# Phenotypic Dispersion of Landrace Lima Bean Varieties Using Multidimensional Scaling

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# Abstract

Studies about phenotypic diversity are common in germplasm banks. The inference about this variability must be realized through several methods. The multidimensional scaling is a multivariate technique that has not yet been well explored in plant breeding programs. The objective of this study was to evaluate the phenotypic dispersion of landrace lima bean varieties using the non-metric multidimensional scaling technique (nMDS) based on seed morphology. Seeds of 25 lima bean accessions were characterized based on the morphological descriptors proposed by the International Plant Genetic Resources Institute. Distance matrices between the accessions were estimated based on the qualitative and quantitative variables, in addition to simultaneous analysis of the qualitative and quantitative data, using the Mahalanobis and Gower distances. The distances were represented by non-metric multidimensional scaling. The adjustment level of the nMDS mapping was calculated using Kruskal's *Stress*. The scaling based on the quantitative and mixed data was efficient to represent the distances of the lima bean accessions in the bidimensional plane presenting *Stress* less than 20%. Divergent accessions, such as 11, 13, 17 and 25 were identified. The inclusion of qualitative character analysis. The nMDS must be used as a complementary technique to those commonly employed in studies of phenotypic diversity in lima beans.

Keywords: Phaseolus lunatus L., multivariate analysis, germplasm, plant breeding

# 1. Introduction

Lima bean (*Phaseolus lunatus* L.) presents rusticity and adaptability to the environment (Vieira, 1992). Its seeds are good sources of nutrients such as proteins, carbohydrates, fibers and minerals (Kathirvel & Kumudha, 2011). In addition, this crop has an important social aspect in many countries in South America.

In Brazil, more specifically in the Northeast region, the lima bean germplasm used by farmers comes from their own crops and trading between rural communities (Penha et al., 2017). Often, this germplasm is maintained in landrace seed banks, presenting great genetic variability for agronomic traits of interest (Santos et al., 2002). However, Camacho-Pérez et al. (2018) emphasize the reduction of genetic diversity in landrace varieties mainly due to changes in traditional agriculture and market preferences, assuming this as a global problem with high risk to the food security of future generations.

The characterization and the study of the genetic divergence of lima bean seeds allow the knowledge of the germplasm and the development of strategies of conservation and improvement of the species (Valls, 2007). The genetic divergence in lima bean was studied using morphoagronomic and molecular markers (Martinéz-Castillo et al., 2004; Guimarães et al., 2007; Carmo et al., 2013; Silva et al., 2015) using mainly grouping methods.

According to Silva et al. (2014), several techniques are necessary to make robust inferences about phenotypic variability.

Multidimensional scaling is a multivariate technique that includes a family of spatial distance models as a way of representing proximity data (Carroll & Arabie, 1980; Davison, 1983). Data of proximity provide information regarding similarity and dissimilarity between individuals as points in the space of low dimension, usually bidimensional or three-dimensional (Scholten & Caldeira, 1997; Manly, 2004; Borg & Groenen, 2005).

According to Silva et al. (2014) the scaling is especially useful when the relationship between individuals is unknown, which is very common in germplasm banks, presenting the potential for use in plant breeding programs. Studies of phenotypic diversity among accessions were only found for garlic (Silva et al., 2014) and cocoa (Leal et al., 2008), no records of the use of this technique in lima beans and other important crops were found. The use of bidimensional scaling has been more common in the study of natural populations (Telles et al., 2001; Souza et al., 2015; Higuchi et al., 2018; Silva et al., 2018), in the construction of genetic linkage maps (Preedy & Hackett, 2016) and not frequent in plant breeding when using molecular data, such as studies of genetic distances between cultivars and varieties of lima bean (*P. lunatus*) and common bean (*P. vulgaris*) (Beebe et al, 1995; Nienhuis et al., 1995, Fuente et al., 2012). However, the routine of conventional breeding programs, are mainly based on phenotypic data based on qualitative and quantitative characters, and scaling may be highly applicable for studies of dispersion between accessions.

The objective of this work was to study the phenotypic dispersion of landrace lima bean varieties using the non-metric multidimensional scaling technique (nMDS) based on seed morphological characters.

# 2. Method

#### 2.1 Plant Material and Characterization

The experiment was carried out at the Laboratório de Melhoramento e Análise de Dados of the *Campus* Professora Cinobelina Elvas of the Universidade Federal do Piauí (CPCE/UFPI), located in Bom Jesus-PI, Brazil. The seeds were collected in private properties and fairs of different municipalities, identified and stored in the Germplasm Bank of CPCE/UFPI (Table 1).

CODE	Popular name	Origin
CPCE 01	Orelha de Vó	Brejo Santo, CE
CPCE 02	Orelha de Vó	Remígio, PB
CPCE 03	Raio de Sol Preta	Brasília, DF
CPCE 04	Raio de Sol Vermelha	Brejo Santo, CE
CPCE 05	Fava Branca	Baixa grande do ribeiro, PI
CPCE 06	Fava Mulatinha	Brejo Santo, CE
CPCE 07	Boca de Moça	Remígio, PB
CPCE 08	Fava moita	Remígio, PB
CPCE 09	Roxinha	Remígio, PB
CPCE 10	Fava Branca	Brejo Santo, CE
CPCE 11	Amarelo Cearense	Remígio, PB
CPCE 12	Rosinha	Remígio, PB
CPCE 13	Non identified*	Mato Grosso
CPCE 14	Branca Gigante	Baixa Grande do Ribeiro, PI
CPCE 15	Branca Gigante	Remígio, PB
CPCE 16	Cana longa	Remígio, PB
CPCE 17	Eucalipto	Remígio, PB
CPCE 18	Branca Pequena	Remígio, PB
CPCE 19	Branca Gigante	Guariba, PI
CPCE 20	Branca Gigante	Tauá, CE
CPCE 21	Orelha de Velho	Brejo Santo, CE
CPCE 22	Fava rajada	Esperança, PB
CPCE 23	Orelha de Vó	Esperança, PB
CPCE 24	Branca Pequena	Cuité, PB
CPCE 25	Cana Longa	Esperança, PB

Table 1. Accessions of lima bean (*Phaseolus Lunatus* L) from the Germplasm Bank of the Universidade Federal do Piauí, Bom Jesus-PI, Brazil

The seeds were characterized according to the following morphological descriptors for *Phaseolus lunatus* L., recommended by the *International Plant Genetic Resources Institute* (IPGRI, 2001): Seed Length (SL), Seed Width (SW), Seed thickness (ST), Seed color (SC), patter color of tegument (CPT), second patter color of tegument (SPC), pattern of seed tegument (SPTG), The seeds were also classified according to their size (Mateo Box, cited by Vilhordo et al., 1996) and shape, by the coefficients Length/width (L/W) and thickness/width (T/W), according to Puerta Romero, cited by Vilhordo et al. (1996).

#### 2.2 Statistical Analysis

Twenty-five accessions of lima bean were evaluated, in a completely randomized design with 15 replicates, where each seed represented a replicate. Based on the quantitative variables, were calculated the generalized distance of Mahalanobis (Mahalanobis, 1936), obtained through the expression:

$$D^{2}_{ii}{}' = \delta' \Psi^{1} \delta \tag{1}$$

Where,  $D_{ii'}^2 = Mahalanobis$  distance among accessions i and i';  $\Psi = is$  the matrix of residual variances and covariance;  $\delta' = [d_1, d_2, ..., d_n]$ , where  $d_n = Y_{ij} - Y_{i'j}$  and  $d_n$  = represents the difference between mean of two accessions i and i'for each evaluated characteristic;  $Y_{ij} =$  mean of the i-th accession relative to the j-th parameter.

A matrix with qualitative data was constructed using Gower's distance (Gower, 1971), employing the equation:

$$S_{ij} = \frac{\sum_{k=1}^{n} W_{ijk} S_{ijk}}{\sum_{k=1}^{n} W_{ijk}}$$
(2)

Where,  $S_{ij}$  is the distance between the accessions i and j;  $W_{ijk}$  is a weight given to the comparison ijk, assigning value 1 for valid comparisons and value 0 for invalid comparisons (when the value of the variable is absent in one or both individuals).  $S_{ijk}$  is the contribution of variable k in the similarity between individuals i and j, having values between 0 and 1. For a nominal variable, if the value of variable k is the same for both individuals, i and j, then  $S_{ijk} = 1$ , otherwise, it is equal to 0. Simultaneous analysis of qualitative and quantitative data was performed on the basis of the two matrices of distance.

It was also applied the non-metric multidimensional scaling (nMDS) for a graphic representation in the bidimensional space of the distance matrices. The analysis of the level of non-fit of the nMDS mapping was calculated using Kruskal's *Stress* (Kruskal, 1964).

$$Stress_{1} = \left[\frac{\sum_{i=1}^{n-1} \sum_{j>1}^{n} (d_{ij} - \tilde{d}_{ij})^{2}}{\sum_{i=1}^{n-1} \sum_{j>1}^{n} \tilde{d}_{ij}^{2}}\right]^{\frac{1}{2}}$$
(3)

Where,  $d_{ij}$  is the distance between the accessions i and j;  $\tilde{d}_{ij}$  is the space distance between the accessions i and j.

The *Stress* values table proposed by Sturrock and Rocha (2000) was used to validate the result obtained with the nMDS solution, comparing the value obtained with the tension value generated from matrices with the same number of objects and the same number of dimensions. All analyses were performed with software R version 3.0.3 (R Core Team, 2014).

#### 3. Results and Discussion

The seed germplasm bank of the UFPI/CPCE, presents a great phenotypic diversity, evidenced by the dispersion represented by multidimensional scaling (Figure 1).

The non-metric multidimensional scaling technique (nMDS) presented the distance between lima bean accesses in the graphical form (Figure 1), in which a large dispersion can be observed for the three representations. Scaling presented 21.77% of *Stress* value for qualitative data (Figure 1A), 4.17% for quantitative data (Figure 1B) and 18.47% when the matrix distance was used for mixed data (qualitative + quantitative) (Figure 1C). According to the table proposed by Sturrock and Rocha (2000), with n = 25 accessions and k = 2 dimensions, there is a probability (p < 0.01) that the accessions are randomly arranged in bi-dimensional space. The lower the *Stress* value, the more real is the position of the points in the generated scaling, with little distortion in the data with the reduction of the dimensions (Clarke & Warwick, 2001).

However, *Stress* values of up to 20.00% are acceptable in this type of analysis (Kruskal et al., 1964; Meyer et al., 2004). According to this criterion, the scaling based on the quantitative and mixed data was efficient to represent the distances of the lima bean accessions in the graphic, indicating a good ordering. Thus, through this analysis, it is possible to identify more divergent materials, which contain phenotype of interest for the genetic improvement of the species. In this experiment, the dispersion of accessions based on qualitative data was unsatisfactory.

Based on the quantitative data, it can be observed that accessions 5, 9, 11, 14, 15 17, 19 and 25 are geographically isolated from the others, and can be considered more divergent. Accession 25 (Cana Longa) from Paraíba state, presented thick seeds, being the only variety classified as full seed. It is important to note that accesses 14, 15 and 19, although belonging to the same variety, known as Branca Gigante, are not close, considering the dimensions of the scaling, also distancing itself from the accession 20, which belongs to the same variety. Accession 20, for example, is closer to accession 01 (Orelha de Vó).

These results show the influence of the sites where the accessions were collected, given the quantitative nature of the variables, the seeds may have been submitted to different edaphoclimatic conditions during their development. In this sense, it can be selected accessions that have characteristics of interest to the breeding program, such as: among the four accessions of Branca gigante, the accessions 14 and 15 from Piauí and Paraiba, respectively, present larger and wider seeds, which would increase production indexes. However, the accessions 19 and 20 present smaller but thicker seeds, which could be desirable for consumers because of their similarity to common beans. Using other multivariate techniques, Carmo et al. (2013) also identified divergent accessions mainly for lima bean grain diameter and thickness.

The other accessions can be grouped since they are closer in the bi-dimensional plane, sharing similarities for the quantitative characters of seeds. It can be highlighted that the proximity of the accessions 23 (Orelha de Vó) and 24 (Branca Pequena), both collected in the state of Paraíba, have a similar form, based on the J coefficient. A larger group could be represented by the accessions 4, 8, 10, 18, 21, 22 that present average length ranging from 11.46 mm (04) to 13.61 mm (22), average width between 8.61 mm (04) to 9.89 mm (21), classified as medium to large seeds (Table 2).

The estimated distances for the mixed data, resulted in a greater dispersion of the accessions, confirming the phenotypic diversity in the lima bean seeds given by the inclusion of the qualitative characters (Figure 1C). According to Vargas et al. (2003) the seeds of this species exhibit a great variety of color and size and these characteristics are used as a criterion to explain the origin and the genetic diversity. This fact can be observed when analyzing the representation of the distances between accessions 1, 2 and 23, all belonging to the variety known as Orelha de Vó when including the qualitative variables, these accessions were close in the bidimensional plane, different from what was observed when considering only quantitative data. Thus, multidimensional scaling for mixed data, with acceptable *Stress* level, may better discriminate the genotypes desirable for selection. Some authors, although did not use multidimensional scaling, have verified the importance of using mixed data, from Gower's distance to analyze the genetic diversity among accessions (Sudré et al., 2010; Oliveira et al., 2015; Mesquita et al., 2016; Upadhyaya et al., 2017).

Again, based on the mixed data, accessions 25 and 17 were more divergent, and also did the accession 13. Despite the randomness of the dispersion based on the qualitative data, the divergence for accessions 13 (unidentified variety) and 17 (Eucalipto) is also observed in Figure 1A. Both accessions are unique specimens in the germplasm bank, differing mainly for seed color, pattern color and second pattern color (Table 2). The color of the seed coat is a factor that can contribute to the good commercialization of the product and this will depend on the preference of the consumers in the different regions (Guimarães et al., 2007). Accession 25 (Cana Longa) presents traits of interest for plant breeding such as normal-sized grains and classified as full. Nienhuis et al. (1995), using scaling based on RAPD markers, grouped lima bean accessions, made an association with the phenotypic data, and verified that these accessions were similar in size and color, emphasizing once again the importance of the qualitative variables.

The success of a breeding program, among other factors, depends on the evaluation carried out with the accesses of the genetic diversity existing in the active germplasm banks in order to determine the potential of higher potential materials that meet the demand of the producer and consumer market. Knowledge about the phenotypic relationship between accessions of germplasm banks may reveal sources of desirable traits for the definition of strategies, planning and optimization of lima-bean breeding programs.

The study of these relationships, as verified in this work, can be performed through nMDS, as a complementary technique to those commonly used, since there may be disagreement between multivariate methods (Carmo et al., 2013). Silva et al. (2014) reported that the representation of the phenotypic distance of garlic cultivars by multidimensional scaling was more effective than the UPGMA clustering method. Therefore, it is necessary to use several methods and the scaling is an option that must be considered, especially when combined with the simultaneous analysis of qualitative and quantitative characters.



Figure 1. Graphical dispersion of 25 lima bean (*Phaseolus lunatus* L.) accessions through multidimensional scaling based on qualitative (A), quantitative (B) and mixed (C) data

Table 2. C	Duantitative and o	qualitative mo	rphological	characteristics	of lima beau	1 (Phaseolus	lunatus L.)
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Acession	SL	SW	ST	J	Н	SC	CPT	SPC	PSTG	SIZE	SHAP1	SHAP2
CPCE 01	16.29	12.05	6.39	1.36	0.53	White	Absent	Black	Pattern 7	Large	Spherical	Flat
CPCE 02	16.25	10.68	5.85	1.52	0.55	White	Absent	Black	Pattern 6	Large	Elliptical	Flat
CPCE 03	15.69	10.84	5.89	1.46	0.55	Gray	Black	Black	Pattern 6	Large	Elliptical	Flat
CPCE 04	11.46	8.61	5.78	1.33	0.67	White	Red-Purple	Brown	Pattern 6	Large	Spherical	Flat
CPCE 05	9.87	8.31	6.36	1.19	0.77	White	Absent	Absent	Absent	Normal	Spherical	Semi-full
CPCE 06	16.63	11.24	5.89	1.48	0.53	Light-Gray	Dark-Brown	Absent	Pattern 1	Normal	Elliptical	Flat
CPCE 07	15.01	10.26	6.02	1.47	0.59	Gray	Red	Red	Pattern 5	Medium	Elliptical	Flat
CPCE 08	12.16	9.09	5.92	1.34	0.65	Gray	Light-Brown/Orange	Brown	Pattern 3	Medium	Spherical	Flat
CPCE 09	13.21	9.61	5.05	1.37	0.53	Red-Purple	Absent	Absent	Absent	Medium	Spherical	Flat
CPCE 10	12.19	8.58	6.07	1.42	0.75	White	Absent	Absent	Absent	Normal	Elliptical	Semi-full
CPCE 11	18.99	13.98	6.65	1.36	0.47	Light-Brown	Absent	Absent	Absent	Large	Spherical	Flat
CPCE 12	17.11	11.89	5.83	1.45	0.49	Orange	Absent	Absent	Absent	Medium	Elliptical	Flat
CPCE 13	15.97	11.69	5.52	1.38	0.48	Light-Brown	Light-Brown/Orange	Absent	Absent	Medium	Spherical	Semi-full
CPCE 14	20.43	14.06	5.09	1.46	0.36	White	Absent	Absent	Absent	Large	Elliptical	Flat
CPCE 15	20.13	13.00	6.95	1.55	0.54	White	Absent	Absent	Pattern 1	Large	Elliptical	Flat
CPCE 16	16.96	10.85	5.71	1.57	0.53	Light-Brown	Dark-Brown	Absent	Pattern 4	Normal	Elliptical	Flat
CPCE 17	11.94	7.93	5.44	1.50	0.69	Light-Brown	Dark-Brown	Black	Pattern 7	Normal	Elliptical	Flat
CPCE 18	12.94	9.63	5.90	1.34	0.61	White	Absent	Absent	Pattern 7	Medium	Spherical	Semi-full
CPCE 19	18.11	13.36	6.06	1.38	0.46	White	Absent	Absent	Absent	Small	Spherical	Flat
CPCE 20	16.50	10.61	6.19	1.56	0.58	White	Absent	Absent	Absent	Large	Elliptical	Flat
CPCE 21	13.45	9.89	6.32	1.36	0.64	White	Absent	Absent	Absent	Large	Elliptical	Flat
CPCE 22	13.61	9.69	6.11	1.40	0.63	White	Red-Purple	Absent	Pattern 5	Large	Spherical	Flat
CPCE 23	10.93	8.13	5.27	1.35	0.65	White	Black	Absent	Pattern 6	Large	Spherical	Flat
CPCE 24	11.64	8.51	5.35	1.37	0.63	White	Light-Brown/Orange	Red-Purple	Pattern 3	Medium	Spherical	Semi-full
CPCE 25	11.20	8.07	8.41	1.39	1.11	Gray	Light-Brown/Orange	Red-Purple	Pattern 3	Normal	Spherical	Cheia

*Note.* SL = Seed Length, SW = Seed Width, ST = Seed thickness, J = coefficients Length/width, H = Coefficients thickness/width, SC = Seed color (SC); CPT = Patter color of tegument; SPC = Second patter color of tegument; PSTG = Pattern of seed tegument.

# 4. Conclusions

The proposed statistical analysis was useful to evaluate the diversity among lima-bean accessions of the CPCE/UFPI germplasm bank. Based on the technique of multidimensional non-metric scheduling, it was possible to identify the accessions 11, 13, 17 and 25 as the most divergent. These accessions have the potential to be used as parents in breeding programs of this species. New acquisitions of germplasm, together with the amplification of morphoagronomic characteristics, with evaluations using molecular markers, cytogenetic and

palynological techniques, will contribute qualitatively and quantitatively to the conservation and selection of new genotypes with the adaptive potential of the southern edaphic conditions of the state of Piauí-Brazil.

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