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Diversity and phenotypic characterization of Bambara groundnut *Vigna subterranea* (L. Verdc.) in Benin

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Abstract

Objective: Bambara groundnut *Vigna subterranea* (L. Verdcourt) is cultivated for its high nutritional value. However, improving the marketability of this crop in local markets is desirable. In the present research, we described the diversity and characteristics of the phenotypes of Bambara groundnut from five Agro-Ecological Zones (AEZs) of Benin. Methodology and Results: A total of 514 farmers were surveyed, and 188 samples of Bambara groundnut were collected from them, reproduced at research station and analyzed in laboratory. Data related to colors and different patterns of integuments were collected. The results showed that, of all the variables measured, 14 allowed to discriminate ($p < 0.05$) at best five groups identified. Among all the phenotypes observed, the cream-colored integument morphotype was the most dominant. Conclusions and application of findings: The present study revealed a great morphological variability of Bambara groundnut collected from the AEZs of Benin. The Bambara groundnut groups identified exhibit different phenotypic characteristics. In addition, the cream-colored of Bambara groundnut seeds were abundant in all the AEZs. These results suggest the use of the characters considered in the present study, as basic criteria for phenotypes differentiation and study of variability between Bambara groundnut phenotypes in Benin.

Keywords: Nutrition, Genetic diversity, Phenotype, *Vigna subterranean*, Benin

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1. Introduction

In many Sub-Saharan African (SSA) countries, agriculture is a major sector of the economy whose intensification and diversification remain a priority (Ebomosele et al., 2008). As a solution to undernutrition, a subsequent

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supply of protein from plant sources is commonly proposed. In fact, this category of proteins is more accessible with a relatively low cost compared to animal proteins. However, in SSA where the nutritional problems are acute, Bambara groundnut *Vigna subterranea* (L. Verdc.), is widely used by indigenous people. This grain legume is an important source of protein, carbohydrate, fiber and essential amino acids (Minka and Bruneteau, 2000; and Amartuifio et al., 2006). Thus, the seed which has an important nutritional value is quite an excellent complement to the cereals and tubers which are the basis of the diet in this region.

In Benin, Bambara groundnut plays a vital role in the diet of both rural and urban populations. On the other hand, it plays an important role in increasing the bioavailability of phosphorus even grown in fertile soils because of its nitrogen-fixing capacity (Andriamananjara, 2011). Unfortunately, this crop is neglected by national research systems despite being a culture of the future, due to its nutritional and economic importance (Dansy et al., 2012). As a result, the assessment of its production status is quite challenging and therefore is not likely to favor the implementation of supportive actions.

The local cultivars used in traditional cropping systems exhibit after harvest, an important phenotypic diversity of the seeds whose integuments are brightly colored. To improve the market value of the product which is generally found in local markets, Bambara groundnut seeds with colored integuments are sometimes sorted by traders who provide them to resource-limited households or processors at a lower cost. Thus, this notorious diversity, which may constitute an asset for the resilience mechanisms of cultivated species in the current context of climate change, is untapped and threatened. Indeed, information on the diversity of these cultivars and the real potential of the different phenotypes are lacking. In most of the production areas, vernacular names are attributed to local cultivars which form very heterogeneous groups. These groups vary greatly from one location to another, from one ethnic area to another or even between locations of the same ethnic area (Mekbib, 2007; Tamiru et al., 2008; Otoo et al., 2009; and Gbaguidi et al., 2016). As a result, the valorization of this potential diversity is challenging due to the difficulty in its capitalization. In general, phenotypic diversity underlies successful genotypes, and can be a source of raw materials in the selection of interesting varieties (Nkogolo et al., 2008). Under such circumstances, it is an important need to implement actions to clarify, document and even enhance the diversity of this important grain legume. Such actions are necessary to lay the ground for improving the productivity of the local cropping systems of this speculation and to ensure the sustainable use of this phylogenetic resource. In the present study, we described the diversity and characterized the phenotypes of Bambara groundnut seeds from different Agro-Ecological Zones (AEZs) of Benin.

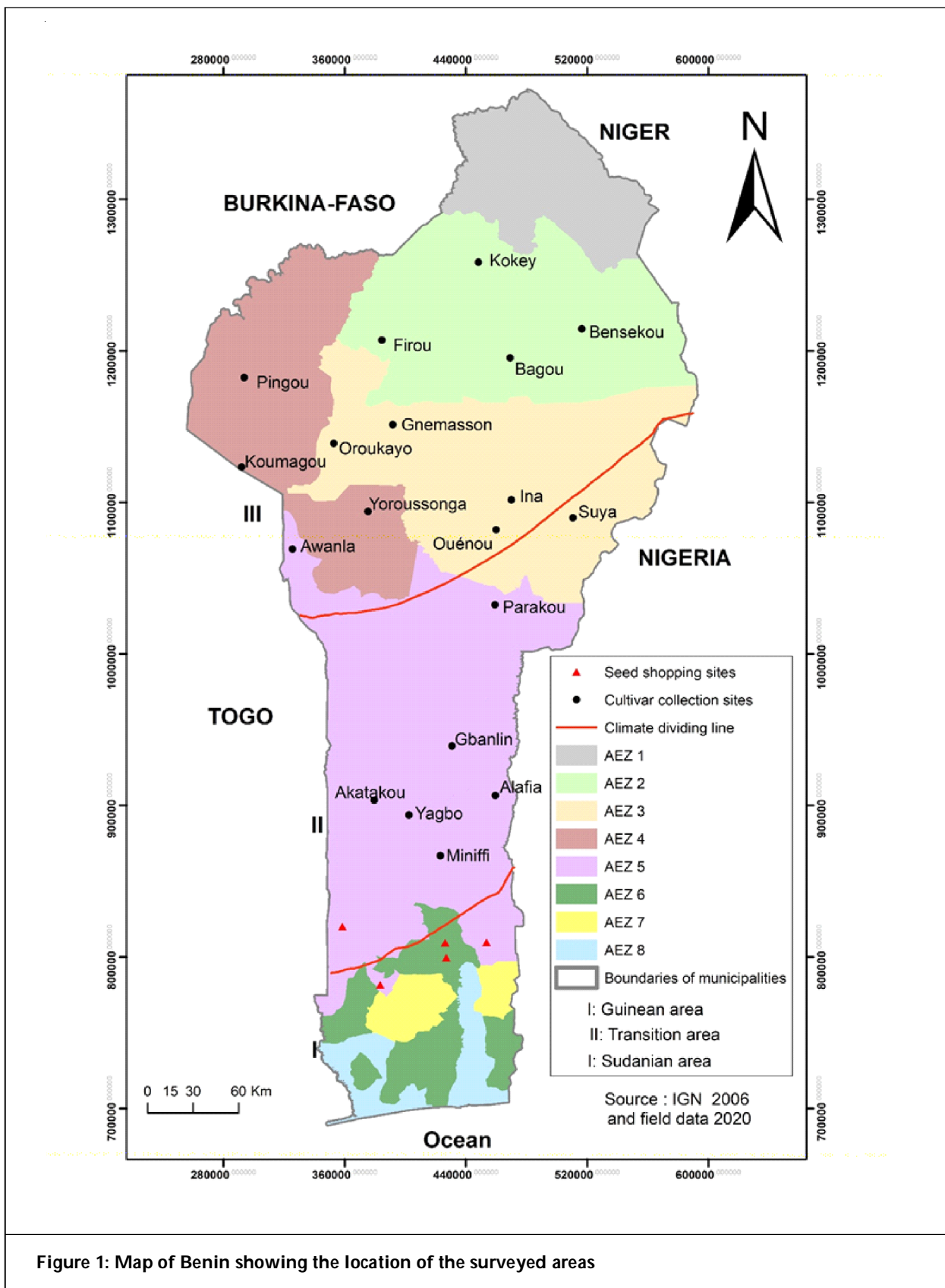
2. Materials and methods

2.1. Study area

The present study was conducted in five AEZs of Benin: AEZ II: Cotton region of North Benin, AEZ III: Food crop region of South Borgou, AEZ IV: West Zone of Atacora, AEZ V: Cotton region of the Centre, and AEZ 6: Zone dominated by laterite soils. The full description of these AEZs was provided by Akossou et al. (2016). The AEZs II, III and IV are characterized by dry tropical climate and bimodal rainfall pattern with an average rainfall of less than 900 mm per year and temperature ranging from 18°C to 38°C. The soils are ferruginous soils on crystalline basement and very fertile alluvial soils from the Niger River. The vegetation is characterized by a shrubby to thorny savannah. The AEZs V and VI are characterized by subequatorial climate with two rainy seasons and annual rainfalls ranging from 900 to 1200 mm and 1100 to 1400 mm in the West and East part respectively, and annual temperature of 26.5°C (Gbemavo et al., 2014). The soils in the both zones are ferrallitic soils on the continental terminal, deep and easy to work on (Gnanglè et al., 2011).

2.2. Sampling method and data collection

Bambara groundnut seeds sampling was performed in two phases. A pool of 60 homogeneous Bambara groundnut seeds was obtained from a pre-collection in markets and commercial centers conducted by the research teams of the National Institute of Agricultural Research of Benin (INRAB) in 2019, and from the study of Gbaguidi et al. (2016) study. Then, a second collection was carried out in June 2020 from Bambara groundnut producers in five AEZs. Furthermore, Bambara groundnut seed samples were purchased from major local and regional markets located within surveyed AEZs which were referred by local actors involved in the sector. In overall, 200 to 500 g of locally available Bambara groundnut seeds were collected and stored in plastic containers previously cleaned and oven-dried. The provenances of the local Bambara groundnut seeds collected were georeferenced using Global Positioning System (GPS).



2.3. Discrimination of Bambara groundnut cultivars

Bambara groundnut seeds collected were sorted, cultivated in field, described and discriminated into phenotypes. The combinations of seed coat/integument colors were identified using the Munsell Codes. The polymorphism of seeds (seed eyes and shapes) was described by referring to IPGRI *et al.* (2000).

3. Chemical analyses

Bambara groundnut seeds cultivated in research station were harvested, sampled and dried in a ventilated oven at 65 °C for 72 h to constant weight. Thereafter, dried sample was ground using a flail mill. The powder obtained, was subjected to chemical analyzes at the “Laboratoire d’Appui à l’Amélioration de la Santé des Sols, de la qualité des Eaux et de la sauvegarde de l’Environnement (2A2S2E)” of the National Institute of Agricultural Research of Benin (INRAB) which was previously called “Laboratoire des Sciences du Sol, Eau et Environnement (LSSEE)”. The different parameters measured were the contents of nitrogen and crude protein, total ash, potassium, calcium, magnesium, iron, zinc, copper and manganese.

Nitrogen was determined by the KJELDAHL method from the micro-distillation of the mineralizate of the sample digested with sulfuric acid in the presence of a selenium-based catalyst. This micro-distillation was performed by steam stripping in the presence of a normal soda solution. The distillate collected in boric acid was titrated with sulfuric acid in the presence of a methyl red indicator. The nitrogen content obtained was then multiplied by a factor of 6.25 to obtain the protein content of the sample.

For mineral elements, the powder obtained after grinding was incinerated in a muffle furnace at 450 °C for 24 h. The ash content was obtained from the mass of ash obtained after incineration divided by the mass of sample incinerated. The ash was dissolved in 5 ml of hydrochloric acid, 6N which was evaporated to dryness on a hotplate at 125 °C. The replicate obtained was dissolved again and added with very dilute nitric acid. The resulting solution was used to measure metals such as iron, zinc, potassium, and copper by Atomic Absorption Spectrophotometry (AAS).

Phosphorus was determined by colorimetry after development of a colored complex using ammonium molybdate added with acidified stannous chloride.

4. Statistical analyses

The description of the distribution of the phenotypes according to the AEZs was carried through Detrended correspondence analysis using the *decorana* function of the *vegan* package. The hierarchical classification on multiple factorial analysis was performed on the agro-morphological and nutritional traits of the varieties using the *FactoMineR* (Le et al., 2008) and *factoextra* (Kassambara and Mundt, 2020) libraries to constitute homogeneous phenotype groups. The difference among identified groups was assessed by measuring the difference between class values and global values. These statistics were converted into a criterion called “*test value*” to select variables, and thus the most characteristic ones (Morineau, 1984; and Husson et al., 2010). The most characteristic variables of a class were those associated with test values greater in absolute value than 2. The variable with positive test value had a high value while that with negative value had a low value in the considered class. The most representative phenotypes of each class were identified as well.

The difference among groups was assessed through Analysis of Variance (ANOVA) and Poisson regression (GLM) followed by the Student-Newman-Keuls test for each discriminating trait. The conditions of application of these tests were assessed prior to analysis. Pearson’s correlation was also calculated and displayed using the *Hmisc* (Frank, 2020) and *Corrplot* (Taiyun and Viliam, 2016) package.

All statistical analyzes were performed with R software version 4.0.2 (R Development Core Team, 2020).

5. Results

5.1. Description of Bambara groundnut phenotypes inventoried from the AEZs of Benin

The results pointed out 32 Bambara groundnut phenotypes inventoried in the five AEZs in Benin. The description of these phenotypes was provided below.

Mtp1 : Integument greyish cream, bold with brown eye in the shape of butterfly with veiled image (without clear contour); Mtp2: Cream or dark yellow integument, bold with brown eye in the shape of very discrete butterfly; Mtp3: Light yellowish-brown integument, bold with eye surrounded by a plug in greyish circle and a well visible butterfly-shaped brown; Mtp4: Pale yellow integument, bold, without eye shape around the hilum; Mtp5: Pale yellow integument spotted with brown, brow eye or surrounded by brown in the shape of



Figure 2: Photos of Bambara groundnut phenotypes inventoried in Benin

discreet butterfly; Mtp6: Integument with longitudinal stripes or marbling of brown color on a cream background and black eye in the shape of well-defined butterfly; Mtp7: Integument on a cream background with black in spots or mottles and grey eye in the shape of obscured butterfly; Mtp8: Integument with cream spots on black background (sometimes brownish black) with a white hilum (without eye); Mtp9: Integument cream in its dorsal region and yellowish-orange in its ventral region; with a yellowish-orange butterfly-shaped eye extended almost to the medial region; Mtp10: Brown, yellow-orange integument on a tabby cream background with a yellowish orange eye in the shape of butterfly; Mtp11: Integument of mixed yellow and orange colors on a cream background with one eye in the shape of an irregular circle of brown color; Mtp12: Integument on mixed-colored cream background; dorsal pale yellow and red; Mtp13: Cream integument, bold (without spot); with circular black eye lined externally with discrete brown butterfly; Mtp14: Cream integument in a clear tone (without spot); with slightly circular greyish-brown eye; Mtp 15: Integument on a cream or brown background with brown streaks; with greyish olive eye in the shape of very discrete butterfly; Mtp16: Black integument, bold without eye shape around the hilum; Mtp17: Red or light brown integument, bold without form around the hilum; Mtp18: Integument on a cream background mixed with greyish olive covered with slightly discrete streaks of brown or greyish color; with greyish circular thinly coated eye around the hilum; Mtp19: Integument with mixed colors of orange, brown and grey with slightly regular stripes of brown or dark brown color with a circular brown eye or in the shape of discrete butterfly; Mtp20: Integument on a cream background with numerous black to brownish spots, usually dense in the dorsal region or tabby; with brownish eye in shape of very discrete butterfly; Mtp21: Integument cream in its dorsal region and ventrally black with butterfly-shaped black eye extending almost to the medial region; Mtp22: Cream integument in the dorsal region with numerous black spots in the shape of varicose veins or longitudinal streaks with eye surrounded by a thin circular black layer lined externally with a brown layer in the shape of discrete butterfly; Mtp23: Brown integument with black eye in the shape of a butterfly; Mtp24: Cream integument with black eye in the form of butterfly; Mtp25: Pale yellow (slightly) spotted brown integument arranged in streaks with a black eyebrow eye in the shape of sharp butterfly and with a few tapered appendages of brown color; Mtp26: Pale yellow integument spotted with brown arranged in streaks with black eye in the shape of clear butterfly with tapered appendages of brown color; Mtp27: Light yellow integument without or with eye in a thin circular grey layer lined with a brown outline in the shape of butterfly; Mtp28: Red integument, bold with numerous small black spots presenting a rusty appearance without individualized eye around a white hilum; Mtp29: Dull orange integument sometimes greyish with streaked spots of brown or brownish brown with greyish circular eye around a slightly prominent hilum; Mtp30: Light yellow-orange integument, bold with discrete brown lines or distinct brown mottling; with a greyish-brown eye in the shape of discreet butterfly; Mtp31: Cream integument, bold with black triangular eye; and Mtp32: Integument with graying olive colors covered with dense marbling of brown or coffee colors; with one eye surrounded by a very thin black layer lined on the outside with another lighter butterfly-shaped layer sometimes bearing a few varicose veins in appendages at one pole of the two poles.

5.2. Phenotypic characterization of Bambara groundnut based on agro-morphological traits

We discriminated ($p < 0.05$) at best the five groups identified based on 14 of all the variables measured. These were terminal leaflet width, petiole length, number of internodes, wingspan 1, number of branches and leaves, and plant height which represent the growth parameters and the manganese, nitrogen, protein, phosphorus, calcium, zinc and iron contents which represent the nutritional parameters (Table 1).

The five groups identified were: Group 1 which comprised eight phenotypes including Mtp2, Mtp6, Mtp9, Mtp11, Mtp12, Mtp17, Mtp21 and the Mtp25; Group 2 with a morphotype Mtp32, Group 3 which gathered 14 phenotypes such as Mtp3, Mtp4, Mtp5, Mtp8, Mtp10, Mtp13, Mtp14, Mtp16, Mtp18, Mtp19, Mtp22, Mtp27, Mtp28 and the Mtp30; Group 4 with 6 phenotypes including Mtp7, Mtp20, Mtp23, Mtp24, Mtp29 and leMtp31 and Group 5 with gathered three phenotypes such as Mtp1, Mtp15 and the Mtp26.

In overall, the phenotypes of the different groups identified were very diverse. The descriptive statistics and the results of the inference tests on each of the discriminant parameters provided in Table 2 indicated the following points:

Table 1: The most discriminating variables of the homogeneous groups identified		
Parameters measured	χ^2	Probability
Manganese	0.75	0.000
Nitrogen	0.72	0.000
Protein	0.72	0.000
Phosphorus	0.58	0.000
Terminal leaflet width	0.50	0.001
Calcium	0.46	0.002
Petiole length	0.42	0.004
Number between nodes	0.42	0.004
Wingspan 1	0.42	0.004
Number of branches	0.41	0.006
Number of leaves	0.36	0.014
Zinc	0.33	0.025
Iron	0.30	0.038
Height	0.29	0.046

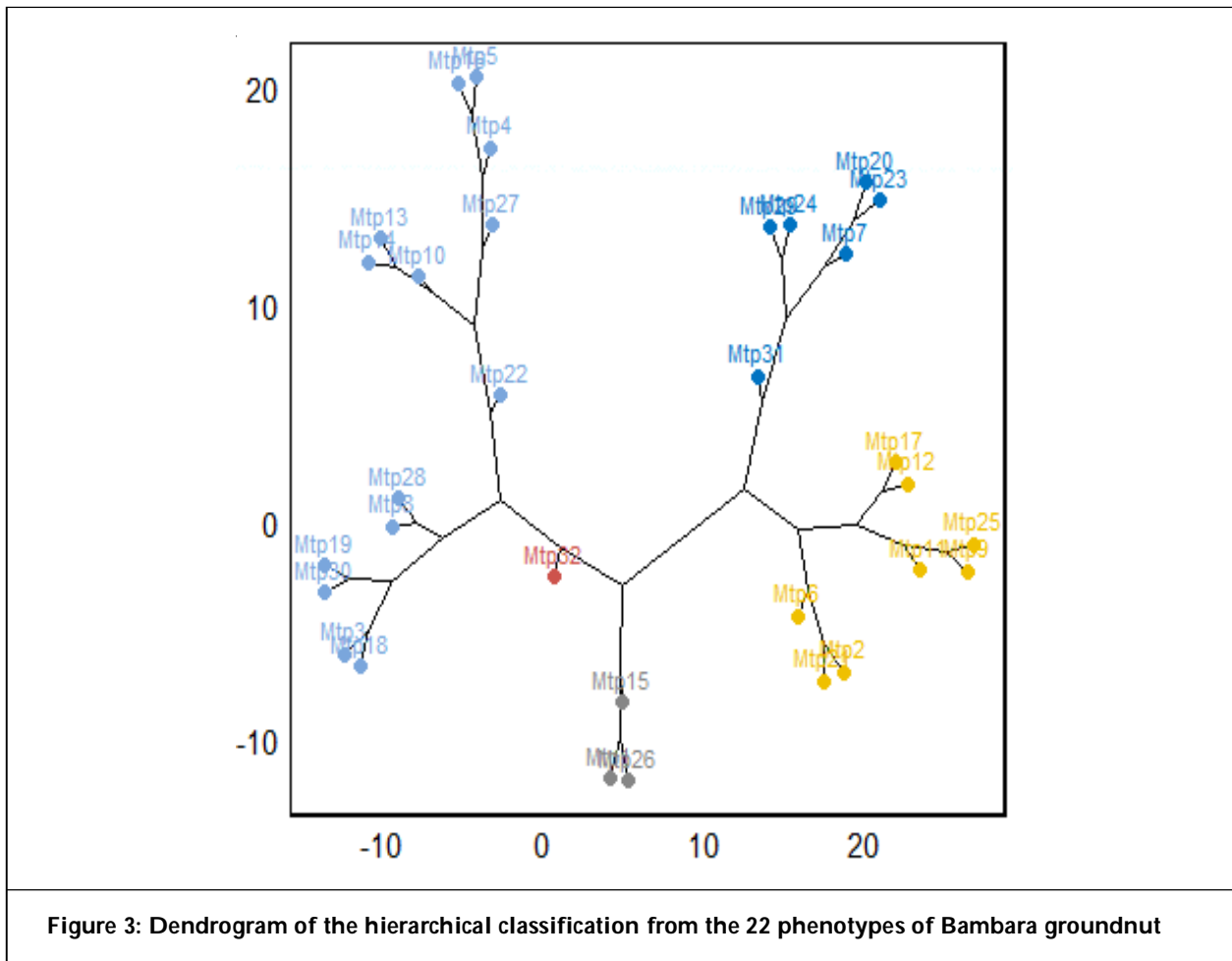
- The first group included the phenotypes with low levels of zinc, nitrogen, proteins and phosphorus (V. test ≤ -2 , Prob < 0.05);
- The second group included phenotypes with high levels of manganese, calcium and iron (V. test ≥ 2 and Prob < 0.05);
- The third group included phenotypes with small petiole length and wingspan (V. test ≤ -2 , Prob < 0.05) with high levels of zinc, nitrogen, proteins (V. test ≥ 2 and Prob < 0.05) but low in manganese (V. test ≤ -2 , Prob < 0.05).
- The fourth group was characterized by large wingspan and terminal leaflet, with many iron leaves (V. test ≥ 2 and Prob < 0.05) with a relatively low number of internodes (V. test ≤ -2 , Prob < 0.05).
- The group 5 was characterized by phenotypes with a developed vegetative trait, with a very important branch and internode production (V. test ≥ 2 and Prob < 0.05) with fewer leaves (V. test ≤ -2 , Prob < 0.05). These phenotypes also have very long petioles and large terminal leaflets and high levels of nitrogen, protein and phosphorus (V. test ≥ 2 and Prob < 0.05).

Besides, the hierarchical classification on principal components pointed out five homogeneous groups comprising respectively: 25, 3.12, 40.62, 18.75 and 12.50% of the initial phenotypes (Figure 2).

6. Correlation between biological and nutritional parameters measured

Overall, Pearson's linear correlation test indicated a weak correlation between growth parameters and nutritional parameters. On the other hand, it indicated a strong correlation between the number of leaves and wingspan 1 ($r = 0.65$), the number of nodes and the number of branches ($r = 0.62$) The terminal leaflet width and the height ($r = 0.53$), the number of branches or the height and petiole length ($r \geq 0.50$), the nitrogen or protein and phosphorus content ($r = 0.50$) and the phosphorus and magnesium content ($r = 0.45$), the content of calcium or iron and manganese ($r \geq 0.50$) (Table 3).

Table 2: Descriptive statistics (mean and standard deviation) of homogeneous groups identified						
Parameters	Group			Global		
	v.test	Moy	ET	Moy	ET	Prob
Group 1						
Zinc	-2.93	25.32	2.18	28.18	3.14	0.003
Nitrogen	-3.74	3.89	0.18	4.20	0.27	0.000
Protein	-3.74	24.30	1.14	26.26	1.68	0.000
Phosphorus	-3.88	0.36	0.02	0.44	0.06	0.000
Group 2						
Manganese	4.62	27.21	0.00	14.53	2.75	0.000
Calcium	3.35	668.12	0.00	422.75	73.34	0.001
Iron	2.66	316.40	0.00	123.71	72.38	0.008
Group 3						
Nitrogen	3.20	4.39	0.13	4.20	0.27	0.001
Protein	3.20	27.42	0.81	26.26	1.68	0.001
Zinc	2.44	29.85	3.41	28.18	3.14	0.015
Wingspan1	-2.02	25.55	2.61	27.39	4.20	0.043
Manganese	-2.03	13.32	1.68	14.53	2.75	0.042
Petiole length	-2.43	101.95	15.85	120.47	35.15	0.015
Group 4						
Wingspan1	3.08	32.22	3.49	27.39	4.20	0.002
Number of leaves	2.34	73.47	9.05	56.26	19.70	0.019
Terminal leaflet width	1.96	26.08	3.02	23.09	4.08	0.050
Number of internodes	-2.66	1.67	0.48	2.06	0.40	0.008
Group 5						
Number of branches	3.35	2.67	0.24	2.16	0.32	0.001
Terminal leaflet width	2.92	28.75	1.44	23.09	4.08	0.004
Number of internodes	2.79	2.58	0.28	2.06	0.40	0.005
Petiole length	2.74	166.17	33.06	120.47	35.15	0.006
Height	2.23	22.33	2.42	18.71	3.42	0.026
Protein	2.10	27.94	0.80	26.26	1.68	0.035
Nitrogen	2.10	4.47	0.13	4.20	0.27	0.035
Phosphorus	2.06	0.50	0.04	0.44	0.06	0.039
Number of leaves	-2.29	34.83	14.38	56.26	19.70	0.022



6.1. Distribution of groups of Bambara groundnut phenotypes among AEZs of Benin

The results of the Detrended correspondence analysis indicated that the first two factor axes accumulate about 70% of the initial information. On the first factorial axis, the phenotypes Mtp13, Mtp30, Mtp32 are more abundant in AEZ 6 while in zones 3 and 4 the phenotypes Mtp6, Mtp9, Mtp12, Mtp16, Mtp27 and Mtp28 were dominant. On the second factorial axis, the phenotypes Mtp5, Mtp14 and Mtp31 were more abundant in AEZ 5 while the Mtp18 morphotype is more representative in AEZ 2 (Figure 2). Moreover, the phenotypes from group 2 were only observed only in the AEZ 5 after classification whereas those from group 3 were recorded in all the Bambara production areas surveyed. However, the phenotypes from groups 4 and 5 were not recorded in AEZ 5.

7. Discussion

Bambara groundnut production is mainly achieved by women in many African countries including Benin, Ivory Coast and Malawi (Yao *et al.*, 2015; and Kamanga *et al.*, 2010). In general, local farmers lack some technical supports in improving seed qualities. As a result, seeds used are relatively heterogeneous mixtures and in most cases exhibit a high phenotypic diversity. The phenotypes inventoried in the present study belong to several groups which are morphologically different and vary among the AEZs surveyed. Indeed, the phenotypes from group 3 were present in local cultivars collected from all the production areas, and therefore appear to be the most cosmopolitan. Even though they have a relatively limited vegetative development, their seeds are highly appreciated by all the local farmers due to their richness in crude protein and zinc. On the other hand, the phenotypes from group 1 were absent only in cultivars from AEZ 2; while those of groups 4 and 5 were missing in the plant materials collected from AEZ 5. In contrast, the phenotypes from group 2 which are generally characterized by low protein contents were observed only in local cultivars collected from AEZ 5 in the southern Benin. According to Danbe *et al.* (2018), this variability observed among phenotypes may be attributed to the exchange of seeds between populations during agricultural campaigns. Besides, the selection of seeds as performed by the population is not sufficiently based on rigorous criteria of differentiation.

Table 3: Correlation matrix (Pearson's linear correlation) between growth parameters and nutritional parameters of the homogeneous groups identified

Parameters	Number of leaves	Terminal leaflet width	Petiole length	Wing-span 1	Number of internodes	Number of branches	Height	Nitrogen	Protien	Phosphorus	Calcium	Iron	Zinc
Terminal leaflet width	0.04												
Petiole length	0.04	0.49											
Wingspan 1	0.65	0.32	0.42										
Number of internodes	-0.27	0.12	-0.09	-0.15									
Number of branches	-0.30	0.26	0.52	0.14	0.62								
Height	0.15	0.53	0.68	0.55	-0.04	0.33							
Nitrogen	-0.22	0.18	0.09	0.01	0.26	0.09	0.25						
Protein	-0.22	0.18	0.09	0.01	0.26	0.09	0.25	1.00					
Phosphorus	-0.23	0.44	0.28	0.03	0.06	0.20	0.26	0.50	0.50				
Calcium	-0.23	0.13	-0.04	-0.12	0.32	0.30	0.01	0.10	0.10	0.03			
Iron	0.07	0.18	0.05	-0.07	0.02	0.02	-0.22	-0.17	-0.17	0.07	0.27		
Zinc	-0.25	0.16	-0.13	-0.12	-0.05	-0.14	-0.10	0.45	0.45	0.47	-0.04	0.15	
Manganese	-0.05	-0.11	-0.04	-0.11	0.12	0.16	-0.14	-0.21	-0.21	-0.07	0.52	0.50	-0.17

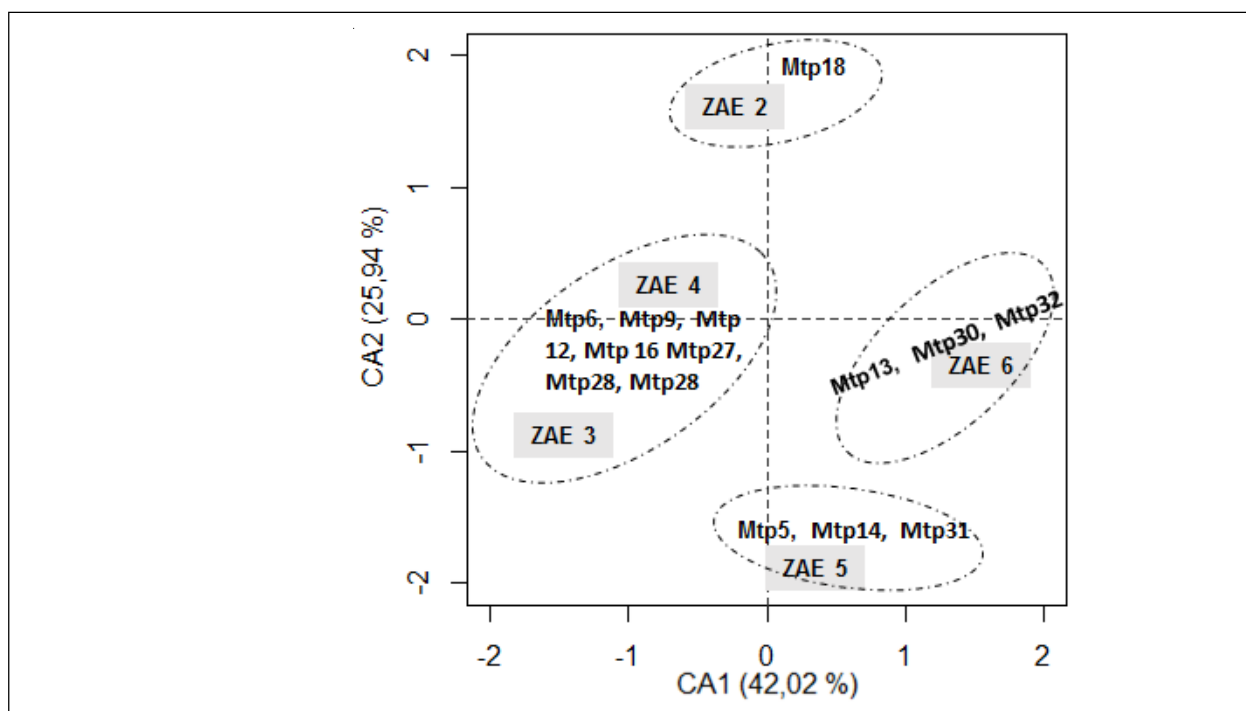


Figure 4: Projection of Bambara groundnut phenotypes among the agro-ecological

This difference in Bambara groundnut phenotypes may also result from the climatic and edaphic conditions prevailing in the production areas (Nebié *et al.*, 2012; and Nerbéwendé *et al.*, 2014). The diversity in Bambara groundnut phenotypes around the world is well documented (Basu *et al.*, 2004; Ntundu *et al.*, 2004; Djè *et al.*, 2005; and Bonny and Djè Yao, 2011). Indeed, Madou *et al.* (2018) reported the specificity of some phenotypes to certain regions and the sporadic distribution of others across production areas. These results provide to the best of our knowledge the existence of a significant phenotypic variability which may not only result from the expression of a strong genotypic heterogeneity but also from the influence of certain environmental factors (Bonny and Djè, 2011).

In the present study, we reported 5 major groups of Bambara groundnut with different characteristics. These morphological differences are not uncommon and have been reported in other producing countries such as Côte d'Ivoire and Malawi (Kamanga *et al.*, 2014 ; and Yao *et al.*, 2015). Our results corroborate those of Touré *et al.* (2013) and Ouoba *et al.* (2018) who identified different phenotypes. Similar results were also obtained by Ramolemana *et al.* (2004) who highlighted cream-colored Bambara groundnut seeds as the most abundant in producing regions of Africa. Ndiang *et al.* (2012) reported that the red color of the petiole, the red color of the stem and the red color of Bambara groundnut seed are intrinsic to the NOR4 variety. The agromorphological traits such as seed size and integument have been used to develop a practical method of improving the seed quality of several plant species, including common bean, cowpea and rape seed (Marwanto, 2004; Zhang *et al.*, 2008; and Possobom *et al.*, 2015). These results suggest that the color of the integument could be an important criterion for determining the quality and commercial values of legume seeds (Yang *et al.* 2010). According to Tiryaki *et al.* (2016), it is a major and even a suitable criterion for the selection of plant material in breeding programs.

8. Conclusion

The present study revealed a great morphological variability of Bambara groundnut collected from the AEZs of Benin. We found five groups of Bambara groundnut with different phenotypic characteristics. In addition, the cream-colored of Bambara groundnut seeds were abundant in all the AEZs. However, the overall analysis of the genetic diversity of Bambara groundnut must consider the production areas and particularly the socio-cultural variability and local names attributed to each morphotype.

9. Acknowledgment

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