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Preliminary morphological characterization and evaluation of selected Bambara groundnut [*Vigna subterranea* (L.) Verdc.] genotypes for yield and yield related traits

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ABSTRACT

Forty nine (49) Bambara groundnut genotypes derived from single plant selection of diverse origin were evaluated for yield and yield components using 26 yield and yield related traits. Highly significant (P<0.001) differences were detected among the genotypes for canopy spread, petiole length, weight of biomass, seed weight and seed height, while seedling emergence, pod weight, seed length and seed width were significantly different (P<0.05). Principal component analysis identified nine influential components whereby PC_1 and PC_2 highly contributed to the total variation at 19% and 14%, respectively. Leaf colour at emergence, petiole colour, leaf joint pigmentation and calyx colour were highly correlated with PC_1, while seed length, seed width and seed height had strong association with PC_2. Both the principal component and cluster analyses displayed common association among most of the genotypes for agronomic and seed yield traits. Genotypes that showed high seed yield performance and greater biomass production can be tested for large-scale production, breeding or germplasm conservation.

Key words: Cluster analysis, Germplasm, Principal component analysis, True-to-type.

INTRODUCTION

Bambara groundnut [*Vigna subterranea* (L.) Verdc.; Syn: *Voandzeia subterranea* (L.) Thouars.] is an under-utilized grain legume grown in sub-Saharan Africa (SSA), mostly by women as a source of protein for subsistence (Ntundu *et al*., 2004). Bambara groundnut is third in importance in the SSA among grain legumes after groundnut (*Arachis hypogea* L.) and cowpea [Vigna unguiculata (L.) Walp.] (Sellschope, 1962; Rachie and Silvestre, 1977; Linnemann and Azam-Ali, 1993).

Bambara groundnut grains make up a complete balanced food (Rowland, 1993). The seeds are rich in protein (16-25%), carbohydrates (~ 63%) and oil (~18%). The predominant fatty acids in the oil include oleic acid, palmitic acid and linolenic acid (Minka and Bruneteau, 2000). Chemical analyses showed that the seed contains 32.50-32.72% of total essential amino acids including lysine, hisidine, arginine, leucine and isoleucine and 66.10-70.80% of the non-essential amino acids such as methionine, glycine, cysteine, tyrosine and proline (Minka and Bruneteau, 2000; Amarteifio *et al*., 2006; Aremu *et al*., 2006).

Various parts of Bambara groundnut are used for human consumption. Fresh seeds in pods may be boiled and eaten as a snack in a manner similar to boiled peanut, while dry seeds can be made into pudding (or steamed-paste) called Moi-Moi or Okpa (bean porridge) in some parts of Nigeria (Okpuzor *et al*., 2009), hence has the potential to enhance nutritional security for humans.

The crop has the potential of producing greater than 3,000 kg ha^{-1} (Collinson *et al*., 2000). Major proportion of Bambara groundnut of the global production comes from West Africa, in which Nigeria is leading with a range of 45-50% (Goli *et al*., 1997).

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Despite its various advantages, to date, the genetic potential of Bambara groundnut remains largely unexploited. Thus far, only farm level selection is being practiced whereby existing landraces consisting of several morhpo-types are evaluated and their seeds multiplied for use by farmers, hence the need to carry out within and between variations of such materials (Mohammed *et al*., 2016) to select desirable genotypes for further studies. As such there are no improved varieties of Bambara groundnut available in the major growing areas. Landraces can provide breeders with sources of genes for biotic and abiotic resistances, adaptability to different environments, nutritional characteristics and yield potential. In view of the above, this

study was aimed to characterize and evaluate for initial yield and yield component responses of 49 genotypes of Bambara groundnut derived from single plant selections made from a diverse germplasm collection using 26 morphological traits.

MATERIALS AND METHODS

Plant material and study site

The 49 genotypes of the Bambara groundnut landraces used in this study are presented in Table 1. The study site was the Research and Training Farm of the University of KwaZulu-Natal, Pietermaritzburg, at Ukulinga, South Africa. The research was conducted in 2014 main season. The site is situated on a Latitude 30° 24′S, Longitude 29° 24′E and is 800 m above sea level.

Experimental design, field management and data collection

For both characterization and yield evaluation, a partially balanced lattice design was used with three replications and was randomised into the seven incomplete blocks across the three replications. The experimental plot comprised of three rows measuring 2.2 m x 3.0 m, with inter and intra row spacing of 0.4 m x 1.0 m. Sowing was done into a flat seedbed, with one seed sown to each stand. Missing stands were replaced within two weeks after sowing. All relevant agronomic practices were carried out to maintain a healthy crop.

Data on the 26 morphological (qualitative and quantitative) traits were generated on five plants selected from the central row of each plot within the incomplete blocks over the three replicates (IPGRI/IITA/BAMNET, 2000). The quantitative field data included number of days to 50% seedling emergence (SDE) by counting number of days from planting to 50% seedling emergence, while plant height (PHT), canopy spread (CNS), terminal leaf length (TLL), terminal leaf width (TLW) and petiole length (PETL) were measured using a measuring ruler and expressed in cm. Qualitative data recorded included leaf colour at emergence (LCE), terminal leave shape (TLS), growth habit (GH), stem pigmentation (SPG), petiole colour (PCL), leaflet joint pigmentation (this is the pigmentation at the point of attachment of petiolule to the petiole) (LJP), calyx colour (CCL), fresh pod colour (FPC), pod shape (PSP), dry pod colour (PCL), pod texture (PTX), seed shape (SSP) and seed eye pattern (SEY). The qualitative data were determined by visual observations.

Post-harvest quantitative data include dry biomass (BMA), pod weight (PDW), seed weight (SDW) were

Table 1: List of sources of Bambara groundnut accessions used in the study.

Genotype	Origin	Seed coat colour	Genotype	Origin	Seed coat colour
211-77	CAPS	cream	211-75	CAPS	Cream
211-87	CAPS	black	211-46-3	CAPS	Red
211-55	CAPS	red	211-83-2	CAPS	Cream
32-1-1	ZM	light brown	712-4	ZM	Tan
45-2	ZM	tan	N211-1	KNG	Cream
211-55-1	CAPS	red	KB 05	ARC	Cream
TV-79-1	IITA (Kenya)*	cream	211-68	CAPS	Cream
211-90	CAPS	black	101-2	ZM	Cream stripe
211-51	CAPS	red	KB 08	ARC	Cream *RBF***
211-91	CAPS	light brown	M12-1	ZIM	Cream
42-2-3	ZM	light brown	712-7	ZM	Tan
84-2	ZM	red	211-45	CAPS	Red
N211K	KNG	cream	101-2-1	ZM	Cream stripe
73-3	ZM	red	42-2	ZM	Light brown
211-76	CAPS	cream	M01-8	ZIM	Cream *RBF*
25-1	ZM	light brown	TV-93	IITA (Kenya)	Cream
B71-2	ARC	cream	M02-3	ZIM	Cream *RBF*
M09-4	ZIM	cream	B71-1	ARC	Cream
N212-5	KNG	brown	73-2	ZM	Red
TV-27	IITA (Nigeria)	dark brown speckle	211-88	CAPS	Black
M09-3-1	ZIM	cream	N212-4	KNG	Brown
011-7	PMB	cream stripe	TV-39	IITA (Sudan)	Dark brown speckle
N212-8	KNG	brown	211-69	CAPS	Cream
211-57	CAPS	red	M09-3	ZIM	Cream
42-1	ZM	light brown			

CAPS= CAPSTONE Seed Company, South Africa; ZM= Zambian National Program; IITA= International Institute of Tropical Agriculture in Ibadan, Nigeria; with a place origin; KNG= Kano, Nigeria; ZIM= Zimbabwean National Program; PMB= Pietermaritzburg; ARC=Agricultural Research Council of South Africa; *RB*F=Red butterfly eye.

recorded in grams (g) using an OHAUS Precision Standard Measuring Scale, while hundred (100) seed weight (HSW) was recorded also in grams using a more sensitive Mettler Scale. Seed length (SDL), seed width (SDW), and seed height (SHT) were determined using a Digital Vernier Calipers (cm) on ten randomly, but well developed and uniform seeds taken from seeds used for 100 seed weight measurement, while qualitative data which were taken visually include kernel shape (PDS), kernel colour (PDC), kernel texture (PDT), seed shape (SDS) and seed eye pattern (SEY).

Data analysis

All the quantitative traits were subjected to analysis of variance based on the lattice procedure using Agrobase statistical software (Agrobase, 2005). Treatments' means were separated by the least significant differences (LSD) at 5% probability. Cluster and principal component analyses were conducted on both quantitative and qualitative traits to determine similarities and dissimilarities among the genotypes using SPSS (SPSS, IBM Statistics 20). Using similarity matrix, a dendrogram was constructed to describe similarities and differences among the Bambara groundnut genotypes.

RESULTS AND DISCUSSION

Morphological and yield attributes analyses

Genetic analysis diversity is one of the criteria for an organised sampling of breeding population/germplasm collection useful for the identification of desirable genotypes for hybridization (Razvi *et al*., 2018). The Bambara groundnut genotypes exhibited considerable variation among the morphological and seed traits. Highly significant (P<0.001) differences were detected for canopy spread, petiole length, weight of biomass, seed weight and seed height, while number of days to seedling emergence, pod weight, seed length and seed width were significantly (P<0.05) different (Table 2). In a similar diversity study using Bambara groundnut landraces in Tanzania, Ntundu *et al*. (2006) and Zenabou *et al*. (2014) reported significant differences among quantitative traits including petiole length, plant spread, plant height, seed length and seed width, among others. In addition, variation in yield related traits have also been reported by Shegro e*t al.* (2013), who showed that cultivar and environment may influence performance. These reports suggested that morphological and seed traits are useful for the characterization of Bambara groundnut and selection of desirable genotypes suitable for breeding, conservation and mass production. Razvi *et al*. (2018) showed that contribution due to character divergence vary from crop to crop.

Principal component analysis

Results of the principal component analyses (PCA) for the 26 agronomic and seed traits among the 49 Bambara groundnut genotypes are presented in Table 3. All 26 traits were grouped under nine components (Eigen values ≥ 1) which accounted for 79% of the variation. Leaf colour at emergence, petiole colour, leaflet joint pigmentation and calyx colour were highly correlated with PC_1, which accounted for 19.7% of the total variation. Seed traits which include seed length, seed width and seed height were correlated with PC_2, while pod weight and weight of biomass correlated with PC_3. Similarly, PC_4 contributed to 8.1% of the available variation and well correlated with terminal leaf length and plant height. Fresh kernel colour correlated positively with dry kernel colour in PC_5 contributing to 7.8% of the variation, suggesting that fresh kernel colour may affect kernel colour in dry condition. However, PCs 6, 7 and 8 had high correlations with 100 seed weight, kernel texture and leaf shape, contributing 6.2, 5.3 and 4.6% to the observed variability, respectively. PC_9 contributed to almost 4.0% of the variability in which stem pigmentation was important. In general, the PC analysis of the 26 traits indicated that PC_1 was composed of a number of traits that contributed for the greatest variation, followed by PC_2. In this study, it was observed that Bambara groundnut farmers may have driven the selection for specific morphological and seed traits. A Similar observation was made by Ntundu *et al*. (2006) who reported that leaf morphology, seed size and colour were morphological criteria used by farmers in Tanzania during selection.

Principal component biplot

The wide variation observed among the Bambara groundnut genotypes used in this study were further expressed by the PCA biplot (Fig 1). The biplot explained relationships and similarities that exist among the genotypes relative to the 26 measured traits. The genotypes scattered within the four quadrants produced by the PC_1 and PC_2 biplot. The genotypes showed a pairing orientation, irrespective of geographical locations within the axes, suggesting that they shared in common most of the features for the 26 traits that were studied. This feature of orientation would mean that movement of Bambara groundnut landraces across the African sub-region was indiscriminate. Using alfalfa germplasm (*Medicago sativa* L. subsp. sativa) (Yin *et al*., 2018) showed that genotype diversity was due to indiscriminate introduction of from various sources. Furthermore, pairing of the genotypes was associated more to individuals with possible common origin. Grouping of Bambara groundnut genotypes from the same region in Tanzania was earlier reported by Ntundu *et al*. (2006). Conversely, genotypes that scattered far apart within the axes would mean that they are distantly related with other genotypes within the same quadrant. PC_1 and PC_2 displayed 20% and 14% of the total variations in the quadrats. The results showed that the Bambara groundnut landraces have sufficient genetic diversity for selection and breeding purposes. Comparing the PC analysis and PCA biplot, the observed associations showed how the landraces share in common certain traits. Similar observations were made by Shegro *et al*. (2013) who suggested additional use of molecular markers to confirm such associations.

Table 2: Summary statistics of mean square and significant differences of agronomic, and pod (kernel) and seed traits among 49 Bambara groundnut genotypes tested using the partially unbalanced lattice design with seven incomplete blocks and three replications.

Source of variation		SEM		PHT		CNS		TLL		TLW		PETL		BMS	
	Df	MS	F-value	MS	F-value	MS	F-value	MS	F-value	MS	F-value	MS	F-value	MS	F-value
Replication	2	27.456	6.86*	122.76	15.20**	371.1	16.48**	149.33	82.14**	104.62	67.33**	166.71	18.62**	34126.9	78.00**
Genotype (Unadjusted)	48	6.545		12.27		64.21		2.02		1.76		26.49		1823.93	
Block (Adjusted)	18	2.807		5.15		12.07		0.61		0.38		6.6		183.39	
RCBD (Residual)	96	4.227		8.3		24.48		2.05		1.78		9.4		485.19	
Genotype (Adjusted)	48	6.545	1.55*	2.27	1.42NS	64.21	2.62**	2.02	0.99NS	1.76	0.99NS	26.49	2.82**	1823.93	3.76**

Table 2: (Continue).

Source of variation		PWT		SWT		HSW		SDL		SDW		SHT	
	Df	MS	F-value	MS	F-value	MS	F-value	MS	F-value	MS	F-value	MS	F-value
Replication	2	12.44	3.61*	2671.4	18.74**	42.74	0.95NS	0.82	1.82NS	0.22	0.66NS	0.22	1.28NS
Genotype (Unadjusted)	48	8.25		444.9		69.03		1.21		0.6		0.47	
Block (Adjusted)	18	2.5		81.22		34.93		0.14		0.23		0.13	
RCBD (Residual)	96	3.62		154.04		46.91		0.51		0.35		0.18	
Genotype (Adjusted)	48	8.25	2.28*	444.9	2.89**	69.03	1.47NS	1.21	2.37*	0.6	1.73*	0.47	2.56**

Legend: SEM (Days to seedling emergence); PHT (plant height); CNS (canopy spread); TLL (terminal leaf length); TLW (terminal leaf width); PETL (petiole length); BMS (weight of biomass); PWT (pod weight); SWT (seed weight); HSW (hundred seed weight); SDL (seed length); SDW (seed width); SHT (seed height); *Significant difference at the 0.05 probability level; ** Significant difference at the 0.01 probability level; Df (degree of freedom); MS (mean square); NS (not significant).

Table 3: Eigen values, proportion of variability and morphological traits that contributed to the nine PCs of Bambara groundnut genotypes.

Traits	PC_1	PC_2	PC_3	PC_4	PC_5	PC_6	PC_7	PC_8	PC_9
Seed emergence (Days count)	-0.1	-0.267	0.112	0.201	-0.065	0.19	-0.425	0.498	0.386
Plant height (cm)	0.018	0.285	0.185	0.814	-0.143	-0.237	-0.15	-0.109	0.035
Canopy spread (cm)	0.211	0.141	0.475	0.475	-0.245	-0.002	0.217	-0.25	-0.057
Terminal leaf length (cm)	0.125	-0.013	-0.232	0.895	-0.049	0.244	0.088	-0.011	-0.022
Terminal leaf width (cm)	-0.179	0.413	0.192	-0.467	-0.193	-0.492	-0.149	-0.192	0.063
Petiole length (cm)	0.16	0.336	0.5	0.629	0.041	-0.11	-0.16	0.008	-0.029
Pod weight (gm)	-0.155	-0.051	0.949	-0.052	-0.066	0.03	-0.052	0.004	-0.021
Seed weight (gm)	0.359	0.329	-0.152	-0.18	0.168	-0.239	0.128	0.06	0.59
Biomass weight (gm)	-0.117	-0.064	0.961	0.044	-0.064	0.025	-0.041	-0.001	-0.067
100 seed weight (gm)	-0.083	0.152	-0.04	-0.172	-0.083	0.768	-0.09	-0.031	-0.245
Seed length (mm)	0.148	0.77	0.112	0.292	0.026	0.328	0.047	0.008	0.016
Seed width (mm)	0.062	0.897	-0.123	0.068	0.03	0.004	-0.003	0.06	0.178
Seed height (mm)	0.219	0.892	-0.016	0.059	-0.12	0.009	0.072	-0.071	-0.1
Leaf colour at emergence	0.946	0.097	-0.049	0.049	0.054	-0.089	-0.014	0.032	0.016
Leaf shape	0.134	0.076	0.01	-0.077	0.117	0.054	0.088	0.83	0.029
Growth habit	-0.008	-0.201	0.082	-0.048	-0.606	0.262	-0.061	-0.344	-0.163
Stem pigmentation	-0.291	-0.016	-0.104	-0.014	0.023	0.002	-0.224	0.003	0.732
Petiole colour	0.949	0.102	-0.105	0.126	0.024	-0.059	0.03	-0.026	-0.04
Leaflet joint pigmentation	0.976	0.097	-0.038	0.063	0.027	-0.042	-0.024	0.021	-0.052
Calyx colour	0.976	0.097	-0.038	0.063	0.027	-0.042	-0.024	0.021	-0.052
Fresh pod colour	0.071	-0.071	-0.054	0.036	0.851	-0.002	0.04	0.075	-0.193
Pod shape	-0.167	-0.072	0.315	-0.016	-0.309	0.038	0.503	0.3	-0.156
Pod colour	0.032	-0.12	-0.064	-0.266	0.714	0.031	-0.135	-0.063	0.197
Pod texture	0.004	0.062	-0.133	0.008	0.032	0.025	0.909	-0.029	-0.044
Seed shape	-0.204	0.073	0.087	0.114	-0.049	0.553	0.07	0.022	0.197
Seed eye pattern	-0.159	-0.158	-0.212	-0.17	0.12	-0.289	-0.103	0.484	-0.38
Eigen-values	5.125	3.736	2.408	2.098	2.031	1.614	1.377	1.205	1.034
Proportion variance (%)	19.711	14.369	9.261	8.069	7.81	6.207	5.297	4.636	3.976
Cumulative variance (%)	19.711	34.079	43.34	51.409	59.219	65.426	70.723	75.359	79.336

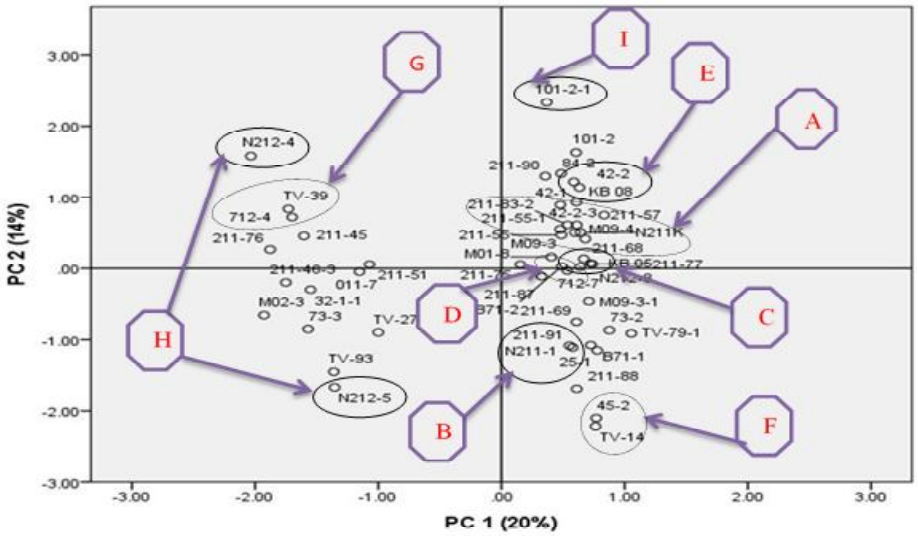

Fig 1: Rotated principal component scores and percentage explained variance of PC_1 versus PC_2 showing similarities among 49 of Bambara groundnut genotypes. Descriptions of the sources of the landraces used are indicated in Table 1 (above).

Cluster analysis

The degree of relatedness and differences among 49 genotypes for the 26 traits are presented in Fig 2. Four major cluster groups were identified, whereby Cluster I consist of three genotypes including two from Zambia (712-4 and 45-2) bearing Tan seed coat colour and one genotype (TV-93) from Sudan (acquired from IITA), which had a cream seed coat colour. The two Zambian genotypes were probably the same genotype, while the inclusion of the genotype from Sudan suggests that the three genotypes have a common origin or exhibit similarities in certain morphological features. The second cluster (Cluster II) was the largest, comprising of 24 genotypes distributed within two sub-clusters II a and II b. Cluster II a, consisted of 19 genotypes, while II b had five genotypes. The first sub-cluster IIa1 had an isolated genotype (TV-79-1) from IITA which originated from Kenya. Cluster II a6 had nine genotypes, with two forming a sub-sub-cluster (II a6-1) comprising of two genotypes (TV-14

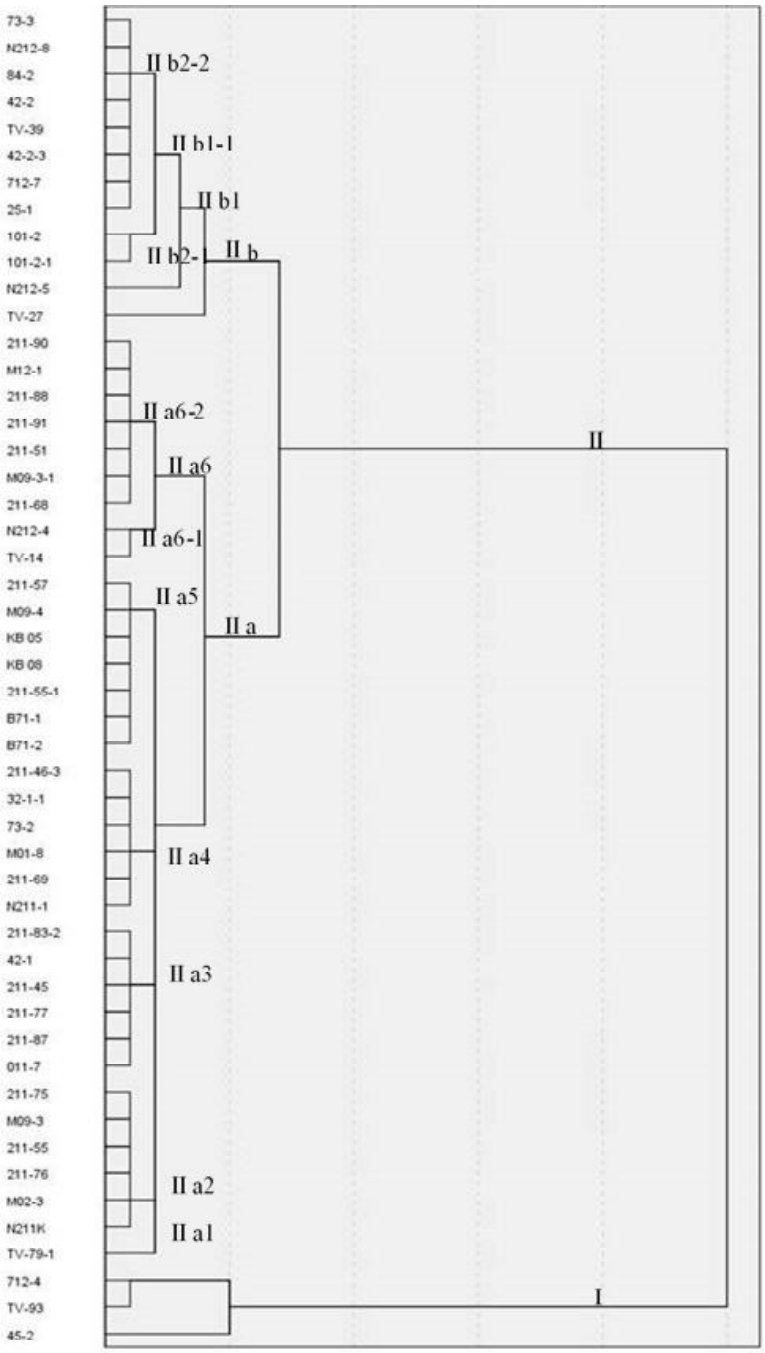

Fig 2: Dendrogram based on average linkage for 13 quantitative and 13 qualitative characters of 49 Bambara groundnut genotypes. Description of the sources of the genotypes used are indicated in Table 1.

and N212-4) from Ghana and Nigeria. Therefore, TV-14 and N212-4 may have come from the same ancestral origin. The other sub-sub-cluster, II a6-2 included six genotypes out of which four (211-68, 211-51, 211-88 and 211-90) were from CAPS, while the remaining two, M09-3-1 and M12-1 both originated from Zimbabwe. Since CAPS manages and sells Bambara groundnut seeds comprising of mixtures of landraces, the inclusion of the last two genotypes from Zimbabwe suggest that the accessions in this cluster may have had the same origin. Similar results were observed by Vyas *et al.* (2018) in cluster analysis using black gram [*Vigna mungo* (L.) Hepper] genotypes.

Cluster II b and II b1 consisted of only one genotype each, TV-27 and N212-5, respectively. TV-27 was from IITA and originates in Nigeria, while N212-5 originated from Kano in Nigeria, as well, suggesting that the two had unique origins in Nigeria. Cluster II b2-1 was made up of two genotypes (101-2 and 101-2-1) from Zambia. The genotype 101-2-1 was a selection from 101-2 and the two had in common their seed coat colour (cream stripe) (Table 1).

The divergence, within Principal component biplot and cluster analysis revealed that some genotypes share common origin, or certain traits among the 26 traits measured. In contrary, morphological diversity of landraces in Tanzania by Ntundu *et al*. (2006) showed that the genotypes were grouped according their regional collection zones, while cluster grouping based on collection location was reported on cowpea in Ghana (Cobbinah *et al.*, 2011).

CONCLUSION

The heterogenic nature with which the landrace collections exhibit would allow for two or more genoytpes to have been the same seed material, but bearing different names depending on where it was grown and/or sourced. Hence a concerted effort for further and advanced morphological and genomic characterization across Africa is important (Amadou *et al*., 2001). From the results in this study, best performing genotypes can be tested for mass production, breeding or germplasm conservation.

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