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Assessment of Twenty Bambara Groundnut (*Vigna subterranea* (L.) Verdcourt) Landraces using Quantitative Morphological Traits

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Abstract *Vigna subterranea* (L) verdcourt is grown for food and income in the savannah zone of Cameroon. However, few studies have been conducted to investigate the variability of the existing landraces. The study aimed to characterize farmer's landraces using quantitative morphological descriptors for further selections in the breeding program. Twenty morphotypes collected from farmers, were planted in pots in the greenhouse during the off season at the Regional Research Centre of Maroua. The experiment was conducted in a Randomised Complete Block Design with four replications using the watering facilities at the station. Ten variables were subjected to analysis of variance on Genstat 12th edition. Multivariate analysis of these variables was performed on XLSTAT version 2013 and interrelationships were established among the descriptors. A significant variability was revealed among the morphotypes. Moreover, it appeared that the landraces could be group into five distinctive classes. In addition the earliness of flowering, number of pod per plant, pod and grain yield per plant were the most discriminant factors, suggesting their consideration when selecting for agronomic superior traits. Significant correlations were shown between number of stems 4WAS and 9WAS (r = 0.56); grain width and length (r = 0.79); pod yield and number per plant (r = 0.90); pod yield and grain yield (r = 0.97) and between grain yield and number of pod per plant (r = 0.91) highlighting the importance of these parameters in selection for the improvement of this crop.

Keywords Bambara Groundnut, Legumes, Morphological Characters, Northern Cameroon

1. Introduction

In the tropical zones of Africa, cereals and legumes are the main sources of food and incomes for farmers[1]. Among the cultivated legumes, Bambara groundnut (*Vigna subterranea* (L) verdcourt) is one of the most important food crops after groundnut and cowpea[2]. It is widely cultivated in the West and Central Africa and the annual production is estimated at 140,198 tonnes. With an annual production of 30,000 tonnes, Cameroon is the second producer of this crop in Africa after Burkina Faso, contributing for more than 21 % of the total production of the continent[3]. Bambara groundnut has the ability to adapt to divers and marginal agro-climatic conditions ([4],[5]) as it is the case of the northern Cameroon. Its seeds are highly nutritious containing 65% of carbohydrates and 18% of proteins[6]. Chemical analyses showed that they contain 32.72% of total essential amino acids and 66.10% of total non-essential amino acids ([7];[8];[9]). Lysine is the major essential amino acid and represents 10.3% of the total essential amino acid. The fodders of Bambara groundnut are used to feed animals[6]. In some communities like Ibos in Nigeria, this plant is used for medicinal purpose, leaves serve as anti-vomiting when eaten in row ([5];[8]). As a legume crop, Bambara groundnut has the ability for nitrogen fixation through its nodules thus contributes to improve soil fertility. Its grains are included in the daily diet to compensate the lack of proteins in the food as it occurs frequently in most populations under the tropics[10]. This cop is mostly grown by female[11] on a small scale, in pure culture without improved techniques. Despite the numerous advantages provided by Bambara groundnut, limited studies have been conducted on this edible crop in Cameron compared to the others such as sorghum, groundnut and cowpea. The similar observations have been made by[12] in the case of Burkina Faso. The production of Bambara groundnut is mainly limited by the lack of improved cultural techniques and the impact of pest insects and diseases ([13];[14]). The use of potential genetic resource

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for plant breeding to control these constraints could help to increase the production and the productivity of this crop[15]. The objective of the present study was to characterize the Bambara groundnut morphotypes found in the local markets of the north Cameroon in the perspective of their conservation and integration into the breeding program. This would enhance their promotion and valorisation in the farming systems, contributing finally to increase food security.

2. Material and Methods

2.1. Experimental Site

The experiment was conducted during the dry season from December 2012 to March 2013 in the greenhouse of the Regional Agricultural Research Centre of Maroua located at Djarengol Station in the savannah zone. The station is at 900 m altitude and 10°59' N; 14°30' E. During the experimental period the temperatures varied from 17 ℃ in November to 42 ℃ in April; with a mean of 34 ℃[16].

2.2. Biological Material

Thirty five Bambara groundnut samples were collected from four most popular markets in the northern part of Cameroon in November 2012 at the end of the raining sea-son campaign, during the harvesting period. Based on the similarities observed in the form, colour, size and texture of the grain, the number was reduced to twenty morphotypes. The characteristics of the samples are presented in table 1.

2.3. Experimental Design

The experiment was conducted in a randomized complete blocks design (RCBD) with four replications where the treatments were the twenty Bambara groundnut landraces and each experimental unit was consisted of a pot of 24 cm in diameter over 23 cm height containing 10 kg of sandy-clay soil[16]. The soil was filled in pots after having covered the small holes made at their bottom to avoid flooding with paper toile. In total, the experiment was consisted of 80 experimental units. Before sowing, the pots were regularly watered as needed using water from a tap in greenhouse. Two seeds were sowed per pods and thinning was performed later to allow 1 plant per pot three weeks after sowing. For the duration of the experiment, the plants were watered every three days during which each pot was filled at its maximum capacity.

Table 1. Characteristics and collection area of the samples

Landraces	Market area	Descriptions		
		Seeds coat colour	Seed eye colour	Seed shape
VZ101	*Maroua*	Light brown with black spots	White to light brown	Oval
VZ102	*Maroua*	Creamy with few light spotted brown	Black	Oval
VZ103	*Maroua*	Creamy with brown stripes	Dark brown surrounded by grey	Round
VZ104	*Maroua*	Mottled	Purple	Oval
VZ105	*Maroua*	Cream	Brown surrounded by sky blue	Oval
VZ106	*Maroua*	Black	Black	Oval
VZ107	*Maroua*	Light brown	Light brown	Oval
VZ108	*Salak*	Purple with black spots	Purple	Oval
VZ109	*Salak*	Creamy spotted with grey	Brown surrounded by grey	Round
VZ110	*Salak*	Black	Black	Oval
VZ111	*Salak*	Creamy spotted with light grey	Brown surrounded by light grey	Round
VZ112	*Salak*	Cream	Dark grey surrounded by sky-blue	Oval
VZ113	*Meskine*	Cream with brown stripes	Black	Oval
VZ114	*Meskine*	Purple spotted with white	Dark brown surrounded by grey	Round
VZ115	*Meskine*	Purple	Dark purple	Oval
VZ116	*Meskine*	Cream	light blue with grey spots	Oval
VZ117	*Meskine*	Cream	Purple	Oval
VZ118	*Gazawa*	Cream	white	Round
VZ119	*Gazawa*	Cream with brown spots	Sky blue	Round
VZ120	*Gazawa*	Black	Black	Oval

2.4. Data Collection

The data were collected per pot and were based on the following 10 parameters (i) number of days for emergence (DEM) from sowing to the apparition of the plant at the soil surface; (ii) number of days to flowering (DFL) from the sowing day, (iii) Leaf area of plant (LAR), (iv) number of Stems 4 weeks after sowing (STEM 1), (v) number of Stems 9 weeks after sowing (STEM 2), (vi) number of pod per plant at the harvesting (PPL), (vii) pod yield (PYD), (viii) grain yield (GYP), (ix) grain width (GW) and (x) grain length (GL). Harvesting was done manually by removing completely the soil from the pots and destroying after wetting to allow pods collection. Then, the pods were hand threshed to remove the seeds. The pods and seeds were weighted per pods and the obtained values were used to determine the mean yield per Bambara groundnut genotype tested. Yield calculation was performed as follow:

$$Y = \frac{W}{NP}$$

Y= Pod or Grain yield (g/plant)
W = Total Pod or Grain weight (g)
NP = Total Number of plant harvested

2.5. Data Analysis

A general analysis of variance (ANOVA) for the recorded data was performed using GenStat statistical package 12th edition to establish differences among the varieties with regard to the quantitative estimates of the morphological traits. Multivariate analysis was made to the component principal analysis using XLSTAT version 2013 based on the means of these quantitative variables to establish the contribution of different traits in the explanation of the total variation. Then, hierarchical cluster analysis was performed to construct a dendogram grouping the twenty varieties into distinctive classes according to the similarly observed ([17];[18]). Finally, computation of Pearson Correlation was performed to establish interrelationships among the descriptors.

3. Results and Discussion

The samples were predominantly creamy for seed coat colour, oval shape seeds with various seed eye colour. Descriptive values (maximum, minimum, mean values standard deviation and coefficient of variation) of the estimated quantitative parameters are shown in table 2. The mean value for the number of days to emergence (9.44) is within the range (7 to 15 days after sowing) used by[19] in the description of the growth and development habit of Bambara groundnut. In addition, the results confirm those from[20] who mentioned the intervals of 6-15 DAS. However, these findings are contrary to those of[21] who found 14-24 DAS for emergence of Bambara groundnut. Furthermore the mean value of close to 44 DAS noted for days to flowering deviated the range of 30 to 35 days reported by this author.

The most varying traits noted were consisted of the pod yield per plant, grain yield per plant and number of pod per plant which are the component of yield. Nevertheless, small variation was observed for the number of days to emergence, number of days to flowering, number of stems 4 weeks after sowing, number of stems 9 weeks after sowing, leaf area, grain length and the grain width. These results infer that there is some level of variability among the Bambara groundnut sampled in term of their yield potential, corroborating with the finding of[22]. Furthermore, the analysis of variance indicated that there were highly significant differences between the genotypes for grain length and width ($P<0.01$). In the meantime significant differences were observed for the number of stems 4 weeks after sowing suggesting variability in growth rate among the genotypes. The same results appeared for number of pod per plant, pod yield per plant and grain yield per plant ($P<0.05$). These variables represent 60% of the total trait considered in this study and the findings deduced the importance of these descriptors for the need of differentiations among Bambara groundnut landraces. The observations are consistent with the past results from[23]. However, no differences were revealed for the following traits: number of days to emergence, number of days to flowering, leaf area and number of stem at 9 weeks after sowing. The principal component analysis grouped the ten variables into various components with the first four components explaining close to 80% of the total variation observed (Table 3).

Table 2. Descriptive Statistics Summary of the Morphological Traits measured

Characters	Observations	Minimum	Maximum	Mean Values	SD	CV (%)
Number of days to emerge	20	9.00	10.25	9.44ns	0.36	3.83
Number of days to flowering	20	42.00	45.33	43.77ns	0.92	2.11
Number of Stems 4WAS	20	12.25	17.25	14.64*	1.44	9.83
Number of Stems 9WAS	20	16.00	24.45	20.59ns	2.36	11.46
Leaf Area (cm^2)	20	10.25	17.40	13.96ns	1.76	12.62
Grain Length (cm)	20	9.36	15.62	12.13**	1.60	13.18
Grain Width (cm)	20	8.33	12.03	10.34**	1.02	9.90
Number of Pod Per Plant	20	2.75	11.67	5.15*	2.23	43.37
Pod Yield (g/plant)	20	1.29	7.00	2.98*	1.34	46.56
Grain Yield (g/plant)	20	0.86	4.24	1.80*	0.84	45.00

SD: Standard deviation; CV: Coefficient of variation; ns: non-significant differences were observed among the means;
*: significant differences were observed among the mean ($P<0.05$); **: highly significant differences were observed among the means ($P<0.01$)

Table 3. Principal Component Analysis of the Genotypes showing the first four Components

	Eigenvectors			
	PC1	PC2	PC3	PC4
Eigenvalue	2.998	2.138	1.692	1.126
Variability (%)	29.980	21.376	16.925	11.264
Cumulative %	29.980	51.356	68.280	79.544

Table 4. Eigen Vectors and Values for the first four Principal Component axes

Characters	Eigenvectors			
	PC1	PC2	PC3	PC4
Number of days to emerge	0.105	0.318	-0.417	0.284
Number of days to flowering	-0.069	-0.269	0.037	0.463
Number of Petioles 4WAS	-0.052	0.424	-0.376	-0.142
Number of Petioles 9WAS	-0.140	0.461	-0.210	-0.378
Leaf Area (cm^2)	0.126	0.323	-0.145	0.697
Grain Length (cm)	0.076	0.417	0.536	-0.050
Grain Width (cm)	0.113	0.382	0.567	0.141
Number of Pod Per Plant	0.544	-0.065	-0.035	-0.124
Pod Yield (g/plant)	0.562	-0.078	-0.046	-0.026
Grain Yield (g/plant)	0.561	-0.003	-0.079	-0.135

Based on the results from tables 3 and 4, it appeared that the principal component 1 (CP1) accounted for close to 30% of the total variation and the characters responsible for genotypes separation along this axis were the number of pods per plant, pod yield per plant and grain yield per plant which are related to the agronomic traits. Thus PC1 was revealed as the most important features in the selection for yield component in this study ([22];[24]). This result is consistent with[23] stating that the most important components of yield are the number of pods and seeds per plant. The second principal component (PC2) associated with the number of stems at 4WAS and number of stems at 9WAS accounting for 21.38% of the total variation. Principal Component 3 (PC3) accounted for close to 17% of the total variation and displayed differences based on the number of days for emergence, the grain length and the grain width. The last principal component (PC4) accounted for 11.26% of the total variation and consisted mostly of the number of days to flowering and the leaf area which are basically related to the phonological traits of the plants.

The Hierarchical Cluster Analysis made of the PCA showed from the variance decomposition for optimum classification that, the twenty landraces are classified into five main classes (Figure 1) displaying 66.20% and 33.80% level of similarity within class and between classes respectively. The predominant classes noted are C1 and C2 with five members each followed by C3 and C4 which was constituted by four members. Finally, C5 was shown with the fewer members of two. The characteristics of the different classes are summarized in table 5.

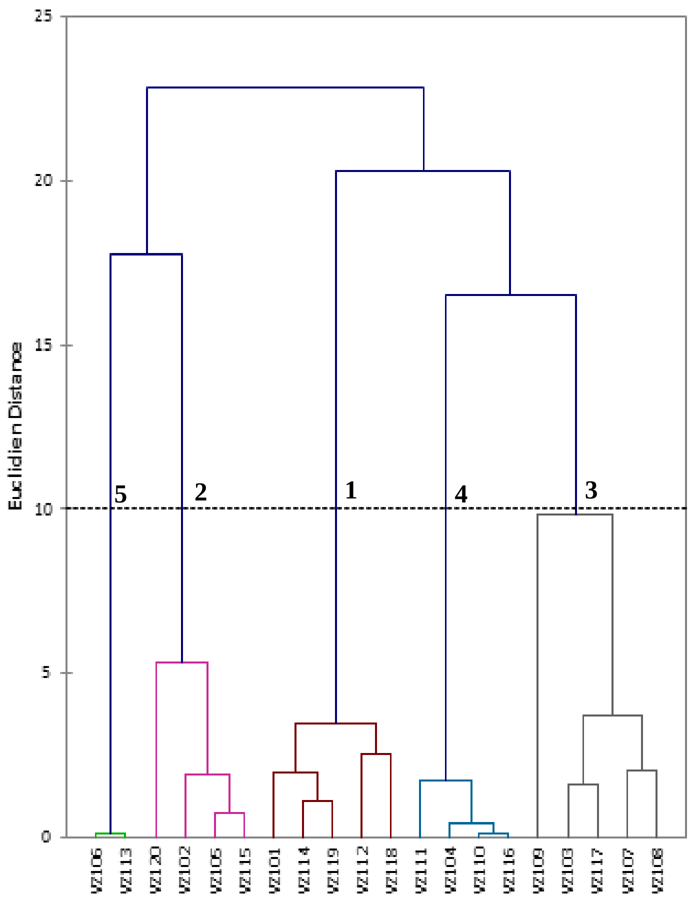

Figure 1. Dendrogram showing the five distinctive classes constructed in the base of the quantitative morphological characters estimated

Table 5. Means values of the characters per class

Characters	Classes				
	C1	C2	C3	C4	C5
DEM	9.450	9.542	9.167	9.500	9.375
DFL	43.667	43.694	43.683	44.000	43.875
STEM 1	15.033	14.431	13.917	14.688	15.000
STEM 2	19.310	21.550	20.617	20.913	20.225
LAR	14.465	14.097	13.377	13.514	14.054
GL	12.180	12.151	12.226	11.764	12.491
GW	10.785	10.326	10.024	10.165	10.095
PPL	5.767	4.486	5.444	4.875	5.667
PYD	3.348	2.598	3.272	2.784	3.196
GYD	2.023	1.511	2.081	1.643	1.999

The results from the above table indicate the mean performances of the selections according to each class. Class 1 represented by VZ101, VZ112, VZ114, VZ118 and VZ119 are characterized by large leaves, early flowering and rapid growth and vegetative development; they seemed to be the most yielding genotypes. They showed the best yield components values with large seeds and mostly creamy in color corroborating with[25]. This author reported that, creamy colored are preferred for home consumption as they are claimed to be tastier[26], consequently such seeds have been favored by farmers when selecting for seeds size. Therefore, seed sizes have been improved over time of cultivation. The lower stems number (19.31) was observed in this group. This could imply that these genotypes have strategies to limit vegetative growth and compensate it by allocating energy to yield production. Also they could have taken advantages of their large leaves to perform better photosynthesis in favor of pod and grain development resulting from more assimilates reserve generated during this physiological activity of the plants. Moreover[20] mentioned that the timing for flowering period is a determinant factor for the final yield. Thus early flowering may have contributed positively to the best yield of the group. Selection for breeding program of Bambara groundnut could explore the accessions from this class. In addition, it has been reported that flowering is indeterminate in Bambara groundnut. However, early flowering has been noted as a good agronomic attribute of crops for early maturity, uniformity of yield and crop production in general[27]. Thus lines that flower early should be considered in the production of Bambara groundnut[28]. Class 2 is formed by VZ102, VZ105, VZ115 and VZ120 characterized by the following traits: the smallest pod and grain yield. In contrast they excelled in vegetative growth with highest stem number at the end of the Cycle. It was also observed that their seeds took more time (9.54 DAS) to emerge compare to the rest of the genotypes. In this group priori seemed to be given to the vegetative development with the detriment of pod and grain production. Class 3 is illustrated by VZ103, VZ107, VZ108, VZ109 and VZ117. These accessions were distinguished by the following characters: earliness in seeds emergence (9.17 DAS), small leaves size, consequently the seeds were small and they bear few pod per plant. The findings contrast the results showed in[20] that pod formation efficiency and number of pod with 2 seeds were obtained from genotypes having reduced leaves area. Class 4 comprised VZ104, VZ110, VZ111 and VZ116. They were individuals exhibiting long cycle by delaying to start flowering and their seeds are the shortest in length. The last class 5, whose members were VZ106 and VZ113 seemed to have long seeds as their most distinctive character.

Correlation analysis between characters has been described to be a great value in determining the most efficient procedures for selection of superior agronomic traits in crops ([29];[30]). Pearson's correlation coefficients in table 6 showed different types of interrelationships between the variables measured and the grain yield. It indicated that there were highly significant positive correlations between grain yield and the yield components principally pod yield (r = 0.97) and number of pods per plant (r = 0.91) suggesting that, these parameters could be used for grain yield prediction. The observations confirmed the study conducted by[30] who reported a significant and positive correlation between grain weight per plant and the number pod per plant. In the meantime highly significant positive correlations were found between number of pod per plant and pod yield (r = 0.90) and between grain width and grain length (r = 0.79) likewise to the study from ([30];[31]). A significant and positive correlation appeared between the number of stems at 4 weeks after sowing and number stems at the end of the crop cycle 9 weeks after sowing (r = 0.56) suggesting the possibility of efficient canopy comparison of the morphotypes at mi-cycle of the crop, 4 weeks after sowing. Furthermore, the study revealed positive correlation between the leaf area and the number of days to emergence (r = 0.42).

Table 6. Interrelationship analysis among the characters (Pearson correlation matrix)

Variables	DEM	DFL	STEM 1	STEM 2	LAR	GL	GW	PPL	PYD	GYD
DEM	1									
DFL	-0.125	1								
STEM 1	0.253	-0.176	1							
STEM 2	0.282	-0.174	**0.563**	1						
LAR	0.427	-0.023	0.259	-0.009	1					
GL	0.047	-0.160	-0.049	0.255	0.069	1				
GW	-0.128	-0.111	0.048	0.041	0.275	**0.791**	1			
PPL	0.165	-0.030	-0.129	-0.154	0.020	0.068	0.079	1		
PYD	0.074	-0.039	-0.067	-0.263	0.167	0.004	0.090	**0.909**	1	
GYD	0.141	-0.164	0.018	-0.145	0.135	0.056	0.092	**0.912**	**0.974**	1

Values in bold are different from 0 with a significance level alpha=0.05

4. Conclusions

Based on the phenological traits, the results revealed that the genotypes were divers but could be grouped into five main classes according to the similarity factors.

The earliness of flowering, number of pod per plant, pod and grain yield per plant were the most discriminant factors, suggesting their consideration when selecting for agronomic superior traits. Significant correlations were shown between number of stems 4WAS and 9WAS (r = 0.56); grain width and length (r = 0.79); pod yield and number per plant (r = 0.90); pod yield and grain yield (r = 0.97) and between grain yield and number of pod per plant (r = 0.91) highlighting the importance of these parameters in selection for the improvement of this crop. The quantitative morphological descriptors provided useful information to characterize the Bambara groundnut landraces for their integration in the breeding program. However, further study should be conduct in field conditions to complete these findings.

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