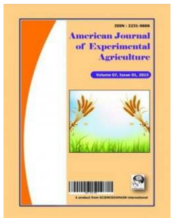

American Journal of Experimental Agriculture
12(2): 1-8, 2016, Article no.AJEA.20592
ISSN: 2231-0606

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Variation in Seeds Physical Traits of Bambara Groundnut (*Vigna subterranea*) Collected in Cameroon

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Authors' contributions

This work was carried out in collaboration between all authors. Author NZ wrote the protocol and wrote the first draft of the manuscript. All authors contributed to the design of the study and authors OB, NHB and MSE completed the literature. Author NZ reviewed the experimental design and all drafts of the manuscript. Author NZ performed the statistical analysis with support from authors OB, BJM and FD. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJEA/2016/20592
Editor(s):
(1) Slawomir Borek, Department of Plant Physiology, Adam Mickiewicz University, Poland.
Reviewers:
(1) O. Peter Bamidele, Obafemi Awolowo University, Osun State, Nigeria.
(2) Norma Almaraz Abarca, Instituto Politecnico Nacional, Mexico.
(3) Niranjalie Perera, Wayamba University of Sri Lanka, Sri Lanka.
Complete Peer review History: http://sciencedomain.org/review-history/14093

Original Research Article

Received 31st July 2015
Accepted 15th February 2016
Published 9th April 2016

ABSTRACT

Vigna subterranea is a leguminous species which produces edible seeds (Bambara groundnut). Its seeds are included in the daily diet to compensate the lack of proteins in the food which occurs frequently in populations under the tropics. However information regarding the extent and pattern of variation in Bambara groundnut accessions in Cameroon is limited. The objective of this study was to estimate variation in seed physical traits of twelve Bambara accessions collected in different regions in Cameroon. Seeds of Twelve Bambara accessions were harvested in Janaury

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2013 and kept for four months in the Laboratory of Botany and Plant Physiology of the University of Douala. Selection was based on their color. Individual seed length, width and thickness were measured as the physical traits. The seed characteristics were also calculated as the geometric mean diameter, sphericity, seed volume, seed surface area and the aspect ratio. Moisture content varied from 8.11 to 13.12%. Results revealed that there was a highly significant difference ($p < 0.001$) for all the studied physical traits and seed characteristics, indicating large variation in seed physical traits of Bambara groundnut in Cameroon. The mean length, width and thickness of Bambara seeds ranged from 9.68 to 11.75 mm; 8.73 to 10.46 mm and 8.12 to 10.30 mm, respectively. Large seeds had higher geometric mean diameters, volume and surface area. However, small seeds had higher sphericity and aspect ratio than large seeds. The seeds were irregular in shape and size, and highly spherical with high aspect ratio and will roll rather than slide. Thus variation between individuals has to be attributed to a high number of additive effects and parameters can be studied through selection as major candidates.

Keywords: Bambara groundnut; seed; physical traits; sphericity; heritability.

1. INTRODUCTION

Vigna subterranea is a leguminous species which produces edible seeds (Bambara groundnut). It’s an indigenous crop that grows across subsaharian Africa [1]. In recent years, it regained a great interest due to its seeds which contain sufficient quantities of proteins (19%), carbohydrates (63%), fats (6.5%) and essential amino acids [2]. Its seeds are included in the daily diet to compensate the lack of proteins in foods which occurs frequently in tropical populations. Despite the numerous advantages provided by Bambara groundnut, limited studies have been conducted on this edible crop in Cameroon as compared to other crops such as sorghum, groundnut and cowpea. Similar observations have been reported in Burkina Faso [3].

In many villages of Cameroon, different accessions of Bambara groundnut are grown and some farmers have several cultivars presenting different traits [4]. However, information regarding the extent and pattern of variation in Bambara groundnut accessions in Cameroon is limited. Previous studies reported variation in agronomic aspect [5,6]. These studies showed high morphological variability between the different accessions. In our knowledge, no work has been carried out on the variation in seed physical traits of Bambara seeds in Cameroon. Assessment of these physical traits can help in characterizing Bambara groundnut accessions as it was the case in others crops [7,8,9].

Moreover, the study of seed physical traits is often considered to be useful in evaluation of genetic variability [10]. It can be considered as a first step in assessing genetic diversity in a specie. The objective of the present study was to evaluate variations in seed physical traits that can be inheritable in twelve Bambara groundnut accessions in Cameroon. This will help 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 Seed Samples

Samples of twelve accessions of Bambara groundnut were chosen from which ten were described by [5] and two from [11]. Selection of the seed samples was based on their seed coat color (Table 1). Seed coat, eye color and pattern were performed using the descriptors list of *V. subterranea* [12]. The seeds were harvested in January 2013 and kept in the Laboratory of Botany and Plant Physiology of the University of Douala. They were stored in plastic buckets with cover [13] and kept in dry storage at room temperature of 25-27℃ for four months before taking any measurements.

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℃ for 72 h [14]. The moisture content, M, in percent was determined using the formula:

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

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

Table 1. Characteristics of Bambara groundnut seeds

N°	Samples	Seeds characteristics	Eco-geographical data
1	NOR1*	Cream testa with grey eye butterfly like eye	Garoua (North region), Agro-ecological zone I (Soudano sahelian), zone between 8° - 13° latitude North, zone characterized by a rainfall of 400-1200 mm/an.
2	NOR2*	Cream testa without eye pattern	
3	NOR4*	Light red testa	
4	NOR5*	Black small dotted spots on brown background without eye	
5	NOR6*	Dark brown small dotted spots on cream background with grey butterfly like eye	
6	NOR7*	Dark brown marbled spots on cream background with grey butterfly like eye	
7	NOR8*	Black marbled spots on cream background with grey butterfly like eye	
8	NOR9*	Cream testa with black irregular eye	
9	NOR1Y*	Cream testa with grey eye butterfly like eye	Yaounde (Centre region), Agro-ecological zone V (Forets at bimodale rainfall), situated between 2° - 4° latitude North, characterized by a rainfall of 1500 – 2000 mm/an.
10	NOR2Y*	Cream testa without eye pattern	
11	MBYO 3	Black testa	Mbalmayo (Centre region), Agro-ecological zone V (Forets at bimodale rainfall), situated between 2° and 4° latitude North, characterized by a rainfall of 1500 – 2000 mm/an.
12	MBYO 10	Light brown testa	

*[6]

2.3 Determination of Seed Physical Traits

The characteristics of the Bambara groundnut 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 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]:

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

Where $B = (WT)^{(0,5)}$

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

$$AR = W/L$$

2.3.2 One hundred seed weight

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

2.4 Data Analysis

The data analyses were done using the software SPSS (2005). The different traits were submitted to an analysis of variance (ANOVA) at 5% level. Duncan's multiple range test was used to separate the means that were significantly different.

The phenotypic and genotypic variances of each trait were calculated from the ANOVA as described by [19]. The phenotypic variation for each seed trait was partitioned into components due to genetic (hereditary) and non-genetic (environmental) factors and estimated using the following formula [19]:

$$Vp = Vg + Ve \quad Vg = [MSG - MSE]/r; \quad Ve = MSE$$

Where MSG and MSE are the mean sum of squares for the genotypes and error in the analysis of variance, respectively; r is the number of replications.

The phenotypic variance (Vp) is the total variance among phenotypes when grown over the range of environments of interest, the genotypic variance (Vg) is the part of the phenotypic variance that can be attributed to genotypic differences among the phenotypes, and the error variance (Ve) is part of the phenotypic variance due to environmental effects. To be able to compare the variation among traits, phenotypic (PCV) and genotypic (GCV) coefficients of variation were computed according to the method suggested by [20]:

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

Vp, Vg and X are the phenotypic variance, genotypic variance and general mean of each seed trait, respectively.

Broad sense heritability (h^2b) was calculated according to [21] as the ratio of the genotypic variance (Vg) to the phenotypic variance (Vp).

$$h^2b = Vg/Vp$$

Expected Genetic Advance (EGA) was calculated as described by [19]. GA as percent of the mean assuming selection of the superior 5% of the genotypes was estimated in accordance with [19] as:

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

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

h^2b is heritability in broad sense,
X is the grand mean for each seed trait.

3. RESULTS AND DISCUSSION

3.1 Bambara Seed Moisture Content and Physical Traits

Moisture content of the seeds was significantly different ($p<0.05$) for all accessions. The moisture content ranged from 8.11% to 13.12% (Table 2). NOR4 seeds had the lowest moisture contents while NOR8 seeds had the highest moisture contents (Table 2). However the conditions of seed storage were homogenous for all accessions in the laboratory with a temperature of 25 - 27℃. Moisture contents of NOR2 and NOR2Y were similar with those recommended for seed storage (12%) of Bambara groundnut by [12].

Significant ($P < 0.001$) differences existed among accessions length, width and thickness (Table 2). The length ranged from 6-16 mm with a mean of 10.81 mm, the width ranged from 6-13.5 mm with a mean of 9.28 mm and the thickness ranged from 6-12.5 mm with a mean of 9.19 mm (Table 2). Majority of the seeds for NOR2, NOR4, NOR5, NOR6, NOR9 and NOR2Y were longer, wider and thicker. The seeds of these accessions were also large in seed size while those of NOR7, NOR8, NOR1, NOR1Y and MBYO3 were shorter. The results of the length, width, and thickness were similar to those obtained by [15]. Our accessions had seeds with high morphometric trait diversity compared to those of [14]. NOR7 and NOR8 had small seed sizes with high moisture contents contrary to NOR2Y and NOR6 which had large seed sizes with high moisture contents.

In reference to [5] who reported similar seed sizes and colors on the parents, after three generations back of self-pollination, we could suppose that Bambara seed sizes and colors are under genetic control. So, these characters may enable the first screening of our accessions.

3.2 Seed Geometric Characteristics

All accessions were significantly different ($p < 0.05$) for weight. The 100-seed weight ranged from 55.5 g (NOR8) to 95.2 g (NOR2Y). These results are in agreement with that (50–80 g) obtained by [15]. This parameter is often useful in agronomy.

Geometric mean diameter (Dg) ranged from 9.12 (NOR7) to 10.75 mm (NOR2Y) (Table 3).

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	10,7cd	8.5	13.5	9,01de	7	11.5	8,64^{e}	6.5	11	11.55±0.07
NOR2	11,08bc	9.5	13	9,32cd	8	11	9,44c	8	11	12.63±0.12
NOR4	11,42ab	8.5	15	9,24cd	6.5	14	9,78b	7.5	12.5	8.18±0.09
NOR5	11,55a	8	15.5	9,05de	7	11	9,47c	7	11	10.84±0.08
NOR6	10,82cd	7	13	9,4c	7.5	11.5	10,3a	8	12.5	11.91±0.03
NOR7	10,53d	7	13	8,73^{e}	6	11	8,26fg	6	10.5	11.42±0.08
NOR8	9,68^{e}	6.5	12.5	8,79^{e}	6	10.5	8,12g	6	10	13.03±0.07
NOR9	11,78a	8	15	9,79b	6	12	9,28c	6.5	12	10.71±0.04
NOR1Y	10,51d	6.5	15	8,76^{e}	6	11	8,49ef	6	10	10.58±0.05
NOR2Y	11,75a	8	14.5	10,46a	8	13.5	10,13a	8	12	12.07±0.10
MBYO3	9,88^{e}	6	14	9,28cd	7	12	8,93d	6	11	9.02±0.07
MBYO10	10,03^{e}	7	16	9,54bc	7	11.5	9,52bc	7	11.5	10.05±0.07
Mean	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

SD = Standard deviation; CV= Coefficient of Variation. Different superscripts denote significant difference (p < 0.05) in the column

Table 3. Geometric characteristics of Bambara groundnut seeds

Seed sources	100-seed weight (g)	Geometric mean diameter (mm)	Sphericity	Volume(mm^3)	Surface (mm^2)	Aspect ratio (%)
NOR1	64,94	9,41de	0,88def	384,30f	2305,79f	0,85de
NOR2	77,55	9,91bc	0,90d	452,78cde	2716,66cde	0,84de
NOR4	72,54	10,09b	0,89de	475,86cd	2855,15cd	0,81ef
NOR5	69,42	9,96bc	0,87ef	442,27de	2653,59de	0,79f
NOR6	81,05	10,14b	0,94b	522,02b	3132,12b	0,87cd
NOR7	58,37	9,12^{e}	0,87ef	343,16g	2058,96g	0,83^{e}
NOR8	55,5	8,83f	0,92c	336,95g	2021,73g	0,92b
NOR9	75,1	10,22b	0,87ef	487,52bc	2925,10bc	0,83^{e}
NOR1Y	70,74	9,20^{e}	0,88def	366,68fg	2200,09fg	0,85de
NOR2Y	95,2	10,75a	0,92c	598,99a	3593,95a	0,89bc
MBYO3	65,84	9,32^{e}	0,96ab	424,23^{e}	2545,39^{e}	0,96a
MBYO10	72,98	9,67cd	0,97a	478,60cd	2871,62cd	0,97a
Mean	71,67±0,54	9,71±1,14	90,52±0,07	442,77±153,42	2656,67±920,55	86,82±0,11
P	< 0.05	< 0.05	< 0.001	< 0.001	< 0.001	< 0.001

Mean ± standard deviation. Different superscripts denote significant difference in the same column

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 seeds in this study (9.71±1.14 mm) was higher than that of [22] and this was in corroboration with those obtained by [15].

Accessions differed significantly (p < 0.001) for seed volume and ranged from 384 (NOR1) to 599 mm^3 (NOR2Y). The seeds differed significantly (P < 0.001) for surface area which ranged from 2021 (NOR8) to 3593 mm^2 (NOR2Y). NOR2Y seeds are significantly higher in volume and surface area as compared to the others. The determination of geometric diameter could be useful in the theoretical estimation of seed volume and seed surface [23,1].

Significant differences (P < 0.001) existed among the accessions for sphericity (Table 3). Sphericity of Bambara groundnut seeds ranged from 87% (NOR5, NOR7 and NOR9) to 97% (MBYO10). [24] indicated that in the jackbean, when sphericity is superior to 70%, shape of the seed was closed to a sphere. Thus, all our accessions seeds have a sphere shape. This high sphericity enables Bambara groundnut 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 [15,23].

The seeds differed significantly for aspect ratio ($P < 0.001$). The aspect ratio of seeds ranged from 79% (NOR5) to 97% (MBYO10). With an aspect ratio superior to 70%, the seed is more likely to roll than slide. A similar trend had been reported for Jackbean [24] and Bambara groundnut [14]. Low aspect ratio indicates the tendency to be oblong in shape [24]. Small sized seeds had higher sphericity and aspect ratio than large seeds. Similar results had been reported by [14,25]. However, large seeds had higher geometric mean diameters, volume and surface area while small seeds had lower ones. A similar results had been reported by [14].

3.3 Genetic Variation

Phenotypic Coefficient of Variability (PCV) was high (7.73%) in seed thickness and low (5.30%) in seed width. Genotypic Coefficient of Variability (GCV) was 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 suggests 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. However, [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] suggested that heritability and 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 for length, width and thickness in the accessions recorded in the test materials could be further exploited through improvement and selection programs.

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 longest seed did not have either the largest width or thickness. A similar trend was reported by [14,23,27] for Bambara groundnut seeds, Locust bean and Guna respectively.

Seed width and thickness were significantly and positively 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.

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	0.52	0.50	6.69	6.56	0.96	16.72
Width	0.24	0.23	5.30	5.17	0.95	10.41
Thickness	0.50	0.49	7.73	7.66	0.98	15.65
Sphericity	0.001	0.001	4.07	4.42	0.96	8.12
Aspect ratio	0.003	0.003	6.55	6.44	0.96	13.05

Table 5. Correlation coefficients among the geometric parameters in Bambara groundnut seeds

	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 $P < 0.01$*

A positive correlation between seed traits would permit selection and improvement of these traits simultaneously. Similar results had been reported by [28]. These results indicated that genes were probably linked or have a pleiotropic effect [28].

4. CONCLUSION

High variation was recorded in shape and size of Bambara groundnut seeds. Longer seeds were not either the widest or the thickest. MBYO10 is more spherical and NOR2Y occupies more volume and area when compared to the other accessions. Bambara groundnut seeds in our study are highly spherical with high aspect ratio and would roll rather than slide. Smaller seeds had higher sphericity and aspect ratio than large seeds. Sphericity seems 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. Thus results indicated that individual plant selection based on seed length, width and thickness could be effectively utilized for the extraction of superior genotypes. There was a substantial amount of variation found in the selected material, and this indicates that there is a great potential for crop improvement. Morpho-metric diversity in this specie 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. These traits seem to be controlled by additive and non-additive genes. Then, recurrent selection might be a useful breeding strategy for these traits.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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