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Future-ready crops, genetic variability in lima bean seeds

Jardel da Silva Souza[1*], Lais Nóbrega Rodrigues[2], Lais Thomaz Ferreira[3], Sandra Helena Unêda-Trevisoli[1] and Naysa Flávia Ferreira do Nascimento[3]

[1]Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Campus de Jaboticabal, Via de acesso Prof. Paulo Donato Castellane, s/n, 14870-900, Jaboticabal, São Paulo, Brazil. [2]Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil. [3]Centro de Ciências Agrárias, Universidade Federal da Paraíba, Areia, Paraíba, Brazil. *Author for correspondence. E-mail: jardel.souza@unesp.br

ABSTRACT. Lima bean cultivation has not received proper national and regional attention by research agencies, impairing the knowledge of the agronomic characteristics and its potential, information that is essential for the selection and launching of superior genotypes. The objective of this work was to characterize the seeds of 17 lima bean genotypes produced by family farmers in Paraíba State. The characterization was based on the descriptors for *Phaseolus lunatus*. A completely randomized design was used with 15 replicates. Data were submitted to analysis of variance with a subsequent grouping of means by the Scott-Knott test at 1% of probability. For analysis of the genetic diversity, the Tocher method was used based on the generalized Mahalanobis distance. There is variability among the genotypes. According to the means test, genotype 10 has a higher seed length, width, and weight. Considering the diversity among the groups and aiming to select light-colored and higher means of the evaluated traits, the selection of genotypes 8, 13, 4, and 10 is recommended to establish a breeding program that satisfies both the consumer and the producer.

Keywords: genetic diversity; *Phaseolus lunatus*; plant breeding.

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Introduction

Creating future-ready crops is the key to sustainably feeding the world. Future-ready crops can provide producers with security from extreme weather events, such as floods, droughts, and heat waves. All this is possible thanks to the genetic improvement of plants. However, it is necessary to have quality genetic material in germplasm banks. And phenotypic characterization is extremely important in the process of genetic improvement. Through characterizations, it is possible to know if genetic variability exists. Genetic information is of great importance to allow a more efficient plant selection (Syukur, Sujiprihati, & Yunianti, 2012). One of the fundamental components of the development of conservation and breeding programs is the characterization of genetic diversity (Penha et al., 2017). This characterization results in better knowledge of the germplasm and is necessary for subsequent steps to develop better conservation strategies for materials and species (Costa & Hiragi, 2010). Genetic diversity has been studied by phenotypic analysis, measurements of agromorphological characteristics, and other studies (Penha et al., 2017).

In this context, cultivation and the conservation of traditional varieties and genetic diversity can be a good strategy to achieve breakthroughs (Salgotra & Chauhan, 2023). One of the most important crops in the northeastern region of Brazil is the lima bean. The Northeast accounts for more than (95%) of national production, with a total crop yield is estimated at 12.061 tons ha^1 and average productivity of 339 kg ha^1 (Instituto Brasileiro de Geografia e Estatística [IBGE], 2022). The highest prevalence of food insecurity was detected in the Northern (61.4% of households) and Northeastern (59.7%) regions of Brazil (ENANI, 2019), where improved subsistence cropping makes a big difference in guaranteeing food security in these regions. The Northeast region of Brazil presents a vulnerability to climate change, especially in the Caatinga biome. Studies indicate that, with climate change, there will be a reduction in river flows and intensification of drought periods, which will lead to a greater demand for irrigation in the region (Ministério da Ciência, Tecnologia e Inovações [MCTI], 2021).

Phaseolus lunatus L. is a leguminous plant of the Fabaceae family, popularly known as the lima bean. It is one of the most important crops of this genus, produced mostly by small producers, with low technology employed, and represents subsistence cultivation. It is a widely used crop in human and animal food, being an important source of protein; the grains are consumed green, dried, or with pods (Silva, Souza, & Silva Souza, 2019). It is also used as green manure or cover crop for soil protection (Pegado, Barbosa, Mendes, Souto, & Souto, 2008). Lima beans are also considered an important source of protein for people in South America, Africa, and (Amorim et al., 2019).

The diverse uses of this crop, combined with the climate of the northeast region of Brazil, among other factors, place this crop as an important alternative to income and a source of food for the population (Guimarães, Martins, Silva, Ferraz, & Oliveira, 2007). Considering the importance of this crop, it can be affirmed that it has received little attention from the research and extension agencies, resulting in a limited collection of agronomic information. The study of several existing varieties is important to register and evaluate traits that may contribute to the selection of plants with good agronomic performance (Oliveira, Torres, & Benedito, 2011; Santos, Corlett, Mendes, & Wanderley Júnior, 2002).

Studies about the variability of species are of fundamental importance, considering that the lima bean is a native species that is not well studied, and the magnitude of diversity is not yet fully known (Freitas et al., 2012). Among the various methods to study genetic characteristics, the variability in seed size, color, and shape is efficient, since these variations are related to the genotype, contributing to the selection process (Nobre et al., 2012). The lima bean seed is its main product of economic value. Seed characteristics are important because there is variability in seed area and seed color, among other factors. These factors are important to study because they influence the final result of all work, the acceptance by the consumer. Therefore, this characteristic we consider one of the most important in the survey and characterization of the germplasm bank. Knowing what genetic material is available can guide which strategy to take to start a breeding program with the species. The seed is a commercial product, and its characterization and classification are necessary. The objective of this study was to characterize the seeds of 17 lima bean genotypes.

Material and methods

This study was carried out at the *Universidade Federal da Paraíba* (UFPB). Seventeen cultivars were cultivated and collected by family farmers in the producer regions in the State of Paraíba (Table 1). The seeds belonged to the AS-PTA germplasm bank in the State of Paraiba and were collected from all over the State of Paraiba. Genotypes belonged to small producers in the state; these are seeds cultivated for decades by these producers in family agriculture. These are seeds known as ‘seeds of passion.’ These are seeds that the producers reserve after the harvest to produce in the next year. Generally, the cultivation is done at the beginning of the rainy season in the northeast region of Brazil, from April to August. They are cultivated together with corn. Samples of 15 seeds per genotype were randomly selected.

Table 1. Lima bean genotypes, common names.

	Genotypes		Genotypes		Genotypes
1	‘Eucalipto Creme'	7	‘Boca de Moça’	13	‘Raio de Sol’
2	‘Vermelha’	8	‘Africana’	14	‘Branca Grande’
3	‘Cavalinha’	9	‘Eucalipto Laranja’	15	‘Branca’
4	‘Coquinho’	10	‘Rainha’	16	NI
5	‘Orelha de Vó’	11	‘Rosinha’	17	‘Cara-Larga’
6	‘Cearense’	12	‘Roxinha’	-	-

NI = non-identified genotype.

The characterization was based on descriptors for *P. lunatus* L. suggested by the International Plant Genetic Resources Institute (IPGRI, 2001). The quantitative characteristics evaluated were the length, width, and weight of the seed. The length was used to define seed size according to the methodology proposed by (Yaguiu, Machado Neto, & Cardoso, 2003), classified as small (8–12.9 mm), medium (13–17.9 mm), or large (18–29.9 mm). The qualitative characteristics evaluated were: seed background color, primary seed coat color, and secondary seed coat color (Yaguiu et al., 2003). The importance of seed variables has already been described in (Johannsen, 1911) studies on pure seed strains. When heavier seeds are selected, the generation average increases. Thus, there is a relationship between the variables diameter, length, and seed weight, which will enable a more efficient selection at the end of the process.

A completely randomized experimental design was used with 15 replicates, where each seed was a replicate. The quantitative data were submitted to analysis of variance (F test), with subsequent clustering of groups by the Skott-Knott test at ($p \geqslant 0.01$). The genetic parameters and their estimators were analyzed for each character using the following mathematical expressions (Cruz, Regazzi, & Carneiro, 2004):

a) Phenotypic variation: $\sigma^2_F = \frac{QMg}{k}$

b) Environmental variation: $\sigma^2_E = \frac{\mathrm{QMr}}{k}$

c) Genotypic variation: $\sigma^2_G = \frac{\mathrm{QMg - QMr}}{k}$

d) Genetic coefficient of variation: $\mathrm{CVg} = \frac{\sqrt{\sigma^2 G}}{m} \times 100$

e) Ratio: $\frac{\mathrm{CVg}}{\mathrm{CVe}}$

For the genetic divergence analysis, the Tocher method was used based on the generalized Mahalanobis distance with the method UPMG. All statistical analyses were performed using the GENES software (Cruz, 2018).

Results and discussion

Variations were observed in the qualitative characteristics (Table 2), indicating that lima beans had variations among different genotypes. This is very important because genetic variability is the main factor that makes selection possible. Information about the variability of any germplasm collection increases the efficiency of breeding studies of cultivated species (Santos et al., 2002; Santonieri & Bustamante, 2016). However, genetic variability needs to be viable; it needs to contain better averages than the existing ones. The background colors varied from white to black. Genotypes 10 and 14 were classified as white, genotypes 3, 5, and 13 were classified as yellow, genotypes 4, 8, 16, and 17 were classified as pink, genotypes 1, 6, 9, and 11 were classified as brown, genotypes 2 and 12 were classified as red, genotype 15 was classified as gray, and genotype 7 was classified as black.

The genetic variation between the genotypes is easily distinguishable with the naked eye with the different color patterns of the seed coat. The differences between the seeds within each genotype were too many or too few spots and stripes. However, these small differences do not indicate contamination in the genotypes but are rather due to incomplete penetrance and variable expressivity, as occurs in carioca beans (Ramalho, Abreu, Santos, & Nunes, 2012). Studies have shown that seed coat color influences water absorption, with darker kernels absorbing water more slowly, probably due to the oxidative reactions of phenolics resulting in hydrophobic substances (Marbach & Mayer, 1974; Marbach & Mayer, 1975; Tully, Musgrave, & Leopold, 1981).

Table 2. Qualitative characterization of lima bean seeds.

Genotypes	Background color	Pattern color	Second color
1	Brown	Dark-brown	Black
2	Red	Red	Black
3	Yellow	Light-brown	Dark-red
4	Pink	Light-brown	Dark-red
5	Yellow	Light-brown	Black
6	Light-brown	Light-brown	Absent
7	Black	Absent	Absent
8	Pink	Light-brown	Red-purple
9	Brown	Dark-brown	Black
10	White	Light-brown	Red-purple
11	Light-brown	Light-brown	Black
12	Red-purple	Absent	Absent
13	Yellow	Light-brown	Dark-red
14	White	Light-brown	Dark-red
15	Gray	Light-brown	Dark-red
16	Pink	Light-brown	Red-purple
17	Pink	Light-brown	Dark-red

Approximately 88% of the genotypes presented two seed coat colors, where the light brown color was the most frequent and present in genotypes 3, 4, 5, 6, 8, 10, 11, 13, 14, 15, 16, and 17). For the secondary seed coat color, it varied from absent to black, where the dark-red colors (genotypes 3, 4, 13, 14, 15, and 17) and black (genotypes 1, 2, 5, 9, and 11) were frequently found.

Northeastern consumers prefer seeds with a white tegument, and some people prefer white seeds with stripes on the tegument (Lopes, Gomes, & Araújo, 2010). However, this does not apply to the choice of restaurants specializing in some traditional northeastern Brazilian dishes that are made with lima beans, requiring large beans with different shapes and pigments located on the front of the seed (Pires et al., 2021). This choice is made by some socioeconomically vulnerable consumers, considering that grains with light teguments have faster Softening than seeds with darker teguments. This represents savings in energy sources [gas, firewood, or sawdust] for the socioeconomically vulnerable population. This characteristic was found in the light seed color genotypes, as found in genotypes 3, 4, 6, 10, 11, 13, 14, 16, and 17. However, we cannot say that this choice for lighter teguments is taken into consideration at planting time by the farmers. All the seeds [with different shades of color in their teguments] that we used in this paper were collected from small farmers.

According to data presented in Table 3, a significant difference ($p \geqslant 0.01$) was found for all analyzed characteristics, indicating that all genotypes analyzed are statistically different, which confirms the variability among them. Genetic variability is important for the maintenance of the species in the environment. Genetic variability or genetic diversity refers to the diversity of alleles present in a species or the difference of alleles in an individual (Biologia Net, 2023). Faleiro (2008) states that, in the last 100 years, there has been a significant reduction in the genetic variability of these plant species, which represents a serious risk to the sustainability of agriculture (Carvalho, Silva, & Medeiros, 2009). One of the actions to avoid, or at least minimize, the process of genetic erosion, is the conservation of genetic variability, which can be both ex-situ and in situ (Carvalho et al., 2009).

The conservation of plant genetic material has the practical objective of guaranteeing a source of new alleles or genes for breeders to develop new cultivars (Borém, 2017). The breeds great genetic variability in the genetic material during the selective processes that may be effective and result in significant genetic gains (Vasconcelos et al., 2010; Oliveira, Martins, Cruz, & Silva, 2014; Martins, Uneda-Trevisoli, Moro, & Vieira, 2016). With climate change advancing and growing, areas are suffering from environmental stresses, such as light, temperature, salinity, and acidification, among other effects. The addition of new genes for resistance to diseases, pests, and stresses caused by the adverse environment has provided a substantial increase in grain yields, as well as an invaluable stock of genetic material from these wild (Mujeeb-Kazi & Kimber, 1985; Prestes & Goulart, 1995). Thus, the lack of variability in crops can mean a loss of productivity and leave the most vulnerable population without food security.

The lowest coefficient of variation was obtained in seed weight, reaching (2.43%). For length and width, the coefficient of variation was (8.82%) and (9.07%), respectively. According to Werner, Motta, Martins, Lima, and Schmildt (2012), Toebe, Cargnelutti Filho, Burin, Casarotto, and Haesbaert (2014), and Toebe et al. (2018) the CV values obtained in length and width are classified as optimal precision, and the CV value of weight is classified as regular precision. The coefficient of variation gives an idea of the experimental accuracy; more accurate results and reliable information are obtained when the effect of the experimental error is reduced (Werner et al., 2012). This result is because each genotype is an individual population, and there is no mixing of genotypes with identical morphology and coloration collected elsewhere. The genotypes we evaluated were a sample of only 15 seeds of each genotype, stored in paper bag packages of approximately 200g of each genotype. The packages of the genotypes were donated samples from ASPTA. And ASPTA collected a small sample from the small growers. Thus, our characterization is a small sample of the whole.

The genetic variation coefficient (CVg) ranged from 7.53 to (38.14%) (Table 3). High values of this coefficient allow the inference of the magnitude of genetic variability present in the population for certain characteristics (Ferrão et al., 2008). When compared to other studies with the same genus by Leite et al. (2016) these values were considered higher. These values give us more precise information about the environmental influence on the selection of superior genotypes. The heritability was higher than (90%) (Table 3) for the three analyzed traits, confirming the low environmental influence, and indicating that there is a prospect of success with the phenotypic selection. High heritability values may be associated with additive genetic variation, lower environmental variation, and lower environmental genotype interaction (Pessoa, Rêgo, Carvalho, Santos, & Rêgo, 2018; Dantas et al., 2019). The genotypic variation for the three evaluated traits corresponded to more than (90%) of the observed phenotypic variation, showing that the genotype and environment interaction is low or null (Table 3).

Table 3. Summary of ANOVA for the evaluated characteristics of the seed of 17 genotypes of lima beans.

SV	DF	MS		
		Length	Width	Weight
Genotypes	16	108.95**	3.08**	0.63**
Residual	238	1.41	0.27	0.01
Total	254	-	-	-
σ^2_F	-	7.26	0.20	0.04
σ^2_E	-	0.09	0.02	0.00
σ^2_G	-	7.17	0.19	0.04
h^2	-	98.70	91.18	97.74
CVg (%)	-	19.88	7.53	38.14
CVg/CVe (%)		2.25	0.83	1.70
C.V	-	8.82	9.07	22.43
Mean	-	13.47	5.74	0.53

σ^2_F: Phenotypic variation; σ^2_E: Environmental variation; σ^2_G: Genotypic variation; h^2: Heritability coefficient; CVg: Genetic coefficient of variation; CVg/CVe: Ratio; **Significant at ($p \geqslant 0,01$) by the F test.

The values obtained in the ratio (CVg/CVe) for length and weight of the seed were higher than 1, indicating that the additive genic action was predominant in the control of these characteristics, which corroborates with the results previously presented. Although the genetic variation for width was superior to the environmental variance, the CVg/CVe ratio was lower than the unit, suggesting that non-additive effects may have a greater influence. These values can be used as an indicative index of the degree of ease of selection of genotypes for each trait (Leite et al., 2016). For the different genotypes, there was variation in the length, width, and weight of the seeds (Figure 1II and III). The length ranged from 9.68 mm in genotype 4 to 18.99 mm in genotype 10. The width of the seed varied from 4.95 mm in genotype 1 to 6.43 mm in genotype 10. The values for weight ranged from 0.33 g in genotype 3 and 11 to 1.01 g in genotype 10.

Genotype 10 presented seeds with the highest length (18.99 mm), width (6.43 mm), and weight (1.01 g) (Figure 1). In addition to genotype 10, genotypes 4 (6.11 mm), 8 (6.27 mm), and 13 (6.27 mm) presented higher values for width (Figure 1I). Considering that seed size is related to higher reserve contents and that these reserves are necessary for seed germination (Barbosa, Smiderle, Alves, Vilarinho, & Sediyama, 2010), the selection of genotypes 10 and 13 is recommended.

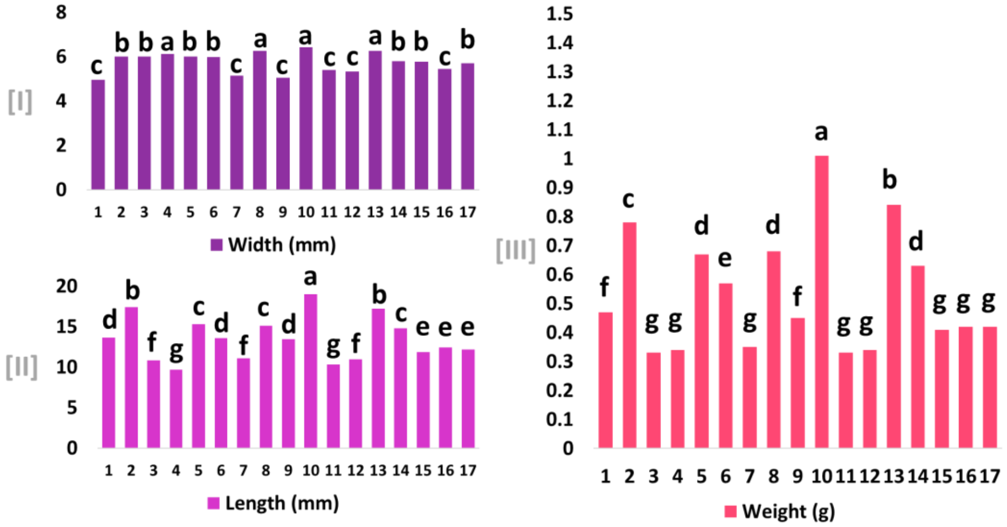

Figure 1. Length, width, and weight of different lima bean genotypes by the Scott-Knott grouping test; *means followed by the same letter in the columns do not differ by the Scott-Knott test at ($p \geqslant 0.01$)

According to the methodology suggested by Yaguiu et al. (2003), the seeds were classified as small, medium, and large. Genotypes 17, 15, 12, 11, 7, 4, and 3 were classified as small, genotypes 1, 2, 5, 6, 8, 9, 13, and 14 as medium, and only genotype 10 was classified as large, and it was indicated for use as a parent plant in breeding programs.

In agreement with the mean test and analysis of variance, the Tocher grouping method presents variation among the genotypes by the formation of seven distinct clusters (Figure 2). The selection of genotypes of different groups or with greater intragroup distance is recommended since genetic variability is necessary for plant breeding programs, which allows a greater expression of the potential of the population for selection (Ramalho et al., 2012b).

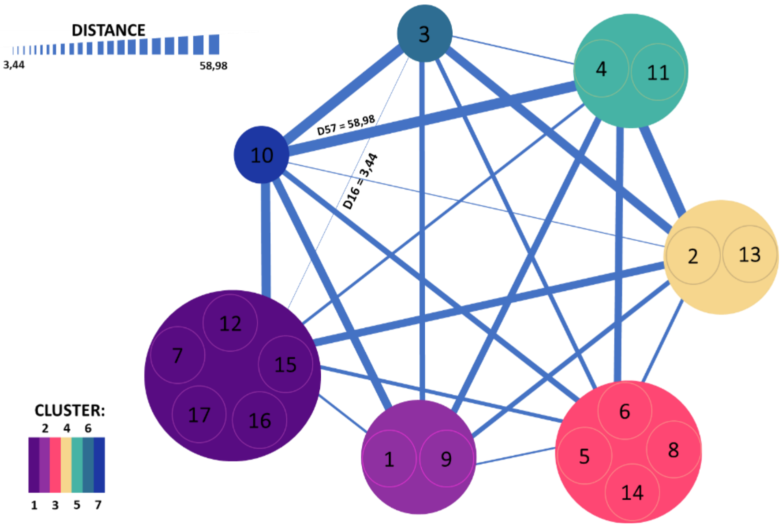

Figure 2. Genotypes clustered in different groups according to the Tocher method.

Considering the diversity among groups and the aim to select light-colored genotypes (Table 2) and higher means of evaluated traits (Figure 1), the selection of genotype 8, which is included in group 3, genotype 13 of group 4, genotype 4 of group 5 and genotype 10 of group 7 are recommended.

Conclusion

A high genetic variability was observed among the genotypes studied, which guarantees the formation of a lima bean germplasm bank. It also guarantees the conservation of this genetic resource to be used in plant breeding programs in the future. To establish a breeding program with lima beans that satisfy the consumer market and the producer producing larger, light-colored, and divergent seeds, the following genotypes are recommended: 4, 8, 10, and 13.

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