

## Morphological and taxonomic diversity of taxa considered as infrataxons of *Phaseolus lunatus* L. in Benin

### Abstract

**Aims:** Parataxonomic work on Lima beans has shown that this plant is subject to taxonomic confusion. The objective of this study is to clarify the taxonomic position of plants grown in Benin as local varieties of *Phaseolus lunatus* L.

**Methods:** Thus an experimental field of 99 accessions, from the 33 local varieties of *P. lunatus* recorded in Benin, was installed. Quantitative and qualitative data were collected from seedling emergence to fruit ripening.

**Results:** A total of 07 species were identified among the taxa considered as local varieties of *P. lunatus* by the 990 farmers surveyed. Of these, 77.58% actually recognized the species but 22.42% cultivated other species that they mistakenly assimilate to local varieties of *P. lunatus*. The latter are mostly dominated by farmers in the Sudanian zone. The seven (07) species identified are: *Phaseolus lunatus*, *Sphenostylis stenocarpa* (A.Rich.) Harms, *Mucuna pruriens* (L.) DC. var. *utilis* (Wall. ex Wight) Baker ex Burck, *Canavalia africana* Dunn, *Canavalia ensiformis* (L.) DC., *Canavalia gladiata* (Jacq.) DC. and *Canavalia* sp. Of the 33 taxa and 99 accessions followed, 25 taxa and 75 accessions are for *Phaseolus lunatus*. Within the 75 accessions of *P. lunatus*, six (06) qualitative morphological characters of which five (05) seed-related showed significant variability (seed coat pattern, seed coat colour, hilum belt colour, seed shape and size). These traits enabled nine (09) morphotypes to be distinguished.

**Conclusion:** The present work has thus shown the importance of morphological traits related to the seed in the discrimination of morphotypes as was the case in the vernacular nomenclature of the species. They are therefore potential indicators in genetic improvement programmes. However, knowledge of their degree of heredity requires molecular genetic analyses.

**Keywords:** Lima beans, morphological traits, morphotypes, taxa, Benin

### 1. Introduction

*Phaseolus lunatus* L. is one of 41 species neglected in Benin [1] despite being an important source of plant protein [2]. Within the genus *Phaseolus* in Benin, [3] reported a strong decline in genetic diversity and threats of extinction of some cultivars due to various biotic, abiotic and anthropogenic constraints. It is therefore necessary to develop and

implement strategies for the exploitation and conservation of these plant genetic resources. As such, [4] found that information on genetic variation is essential for developing effective conservation strategies for rare and threatened species. Indeed, the more efficient use of plant resources requires detailed knowledge of their genetic characteristics and even cytogenetic and agro-morphological characteristics [5]. Morphological analyses remain essential for molecular genetic studies to accelerate the identification of traits and selection of the best morphotypes [6]. Better, the exploitation of morphological parameters increases the knowledge on the genetic variability available and greatly facilitates the cultivation for a wider geographical adaptability, in relation to biotic and abiotic stresses [7].

In Benin, ethnobotanical inventories of taxa of the genus *Phaseolus* have highlighted some inaccuracies and taxonomic confusions. Indeed, the work of [3] and [8] revealed the existence of 17 local varieties without being able to locate each of these varieties under one species. In addition, those of [9, 10] were devoted to *Phaseolus vulgaris* L. with the mistaken assimilation of certain varieties of *Phaseolus lunatus* to those of *Phaseolus vulgaris* [11]. Better still, the taxa cited by farmers such as *Phaseolus vulgaris* by [9] are also, for the most part, cited by farmers interviewed by [12] as taxa of *Phaseolus lunatus*. This raises questions about the level of knowledge of local populations in terms of differentiation of taxa of the genus *Phaseolus* in Benin. According to [12], the high use of seed morphological traits in parataxonomy and the shared uses of *Phaseolus lunatus* seeds with other neighbouring taxa would justify the observed taxonomic inconsistency or confusion. These authors gave as an example, the case of *Vitellaria paradoxa* C.F.Gaertn. called *kotoblè* en fon and *Lophira lanceolata* Tiegh. ex Keay. called *kotoblè assou* (the male of the shea tree) which are two species of different botanical families but considered as the same species by the local populations. [8] rightly pointed out that in Benin, the clarification of species of the genus *Phaseolus* remains a stage without which the lines of research on this speculation would not be discussed without limits.

Morphological characterisation will thus make it possible to clarify the taxonomic position of taxa considered to be local varieties of *Phaseolus lunatus* or *Phaseolus vulgaris* in Benin on the one hand and to identify local varieties with positive morphological traits on the other hand for the promotion of culture. The objective of this study is therefore to evaluate the morphological and taxonomic diversity of the taxa considered to be local varieties of *Phaseolus lunatus* cultivated in Benin.

## 2. Methods

## 2.1. Data Collection

An experimental field was set up in the village Sodohomè in Bohicon, located in the department of Zou. The climate is subtropical with 4 climatic seasons and an average annual rainfall of 1200 mm. The soil is of the ferrallitic type.

During the census of the local varieties of *Phaseolus lunatus* cultivated in Benin, accessions were collected from 990 farmers throughout the territory of Benin. They have been divided into 33 taxa considered by the population as local varieties of *Phaseolus lunatus*. The plant material used for this study consists of 99 accessions of taxa, with 3 accessions, randomly selected by taxa, from those collected during ethnobotanical surveys.

Mounds were made in rows, 9 mounds per row and 11 rows in total. The spacing between two consecutive mounds is 2 m. Seeds were sown on June 13, 2021. The three accessions of the same taxon are sown consecutively. Each row of mounds therefore bears 3 different taxa. The field was regularly weeded and the seedlings were all supported as recommended by [13] as they are lianas (Figure 1).



**Figure 1.** Photos showing two *P. lunatus* plants wrapped in support (left: flowering plant; right: fruiting plant)

Data were collected from seedling emergence to fruit ripening. The variables collected are those proposed by [14] as the morphological descriptors of *Phaseolus lunatus*. These descriptors take into account 30 parameters including 18 qualitative and 12 quantitative plant parameters (Table 1).

For the collection of organ-related data such as leaf, flower and fruit, sampling took into account 20 organs randomly selected plants. For the hypocotyl variables, the stem, the plant itself, the data were collected per individual. The length, width and thickness of the fruit were measured using a caliper. The fruit mass was recorded using an electronic scale (OHAUS CL 501 range 500 g x 0.1).

**Table 1.** Morphological descriptors for *Phaseolus lunatus*

N°	Qualitative variables	N°	Quantitative variables
1	Type of germination	19	Length of the vegetative cycle
2	Colour of cotyledon after germination	20	Length of reproductive cycle
3	Stem pigmentation	21	Terminal leaflet length
4	Stem colour	22	Terminal leaflet width
5	Terminal leaflet shape	23	Number of pods per plant
6	Terminal leaflet colour	24	Pod length
7	Corolla colour	25	Pod width
8	Chalice colour	26	Number of seeds per pod
9	Pod curvature	27	Seed length
10	Shape of the beak of the pod	28	Seed width
11	Pod colour at maturity	29	Seed thickness
12	Immature pod colour	30	Mass of 20 seeds
13	Seed coat pattern		
14	Seed coat colour		
15	Colouring around the hilum		
16	Cotyledon colour		
17	Seed shape		
18	Plant growth		

## 2.2. Data analysis

The taxonomic position of accessions was determined as botanical species and varieties using Flores and other taxonomic documents [15, 16, 17, 18]. The morphological characteristics of the plants at various stages of their development made it possible to develop the key for determining the taxa considered as *Phaseolus lunatus* in Benin by local populations.

The morphotypes of *Phaseolus lunatus* were discriminated on the basis of the classification of [19] and [20]. The latter being based on quantitative morphological traits of the seeds, it was supplemented by qualitative discriminating morphological traits. The different morphotypes of Lima beans based on seed mass, length and width are:

- Big Lima (100-110 g, 25 mm of length, 8 mm wide) ;
- Sieva (30-45.3 g, 12 mm of length, 8 mm wide) ;

- Potato (35.5 g, 9 mm of length, 8 mm wide) ;
- Potato-Sieva (36.3 g, 11 mm of length, 8 mm wide) ;
- Sieva-Big (77.5 g, 17 mm of length, 11 mm wide) ;

Quantitative data were subjected to descriptive statistics (mean, standard deviation and coefficient of variation). The Student-Newman and Keuls test was used to compare averages using MINITAB 18.0 software. The coefficient of variation (cv) rating scale proposed by [21] was used: (1) low variation (cv = 0-10%); (2) medium variation (cv = 10-15%); (3) fairly large variation (cv = 15-44%); and (4) large variation (cv > 44%).

### 3. Results

#### 3.1. Taxonomic diversity of plants considered *Phaseolus lunatus* in Benin

A total of seven (07) species have been identified among taxa considered as local varieties of *Phaseolus lunatus* by local populations in Benin (Table 2). These 7 species belong to 4 genera, namely : *Phaseolus*, *Sphenostylis*, *Mucuna* and *Canavalia*.

**Table 2. List of species identified within accessions of plants considered as local varieties of *Phaseolus lunatus* in Benin, with their frequency of citation**

Species	Taxa indicated by farmers as local varieties of <i>Phaseolus lunatus</i>		
	Taxa code	Number of taxa	Frequency of citation
<i>Phaseolus lunatus</i>	V1-V11 ; V13-V26	25	77.58
<i>Sphenostylis stenocarpa</i>	V12	1	0.10
<i>Mucuna pruriens</i> var. <i>utilis</i>	V27, V28	2	0.30
<i>Canavalia ensiformis</i>	V29	1	10.51
<i>Canavalia africana</i>	V30	1	0.20
<i>Canavalia gladiata</i>	V31, V32	2	0.40
<i>Canavalia</i> sp.	V33	1	10.91

Of the 990 *Phaseolus lunatus* farmers surveyed in Benin, 77.58% actually recognized the species (Table 2). The remaining 22.42% grow other species that they mistakenly assimilate to local varieties of *P. lunatus*. Thus, *Canavalia ensiformis* and *Canavalia* sp. are confounded at *Phaseolus lunatus* by 10.51% and 10.91% respectively. The confusion between

*P. lunatus* and the other 4 species is made for *Canavalia gladiata* by 0.40% of informants, for *Canavalia africana* by 0.20% of farmers, for *Mucuna pruriens* var. *utilis* by 0.30% of informants and for *Sphenostylis stenocarpa* by 0.10% of farmers surveyed.

### 3.2. Variability of morphological parameters of plants considered as *Phaseolus lunatus* in Benin

#### 3.2.1. Variability of foliar parameters

The leaf characteristics of each species were presented in Table 3. *Mucuna pruriens* has the highest mean values of length ( $17.1 \pm 3.3$  cm) and width ( $11.7 \pm 2.5$  cm) of terminal leaflets. In contrast, the smallest leaf sizes are observed in *Phaseolus lunatus* ( $7.9 \pm 2.1$  cm long by  $4.6 \pm 1.1$  cm wide) and *Sphenostylis stenocarpa* ( $7.4 \pm 0.8$  cm long by  $2.9 \pm 0.3$  cm wide). Average lengths and widths differ significantly from one species to another, except for 3 *Canavalia* (*C. africana*, *C. gladiata*, *C. sp.*) which are similar ( $p < 0.001$ ).

The variations observed in these leaf parameters are quite significant in *Phaseolus lunatus*, *Mucuna pruriens* and *Canavalia ensiformis* ( $15 < cv < 44\%$ ). They are medium in *Sphenostylis stenocarpa*, *Canavalia gladiata* and *Canavalia sp.* ( $10 < cv < 15\%$ ) then low in *Canavalia africana* ( $0 < cv < 10\%$ ).

**Table 3.** Variability of foliar parameters of plants considered as *Phaseolus lunatus* in Benin

Species	Terminal leaflet length (cm)			Terminal leaflet width (cm)		
	Range of variation	Mean	CV (%)	Range of variation	Mean	CV (%)
<i>Phaseolus lunatus</i>	4.1 - 17.6	7.9±2.1d	26.1	2.4 - 8.6	4.6±1.1d	24.4
<i>Sphenostylis stenocarpa</i>	6.3 - 8.4	7.4±0.8d	10.4	2.1 - 3.2	2.9±0.3e	11.9
<i>Mucuna pruriens</i> var. <i>utilis</i>	11.3 - 21.6	17.1±3.3a	19.2	7.4 - 16.1	11.7±2.5a	21.1
<i>Canavalia ensiformis</i>	10.5 - 20	15.7±3.9b	22.2	6.6 - 13.5	10.2±2.4b	23.0
<i>Canavalia africana</i>	9.2 - 11.5	10.7±0.8c	7.5	7.1 - 9.2	7.9±0.6c	7.4
<i>Canavalia gladiata</i>	7.5 - 14.7	11.2±2.1c	14.7	5.9 - 11.3	8.7±1.4c	14.1
<i>Canavalia sp.</i>	8 - 14.4	11.6±1.9c	15.9	6.1 - 10.2	8.2±1.1c	13.6

Values with different letters in the same column are significantly different ( $p < 0.001$ )

#### 3.2.2. Variability of fruit-related parameters

The pod lengths and widths, as well as the number of seeds per taxon pod, are recorded in Table 4.

The average pod length ranged from  $6.2 \pm 1.6$  cm for *Phaseolus lunatus* to  $38.3 \pm 2.3$  cm for *Canavalia ensiformis*. Mean pod length values are significantly different for each species except for *Sphenostylis stenocarpa* ( $19.6 \pm 1.3$  cm) and *Canavalia* sp. ( $17.7 \pm 1.3$  cm) where they are similar ( $p < 0.001$ ). The highest coefficient of variation (26.1%) was recorded for pod length in *Phaseolus lunatus*. The latter therefore has quite a large variation within its individuals ( $15 < cv < 44\%$ ), in terms of the length of the pods. In contrast, the coefficients of variation indicated moderate variation in *Canavalia ensiformis* ( $10 < cv < 15\%$ ) and low variation in other species ( $0 < cv < 10\%$ ).

In terms of fruit width, the widest pods are those of *C. gladiata* and *Canavalia* sp., with similar mean values (4.0 cm and 3.8 cm respectively). *C. ensiformis* and *C. africana* also have relatively large and similar medium-wide pods (2.8 cm and 2.5 cm respectively). The smaller pods are *S. stenocarpa* (0.4 cm on average), followed by *P. lunatus* (1.6 cm) and *M. pruriens* var. *utilis* (1.6 cm). Only *S. stenocarpa* showed average pod width values that were statistically different from those of all other species ( $p < 0.001$ ), in which similarity is observed between some of them. *Phaseolus lunatus* also has the highest coefficient of variation (20.9%) for pod width ( $15 < cv < 44\%$ ). This therefore reflects a fairly large variability of its individuals, in terms of the width of the pods. In contrast, the coefficients of variation indicated a medium variation in *Sphenostylis stenocarpa* ( $10 < cv < 15\%$ ) and a low variation in other species ( $0 < cv < 10\%$ ).

As regards the number of seeds per pod, *Phaseolus lunatus* is the very small taxon compared to other species (Table 4). It has 1 to 4 seeds per pod, with an average of  $3 \pm 1$  seeds/pod. The species with the highest number of seeds per fruit are *S. stenocarpa* (11-17 seeds/pod), *C. ensiformis* (10-15 seeds/pod) and *C. gladiata* (11-13 seeds/pod). For this parameter, *P. lunatus* also showed a fairly large variation within its individuals ( $15 < cv < 44\%$ ). Coefficients of variation indicated a medium variation in *C. ensiformis*, *C. africana* and *S. stenocarpa* ( $10 < cv < 15\%$ ) and low variation in *M. pruriens* ( $0 < cv < 10\%$ ).

**Table 4.** Variability of the pod parameters of plants considered as *Phaseolus lunatus* in Benin

Species	Pod length (cm)			Pod width (cm)			Number of seeds per pod		
	Range of variation	Mean	CV (%)	Range of variation	Mean	CV (%)	Range of variation	Mean	CV (%)
<i>Phaseolus lunatus</i>	3 - 11.3	6.2±1.6 <sup>f</sup>	26.1	1.2 - 2.5	1.6±0.3 <sup>c</sup>	20.9	1 - 4	3±1 <sup>d</sup>	34.4
<i>Sphenostylis stenocarpa</i>	17.8 - 22.9	19.6±1.3 <sup>c</sup>	6.6	0.3 - 0.4	0.4±0.1 <sup>d</sup>	14.7	11 - 17	15±2 <sup>a</sup>	11.6
<i>Mucuna pruriens</i> var. <i>utilis</i>	9.6 - 10.8	9.8±0 <sup>e</sup>	3.0	1.5 - 1.8	1.6±0 <sup>c</sup>	0.3	4 - 6	5±0 <sup>c</sup>	3
<i>Canavalia ensiformis</i>	24 - 32.8	27.3±2.9 <sup>b</sup>	10.9	2.6 - 3.5	2.8±0.3 <sup>b</sup>	9.7	10 - 15	12±1 <sup>b</sup>	11.3
<i>Canavalia africana</i>	11.4 - 14.3	12.9±1.0 <sup>d</sup>	7.8	2.2 - 2.7	2.5±0.2 <sup>b</sup>	6.7	5 - 8	7±1 <sup>c</sup>	14.3
<i>Canavalia gladiata</i>	35.1 - 38.3	38.3±2.3 <sup>a</sup>	1.2	3.1 - 4.7	4.0±0 <sup>a</sup>	5.6	11 - 13	12±0.3 <sup>abc</sup>	4.7
<i>Canavalia</i> sp.	15 - 19.5	17.7±1.3 <sup>c</sup>	7.4	3.5 - 4	3.8±0.2 <sup>a</sup>	4.3	4 - 7	6±1 <sup>c</sup>	18.1

Values with different letters in the same column are significantly different ( $p < 0.001$ )

### 3.2.3. Variability of seed-related parameters

Parameters such as seed length, width, thickness and mass are summarized in Table 5. The average seed length ranged from  $2.93 \pm 0.19$  cm for *Canavalia gladiata* to  $0.81 \pm 0.04$  cm for *Sphenostylis stenocarpa*. Only *S. stenocarpa* showed statistically different mean seed length values from all other species ( $p < 0.001$ ), in which there is no significant difference between some of them. The highest coefficient of variation (30.07%) is recorded for seed length in *Phaseolus lunatus*. The latter therefore has a fairly large variation within its individuals ( $15 < cv < 44$  %), in terms of the length of the seeds. In contrast, the coefficients of variation indicated a low variation in other species ( $0 < cv < 10$ %).

For seed width, the widest seeds are *Canavalia* sp. ( $1.88 \pm 0.09$  cm) and *C. gladiata* ( $1.78 \pm 0.1$  cm). The smallest seeds are *S. stenocarpa* ( $0.67 \pm 0.03$  cm), followed by *P. lunatus* ( $0.97 \pm 0.27$  cm). Only *S. stenocarpa* showed statistically different mean seed width values from all other species ( $p < 0.001$ ), in which no significant difference was observed between some of them. The highest coefficient of variation (28.07%) is recorded for seed width in *Phaseolus lunatus*. The latter therefore has a fairly large variation within its individuals ( $15 < cv < 44$  %), in terms of the length of the seeds. In contrast, the coefficients of variation indicated a low variation in other species ( $0 < cv < 10$ %).

Seed thickness ranged on average from  $0.51 \pm 0.07$  cm for *Phaseolus lunatus* to  $1.30 \pm 0.13$  cm for *Canavalia* sp. (Table 5). The mean values of seed thicknesses are significantly different from one species to another, except in *S. stenocarpa* and *M. pruriens* where it is noted that these averages are similar ( $p < 0.001$ ). A fairly large variation in seed thicknesses is observed in its individuals of *P. lunatus*, *M. pruriens* and *C. ensiformis* ( $10 < cv < 15$ %). In the other taxa, the variation in seed thicknesses is low ( $0 < cv < 10$  %).

The mean seed masses are lower for *Phaseolus lunatus* ( $0.50 \pm 0.31$  g) and *Sphenostylis stenocarpa* ( $0.21 \pm 0.03$  g). They are higher in *Canavalia gladiata* ( $2.98 \pm 0.32$  g) and *Canavalia* sp. ( $3.00 \pm 0.53$  g). Apart from these last two species which showed similar mean masses, there is a significant difference in masses from one species to another ( $p < 0.001$ ). For this parameter, *P. lunatus* also showed a large variation within its individuals ( $cv > 44$ %). The coefficients of variation indicated a fairly large variation in *Mucuna pruriens*, *Canavalia ensiformis* and *Canavalia* sp. ( $15 < cv < 44$ %), medium variation in *C. gladiata* and *S. stenocarpa* ( $10 < cv < 15$ %) and low in *C. africana* ( $0 < cv < 10$ %).

**Table 5.** Variability of seed-related parameters of plants considered as *Phaseolus lunatus* in Benin

Species	Seed length (cm)			Seed width (cm)			Seed thickness (cm)			Seed mass (g)		
	Range of variation	Mean	CV (%)	Range of variation	Mean	CV (%)	Range of variation	Mean	CV (%)	Range of variation	Mean	CV (%)
<i>Phaseolus lunatus</i>	0.17 - 2.53	1.37±0.41d	30.07	0.57 - 1.9	0.97±0.27c	28.07	0.3 - 0.77	0.51±0.07f	14.4	0.16 - 1.6	0.50±0.31e	62.7
<i>Sphenostylis stenocarpa</i>	0.72 - 0.85	0.81±0.04e	4.37	0.6 - 0.71	0.67±0.03d	4.44	0.58 - 0.7	0.65±0.03e	4.9	0.14 - 0.3	0.21±0.03f	14.9
<i>Mucuna pruriens</i> var. <i>utilis</i>	1.3 - 1.6	1.45±0.11cd	7.91	1 - 1.2	1.09±0.07bc	6.59	0.5 - 0.7	0.66±0.07e	10.3	0.6 - 1.2	0.95±0.18d	18.9
<i>Canavalia ensiformis</i>	1.5 - 1.7	1.65±0.08bc	5.01	1.2 - 1.3	1.23±0.05b	3.82	0.6 - 1	0.74±0.11d	14.4	0.9 - 1.6	1.30±0.23c	17.65
<i>Canavalia africana</i>	1.7 - 1.9	1.78±0.08b	4.31	1 - 1.2	1.09±0.06bc	5.07	1.1 - 1.2	1.11±0.03c	2.8	1.7 - 2	1.81±0.13b	7.37
<i>Canavalia gladiata</i>	2.6 - 3.2	2.93±0.19a	6.47	1.6 - 1.9	1.78±0.1a	5.65	1 - 1.4	1.23±0.1b	8.4	2.2 - 3.5	2.98±0.32a	10.76
<i>Canavalia</i> sp.	2.3 - 3	2.81±0.19a	6.62	1.7 - 2	1.88±0.09a	4.76	1.1 - 1.5	1.30±0.13a	9.9	1.8 - 3.8	3.00±0.53a	17.64

Values with different letters in the same column are significantly different (p < 0.001)

### 3.3. Identification key for plants considered as *Phaseolus lunatus* in Benin

The established identification key is as below.

- 1a-** Annual plant with trifoliolate leaves, pure green; young stem or scabby tendril; arching pod, 3-12 cm long, containing 1-4 seeds; terminal leaflet, 6-9 cm long and 3-5 cm broad ..... *Phaseolus lunatus*
- 1b-** Annual or perennial plant with tri-leafy, dark green, sky green or glaucous leaves; young, smooth stem; more or less straight or arching pod, 12 cm long and containing more than 4 seeds; terminal leaflet, narrowly elliptical or oval ..... **2a**
- 2a-** Annual plant, slender stem; sky-green leaves, papyraceous; terminal leaflet, 6-9 cm long by 2-3.5 cm wide; pods straight and small, 17-23 cm long, 3-4 mm wide, containing 11-17 seeds ..... *Sphenostylis stenocarpa*
- 2b-** Perennial plant, robust stem; leathery and dark green leaves or papyraceous and pure green with glaucous beaches; terminal leaflet oval, 8-22 cm long by 5-17 cm wide; pods longer than 9 cm, 1.5-4 cm broad and containing 4-15 seeds ..... **3a**
- 3a-** Papyraceous and pure green leaves with glaucous beaches; terminal leaflet oval, 11-22 cm long by 7-17 cm wide; pods longer than 9-11 cm, 1.5-2 cm broad and containing 4-6 seeds ..... *Mucuna pruriens var. utilis*
- 3b-** Perennial, robust stem; leathery, dark green leaves; terminal leaflet, oval, 11-20 cm long by 5-14 cm wide; pods, 11-40 cm long, 2-5 cm broad and containing 5-15 seeds **4a**
- 4a-** Pods containing at least 10 seeds ..... **5a**
- 4b-** Pod with less than 10 seeds ..... **6a**
- 5a-** Terminal leaflet 10-20 cm long and 6-14 cm wide; pod 24-30 cm long, 2.6-3.5 cm wide and containing 10-15 seeds; white seeds, 1.5-1.7 cm long by 1.2-1.3 cm wide and 0.6-1 cm thick; hilum 0.7-1 cm long and 2 mm wide ..... *Canavalia ensiformis*
- 5b-** Terminal leaflet 38 cm long and 4 cm wide; pod 38 cm long, 4 cm wide and containing 12 seeds; seeds red, brown or black, 2.8-3.2 cm long by 1.6-1.9 cm wide and 1-1.4 cm thick; hilum 1.2-1.4 cm long and 2 mm wide ..... *Canavalia gladiata*
- 6a-** ..... Terminal leaflet 9-12 cm long and 7-9.5 cm wide; pod 11-14.5 cm long, 2-2.7 cm wide and containing 5-8 seeds; red seeds with golden yellow beaches, 1.7-1.9 cm long on 0.9-1.2 cm wide and 1.1-1.2 cm thick; hilum 0.8-1 cm long and 2 mm wide ..... *Canavalia africana*
- 6b-** ... Terminal leaflet 8-15 cm long and 6-10 cm wide; 15-20 cm long pod, 3.5-4 cm wide

and containing 4-7 seeds; grey-purplish seeds with white spots, 2-3 cm long by 1,5-2 cm wide and 1,1-1,4 cm thick; hilum 1-1,3 cm long and 2 mm wide.....*Canavalia* sp

### **3.4. Relationships between species knowledge and socio-demographic characteristics of farmers**

Table 6 summarises the results of the chi-square tests on the dependence or not of the knowledge of the species and the socio-demographic profiles of the farmers of the taxa considered as local varieties of *Phaseolus lunatus*. It highlights, at the statistical level, the existence of a very significant dependence between the knowledge of the species (*Phaseolus lunatus* and *others*) and the socio-demographic profiles of informants (phytogeographical zones, age categories, education levels, main activities and sociolinguistic groups) ( $p < 0.001$ ).

The comparison tests of the percentages of farmers who recognized or not the species *Phaseolus lunatus* showed that farmers in the Sudanian zone or those Muslims or those of Bariba and related parties sociolinguistic or Dendi and related parties sociolinguistic groups appear in the same proportions. On the other hand, at the modalities of the socio-demographic profiles, the percentages of farmers who recognized the species *Phaseolus lunatus* are significantly different and very high compared to those who assimilated other taxa to *P. lunatus*. For example, 95% of farmers in the Guinean zone have accurate knowledge of *P. lunatus*. The same is true of women (93.11%), primary school educated (91.45%), animists (91.85%) and christians (90%), traders (94.94%), Adja and related parties (99.42%), Yoruba and related parties (94.96%) and Fon and related parties (91.54%).

**Table 6.** Relationships between species knowledge and socio-demographic characteristics of farmers

Socio-demographic parameters / Modalities	Total number of informants	Number of informants who recognized <i>Phaseolus lunatus</i>	Number of informants citing other species instead of <i>Phaseolus lunatus</i>	Percentage of informants who recognized <i>Phaseolus lunatus</i>	Percentage of informants citing other species instead of <i>Phaseolus lunatus</i>	Khi-deux test results
<b>Phytogeographical zones</b>						
Guinean zone	540	514	26	95,19	4,81	X <sup>2</sup> = 212,89 DDL = 2 P = 0,000
Sudano-Guinean Zone	270	157	113	58,15	41,85	
Sudanian zone	180	97	83	53,89 <sup>a</sup>	46,11 <sup>a</sup>	
<b>Gender</b>						
Women	305	284	21	93,11	6,89	X <sup>2</sup> = 6,185 DDL = 1 P = 0,000
Men	685	484	201	70,66	29,34	
<b>Age categories</b>						
Young (age < 40)	210	157	53	74,76	25,24	X <sup>2</sup> = 32,070 DDL = 2 P = 0,000
Adult (40 ≤ age ≤ 60)	612	506	106	82,68	17,32	
Old (age > 60)	168	105	63	62,50	37,50	
<b>Education levels</b>						
Uneducated	627	439	188	70,02	29,98	X <sup>2</sup> = 56,891 DDL = 2 P = 0,000
Primary school educated	304	278	26	91,45	8,55	
Secondary school educated and over	59	51	8	86,44	13,56	
<b>Religions</b>						
Animists	319	293	26	91,85	8,15	X <sup>2</sup> = 201,33 DDL = 2 P = 0,000
Christians	340	306	34	90,00	10,00	
Muslims	331	169	162	51,06 <sup>a</sup>	48,94 <sup>a</sup>	
<b>Main activities</b>						
Farmers	890	674	216	75,73	24,27	X <sup>2</sup> = 17,439 DDL = 2 P = 0,000
Traders	79	75	4	94,94	5,06	
Other	21	19	2	90,48	9,52	
<b>Socio-linguistic groups</b>						
Adja and related parties	172	171	1	99,42	0,58	X <sup>2</sup> = 372,43 DDL = 8 P = 0,000
Bariba and related parties	82	35	47	42,68 <sup>a</sup>	57,32 <sup>a</sup>	
Dendi and related parties	25	15	10	60,00 <sup>a</sup>	40,00 <sup>a</sup>	
Fon and related parties	331	303	28	91,54	8,46	
Ottamari and related parties	127	84	43	66,14	33,86	
Peulh and related parties	58	9	49	15,52	84,48	
Yoa-Lokpa	47	14	33	29,79	70,21	
Yoruba	139	132	7	94,96	5,04	
Other	9	5	4	55,56	44,44	

X<sup>2</sup>: Khi-deux, DDL: degree of freedom, P: associated probability. Percentages followed by the same letter at the same line level are similar (p < 0.001)

### 3.5. Variability of phenotypic traits within local varieties of *Phaseolus lunatus*

Of the 75 accessions of *P. lunatus*, 13 qualitative morphological characters are constant and 6 showed variability (Table 7). Thus, 100% of accessions have an epigeal germination, a whitish cotyledon before and after germination, a greenish stem, greenish terminal leaflet, flowers with greenish calyx and whitish corolla, curved and greenish pods at the immature stage and khaki at maturity, beaks of curved pods in the opposite direction of the curvature of the pods and of lianescent and voluble plants.

On the other hand, the 6 qualitative traits presenting variability within the accessions are: the shape of the terminal leaflets, the seed coat pattern, the seed coat colour, the colour of the hilum belt, the shape and size of the seed. Seed coat colour showed the greatest dispersal with 14 modalities, the most important of which are the reddish colour observed in the seeds of 28% of accessions and the cream colour (16% of accessions). Two sets of accessions are distinguished by considering the seed coat pattern: seed with variegated coat (60%) and seed with one-colour coat (40%).

**Table 7.** Variability of qualitative morphological characteristics of accessions of *Phaseolus lunatus*

N°	Morphological parameters	Modalities	Relative frequency (%)
1	Type of germination	spotted	100
2	Colour of cotyledon before germination	white	100
3	Colour of cotyledon after germination	white	100
4	Stem pigmentation	absent	100
5	Stem colour	greenish	100
6	Terminal leaflet shape	lanceolate	94
		oval-lanceolate	6
7	Terminal leaflet colour	greenish	100
8	Corolla colour	whitish	100
9	Chalice colour	greenish	100
10	Pod curvature	curved	100
11	Shape of the beak of the pod	opposite direction of pod curvature	100
12	Pod colour at maturity	khaki	100
13	Immature pod colour	green	100
14	Seed coat pattern	one-colour	40
		variegated	60
15	Seed coat colour	creme	16
		orange-yellow crest	4

		black crest	4
		red crest	4
		dark red crest	4
		light grey	4
		grey ash stained with red	8
		khaki	4
		black	4
		yellow light red	4
		dark red	4
		deep red yellow	4
		black-spotted red	8
		reddish	28
16	Colouring around the hilum	absent	76
		yellow	4
		black	8
		red	12
17	Seed shape	flattened	48
		arched	4
		spherical or spherical	48
18	Seed size	small	40
		medium	36
		high	24
19	Plant growth	indefinite	100

From the combination of the 6 qualitative discriminating variables, the 25 local varieties of *P. lunatus*, representing the 75 accessions, are grouped into 9 morphotypes (Figure 2). The qualitative and quantitative morphological characteristics of the seeds of the different morphotypes are summarized in Table 8.

**Table 8.** Phenotypic Characteristics of distinguished *P. lunatus* morphotypes

Morphotypes	Local varieties	Qualitative morphological traits	Seed length (cm)	Seed width (cm)	Seed thickness (cm)	Seed mass (g)
M1 Potato-Sieva 1	V1, V2, V3, V4, V5, V6, V7, V18	Small seed, one-colour coat, reddish, hilum not surrounded by colouring	1.13±0.11	0.84±0.11	0.51±0.06	0.34±0.08
M2 Potato-Sieva 2	V8	Small seed, one-colour coat, black, hilum not surrounded by colouring	1.03±0.04	0.84±0.04	0.54±0.03	0.32±0.03
M3 Potato-Sieva 3	V9, V10	Small seed, with one-colour coat, cream, hilum surrounded by a red or black colour	1.18±0.17	0.81±0.70	0.58±0.05	0.38±0.09
M4	V11, V20	Small seed, one-	1.21±0.12	0.80±0.10	0.47±0.07	0.34±0.06

Potato-Sieva 4		colour coat, cream, hilum not surrounded by colouring				
M5 Sieva 1	V13, V14, V15, V16, V17	Medium seed, variegated coat, hilum not surrounded by colouring	1.19±0.16	0.86±0.06	0.49±0.06	0.34±0.07
M6 Sieva 2	V19	Medium seed, one- colour coat, reddish, hilum not surrounded by colouring	1.43±0.04	1.00±0.07	0.45±0.05	0.48±0.04
M7 Big Lima	V21, V22, V23, V24	Seed large, flattened, variegated coat, hilum not surrounded by colouring	2.16±0.21	1.49±0.23	0.57±0.09	1.11±0.29
M8 Big Sieva 1	V25	Seed large, flattened, single-coloured coat, whitish, hilum not surrounded by colouring	2.03±0.11	1.36±0.07	0.52±0.04	0.95±0.12
M9 Big Sieva 2	V26	Seed large, flattened, one-colour coat, reddish, hilum not surrounded by colouring	1.94±0.11	1.31±0.06	0.44±0.05	0.88±0.13



**Figure 2.** Morphotypes of *Phaseolus lunatus* grown in Benin

#### 4. Discussion

The results of this study on the morphological and taxonomic diversity of the taxa considered as those of *Phaseolus lunatus* constitute a very good contribution to the knowledge of the flora of Benin. Indeed, they have made it possible to identify within cultivated taxa such as the Lima bean, 7 species namely: *Phaseolus lunatus*, *Sphenostylis stenocarpa*, *Mucuna pruriens* var. *utilis*, *Canavalia africana*, *Canavalia ensiformis*, *Canavalia gladiata* and *Canavalia* sp. Among these species, *Canavalia gladiata* and *Canavalia* sp. have not previously been recorded [15], so these two (02) taxa are new species for Benin.

These results also highlight the limitations of parataxonomy that have led local populations to consider the seven species as *Phaseolus lunatus* while, apart from the latter, the other six (06) even belong to other genera and have completely different morphological characteristics (leaves, stem, flowers, pods and seeds). These results show that the confusion between taxa would not be apparent but full of substance. In fact, one might think that it is because of the same shared uses that these species are designated by the same names from one phytogeographical zone to another or from a set of sociolinguistic groups sharing the same cultural areas with each other, as highlighted by [15].

The degree of taxonomic confusion is not the same from one phytogeographical zone to another. Indeed, the sociolinguistic groups at which *Phaseolus lunatus* is most recognized are adja, fon and yoruba with their related parties. These groups are those strongly represented in Guinean zone where the number of farmers recognizing the species is also very high compared to the other phytogeographical zones of Benin. On the other hand, the sociolinguistic groups in which the greatest numbers of informants who cited other species instead of *P. lunatus* are those who share the Sudanian-Guinean and Sudanian zones. This would reflect an affinity of farmers in the Guinean zone to *Phaseolus lunatus* compared to those in the Sudanian zone. This differential attachment to taxa considered as local varieties of *P. lunatus* could be linked to the migratory flow of peoples; the sociolinguistic groups of the Guinean zone having come from Nigeria and Togo and those of the Sudanian zone of Burkina and Niger [22]. This hypothesis is supported by the fact that the Adja and Fon farmers, established in the Sudanian zone have the same parataxonomic performance and cultivate exactly *Phaseolus lunatus* as their parents in the Guinean zone of the country.

The results from the descriptive statistics of the quantitative morphological parameters of the 7 taxa considered as local varieties of Lima beans showed high values of the coefficient

of variation of all parameters measured in *Phaseolus lunatus*. This indicates phenotypic and genotypic heterogeneity of *P. lunatus* individuals and confirms the perception of local populations that find several local varieties. This study ranked the local varieties grown in 9 morphotypes on the basis of the morphological traits related to the seeds since the latter are those that showed variability among the 18 qualitative morphological traits collected. It thus highlights the importance of seed characteristics as indicators of morphotypes of *Phaseolus lunatus* and would justify parataxonomic knowledge of local populations that are based mainly on the colour, shape and size of seeds. Similar observations have also been made in parataxonomic and morphological studies of *Phaseolus lunatus* [23, 24] and also of *Phaseolus vulgaris* [25, 10]. Morphological traits could be hereditary characteristics and therefore deserve special attention during breeding programs.

The nine (09) morphotypes discriminated in this study took into account all the 05 morphotypes of [19] and [20] classified on the basis of seed mass, length and width. The lack of limitation to the quantitative traits of seeds (mass, length and width) in distinguishing morphotypes is related to the fact that these characters proposed by [19] and [20] are subjective characters because they can be influenced by environmental conditions. The subjectivity in the exclusive use of this classification based on quantitative traits is increased all the more because the intervals of variation of the quantitative values are indicated whereas they are sometimes close to each other from one morphotype to another. The coupling of the qualitative traits of the seeds with those quantitative in the discrimination of the morphotypes thus finds its basis in its concordance with the bases of vernacular nomenclature of the species. The level of heredity of these two categories of morphological parameters remains to be determined in order to further refine the varietal groups within *Phaseolus lunatus*.

## **5. Conclusion**

The present study has made it possible to remove the taxonomic confusions maintained by farmers at the level of *Phaseolus lunatus*. The taxa grown as such by these farmers have been identified and characterized. They contain seven (07) species, namely *Phaseolus lunatus*, *Sphenostylis stenocarpa*, *Mucuna pruriens* var. *utilis*, *Canavalia africana*, *Canavalia ensiformis*, *Canavalia gladiata* and *Canavalia* sp. The last two species are new to the Analytical Flora of Benin and constitute a good contribution to the knowledge of the flora of Benin. Morphological characteristics, with a taxonomic determination key, have been

reported. They will allow farmers and researchers to access the exact taxonomic position of each of these taxa, all grown in Benin as local varieties of *Phaseolus lunatus*.

The quantitative variables of *Phaseolus lunatus* all showed great variability within the accessions collected. However, of the 19 qualitative variables, only 6 showed variability. Of these 6 variables, 5 are seed-related and constitute the most important discrimination traits of the 9 identified morphotypes. Molecular genetic analysis is therefore essential to understand the level of inheritance of these morphological parameters in order to further refine the varietal groups within *Phaseolus lunatus*.

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