Original Research Article

Genetic diversity in Bambara groundnut {*Vigna subterranea* (L.) Verdc.}

Nwakuche Chinenye **Onwubiko**

Department of Crop Science and Technology, Federal University of Technology, Owerri, Nigeria

Correspondence to:
N.C. Onwubiko, Dept. of Crop Science and Technology, Federal University of Technology, Owerri, Nigeria,
Email: nwakuche.onwubiko@futo.edu.ng

Abstract

Bambara groundnut is a grain legume with enormous morphological variability. In order to genetically establish the variation that exists in this crop, an assessment of genetic diversity was therefore carried out with 20 accessions of Bambara groundnut collected from International Institute of Tropical Agriculture (IITA) Ibadan. The design of the experiment was randomised complete block design with three replications. Results from analysis of variance (ANOVA), and principal component analysis (PCA) showed outstanding genetic diversity among the collections. The first four principal components accounted for 91.89% of the total variability. Cluster analysis and the dendrogram discretely grouped the accessions into four genetically distinct groups. One accession TVSU 353 singly formed a group in cluster analysis and dendrogram, which implies that TVSU 353 was genetically distinct from the rest of the accessions. Morphological characters assessed provided a useful measure of genetic differences among Bambara groundnut accessions, which can facilitate identification and selection of potential breeding lines for crop improvement as well as germplasm conservation.

Keywords: Accessions; genetic distinction; morphological descriptors; variability

INTRODUCTION

Differences exist in crop species which discriminate one crop from another. The extent to which one crop is distinguished from another is an expression of diversity. This can be called species diversity or also referred to as species richness. Differences can sometimes be found within genotypes of the same species. And the discrimination of one genotype from another can be traced down to differences in their genetic make-up. This type of diversity is called genetic diversity (Bhandari et al., 2017), and it is the basis for the continued natural existence of crop species. The fitness of species and adaptability in an environment is possible because of substantial different genetic forms of crop species. Both artificial and natural selection depends on genetic diversity to function (Kelly, 2011), and it is the amount of genetic diversity that exists in crop species that determine efficiency of artificial and natural selection. Artificial selection acts on the existing genetic diversity to select superior genotypes that can be released as new varieties. Parents used for hybridisation programme to develop new lines are also selected from diverse genetic forms that exist in crop species. Genetic diversity in crop species is fascinating. It expresses phenotypic differences in the genetic make-up of all the individuals in the same species or other species, such as variations in shapes, colours and sizes of leaves, flowers, fruits, seeds, growth habit and pigmentation. Plant breeders rely absolutely on existing genetic diversity for resources to improve the existing crops and to create new varieties.

The exploitation of genetic diversity in crops to meet needs is as old as agriculture (Govindaraj et al., 2015), although in the past, there were little or no emphasises

on conservation of genetic diversity in crops, which may have led to massive loss of genetic diversity (otherwise called genetic erosion) in crop species. Scholars in life sciences in recent times guard against extinction of species especially those endowed with desirable genes that can be used for crop improvement. Endangered, threatened, and vulnerable species are terms used in recent times to increase awareness on the need to conserve genetic diversity and to avoid further loss of genetic resources. Further, there is increasing awareness on the importance of assessment of genetic diversity in crops. This has led to the use of other methods rather than the traditional use of morphological characters in assessment of genetic diversity. Scholars have reported the use of biochemical markers (Ahmed et al., 2014), cytological markers (Nayak et al., 2005; Egbucha and Malgwi, 2014), and molecular markers (Ntundu et al., 2004; Odongo et al., 2015) in the assessment of genetic diversity in crops. Kone et al. (2007) reported a study on tissue culture to assess genetic diversity in Bambara groundnut. Recent studies combine two or more markers to assess genetic diversity in crops (Siise and Massawe, 2013; Han et al., 2020).

Scholars have reported enormous morphological diversity that exists in Bambara groundnut (Ntundu et al., 2006; Mohammed, 2014; Unigwe et al., 2016; Onwubiko, 2020). However, there are few reports on the estimation of genetic diversity that exists in Bambara groundnut (Amadou et al., 2001; Massawe et al., 2003; Molosiwa et al., 2013), moreover most of these reports were carried out with molecular markers. Hence this study was set up to estimate genetic diversity in Bambara groundnut with morphological markers.

MATERIALS AND METHOD

Seeds of 20 accessions of Bambara groundnut used for the study were collected from IITA (International Institute of Tropical Agriculture), Ibadan, Nigeria. The field experiment was conducted at the Teaching and Research Farm of Department of Crop Science and Technology, Federal University of Technology, Owerri, between 2018 and 2019 cropping seasons. The farm is located on latitude 5 27' 50.23" North and longitude 70 02' 49. 33" East. This area had an altitude of 55 m above sea level, a mean annual rainfall of 2500 mm and a relative humidity of 88.6%. Experiment design was a randomised complete block (RCBD) with three replications, and the size of the experimental field was 20 m × 12 m. Each block had a size of 20 m × 4 m and the distance in-between plots were 1 m. The field was disc-harrowed and ridged before planting. Two seeds of each accession were planted and were later thinned down to 1 at 3 weeks after planting (WAP). The intra- and inter-seed planting distance was 30 cm × 100 cm, which gave a planting density of 120 plants per plot. Standard cultural practices were applied to ensure optimum crop production like thinning, weeding, earthing up at 9 weeks after planting and application of poultry manure at 15 t/ha.

The accessions were evaluated for 23 characters considered relevant for genetic diversity assessment. These traits were outlined in descriptor for Bambara groundnut (IPGRI, IITA, BAMNET, 2000). Traits evaluated were days to emergence, days to first flowering, days to 50% flowering, days to maturity, vigour index, plant height at flowering, canopy width (spread of plant) at flowering, number of leaves per plant, terminal leaflet length, terminal leaflet width, petiole length, number of stems per plant, number of branches per stem, number of nodes, internode length, pod length, pod width, shelling percentage, seed length, seed width, number of pods per plant, number of seeds per pod, 100-seed weight, and seed yield. Data collected were subjected to analysis of variance (ANOVA). It was also used to perform principal component analysis (PCA), cluster analysis and dendrogram using GenStat statistical package software (Discovery edition 3).

RESULTS

Analysis of variance results showed highly significant differences ($p < 0.01$) for 17 out of the 23 characters used to discriminate the Bambara groundnut lines. These characters were number of days to first flowering, number of days to 50% flowering, number of days to maturity, canopy width (spread of plant) at flowering, number of leaves per plant, terminal leaflet length, terminal leaflet width, petiole length, number of stems per plant, number of nodes, internode length, pod length, seed length, seed width, number of pods per plant, shelling percentage, 100-seed weight, and seed yield. Further, other characters that showed significant differences ($p < 0.05$) among the Bambara groundnut lines evaluated were number of branches per plant, number of stems per plant, and vigour index. There were no significant differences ($p > 0.05$) for days to emergence, plant height, and pod width (Table 1).

The first principal component (PC) accounted for 61.78% of the total phenotypic variation among the genotypes and was found to be closely related to the number of leaves per plant, and petiole length. The second, third, and fourth PCs accounted for 12.36%, 11.02%, and 6.73%, respectively, of the total variation among the genotypes, and the characters responsible

Table 1. Analysis of variance (ANOVA) of the Bambara groundnut accessions

Character	Sum of squares	Df	Mean square	F	Sig
Canopy width	19	4212.67	221.72	258.18	0.001
Days to 50% flowering	19	1420.73	74.77	42.12	0.001
Days to emergence	19	22.40	1.17	1.78	0.063
Days to flowering	19	105.67	5.56	7.49	0.001
Days to maturity	19	6630.32	348.96	356.15	0.001
Internode length	19	228.85	12.04	29.92	0.001
Number of branches per plant	19	31.92	1.67	2.59	0.006
Number of leaves per plant	19	62987.92	3315.15	2040.65	0.001
Number of nodes per stem	19	669.25	35.22	69.35	0.001
Number of pods per plant	19	11805.60	621.34	1068.38	0.001
Number of stems per plant	19	52.33	2.75	2.82	0.003
Petiole length	19	45528.20	2396.2	13.37	0.001
Plant height	19	311.65	16.40	1.46	0.158
Pod length	19	375.65	19.77	24.80	0.001
Pod width	19	392.93	20.68	2.03	0.031
Seed yield	19	6364.60	334.97	659.54	0.001
Seed length	19	98.56	5.18	171.47	0.001
100-seed weight	19	12175.40	640.81	962.48	0.001
Seed width	19	33.16	1.77	70.74	0.001
Shelling percentage	19	2011.65	105.87	212.87	0.001
Terminal leaflet length	19	5195.25	273.43	192.77	0.001
Terminal leaflet width	19	1054.27	55.48	89.22	0.001
Vigour index	19	19.9333	1.0491	2.65	0.005

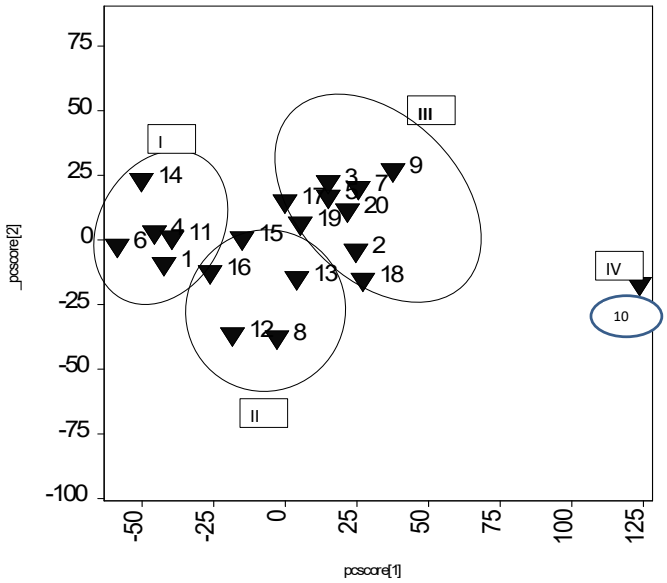
Table 2. Eigenvalues, percentage variance and cumulative variance of the first four principal component axes (PCA)

PC	Eigenvalue	% variance	Cumulative variance (%)
1	–0.28	61.78	61.78
2	0.00	12.36	74.14
3	–0.01	11.02	85.16
4	–0.03	6.73	91.89

for the variability were number of stems per plant, seed yield, seed weight and days to maturity. In total, the first four PCs accounted for 91.89% of the total variation (Tables 2 and 3).

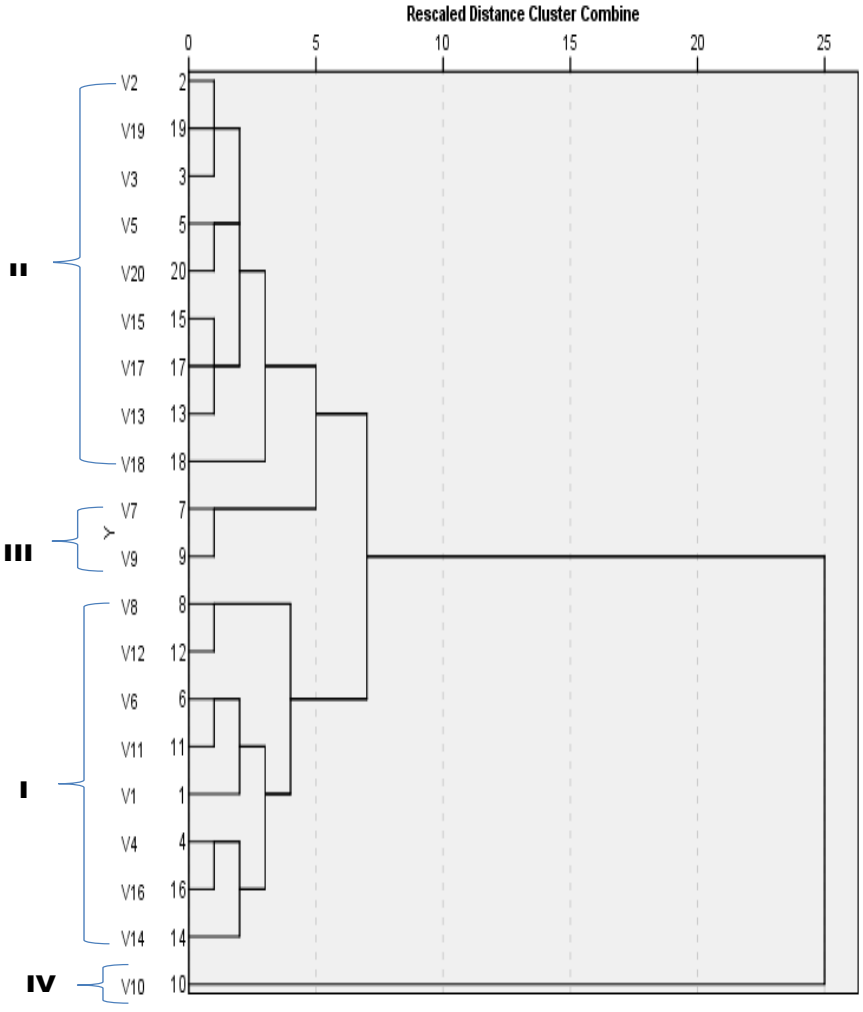
Cluster analysis resolved the genotypes into four groups. Group 1 consisted of five genotypes and these were TVSU 436, TVSU 134, TVSU 275, TVSU 356, and TVSU 129. In this group the average intra-cluster distance was 36.69. The accession that had the highest cluster distance of 28.83 was TVSU 129 whereas the least distance of 23.08 was obtained for TVSU 436. The five genotypes in Group 2 were TVSU 437, TVSU 438, TVSU 434, TVSU 358, and TVSU 350. Average intra cluster distance in this group was 24.63, and the genotypes that had the highest and the least cluster distance of 30.38 and 20.49 was TVSU 437 and TVSU 495, respectively. The highest number of genotypes (9) was resolved in Goup 3, and the accessions were TVSU 439, TVSU 131, TVSU 272, TVSU 277, TVSU 352, TVSU 442, TVSU 447, TVSU 130 and TVSU 440. This group had an intra cluster distance of 32.92. Accessions TVSU 277 had the highest cluster distance of 48.64 whereas the least cluster distance of 20.28 was for TVSU 440. Group 4 had only one genotype; TVSU 353 (Table 4). In summary, in the cluster analysis result, TVSU 277 and TVSU 440 had the highest and the least cluster distance, respectively, among all the accessions.

The dendrogram resolved the accessions into four groups which were close to the result on cluster analysis. The seven accessions in Group 1 of the dendrogram were TVSU 272, TVSU 277, TVSU 352, TVSU 275, TVSU 350, TVSU 358, and TVSU 356. More accessions (eight) were clustered in Group 2, and these were TVSU 434, TVSU 437, TVSU 439, TVSU 442, TVSU 447, TVSU 440, TVSU 436, and TVSU 438, while only one accession (TVSU 353) was resolved in Group 3. Further four accessions were gathered in Group 4, and these were TVSU 134, TVSU 129, TVSU 130 and TVSU 131.



Legend: I Group 1, II. Group 2, III Group 3, IV Group 4

Figure 1. Cluster analysis of the Bambara groundnut accessions evaluated



Legend: I Group 1, II Group 2, III Group 3, IV Group 4

Figure 2. Dendrogram of 20 accessions of Bambara groundnut evaluated

Table 3. Principal component analysis of the Bambara groundnut accessions used for the study

Characters	PC 1	PC 2	PC 3	PC 4
Canopy width	0.17451	0.06149	–0.05981	0.00480
Days to 50% flowering	0.00056	0.07422	–0.13153	–0.02106
Days to emergence	0.00076	0.00209	–0.00244	–0.01662
Days to flowering	–0.00475	0.00487	–0.02761	–0.00299
Days to maturity	–0.01725	0.17394	0.02069	–0.62504
Internode length	0.01115	–0.02904	–0.00905	0.04597
No of branches per plant	0.00065	–0.00335	0.00636	0.02570
No of leaves per plant	0.75173	0.47635	0.27308	–0.17829
No of nodes per stem	–0.00781	–0.06097	0.07956	–0.04883
No of pod per plant	0.13675	0.34332	–0.46710	0.53592
No of stem per plant	0.00411	–0.00353	0.01691	–0.01549
Petiole length	0.58482	–0.70733	–0.22066	–0.03072
Plant height	0.01476	–0.00193	–0.02585	0.00225
Pod length	–0.01279	0.01821	–0.02427	–0.01731
Pod width	0.01289	0.00685	0.01585	0.03582
Seed yield	0.14268	0.21999	**–0.39414**	0.10415
Seed length	–0.00183	0.00215	0.00923	0.01423
Seed weight	–0.06836	0.02440	**–0.67780**	–0.47895
Seed width	–0.00147	0.00197	0.01976	0.00808
Shelling percentage	0.05002	0.02380	0.09549	0.13287
Terminal leaflet length	0.12068	–0.23310	0.02270	0.06721
Terminal leaflet width	0.02274	–0.08015	0.05801	0.13660
Vigour index	0.00566	–0.00625	0.00131	0.01742

Table 4. Cluster analysis of the Bambara groundnut accessions evaluated

No	Treatment	Cluster	Distance	Country of origin
1	TVSU 129	1	23.083	Nigeria
2	TVSU 134	1	26.802	Ghana
3	TVSU 275	1	26.921	Nigeria
4	TVSU 356	1	31.309	Nigeria
5	TVSU 436	1	36.697	Cameroon
6	TVSU 350	2	30.380	Nigeria
7	TVSU 358	2	20.495	Nigeria
8	TVSU 434	2	21.507	Cameroon
9	TVSU 437	2	28.821	Cameroon
10	TVSU 438	2	21.980	Cameroon
11	TVSU 130	3	24.482	Nigeria
12	TVSU 131	3	29.873	Nigeria
13	TVSU 272	3	28.649	Nigeria
14	TVSU 277	3	48.646	Nigeria
15	TVSU 352	3	40.663	Nigeria
16	TVSU 439	3	29.663	Cameroon
17	TVSU 440	3	44.814	Cameroon
18	TVSU 442	3	29.678	Cameroon
18	TVSU 447	3	20.282	Cameroon
20	TVSU 353	4	.000	Nigeria

DISCUSSION

Variation within phenotypes in a species and between different crop species may be genetic or due to environmental influence. When the expression of a character is stable in an environment over the years, it is more likely to be genetic. Similarly, a character whose expression is stable in different environments is mostly genetic (Olaoye and Atande, 2000). In this study distinctive variation was found among the 20 Bambara groundnut accessions used for the study. Highly significant differences ($p < 0.01$) were observed for 17 out of the 23 morphological characters evaluated. It was only 3 characters: day to emergence, plant height and pod width that did not differ significantly among the accessions. The expression of these characters was stable over the two years of field investigation. Variability in the environment during the years of field trails had negligible influence on the expression of these characters, which implies that the observed distinctive differences among the accessions were mostly genetic. Invariably, this result implicates a wide range of genetic diversity among the Bambara groundnut lines used for the study. Previous studies have reported varying levels of genetic diversity in Bambara groundnut (Massawe, 2000; Olukolu et al., 2012; Shegro et al., 2013; Onwubiko et al., 2019).

The characters that accounted for 91.89% of the total variation for the first four principal component axes were number of leaves per plant, petiole length, number of stems per plant, seed yield, seed weight and number of days to maturity. Correspondingly these characters were among those that showed highly significant differences between lines ($p < 0.01$) in the ANOVA result. This implies that the expression of these characters was significantly under genetic control. Influence of the environment on the expression of these characters was negligible. Literature reports from other studies on genetic diversity of Bambara groundnut have also revealed these characters to be responsible for distinctive genetic variations (Siise and Massawe, 2013; Mohammed, 2014).

Cluster analysis grouped the accessions into four groups. In each group were accessions from different geographical areas (like Nigeria, Cameroon, and Ghana), except for group four that had only one accession TVSU 353 (from Nigeria). The pattern of clustering showed that accessions collected from the same geographical area did not form a separate group, rather accessions with close cluster distance were gathered into a group. This indicates that genetically similar genotypes were resolved into one group. This result may have some implication on the origin and classification of the evaluated Bambara groundnut lines. Previous study has reported a similar result (Omokhafe and Alika, 2001). Further, it can be deduced from the cluster analysis that the accession with the least distinctive features of each group had the highest cluster distance. In group 1 was TVSU 129 while for groups 2 and 3 were TVSU 347 and TVSU 277, respectively. Similarly, accession with the least cluster distance for each group (TVSU 436 for Group 1, TVSU 495 Group 2 and TVSU 440 Group 3) was the most outstanding genotype of the group. Ideal accession(s) of each group had their cluster distance(s) close to the average cluster distance of their group. This result has some implication in selection of genotypes for crop improvement.

The dendrogram resolved the Bambara groundnut accession into four groups, similar to the result on cluster analysis. Accessions from geographically distinct areas were resolved into the same group, which implies that similar genetic lines were gathered to form a group. There was another similarity in the pattern of resolving the accessions into groups in the two results (cluster and dendrogram). Accession TVSU 353 was resolved into a distinct group alone in the dendrogram, as was the case in the cluster analysis. In the dendrogram TVSU 353 was resolved alone in Group 3 which suggests that TVSU 353 was genetically distinct from other accessions used in the study. Although the depth of this study was unable to unravel why only TVSU 353 formed a group in the two results, however, it can be inferred that TVSU 353 possesses unique qualities. Typically, such genetically divergent accession can enhance the improvement of Bambara groundnut through various breeding programmes. Ntundu et al. (2004) reported a similar result.

Apparently, a wide range of genetic diversity was revealed among the evaluated accessions of Bambara groundnut. The passport data of the lines showed that they were mostly collected from Nigeria and Cameroon. This result has some implication on reports on the origin of Bambara groundnut. Patra et al. (2016) reported that evidence of extensive diversity of a crop in an area is one of the criteria used in the determination or confirmation of centre of origin. Therefore, this result corresponds with the report of other workers who observed that Bambara groundnut originated from West Africa, precisely from North-Eastern Nigeria and Northern Cameroon (Dalziel, 1937; Hepper, 1963; Begemann 1988; FAO, 2020).

CONCLUSION

Genetic diversity in Bambara groundnut as assessed with morphological characters showed outstanding

distinguishing differences among the accessions. ANOVA and PCA results showed that observed variations (diversity) was genetic; clearly an expression of inherent characters of the Bambara groundnut lines. Genetic effect of variability was further strengthened by the results of cluster analysis and the dendrogram. These two results also established a wide range of genetic diversity among the accessions. Each of these results independently resolved the accessions into four genetically distinct groups. Further the two results (cluster analysis and dendrogram) independently resolved accession TVSU 353 into a separate group alone. Cluster analysis had TVSU 353 alone in Group 4 while dendrogram separately resolved accession TVSU 353 in Group 3. Apparently TVSU 353 was genetically distinct from other Bambara groundnut accessions used in the study. Meticulous study on TVSU 353 is imperative to uncover its potentials.

CONFLICT OF INTEREST

The authors declared no conflicts of interest with respect to research, authorship and publication of this article.

ETHICAL COMPLIANCE

The authors have followed the ethical standards in conducting the research and preparing the manuscript.

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