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Research Article

Morphological characterisation of Bambara groundnut (*Vigna subterranea* (L.) Verdc.) germplasm collections: A basis for crop improvement

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Abstract

Bambara groundnut is one of the underutilised legume crops adapted to Sub-Saharan Africa's harsh, semi-arid tropical regions, providing nutrition and cash income among resource-poor farmers. A field study comprising 25 Bambara groundnut accessions was carried out during the 2018/2019 cropping season at the Crop Science Experimental Field, University of Namibia-Ogongo Campus, to characterise the accessions using qualitative traits. The experimental design used was an alpha lattice with three replications. Based on the visual observation techniques, the results showed morphological variation among the genotypes. The Bambara groundnut germplasm studied showed high phenotypic variations. The germplasm had a high proportion of the bunch-type growth habit (44% accessions), oval leaflet shape (64%), sparse hairiness (56%), and absence of eye pattern colour (70%). Also, cluster analysis grouped the accessions into five super classes. Shanon-Wennier index indicated a significant level of diversity among the Bambara groundnut accessions for most traits studied, with plant growth habits having the highest index of 2.15, followed by for pod colour (1.45) and seed eye pattern colour (1.22). These results may be useful in formulating national Bambara groundnut breeding programs in semi-arid Sub-Saharan African regions. However, molecular analysis is required to determine the genetic variations among the accessions.

Introduction

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) is a diploid (2n=22) neglected and underutilised warm-season legume crop grown in semi-arid regions of Africa (Azam-Ali et al., 2001; Mayes et al., 2019). It is also grown in Asian countries such as Indonesia, Thailand and Malaysia (Ellstrand & Elam, 1993; Redjeki et al., 2013). The global population is estimated to be 10 billion by 2050, implying that the demand for food to feed the growing population would also increase. Hence, breeding orphan crops, such as Bambara groundnut, is essential, as the food supply faces climate, environmental, and health (Sarkar et al., 2019; Takahashi et al., 2016). Therefore, plant breeders are

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working on crop improvement to diversify crops, enhance crop climate change adaptation, and upgrade orphan crops to address the food shortage problem (Garnett, 2014; Godfray & Garnett, 2014; Muhammad et al., 2020).

Bambara groundnut is one crop that has not received research attention in the past (Berchie et al., 2012); however, there are organisations and research institutions that support Bambara groundnut research as listed as an orphan crop. The crop is grown for food, nutrition, fodder, medicine and income generation (Minka & Bruneteau, 2000; Muhammad et al., 2020). It is a drought-tolerant legume crop adapted to infertile poor sandy soil conditions (Chai et al., 2016; Mabhaudhi et al., 2016; Mkwandawire, 2007; Sarkar et al., 2019) and can thrive under the changing climate (Hillocks et al., 2012). Regardless of its benefits, the qualitative morphological characteristics of Bambara groundnut still need to be established as most of the cultivars used by farmers have unknown characters (Massawe et al., 2005; Mubaiwa et al., 2018). Besides, there are many uncharacterised crop germplasm in national genetic resource centres (Dwivedi et al., 2017).

The morphology characterisation is a standard procedure used to identify the most critical germplasm collection traits for many crop species (Muhammad et al., 2020; Olukolu et al., 2012). Various qualitative characterization studies have been conducted in different countries. For example, Goli (1997) characterised 1384 Bambara groundnut accessions at Nigeria's International Institute for Tropical Agriculture (IITA). He reported that 45 and 47% of the accessions were of the bunch and semi-bunchy conformation, while few accessions (8%) were the spreading type. Ntundu et al. (2006) characterised 100 accessions using qualitative traits: growth habit, terminal leaflet shape, terminal leaflet colour, seed shape, pod colour, pod texture, and pod shape in Tanzania. The results showed a diversity of morphological traits useful for germplasm management and utilisation in crop improvement. In Benin, Gbaguidi et al. (2018) qualitatively characterised 52 Bambara groundnut germplasm accessions, which showed large variations in terminal leaflet colour, bunch type, stem hairiness, leaflet shape, pod colour, pod texture, seed colour, eye colour, seed shape, pod texture in the collection of Bambara groundnut. Molosiwa et al., (2015) characterised the crop using growth habit, terminal leaflet shape, the colour of a fully expanded terminal leaflet, pod colour, pod texture, all of which showed variation. Mohammed et al., (2016b) studied Bambara groundnut seed morphology, assessing seed coat colour and pattern, seed hilum colour and pattern, seed eye colour and pattern. Their results indicated that variation among traits and generated the seed morphology baseline of the crop. Bonny et al., (2019) evaluated the diversity of qualitative traits in 101 Bambara groundnut accessions in Cote d'Ivoire, which showed high morphological diversity among traits studied. The germplasm was collected from different regions, and for each region, the crop showed a high morphological diversity

In Namibia, few studies have evaluated and characterised Bambara groundnut quantitative traits. Mukakalisa (2006) evaluated six landraces for genetic diversity using molecular markers, random amplified polymorphic DNA (RAPDs), and simple sequence repeat (SSR). Fleißner (2006) evaluated Bambara groundnut accessions using quantitative traits, such as yield and yield components and the results showed variations among the accessions. Valombola et al., (2019) evaluated quantitative traits in Bambara groundnut and observed variation among the accessions. However, the qualitative traits of the Bambara groundnut germplasm in Namibia are not fully characterised. Characterisation of different Bambara groundnut germplasm collections is essential for variety improvement (Ndiang et al., 2014). Qualitative trait studies are important since farmers use seed coat colours, growth habits, and leaf shapes to distinguish, classify and name Bambara groundnut landraces in their areas. Therefore, we carried out morphological diversity characterisation of Bambara groundnut landraces in this study using qualitative traits related to leaves, pod, leaves, and seed traits to generate information needed to establish the Bambara groundnut breeding program for Namibia.

Materials and methods

A total of 25 Bambara groundnut germplasm accessions (Table 1) were collected and characterised at the University of Namibia-Ogongo Campus, Omusati Region (latitude of 17°40′37 .6″S, longitude 15°17′43.0″E and altitude of 1109 m above sea level) during the 2017/2018 cropping season. The region is characterised by low annual precipitation with an average of 400–500 mm (Awala et al., 2019), and the average temperature is greater than 22 °C. Bambara groundnut accessions were acquired from Namibia Botanical Research Institute (NBRI) (10 accessions), Omahenene Research Station, Namibia (10); Kitwe, Zambia (2); and Chitedze Research Station in Malawi (3).

Table 1. Bambara groundnut germplasm accessions used in the study

S/N	Accession ID	Source
1	MW791, MW2875, MW 266	Chitedze Research Station
2	NAMFA	Local Bambara groundnut farmers, northern Namibia
3	ZAM01, ZAM02	Kitwe- Zambia
4	NAM 1754/3, NAM 3804, NAM 1195/2, NAM 1762/2, NAM 1866, NAM 1758/3, NAM 1084/3, NAM 1156/3, NAM 959/4	Namibia Botanical Research Institute (NBRI)
5	LR4, LR6, NAM 3900, AHM 968, NAMBLACK, UNISWA RED, NYAKC, NAM RED, DIPC, KFBN	Omahenene Research Station

Experiment design

The experimental design was an alpha lattice with 25 accessions in three replications and 15 blocks and 75 experimental units. The experimental area was 1018 m^2 (68 m long × 15 m wide). The size of individual plots was 8.1 m^2 (3.6 m long × 2.25 m wide). The inter-row spacing was 0.75 m while intra-row spacing was 0.45 m, giving three rows per plot with ten plant hills totalling a population of 30 plants per plot. The experimental field was weeded manually and scouted for any insects from the first day of field emergence.

Data collection

Bambara groundnut descriptors (Institute International Plant Genetic Resources, 2000), were used to characterize the accessions based on ten qualitative traits as described in Table 2. The characterisation was done at a particular growth stages and after harvest in the case of seeds. Growth habits and terminal leaflet shape were recorded in the tenth week after planting. Stem hairiness was recorded after harvest. Pod shape, pod colour, pod texture, seed shape, seed testa colour, and eye pattern were recorded two months after harvest.

Data analysis

The phenotypic percentage classes of the qualitative traits were estimated using all 25 accessions. The accession similarity was calculated using the agglomeration method—the unweighted pair-group method with the arithmetic averages (UPGMA). The data were analysed using the GenStat 19th edition software program. The results were recorded as nominal. The diversity of qualitative traits was calculated using the Shannon Wiener Index formula:

$$-\sum_{i-1}^{s}(PilnPi)$$

Where P_i is the proportion of accessions (n/N) of individuals of one particular character found (n) divided by the number of individuals found (N), ln is the natural logarithm, Summation of the calculation, and s is the number of traits.

Table 2. Some descriptors of Bambara groundnut qualitative traits

Morphological traits	Recording description/method
1. Seed shape	1) Round, 2) Oval, 99 Other
2. Seed testa colour	1) Cream, 2) Grey, 3) Light red, 4) Dark red, 5) Light brownish red, 6) Dark brown, 7) Dark purple, 8) Black, 99) Other
3. Seed eye pattern colour	1) Cream testa butterfly-like eye, 4) Cream testa triangular eye, 10) Light brown testa with a butterfly-like eye, 99) Other
4. Seed hilum colour	1) White, 99) Other
5. Growth habit	1) Bunch type, 2) Semi-bunch type, 3) Spreading type
6. Terminal leaflet shape	1) Round, 2) Oval, 3) Lanceolate, 4) Elliptic, 99) Other
7. Stem hairiness	0) Absent, 3) Sparse, 7) Dense
8. Pod texture	1) Smooth, 2) Little grooves, 3) Much grooved
9. Pod colour	1) Yellowish-brown, 2) Brown, 3) Reddish-brown, 4) Purple, 5) Black, 99) Other
10. Pod shape	1) Without a point, 2) Round on the other side, 3) With nook on the other side, 4) Two points on each side

Source:(Institute International Plant Genetic Resources, 2000)

Results

Phenotypic diversity

The 10 qualitative traits studied showed variations among the Bambara groundnut accessions (Table 3). Among the accessions studied, 60% had oval-shaped seeds, while 40% had round seed shapes. Seed testa colour/pattern was 20% for the tan colour, 12% for the red and cream, 8% for each of the dark tan and purple, and a minimum of 4% for speckled brown, cream, brown, and black (Figure 4). Regarding seed eye pattern colour, 64% of the accessions had no eye colour, 16% had amber colour, and 4% had brownish, brown, grey, black, and purple colour (Figure 4). Growth habit showed that 44% of the germplasm accessions were bunch type, 24% were semi-bunch, and 32% were slightly spread. Terminal leaflet shape indicated that many were oval, representing 64%, 24% elliptic, and 12% were lanceolate (Figure 2). Stem hairiness was sparse (56%), absent (40%), and dense (4%) among the accessions. For the pod shapes, 70% of the accessions had ending points and round on the other side, while 28% had ending points with nook on the other side. Pod colours were brown (40%), reddish-brown (4%), yellow (24%), yellowish-brown (24%) and yellowish (8%); whereas the pod textures were little groove (36%), much grooved (32%), and smooth (32%) (Figure 1).

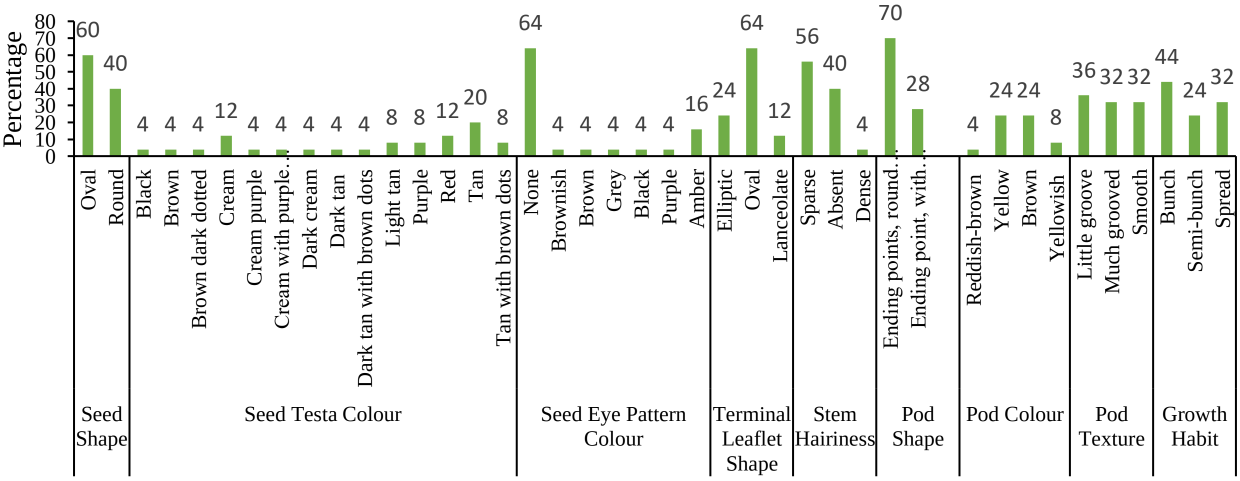

Figure 1. Percentage distribution of qualitative traits in the Bambara groundnut germplasm

Elliptic **Oval** **Lanceolate**

Figure 2. Terminal leaflet shapes in the Bambara groundnut germplasm

Figure 3 Pod colour in some Bambara groundnut accessions

Figure 4. Seed coat colour of the Bambara groundnut germplasm

Cluster analysis

Twenty-five germplasm accessions were used for the hierarchical cluster analysis of qualitative traits. The dendrogram revealed five main clusters for the germplasm accessions (Figure 5). Two accessions from Malawi showed similarity as they belong to the same cluster, including some of Namibia's accessions. ZAM 01 from Kitwe, Zambia, revealed a close relationship with Namibian accession DIPC originally obtained from Botswana and sourced from Omahenene Research Station. Some Namibian accessions were in the same cluster as Malawian and Zambian accessions, which may have been resulted from the exchange of seeds across the border. The distance ranged from 1.00 to 0.65, with a decrease in similarity.

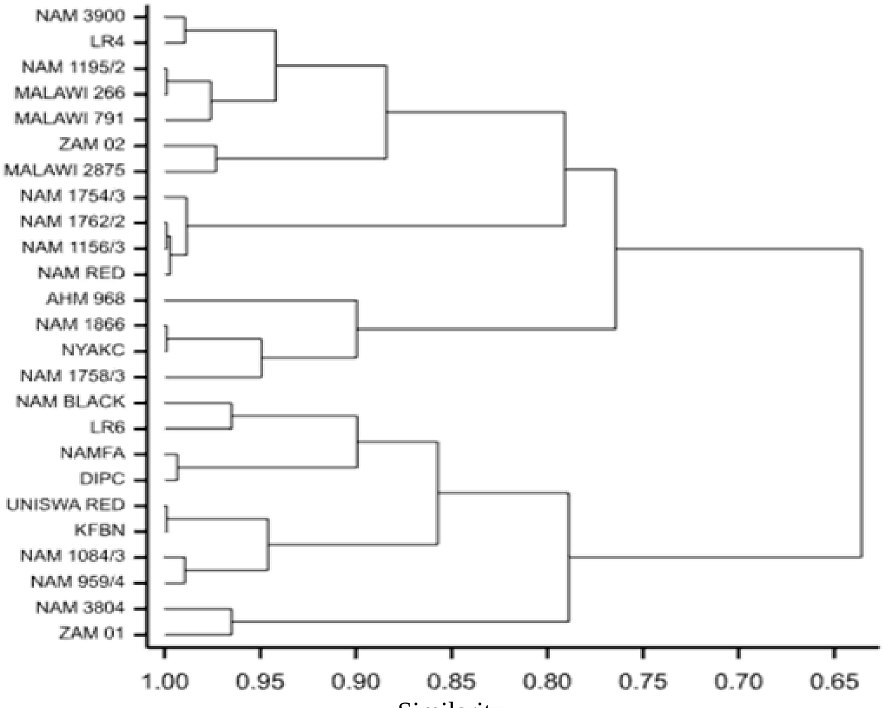

Figure 5. Phenogram showing the relationship among Bambara groundnut accessions based on similarity

Diversity index

Estimated Shannon-Weiner diversity index (H') for the Bambara groundnut qualitative traits are shown in Table 3, which were generally high. All traits showed a high level of polymorphism (H'>0.500). The indices ranged from H'=0.58 (pod texture) to H'=2.15 (growth habit) with an average of H'= 0.92. The polymorphism is shown in all traits except in seed hilum H'= 0 that showed monomorphism.

Table 3. Diversity index values of qualitative traits among Bambara groundnut accessions

Qualitative trait	Shannon-Weiner index(H')
Seed shape	0.67
Seed testa colour	0.75
Seed eye pattern colour	1.22
Growth habit	2.15
Terminal leaflet shape	0.88
Seed hilum	0.00
Stem hairiness	0.82
Pod colour	1.49
Pod texture	0.58
Pod shape	0.59
Average diversity index	0.92

Discussion

Phenotypic diversity

To our knowledge, this is the first study presenting scientific data on the qualitative traits of the Bambara groundnut germplasm in Namibia. The germplasm studied showed variation in morphological traits. The trait growth habit exhibited high variability as many accessions were highly bunch type, followed by spread type and then by semi-bunch type. In terms of harvesting, the bunch type and semi-bunch type may be advantageous during harvesting; the pods are attached to the stem crown, and only a few pods remain in the soil when the plant is pulled up, reducing labour to search for the pods remaining in the soil. Nonetheless, the bunch type growth habit, on the other hand, seems to be more advantageous during crop podding; the pods firmly attach to the stem around the plant. The bunch type can also increase plant population per unit area as one plant occupies less plant area under a monoculture cropping system. The bunch type may benefit farmers if the crop is earthed or unearthed; the roots and stems can stand and bear pods. These findings are similar to that of Bonny et al. (2019) and Gbaguidi et al. (2018). The spread type was the second, followed by bunch type growth habit in our germplasm and can be beneficial in intercropping cropping system in which it may suppress weed growth by covering the ground. However, the results disagree with Ntundu et al. (2006), which showed many semi bunch type accessions compared to the spread type ones.

The highest seed coat colour percentage observed was tan, followed by cream and red seed coat. Light coloured seeds and cream and tan seeds have lower tannin content than dark seeded, red and black (Mubaiwa et al., 2018). The tannin content in seeds makes seeds unpalatable to insects and poisonous to the beetle. In contrast, the dark coloured Bambara groundnut seeds have a low chance of being attacked by insects (Lattanzio et al., 2005). Dark coloured seeds have higher emergence and germination rates than light coloured seeds (Chibarabada, 2014). The seed coat colour in Bambara groundnut is theorised to tolerate drought more than light coloured seeds because of polyphenols that act as antioxidants under stress conditions (Mabhaudhi et al., 2013). These findings are similar to those of Amarteifio et al. (1998); Lungelwa (2012) and Mayes et al. (2019).

For seed eye colour or patterns, 64% of the accessions had no eye colour or patterns. Mohammed et al. (2016a) observed that 51% of their study's accessions have no eye colour or patterns. The terminal leaf shapes were observed as oval, elliptic and lanceolate. Narrow-leaved type crops are likely to adapt in harsh environments, for instance, drought (Chai et al., 2016). The germplasm with lanceolate leaf was 12%, implying that the accessions can survive in semi-arid areas. These results agree with those of Bamshaiye et al. (2011), Ghafoor et al. (2001) and Ntundu et al. (2006). The accessions with oval-shaped terminal leaflets dominated the Bambara groundnut germplasm, followed by elliptic terminal leaflets. Oval shaped leaves are big and preferable as fodders for livestock (Unigwe et al., 2016). The oval and elliptic terminal leaflets may help capture sunlight and carry out photosynthesis efficiently; Kidner & Umbreen (2010) and Nicotra et al. (2011) reported similar results. Stem hairiness was mainly sparse or absent among the accessions, but some had dense stem hairiness. Stem hairiness is generally valuable for crop protection against ultra-violet light, herbivores, insects and drought. Therefore, this trait is important in crop breeding, aiding in curbing crop stress and aiding accession identification (Gbaguidi et al., 2018).

Seed shape is vital in seed classification and identification. The trait is also a good determinant of quality, market price and yield. The seed shape also reflects a particular seed's genetic, physiological, and ecological components (Cervantes et al., 2016). Pod colour and texture are the main factors determining the Bambara groundnut as a fresh vegetable in the market (Mustapha, 2009; Mustapha & Singh, 2008; Yuste-Lisbona et al., 2014). Further, Bonny et al. (2019), Goli (1997), Ntundu et al. (2006) and Ndiang et al. (2014) observed morphological qualitative traits variation among Bambara groundnut accessions.

Cluster analysis

Cluster analysis revealed the importance of morphological markers to differentiate and classify Bambara groundnut germplasms. Germplasm accessions with common traits were grouped in the same clusters. The dendrogram showed variation among the accessions for the traits studied. This information would help a plant breeder to make the right decision for the crop improvement program. The similarity detected among the Bambara groundnut accessions could have resulted from seed exchange among farmers and farmers' seed selection practice, which is the same from year to year. Also, the accessions in the same cluster might have similar genetic makeup because Bambara groundnut is a self-pollinated crop. These results are similar to those of Bonny et al. (2019), who noted a 30% similarity among the accessions studied and observed diversity by cluster analysis.

Diversity index

Shannon-Weiner diversity (H') indices for the Bambara groundnut qualitative traits assessed in the study were generally high. The diversity among the traits could be due to the new introduction of seeds from other countries, phenotypic plasticity and selection pressure among individual farmers. These factors may contribute to significant qualitative trait diversity by creating or maintaining polymorphism. The Bambara groundnut morphological diversity observed in this study should be genetically conserved correctly in germplasm bank or in-situ genetic conservation, as suggested by Bonny et al. (2019). Germplasm conservation is essential to maintain genetic diversity, which allows species adaptation to variable environmental conditions (Aliyu et al., 2016).

Conclusion

The Bambara groundnut germplasm studied showed high phenotypic variations. The germplasm had a high percentage of the bunch-type growth habit (44% accessions), oval leaflet shape (64%), sparse hairiness (56%), and absence of eye pattern colour (70%). Also, cluster analysis grouped the accessions into five super classes. Shanon-Wennier index indicated a significant level of diversity among the Bambara groundnut accessions for most traits studied, with plant growth habits having the highest index of 2.15, followed by for pod colour (1.45) and seed eye pattern colour (1.22). The observed variations are crucial for adaptation to variable environments; the diverse traits could reduce the risks of total crop failure due to biotic and abiotic stresses. These results may be useful in formulating national Bambara groundnut breeding programs. However, molecular analysis is required to determine the genetic variations among the accessions. Moreover, more accessions should be collected from local and international genetic sources for further scientific study.

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Author’s declaration and contribution

The author declare no conflict of interest. JSV is the principal author, wrote and contributed to the article from introduction to conclusions. SKA the author contributed to the agronomic section of the article in the introduction, results and discussions. KH, the author worked on data arrangement, analysis and article editing.

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