

Morphological Characterization and Diversity of Bambara Groundnuts in Uganda

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Abstract

Sixty nine Bambara groundnut accessions were evaluated at the National Crops Resources Research Institute (NaCRRI), Namulonge, Uganda to determine their morphological variability in a randomized complete block design with three replications. Analysis of variance showed significant ($P < 0.01$) divergence among accessions for all traits. Cluster analysis exhibited six distinct clusters with the highest intra-cluster distance (8.09) observed in cluster II and the lowest distance (0.00) in cluster VI. Maximum inter-cluster distance was observed between cluster VI and IV and minimum distance between cluster II and IV. Inter-cluster distance was much higher than intra-cluster distance suggesting a wider variability among accessions. All late maturing accessions with high yield were grouped in cluster V while early maturing accessions were grouped in cluster III. Results of principal component analysis indicated that both yield and vegetative traits were the principal discriminatory characteristics. The accessions evaluated exhibited high diversity for most traits indicating that they can be used in breeding programs to develop varieties with desirable traits.

Keywords: *Vigna subterranea*, accessions, cluster, variability

1. Introduction

Bambara groundnut (*Vigna subterranea*) is the third most important food legume crop after peanut and cowpea in Africa (Forrester et al., 2015). The crop is a good source of dietary protein especially for poor communities who cannot afford animal protein (Baryeh, 2001). Based on its nutritional value, Bambara groundnut (BG) has the potential to reduce food and nutrition insecurity in Africa.

Despite its nutritional value, BG yields remain as low as 0.2 t ha^{-1} , largely because farmers still use traditional cultivars whose yield potential is low (Odireleng, 2012). The continued use of low yielding BG cultivars has been attributed to lack of improved varieties. Presently, few studies have been undertaken to understand yield inheritance with its related traits in BG (Basu et al., 2007). This limited understanding of yield inheritance has hindered breeding programs from developing high yielding BG varieties (Oyiga et al., 2010).

Apart from using low yielding BG cultivars, farmers continue to grow only local cultivars with preferred taste posing a risk of genetic erosion to the non-preferred cultivars which may possess other good traits which can be utilized by plant breeders. Over generations, the practice of using preferred cultivars has led to considerable loss of BG genetic resources in Uganda; yet the national research institutes have not taken bold steps to conserve it.

The situation is further worsened by the fact that currently, there is no Consultative Group on International Agricultural Research Institution (CGIAR) in Uganda mandated to do research on BG (Mayes et al., 2009). This has made avoiding genetic erosion through conservation of BG difficult. Hence, there is need to save the remaining BG genetic resources from extinction through collection and conservation.

Germplasm conservation alone is not enough to rescue BG from extinction but efforts to start a breeding program to support the development of improved BG varieties is critical. Setting up of a breeding program for

BG requires a clear understanding of its variability in order to guide its improvement process as well as setting priorities for future germplasm collection expeditions. Bambara improvement can be achieved through breeding but the success in breeding depends on the choice of appropriate parental lines and how diverse the parental lines are (CIMMYT, 1997). The likelihood of attaining a wide range of variability in segregating generations can be increased by the inclusion of more diverse parents in hybridization program (Joshi, 1996). Therefore evaluating the levels and patterns of variability facilitates the investigation of diversity in germplasm and further identifies diverse parental combinations that segregate with maximum genetic variability for appropriate selection (Barret et al., 1998) and introgression of desirable genes (Thompson, 1998). The information about the variability of the materials will simplify the selection of parental genotypes from random populations before attempting crosses and save time and resources (Hallauer & Miranda, 1988) and also will help to make decisions on management procedures of the BG germplasm.

A number of methods are now available to study crop variability, but morphological method is considered as the first step in description and classification of germplasm (Hedrick, 2005). This method is inexpensive and direct, and is one of the standard ways of assessing genetic variation for many species, especially for under-researched crops such as BG (Azam-Ali et al., 2001). This method has been used by (Odireleng, 2012) to characterize legume crops based on quantitative and qualitative traits. The same method also revealed substantial agro-morphological diversity and distinguished rice genotypes (Ahasanul et al., 2015). In addition, morphological characters which can be highly correlated to grain yield give breeders the choice to make decisions as to which traits to select for crops being evaluated (Odireleng, 2012). This study, therefore, purposed to determine the diversity of BG accessions in Uganda based on morphological characters.

2. Materials and Methods

2.1 Germplasm Collection

Bambara groundnut germplasm was collected from four regions of Uganda (Figure 1) namely south western, Central, Eastern and Northern. This constituted 15 districts including Kabale and Kisoro (South west), Mbale, Sironko, Kamuli, Pallisa, Kumi and Soroti (East) Lira, Dokolo, Apac, and Gulu (North) Bukomansimbi, Lwengo, and Rakai (Central). The districts were selected based on their previous history of BG cultivation and their location within the agro-ecological zoning of Uganda. The germplasm was collected in form of seed from farmers and local markets which were 10 km apart where possible. For each sample collected its identity information based on IPGRI descriptors (IPGRI, 2000) and the Global Positioning System (GPS) reading for that particular site were recorded. The samples collected were placed in paper bags, labelled and transported to the National Crops Resources Research Institute (NaCRRI), Namulonge for characterization.



Figure 1. Map of Uganda indicating districts where Bambara groundnuts accessions were collected

2.2 Location and Establishment of the Experiment

A field experiment was conducted at NaCRRRI, Namulonge to characterize BG germplasm collected from Uganda. Namulonge lies at an altitude of 1150 metres above sea level with a bimodal rainfall pattern. It receives an average 1,270 mm of rainfall annually with a mean temperature of 22 °C. The soils are red sandy clay loam type with a pH range of 4.9-5.0. Sixty nine accessions of BG were assembled for field evaluation during 2014B and 2015A rainy seasons. A randomized complete block design was used with three replications. Three BG seeds for each accession were planted per hole in 2m² plots at a spacing of 50 × 20 cm. Each plot was surrounded by two guard rows to safeguard the plants in demarcated plots from external interference. Three weeks later the seedlings were thinned to one seedling per hole. The plots were maintained weed free with a monthly weeding interval. Each weeding was accompanied with mounding of soil around each plant to create sufficient space for root and pod expansion.

2.3 Data Collection

Ten weeks after planting, five plants per accession were randomly selected from each plot to record their quantitative data which included; terminal leaflet length (mm), terminal leaflet width (mm), petiole length (mm), plant spread (cm), plant height (cm), internodes length (mm), peduncle length (mm), number of flowers per peduncle, number of leaves, number of nodes per stem, number of branches per stem and number of stems per plant. Furthermore, traits related to growth duration such as days to 50% flowering and days to maturity were also recorded. Yield characters which were scored during and after harvest included, number of pods per plant, 100-seed weight (g), number of seeds per pod, and total yield were computed on a plot basis and extrapolated to yield per hectare. However, for qualitative data, five individual plants were recorded per plot to represent each BG accession and nine qualitative characters which were recorded included, testa colour, eye pattern, pod colour, pod texture, pod shape, seed shape, growth habit and stem hairiness.

2.4 Statistical Analysis

The germplasm collection map was developed using survey data points that were geo-referenced with a global positioning system (GPS). Data points were transformed into a point map using Ilwis 3.2 software and the map

exported and visualized in Arc View® GIS3.2 software (Rockware Inc). In order to test the significance of variation among BG accessions for each trait, analysis of variance (ANOVA) was performed using Genstat version 14 (Payne, 2014). The Mahalanobis's generalized distance (D^2) and principal component analysis were done also using Genstat statistical software, version 14. The clustering of the accessions was done following the Tocher's method (Rao, 1952).

3. Results and Discussion

3.1 Observable Characters

Twenty six characteristics were studied in sixty nine BG accessions. Based on the qualitative characteristics, most accessions had round shaped seeds (84%) whereas a few were oval shaped (16%). Cream (75%) and cream testa with grey butterfly-like eyes (53%) were the most dominant seed and eye colour respectively (Table 1). Though cream seeded accessions with grey double three lines on both sides of the eye (30%) were also observed.

Table 1. Grouping of *Vigna subterranea* accessions based on qualitative parameters

Trait	Category
Growth habit	A. bunch type (0%), B. semi bunch type (19%), C. spreading type (81%)
Terminal leaflet shape	A. round (0%), B. oval (100%), B. lanceolate (0%)
Terminal leaf color	A. green (100%), B. red (0%), B. purple (0%)
Stem hairiness	A. absent (0%), B. sparse (100%), C. dense (0%)
Pod color	A. yellowish brown (64%), B. brown (16), C. reddish brown (20), D. purple (0%), E. black (0%)
Pod texture	A. smooth (2%), B. little grooved (60%), C. much grooved (38%)
Seed shape	A. round (84%), B. oval (16%)
Seed color	A. cream (75%), B. grey (15%), C. light red (10%),
Eye pattern	A. cream testa with black butterfly-like eye (17%), B. cream testa with dark red butterfly-like eye (30%), C. cream testa with grey butterfly-like eye (53%)

Cream colour being dominant in the studied BG accessions indicated that most farmers select for cream colour during planting. Although some farmers also plant a mixture of colours, but a survey done in Swaziland revealed that the most preferred BG landraces were of cream colour (Oyiga et al., 2010; Brink and Belay, 2006), which further confirms that in most African countries that grow BG cream was the most preferred colour by most farmers. Bambara groundnut pod colour ranged from brown to yellowish brown with little grooved pod texture (60%) and much grooved pod (38%). However, for pod shape, most BG accessions had round pods with pointed ending (81%), while others were pointed with a nook (19%). Two growth habits; spreading type (81%) and semi-bunch type (19%) were observed indicating that farmers mostly select for two growth habits. The two growth habits also reflected the type of cropping systems used by farmers to cultivate BG. According to Odireleng (2012), the spreading type accessions are useful in mixed cropping with other crops while the semi-bunch types are for monoculture. On the other hand, the following qualitative characters; terminal leaf colour, shape and stem hairiness did not vary among BG accessions studied.

3.2 Intra and Inter-Cluster Divergence

Prior knowledge on the variation based on agro-morphological characters is important in initiating any crop breeding work. The analysis of variance revealed that BG accessions differed significantly ($P < 0.05$) for 17 quantitative traits studied (Table 2). This indicated the presence of variability among accessions, which signified that there is good opportunity for BG improvement if breeding work was initiated. These findings were in line with (Odireleng, 2012), who also reported significant differences in 23 characters among 24 characters that were studied to determine the diversity and population structure of BG landraces in Swaziland. Similarly, Ntundu et al. (2006) observed significant morphological variation for 13 BG characters measured for two seasons in Tanzania.

Table 2. Analysis of variance for 17 quantitative traits of 69 *Bambara groundnut* accessions in Uganda

Traits	Source of variation (Mean square)		
	Replication (d.f. = 2)	Accessions (d.f. = 68)	Error (d.f. = 136)
Peduncle length	6.8	354.8**	0.041
Number of leaves	85.1	1258**	1.11
Terminal leaflet length	353.0	221.5**	2.26
Terminal leaflet width	50.2	65.9*	0.85
Petiole length	156.8	1,160.5**	1.51
Plant spread	20,798	4,3836**	17.36
Plant height	137.6	3,094.2**	1.41
Internode length	58.4	1,096.8**	0.92
Number of nodes per stem	0.121	1.49**	0.04
Number of branches per stem	0.47	3.05**	0.08
Number of stems per plant	1.68	37.87**	0.16
Pod/plant	2,717	265.2**	6.27
Seed/pod	0.1125	2.784**	1.00
Days to 50% flowering	4.69	7.49**	0.26
Days to maturity	4.49	37.67**	0.25
100 seed weight	17.8	380.5**	0.51
Yield (Kgha ⁻¹)	57,401	2,610,374**	28.8

Note. d.f. = degree of freedom; ** significance at 5% level.

Due to significant variability observed among the BG accessions, it was necessary to classify the accessions based on the degree of divergence considering all the 17 traits. Based on diversity range, 69 BG accessions were grouped into six clusters (Table 3). However, the clustering did not depend on the districts or agro-ecologies where BG was collected. The distribution pattern among clusters revealed that the highest number of accessions were found in cluster I while the least number of accessions were in cluster VI. Clusters II III IV and V comprised of 16, 5, 3 and 3 BG accessions, respectively.

Table 3. Clustering of Bambara groundnut accessions based on D² statistics

Cluster No.	No. of accessions	Accessions
I	40	A3, A4, A5, A7, A8, A11, A13, A14, A16, A19, A21, A22, A23, A24, A25, A26, A28A29, A30, A31, A32, A33, A34, A38, A41, A42, A44, A45, A47, A48, A50, A52, A53, A56, A57, A59, A62, A64, A67, A68
II	16	A2, A10, A12, A17, A18, A27, A40, A46, A49, A51, A54, A58, A60, A63, A65, A66
III	5	A1, A37, A39, A61, A69
IV	3	A43, A9, A55
V	3	A20, A6, A35
VI	2	A15, A36

The less number of accessions in clusters IV, V and VI was probably due to small number of traits and the duplication effect of traits included in the study. Earlier work by Ahasanul et al. (2015) in rice crop indicated that in hierarchical and dynamic clustering, the frequency of genotypes in a given cluster is increased by increasing the number of traits under study.

The intra-cluster divergence was maximum in cluster II (8.09) and minimum in clusters V and VI (0.00), indicating that BG accessions in cluster II were more diverse than their counterparts in clusters V and VI (Table 4). The intra-cluster distances were only high for I, II and IV clusters and ranged from 1.49 to 8.09, indicating the heterogeneous nature of BG accessions within each of the three clusters. The high intra-cluster divergence observed in cluster I, II and IV in the study were in line with findings by Forrester et al. (2015) who attributed the high divergence to seed exchange between farmers and the geographical proximity of the areas where the accessions are collected. On the other hand, the zero intra-cluster distance in clusters V and VI, indicated that BG accessions in these clusters were homogenous.

In terms of inter cluster distance, cluster VI showed maximum distance from cluster IV (24.93) followed by the distance between cluster IV and III (24.62), cluster I and IV (22.75), and cluster II and IV (21.42) (Table 4) which reflected wider variability among these clusters. Minimum distance was found between BG accessions of the cluster II and VI (4.14) followed by the distance between clusters III and VI (1.50) (Table 4). However, in some cases the inter-cluster distances were observed to be greater than the intra-cluster distances suggesting wider variability among accessions of the distant groups. Similar variability based inter- and intra-cluster distances have been described in other crops rather than BG by various researchers (Panigrahi et al., 2014; Zai-ul-Qamar et al., 2012).

Table 4. Intra and inter-cluster average distances among 69 Bambara groundnut accessions

Cluster No.	I	II	III	IV	V	VI
I	7.060	14.52	1.640	22.75	14.31	17.80
II		8.070	3.300	21.42	5.800	4.140
III			1.490	24.62	9.020	1.500
IV				7.620	15.62	24.93
V					0.000	9.480
VI						0.000

Note. NB: Values in bold illustrate the intra cluster distance and others show inter cluster distance.

3.3 Cluster Mean Performance and Their Contribution Towards Divergence

The mean performance of different clusters for the traits evaluated indicated that the early maturing BG accessions were grouped into cluster III while cluster V included late maturing BG accessions indicating maximum contribution of growth durations towards the divergence between cluster III and V (Table 5).

Table 5. Cluster mean of different traits among 69 Bambara groundnut germplasm

Traits	Cluster numbers					
	I	II	III	IV	V	VI
Peduncle length	37.2	35.1	39.6	39.2	39.5	45.7
Number of flowers per peduncle	1.4	1.3	1.2	1.4	1.3	1.4
Number of leaves	60.9	45.9	45.6	76.6	54.0	57.0
Terminal leaflet length	68.2	57.0	52.3	57.2	60.9	60.6
Terminal leaflet width	33.0	23.5	22.0	28.0	24.3	24.7
Petiole length	140.1	156.3	133.7	146.8	136.2	141.3
Plant spread	428.1	323.7	341.7	328.1	335.4	348.4
Plant height	187.1	213.4	170.5	185.3	180.8	176.6
Internode length	22.3	18.7	14.4	19.7	16.0	17.5
Number of nodes per stem	5.6	3.1	3.7	3.1	3.4	4.7
Number of branches per stem	2.0	2.4	1.9	2.9	3.2	3.9
Number of stems per plant	8.4	7.6	7.7	14.3	10.4	8.7
Pod/plant	30.1	25.0	29.9	32.3	40.5	31.8
Seed/pod	1.8	1.4	1.7	1.4	1.5	1.6
Days to 50% flowering	61.2	63.4	60.7	61.5	62.5	60.6
Days to maturity	126.6	128.0	125.8	127.6	129.1	128.2
100 seed weight	19.4	14.7	16.3	19.7	21.0	20.3
Yield (Kgha ⁻¹)	1648	1023	1968	1693	2922	2116

Again all the high yielding accessions were grouped into cluster V whereas cluster II included low yielding accessions, implying maximum contribution of yield towards the divergence between cluster V and II. Cluster I was divergent from cluster IV mainly due to seed per pod, plant spread, number of nodes and stems per plant, indicating maximum contribution of these traits towards the divergence between the two clusters. On the other hand, cluster IV was divergent from cluster VI mainly due to yield and number of stems per plant. Based on these findings, the traits contributing maximally towards the divergence should be used as a basis for deciding which type of cluster would be considered for selection of best parents for hybridization (Latif et al., 2011). As a

result, the first component differentiated those accessions that flower and mature earlier and also differentiated high yielding accessions.

The contribution of different traits towards the divergence was measured through principal component analysis (Table 6). The first principal component accounted for more than 21.8% of total variance, whereby number of flower peduncle, pod per plant, days to 50% flowering, days to maturity, 100 seed weight and yield were the variables that contributed most negatively (Table 6).

Table 6. Principal components for seventeen traits in Bambara groundnut accessions

	PC1	PC2	PC3
Eigen value	8.95	3.28	1.12
% Variance	21.8	14.0	11.6
Cumulative (%) total variation	21.8	27.5	29.7
<i>Coefficient vector</i>			
Peduncle length	0.2429	-0.1578	-0.3130
Number of flowers per peduncle	-0.0747	0.0378	-0.1079
Number of leaves	0.1982	0.4231	-0.2283
Terminal leaflet length	0.4143	-0.0341	0.1576
Terminal leaflet width	0.3832	0.0667	0.1930
Petiole length	0.2805	0.0648	-0.1453
Plant spread	0.4051	0.0544	0.0709
Plant height	0.3160	-0.0176	-0.2019
Internode length	0.2029	0.1645	0.3849
Number of nodes per stem	0.3669	-0.2736	0.1287
Number of branches per stem	0.1959	-0.0105	-0.1045
Number of stems per plant	0.0439	0.5152	-0.2455
Pod/plant	-0.0239	0.1565	0.3027
Seed/pod	0.0459	-0.3013	0.3564
Days to 50% flowering	-0.0090	0.4143	0.1192
Days to maturity	-0.0201	0.3371	0.3302
100 seed weight	-0.0239	0.1565	0.3027
Yield (Kg ha ⁻¹)	-0.1253	0.1166	0.3633
% variation explained	21.8	14.0	11.6

The first component identified mainly phenological variables presenting negative contributions. The second principal component accounted for more than 14.0% of total variance. Variables highly and negatively correlated were peduncle length, terminal leaflet length, plant height, number of nodes per plant and number of branches per plant, thus differentiating those accessions by vegetative characters which contribute to good architecture. Ntundu (2006) observed similar patterns of loading in their study on 100 BG landraces in Tanzania, whereby the high loading within principal component one was mainly due to vegetative characters. The third principal component accounted for 11.5% of total variance and was associated with peduncle length, numbers of flowers per peduncle, number leaves, petiole length, plant height, number of branches, and stems per plant, hence differentiating those accessions by vegetative and flowering characteristics. This indicated the importance of these characters in identifying BG accessions that follow in the same cluster. These findings agree with Ntundu (2006), who reported that vegetative characters were major factors contributing to the variation of BG germplasm.

4. Conclusion

The study exhibited that BG accessions from Uganda are morphologically diverse and agro-morphometric characters can be employed to assess variability and measure the extent of relationship among the accessions. This study also revealed that Ugandan BG accessions constituted six major clusters based on yield, growth duration, vegetative and flowering traits. The clustering was further confirmed by PCA which also exhibited that all the four major traits revealed during clustering were the basis for principal discriminatory characteristics. The information generated on the degree of BG variability will be of importance for its genetic improvement.

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