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Evaluation of the diversity in qualitative traits of Bambara groundnut germplasm (*Vigna subterranea* (L.) Verdc.) of Côte d'Ivoire

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The objectives of this study were to assess phenotypic diversity of Bambara groundnut germplasm from Côte d'Ivoire using qualitative traits and to understand the genetic diversity at different levels. Hundred and one accessions collected from four agro-ecological zones (central, eastern, northern, western) were characterized in a randomised complete block design with three replications. Thirteen qualitative traits were recorded from seedling emergence to physiological maturity of the crop species. All recorded traits were found to be polymorphic with three or four phenotypic classes. The results revealed a considerable amount of phenotypic variation in the germplasm studied. The phenotypic variation was expressed in color, shape, texture, flexibility, growth habit, pilosity and hardness in both the aerial organs and the underground pods. Cluster analysis grouped together accessions into six genetically distinct groups independently to their geographical origin, suggesting seeds exchanges between growing-zones. The chi-square analysis highlighted the presence of phenotypic variability within and between accessions from each agro-climatic zone for most of the traits evaluated indicating some adaptive forms related to the four zones. Estimates of Shanon-Weaver diversity index (*H'*) for all agro-climatic zones ranged from 0.32 to 0.66 with a mean of 0.46. The northern zone appeared phenotypically more diversified (*H'* = 0.66) than the others. These results are useful to ensure efficient germplasm collection, conservation and management strategies.

Key words: Bambara groundnut, accession, agroclimatic zones, phenotypic diversity, qualitative traits.

INTRODUCTION

Bambara groundnut (*Vigna subterranea)* is an African crop widely grown by subsistence farmers. It is an under-utilised food legume (Azam-Ali et al., 2001) that occupies a prominent place in the strategies to ensure food security in sub-Saharan Africa (Koné et al., 2015). Its edible seeds are an important source of calories, vitamins

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and vegetable proteins (Amarteifio and Moholo, 1998; Minka and Bruneteau, 2000). In addition to its nutritional values, the roots, leaves and seeds are used in traditional medicine (Basu et al., 2007; Jideani and Diedericks, 2014; Maphosa and Jideani, 2016). Only grown as landraces (Zeven, 1998), the crop has a potential for economic exploitation (Ahmad et al., 2016) and offers several agronomic advantages to rural communities in most sub-Saharan countries. It is adapted to various agro-ecosystems, has greater tolerance to drought and yields well on poor soils (Collinson et al., 1997; Mabhaudhi and Modi, 2013). That makes it a useful legume to include in climate change adaptation strategies (Hillocks et al., 2012). The Bambara groundnut landraces are generally grown on small areas in rotation or intercropped with cereals (maize, sorghum, pearl millet), root and tuber crops (cassava, yam). As a nitrogen-fixing legume, they contribute to the maintenance of the soil fertility. They particularly are a remedy to the intensive use of infertile soils (Kumaga et al., 1994).

In Côte d'Ivoire, Bambara groundnut is grown in contrasted agro-climatic zones including tropical rain forest and dry savannah. Rainfall and temperature are the most important climatic factors in Côte d'Ivoire, and vary significantly from one region to another (Mobio et al., 2017). Due to the mountainous zone in the west of the country, rainfall decreases along a South-west/North-east gradient while thermal gradient is oriented from the South to the North where temperatures are higher (Yao et al., 2013). These strong climatic gradients affect the genetic structure of plant populations in different agro-climatic regions (Hamasha et al., 2012).

In these zones, farmers possess considerable indigenous knowledges arising from their long habit of farming Bambara groundnut. Farmers grow the crop for immediate consumption and sale in local markets. It is therefore closely integrated in various traditional intercropping systems and plays a key role in both nutrition and the culture of peoples (Djè et al., 2005). However, the plant is now an endangered crop species (Ahoussou et al., 1995) because of the priority given to major crops such as cassava, cashew, peanut and maize in its growing areas. As a result, preliminary surveys were carried out in different agro-climatic growing regions in Côte d'Ivoire to collect Bambara groundnut germplasm. Accessions thus sampled are maintained in the seed bank of Nangui Abrogoua University. Nevertheless, the collected germplasm needs to be evaluated for various quantitative and qualitative traits. Thereby, like quantitative traits which are usually the target of the selection, qualitative traits are also essential to assess plant diversity and intraspecific variation. Furthermore, qualitative traits allow easy and rapid differentiation between phenotypes. They generally have high heritability, can be easily observed with the naked eye, and are also expressed in all environments (IPGRI et al., 2000). Both of these kinds of characters are appreciated for different objectives, and assessed with different approaches and techniques. Genetic analysis of qualitative traits often requires the interpretation of numbers in various phenotypic classes. Statistical methods such as chi-square tests (χ^2) and Shannon-Weaver diversity index (H) or it relative index (H') are extensively used to respectively assess qualitative variation and level of diversity in several species (Ayana and Bekele, 1998; Arshad et al., 2005; Yirga and Tsegay, 2013). Morphological variability in Bambara groundnut is usually analyzed based on quantitative traits. Analysis of these quantitative traits revealed a significant genetic variability among and within indigenous accessions of Bambara groundnut from Côte d'Ivoire (Bonny and Djè, 2011; Touré et al., 2012). But little is known about their qualitative morphological traits and no work on the variability across regions and on the distribution of these traits was ever conducted. Analysis of the qualitative characteristics could also provide useful information for breeders and managers of genetic resources. So quantifying the variation and the distribution of qualitative traits of local Bambara groundnut landraces is necessary for the collection and the selection of varieties, and the effective management of its conserved germplasm. In Côte d'Ivoire, the growth habit and the color of the seed coat are the main qualitative criteria used by farmers to distinguish, to name and to easily classify Bambara groundnut landraces in the growing areas. Germplasm analysis requires however more characters. The wide range of variation in rainfall and temperature might shape the patterns of qualitative traits variation and their distribution in the different agro-climatic growing areas of Bambara groundnut in Côte d'Ivoire. It is therefore useful to know if some specially adapted forms occur, particularly in these areas. Indeed, the identification of the genetic diversity occurring between and within populations from different geographic areas and the classification of germplasm are necessary for sustainable use of a crop species for different purposes (Rao and Hodgkin, 2002).

The objectives of this research were: i) to describe the variation in 13 qualitative traits of Bambara groundnut landraces; ii) to evaluate the genetic variability and the relationship among accessions; iii) to estimate the distribution of these qualitative traits; and; iv) to assess the level of diversity linked to the main growing zones of Côte d'Ivoire in order to provide a useful qualitative database for Bambara groundnut germplasm collection and conservation.

MATERIALS AND METHODS

Plant

One hundred and one accessions of *V. subterranea* randomly selected from the seed bank of Nangui Abrogoua University were used. They were sampled in four contrasted agro-climatic zones (northern: 29; central: 24; eastern: 27; and western: 21) in Côte

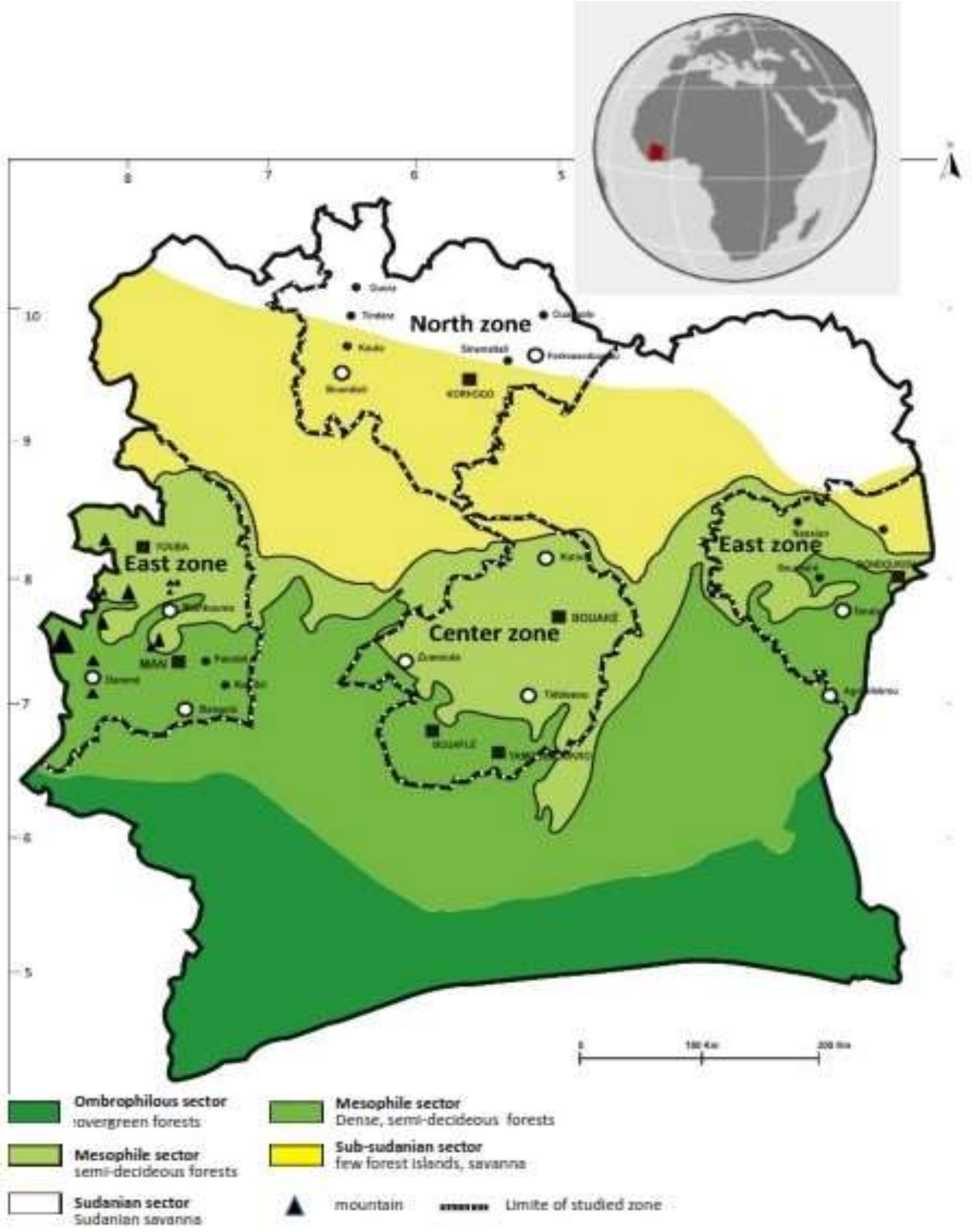

Figure 1. Map of Côte d'Ivoire depicting areas from which the bambarar groundnut germplasm was collected.

d'Ivoire (Figure 1). In this study, each accession is identified by its introduction number and geographical origin. All accessions of a given agro-climatic zone represent a population.

Study area and experimental design

Seeds from the accessions were sown at the Research Station of Nangui Abrogoua University at Abidjan, in the southern region of Côte d'Ivoire. Rainfall in that region is abundant with annual mean greater than 2000 mm. There are two rainy seasons extending from March to July and September to November. The mean monthly temperature ranges from 25 to 28°C. Vegetation is mainly represented by the tropical rain forest (Konaté and Kampmann, 2010). The ferralitic soil is deeper, sandy, well drained and rich in organic matter.

The experiment was conducted according to a randomized complete block design with three replications. The blocks, spaced about 4 m from each over, were ploughed and leveled at the onset of the rainfall season. Each block (52 m × 10 m) consisted of rows, 10 m long spaced 1 m apart. Spacing between plants within a row was adjusted to 0.9 m. Five seeds of each accession were sown randomly in each block on June 20th, 2015. Thus, each accession was represented by 15 plants for a total of 1515 plants for all the blocks. The field was evenly kept weed-free throughout the experimentation and neither insecticide nor herbicide was applied. Harvests were done during the dry season in February 2016.

Data collection

The accessions were characterized based on 13 qualitative characters selected essentially from the descriptors of Bambara groundnut (IPGRI et al., 2000). Data were collected from seedling to harvest. The phenotypic classes found for the examined 13 qualitative traits and their codes are presented in Table 1. For each plant of an accession, the phenotypic classes were recorded according to the binary method: 1 for presence and 0 for absence. Then, each accession was scored for the most frequent phenotypic class for each qualitative trait. And phenotypic frequencies for all traits were computed for each agro-climatic zone.

Statistical analysis

Phenotypic frequency distributions of the qualitative traits were estimated using a sample of all the germplasm. Based on the qualitative data of these accessions, a cluster analysis was performed to establish groups' relationship among the accessions. The Ward method based on Euclidean distances was carried out after standardizing the data to mean zero and unity variance (Van der Berg et al., 2006). The software program Statistica version 7.1 (StatSoft Inc, 2005) was used for all statistical analyses.

Chi-square test

Data were analyzed with chi-square tests (Bolboacă et al., 2011). First, a chi-square test of independence was applied to determine whether or not characters and agro-climatic zones were dependent. Then, a chi-square test of homogeneity was performed to test the homogeneity of the populations from different agro-climatic zones. Calculations were performed using XLStat software 2016.2 (Addinsoft, 2016).

The Shannon-Weaver diversity index

The Shannon-Weaver diversity index (*H'*) (Hennink and Zeven, 1991) is given by:

$$H' = \frac{-\sum_{i=1}^{n} P_i * \ln(P_i)}{\ln(n)}$$

Where, P_i is the frequency of the i^{th} phenotypic class and *n* is the number of phenotypic classes for a given phenotypic trait. The Shannon-Weaver diversity index was used to assess the phenotypic diversity for each trait by agro-climatic zone. Using the additive properties of *H'*, an analysis of variance (ANOVA) was conducted and means of *H'* for the agro-climatic zones were compared by the least significance difference (LSD) at 0.05 probability level using statistical software program Statistica version 7.1 (StatSoft Inc, 2005).

RESULTS AND DISCUSSION

Phenotypic diversity

The evaluation of available genetic diversity is a pre-requisite for genetic improvement in crop plants (Olukolu et al., 2012). Scoring qualitative traits is an important alternative to molecular technique to assess genetic variation on plant germplasm (Ngompe-Deffo et al., 2017). Based on 13 qualitative traits, a sample of 101 accessions was evaluated. The observations revealed a considerable amount of variation in all the traits as well as their frequency distribution (Table 1). The number of phenotypic classes observed for each trait ranged from 3 to 4 indicating that all the traits examined were polymorphic. Thus, a total of 43 phenotypic classes were identified in the current study. The number and nature of the phenotypic classes indicated a high phenotypic variability in the conserved germplam suggesting the presence of genetically distinct accessions. Moreover, a predominance of some phenotypic classes was observed for all the traits.

In the early stages of seedling emergence, three categories of seedling color were detected in the accessions studied. The phenotypic variance for this trait showed significantly higher frequencies of purplish red (48.52%) and green (47.52%) seedlings than dark green (3.96%) seedlings (Table 1). Regarding the leaflet color, four phenotypic classes were observed. Among them, a high frequency of accessions with green leaflets (69.31%) was recorded, followed by accessions with light green (12.87%) and dark green (11.88%) leaflets. Only 5.94% of accessions had yellowish green leaflets. In addition, three phenotypic classes were identified for leaflet flexibility.

The majority of the accessions studied had soft leaflets (89.11%), while accessions with moderately soft and hard leaflets were poorly represented at 5.94 and 4.95%, respectively. The leaflet shape of the accessions also differed during plant growth. It was found that most of the accessions produced elliptic leaflets (74.26%) followed by the accessions with oval leaflets (17.82%). Only 6.93% of the accessions had lanceolate leaflets and accessions with round leaflets were rare (0.99%). The variation in the colors of the petioles also was noticeable. Out of the accessions analysed, 76.24% had ligth green petiole, 22.77% showed green petiole and only 0.99% had pale green petiole. Observed variations found among the local landraces regarding the leaflet color, shape and flexibility may be a useful tool in future collection missions.

The majority of the accessions in the sample showed a dark green stem color (78.22%). The two other stem colors observed were green and pale green in the respective proportions of 19.80 and 1.98%. Furthermore, three distinct types of stem hairiness were recorded among the accessions. With the exception of some accessions which showed absent hairiness (6.93%) or

Table 1. Qualitative traits, codes and phenotypic classes and frequency (%) distribution of Bambara groundnut accessions.

Qualitative trait	Code and phenotypic class		Frequency
Seedling color at emergence	1	Purplish red	48.52
	2	Green	47.52
	3	Dark green	3.96
Fully expanded leaflet color	1	Dark green	11.88
	2	Green	69.31
	3	Ligth green	12.87
	4	Yellowish green	5.94
Leaflet flexibility	1	Soft	89.11
	2	Moderately soft	4.95
	3	hard	5.94
Terminal leaflet shape	1	Round	0.99
	2	Oval	17.82
	3	Lanceolate	6.93
	4	Elliptic	74.26
Petiole color	1	Green	22.77
	2	Pale green	0.99
	3	Ligth green	76.24
Stem color	1	Green	19.80
	2	Pale Green	1.98
	3	Dark green	78.22
Stem hairiness	1	Absent	6.93
	2	Sparse	2.97
	3	Dense	90.10
Growth habit	1	Bunch type	31.68
	2	Semibunch type	8.91
	3	Spreading type	59.41
Pod shape	1	Round without point	22.77
	2	Rounded base with a point at the top	76.24
	3	Elongated with rounded base and a point at the top	0.99
Pod color	1	Yellowish-brown	2.97
	2	Brown	89.11
	3	Ligth brown	2.97
	4	Beige	4.95
Pod texture	1	Smooth	6.93
	2	Wrinkled with little grooves	12.87
	3	Wrinkled with much grooves	53.47
	4	Rough	26.73
Pod hardness	1	Low	11.88
	2	Moderately hard	41.58
	3	Hard	46.54

Table 1. Contd.

Seed shape	1	Round	10.89
	2	Oval	86.14
	3	Oblong	2.97

sparse hairiness (2.97%), most of the accessions produced dense hairiness stem (90.10%).

Bambara groundnut varied in vegetative growth. Our observation was in agreement with those of Doku (1969) who categorized the vegetative growth of Bambara groundnut in three distinct growth habits, namely, bunch, semi-bunch and spreading types. These three growth habits were found in our germplasm in different proportions. Among the accessions studied, the spreading type was the most observed (59.41%) followed by the bunch type (31.68%). The semi-bunch type was less frequent (8.91%). The growth habit of crops is of high significance to the cropping system (Egbadzor et al., 2014). Each type of growth habit could be subject to different cultivation procedures in the traditional cropping system. They could be chosen judiciously as cover crop in phytotechnical procedures involving various cultivated species of locale tubers and cereals crops.

The pod of the Bambara groundnut is an important underground organ of the crop species. The pod was a qualitative trait that differed according to genotype. The accessions showed phenotypic variations with respect to color, shape, texture and hardness of pods. In this study, the majority of accessions expressed brown pod color (89.11%). The color of the pods of the remaining accessions was almost equally distributed as yellowish-brown (2.97%), ligth brown (2.97%) and beige (4.95%). The pod texture varied considerably among the accessions. Four patterns of pod texture were identified in the present study: smooth, rough, and wrinkled with little grooves or more grooves. Most of the accessions had wrinkled pods with more grooves (53.47%). These were followed by accessions producing rough pods (26.73%), wrinkled pods with little grooves (12.87%) and smooth pods (6.93%). Pod shape varied from accession to accession and phenotypic variations included three patterns. Accessions producing pods characterized by rounded base with a point at the top were most frequent (76.24%) followed by those producing rounded pods without point (22.77%). Accessions producing elongated pods with rounded base and a point at the top were rare (0.99%). Concerning the proportion of pod hardness in all the material studied, three categories of hardness were observed as follow: 46.54% of the accessions showed hard pods, while 41.58% had moderately hard pods. Only 11.88% of the accessions had soft pods.

With regard to seed shape, three categories were observed. A large proportion of the accessions produced oval seed (86.14%), while 10.89% of accessions had round seed. Only 2.97% of the accessions studied produced oblong seed.

The phenotypic variations expressed in color, shape, texture, flexibility, growth habit, pilosity and hardness in both the aerial organs and underground fruits of Bambara groundnut landraces suggest that a high degree of diversity is still maintained within this species. According to Joshi and Baniya (2006), the existence of such diversity in cultivated crops may be due to genetic drift, spontaneous variations and natural or human selections. These preliminary results point to the need for more studies in order to identify useful germplasm with known characteristics.

Cluster analysis

The dendrogram (Figure 2) resulting from the cluster analysis shows the phylogenetic relationships between the accessions from all the zones. This analysis helped to identify related and genetically distinct groups of accessions. Six groups were formed at 30% similarity level. Except, the other traits, the phenotypic traits related to seedling color, petiole color and stem color were observed in all groups.

For example, group I contained 15 accessions from all collection zones and was further subdivided into two subgroups. The common traits of the accessions of both subgroups were green and soft leaflets. Furthermore, their plants had a bunch type growth habit with dense hairiness stems and produced oval seed. The pods of these accessions have a rounded base with a tip at the top, and wrinkled with more grooves on their surface. These pods have a low hardness and a brown color. The difference between the two subgroups involved the leaflet shape, which was oval for the first subgroup and lanceolate for the second subgroup.

Group II consisted of 21 accessions collected in three zones (central, eastern, and western zones). Those accessions clustered into two subgroups. Accessions from group II are similar for their oval seed and differ in the pod shape. The pods of the first subgroup have a rounded base with a tip at the top while those of the second subgroup are rounded without a tip at the top. Moreover, all the acccessions of group II have a green color, soft and elliptic leaflets, produced plants with a spreading growth habit and dense hairiness stems. The pods of this group had more grooves, were hard and brown in color.

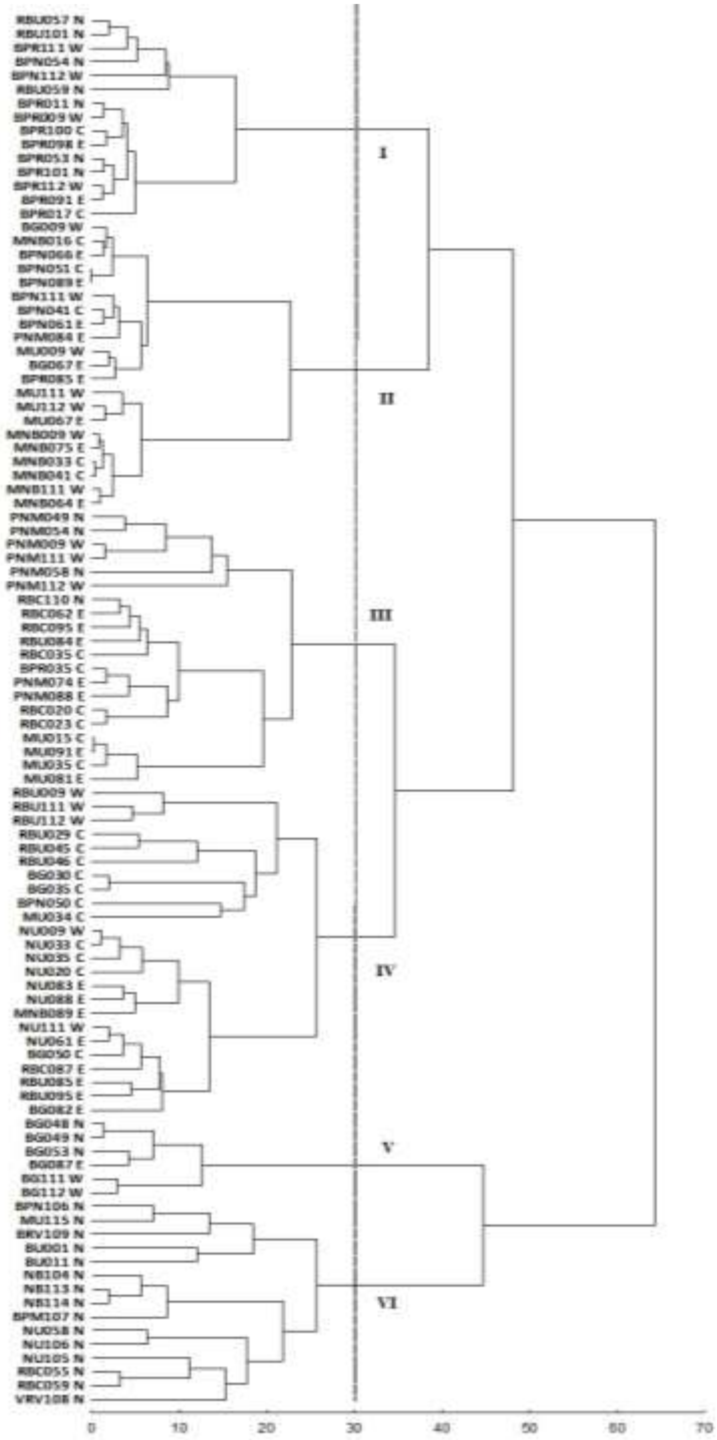

Figure 2. Dendogram for the 101 accessions of *Vigna subterranea* from four agroclimatic zones [central (C), eastern (E), northern (N), western (W)] of Côte d'Ivoire, constructed by Ward's method.

Group III included 20 accesions from the four collection zones. It is subdivided into three subgroups. Accessions from these subgroups were identical for the following phenotypic traits: green, soft and elliptic leaflet, spreading growth habit, dense hairiness stem, brown and hard pod, and oval seed. The first subgroup additionally contains accessions with oval leaflets, a bunched growth habit, rounded base pod with a tip at the top and rough.

Accessions from the second subgroup produced rounded base pods with a tip at the top but wrinkled with more grooves. Accessions from the third subgroup produced only rounded pods without a tip at the top and wrinkled with more grooves.

Group IV consisted of 24 accessions originating from the western, central and eastern zones. It is structured into three subgroups. All the accessions that belong to this group have mainly light green color, soft and elliptic leaflets, a spreading growth habit, dense hairiness stem and produce oval seeds. Pods produced by these accessions are brown, rough and hard but differ in their shape. Thus, accessions in the first and second subgroups have rounded base pods with a tip at the top while those in the third subgroup have rounded pods without a tip at the top.

The group V gathered 6 accessions originating from three zones (northern, western and eastern). The leaflets of these accessions are yellowish-green, relatively hard and oval. Their plants have semi-bunch growth habit and dense hairiness stems. Their pods have a rounded base with a tip at the top, wrinkled with little grooves on the surface, hard and brown in color. The pods contained oval seeds.

The group VI was more heterogeneous. It was composed of 15 accessions collected exclusively in the northern zone. It is further subdivided into two subgroups. Accessions belonging to this group are phenotypically more varied with respect to the leaflet color and shape, and also the color and texture of the pod. For example, the leaflet color varied from ligth green to dark green, the leaflet shape was oval, elliptic or lanceolate. All the plants of the accessions had a bunch growth habit, hairiness stems and produced rounded base pods with a tip at the top. The differences between accessions of the two subgroups concerned mainly the texture, the hardness and the color of the pods and the shape of the seed. In the first subgroup, the pods of the accessions have a smooth surface, a beige color and were soft; their seeds were round. In the second subgroup, the pods had a roughed surface, a ligth brown color and were moderately hard; the seeds shape was round or oblong.

The cluster analysis revealed that qualitative traits are also useful markers to distinguish and classify local Bambara groundnut accessions. Genetic patterns obtained from this study of Bambara groundnut germplasm could be of great importance. It can help Bambara groundnut breeders to make better choices during selection and gene bank management. The qualitative traits evaluated in this study could be used to easily distinguish varieties with simple and inexpensive selection methods if their high heritability was proven.

The accessions from different geographical origins were clustered into the same group, and those from the same geographical zone were clustered into different groups. These results suggest that for a given traits, farmer's selection criteria could be uniform. That could also be due to seed exchanges among the different growing-regions.

Distribution of traits in the agro-climatic zones

Characterizing the geographical patterns of variation is useful for efficient conservation strategies and use of agricultural genetic resources (Dulloo et al., 2010). In this study, the frequency distribution for the thirteen qualitative traits was evaluated among the main growing zones of Bambara groundnut landraces. Tables 2 and 3 show the observed and relative frequency distributions of phenotypic classes by agro-climatic zones of collection. Out of the thirteen qualitative traits examined, four traits, namely seedling color, leaflet flexibility, petiole color and stem color showed an independant distribution of the agro-climatic zones ($\chi^2_{gk} < 12.59$ for a *ddl* = 6 and $\chi^2_{gk} < 16.92$ for *ddl*= 9; P > 0.05). These results indicate that collection zones shared strong similarity in reference to all phenotypic classes for these traits. The nine other traits, which expressed together 31 phenotypic classes, presented a dependant distribution of the agro-climatic zones ($\chi^2_{gk} > 12.59$ for a *ddl* = 6 and $\chi^2_{gk} > 16.92$ for *ddl*= 9; P < 0.05). Overall, these data demonstrated the existence of different populations of Bambara groundnut in Côte d'Ivoire related to agro-climatic zones. This spatial distribution of populations could be an essential factor for the long-term maintenance of genetic variation (Cruzan, 2001).

The homogeneity tests corroborated the differences among accessions of the different zones with regard to the expression of 31 phenotypic traits. Geographical origin likely contributed to the genetic variability among the accessions (Geleta et al., 2005). Out of the 31 phenotypic classes, the most remarkable results were achieved with only 15 phenotypic traits ($\chi^2_g > 7.81$ for *ddl* = 3). The identified phenotypic classes were dark green (for Leaflet color), oval, lanceolate and elliptic (for leaflet shape), absent (for stem hairiness), bunch, semi-bunch and spreadind (for growth habit), round without point at the top (for pod shape), ligth brown and beige (for pod color), smough (for pod texture), low and hard (for pod hardness) and round (for seed shape). These phenotypic traits that showed different occurrences depending on the agro-ecological zone could serve as morphological markers during future collection missions.

All phenotypic classes of a given qualitative trait occurred with varied frequencies within and between accessions from collection zones. Overall, the central, eastern and western zones showed, in general, the same trend of frequency distribution for almost all phenotypic classes, unlike the northern zone.

Within each zone, an unequal frequency distribution of phenotypic classes was recorded among accessions ($\chi^2_g > 7.81$ for a *ddl* = 3 or $\chi^2_g > 9.48$ for *ddl* = 4, data not

Table 2. Frequencies of Bambara groundnut accessions from four agroclimatic zones (central, eastern, northern, western) of Côte d'Ivoire by phenotypic classes for thirteen qualitative traits.

Qualitative trait		Northern	Western	Central	Eastern	χ^2_g	χ^2_{gk}	*p*
Seedling color at emergence	1	10	12	13	14	1.72	3.69	0.718
	2	18	8	10	12	1.91		
	3	1	1	1	1	0.06		
Fully expanded leaflet color	1	10	1	0	1	17.74	27.20	0.001
	2	14	17	19	20	2.69		
	3	2	1	5	5	3.73		
	4	3	2	0	1	3.05		
Leaflet flexibility	1	23	18	24	25	0.70	6.73	0.347
	2	3	1	0	1	2.98		
	3	3	2	0	1	3.05		
Leaflet shape	1	1	0	0	0	2.48	43.74	< 0.0001
	2	10	7	0	1	14.65		
	3	7	0	0	0	17.38		
	4	11	14	24	26	9.22		
Petiole color	1	8	4	5	6	0.47	3.25	0.777
	2	1	0	0	0	2.48		
	3	20	17	19	21	0.30		
Stem color	1	6	4	5	5	0.05	5.20	0.518
	2	2	0	0	0	4.97		
	3	21	17	19	22	0.18		
Stem hairyness	1	7	0	0	0	17.38	19.55	0.003
	2	0	1	1	1	1.25		
	3	22	20	23	26	0.92		
Growth habit	1	21	4	2	5	21.85	54.78	< 0.0001
	2	7	1	0	1	10.91		
	3	1	16	22	21	22.02		
Pod shape	1	0	5	10	8	10.93	16.12	0.013
	2	28	16	14	19	2.70		
	3	1	0	0	0	2.48		
Pod color	1	1	0	2	0	3.77	28.33	< 0.001
	2	23	18	22	27	0.72		
	3	0	3	0	0	11.43		
	4	5	0	0	0	12.41		
Pod texture	1	7	0	0	0	17.38	31.12	< 0.001
	2	2	5	0	6	7.68		
	3	10	10	17	17	3.90		
	4	10	6	7	4	2.17		

Table 2. Contd.

Pod hardness	1	12	0	0	0	29.79	49.82	< 0.001
	2	15	11	5	11	3.80		
	3	2	10	19	16	16.23		
Seed shape	1	11	0	0	0	27.31	40.35	< 0.001
	2	15	21	24	27	5.59		
	3	3	0	0	0	7.45		

$\chi^2_g > \chi^2_\alpha = 7.81$ (*ddl*= 3) is significant at the level α = 0.05.and indicates that column (i.e. zone) frequencies are significantly different for a particular phenotypic trait; $\chi^2_{gk} > \chi^2_\alpha = 12.59$ (*ddl*= 6) or $\chi^2_{gk} > \chi^2_\alpha = 16.92$ (*ddl*= 9) is significant at the level α = 0.05 and indicates that zone frequencies are significantly independant for a particular trait.

presented here) with a preponderance of certain phenotypic traits. These results would undoubtedly be the consequence of anthropic pressure resulting from a localized propagation of certain types of Bambara groundnut seeds. But for some phenotypic classes, the difference of frequency distributions among the four collection zones could be attributed to the unequal sizes of samples of accessions used in this study (Bolboacă et al., 2011). For all material, a wide range of variation was highlighted by varied degrees of polymorphism and irregular frequency distributions of qualitative traits among the collection zones. Concerning seedling color, three colors were distinguishable in this work. Most of the accessions from the four agro-climatic zones were purplish red or green in seedling color, except one accession for each zone, which was dark green in seedling color. However, the green color had the highest frequency among accessions from northern zone while the purplish red color was the dominant one in the other zones.

It was found that accessions from each agro-climatic zone presented different expression for the examined qualitative traits associated with the leaves. Four different leaflet colors were found for all the zones, except the central zone where only green and light green leaflets were observed. However, the green leaflet was the most frequent in all zones, with a predominence in the western (80.95%), central (79.17%) and eastern (74.07%) zones. The dark green leaflet was more observed in the northern accessions than the central, western and eastern ones. The proportions of light green and yellowish green leaflets were relatively low among accessions from each agro-climatic zones studied.

The three phenotypic classes (soft, moderately soft and hard) for leaflet flexibility detected in this study were found among accessions from northern, western and eastern zones. Accessions of the central zone only had the soft leaflets. Soft leaflet was the dominant leaflet flexibility over all zones, unlike the moderately soft and hard leaflets which were less frequent.

The four leaflet shapes (round, oval, lanceolate and elliptic) were present in accessions taken from the northern zone while only two (oval and elliptic) and one (elliptic) were recorded among samples from western and eastern zones, respectively. The elliptic leaflet was the most frequent in all zones, with preponderance in accessions from central (100%) and eastern (96.3%) zones. The lanceolate and round leaflets were observed only in the northern zone. These two shapes may have evolved due to adaptation to the northern zone.

Green, light-green and pale-green colors were the petiole colors found among all samples. The light-green type was predominant in all agro-climatic zones (Table 2) followed by the green type. The pale-green type was less present in the northern zone and absent among accessions from the other zones.

Dark-green stem was predominant in the samples of each agro-climatic zone, followed by green stem. The pale-green stem was only observed among accessions from northern zone and was less frequent. Stem hairiness were classified as absent, sparse and dense. In all samples from the four agro-climatic zones, the dense type of stem hairiness was the most abundant. In this study, absent stem hairiness was limited to the northern zone whereas sparse stem hairiness was present in the other zones.

As noted earlier, the three types of growth habits (bunch, semi-bunch and spreading types) were present in accessions from northern, western and eastern zones but with a mix of different proportions. The sample of central zone contained only bunch and spreading types. The frequency distributions of the three phenotypes differed within and among the four agro-climatic zones. The majority of the accessions (21 out of 29) from the northern zone were the bunch type, while the semi-bunch and spreading types were less represented. The three other agro-climatic zones showed more or less the same trend of distribution for the three growth habits with a relatively high frequency for the spreading type followed by the bunch and semi-bunch types (Table 2).

Table 3. Percentage of Bambara groundnut accessions from four agroclimatic zones of Côte d'Ivoire by phenotypic classes for thirteen qualitative traits.

Qualitative trait		Agro-climatic zones			
		Northern	**Western**	**Central**	**Eastern**
Seedling color at emergence	1	34.48	57.14	54.17	51.85
	2	62.07	38.1	41.67	44.44
	3	3.45	4.76	4.17	3.7
Fully expanded leaflet color	1	34.48	4.76	0	3.7
	2	48.28	80.95	79.17	74.07
	3	6.9	4.76	20.83	18.52
	4	10.34	9.52	0	3.7
Leaflet flexibility	1	79.31	85.71	100	92.59
	2	10.34	4.76	0	3.7
	3	10.34	9.52	0	3.7
Leaflet shape	1	3.45	0	0	0
	2	34.48	33.33	0	3.7
	3	24.14	0	0	0
	4	37.93	66.67	100	96.3
Petiole color	1	27.59	19.05	20.83	22.22
	2	3.45	0	0	0
	3	68.97	80.95	79.17	77.78
Stem color	1	20.69	19.05	20.83	18.52
	2	6.9	0	0	0
	3	72.41	80.95	79.17	81.48
Stem hairyness	1	24.14	0	0	0
	2	0	4.76	4.17	3.7
	3	75.86	95.24	95.83	96.3
Growth habit	1	72.41	19.05	8.33	18.52
	2	24.14	4.76	0	3.7
	3	3.45	76.19	91.67	77.78
Pod shape	1	0	23.81	41.67	29.63
	2	96.55	76.19	58.33	70.37
	3	3.45	0	0	0
Pod color	1	3.45	0	8.33	0
	2	79.31	85.71	91.67	100
	3	0	14.29	0	0
	4	17.24	0	0	0
Pod texture	1	24.14	0	0	0
	2	6.9	23.81	0	22.22
	3	34.48	47.62	70.83	62.96
	4	34.48	28.57	29.17	14.81
Pod hardness	1	41.38	0	0	0
	2	51.72	52.38	20.83	40.74
	3	6.9	47.62	79.17	59.26
Seed shape	1	37.93	0	0	0
	2	51.72	100	100	100
	3	10.34	0	0	0

Lule et al. (2012) reported that besides genetic factors, edaphic factors or other environmental conditions can influence the adaptive role of somes qualitative traits.

Therefore, it is probable that differences in the phenotypic expression of some qualitative traits across contrasted environments could be more or less induced by climatic factors. For exemple, the predominance of bunch type in the dry northern zone compared to the predominance of spreading type in the western, central and eastern zones indicates adaptative characters. It can be concluded that variation for growth is under the control of genetic and environmental factors. This could also be the case of other aerial traits such as leaflet shape and color.

The pods' qualitative traits showed divergences from one agro-climatic zone to the other. In this work, out of the three pod shapes identified, two were observed in each sample from the agro-climatic zones. Pods characterized by a rounded base with a point at the top were the most frequent in all four zones with predominance in accessions from the northern zone. The rounded pods without point were expressed only in few accessions from central, eastern and western zones (Table 2). Only one accession from the northern zone had elongated pods with rounded base and a point at the top.

Among the four pod colors, brown pods were the most frequent in the accessions of all collection zones (Table 2). Yellowish brown pods were present only among the accessions from the northern and central zones. Beige and ligth brown pods were present only in accessions from the northern and western zones, respectively.

All the four types of pod texture were present in the sample from the northern zone. Eastern and western zones comprised three types and the central zone, two types (Table 2). Wrinkled pods with more grooves were the most frequent among the accessions from central, eastern and western zones, whereas wrinkled and rough pods with more grooves were the most abundant among accessions from the northern zone. However, smooth pod was limited to the northern zone where pods with little grooves were less frequent.

In the central and eastern zones, most of the accessions had hard pods followed only by accessions showing moderately hard pods. Accessions collected from the northern zone produced all three textures of pods. But, the accessions with moderately hard pods were the most frequent, followed by the accessions with the soft and the hard pods. Only moderately hard and hard pods were found among accesions from central zone with equal proportions.

The three phenotypic classes of seed shape (round, oval and oblong) were observed among the accessions from the northern zone with the predominance of oval seed followed by round and oblong ones (Table 2). In the present study, round and oblong seeds were absent from all the accessions from central, eastern and western zones.

Estimates of Shannon-Weaver diversity

The estimates of the Shannon-Weaver diversity index (H') for the thirteen traits by agro-climatic zone are shown in Table 4. For all the accessions sampled, the mean value of H' varied from 0.21 for seed shape to 0.74 for seedling color with an overall mean of 0.46. The qualitative traits also showed different levels of diversity within and between the four agro-climatic zones. The northern zone presented the highest H' values for all traits, except for the seedling color and the pod shape, which were higher in the western (H' = 0.71) and the central (H' = 0.62) zones, respectively. Moreover, all traits appeared polymorphic in the northern zone. Conversely, a monomorphism (H'= 0) was recorded in three traits (leaflet flexibility, leaflet shape, and seed shape) for the central zone, two traits (pod color and seed shape) for the eastern zone and one trait (seed shape) for the western zone. According to Hammer et al. (1996), monomorphism of some traits occurring within a given zone could be either a drift or a loss of genetic integrity caused by selection forces.

A highly significant difference (F = 5.06; P = 0.003) of mean values of H' was depicted between the agro-climatic zones. The level of genetic diversity in northern zone (H' = 0.66 ± 0.21) was high compared to the other zones. Relatively, intermediate values of H' were recorded for western (H'= 0.46 ± 0.21) and eastern (H'= 0.40 ± 0.26) zones, while the lowest was recorded for the central zone (H' = 0.32 ± 0.24).

The Northern zone with the highest genetic diversity appeared genetically more diversified than the other zones. That is demonstrated by the specific phenotypic traits that it contained. Nevertheless, mean values of H' of the central, eastern and western zones appeared statiscally identical (Table 4), suggesting a similar genetic diversity.

Bambarra groundnut is a self-pollinated species. Therefore, the level of diversity found in each zone could result from farmers' agricultural practices and seed management methods such as recycling, sorting, exchange and new introductions. According to Alvarez et al., (2005), Robert et al. (2005) and Thomas et al. (2012), these factors may favor a diversifying selection leading to the maintenance, or even the creation of a significant morphological diversity. The overall species vitality and the potential for evolutionary responses to environmental change rely on the level of genetic variation within populations (Ellstrand and Elam, 1993). Based on this assumption, a particular attention should be given to all these Côte d'Ivoire's main growing-regions. The northern zone specially seems to be the appropriate region for maximum diversity and *in situ* genetic conservation of Bambara groundnut landraces.

Conclusion

The present study revealed high phenotypic variability of

Table 4. Estimates of the Shannon-Weaver diversity index (H') for thirteen qualitative traits in Bambara groundnut by agroclimatic zones.

Character	Agroclimatic zones				H' values
	Northern	Western	Central	Eastern	Mean ± SD
Seedling color	0.71	0.76	0.75	0.75	0.74 ± 0.02
Leaflet color	0.82	0.49	0.37	0.56	0.56 ± 0.19
Leaflet flexibility	0.59	0.46	0.00	0.29	0.33 ± 0.26
Leaflet shape	0.86	0.46	0.00	0.11	0.36 ± 0.39
Petiole color	0.66	0.44	0.47	0.48	0.51 ± 0.10
Stem color	0.68	0.44	0.47	0.44	0.51 ± 0.12
Stem hairyness	0.50	0.17	0.16	0.14	0.24 ± 0.17
Growth habit	0.63	0.61	0.26	0.57	0.52 ± 0.17
Pod shape	0.14	0.50	0.62	0.55	0.45 ± 0.22
Pod color	0.44	0.30	0.21	0.00	0.23 ± 0.18
Pod texture	0.91	0.76	0.44	0.66	0.69 ± 0.20
Pod hardness	0.81	0.63	0.47	0.62	0.63 ± 0.14
Seed shape	0.86	0.00	0.00	0.00	0.21 ± 0.43
Mean ± SD (H')	0.66 ± 0.21^{a}	0.46 ± 0.21^{b}	0.32 ± 0.24^{b}	0.40 ± 0.26^{b}	0.46 ± 0.17

Côte d'Ivoire local Bambara groundnut germplasm. This work indicated that accessions can be clustered into six groups based on the qualitative traits. The distribution of most of the phenotypic traits was related to agro-climatic zones and indicated the adaptive forms in the expression of these traits. In addition, highly significant difference of levels of diversity was found among the agro-climatic zones of origin. The results represent an important database for many purposes. Phenotypic traits could be useful tools for identifying varieties in breeding programs. Data could serve for future prospection and collection missions. They will largely determine the location of areas and conservation strategies, as well as the management of Bambara groundnut genetic resources in Côte d'Ivoire.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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