

1 **VARIATION IN SEEDS PHYSICAL TRAITS OF BAMBARA GROUNDNUT (*Vigna***
2 ***subterranea* (L.) Verdc.) COLLECTED IN CAMEROON**

3

4 **ABSTRACT**

5 Bambara groundnut (*Vigna subterranea* (L.) verdc.) is a leguminous species which produces
6 edible seeds. Its seeds are included in the daily diet to compensate the lack of proteins in the
7 food which occurs frequently in populations under the tropics. Information regarding the
8 extent and pattern of variation in bambara groundnut populations in Cameroon is limited. The
9 objective of this study was to estimate variation in seed physical traits of twelve bambara
10 accessions collected in different regions in Cameroon. Seeds of Twelve bambara accessions
11 harvested four months ago and keep at laboratory were used and their selection was based on
12 their color. Various physical traits were measured on seeds with 8.11 to 13.12% moisture
13 content. The results revealed highly significant differences ($p < 0.001$) for all the studied
14 characters, indicating large variation in seed physical traits of bambara groundnut in
15 Cameroon. The mean value for length, width and thickness of bambara seeds ranged from
16 9.68 to 11.75 mm; 8.73 to 10.46 mm and 8.12 to 10.30 mm, respectively. Large seeds had
17 higher geometric mean diameters, volume and surface area. However, small sized seeds had
18 higher sphericity and aspect ratio than large seeds. The seeds are irregular in shape and size,
19 are highly spherical with high aspect ratio and will roll rather than slide. Variation between
20 individuals has to be attributed to high number of additive effects and parameters can be
21 studied through selection as major candidates.

22 **Key words:** Bambara groundnut, seed, physical traits, sphericity, heritability.

23

24 **1. Introduction**

25 Bambara groundnut (*Vigna subterranea* (L.) verdc.) is a leguminous species which produces
26 edible seeds. It's an indigenous African crop that grows across the continent from Senegal to
27 Kenya and from the Sahara to South Africa [1]. Bambara groundnut production was reduced
28 in many parts of Africa because of the expansion of groundnut production. However, in recent
29 years, it regained a great interest due to its seeds which contain sufficient quantities of
30 proteins (19%), carbohydrates (63%), fats (6.5%) and essential amino acids [2]. Its seeds are
31 included in the daily diet to compensate the lack of proteins in the food which occurs

frequently in populations under the tropics. Despite the numerous advantages provided by bambara groundnut, limited studies have been conducted on this edible crop in Cameroon compared to others crops such as sorghum, groundnut and cowpea. Similar observations have been reported in Burkina Faso [3]. In addition, this crop is mostly grown by female, on a small scale, in pure culture without improved techniques [4].

In many villages of Cameroon, different accessions of bambara groundnut are grown and some farmers have several cultivars presenting different traits [5]. However, information regarding the extent and pattern of variation in bambara groundnut populations in Cameroon is limited. Previous studies reported variation in agronomic aspect [6, 7]. These studies showed high morphological variability between the different accessions. In our knowledge, no work has been carried out on the physical characteristics of Bambara seeds in Cameroon. Assessment of these properties can help in characterizing Bambara groundnut populations as it was the case in others crops [8, 9, 10]. Moreover, the study of seed traits is often considered to be useful in evaluation of genetic variability [11]. It can be considered as a first step in assessing genetic diversity in a specie. The present work was carried out to evaluate variation in seed physical traits of twelve bambara accessions from Cameroon. It can help in identifying desired traits that can be used in breeding programs. It can also be useful in the management and utilization of bambara groundnut germplasm in Cameroon.

2. Materials and methods

2.1. Bambara groundnut seed samples

Seeds of Twelve bambara accessions harvested four months ago and keep at laboratory were used. Twelve accessions of bambara groundnut were chosen from which ten were described by [6] and two from [12]. Selection of twelve accessions was based on their color (Table 1). Seed coat and eye color and pattern were performed using the descriptors list of *V. subterranea* [13]. The seeds were stored in plastic buckets and kept in dry storage at room temperature of 25-27°C before making any measurements.

Table 1. Characteristics of bambara groundnut seeds

N°	Denomination	Seeds traits
1	NOR1*	cream testa with grey eye butterfly like eye
2	NOR1Y*	
3	NOR2*	Cream testa without eye pattern
4	NOR2Y*	
5	NOR4*	light red testa

6	NOR5*	Black small dotted spots on brown background without eye
7	NOR6*	Dark brown small dotted spots on cream background with grey butterfly like eye
8	NOR7*	Dark brown marbled spots on cream background with grey butterfly like eye
9	NOR8*	Black marbled spots on cream background with grey butterfly like eye
10	NOR9*	Cream testa with black irregular eye
11	MBYO 3	Black testa
12	MBYO 10	Light brown testa

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2.2. Moisture content

The moisture content of bambara groundnut seeds was determined through the standard oven-drying method [14]. Seeds (5 g), in triplicates, were oven dried at 103°C for 72 h. The moisture content, M, in percent was determined using the formula:

$$M (\%) = [(WF-WD)/WD] \times 100$$

Where WF is the fresh weight before drying and WD is the dry weight.

2.3. Determination of seeds traits

The characteristics of the Bambara seeds were determined according to [15] and [16]. For each Bambara groundnut source, triplicate of one hundred (100) undamaged seeds were randomly selected. For each 100 seeds, individual seed length, width and thickness were measured using an electronic vernier caliper.

T was defined as the distance from the eye's seed to the opposite end, while L and W taking in the two opposite perpendicular direction of eye seed represented the major and the minor seed diameters.

2.3.1. Seed characteristics calculation

Geometric mean diameter (D_g) of the seed was calculated using the following relationship [17]:

$$D_g = (LWT)^{1/3}$$

Where the L = length, W= width and T = thickness

Sphericity, ϕ of bambara seeds was calculated using the formula [17]:

$$\phi = D_g/L$$

Seed volume (V) and **seed surface area (S)** were calculated using the formula stated by [18]:

$$V = \frac{\pi B^2 L^2}{6(2L - B)} \quad \text{And} \quad S = \frac{\pi B^2 L^2}{2L - B}$$

$$\text{Where } B = (WT)^{(0.5)}$$

77 **Aspect ratio (AR)** was calculated using equation [17]:

$$78 \quad AR = W/L$$

79 **2.3.2. One hundred seed weight**

80 For each accession, triplicate of one hundred seed weight was obtained using an electronic
81 balance.

82 **2.4. Data analysis**

83 The different traits were submitted to an analysis of variance (ANOVA) at 5% level.
84 Duncan's multiple range test (SPSS, 2005) was used to separate the means when differences
85 were significant.

86 The phenotypic variation for each seed trait was partitioned into components due to genetic
87 and environmental factors using the following formula [19]:

88

$$V_p = MSG/r; \quad V_g = [MSG - MSE]/r; \quad V_e = MSE$$

Where MSG, MSE and r are the mean squares of Bambara groundnut accessions, mean squares of error and number of replications, respectively. V_p is the phenotypic variance, V_g the genotype variance and V_e the environmental variance.

89 To be able to compare the variation among traits, phenotypic (PCV) and genotypic (GCV)
90 coefficients of variation were computed according to the method suggested by [20]:

91

$$PCV = (\sqrt{V_p}/X) \times 100; \quad GCV = (\sqrt{V_g}/X) \times 100$$

V_p , V_g and X are the phenotypic variance, genotypic variance and grand mean for each seed trait, respectively.

92 Broad sense heritability (h^2_B) was calculated according to [21] as the ratio of the genotypic
93 variance (V_g) to the phenotypic variance (V_p). Genetic advance (GA) expected and GA as per
94 cent of the mean assuming selection of the superior 5% of the genotypes were estimated in
95 accordance with [19] as:

$$GA = K \cdot h^2_B \cdot \sqrt{V_p}; \quad GA \text{ (as \% of the mean)} = (GA/X) \times 100$$

K is the selection differential (2.06 for selecting 5% of the genotypes).

3. Results and discussion

3.1. Bambara seeds traits and moisture content

The seeds are heterogeneous in color with very segregation into different shades of colors. NOR2 and NOR2Y seeds have similar uniform Cream testa without eye pattern while NOR1Y and NOR1 seeds have cream testa with grey eye butterfly like eye. NOR7 and NOR8 have the characteristics marbled spots on cream background, with grey butterfly like eye around hilum and have been classified into two genotypes: dark brown and black marbled, respectively. The others accessions are different in their shades of colors. The same observations were reported by [6] and [12] who worked on the accessions parents, three generations back of self-pollination.

The moisture content of the seeds ranged from 8.11 % (NOR4) to 13.12 % (NOR8) (Table 2). Black (MBYO3) and red (NOR4) seeds had low moisture contents compared to white seed (Table 2). The conditions of seed storage were homogenous for all accessions in the laboratory with a room temperature about 27°C. These moisture contents have not far with that recommended for seed storage (12%) in bambara groundnut.

Values were ranged for length in 6-16 mm with mean of 10.81 mm, for width in 6-13.5 mm with mean of 9.28 mm and for thickness in 6-12.5 mm with mean of 9.19 mm. Significant ($P < 0,001$) differences existed among accessions length, width and thickness (Table 2). Majority of the seeds for NOR2, NOR4, NOR5, NOR6, NOR9 and NOR2Y have high length, width and thickness. The seeds of these accessions are large in seed size while those of NOR7, NOR8, NOR1, NOR1Y and MBYO3 are shorter. [15] reported that range values of L, W and T, on bambara seeds were 10.1-15.2 mm, 9.5-11.5 mm and 8.2-11.0 mm, respectively. Our accessions have seeds with high morphometric trait diversity compared to those of [15]. NOR7 and NOR8 have small seed sizes with high moisture contents contrary to NOR2Y and NOR6 which have large seed sizes and high moisture contents.

In reference to [6] who reported similar seed sizes and colors on the parents, three generations back of self-pollination, we could suppose that bambara seed sizes and colors are under genetic control. So, these characters may permit to do a first screening for our accessions.

Table 2: Bambara groundnut seeds traits and moisture content (%)

	Length (mm)			Width (mm)			Thickness (mm)			Moisture content (%)
	Mean	Min	max	Mean	min	Max	Mean	Min	Max	
NOR1	10.7	8.5	13.5	9.01	7	11.5	8.64	6.5	11	11.55±0.07

NOR2	11.08	9.5	13	9.32	8	11	9.44	8	11	12.63±0.12
NOR4	11.42	8.5	15	9.24	6.5	14	9.78	7.5	12.5	8.18±0.09
NOR5	11.55	8	15.5	9.05	7	11	9.47	7	11	10.84±0.08
NOR6	10.82	7	13	9.4	7.5	11.5	10.3	8	12.5	11.91±0.03
NOR7	10.53	7	13	8.73	6	11	8.26	6	10.5	11.42±0.08
NOR8	9.68	6.5	12.5	8.79	6	10.5	8.12	6	10	13.03±0.07
NOR9	11.78	8	15	9.79	6	12	9.28	6.5	12	10.71±0.04
NOR1Y	10.51	6.5	15	8.76	6	11	8.49	6	10	10.58±0.05
NOR2Y	11.75	8	14.5	10.46	8	13.5	10.13	8	12	12.07±0.10
MBYO3	9.88	6	14	9.28	7	12	8.93	6	11	9.02±0.07
MBYO10	10.03	7	16	9.54	7	11.5	9.52	7	11.5	10.05±0.07
Total	10.81	6	16	9.28	6	13.5	9.19	6	12.5	10.75
SD	1.58			1.16			1.16			1.22
CV (%)	13.25			11.55			10.38			13.99
P	<0.001			<0.001			<0.001			<0.05

3.2. Seed geometric characteristics

Geometric mean diameter (Dg) ranged from 9.12 (NOR7) to 10.75 mm (NOR2Y) (Table 3). It differed significantly among the accessions ($P < 0.05$). Dg of NOR2, NOR2Y, NOR9, NOR6 and NOR4 were significantly higher compared to others accessions. Geometric diameter of bambara seeds in this study (9.71 ± 1.14 mm) was higher than that of [22] and was conform to those obtained by [15].

Accessions differed significantly ($p < 0.001$) in seed volume and ranged from 384 (NOR1) to 599 mm³ (NOR2Y). The seeds differed significantly ($P < 0.001$) in surface area which ranged from 2021 (NOR8) to 3593 mm² (NOR2Y). NOR2Y and NOR6 are significantly higher in volume and surface area comparatively with the others. The determination of geometric diameter could be useful in the theoretical estimation of seed volume [23, 1] and seed surface. Sphericity of Bambara seeds ranged from 87% (NOR5, NOR7 and NOR9) to 97% (MBYO10). Significant differences ($P < 0.001$) existed among the accessions in sphericity (Table 3). In the jackbean, when sphericity was superior to 70% shape of the seed was closed to a sphere [24]. Then, all our accessions seeds have sphere shape. This high sphericity enables bambara seeds to roll rather than slide. In addition, bambara groundnut has a more rounded shape as referred to the degree of sphericity. This property is required in the design of hoppers, chutes and other storage facilities [24]. However, elliptical shapes of Bambara seeds had been reported by [15] and [22]. The aspect ratio of bambara seeds ranged from 79% (NOR5) to 97% (MBYO10). The bambara seeds differed significantly in aspect ratio ($P < 0.001$). With an aspect ratio superior to 70%, the Bambara seed is more likely to roll than

slide. A similar trend had been reported for jackbean [24] and bambara groundnut [25]. Low aspect ratio indicates the tendency to be oblong in shape [24]. Our results show that sphericity and aspect ratio increased with decrease in seed size, whereas volume, surface area and geometric mean diameter increased with increase in seed size.

The 100-seed weight of bambara ranged from 55.5 (NOR8) to 95.2 g (NOR2Y) and was significantly different ($p < 0.05$) among the accessions. This result is in agreement with that (50–80 g) obtained by [15]. NOR2Y and NOR6 seeds are significantly heavier than the others. This parameter is often useful in agronomy.

Table 3: Geometric characteristics of Bambara seeds

Seed sources	100-seed Weight (g)	Geometric mean diameter(mm)	Sphericity	Volume(mm ³)	Surface(mm ²)	Aspect Ratio
NOR1	64.94	9.41	0.88	384.30	2305.79	0.85
NOR2	77.55	9.91	0.90	452.78	2716.66	0.84
NOR4	72.54	10.09	0.89	475.86	2855.15	0.81
NOR5	69.42	9.96	0.87	442.27	2653.59	0.79
NOR6	81.05	10.14	0.94	522.02	3132.12	0.87
NOR7	58.37	9.12	0.87	343.16	2058.96	0.83
NOR8	55.5	8.83	0.92	336.95	2021.73	0.92
NOR9	75.1	10.22	0.87	487.52	2925.10	0.83
NOR1Y	70.74	9.20	0.88	366.68	2200.09	0.85
NOR2Y	95.2	10.75	0.92	598.99	3593.95	0.89
MBYO3	65.84	9.32	0.96	424.23	2545.39	0.96
MBYO10	72.98	9.67	0.97	478.60	2871.62	0.97
Mean	71.67±1.74	9.71±1.14	0.90±0.07	442.77±153.42	2656.67±920.55	86.82±0.11

3.3. Genetic variation

Phenotypic coefficient of variability (PCV) were high (7.73%) in seed thickness and low (5.30%) in seed width. Genotypic coefficient of variability (GCV) were high (7.66%) in seed thickness and low (5.17%) in seed width (Table 4). The magnitudes of PCV for most of the characters were relatively close to the corresponding magnitudes of GCV. This suggested that environmental components have relatively less influence on the expression of these traits. High heritability in broad sense ($h^2b > 95\%$) was recorded in all seed traits. A high heritability of a trait provides a measure of the effectiveness of selection on phenotypic basis for that particular trait. Whatever, [26] indicated the limitations of estimating heritability in broad sense, as it included both additive and epistatic gene effects and suggested that heritability estimates in broad sense will be reliable if accompanied by a high genetic advance. [19] have suggested that heritability and followed with genetic advance provide a better picture than heritability alone. Genetic advance (GAM) varies from 8.12% for seed sphericity to 16.71%

observed in seed length. GAM is moderated (10-20%) for all seed traits except for sphericity (8.12%). Thus, moderate progress, through selection, would be expected for these traits between individual genotypes. Based on the findings, it can be concluded that selection could be effective in the test materials used in this study as revealed by the significant substantial variations among the accessions for all the traits. Therefore, the significant genetic variability

Table 5: Correlation coefficients among the geometric parameters in bambara seeds

for length, width and thickness in the accessions recorded in the test materials could be further exploited through improvement and selection programs.

Table 4. Estimation of genetic variables for seeds traits in Bambara groundnut.

Traits	Variance		Coefficient of variation (%)		Heritability (broad sense)	Genetic Advance as % mean (GAM)
	Phenotypic	Genotypic	Phenotypic	Genotypic		
Length	0.5236	0.5031	6.6925	6.5600	0.9607	16.7183
Width	0.2424	0.2309	5.3038	5.1765	0.9525	10.4078
Thickness	0.5064	0.4973	7.7363	7.6662	0.9819	15.6489
Sphericity	0.00135	0.00131	4.0731	4.4285	0.9682	8.12
Aspect Ratio	0.00323	0.00312	6.5515	6.4429	0.9671	13.0495

3.4. Correlation between seed size and geometric traits

Significative correlations ($r > 0.60$) have been recorded between seed traits (Table 5). Seed length was significantly correlated with all measured traits. The seed with the longer length did not have either the largest width or thickness. A similar trend was reported by [23], [27] and [25] for locust bean, guna and bambara seeds, respectively. Seed length was significantly correlated with all traits. Seed width and thickness were significantly correlated with all traits except for sphericity and aspect ratio. Sphericity and aspect ratio were negatively correlated with length. Length could be an interest trait when selection is based on seed traits.

Width and thickness were positively and significantly correlated with seed volume and surface.

Positive correlation between seed traits would permit selection and improvement of these traits simultaneously. These results indicated that genes were probably linked or have a pleiotropic effect [28].

	L	W	T	ϕ	V	S	AR	Dg
Length (L)	1							
Width (W)	0,62**	1						
Thickness (T)	0,61**	0,78**	1					
Sphericity (ϕ)	-0,59**	0,18	0,19	1				
Volume (V)	0,66**	0,93**	0,92**	0,17	1			
Surface area (S)	0,66**	0,93**	0,92**	0,17	1,00**	1		
Aspect Ratio (AR)	-0,56**	0,26	0,06	0,94**	0,16	0,16	1	
Geometric mean diameter (Dg)	0,86**	0,89**	0,89**	-0,12	0,93**	0,93**	-0,13	1

**Correlation is significant at $P < 0.01$

4. Conclusion

High variation was recorded in shape and size of Bambara groundnut seeds. The seeds of NOR2Y, NOR5 and NOR9 are longer while seeds of NOR8, MBYO3 and MBYO10 are shorter in length. Seeds of NOR2Y are wider and thicker while seeds of NOR7, NOR8 and NOR1Y are less broad and thicker than others. The seed with the longer length did not have either the largest width or thickness. MBYO10 is more spherical and NOR2Y occupies more volume and area when compared to the other accessions. Bambara seeds in our study are highly spherical with high aspect ratio and would roll rather than slide. Small sized seeds had higher sphericity and aspect ratio than large seeds. Sphericity seem to be influenced by width and thickness. Broad sense heritability were high for all traits, accompanied with moderate genetic advance indicate that traits can be improved through selection. Morpho-metric diversity in this species is of great interest as it forms the basis for selection and further improvement. The estimates of heritability and genetic advance would no doubt help in drawing conclusion about the nature of gene action governing a particular trait.

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