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Phenotypic Identification of Landraces of *Phaseolus lunatus* L. from the Northeastern Region of Brazil Using Morpho-Colorimetric Analysis of Seeds

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Abstract: *Phaseolus lunatus* L. is a species of landrace bean widely cultivated in Northeast Brazil. The integration of new technologies in the agricultural sector has highlighted the significance of seed images analysis as a valuable asset in the characterization process. The objective was to assess the morphology of 18 *P. lunatus* varieties gathered from four states in the Brazilian Northeast. To achieve this, 100 seeds from each variety were utilized, and their images were acquired using a flatbed scanner with a digital resolution of 400 dpi. Subsequently, the images were processed using the ImageJ software package for analyzing seed size, shape and color characteristics. Statistical analyses were performed with SPSS software applying stepwise Linear Discriminant Analysis (LDA). The overall accuracy rate for correct identification was 80.5%. Among the varieties, the lowest classification percentage was attributed to the 'Coquinho Vermelha' variety (39%), while the highest rates were observed for 'Fava Roxa' and 'Fava de Moita' (98%). The morpho-colorimetric classification system successfully discriminated the varieties of *P. lunatus* produced in the northeastern region of Brazil, highlighting the -+*/high degree of diversity within them. In particular, seeds with uniform coloring or clearly defined secondary color patterns were easier to classify. The varieties showed low correlation, forming distinct groups based on background color, secondary color, or seed size.

Keywords: agrobiodiversity; fabaceae; lima bean; seed image analysis

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1. Introduction

Phaseolus lunatus L., commonly known as lima bean, is a species of creole bean, widely cultivated by small farmers in Northeast Brazil [1]. This Brazilian region is responsible for 99% of national production, with the states of Ceará, Paraíba, Pernambuco, and Piauí standing out as the largest producers [2]. *P. lunatus*, in addition to being a source of nutrients in the diet of rural populations in South America and Africa, also contributes to the generation of income and maintenance of people in the countryside [3].

Although *P. lunatus* lacks official varieties registered in Brazil, it is recognized by various popular names, varying across regions and communities, bestowed by traditional populations based on plant and seed characteristics such as size, shape, color, pattern, and weight. Plant sizes can be either determined or indeterminate in growth, reflecting the genetic diversity distributed among gene pools, evident in the morphological traits of plants and seeds [4,5].

In the use of new technologies that assist in the morphological characterization of plant varieties, one of the tools that has stood out is the analysis of digital images. This technique involves appropriate software for digital seed analysis and has been effective in characterizing different plant species. Using this technique, researchers have obtained good results in characterizing various wild species, including, e.g., *Linaria purpurea* group [6] and *Santolina* L. [7]. Consistent results have also been obtained in the characterization of cultivated species of *Phaseolus vulgaris* L. [8] and *P. lunatus* L. [9].

In this research, the scientific objective was to evaluate the morphological diversity of *P. lunatus* varieties collected in four states of northeastern Brazil (Ceará, Paraíba, Piauí, and Rio Grande do Norte) using the morpho-colorimetric analysis system already used on other species to verify its efficiency.

2. Materials and Methods

The research was conducted at the Seed Analysis Laboratory (LAS) of the Federal Rural University of the Semi-Arid (UFERSA), Mossoró campus, RN, Brazil and at the Laboratory of Microscopy and Digital Morphometric Analysis of the University of Cagliari, Italy. The materials consisted of 18 seed varieties of *P. lunatus* L. from the States of Ceará (CE), Paraíba (PB), Piauí (PI), and Rio Grande do Norte (RN), harvested in 2021 and 2022, and stored for seven months in controlled environments (18 °C and 50% relative humidity) (Figure 1, Table 1).

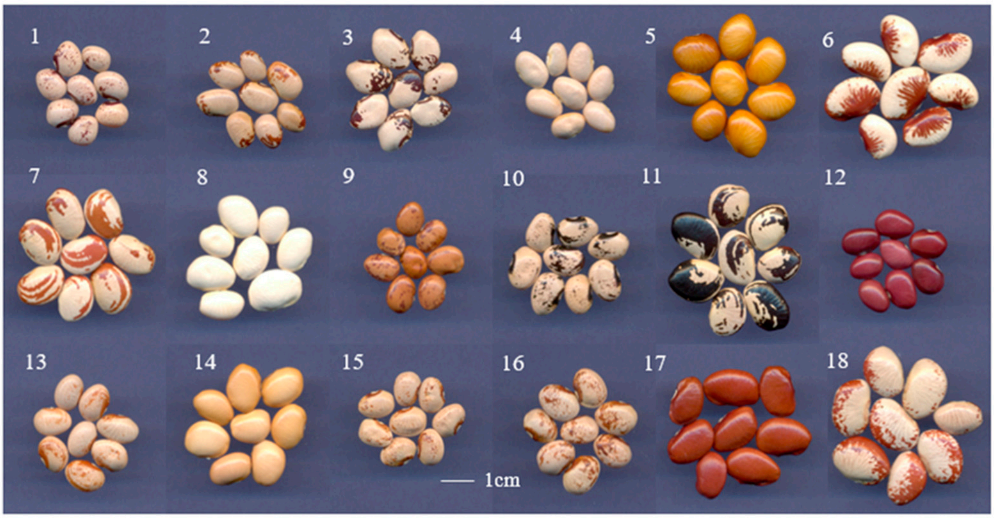

Figure 1. Varieties of *P. lunatus* L. collected in Northeast Brazil.

Table 1. Popular denominations of *P. lunatus* L. varieties and collection location in the northeastern region of Brazil.

Variety	Popular Name	Collection Location	Coordinates
1	Fava Guandu	Tenente Laurentino Cruz–RN	6°08′52.0′ S; 36°43′08.0′ W
2	Coquinho Vermelha	Belém–PB	6°44′49.0′ S; 35°31′08.0′ W
3	Coquinho Preta	Barra de Santa Rosa–PB	6°43′12.0′ S; 36°03′39.0′ W
4	Branca Miúda	Barra de Santa Rosa–PB	6°43′12.0′ S; 36°03′39.0′ W
5	Amarela Cearense	Jucás–CE	6°31′30.0′ S; 39°31′40.0′ W
6	Raio de Sol	Iguatu–CE	6°21′32.0′ S; 39°17′56.0′ W
7	Orelha de Vó Vermelha	Catolé do Rocha–PB	6°20′38.0′ S; 37°44′49.0′ W
8	Branca	Jucás–CE	6°31′30.0′ S; 39°31′40.0′ W
9	Bacural	Cacimba de Dentro–PB	6°38′31.0′ S; 35°47′24.0′ W
10	Cara Larga Preta	Cacimba de Dentro–PB	6°38′31.0′ S; 35°47′24.0′ W
11	Fava Vovó	Catolé do Rocha–PB	6°20′38.0′ S; 37°44′49.0′ W

Table 1. *Cont.*

Variety	Popular Name	Collection Location	Coordinates
12	Fava Roxa	Cacimba de Dentro–PB	6°38′31.0′ S; 35°47′24.0′ W
13	Cara Larga de Moita	Cacimba de Dentro–PB	6°38′31.0′ S; 35°47′24.0′ W
14	Mulata	Iguatu–CE	6°21′32.0′ S; 39°17′56.0′ W
15	Fava de Moita	Cacimba de Dentro–PB	6°38′31.0′ S; 35°47′24.0′ W
16	Cara Larga	Cacimba de Dentro–PB	6°38′31.0′ S; 35°47′24.0′ W
17	Fava de Sopa	São Raimundo Nonato–PI	9°00′54.0′ S; 42°41′56.0′ W
18	Raio de Sol	Cruzeta–RN	6°24′43.0′ S; 36°47′24.0′ W

2.1. Image Analysis System

Digital images of 100 seeds from each variety were acquired using a flatbed scanner (HP Scanjet G2410, Palo Alto, CA, USA) with a digital resolution of 400 dpi and a scanning area not exceeding 1024 × 1024 pixels that worked with 2D images. To do this, the seeds were randomly arranged in the scanner tray, so that they did not touch each other, and covered with a paper box with a blue background to avoid interference from external light. Images were processed using the ImageJ v 1.54f software package (http://rsb.info.nih.gov/ij (accessed on 30 June 2024). Descriptions of seed size, shape, and color characteristics were measured and analyzed according to the protocol of Loddo et al. [10].

2.2. Statistical Analysis

The results were used to build a database that included morpho-colorimetric descriptors. Statistical elaborations were conducted using SPSS version 29 software (SPSS 29.0 for Windows; SPSS Inc., Chicago, IL, USA) applying stepwise Linear Discriminant Analysis (LDA).

LDA operates by generating linear combinations of predictors with a new latent variable for each function, called discriminant function. The first function maximizes differences between groups, and subsequent functions maximize differences while remaining uncorrelated with previous ones. This continues with subsequent roles, with the requirement that new roles are not correlated with any of the previous ones. Three statistical variables—Tolerance, F-to-enter, and F-to-remove—were considered. Tolerance indicates the proportion of a factor's variance unaccounted for by other independent variables, while F-to-enter and F-to-remove values define a variable's influence in the model, describing changes upon insertion or removal.

A cross-validation procedure, specifically Leave-One-Out Cross-Validation (LOOCV), was applied to assess the identification system's performance. Before statistical analyses, raw data were standardized. Box's M test was used to assess equality of multiple variance–covariance matrices, and analysis of standardized residuals verified homoscedasticity [11,12]. The Kolmogorov–Smirnov test was used to compare the empirical distribution of discriminant functions with the reference probability distribution cumulative, while Levene's test was used to evaluate discriminant functions' equality of variances for group membership determination (Figure 2) [13–15].

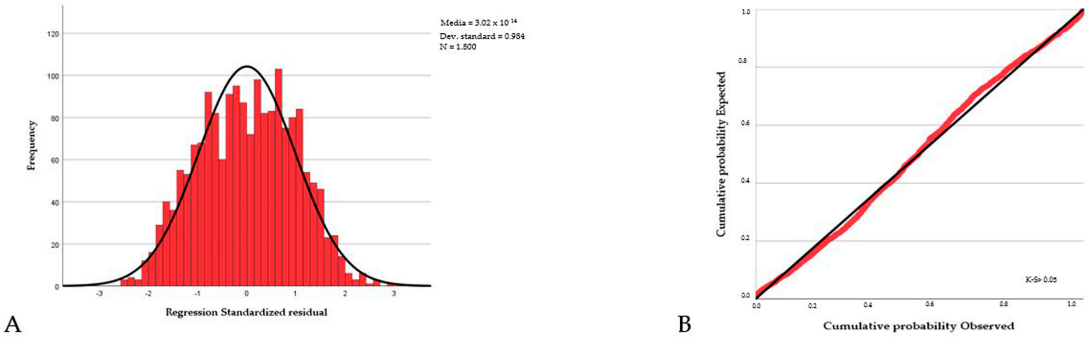

Figure 2. *Cont.*

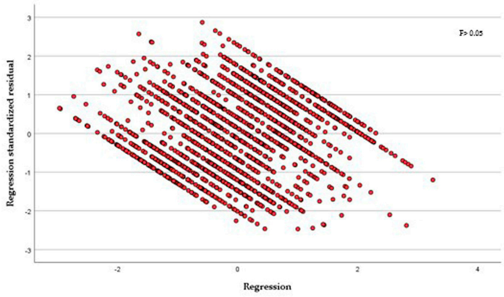

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Figure 2. (**A**) Histogram of the standardized residuals, (**B**) normal probability plot (P-P) tested with the Kolmogorov–Smirnov test (K–S), and (**C**) dispersion plot of the standardized residuals tested with Levene's test (F).

3. Results

The results of the qualitative analysis for the color of *P. lanatus* varieties were compared using morpho-colorimetry analysis (Table 2, Figure 3), achieving an overall correct identification rate of 80.5%. The lowest classification percentage was for the 'Coquinho Vermelha' variety at 39%, while the highest were for 'Fava Roxa' and 'Fava de Moita', both with 98% (Table 2, Figure 3). Although considering all varieties together significantly reduced identification performance, there was notable mutual misidentification between the 'Coquinho Vermelha' and 'Cara Larga de Moita' varieties, with misidentification rates of 39% and 22%, respectively (Table 2, Figure 4). Additionally, high mutual misattribution occurred between 'Coquinho Vermelha' and 'Cara Larga de Moita' at rates of 18% and 22%, respectively.

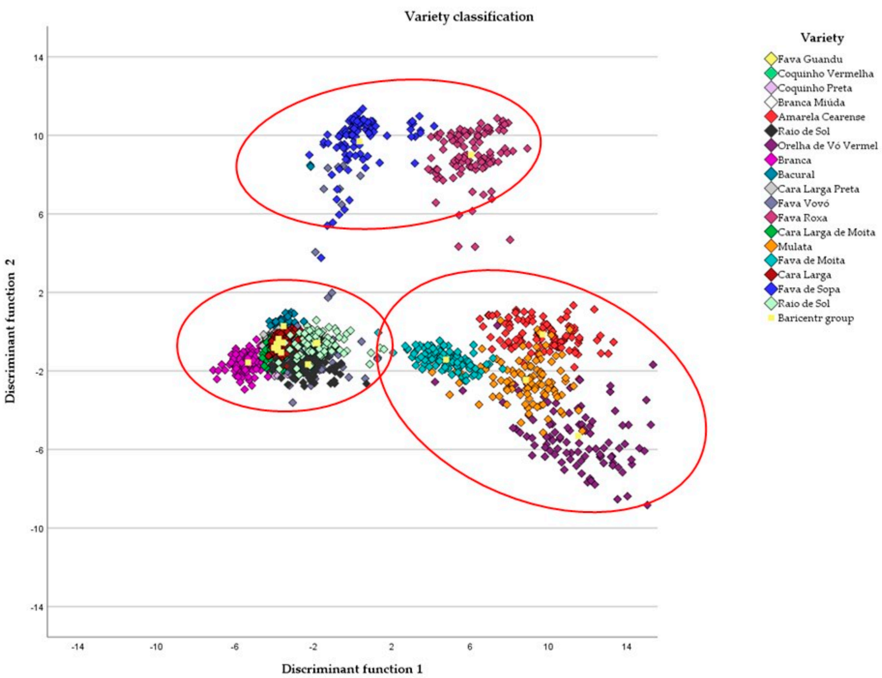

Figure 3. Graphical representation of the discriminant analysis of the *P. lunatus* L. varieties.

Table 2. Percentage of correct classification of *P. lunatus* L. varieties.

Varieties	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Total
Fava Guandu (1)	83.0	6.0	5.0	-	-	-	-	-	-	1.0	-	-	-	-	-	5.0	-	-	100.0
Coquinho Vermelha (2)	3.0	39.0	12.0	9.0	-	1.0	-	-	-	5.0	-	-	18.0	-	-	11.0	-	2.0	100.0
Coquinho Preta (3)	3.0	3.0	71.0	3.0	-	2.0	-	-	-	8.0	3.0	-	3.0	-	-	3.0	-	1.0	100.0
Branca Miúda (4)	-	-	-	87.0	-	-	-	-	-	-	-	-	11.0	-	-	2.0	-	-	100.0

Table 2. *Cont.*

Varieties	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Total
Amarela Cearense (5)	-	-	-	-	97.0	-	-	-	-	-	-	-	-	3.0	-	-	-	-	100.0
Raio de Sol (6)	-	-	4.0	-	-	90.0	-	-	-	1.0	1.0	-	-	-	-	-	-	4.0	100.0
Orelha de Vó Vermelha (7)	-	-	-	-	2.0	-	92.0	-	-	-	-	-	-	4.0	2.0	-	-	-	100.0
Branca (8)	-	-	2.0	-	-	-	-	97.0	-	-	-	-	1.0	-	-	-	-	-	100.0
Bacural (9)	2.0	1.0	-	8.0	-	-	-	-	68.0	-	-	-	5.0	-	-	14.0	2.0	-	100.0
Cara Larga Preta (10)	1.0	8.0	11.0	4.0	-	-	-	-	2.0	67.0	-	-	-	-	-	7.0	-	-	100.0
Fava Vovó (11)	1.0	-	-	-	-	6.0	-	-	-	-	82.0	-	-	-	1.0	1.0	6.0	3.0	100.0
Fava Roxa (12)	-	-	-	-	2.0	-	-	-	-	-	-	98.0	-	-	-	-	-	-	100.0
Cara Larga de Moita (13)	1.0	22.0	3.0	9.0	-	-	-	-	-	3.0	-	-	45.0	-	-	16.0	-	1.0	100.0
Mulata (14)	-	-	-	-	8.0	-	1.0	-	-	-	-	-	-	82.0	9.0	-	-	-	100.0
Fava de Moita (15)	-	-	-	-	-	-	-	-	-	-	-	-	-	2.0	98.0	-	-	-	100.0
Cara Larga (16)	2.0	5.0	-	5.0	-	-	-	-	2.0	8.0	-	-	3.0	-	-	73.0	-	2.0	100.0
Fava de Sopa (17)	-	-	-	-	-	-	-	-	-	-	-	3.0	-	-	-	-	96.0	1.0	100.0
Raio de Sol (18)	-	1.0	3.0	-	-	3.0	-	-	-	-	-	-	3.0	-	3.0	3.0	-	84.0	100.0
Overall																			80.5%

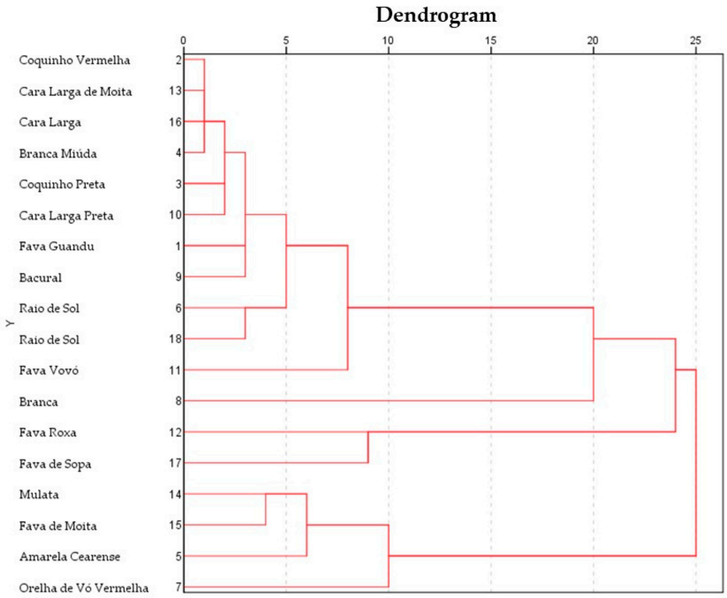

Figure 4. Dendrogram of distribution of the studied *P. lanatus* L. varieties.

Of the parameters evaluated, 17 of the 31 variables selected and used by stepwise LDA were descriptive of colors.

4. Discussion

The classification system based on morpho-colorimetry was able to correctly discriminate *P. lunatus* varieties, highlighting the high degree of morphological diversity among them.

The varieties 'Amarela Cearense', 'Branca', 'Fava Roxa', 'Fava de Moita', and 'Fava de Sopa' achieved a classification accuracy above 96%. Most displayed a uniform color, except for 'Fava de Moita', which exhibited two distinct colors. Notably, the high classification rates of 'Fava Roxa' and 'Fava de Sopa' suggest their status as autochthonous varieties. While differing in shape and size, they share similar brown tones and predominantly monochromatic characteristics. Seeds with uniform coloration or clearly defined secondary color patterns are more straightforward to classify using colorimetric evaluation methods [9]. According to Nobre et al. [16], seed color is an important descriptor in analyzing genetic variability between varieties. Furthermore, it assists researchers in the botanical characterization of different cultivars, also allowing the evaluation of the product's acceptance in the market [17].

Some of the qualitative traits such as seed color and seed coat pattern are important to farmers and consumers because different ethnicities have different preferences to these traits. The differences in these features, particularly in the color of the seed and the pattern of its tegument, relate to the preference of Brazilian consumers for bicolored beans [4]. The differences in the bean patterns are important for breeders, who aim to improve the bean to conform to consumer preferences. Also using agromorphological characters to verify genetic diversity among *P. lunatus* accessions, Bria et al. [18] found that the color and coloring pattern of the seeds showed great variation.

The varieties exhibit low correlation, as shown in Table 2, corroborated by the distance dendrogram in Figure 3, where groups emerge based on seed background color, secondary color, or size. The classification resulted in three distinct groups, as illustrated in Figure 2. The largest group encompasses 12 varieties, followed by a group of four varieties, and a third group comprising two varieties. In a previous study involving the same species, [19] classified seeds into two groups based on size and shape: small (I) and large (II). However, varieties like 'Raio de Sol-Iguatu', 'Raio de Sol-Cruzeta', and 'Fava Vovó', assigned to category I, display colors reminiscent of those in other groups, despite sharing similarities in size and shape with varieties categorized in groups II and III. Varieties such as 'Coquinho Vermelha', 'Cara Larga de Moita', and 'Cara Larga' share similar shapes and colors, differing primarily in secondary tones and geometric patterns formed on the seeds. Consequently, 'Cara Larga de Moita' and 'Cara Larga' may be synonymous of the same variety, distinguished primarily by their secondary coloration. The high similarity values observed in morpho-colorimetric analysis are attributed to both primary and secondary color characteristics and their intensities [5].

The observed degree of similarity among *P. lunatus* seeds is significant. In fact, producers note differences in plant sizes. For instance, the 'Cara Larga de Moita' variety has a determinate size, while the 'Cara Larga' variety exhibits indeterminate growth, typically associated with higher productivity. Morpho-geometric analyses confirm their distinction as different genetic materials [20].

The *P. lunatus* L. varieties in Northeast Brazil remain geographically isolated from other global production gene pools, situated thousands of kilometers away from other countries. Additionally, producers resist the introduction of new varieties, considering cultivated materials as family heritage. These family farmers employ their own selection methods, preserving materials noted for productivity and superior agronomic and sensory traits, including low cyanide content. Typically, these producers belong to communities that maintain rural seed banks, which serve to store selected materials for future sowings. Collective storage offers advantages, such as species preservation and mitigating post-harvest seed quality issues [1,21].

Due to the limited agronomic and commercial knowledge about *P. lunatus* in the northeastern region of Brazil, it is essential to evaluate the different varieties available. To achieve this, it is extremely important to comprehensively document the characteristics that enable the precise identification of this species. In this context, it is important to characterize the seeds of different varieties, classifying them by popular names/varieties, as well as understanding the characteristics that distinguish them during the vegetative cycle. In addition, studies related to physiological potential are important, as they play a determining role in the production, storage, and commercialization of seeds.

5. Conclusions

The morpho-colorimetric classification system effectively discriminated between *P. lunatus* varieties produced in Northeast Brazil, revealing a high degree of diversity among them. Seeds with uniform coloring or clearly defined secondary color patterns were particularly easy to classify. The varieties exhibited low correlation, forming distinct groups based on background color, secondary color, or seed size.

Among the varieties, the lowest classification percentage was attributed to variety 'Coquinho Vermelha', while the highest rates were observed for 'Fava Roxa' and 'Fava de Moita'.

These findings demonstrate that image analysis techniques are valuable for taxonomic investigations, even at the varietal level, offering a rapid and cost-effective method for identifying traditional beans, enhancing consumer satisfaction, and distinguishing economically valuable varieties. Additionally, this system is beneficial for germplasm banks, nurseries, and institutions dedicated to the ex situ conservation of plant biodiversity.

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