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Morphological Characterization of Bambara Groundnut [*Vigna subterranea* (L.) Verdc.] Landraces in Kenya

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ABSTRACT

Background: Bambara groundnut [*Vigna subterranea* (L.) Verdc.], an indigenous drought tolerant crop of African origin is one of most important leguminous crop in Sub-Saharan Africa. Small-scale farmers continue cultivating unimproved landraces over the production areas in Kenya. Bambara exist variously as mixtures of seeds, which contain variable types of seed morphology which need to be agronomically and phenotypically differentiated. The study aimed at characterizing Bambara groundnuts collected in Kenya using morphological markers.

Methods: One hundred and five germplasm assembled from four major growing agro-ecologies (Busia, Kakamega, Bungom and Vihiga Counties) and Kenya National Gene bank, were evaluated at the Kenya Agricultural and Livestock Research Organization (KALRO) - Alupe (0.4347° N, 34.2422° E) in a randomized complete block design with three replications in the long and short rains of 2015. Nineteen quantitative traits and seven qualitative traits were observed and measured at different growth stages and during harvesting.

Result: Many landraces displayed pointed, round and yellowish pod, with grooved and oval seeds. About 49.4% had round leaves, 21.5% had elliptical leaves, while 55.7% were heterogeneous for leaf shape. Quantitative traits were significant ($p \leq 0.05$) except for seed weight, seed number per plant and number of stems. The first four principal components accounted for a total of 73.1% of the variations. germplasm were divided into two distinct clusters. Leaf morphology variations could be used as a reliable phenotypic marker in Bambara breeding.

Key words: Bambara, Landraces, Morphological traits, Variation.

INTRODUCTION

Strategic collection, characterization and preservation of genetic resources are critical practices in plant breeding especially for new and under-utilized crops (Olukolu *et al.,* 2012). Knowledge on morphological differences of germplasm assembled is important in generating knowledge of the relationship between the plants structural morphology and how these morphological differences affect corresponding functional botany (Lauri and Normand, 2017). Phenotypic characterization is the oldest and first step in describing and classifying plant germplasm (Hedrick, 2005; Lauri and Normand, 2017). Phenotypic characterization is the oldest and first step in describing and classifying plant germplasm (Hedrick, 2005). Morphological variability depends on a few genes and these may not access much of the potential variability for the agronomic traits available in a crop (Mayes *et al.,* 2019). Morphological and agronomic traits usage is a standard approach of assessing genetic variation for many under-researched crops (Azam-Ali *et al.,* 2001). Goli *et al.,* (1995) characterized and evaluated over 1000 Bambara groundnut accessions based on quantitative and qualitative traits revealing agro-morphological diversity. Ntundu *et al.* (2006) identified vegetative traits that had prominent loadings in principal components analysis and which were useful in distinguishing *Bambara* groundnut landraces. Seed weight, internode length, petiole length, leaflet length, leaflet width, were reported as major traits in distinguishing between wild and domesticated *Bambara* groundnuts when analysed using isozyme markers (Pasquet *et al.,* 1999). Bambara phenotypes which are highly correlated to grain yield give breeders the options on traits selection in Bambara breeding (Karikari, 2000).

Numerical taxonomic techniques have been employed to classify and measure the patterns of genetic diversity in the germplasm collections (Ghafoor *et al.,* 2001, Cui *et al.,* 2001). The comparison of phenotypic and genotypic variation within and between crops has been examined to provide accurate taxonomic and genetic differentiation in *musa* spp. (Crouch *et al.,* 2000). Agronomic and morphological traits have been used to identify characters contributing to important traits like yield in soybean [*Glycine max* (L.) Merr] (Malik *et al.,* 2007) and Bambara groundnut (Cui *et al.,* 2001) in a study the phenotypic diversity of

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Chinese and North American soybean revealed more phenotypic diversity among the Chinese cultivars, than the North American cultivars.. A study on phenotypic diversity study identified traits with higher loadings in principal component analysis (PCA) in a collection of Asian groundnuts (Swamy *et al.,* 2003).

Morphological variability within Bambara landraces have been reported (Ouedrago *et al.,* 2008) which however useful, are affected by the stage of growth of the plant and environmental conditions. Ntundu *et al.* (2006) observed that quantitative traits contributed much of the Bambara diversity. Genetic diversity evaluation is the first step in crop improvement. This can be followed by deployment of molecular markers to accurately and efficiently assess level of polymorphism so as to harness the huge genetic pool of Bambara groundnut landraces (Massawe *et al.,* 2005).

Studies of genetic and morphological diversity of Bambara are scarce and there is limited or no reports on the morphological evaluation of Bambara nuts accessions in Kenya. The present study analyzed the level of diversity in 105 Kenyan Bambara groundnut using morphological differences.

MATERIALS AND METHODS

A total of 105 Bambara landraces were collected from varied agro-ecological zones in Kenyan counties; (Busia (44), Kakamega (21), Bungoma (6) Vihiga (2) and Genetic Resources Research Institute of Kenya (32). The study was conducted at the Kenya Agricultural and Livestock Research Organization (KALRO)- Alupe (0.4347° N, 34.2422° E) with an of elevation of 1220 m above sea-level, mean annual temperature of 22.2°C. The area has sandy clay loam to clay petroplinthite and strongly acidic soil.

Trial was laid out in a randomized complete block design with three replications in the long and short rains of 2015. Field was ploughed and harrowed to a fine tilth. The trial plots measured 2.2 m x 3.0 m. Spacing was 30 cm in three rows, 50 cm apart. Weeds were controlled manually. Di-ammonium phosphate (18:46:0) was applied before sowing at the rate of 130 Kg ha^{-1} to supply nitrogen and phosphorous at an equivalent rate of 23.4 Kg N and 59.8 Kg P ha^{-1}, respectively.

Data collection and analysis

Nineteen quantitative traits and ten qualitative traits were recorded according to Bambara groundnut descriptors note (IPGRI and BAMNET, 2000). Traits evaluated included: pod texture, pod colour, pod shape, terminal leaflet colour, terminal leaflet shape, growth habit, seed shape, peduncle length, number of leaves per plant, terminal leaflet width, terminal leaflet length, petiole length, plant spread, plant height, internode length, number of stems per plant, pod length, pod width, seed length, seed width, 100-seed weight, days to 50% flowering and days to physiological maturity. Combined analysis of variance was conducted on qualitative traits using general linear model and means separated using SNK-Test. Traits with significant differences ($p \leq 0.05$) were subjected to principal component and cluster analysis conducted using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) with DARwin using simple matching coefficient.

RESULTS AND DISCUSSION

Variability in qualitative traits

Frequency distribution of qualitative characters is given in Fig 1. There were differences on qualitative features in growth habit, pod shape and colour. Oval and round leaf shapes dominated with 83% and 17%, respectively. Terminal

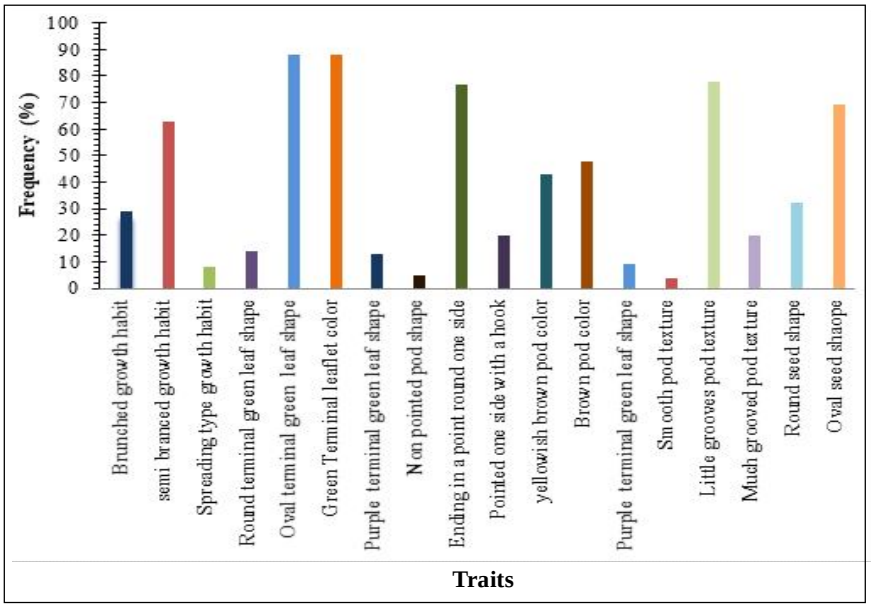

Fig 1: Distribution of qualitative features among the Kenyan Bambara groundnuts.

leaflet colour was largely green (85%) and purple (15%). Bunched, semi-bunched and spreading growth habits existed. Short stems and internodes characterized bunched growth habit resulting in plants with tightly clustered leaves. Plants with spreading (open) growth habit had stems with long internodes resulting in plants with larger diameter of the foliar crown. Semi-bunched type was the most frequent (62%) followed by bunched types (30%) and only 8% were of the spreading type.

About 77% of plants had pod shape ending in a point at the dorsal and round on the ventral side, while 20% had a pod shape ending in a point with a hook on the other side. Only 3% plants had a pod shape without a point. About 44% and 46% of the accessions had yellowish-brown and brown pods, respectively. Nearly 10% of the accessions had pods with purple colour. Terminal leaflet shape, terminal leaflet colour, pod texture and pod shape, displayed little variation. Oval and round terminal leaflet shapes were observed. Landraces with oval leaflet shape were most common (87%), while 13% had round terminal leaflet shape. Most accessions (88%) had green terminal leaflet colour while 12% had purple terminal leaflet colour. Smooth, little grooves and much grooved pod textures were seen. Some 78% of the accessions had a pod texture with little grooves, 20% were much grooved and only 2% were smooth.

Oval shaped seeds were more frequent (66%) compared to round shaped (34%). Grey seed coat predominated (61%) as opposed to red (22%), black (15%) and spotted (2%). There was variability for qualitative characteristics like growth habit, pod shapes, pod colour, pod texture and seed color. These differences could be attributed to genetic, growth environment and genotype by environment interaction in the manifestation of characters. These observations were consistent with those of Mohammed *et al.* (2016). In this work, bunch, semi-bunch and spreading (open) were observed, however with a low proportion, suggesting that farmers selected against these traits. Bunched and semi-bunched types were common, indicating their popularity among farmers. Short stems and internodes that produce plants with tightly clustered leaves are typical characteristics of these plants (Ntundu *et al.,* 2006). This is important during harvesting because most of the pods remain attached to the stem crown after the plant is pulled up. Both bunched and semi-bunched types could have been selected by farmers for ease of management in mixed cropping systems. Spreading growth habit could be used in intercropping to form a more rapid ground cover to help suppress weeds.

Analysis of variance (ANOVA) for quantitative morphological traits

Results were statistically significant at $p \leq 0.05$ except for seed number per pod, number of stems per plant, 100-seed weight and number of days to maturity (Table 1).

Seedling emergence, peduncle length, number of leaves per plant, petiole length, internode length, number of stems per plant and pod width showed significantly differences ($p \leq 0.001$ or $p \leq 0.05$) (Table 2). Draweel *et al.* (2020) also reported that Bambara groundnut genotypes exhibited considerable variation 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. These variations could be due to genetic, environment or genotype by environment interactions. N and P fertilizer were found to play a dominating role in increasing the vegetative growth, yield and yield components of Bambara as these attributes increased with the increased with the application of N and P (Hasan *et al*., 2020).

There were no significant variation for number of stems per plant, 100-seed weight and days to maturity (Table 3). Gene bank accessions were largest sources to assessing morphological variations in plant spread, plant height, internode length, terminal leaflet length and petiole length.

Table 1: Mean of squares for quantitative morphological traits of Bambara evaluated in Kenya.

Source of variation	df	DAE	PDL	SWT	NLP	TLW	TLL	PTL	PTS	PHT
Replication season	21	4.8**	1.37	3.21	54.91**	0.02	0.02	0.27	0.29	0.34
Genotype	104	23.27**	39.30**	509.12	873.08**	1.11*	2.91**	19.80*	271.45**	32.71*
Genotype* season	104	0.66**	0.33	1.34	2.18	0.01	0.02**	0.02	0.072	0.04
Error		0.047	0.33	1.21	1.99	0.01	0.01	0.02	2.35	1.10
CV (%)		2.15	15.82	26.73	22.97	13.18	9.69	16.32	17.40	13.56
Source of variation	df	POL	PDW	SDL	SDW	SNP	DTM	INL	NSP	
Season	1	0.57	0.85	0.16	0.00	0.01	1.63	0.55	120.91*	
Genotype	104	52.33**	10.23**	9.62**	14.77**	0.55	154.07	17.93*	8.49*	
G*S	104	1.73	0.21	0.06	0.002	0.01	0.75	0.07	1.4	
Error		1.45	0.20	0.06	0.02	0.01	0.73	0.07	3.95	
CV (%)		9.63	5.67	14.19	13.82	4.21	1.50	14.97	19.49	

**, *Significant at $P \leq 0.001$ and 0.05 respectively.

Key: DAE-Days after emergence, PDL- Petiole length, TLW- Terminal leaflet width, TLL- Terminal leaflet length, PTL- Petiole length, PTS- Plant spread, PHT- Plant height, INL- Internode length, NSP- Number of stems per plant, POL- Pod length, PDW- Pod width, SDL- Seed length, NPP- Number of pods per plant, SWT -100-Seed weight, DTM- Number of days from sowing to maturity.

Vihiga and Bungoma accessions were generally smaller in architecture while Genebank materials were characterized by low 100-seed weight, pod length and pod width by low 100-seed weight, pod length and pod width (Table 3).

Significant variation was displayed in peduncle length, number of leaves per plant, terminal leaflet width, terminal leaflet length, petiole length, plant spread, plant height, internode length, pod length, pod width, seed length, seed width and number of pods per plant. These traits could be considered as the most useful for the characterization of Bambara in Kenya.

Principal component analysis

Four principal components (PCs) accounted for 73.16% of the total variations (Table 4), explaining 33.28%, 18.39%, 13.32% and 8.17% of the variations, respectively. The first four traits with the highest loadings for both PC1 and PC2 are all quantitative implying that qualitative features accounted for less of the variations. PCA failed to differentiate accessions according to their area of origin with most of the accessions overlapped demonstrating close morphological relationships. Mohammed *et al.* (2019) also reported that Bambara 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. They suggested this may be because movement of Bambara groundnut landraces across the African sub-region could be indiscriminate. PCoA plot (Fig 2), principal axes 1 and 2 showed that KE/BN/2/2 (4) (Kakamega), KE/BN/10 (14) (Vihiga) KE/BN/8/1 (11), KE/BN/16/3 (28), KE/BN/30/2 (51) and KE/BN/48 (105) (Busia), GBK/050491 (65) from the (National Genebank of Kenya) and KE/BN/3/1 (5) (Bungoma) were the most distinct.

Terminal leaflet width, terminal leaflet length, petiole length, plant spread, plant height, pod length, pod width,

Table 2: Mean separation of quantitative features of Bambara in Kenya.

Season	DAE	PDL	NLP	TLW	TLL	PTL	PTS	PHT	INL	NSP
1	10.13a	11.88b	71.98a	2.54a	6.44a	11.17b	32.40a	22.68a	13.59a	7.64a
2	9.95b	11.98a	71.39b	2.55a	6.45a	11.21a	32.37a	22.59a	13.53b	6.76b
Season	POL	PDW	SDL	SDW	NPP	SNP	SWT	DTF	DTM	
1	21.42a	12.01b	11.19a	8.81a	16.11a	1.89a	42.60a	46.32a	119.66a	
2	21.36a	12.09a	11.22a	8.81a	16.12a	1.90a	42.61a	46.31a	119.56a	

*Means followed by the same letters are not significantly different according to SNK at p≤0.05 Key: DAE-Days after emergence, PDL-Petiole length, TLW- Terminal leaflet width, TLL- Terminal leaflet length, PTL- Petiole length, PTS- Plant spread, PHT- Plant height, INL-Internode length, NSP- Number of stems per plant, POL- Pod length, PDW- Pod width, SDL- Seed length, NPP- Number of pods per plant, SWT -100-Seed weight, DTM- Number of days from sowing to maturity.

Table 3: Mean values of quantitative traits for Bambara landraces evaluated in Kenya.

Descriptor	Bungoma	Busia	Gene bank	Kakamega	Vihiga
Days to emergence	10.36 ± 0.45	9.92 ± 0.13	10.55 ± 0.14	9.47 ± 0.13	9.58 ± 0.31
Peduncle length	10.39 ± 0.34	11.97 ± 0.17	12.15 ± 0.15	12.00 ± 0.27	11.46 ± 0.91
Number of leaves per plant	74.33 ± 1.67	71.82 ± 0.73	67.05 ± 0.82	77.48 ± 1.02	73.92 ± 3.36
Terminal leaflet width	2.45 ± 0.06	2.63 ± 0.03	2.43 ± 0.02	2.55 ± 0.05	2.53 ± 0.08
Terminal leaflet length	6.25 ± 0.08	6.36 ± 0.05	6.62 ± 0.05	6.51 ± 0.06	6.29 ± 0.06
Petiole length	10.22 ± 0.28	11.12 ± 0.13	11.82 ± 0.10	11.57 ± 0.16	11.06 ± 0.37
Plant spread	31.00 ± 0.99	31.53 ± 0.42	36.39 ± 0.44	31.78 ± 0.37	30.20 ± 1.81
Plant height	21.19 ± 0.27	22.84 ± 0.14	23.50 ± 0.14	23.02 ± 0.25	22.70 ± 0.60
Internode length	12.20 ± 0.12	13.30 ± 0.10	14.20 ± 0.12	13.95 ± 0.18	13.16 ± 0.30
Number of stems per plant	7.03 ± 0.25	7.15 ± 0.11	6.85 ± 0.15	7.86 ± 0.15	7.58 ± 0.48
Pod length	21.64 ± 0.71	22.08 ± 0.19	20.54 ± 0.17	20.92 ± 0.30	24.17 ± 1.20
Pod width	11.79 ± 0.25	12.10 ± 0.09	11.38 ± 0.09	11.47 ± 0.11	12.52 ± 0.23
Seed length	11.58 ± 0.16	11.15 ± 0.08	11.25 ± 0.08	11.06 ± 0.13	12.00 ± 0.30
Seed width	8.97 ± 0.20	8.68 ± 0.09	9.28 ± 0.12	8.43 ± 0.13	8.00 ± 0.00
Seed number per pod	2.00 ± 0.00	1.81 ± 0.02	1.94 ± 0.02	1.94 ± 0.02	2.00 ± 0.00
100-Seed weight	55.08 ± 5.74	44.65 ± 1.83	20.77 ± 0.24	44.45 ± 2.17	79.95 ± 5.62
No. of days to 50% flowering	51.42 ± 1.53	46.59 ±0.40	45.75 ± 0.42	45.65 ± 0.45	41.17 ± 0.54
Number of days to maturity	123.92 ± 0.63	123.04 ±0.31	117.45±0.37	121.02 ±0.37	117.08 ±1.08
Number of pods per plant	13.83 ± 0.94	13.23 ± 0.51	11.66 ± 0.53	12.81 ± 0.41	16.50 ± 0.75

†† The values are the means ± SE of the three replicates. Figures within a column whose SE values do not overlap are statistically different at p≤0.05.

Table 4: PCA for morphological traits of the Bambara landraces evaluated in Kenya.

Traits	PC 1	PC 2	PC 3	PC 4
Latent roots (Eigen values)	5.63	2.41	1.79	1.23
Percentage variation	33.28	18.39	13.32	8.17
Cumulative % variation	33.28	51.67	64.99	73.16
Peduncle length	0.24	0.06	0.23	-0.48
Number of leaves per plant	0.31	-0.09	0.37	-0.23
Terminal leaflet width	0.29	0.14	-0.03	0.26
Terminal leaflet length	0.42	0.04	-0.06	0.24
Petiole length	0.36	0.38	-0.29	0.12
Plant spread	0.41	-0.68	0.14	0.03
Plant height	0.34	0.36	-0.21	0.07
Internode length	0.25	0.05	-0.02	-0.29
Pod length	0.25	0.51	-0.22	-0.35
Pod width	0.09	0.50	0.03	0.08
Seed length	0.21	0.42	0.35	-0.2
Seed width	-0.06	0.58	0.35	-0.05
Seed number per pod	-0.08	0.61	0.47	0.02
Number of days from sowing to 50% flowering	-0.17	-0.36	0.25	-0.21
Number of pods per plant	-0.05	0.54	0.42	0.02

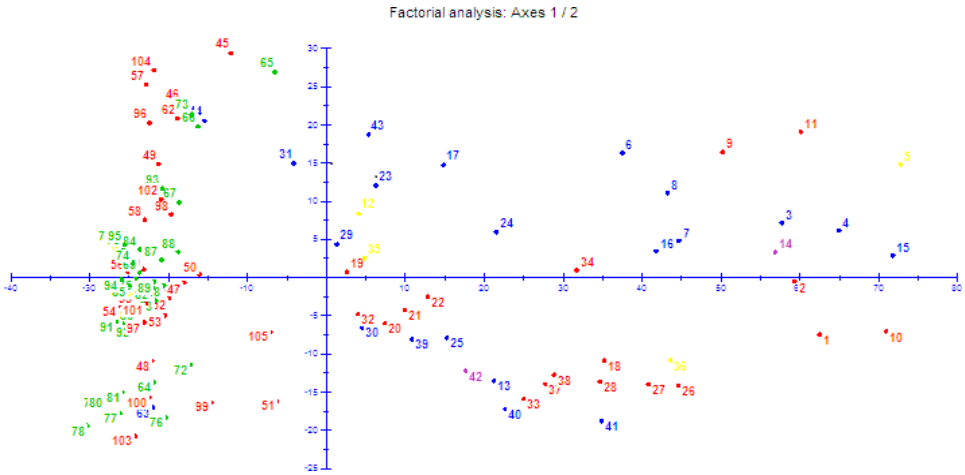

Fig 2: PCoA of axes 1 and 2 based on the dissimilarity of Bambara from Busia (red), Kakamega (blue), Vihiga (purple), Bungoma (yellow) and Genebank (green).

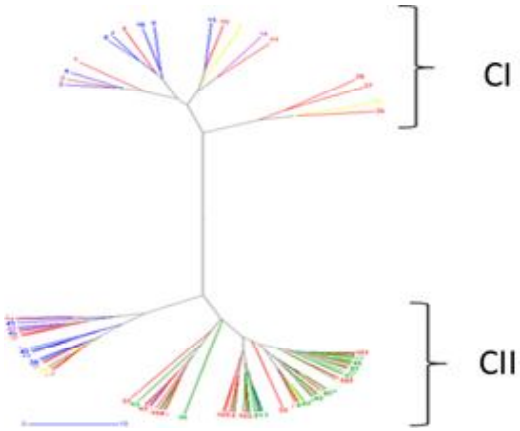

Fig 3: Morphological relationships generated by Jaccard's similarity coefficients among *Bambara* from Busia (red), Kakamega (blue), Vihiga (purple), Bungoma (yellow) and Genebank (green).

seed length, seed width, number of pods per plant accounted for more variability in both PC 1 and PC 2. Accessions with high PC 1 and PC 2 had high vegetative scores with large seeds. The main loadings for the vegetative and seed characteristics observed for PC 1 and PC 2, respectively, implied that farmers preferred leaf size and shape (Ntundu *et al.,* 2002; 2006).

Unweighted pair group method with arithmetic mean

Accessions from different counties overlapped. However, germplasm from Busia and the National Genebank tended to agglomerate together in cluster II in all the sub-clusters. All plants were grouped into two main clusters (I, II) (Fig 3). Cluster I and II had sub- clusters with all the accessions from different regions present in sub-cluster I except those from the Genebank Accessions from the Genebank and Busia tended to group together (cluster II). Kakamega collections were more in cluster I. Vihiga germplasm were presented in cluster I only.

Cluster analysis and PCoA failed to group accessions based on their origin. This can be attributed to the high frequency of Bambara seed exchange among farmers over wide geographic-ethnic areas as well as the different informal naming of the same landraces regionally, permitting genotype duplications (Hudu and Saaka, 2011). This was also noted for coconut studies in Kenya (Oyoo *et al.,* 2015). Yin *et al.* (2018) showed that genotype diversity was due to indiscriminate introduction of from various sources. Furthermore, reporting that pairing of the genotypes was associated more to individuals with possible common origin.

Divergent accessions can be evaluated for their breeding value, applied in direct selection or as a parent stock for hybridization. The mixture of accessions in cluster II showed the group was heterogenic.

CONCLUSION

Bambara germplasm from Kenya displayed a considerable diversity for most of the phenotypes studied. However, cluster analysis failed to group materials by origin, suggesting material exchange among farmers from different regions.

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