and wheat. They indicted number of genotypes and environments to be responsible for the magnitude of the environmental variation and remarked that testing larger number of genotypes in fewer environments often result in lower proportion of the environmental variance and vice versa. However, regardless of the above, the universal phenomenon in all multi-environment trials is that the environment is always the predominant sources of variation (Gauch and Zobel, 1996; cited by Yan and Kang (2003)).

From an initial study on the morphological classification of 80 AYB accessions by Adewale *et al.* (2012), the most similar accessions were TSs112 and TSs128. The identification of TSs33 and TSs84 in the present study as the most similar accessions could be due to non-uniformity in the number of the genetic materials used in the two studies. The pattern of classification and

grouping of genetic materials by similarity in multivariate analysis is dependent on the phenotypic descriptors used. Adewale *et al.* (2012) and Popoola *et al.* (2011) observed definite grouping pattern with different sets of phenotypic descriptors for some AYB samples. The inconsistencies in the pattern of classification of genotypes when sets of descriptor vary have been indicted as a major shortcoming of phenotypic characterization scheme as against DNA finger printing (Mohammadi and Prassanna, 2003). However, some synchronies have been established between morphological and DNA-markers descriptors (Arbelbide and Bernardo, 2006; Semagn *et al.*, 2010). Phenotypic characterization, despite shortcomings from environmental influences (Mohammadi and Prassanna, 2003) has provided basis for different selection strategies and targets for specific breeding programmes (Nassir and Ariyo, 2007; Aremu *et al.*, 2010).

The ANOVA result proved the 30 AYB accessions to be characteristically different, justifying their use in research as different genetic materials of AYB. The GCV and the PCV for the twelve parameters ranged between low to moderate according to Sivasubramanian and Menon (1973). The significant uniformity within each cluster for most seed characters would enhance intra and



inter-cluster selection process. For instance, accessions in cluster I and V oppositely and respectively displayed the minimum and the maximum values for SSW, SV, GMD and S100. Concerted mating among accessions within a cluster may foster pure-line breeding for some quantitative characters. Moreover, accessions within the same cluster could be further evaluated under multi-environment to ascertain their adaptability and suitability for various ecologies. Generation of hybrids through cross breeding for any of these traits for example, can be done across clusters. A proposed breeding programme to generate AYB hybrids with longer seed length, wider width and thickness can be achieved through cross breeding programme between TSs69 (cluster III) and TSs104B (cluster V).

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