Characterization of genetic variation of Bambara groundnut [Vigna subterranea (L.) verdc.] under different environmental conditions for yields and yield component performances

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Article

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Abstract

Bambara groundnut has significant roles to play in terms of food security even though researchers have paid very little attention to the crop in the past. The aim of this study was to investigate the high yielding accessions in three environments. A total of 34 morphological, phenological and agronomic traits were measured and analyzed statistically with R software. There were significant differences in all the traits except for plant height, initial plant stand, panicle length per stem, and petiole length. Across the three environments, TVSU-455 gave the highest values for total number of pods (42.67 ± 13.37), final plant stand (7.67 ± 1.58), fresh seed weight of (45.83 ± 14.82), number of seed per plants of (46.62 ± 14.89), hundred seed weight with a value of (124.56 ± 18.99), dry seed weight (27.14 ± 8.91), fresh pod weight, a value of (92.65 ± 30.96), harvest index of (0.57 ± 0.25), yield per plot (45.83 ± 14.82) and unshelled yield per plot (550.26 ± 117.89). TVSU-455 was the only accessions in cluster I of the dendogram based on its superiority over other accessions. The clustering analysis produced a dendogram categorizing the 15 accessions into 4 groups based on the morphological, phenological and agronomic traits. There were significant differences among the correlations of the 34 traits. The first two principle components explained 56.16% of the total variation with each dimension accounting for 39.85% and 16. 31% variation, respectively. TVSU-455 can be used in breeding programs to improve Bambara groundnut.

Introduction

Bambara groundnut *(Vigna subterranea L. Verdc)* is an annual legume belonging to the family of *Fabaceae*, subfamily of Faboidea and genus Vigna[1]. The seed qualities, pod, color, texture, leaf shape, plant vigor, nutritional and anti-nutritional qualities of the crop differ from one variety to another. It is said to have originated from West Africa (Chad, Central African Republic, Nigeria and Cameroon). This crop can also be found in some tropical parts like America, Australia, and Asia but the level of its cultivation is very low[2].

Bambara groundnut ranks third as the most common essential crop behind peanuts *(Arachis hypogea)* and cowpeas *(Vigna unguiculata)* in Africa but not classified globally in the world's trading scheme because it is underutilized and researchers have paid very little attention to the crop because its rank in the production percentages were low[3]. This crop is said to be underutilized and often times regarded as "poor man's crop "and/or "women's crop" simply because all the pre field, field, and post field activities were being performed by women and this was reported by Oyugi et al.[4] and it is grown at the subsistence level to meet family's immediate needs[1]. Also, it was recently noticed and declared as a crop for the new millennium due to its characteristics as crop rich in nutrients and most resilient crop to climate change[5].

Bambara groundnut has significant roles to play in terms of food security. It can be used in different ways or processed into divergent things by different people and dialect across different locations. The recorded setbacks to the use of the crop are in its hard to cook and hard to mill phenomenon which is brought about by an extended cooking time before the adequate softening texture for consumption is achieved[6], the lack of practicing modern production techniques and the fact that breeders still have to use the local variety or natural breed of the crop[7].

The green freshly harvested nuts can be cooked with spice and salt and then be consumed by West Africans[8]. Although, Mazahib et al[9] reported that the methods to shorten the length of cooking of the crop has been a source of major concern for researchers and farmers, the results of a survey revealed that the seeds could be roasted or soaked as a pretreatment means before milling into flour to combat the hard to cook problem[10]. Also, in previous surveys before this, soaking and roasting of the seeds were also employed to bypass the hard to mill issue[11]. Bambara groundnut have been reported to be highly nutritious compared to other legumes and is the preferred food crop of many local people and people who cannot afford to buy the valuable protein in animal products[12].

Bambara nut can be grilled or steamed and afterwards served as tidbits [13], the seeds contain a high percentage of calorie and the flour after it has been milled can be used to prepare porridge[14], prepare soup and also be used as coffee substitute as noted by Hillocks et al[8], the dehydrated seeds can be crushed into powder to bake cakes and breads[15], and as reported by FAO[16] it can be used to make dumplings. According to a study review, it was observed that the milk derived from Bambara nuts is similar to that of soybean milk and it can be used as a baby food supplement[17] because it has a higher rate of acceptance amongst soybean and cowpea milk[18]. The protein which was obtained from the shafts of Bambara nuts and the leaves can be used in making animal feeds like fishes, cattle and goats[19]. The total world supply of protein is about 65% and Bambara contributes up to 25% of protein[20]. It was also reported by some inhabitants of Congo according to a survey carried out by Doku and Karikari[21] that oil can also be extracted from Bambara seeds.

Aside from its nutritional benefits, Bambara also has diverse health benefit which ranges from being used to cure diarrhea[22], prevent high blood pressure[23], treat wounds and heal epilepsy[20], to control of vomiting during pregnancy when chewed and swallowed[24].

Because Bambara is a leguminous crop highly rich in nutrient, it is referred to "a complete balanced diet "due to its high carbohydrate content (49-64.5%), protein (15-25.5%), fat (4.5–7.4%), fiber (5.2–6.4%), ash (3.2–4.4%), minerals (2%) and it also contains micronutrients such as K (11.45–19.35 mg/100 g), Fe (5.1–9.2 mg/100 g), Na (2.9–12.0 mg/100 g), and Ca (95.8–99 mg/100 g) present in its seed[25]. The biochemical investigation that was also carried out revealed that Bambara groundnut also contains nonessential amino acids of (66.69%) and essential amino acids of (33.31%) of the content[26]. Just like other leguminous crops, it can help fix the atmospheric nitrogen[27].

Bambara groundnut is drought resistance and it has the ability to thrive well in area of poor soil composition even while some other crops fail[28]. It has the ability to safeguard the future food and dietary needs in face of climate change due to the crop's intrinsic resistance to stress[29]. To small scale or subsistence farmers, in situations of low agricultural inputs like fertilizers and pesticides for production, Bambara groundnut can still thrive well. The roots of Bambara groundnut help to fix nitrogen in the soil thereby replenishing and repairing the nutrients in the soil and this attribute makes it highly suitable to be rotated or intercropped with rice, maize, millet, yam or cassava to mention few[20]. The leaves of the crop are rich in Nitrogen and Potassium which also renders it suitable for animal feed.

Bambara groundnut produced annually in the world is estimated approximately at 160,378 tons out of which 111,562 tons were produced in Africa[30] and the quantity of Bambara groundnut produced in Nigeria remains the first with 100,000 metric tons per annum while Burkina Faso ranked first for producing highest yield of the crop[8]. It should be noted that the existing natural breeds produced low yield of 650kg/ha due to unfavorable conditions such as diseases and pests' outbreak and lack of improving the genotype best modified to climate change[31]. On the other hand, according to Azam-Ali[13] some researchers have noted that improved Bambara groundnut genotypes can produce yield from 3.0t/ha to 4.5t/ha when all factors related to yield are correlated. Diverse methods have been employed in crop advancement in order to make use of better cropping methods like mutation, genome editing, proteomics profiling and mutagenesis. The primary motive of plant breeders is to create varieties that are rich and able to generate genetic difference in the traits of crops. After such a variety has been developed, identification and choosing of the best traits and/or a combination of such traits are also employed. After this, the chosen traits must be established and popularized for general and global use and understanding. However, the use of different environments alone cannot provide breeders with all the necessary needed information about the traits therefore, a genotype x environment interaction (GEI) analysis will help to provide a better phenotype of the traits[32]. The presence of GEI points out that the genotypic and environmental factors both have impact on the phenotypic expression of a trait. In order to be able to accurately identify and pick out the genotypes for the targeted environments, this approach can be carried out in plant breeding. Most of the times, the genotypes are tested over different environments like location, season of planting and different genotypes are also involved. The most important activity in improving the status of crops is, but not limited to how to maximize revenue, minimize cost, maximize yield stability and minimize risk[33]. Also, some factors such as soil composition, soil type, soil structure, temperature, rainfall, level of atmospheric humidity, presence and effects of pathogens and precipitation may all bring about differences in the yield, yield stability and yield quality of the crop as a way of responding to the different environment[34] and these differences are known as genotype x environment interactions. In plant breeding, yield is the most common trait that is observed by using the GEI[35] but reports from previous researches have been given for traits like quality and biomass production. It is of paramount importance for plant breeders to carry out multi-locations experiments on crop genotypes to admittedly authenticate the stable and superior of these crop genotypes. Therefore, the objectives of this study were to (i) determine the phenological, morphological, agronomic and qualitative traits performances of 15 Bambara groundnut accessions across three different environments. (ii) investigate the high yielding accessions in these environments.

Results

Soil analysis.

Higher amount of sand, bray P, %OC, Mn, Na and Mg was in Bowen, higher amount of clay and Cu were found in Ibadan while higher amount of silt, %N, Zn, Fe, K and Ca were found in Odeda (Table 1). The pH at Bowen, Ibadan and Odeda were 7.20, 7.64 and 6.79, respectively. There was different types of soils in terms of texture, physical and chemical properties from the three environments under study. It is ascertained that the climatic and soil conditions influence the growth, development and yield of crops. Crops also respond differently to different types of soils. It has been observed that Bambara groundnut has high yield in sandy soils because it bears fruits underground and sandy soil has porous and loose structures with large pores which allows for pods to grow and smooth harvest. When the sandy soil gets dried, they produce thin, loose fissures which are of advantages especially in the semi-arid tropics where there's an uneven rainfall pattern and long droughts. Clay soil has a high water retention ability and it expands when wet and vice versa when dry.

Table 1

The physio-chemical properties of soil for the 3 experimental locations

PROPERTIES	BOWEN	IBADAN	ODEDA
Sand %	71.00	60.22	56.22
Clay %	10.90	29.14	13.63
Silt %	18.10	10.64	30.15
% N	0.19	0.20	1.12
Bray P	23.11	14.84	17.26
% OC	1.19	0.99	0.84
Zn (ppm)	1.38	1.30	2.30
Cu (ppm)	0.68	1.20	0.98
Fe (ppm)	77.96	82.40	89.62
Mn (ppm)	169.42	108.17	117.64
Na (Cmol/kg)	0.09	0.03	0.08
K (Cmol/kg)	0.43	0.27	1.23
Mg (Cmol/kg)	2.50	2.00	0.75
Ca (Cmol/kg)	4.75	2.78	5.13
pH	7.20	7.64	6.79

Characterization of Bambara groundnut accessions using morphological, phenological and agronomic traits

The ANOVA for the morphological is presented in Tables 5. The least internode length (2.49±0.63 cm) was recorded with V2 while V11 had the longest internode length (2.95±0.64) across the environments, it had a grand mean value of 2.69cm. The Leaf length ranged from 6.96±1.44 (V5) to 8.47±1.53 (V11) while the minimum value across environments was 3.50cm and the maximum length was 12.60cm with a grand mean value of 7.53cm. The Leaf width was recorded with the lowest value of 2.83±0.55 and 2.83±0.60 in V7 and V10 respectively, and the highest width (3.27±0.71) was seen in V3 where the recorded grand mean was 2.98cm. Additionally, the plant height ranged from 24.41±3.14 (V9) to 29.53±4.19 (V6) and a grand mean value of 27.12cm across environments. V13 was observed to show the lowest initial plant stand with a value of 4.67±2.06 while V8 showed the most initial plant with a grand mean of 6.02. The values for the number of branches varied from 11.85±5.00 (V6) to 20.24±4.89 (12) although, the maximum across environments was 35.00 while the minimum was 5.00. For the number of nodes, V13 performed least while V8 performed best with means of 9.67±2.78 and 12.87±2.84, respectively. Also, V15 gave the least panicle length of 2.38±0.70 while the longest panicle length (2.77±0.80 cm) was observed in V4. The biomass fresh weight ranged from 53.64±22.97 (V3) to 74.32±34.47 (V14) with a grand mean of 64.41, while the biomass dry weight varied from 25.85±9.90 (V4) to 33.20±12.15 (V10). V13 recorded the least biomass per plant of 44.33±8.23 while the most biomass per plant of 50.89±8.39 was found in V9. The least number of stems was observed in V9, while V8 produced the highest number of stems which ranged from 60.29±15.56 to 79.67±21.68. Two different varieties V2 and V5 showed equal number of leaves (204.05 and 204.05) but the least number of leaves was found in V9 (179.37±47.51) while the highest was recorded in V8 (236.93±65.21). Lastly, the length of petioles ranged between 1.66±0.38 (V1) and 1.87±0.42 (V4) with a grand mean of 1.77 cm.

The phonological traits showed very highly significant differences between the accessions subjected to the experiment. The coefficient of variation (CV%) in this present research ranged from 4.22% to 8.60% while the Pr(<F) ranged from 7.44e-14*** to <2e-16***which indicates a very highly significant difference ($p \leq 0.0001$) and this was observed in days to emergence, days to flowering, days to maturity and days to harvest (Table 7).

The yield and yield component traits are shown in Table 6. There were very highly significant differences among all the means recorded. In ten out of the 17 agronomic traits, TVSU-455 was observed and recorded to be the best genotype across the three environments. TVSU-455 gave the highest values for total number of pods (42.67±13.37), final plant stand (7.67±1.58), fresh seed weight of (45.83±14.82), number of seed per plants of (46.62±14.89), hundred seed weight with a value of (124.56±18.99), dry seed weight (27.14±8.91), fresh pod weight, a value of (92.65±30.96), harvest index of (0.57±0.25), yield per plot (45.83±14.82) and unshelled yield per plot (550.26±117.89). Additionally, it was observed that TVSU-455 gave higher values than the grand means for those 10 traits that it performed best at. Although, V14 recorded 100 number of pods in a single environment i.e. Bowen. In the alternative, V5 gave the least values for the traits which include final plant stand (3.77±1.99), yield per plant (20.25±10.87), hundred seed weight (76.15±16.58), yield per plot (95.33±52.95), dry seed weight (11.57±5.54), width of seed (9.12±1.65) and harvest index (0.25±0.2). Also, V14 gave the least values for fresh pod weight (46.66±23.13), length of pod (17.56±2.82), width of pod (12.87±2.33) and unshelled yield per plot (220.10±87.20). V15 gave the lowest value for final plant stand (3.77±1.99). V1 gave the least value for number of seeds per plant (26.08±7.40). V11 gave the lowest value for shelling percentage (41.91±12.98) and it gave the highest value for the width of pods (16.29±2.16). V2 gave the highest value for length of pods (23.40±3.53). V4 gave the highest value for width of seed (11.29±2.26). V13 also reported the highest value in length of seed (14.02±1.64) and V6 was observed to perform best in shelling percentage (52.78±14.17).

Table 5

ANOVA for morphological parameters after 82 days of planting

VARIETY	IL50%	LL50%	LW50%	PH50%	IPS50%	BFW	BDW	BPP
TVSU-454	2.64±0.43bcd	7.69±1.18bcde	3.14±0.56abc	29.29±4.03ab	5.56±2.46cd	69.90±24.39abc	33.00±11.57abc	45.11±9.7
TVSU-158	2.49±0.63d	7.54±1.09bcdef	2.88±0.42defg	26.41±3.24cd	5.89±2.15abcd	55.43±24.46de	26.61±12.19f	48.24±9.3
TVSU-438	2.54±0.64d	7.70±1.34bcd	3.27±0.71a	25.85±.63de	6.78±2.44abc	53.64±22.97e	26.16±11.02f	48.97±7.4
TVSU-633	2.84±0.74ab	7.30±1.32cdefg	2.99±0.39cde	26.07±3.69de	5.11±1.96cd	58.33±22.09de	25.85±9.90f	45.15±10.
TVSU-1520	2.53±0.53d	6.96±1.44g	2.89±0.53cdefg	26.15±4.39cd	5.25±1.16cd	73.69±31.55ab	32.81±11.52abcd	46.77±10.
TVSU-939	2.61±0.65bcd	7.81±1.13bd	2.98±0.56cdef	29.53±4.19a	6.11±2.80abcd	77.23±35.69a	31.70±10.80abcde	44.41±12.
TVSU-513	2.52±0.58d	7.07±1.12fg	2.83±0.55efg	25.86±2.61de	6.11±1.27abcd	56.48±21.86de	27.81±11.88f	49.21±7.4
TVSU-455	2.91±0.63a	7.96±1.29b	3.24±0.67ab	29.04±4.21ab	7.67±2.45a	71.78±21.17abc	35.21±11.56a	49.18±8.0
TVSU-643	2.63±0.64bcd	7.28±1.02defg	2.74±0.56fg	24.41±3.14e	5.78±2.17bcd	56.07±20.38de	27.98±9.43def	50.89±8.3
TVSU-2096	2.79±0.52abc	7.35±1.10cdefg	2.83±0.60efg	26.98±2.84cd	6.56±2.79abc	65.41±21.47bcd	33.20±12.15ab	50.63±8.5
TVSU-194	2.95±0.64a	8.47±1.53a	3.10±0.64abcd	27.81±3.88bc	7.44±2.35ab	65.07±19.89bcd	31.82±10.17abcde	50.21±11.
TVSU-1611	2.55±0.37cd	7.19±0.92efg	3.05±0.61abcde	25.94±3.87de	5.78±2.22bcd	62.40±19.43cde	29.10±10.28bcdef	49.26±11.
TVSU-1920	2.86±0.58ab	7.36±0.66cdefg	3.01±0.58bcde	29.43±3.27ab	4.67±2.06d	72.79±25.99abc	31.63±10.13abcde	44.33±8.2
TVSU-1531	2.85±0.59ab	7.72±1.26bcd	3.04±0.58abcde	28.90±3.43ab	5.89±2.15abcd	74.32±34.47ab	32.40±12.92abcde	45.04±8.8
TVSU-1392	2.59±0.51cd	7.32±0.96cdefg	2.70±0.45g	25.46±3.29de	5.67±2.91bcd	56.55±20.60de	28.24±9.99cdef	49.45±7.8
MIN	1.10	3.50	1.60	14.40	1.00	16.40	5.80	22.06
MAX	5.50	12.60	5.40	40.80	11.00	181.80	65.10	83.56
Grand means	2.69	7.53	2.98	27.12	6.02	64.41	30.27	48.07
CV	21.48	15.43	19.00	14.36	33.22	38.38	36.57	19.67
Pr(<F)	4.56e-05 ***	1.82e-08 ***	2.84e-06 ***	0.701419	0.115	1.89e-07 ***	0.000217 ***	0.00495 *

IL50%: Internode Length (cm), IPS50%: Initial plant stand, LL50%: Leaf length (cm), LW50%: Leaf width (cm), PH50%: Plant height (cm), NL50%: Number of leaves per plant, NS: Number of stem per plant, LP50%: Petiole length per stem (cm), Min: Minimum across environment, Max: Maximum across environment, *Significant at $p \leq 0.05$; **highly significant at $p \leq 0.01$; ns = non-significant $p > 0.05$ and very highly significant at ***. CV: Coefficient of variation, BFW: Biomass fresh weight per plant (g), BDW: Biomass dry weight per plant (g), BPP: Biomass per plant (BDW/BFW X 100).

Table 6

ANOVA for agronomic parameters

VARIETY	TNP	FPS	FSW	NSPP	YPP	HSW	YPPL	D
TVSU-454	26.22±6.86e	4.86±2.10bcde	25.14±6.40efgh	26.08±7.40f	25.14±6.40efgh	95.41±8.94cdef	117.56±43.43def	14
TVSU-158	28.32±10.52de	5.33±1.94bcd	28.74±10.64def	30.15±9.97cdef	28.74±10.64def	102.14±15.45bcd	152.77±77.44def	17
TVSU-438	34.09±10.72bc	6.33±1.58ab	38.63±11.41b	37.05±10.32b	38.63±11.41b	102.27±8.65bcd	226.91±62.62b	22
TVSU-633	26.41±11.10e	4.44±1.67de	31.62±11.86cd	28.81±11.34cdef	31.62±11.86cd	111.71±21.40ab	141.21±63.06def	18
TVSU-1520	26.26±8.88e	4.63±1.19cde	20.25±10.87h	26.63±8.89ef	20.25±10.87h	76.15±16.58g	95.33±52.95f	11
TVSU-939	35.25±12.24b	5.33±1.80bcd	31.04±9.97cd	33.58±11.57bc	31.04±9.97cd	101.52±15.37bcd	158.63±53.81cde	18
TVSU-513	27.91±8.50de	5.56±1.13bcd	25.02±9.67fgh	29.33±8.46cdef	25.02±9.67fgh	98.78±8.21bcde	139.51±44.80def	15
TVSU-455	42.67±13.37a	7.67±1.58a	45.83±14.82a	46.62±14.89a	45.83±14.82a	124.56±18.89a	319.51±44.80a	27
TVSU-643	25.37±8.97e	4.78±1.79cde	25.27±10.41efgh	26.74±8.83ef	25.27±10.41efgh	97.09±16.62cdef	120.76±65.53def	15
TVSU-2096	29.59±13.45cde	6.30±2.75ab	34.93±17.02bc	31.12±13.90cde	34.93±17.02bc	104.43±16.14bc	208.41±116.67bc	20
TVSU-194	28.14±9.98de	6.00±2.00bc	29.97±13.16de	28.75±12.27def	29.97±13.16de	103.01±15.84bcd	175.58±91.31bcd	18
TVSU-1611	26.95±10.61de	4.67±1.80cde	23.29±10.12gh	29.05±10.87cdef	23.29±10.12gh	90.40±7.80defg	116.10±65.79ef	14
TVSU-1920	31.58±8.24cd	4.56±1.94cde	35.10±9.96bc	32.26±8.93bcd	35.10±9.96bc	105.26±18.94bc	159.84±79.31cde	22
TVSU-1531	29.20±16.29de	5.33±2.00bcd	21.38±11.16h	30.98±14.70cdef	21.38±11.16h	83.60±13.50fg	107.87±37.86ef	12
TVSU-1392	28.44±9.26e	3.77±1.99e	28.51±10.42defg	29.91±9.70cdef	28.51±10.42defg	86.39±13.10efg	105.11±61.55ef	17
MIN	5.00	1.00	1.80	2.00	1.80	50.20	11.2	0.
MAX	100.00	5.32	85.50	97.00	85.50	163.40	408.90	55
Grand means	29.96	11.00	29.93	31.38	29.93	99.10	157.50	18
CV	36.32	30.46	38.29	35.38	38.29	15.20	38.74	38
Pr(<F)	1.23e-14 ***	0.000191 ***	<2e-16 ***	<2e-16 ***	<2e-16 ***	8.19e-08 ***	2.98e-12 ***	<2

VARIETY	FPW	MPN	LOP	WOP	LOS	WOS	SP	HI
TVSU-454	59.54±15.42ef	24.57±6.91de	20.19±3.60def	14.32±2.20cde	12.45±1.78fg	9.64±1.21fgh	42.55±4.62ef	0.3
TVSU-158	59.61±26.13ef	26.88±10.58de	23.40±3.53a	14.23±2.04de	13.07±1.35cdef	10.15±1.88bcdef	51.29±12.87ab	0.3
TVSU-438	77.54±24.04b	31.66±10.68bc	22.16±3.95abc	14.28±2.33cde	12.93±1.95def	9.88±1.77defg	51.16±10.48ab	0.5
TVSU-633	72.14±26.11bc	25.54±10.79de	21.02±2.88cde	15.19±1.64bc	14.03±2.14ab	11.29±2.26a	45.24±13.61cde	0.4
TVSU-1520	50.99±22.12efg	24.03±7.92e	20.30±2.57def	14.10±1.77de	12.52±1.84fg	9.12±1.65h	38.49±12.24f	0.2
TVSU-939	60.71±20.97de	32.53±11.75b	18.82±3.06fg	14.07±2.39de	13.45±1.87bcde	10.01±2.04bcdefg	52.78±14.17a	0.4
TVSU-513	57.21±18.21ef	25.95±8.26de	19.96±3.55ef	14.33±2.44cde	13.05±1.17cdef	9.90±1.28defg	44.14±10.71de	0.3
TVSU-455	92.65±30.96a	41.09±12.66a	21.60±3.01cd	15.15±2.19bc	13.75±1.86abc	10.74±2.28ab	52.48±14.99a	0.5
TVSU-643	56.73±22.02efg	23.68±8.61e	20.87±3.92cde	14.87±2.32bcd	13.44±1.75bcde	10.76±1.68ab	45.43±11.62cde	0.3
TVSU-2096	71.12±31.43bcd	27.95±13.59bcde	23.07±3.54a	15.39±1.61ab	13.63±1.38abcd	10.42±1.11bcde	49.04±11.42abcd	0.4
TVSU-194	71.19±28.85bc	26.77±9.82de	22.47±3.10ab	16.29±2.16a	14.30±2.12a	10.71±1.88abc	41.91±12.98ef	0.3
TVSU-1611	49.88±20.34fg	25.03±10.09de	20.86±3.90cde	13.44±2.22ef	12.92±1.61def	9.98±1.77cdefg	49.86±13.77abc	0.3
TVSU-1920	80.42±21.98b	29.19±8.40bcd	20.85±2.00cde	15.55±2.09b	14.02±1.64ab	10.61±1.25abcd	44.59±9.41cde	0.5
TVSU-1531	46.66±23.13g	27.58±15.55cde	17.56±2.82g	12.87±2.33	12.14±1.64g	9.32±1.40gh	49.82±14.25abc	0.3
TVSU-1392	62.24±19.55cde	26.16±9.24de	20.31±3.15def	14.64±1.73bcd	12.70±1.36efg	9.76±1.25efgh	45.86±9.29bcde	0.3
MIN	9.30	5.00	12.04	7.05	9.02	7.00	13.97	0.0
MAX	176.40	100.0	31.15	21.08	20.00	18.01	94.59	1.8
Grand means	64.99	28.12	20.94	14.59	13.24	10.16	47.15	0.3
CV	36.82	37.65	15.64	14.59	13.02	16.68	25.55	46.
Pr(<F)	<2e-16 ***	8.57e-15 ***	<2e-16 ***	1.37e-12 ***	8.84e-10 ***	3.56e-08 ***	6.89e-09 ***	< 2

TNP: Total number of pods, FPS: Final plant stand, FSW: Fresh seed weight (g),NSPP: Number of seeds per pod, YPP: Yield per plant (g), HSW: Hundred seed weight (g), YPPL: Yield per plot (g), DSW: Dry seed weight (g), FPW: Fresh pod weight (g), MPN: Mature pod number per plant, LOP: Length of pods(mm), WOP: Width of pods(mm), LOS: Length of seeds (mm), WOS: With of seeds (mm), SP: Shelling percentage (%), HI: Harvest index, YPPU: Yield per plot of unshelled.

Table 7

ANOVA for phenological traits

VARIETY	DTE	DTF	DTM	DTH	NB	NN	PL
TVSU-454	7.44±0.73ef	36.67±4.09ghi	92.44±1.67hi	105.44±5.46fg	12.97±6.93g	12.05±2.88abc	2.67±0.44ab
TVSU-158	8.22±0.44d	42.56±3.64cde	93.67±1.66gh	108.78±5.97def	17.39±3.65bcdef	10.32±2.05fgh	2.50±0.57bc
TVSU-438	7.33±0.50f	39.78±1.79efg	91.44±2.16hi	104.78±6.57fg	19.34±3.90ab	11.59±2.20bcde	2.71±0.58ab
TVSU-633	8.00±0.00de	43.00±3.54cde	101.89±6.49de	110.33±4.09cde	16.38±5.19ef	10.49±2.99efgh	2.77±0.80a
TVSU-1520	9.00±1.22c	53.67±4.80a	117.78±1.64a	120.67±2.50a	18.87±3.97abc	10.82±2.99defgh	2.67±0.71ab
TVSU-939	7.44±0.53ef	35.89±3.06hi	89.56±2.13i	103.33±4.21g	11.85±5.00g	11.38±2.00bcdef	2.70±0.60ab
TVSU-513	11.44±0.73a	43.22±3.77cd	109.67±5.10b	112.56±4.98cd	18.84±3.72abc	10.18±2.50gh	2.47±0.83bc
TVSU-455	10.67±0.50b	48.33±2.60b	114.33±2.40a	119.44±2.51ab	17.82±3.98bcde	12.87±2.84a	2.67±0.60ab
TVSU-643	8.11±0.33d	36.00±2.29hi	97.00±7.89fg	106.33±8.79efg	18.29±3.89abcde	11.98±2.51abc	2.60±053abc
TVSU-2096	8.89±0.33c	39.89±4.81efg	92.22±3.90hi	103.44±4.56g	16.23±6.87def	10.95±3.26cdefg	2.58±0.69abc
TVSU-194	8.89±1.05c	45.56±4.75bc	108.56±7.33b	115.00±5.68bc	15.71±3.84f	11.84±3.05abcd	2.40±0.51c
TVSU-1611	8.00±0.00de	34.56±1.67i	101.22±4.76de	108.78±4.79def	20.24±4.89a	11.63±1.87bcde	2.62±0.46abc
TVSU-1920	9.11±0.33c	37.89±4.34fgh	106.56±4.28bc	113.56±4.98cd	17.17±5.15cdef	9.67±2.78h	2.56±0.58abc
TVSU-1531	7.44±0.53ef	42.89±3.82cde	98.00±2.65ef	110.78±4.94de	17.07±4.53cdef	11.12±2.85bcdefg	2.48±0.47bc
TVSU-1392	11.78±0.67a	41.11±2.09def	103.89±2.85cd	113.11±4.88cd	18.64±4.89abcd	12.24±3.03ab	2.38±0.70c
MIN	7.00	30.00	86.00	95.00	5.00	4.00	1.20
MAX	12.00	60.00	120.00	125.00	35.00	19.00	5.60
Grand means	8.79	41.40	101.21	110.4	17.19	11.30	2.58
CV	7.04	8.60	4.22	4.73	27.63	23.14	23.67
Pr(<F)	<2e-16 ***	<2e-16 ***	<2e-16 ***	7.44e-14 ***	<2e-16 ***	5.74e-08 ***	0.0864.

DTE: Days to emergence, DTF: Days to flowering, DTM: Days to maturity, DTH: Days to harvest, NB: Number of branches per plant, PL: Panicle length per stem (cm), NN: Number of nodes per stem

Principal Component Analysis

The Principal Component Analysis (PCA) with combined morphological, phenological and agronomic characters allows us to measure the relationships between variables and thus identified 10 dimensions (PCA), which significantly explained up to 96.76% of the variance in the data resulting in strong contribution to the total variation (Figures 1 and 3). The first two dimensions explained 56.16% of the variation (Fig. 1 and Figure 2) with each dimension accounting for 39.85% and 16. 31% variation, respectively. Total number of pods, Final plant stand, Fresh seed weight, Number of seeds per pod, Yield per plant, Hundred seed weight, Yield per plot, Dry seed weight, Fresh pod weight, Mature pod number per plant, Width of pods, Length of seeds, Width of seeds, Harvest index, Yield per plot, Internode Length, Initial plant stand, Leaf length and Leaf width had a positive correlation with Dim1 while Biomass fresh weight per plant, plant height, Number of leaves per plant, Number of stem per plant, and Biomass dry weight per plant had a positive correlation with Dim2 (**Supplementary Table S1**, Table 8, Figures 1 and 2). Also, the following traits (Days to emergence, Days to flowering, Days to maturity, and Days to harvest), (Width of pods, Length of seeds, and Width of seeds) and (Leaf length and Number of nodes per stem) had a positive correlation with PC3, PC4 and PC5, respectively (**Supplementary Table S1**, Table 8, Figures 1 and 2). Among the variables, Total number of pods, Final plant stand, Fresh seed weight, Number of seeds per pod, Yield per plant, hundred seed weight, Yield per plot, Dry seed weight, Fresh pod weight, Mature pod number per plant, Width of pods, Length of seeds, Width of seeds, Harvest index, Yield per plot, Internode Length, Initial plant stand, Leaf length and Leaf width were the major contributing characters in Dim1, for PC2 (Biomass fresh weight per plant, plant height, Number of leaves per plant, Number of stem per plant, and Biomass dry weight per plant), for PC3 (Days to emergence, Days to flowering, Days to maturity, and Days to harvest), for PC4 (Width of pods, Length of seeds, and Width of seeds) and PC5 (Leaf length and Number of nodes per stem), highly contributed to the respective PCs (Table 8, Figures 1 and 2). The rapport among dimensions and their proportion of variation and eigenvalues are presented in Figures 3 and 4 and Table 8. For the first five principal components, the eigenvalues range from 13.5 for the PC1 with the highest value to 2 for the PC5, which recorded the lowest (Figure 4 and Table 8).

In PCA biplot (Figure 2), both individual variables and genotypes are loaded at the same times indicating the relationship among traits and the distances between genotypes. The closer the vectors, the stronger the correlation. The PC 1 includes individual varieties accessions TVSU-455, TVSU-438, TVSU-2096, TVSU-194 and TVSU-1920. TVSU-455 was diametrically opposed to TVSU-1520.

IL50%: Internode Length, IPS50%: Initial plant stand, LL50%: Leaf length, LW50%: Leaf width, PH50%: Plant height, NL50%: Number of leaves per plant, NS: Number of stem per plant, LP50%: Petiole length per stem, Min: Minimum across environment, Max: Maximum across environment, BFW: Biomass fresh weight per plant, BDW: Biomass dry weight per plant, BPP: Biomass per plant (BDW/BFW X 100). TNP: Total number of pods, FPS: Final plant stand, FSW:

Fresh seed weight, NSPP: Number of seeds per plant, YPP: Yield per plant, HSW: Hundred seed weight, YPPL: Yield per plot, DSW: Dry seed weight, FPW: Fresh pod weight, MPN: Mature pod number per plant, LOP: Length of pods(mm), WOP: Width of pods, LOS: Length of seeds, WOS: With of seeds, SP: Shelling percentage, HI: Harvest index, YPPU: Yield per plot of unshelled. DTE: Days to emergence, DTF: Days to flowering, DTM: Days to maturity, DTH: Days to harvest, NB: Number of branches per plant, PL: Panicle length per stem, NN: Number of nodes per stem.

Table 8

Eigenvalues, Proportion of variance (%), Cumulative variance (%) and trait contributions for the first six principal component axes for 34 phenotypic traits of Bambara groundnut accessions

	Dim.1	Dim.2	Dim.3	Dim.4	Dim.5
Eigenvalue	13.50	5.50	4	3.50	2
Proportion of variance (%)	39.9	16.3	12.1	9.8	6.2
Cumulative variance (%)	39.9	56.2	68.3	78.1	84.3
IL	2.5076	1.2372	0.1822	7.1055	2.5453
LL	2.6761	0.9580	0.4381	0.2082	15.4599
LW	2.4033	1.4910	0.3746	1.9520	0.6538
PH	1.6441	12.4625	0.6886	0.5301	0.0931
IPS	3.1615	0.3478	0.7500	6.0014	9.6942
BFW	0.2786	14.7567	0.1627	0.3180	0.0049
BDW	0.9441	9.2618	2.3255	0.0042	3.1493
BPP	0.0690	9.0905	2.5155	2.7758	6.0731
LP	0.1424	0.8390	6.9714	5.3888	4.9235
NL	1.2802	11.2003	0.0145	1.2993	0.0738
NS	1.7153	9.5658	0.1843	1.6882	0.6394
NB	0.2572	5.7601	5.2549	2.2309	6.8996
NN	0.5685	0.0017	0.5817	9.0235	12.9638
TNP	5.5460	0.6649	0.0117	3.1969	3.1233
FPS	4.6115	0.0030	0.2067	3.7825	1.3241
FSW	6.5489	0.8320	0.1721	0.0002	1.0494
NSPP	5.5910	0.0304	0.1426	3.9456	4.1998
YPP	6.6631	0.6604	0.1050	0.0430	0.9720
HSW	5.4847	0.9075	0.7216	1.4552	0.0066
YPPL	6.7077	0.4069	0.0558	1.0582	0.0154
DSW	6.4406	0.8305	0.2232	0.0194	1.3834
FPW	6.0640	0.7858	0.0754	1.3917	0.3516
MPN	5.9335	0.5492	0.0189	2.6935	2.3925
LOP	1.0635	8.1168	0.0212	0.4537	2.3541
WOP	1.9888	2.1320	0.9502	12.0161	5.3763
LOS	2.7015	1.6810	0.0329	11.9623	1.1329
WOS	2.1424	3.5400	0.6266	9.6921	0.7355
SP	1.6487	0.0307	6.7837	7.4972	3.6574
HI	6.0303	0.1512	1.0844	0.1401	3.1926
YPPU	6.7387	0.5115	0.3659	0.1110	0.0345
DTE	0.1610	0.6616	12.2666	0.1166	2.7002
DTF	0.1322	0.0991	14.8025	0.0127	0.2793
DTM	0.0469	0.0659	21.0259	1.2598	1.1323
DTH	0.1073	0.3673	19.8633	0.6264	1.4131

Correlation Analysis of the Traits

The correlation coefficients for 34 traits including morphological, phenological and agronomic characters are presented in Figure 5. There were significant differences among the correlations of the 34 traits. According to the scale in Figure 5 the non-significant or significant differences ($p < 0.05$) are shown with blue or red rounds which represent negative and positive correlation, respectively. The more intense the colors of relationship, the stronger the correlations.

Internode length had a positive correlation with leaf length (r = 0.56, p = 0.03*), leaf width (r = 0.39, p = 0.15), plant height (r = 0.51, p = 0.04*), Initial plant stand (r = 0.11, p = 0.69), Biomass fresh weight per plant (r = 0.47, p = 0.08), Biomass dry weight per plant (r = 0.475, p = 0.07), Petiole length per stem (r = 0.22, p = 0.42), Number of leaves per plant (r = 0.57, p = 0.03*), Number of stem per plant (r = 0.59, p = 0.02*), Number of nodes per stem (r = 0.28, p = 0.32), Total number of pods (r = 0.25, p= 0.38), Final plant stand (r = 0.12, p = 0.67), Fresh seed weight (r = 0.41, p = 0.13), Number of seeds per pod (r = 0.14, p = 0.63), yield (r = 0.41, p = 0.13), Hundred seed weight (r = 0.48, p = 0.07), Yield per plot (r = 0.37, p = 0.17), Dry seed weight (r = 0.36, p = 0.18), Fresh pod weight (r = 0.45, p = 0.09), Mature pod number per plant (r = 0.30, p = 0.27), Length of pods (r= 0.09, p = 0.76), Width of pods (r = 0.57, p = 0.03*), Length of seeds (r = 0.51, p = 0.05*), Width of seeds (r = 0.46, p = 0.08), Harvest index (r = 0.40, p = 0.14), Yield per plot of unshelled (r = 0.38, p = 0.16), Days to flowering (r = 0.13, p = 0.65), Days to emergence (r = 0.02, p = 0.93), Days to maturity (r = 0.17, p = 0.65) and Days to harvest(r = 0.24, p = 0.38) but negatively correlates with Biomass per plant, Number of branches per, plant Shelling percentage, (**Supplementary Tables S2, S3**). Among the positive correlations however, correlations with leaf length, leaf width, petiole length, and chlorophyll content were not significant (**Supplementary Tables S2, S3**). Leaf length had strong and positive correlation with leaf width (r = 0.58, p = 0.02*), plant height (0.61, p = 0.01*), Initial plant stand (r = 0.57, p = 0.03*), Number of stem per plant (r = 0.51, p = 0.05*), Total number of pods (r = 0.62, p = 0.01*), Final plant stand (r = 0.56, p = 0.03*), Fresh seed weight (r = 0.51, p = 0.05*), Yield per plant (r = 0.51, p = 0.05*), Yield per plot (r = 0.58, p = 0.02*), MPN (r = 0.7, p =0.004**), and Harvest index (r = 0.63, p = 0.01*). Plant height had positive correlation with internode length (r= 0.51,p=0.05*), Leaf length (r= 0.61, p= 0.02*), leaf width (r= 0.42, p= 0.12), Biomass fresh weight per plant (r= 0.79, p = 0.0005****), Biomass dry weight per plant (r= 0.61, p = 0.02*), Number of leaves per stem (r= 0.84, p = 0.00009***), Number of stem per plant (r= 0.86, p = 0.00004***), Total number of pods (r= 0.425, p = 0.11), Final plant stand (r= 0.20, p = 0.11), Fresh seed weight (r= 0.24, p = 0.48), Number of seeds per pod (r= 0.29, p = 0.30), Yield per plant (r= 0.24, p = 0.39), Hundred seed weight (r= 0.29, p= 0.30), Yield per plot (r= 0.31, p= 0.27), Dry seed weight (r= 0.23, p = 0.41), Fresh pod weight (r= 0.23, p = 0.41), Mature pod number per plant (r= 0.5, p = 0.06), Length of seeds (r=0.21, p= 0.45), Harvest index (r= 0.42, p = 0.12), and Yield per plot of unshelled (r=0.27,p= 0.37). TNP: Total number of pods had a strong, positive, and highly significant correlation with Final plant stand (r= 0.49, p= 0.06), Fresh seed weight (r= 0.67, p= 0.006***), Number of seeds per pod (r= 0.96, p= 1.30E-08*****), Yield per plant (r= 0.67, p= 0.006***), Hundred seed weight (r= 0.46, p= 0.08), Yield per plot (r= 0.64, p= 0.01**), Dry seed 57weight (r= 0.74, p= 0.002***), FPW: Fresh pod weight (r= 0.58, p= 0.03*), Mature pod number per plant (r=0.98, p= 8.16E-11*****), Shelling percentage (r= 0.66, p= 0.008***), HI: Harvest index (r= 0.83, p= 0.0001****), and YPPU: Yield per plot of unshelled (r= 0.52, p = 0.05*). A perfect positive significant correlation (r=1.00) was observed between Yield per plant and Hundred seed weight, meanwhile a positive and moderate and equal correlation was recorded with the characters of harvest index and leaf width (r=0.36); Fresh seed weight and Initial plant stand (r=0.35), yield per plant and Initial plant stand (r=0.35), Mature pod number per plant and Length of seeds (r=0.35). Fresh seed weight had a very strong, positive, and highly significant correlation with Number of seeds per pod (r=0.68, p=0.005), Yield per plant (r=1, p=0.000), HSW: Hundred seed weight (r=0.89, p=7.49E-06****), YPPL: Yield per plot (r=0.98, p=7.49E-06****), DSW: Dry seed weight (r=0.94, p=8.16E-11****), FPW: Fresh pod weight (r=0.94, p=1.42E-07****), Mature pod number per plant (r=0.72, p=0.002***). Yield per plant had a very strong, positive, and very highly significant correlation with Hundred seed weight (r=0.89, p=7.49E-06****), YPPL: Yield per plot (r=0.98, p=7.49E-06****), DSW: Dry seed weight (r=0.94, p=8.16E-11****), FPW: Fresh pod weight (r=0.94, p=1.42E-07****), Mature pod number per plant (r=0.72, p=0.002***), Harvest index (r=0.85, p=8.81E-08), Yield per plot of unshelled (r=0.90, p=2.32E-05****) (**Supplementary Tables S1, S2**).

The clustering analysis produced a dendogram categorizing the 15 accessions into 4 groups based on the morphological, phenological and agronomic traits (Figure 6). Cluster I consisted of one accession (TVSU-455). The second cluster comprised six accessions including TVSU-2096, TVSU-194, TVSU-1920, TVSU-158, TVSU-438, and TVSU-633. The third cluster is made up of three accessions, namely TVSU-1531, TVSU-454, and TVSU-939. The last cluster included four accessions including TVSU-1520, TVSU-513, TVSU-643, TVSU-1392, and TVSU-1611.

Cluster I illustrated by only one accession TVSU-455 was distinguished by the highest mean values for total number of pods, final plant stand, Fresh seed weight, Number of seeds per pod, Yield per plant, hundred seed weight, yield per plot, Dry seed weight, Fresh pod weight, Mature pod number per plant, width of pods, with of seeds, Shelling percentage, Harvest index, yield per plot of unshelled, initial plant stand, number of leaves per plant, number of stem per plant, biomass dry weight per plant, and biomass per plant. On the other hand, cluster II was characterized by the highest mean values for Length of pods, Length of seeds, Width of pods, Internode Length, Leaf length, Leaf width, Petiole length per stem and Biomass per plant apart from these characteristics were next best to cluster I in terms of yields and yield components. However, cluster IV were characterized by low mean values of Yield per plant, Hundred seed weight, Yield per plot, Dry seed weight, Mature pod number per plant, Length of seeds, With of seeds, and Yield per plot of unshelled. This cluster IV was majorly made up of accessions from unknown origin while the best accession TVSU-455 performing across the three environment is from Cameroon.

Qualitative trait analysis

Out of the 15 accessions used during this research, 66.67% had a bunch type of growing habit while the remaining 33.33% had a semi-bunch type of growing habit. It was also observed that 40% had hair on their stems, 33.33%had a large amount of hair on the stem while the remaining 26.67% didn't have hair on their stems (Table 9). In addition, most of the accessions had a green first stem color of 53.33% followed by stripped 26.67%, then reddish green 13.33% while little accessions had a brownish 6.67 first stem color. All the accessions exhibited 100% terminal leaflet color of Green. The terminal leaflet shape was oval, round, elliptical and lanceolate with frequencies of 40%, 20%,20% and 20%, respectively. Most accessions had a green petiole color of 40%, followed by 26.67% for brown petiole, then 20% for reddish brown petiole and few had a reddish green petiole with a frequency of 13.33. 60% had pods that ended in a point and round on the other side, 20% was without point while the last 20% had point but ended with nook on the other side. The color of pod varied from yellowish brown 60%, reddish brown 13.33%, brown 20% and to cream with brown patches 6.67%. The accessions had different texture of pods after they were harvested. 53.33% had a much grooved pod, 26.67% had a much folded pod, 13.33% had a smooth pod and few percent, 6.67% had little grooved pod. In this regard also, they exhibited two different shape of seeds which was oval and round with frequencies of 73.11% and 26.67%, respectively. The seed color and their frequency ranged from cream 60%, light red 6.67%, light brownish red 13.33%, dark purple 6.67%, light brown 6.67% and to purplish red 6.67%. Some accessions 13.33% did not have an eye color, 26.67% had grey, 20% had light red, 13.33% had black eye color, the next 13.33% had cream and the last 13.33%

had a brown eye color. Similarly, 60% of the accessions didn't have a testa pattern, 6.67% had cream marbled pattern, 6.67% had black stripes, 13.33% had brownish red strips and the last 13.33% had light red stripes. Observations was also noted on their testa color and eye pattern around the hilum. 20% had cream and triangular, 6.67% had light red and butterfly like, 26.67% had cream and butterfly like, 13.33% had no eye pattern, 13.33% had cream and reddish patches, 6.67% had light brown and butterfly like pattern and the remaining 13.33% had a black and cream testa

Table 9

Qualitative trait occurrence and frequency

	Parameters	S/N	Traits	Occurrence	Frequency	QT	Parameters	S/N	Traits	Occurrence	Frequency
A	GrH	1	Bunch	10	66.67	J	SeS	1	Oval	11	73.33
		2	Semi-bunch	5	33.33			2	Round	4	26.67
B	StH	1	Absent	6	40	K	SeC	1	Cream	9	60
		2	Sparse	4	26.67			2	Light red	1	6.67
		3	Dense	5	33.33			3	Light brownish red	2	13.33
C	FSC	1	Green	8	53.33			4	Dark purple	1	6.67
		2	Stripped	4	26.67			5	Light brown	1	6.67
		3	Brownish	1	6.67			6	Purplish red	1	6.67
		4	Reddish green	2	13.33	L	EyC	1	Grey	4	26.67
D	TLC	1	Green	15	100			2	Light red	3	20
E	TLS	1	Oval	6	40			3	Black	2	13.33
		2	Round	3	20			4	No eye	2	13.33
		3	Elliptical	3	20			5	Cream	2	13.33
		4	Lanceolate	3	20			6	Brown	2	13.33
F	PeP	1	Green	6	40	M	TeP	1	No testa pattern	9	60
		2	Brown	4	26.67			2	Cream marbled	1	6.67
		3	Reddish green	2	13.33			3	Black strips	1	6.67
		4	Reddish brown	3	20			4	Brownish red strips	2	13.33
G	SoP	1	Ending in a point round on other side	9	60			5	Light red strips	2	13.33
		2	Without point	3	20	N	Tc + EyP	1	Cream +triangular	3	20
		3	Ending in a point with nook on other side	3	20			2	Light red+ butterfly like	1	6.67
H	CoP	1	Yellowish brown	9	60			3	Cream + butterfly like	4	26.67
		2	Reddish brown	2	13.33			4	No eye pattern	2	13.33
		3	Brown	3	20			5	Cream + reddish patches	2	13.33
		4	Cream with brown patches	1	6.67			6	Light brown + butterfly like	1	6.67
I	PoT	1	Much grooved	8	53.33			7	Black + Cream	2	13.33
		2	Much folded	4	26.67						
		3	Smooth	2	13.33						
		4	Little grooved	1	6.67						

Growth Habit GrH; Stem Hairiness StH; First Stem Color FSC; Terminal leaflet color TLC; Terminal leaflet shape TLS; Petiole pigmentation PetP; Shape of pods SoP; Color of pods CoP; Pod texture PoT; Seed shape SeS; Seed color SeC; Eyes color EyC; Testa pattern TeP; Testa color + eye pattern TC+EyP.

Discussion

Agro-morphological characteristics

With other required factors kept in place, the ability of a crop to compete with weed, withstand drought and other harsh climatic and environmental conditions, yield improvement and high nutrient uptake and utilization are some of the factors to be considered for breeding and developing high yielding varieties of crops[36]. The significant difference that was observed throughout the accessions indicates that there is a high level of variation in the selected population and it can be effectively studied for an improved breeding program. The variations in traits bring about the selection of best lines for improvement[37]. Different other studies have also shown the effect of GEI on several other crops like cereal crops like Rice[38] and in root and tuber crops like sweet potato[39]. It was observed in 2003 by Yan and Kang that the number of genotypes and environments tested will determine the degree of environmental variations and so therefore, when there are few environments with many genotypes, the environmental variance will be reduced and vice versa. However, according to Aremu et al[40], the most dominant source of variation is the environment and it is of high importance in plant breeding. There is a high level of variation among the germplasm of plant itself and these must be evaluated in order to develop cultivars of those germplasms[41]. Also, there is a high level of variation even within the same accessions and across different accessions and these variations are further manifested in the environments in which they are grown[36].

Bambara groundnut has been localized in various environments and its significance especially in the Sub Saharan part of Africa is increasing because it is a crop that has a rich source of diversity. In this current study, all the 15 accessions of Bambara groundnut showed a very high level of diversity and variability for all the parameters studied. This report support that of the studies of[42,43,44,45]. In the publication of these authors, they all reported a coefficient of variation (CV %) ≥ 20% for traits like petiole length, number of pods, hundred seed weight and yield. This report also confirms a coefficient of variation (CV %) ≥ 20% for the same traits of petiole length (38.46%), number of pods (36.32%) and yield (38.74%) except for hundred seed weight which has a CV % of (15.20) < 20%. The high coefficient of variation observed in some of the traits in traits in this study shows that there is a high level of heterogeneity across the studied environments. This high heterogeneity in Bambara groundnut was also reported by Goli et al.[46], Khan et al.[2] and Khan et al[28]. The variations in the phonological traits and morphological traits are as a result of the differences in the genetic makeup of the accessions and planting seasons[47]. For instance, in this present study, days to flowering ranges from 34 to 53 days and Khan et al.[28] reported 36 to 53 days which is still in the present range which are both comparably lower to those reported by Masindeni[48] 43 to 80 days, Goli et al.[46] 38 to 68 days and comparably higher than those reported by Quadraogo et äl.[49], 32 to 42 days. A significant difference was reported by this present study in the number of days to maturity which ranges from 89 to 118 days and it agrees with the reports from Goli et al.[46] and Masindeni et al.[48].

For an effective breeding for Bambara groundnut, there is need to study the GEI for the crop in order for the breeders to identify the stable genotypes across the locations or the particular genotypes that will do better in particular environments[50]. There are various factors which affect the responses of genotypes to locations and planting seasons some of which include soil fertility, pests and diseases, rainfall, humidity and temperature. This present study showed that the responses of growth traits especially the morphological traits are strongly affected by accessions and locations and this is in accordance with the research of [51] who also performed the experiment at three different locations. This very highly significant effect observed for traits like number of branches, number of nodes, leaf length, leaf width, number of stems, number of leaves and internode length can be attributed to differences in climatic and soil conditions exhibited at the three locations. This further buttresses the need for accessions to be evaluated under different environment to identify the most stable and the highest yielding varieties like TVSU-455 and this agrees with the reports of researchers like Rubilar et al.[52], and Olanrewaju et al.[53] Also, the accessions in this present research showed that there is no significant variation in plant height, which is absolutely in support of [1,54]. All the seventeen yields and yield related traits evaluated in this study showed a very high significant genetic discrepancy. A similar report was given by Shegro et al.[54], (2013), and these variations were accredited to the effect of genotype by environment interaction on Bambara groundnut yield. The traits like total number of pods, fresh seed weight, dry seed weight, fresh pod weight, hundred seed weight, number of seeds per plant and harvest index show very high significant differences and this was similarly reported by[54]. The hundred seed weight ranged from 76g to 125g and this is a critical factor that is usually used to determine the morphological traits relating to plant yield[55,28]. The hundred seed weight also influences yield directly. In this study, the variations in seed length and seed width may be attributed to the different seed shapes, sizes and shapes of the pods, while the variations in hundred seed weight can also be attributed to the size of the seeds and nutrient contents. The yield of Bambara groundnut was recorded from 146.6 to 2678.6kg ha^{-1} by[44], 1,058.8kg ha^{-1} by[56] and from 0 to 1,266.77kg ha^{-1} by[53] whereas in this study we reported from 997.3 to 1,106.4kg ha^{-1} for the shelled yield and from 1,912.9 to 2,300.8kg ha^{-1} for the unshelled yield. The findings from this study and other studies by previous researchers show that there is a high level of diversity and a high level of influence of environment on the growth, development, maturity and yield of Bambara groundnut.

Principal Component Analysis

The principle components allow to identify quantitative traits that are highly and strongly correlated with each component. Additionally, PC is for classification of genetically similar accessions into the same groups playing a similar function as cluster analysis[57]. Additionally, Mercati et al.[58]; Figàs et al.[59]; Nankar et al.[60] reported that cluster analysis is very useful in the classification of genotypes based on their similarity and affiliation. Valombola et al.[61] demonstrated that resemblances of accessions could be due to the fact that they might be the same accessions but having different names given by different ethnics or cultivated from agro-ecological zones. The breeding material should be selected from different clusters for the reason that each cluster has its specificity and this could help optimize the betterment of the new developed varieties and hybrids in terms of performance.

Moreover, many studies[62, 53] have used PCA clustering analysis and multidimensional scaling to evaluate genetic variability and genetic diversity in crop accessions including Bambara. As in the previous studies[53,28], we also found that the Dim 1 accounted for the highest percentage of variance which was followed by the PC2 and this pattern was observed in descending order in the remaining 8 dimensions. Khan et al.[28] reported a total variation at 45.88% for PC1 and 10.68% for PC2 while Olanrewaju et al.[53] found 24.67% for PC1 and 17.63% for PC2, the two authors worked on Bambara. In this study, the PC1 accounted for 39.85% and the PC2 represented 16. 31% of the total variation.

Correlation analysis

To select a genotype, it is of paramount importance to go through screening of genotypes and identifying of the traits that are strongly and positively correlated. Karikari and Tabore[63] reported that the understanding of variation and inter-correlation between traits is fundamental for fruitful selection. Similarly, Adebisi et al.[64] are of the opinion that one should take into consideration the strong correlation of variables in the selection process of superior genotypes for crop improvement. The correlation coefficients for 34 traits including morphological, phenological and agronomic traits were assessed in this study. The R software packages provides r-values and the level of probability for their significance. Plant height had positive correlation with internode length, leaf length, leaf width, biomass fresh weight per plant, biomass dry weight per plant, number of leaves per stem, number of stem per plant, total number of pods, final plant stand, fresh seed weight, number of seeds per pod, yield per plant, hundred seed weight, yield per plot, dry seed weight, fresh pod weight, mature pod number per plant, length of seeds, harvest index, and yield per plot of unshelled. This indicates that plant height is interrelated with vegetative and reproductive traits. Similar results were reported by many authors including Khan et al.[28], Olanrewaju et al.[53]. Total number of pods had a strong, positive, and highly significant correlation with final plant stand, fresh seed weight, number of seeds per pod, yield per plant, hundred seed weight, yield per plot, dry seed weight, Fresh pod weight, mature pod number per plant, shelling percentage, harvest index, and yield per plot of unshelled. Similar observations were made by Khan et al.[2]

In this study, we observed strong and positive and high contribution between hundred seed weight and yield (for yield per plant and yield per plot 0.89 and for yield per plot of unshelled 0.90), which is contrary to the results of Khan et al.[28] but similar to those of Karikari and Tabore[63], Misangu et al.[65]. The correlation matrix in this study also showed that yield was strongly and positively correlated with seed width, seed length, pod width, pod length, harvest index, shelling percentage, mature pod number, fresh pod weight, dry seed weight, number of seed per plant, fresh seed weight, fresh pod weight, total number of pods, leaf length, internode length, plant height, petiole length, number of leaf. The correlation between yield and leaf length and number of leaves demonstrated the ability of the plant to efficiently intercept light for photosynthesis but the yield was negatively correlated with biomass fresh, biomass dry weight and biomass per plant, which could be due to the fact that plant during seed development prioritized seed filling to the other plant organs. Similar results were reported by Evans[66], Carter [67], Helms[68] who revealed that yield and photosynthesis are often poorly correlated, both in field crops and in forest trees., while the results of Khan et al.[1] are contradictory because they reported that biomass fresh weight and biomass dry weight were correlated with yield. On the other hand, in our study seed width, seed length, pod width, pod length, harvest index, shelling percentage, mature pod number, fresh pod weight, dry seed weight, number of seed per plant, fresh seed weight, fresh pod weight, total number of pods greatly contributed to the yield. Similar results were obtained by Karikari and Tabore[63] who reported that the number of pods, number of seeds and seed weight per plant had strong influence on final seed yield while the results of [53] were contradictory because they found that numbers of pods, number of seeds, and total seed weight were positively correlated but negatively correlated with the yield. The seed size contribution to high yield cannot be overemphasized as farmers and consumers always seek for big seed and fruits, Duncan et al.[69], Pathirana[70], and Karikari and Tabore[63] demonstrated that the size of seed is well considered in the market either locally and internationally as an essential factor worldwide.

Materials And Methods

2.1 Experiment Location.

The research was conducted from August 2021 to December 2021 at three different environments. The experiments were conducted at Bowen University teaching and research farm Iwo, Osun State, Nigeria (7°38'N, 4°11'E) with an altitude of 322 m above the sea level, a leased farmland in Ologuneru Ibadan, Oyo State, Nigeria (7°44'N, 3°83'E) with an altitude of 275 m above sea level and a leased farmland in Odeda, Ogun state, Nigeria (7°23'N, 3°53'E) with an altitude of 162 m above the sea level. The seeds of the accession were sown in an open field across all environments during the 2021 cropping season.

2.2 Soil sampling and analysis.

Top soil was collected from the field for sampling at a depth of 0 – 15 cm randomly over the entire plot in the three environments. Cutlass and hand trowel was used to dig and collect soil samples. The collected samples were put together and sorted per location in order to obtain a composite sample after the experiment was carried out. The collected soil sample was then taken to the university laboratory for analysis. The samples were dried under shade and grinded in a glass mortal and pistil to ensure uniformity in nutrient distribution and for samples to be a true representation of the plots. After this process, the sample was sieved and the procedures for the chemical analysis and particle size distribution were carried out (sand, clay, silt, pH, organic carbon (OC), total N, exchangeable Ca, Mg, K, available P, Na, Mn, Cu, Fe, and Zn).

2.3 Plant materials.

Fifteen (15) accessions of Bambara groundnut were selected for this research work out of the Bambara groundnut germplasm that is located at the Genetic Resources Center, IITA, Ibadan Nigeria. The list of Bambara groundnut accessions used in this research are presented in Table 2. Five plants in the middle were selected to ensure uniformity across all beds and these five plants were used for data collection.

Table 2

The Accessions of Bambara Groundnut and their Countries of Origin.

S/N	Accessions	Origin
1.	TVSU-454	Cameroon
2.	TVSU-158	Ghana
3.	TVSU-438	Cameroon
4.	TVSU-633	Nigeria
5.	TVSU-1520	Unknown
6.	TVSU-939	Zambia
7.	TVSU-513	Cameroon
8.	TVSU-455	Cameroon
9.	TVSU-643	Nigeria
10.	TVSU-2096	Unknown
11.	TVSU-194	Benin
12.	TVSU-1611	Unknown
13.	TVSU-1920	Cameroon
14.	TVSU-1531	Unknown
15.	TVSU-1392	Unknown

2.4 Experimental Design and Intercultural Practice.

The experiment was conducted by using a randomized complete block design (RCBD) with three replications across all locations. In each replication, there were 15 plots/beds with each bed measuring 3 m X 0.5 m. The furrow spacing between each bed was 30 cm and the intra spacing distance plants were 30 cm while the interspacing distance between plants was 50 cm. The replications were separated from each other by a distance of 1 m. The total size of the experiment plot was 13 m X 12 m leaving 1 m of spacing before the first replication and 1 m spacing after the third replication and with 15 beds per replications and a total of 45 beds across all locations. Each replication had 11 plants per plot.

2.5 Measurement of Parameters for data analysis

For this study, the phonological, morphological, Agronomical and qualitative data were taken (Table 3 and Table 4).

Table 3

The list of 34 phenological, morphological and agronomical traits considered according to IPGRI, IITA, and BAMNET. (No 1-4 are the phonological traits, No 5-17 are the morphological traits while No18-37 are the agronomic traits.

S/N	NAME OF TRAITS	CODE	DESCRIPTION AND MEASUREMENT TYPE
1	Days to emergence	DTE	The no of days from planting to the arrival of first typical leaf on the soil surface.
2	Days to 50% flowering	D50%F	Measured from seed germination to arrival of 50% flowerings.
3	Days to 50% maturity	D50M	The number of days from sowing to 50% maturity
4	Days to harvest	DTH	The number of days from sowing to the period of harvest
5	Plant height	PH	Taken at 30 days and 50%F. Measured from soil level to the tip of terminal leaflet
6	No of branches per plant	NBPP	Data counted at time of harvest from the stems of the 5 measuring middle plants.
7	No of stems per plant	NSPP	Taken at 30 days and 50%F. Data counted from five middle healthy plants
8	Length of petioles per stem	LPPS	Taken at 30 days and 50%F. Data counted from five middle healthy plants.
9	No of leaves per plant	NLPP	Taken at 30 days and 50%F. Data counted from five middle healthy plants.
10	No of nodes per plant	NNPP	Data counted at the time of harvest from the stems of middle healthy plants.
11	Leaf length	LL	Taken at 30 days and 50%F. Measured from the longest leaf on the middle stem.
12	Leaf width	LW	Taken at 30 days and 50%F. Measured from the widest leaf on the middle stem
13	Inter node ode length	IL	Taken at 30 days and 50%F. Average internode length of 3 middle stems of 5 plants.
14	Panicle length	PL	Data measured at 50%F from length of 2 flowers on stems of 5 healthy middle plants.
15	Biomass fresh weight	BFW	Weight recorded at harvest of five healthy plants that were used in measuring
16	Biomass dry weight	BDW	Weight of dried plants in oven at 35°C for 2 hours from the same harvested plants.
17	Biomass per plant	BPP	Obtained by dividing dry by wet biomass and multiplying by 100 for same plants used.
18	Total no of pods per plant	TNPPP	Data counted at harvest from the five middle plants that were used during morphology.
19	Mature pod no per plant	MPNP	Data was counted after harvest and was recorded and separated from immature pods
20	Immature pod no per plant	IMPNP	Data was counted after harvest and separated from five healthy middle plants.
21	Fresh pod weight	FPW	Recorded at the time of harvest by using an Atom A-120 standard measuring scale.
22	Fresh seed weight	FSW	Recorded after breaking the pods and weighed using the same measuring scale
23	Dry seed weight	DSW	Weighed and recorded after seeds have been oven dried and set at 12% moisture.
24	Length of pods	LOP.	Measured within 3 days of harvesting by using a manual caliper. Pods were set horizontally.
25	Width of pods	WOP	Measured within 3 days of harvesting by using a manual caliper. pods were set vertically
26	Length of seeds	LOS	Measured for 5 plants by using a manual caliper and the seeds were placed horizontally
27	Width of seeds	WOS	Seeds were placed vertically and were measured by using a manually operating caliper.
28	Shelling percentage	SP	Measured by dividing the fresh seed weight with the fresh pod weight multiplied by100.
29	Number of seeds per pod	NoSP	Counted and recorded at harvest after breaking of pods
30	Yield per plant	YPP	Weighed after breaking the pods per plant by using a weighing scale
31	Hundred seed weight	HSW	Data weighing of 100 fresh seeds by using a weighing scale at harvest
32		YPPs	Yield of all the harvested plants on each plot after breaking the pods
33		Yppu	Yield of all the harvested plants on each plot before breaking the pods
		HI	
		Yield(Kg/ha)	

34	Yield per plot (Shelled)	Measured using the formula of ratio of grain yield and biological yield
35	Yield per plot (Unshelled)	The weight of all the harvested plants (Yields) across all blocks and replications and then converting into kg/ha
	Harvest index	
	Yield kg per hectare	

Table 4

The qualitative traits measured

S/N	QUALITATIVE TRAITS	CODE	DESCRIPTION	SCALE
1	Growth habit	GrH	Data was taken after 8 weeks of sowing based on the petiole and internode length of the middle stem of 5 middle plants per plot	(1) Accession of bunch type (P/I≥9) (2) Accession of semi-bunch type (P/I=7-9) (3) Accession of Spreading type (P/I≤7)
			Data was recorded at harvest	
2	Stem hairiness	StH	Recorded after 2 weeks of planting	(1) Absent. (2) Sparse. (3) Dense.
3	First stem color	FSC	Data were recorded 8 weeks after sowing	(1) Green (2) Reddish (3) Stripped (4) Reddish green (5) Brownish
4		TLS	Data were recorded after 8 weeks of sowing	(1) Round (2) Oval (3) Elliptical (4) Lanceolate
	Terminal leaflet shape			
5		TLC	Data were recorded after 2 weeks of sowing	(1) Green (2). Red (3) Purple
	Terminal leaflet color		Data were taken based on 1 seeded pods at harvest	
6		PetP	Data was taken using color wheel and done at harvest	(1) Green (2) Reddish-brown (3) Brown (4) Reddish-green
7	Petiole pigmentation	PoS	Data were taken within 3-5 days of harvesting	(1) Hook with ending point on the opposite side (2) Round with ending point on the opposite side (3) Hook with ending point on both sides
				(1) Yellowish-brown (2) Reddish-brown (3) Brown (4) Cream with brown patches
8	Shape of pod	PoC	Data were taken from seed of 1 seeded pods at harvest	
				(1) Much grooved (2) Much folded (3) Little grooved (4) Smooth
			Data were taken using color wheel and done at harvest	
9	Color of pod	PoT		
				(1) Oval. (2) Round (3) Other
			Data taken based on Bambara groundnut descriptor at harvest	
10	Pod texture	SeS		
			Data taken based on Bambara groundnut Descriptors at harvest	(1) Cream. (2) Light red (3) Light brownish red (4) Dark purple (5) Light brown (6) Purplish red
11	Seed shape	SeC		
				(1) Absent (2) Brown (3) Light red (4) Black (5) Grey
			Described in a combination of the testa background with eye color	
	Seed color	EyC		
12				(1). No testa pattern. (2) Black strips on both micopylar end (3) Cream marbled (4) Light red strips on one micopylar end (5) Brownish red strips on both micopylar
		TeP		
13				
	Eye color	(TC+EyP)		(1) Light red/brown testa with light red /brown butterfly like eye (2) No eye pattern (3) Cream testa with black/Brown butterfly like eye (4) Cream testa with grey/Light red triangular eye (5) Cream testa with light red patches (6) Purplish red testa with cream butterfly like eye
	Testa pattern			
14				

Testa color
with eye
pattern
around hilum

2.6 Statistical Analysis for ANOVA

The morphological, agronomic and phenological traits were examined using the ANOVA to determine if variations existed among the accessions and locations by making use of the R statistical packages version R-4.0.5. Fischer's least significant difference (F-LSD) was used to separate means at a probability level of 5%. PCA was performed using the FactoMineR and factoextra packages and Pearson correlation was done using the corr. Functions in R. A hierarchical cluster analysis was performed using the ward D2 method with cluster factoextra package in R.

Declarations

Author contributions

The concept, design and methods of the paper was constructed by V. I. E. Data collection was carried out by G. O. O. Statistical R analysis and interpretation were undertaken by V. I. E., and G. O. O. and T. O. O. Original draft preparation of the manuscript was carried out by V. I. E. and G. O. O. Review and editing by V. I. E., and T. O. O. All authors have read and agreed to the published version of the manuscript.

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Figures

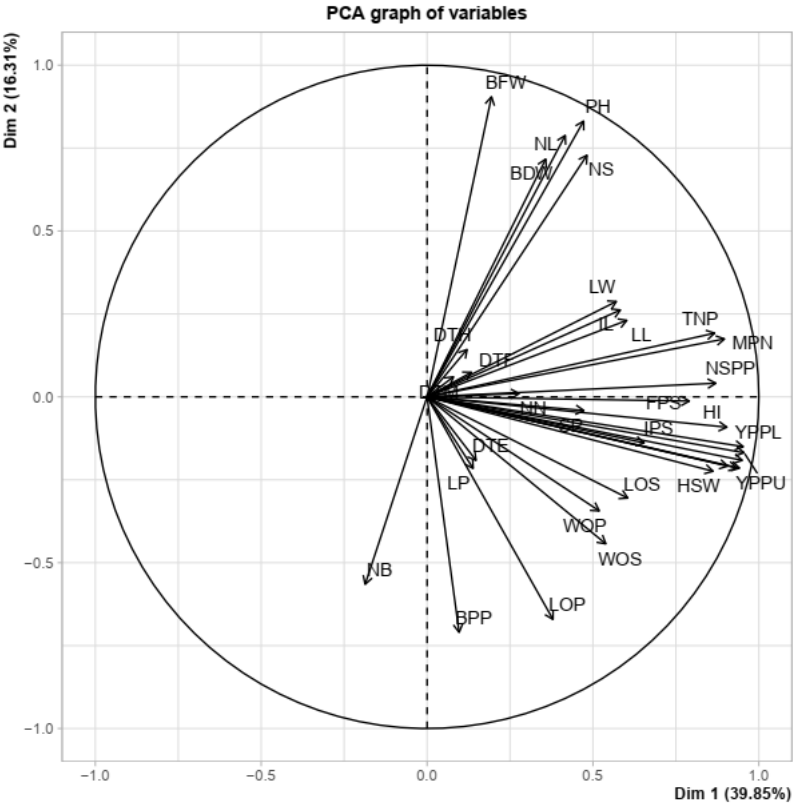

Figure 1

Principal Component Analysis (PCA) of quantitative trait scores

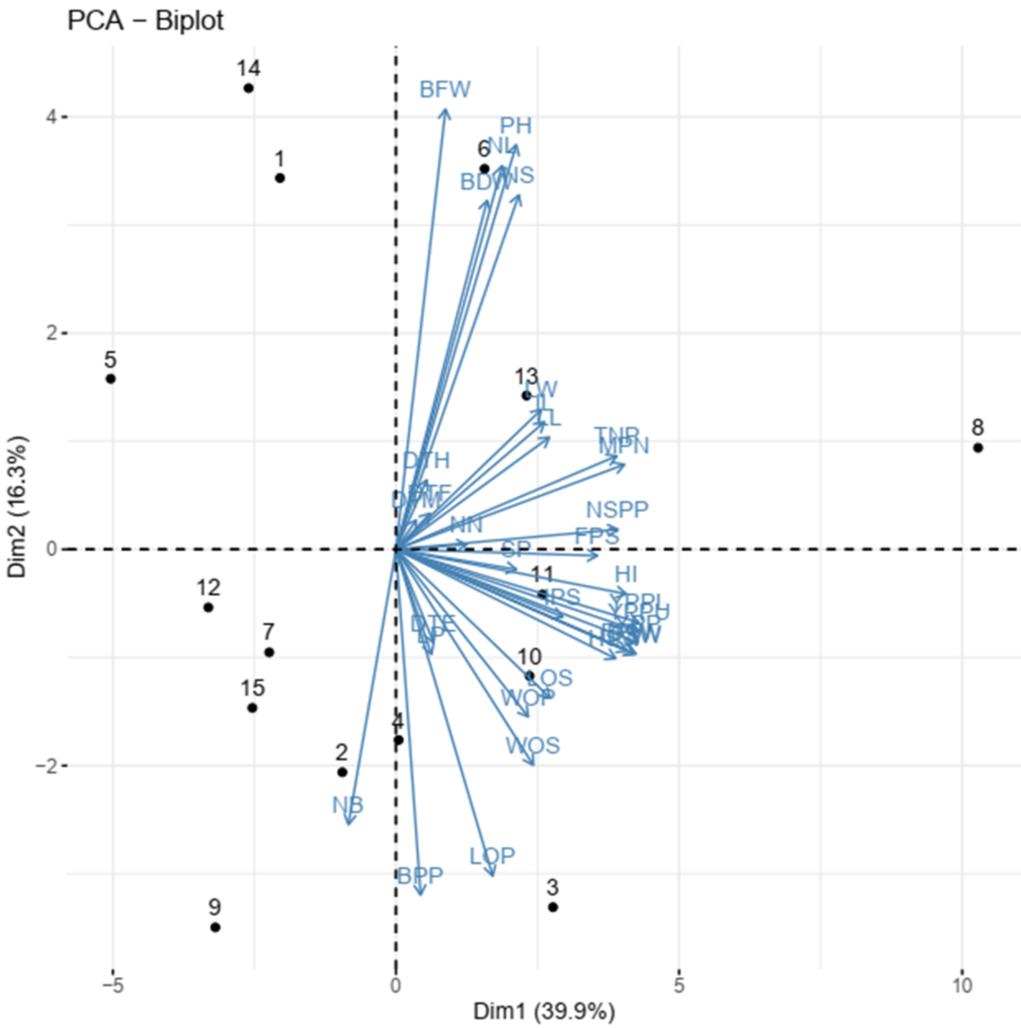

Figure 2

Dim 1 and Dim 2 biplot using morphological, phenological and agronomic trait scores of the Bambara groundnut accessions.

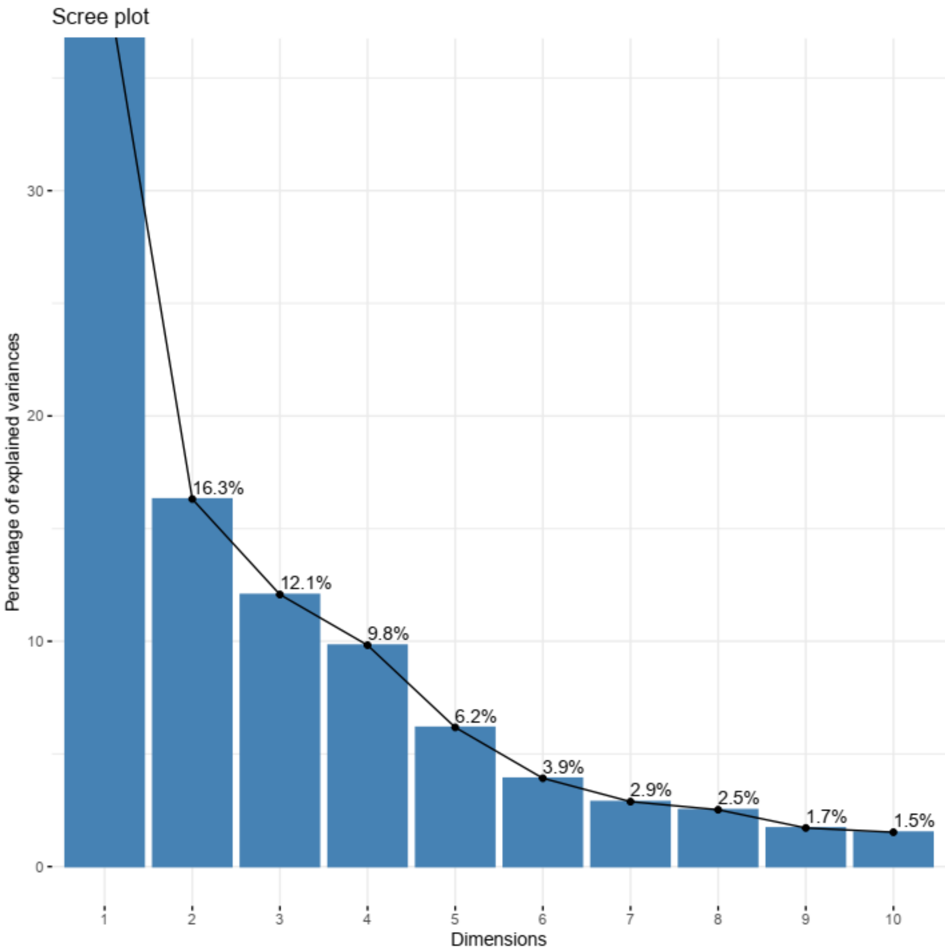

Figure 3

Percentage of explained variances of 10 PCs using quantitative trait scores of the 15 Bambara groundnut accessions

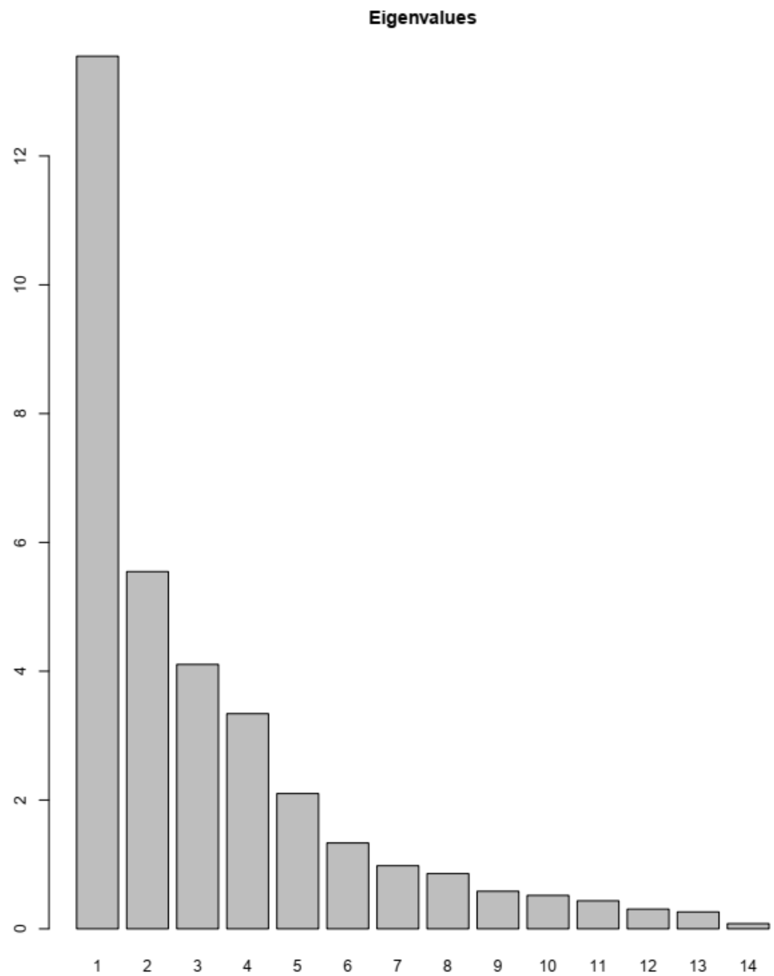

Figure 4

Eigenvalues of the quantitative parameters on the axes

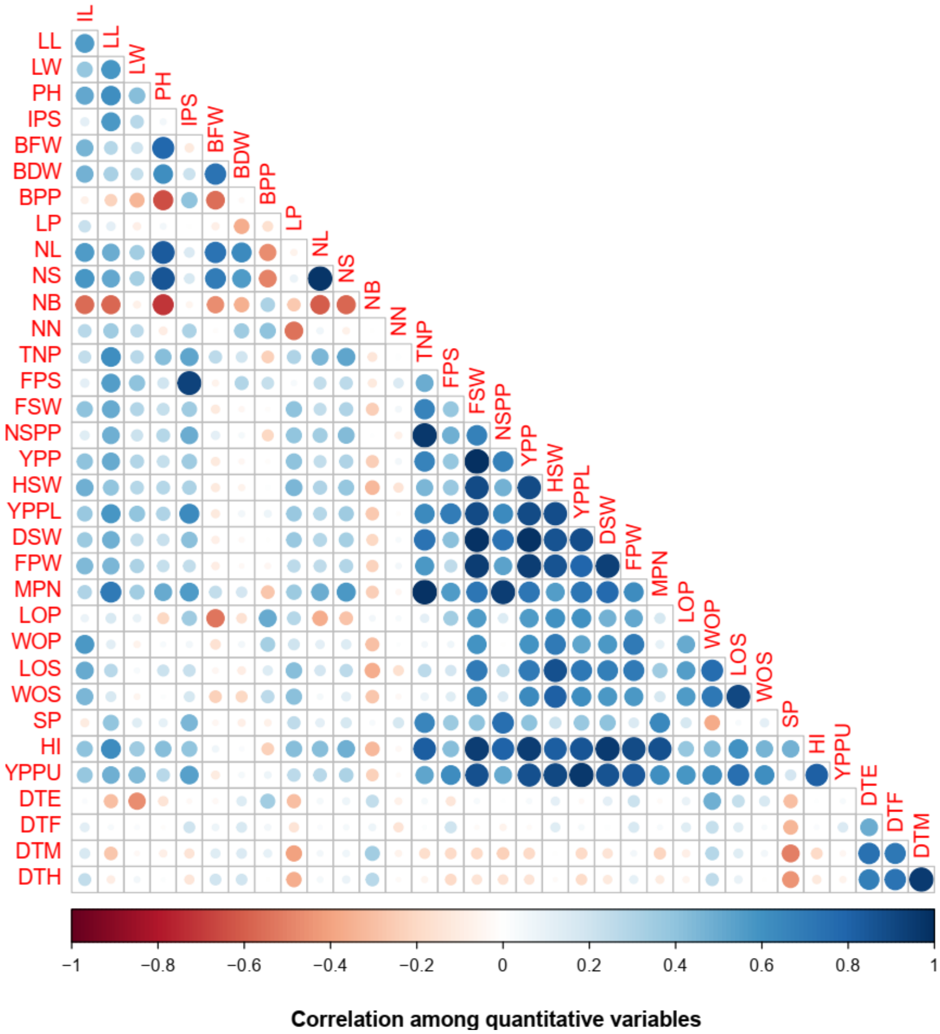

Figure 5

Correlations among the phenological, morphological and agronomic traits; Pearson's rank correlation matrix indicating the correlation among variables of Bambara accessions grown across three environments.

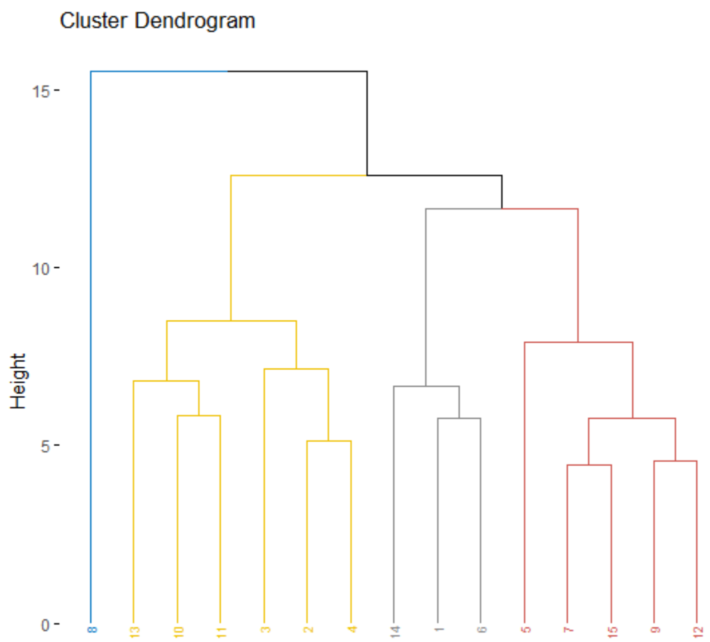

Figure 6

Hierarchical clustering dendrogram of the 15 Bambara groundnut accessions based on quantitative traits

1=TVSU-454, 2=TVSU-158, 3=TVSU-438, 4=TVSU-633, 5=TVSU-1520, 6=TVSU-939, 7=TVSU-513, 8=TVSU-455, 9=TVSU-643, 10=TVSU-2096, 11=TVSU-194, 12=TVSU-1611, 13=TVSU-1920, 14=TVSU-1531, 15=TVSU-1392

Supplementary Files

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- SupplementaryTableS1.xlsx