ARTICLE

Cytogenetic and morphological characterization of lima bean germplasm from the Brazilian Northeast region with a focus on genetic resource conservation

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Abstract: *In Brazil, lima bean is mainly grown in the Northeast region, where it is widely consumed and is of major economic importance. We evaluated different Phaseolus lunatus accessions from the Northeast of Brazil using agromorphological markers and CMA/DAPI fluorochrome banding techniques. All the accessions showed CMA$^+$ blocks of pericentromeric constitutive heterochromatin (CH). At least one pair of CMA^{++} terminal marks corresponds to the nucleolus organizer region (NORs). Characterization of the seeds suggests that the two Andean and Mesoamerican Phaseolus cultigroups are represented in the Brazilian germplasm analyzed. Most of them belong to the "Big Lima" group. Characterization studies of lima bean germplasm are important for increasing knowledge of the diversity and variability of the species and for generating useful information for breeding and conservation of this economically important legume.*

Keywords: Phaseolus lunatus, *karyotype analysis, CMA and DAPI fluorochromes, heterochromatin, seed characterization*

INTRODUCTION

The *Phaseolus* L. genus, composed of ~90 species, is especially important among the numerous leguminous plants because five of its species are grown for food (Bitocchi et al. 2017). At least seven independent domestication events have occurred within the genus, which have generated the reproductive isolation of five domesticated species: *P. acutifolius* A. Gray, *P. coccineus* L., *P. polyanthus* Greeman, *P. lunatus* L., and *P. vulgaris* L. (Delgado-Salinas et al. 2006). *Phaseolus lunatus* L. (lima bean) is the second most economically important protein source of the genus (Ormeño-Orrilloet al. 2015).

Mexico is the main diversity center of lima bean (with 90% of the species), while Europe and Brazil are considered secondary diversification centers (revised by Martínez-Nieto et al. 2020). In Brazil, the Northeast region is the main producer of lima bean, where it has a positive economic impact on family farmers, since they are able to sell surplus production. It also has social importance, due to

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its nutritional benefits, improving quality of life (Assunção-Filho et al. 2022). The seeds of the *P. lunatus* L. species have high protein content, as well as carbohydrates, vitamins, and minerals, which make them a nutrient source for humans, especially in developing countries such as Africa and Latin America (Adebo 2023).

Just as in other centers of *P. lunatus*, the Brazilian Northeast region could be a center of genetic diversity in which breeding programs could be generated for this crop, and this represents a great responsibility for local governments and research institutions. Diversity centers are subject to various risks, including loss of genetic diversity (Lustosa-Silva et al. 2022). This affects the evolutionary potential of the species, leading to genetic vulnerability or reduction in the ability to adapt to environmental changes, thus increasing the possibility of extinction (Adebo 2023, Lustosa-Silva et al. 2023). Therefore, characterization of accessions conserved in germplasm banks is crucial for maintaining genetic resources in diversity centers (Maxted et al. 2012).

Morphological characterization of seeds is one of the first steps to be carried out in germplasm banks or research centers; it helps in classification and making inferences regarding the origin and diversity of the accessions. Based on seed size, morphology, and variance in 100-seed weight, three commercial lima bean types have been described: Sieva and Potato seeds (with small, rounded seeds that are globular, flat, and kidney-shaped), both of Mesoamerican origin, and Big Lima seeds (which are larger and flat), of Andean origin (Mackie 1943, Baudet 1977, Castiñeiras et al. 1991). For example, seed morphological characteristics (size, shape, and color) allowed differentiation of the two Potato and Sieva cultigroups, confirming the presence of both cultigroups in the landraces of lima bean grown in the Yucatan Peninsula (Esquivel-Martínez et al. 2023). In Brazil, Silva et al. (2017) used qualitative and quantitative descriptors through the Ward-MLM (Modified Location Model) and characterized several accessions of cultivated lima bean. They showed the presence of accessions with characteristics typical of the Mesoamerican and Andean cultigroups. Jesús-Pires et al. (2022) also investigated the genetic diversity of lima bean landraces using agromorphological and microsatellite markers (SSRs) from the Northeast, West Central, and Southeast regions, and data indicated a considerable diversity in traits related to agronomic performance, such as number of seeds per pod, and 100-seed weight. However, although some studies have evaluated the genetic diversity of lima bean in Brazil using agromorphological and molecular markers (Silva et al. 2017, Jesús-Pires et al. 2022) and cytogenetics (Bonifácio et al. 2012, Almeida and Pedrosa-Harand 2013, Feitoza et al. 2017), they included only a few or no genotypes collected from the Northeast of Brazil.

Cytogenetics provides the cytological features of a target species, such as number of chromosomes and morphology, the constitutive heterochromatin (CH) distribution pattern, ribosomal DNA sites, among others. Like most *Phaseolus* species, *P. lunatus* is a diploid, with $2n = 2x = 22$ and mostly metacentric and submetacentric chromosomes. The mapping of 5S and 35S rDNA sites in four cultivated *Phaseolus* species revealed the presence of one or two 5S rDNA sites and from one to seven 35S rDNA sites located in chromosomes 6, 9, and 10 (Moscone et al. 1999, Pedrosa-Harand et al. 2006). Bonifácio et al. (2012) constructed the first comparative cytogenetic map of *P. lunatus*, using previously mapped markers from *P. vulgaris*, and results showed a significant conservation of synteny among species. In addition, chromomycin A3 (CMA) fluorochrome reveled that all the pericentromeric regions of lima bean chromosomes are rich in GC (guanine and cytosine). Feitoza et al. (2017) showed condensation patterns of the prophase/prometaphase chromosome correlated with H4K5 histone acetylation and CMA in some species, among them *P. lunatus* and *P. vulgaris*. The CMA^+ bands in both species were found to be located in the proximal regions of all the chromosomes, and immunodetection of H4K5ac, an epigenetic mark universally associated with gene expression, revealed signals in the low-condensing region terminal, but not in the pericentromeric CMA^+ bands.

Thus, considering that knowledge about the genetic diversity of a germplasm collection is essential for plant breeding programs and for conservation of genetic resources by allowing planning and execution of appropriate strategies, the aim of the present study was to cytogenetically characterize and morphologically classify the seeds of *Phaseolus lunatus* (lima bean) accessions, a species of great economic importance for the Brazilian Northeast region. The results generated have increased our knowledge about the diversity and variability of the *P. lunatus* accessions analyzed here and have provided additional assistance regarding the accessions in the germplasm banks of Brazil.

MATERIAL AND METHODS

Plant materials

Twenty cultivated lima bean accessions collected in several states of the Brazilian Northeast and part of the *Phaseolus* Germplasm Bank of the Universidade Federal de Piauí (PGB-UFPI, Brazil) were used in this study (Table 1). The seeds from the accessions were samples from the bank and were replicated in a greenhouse without cross-pollination for this and further studies.

CMA/DAPI fluorochrome staining

Root tips of different lima bean accessions were pretreated in 0.002M 8-hydroxyquinoline for 24 hours at 10 ºC, fixed in a 3:1 ethanol-acetic acid solution, and stored at -20 ºC until use. For the CMA/DAPI fluorochrome staining, we followed Schweizer and Ambros (1994), with minor modifications. Root tips were digested with an enzymatic solution of 2% cellulase (Onozuka R-10) and 20% pectinase (Sigma). The slides were stained with 10 µL of CMA (0.5 mg mL^{-1}) for 1h, counterstained with 10 µl of DAPI (2 mg mL^{-1}) for 30 min, mounted in glycerol/McIlvaine (1:1), and stored for three days before analysis.

Image analyses and morphometry

The five metaphases of each accession were photographed using a DF7000GT digital camera coupled to a Leica DM4B microscope. The images were optimized for brightness and contrast using Adobe Photoshop CS3. Chromosome sizes were measured using the Drawid v0.26 (Kirov et al. 2017). Idiograms were constructed using Corel DRAW (2017) and chromosome morphologies and parameters (Table 1) were classified according to Guerra (2002).

Morphological characterization of the seeds

We measured the length, width, and thickness of 10 randomly selected seeds for each of 16 accessions. The measurements were performed using a digital caliper (in mm), according to IPGRI (2001). The 100-seed weight was

Table 1. Accession, Provenance, Range of Chromosome Size (RCS), Karyotype Formula (KF), Total Chromosome Length (TCL), Mean Chromosome Length (MCL), Haploid Karyotype Length (HKL), and number of CMA/DAPI bands (CMA_3/DAPI)

Accession	**Provenance**	**RCS** (µm)	**KF**	**TCL** (µm)	**MCL** (µm)	**HKL** (µm)	**CMA_3/DAPI**
PGB-UFPI 790	Teresina-PI	0.85 –1.60	10 M + 1 SM	26.10	1.19	13.05	2 CMA_3^{++}/DAPI and 20 CMA^{+}/$DAPI^{-}$
PGB-UFPI 793	Buriti Bravo-MA	1.31 – 2.14	11 M	38.22	1.74	19.11	-
PGB-UFPI 794	Tanque-PI	1.10 – 1.92	11 M	32.89	1.49	16.44	-
PGB-UFPI 795	Esperantina-PI	1.03 – 1.73	10 M + 1 SM	29.23	1.33	14.62	-
PGB-UFPI 796	Riachão-MA	0.90 – 1.49	11 M	26.71	1.21	13.36	-
PGB-UFPI 797	Riachão-MA	1.15 – 2.36	9 M +2 SM	38.11	1.73	19.06	-
PGB-UFPI 799	Nova Colina-MA	1.81 – 3.14	11 M	52.91	2.41	26.46	-
PGB-UFPI 800	José de Freitas-PI	1.54 – 2.42	11 M	43.86	1.99	21.93	-
PGB-UFPI 801	São Pedro-PI	1.41 – 2.47	11 M	41.87	1.90	20.93	-
PGB-UFPI 803	Água Branca-PI	1.69 – 2.74	11 M	48.57	2.21	24.29	-
PGB-UFPI 804	Angical-PI	1.51 – 2.79	10 M + 1SM	46.48	2.12	23.29	-
PGB-UFPI 806	Palmeiras-PI	1.59 – 2.81	9 M + 2 SM	48.64	2.21	24.32	-
PGB-UFPI 807	Amarante-PI	1.61 – 2.88	11 M	49.42	2.25	24.71	-
PGB-UFPI 809	Angical-PI	0.92 – 1.60	10 M + 1 SM	27.02	1.23	13.51	-
PGB-UFPI 810	Água Branca-PI	1.61 – 2.78	11 M	48.06	2.18	24.03	-
PGB-UFPI 814	Remígio-PB	1.31 – 2.12	11M	37.73	1.71	18.86	-
PGB-UFPI 815	Picuí-PB	1.34 – 2.42	9M + 2SM	40.97	1.86	20.48	-
PGB-UFPI 816	Remígio-PB	1.43 – 2.69	9M + 2SM	45.66	2.08	22.83	-
PGB-UFPI 817	Remígio-PB	1.65 – 2.97	9M + 2SM	51.27	2.33	25.64	-$^{-}$
PGB-UFPI 857	Campos Sales-CE	1.61 – 2.52	10 M + 1 SM	44.63	2.03	22.31	-

$^{++}$ represents CMA bands more strongly stained and $^{-}$AT reduced bands determined, and the values were established in grams (g) according to Mackie (1943). Seed shape was based on the J and H coefficients, following the established protocols of Puerta Romero (1961) and Vilhordo (1996), and seeds were classified as spherical (J = 1.16 – 1.42), elliptical (J = 1.43 to 1.65), oblong/short reniform (J = 1.66 to 1.85), oblong/mean reniform (J = 1.86 to 2.00), and oblong/long reniform (J > 2.00). According to the H coefficient, seeds were classified as flattened (H < 0.69), semi-flattened (H =0.70 to 0.79), and full (H > 0.80). Statistical analysis was performed by the Scott-Knott test, with 5% probability.

RESULTS AND DISCUSSION

All lima bean accessions showed 2*n*=22 predominantly metacentric chromosomes, and late-condensed terminal chromatin was found in most of the accessions analyzed (Figures 1 and 2). According to the Guerra (1983) nomenclature proposal, chromosomes were classified as metacentric (m) and submetacentric (sm). Most of the *Phaseolus* Germplasm Bank accessions (PGB-UFPI 793, 794, 796, 799, 800, 801, 803, 807, 810, and 814) showed karyotype formula 11M, while

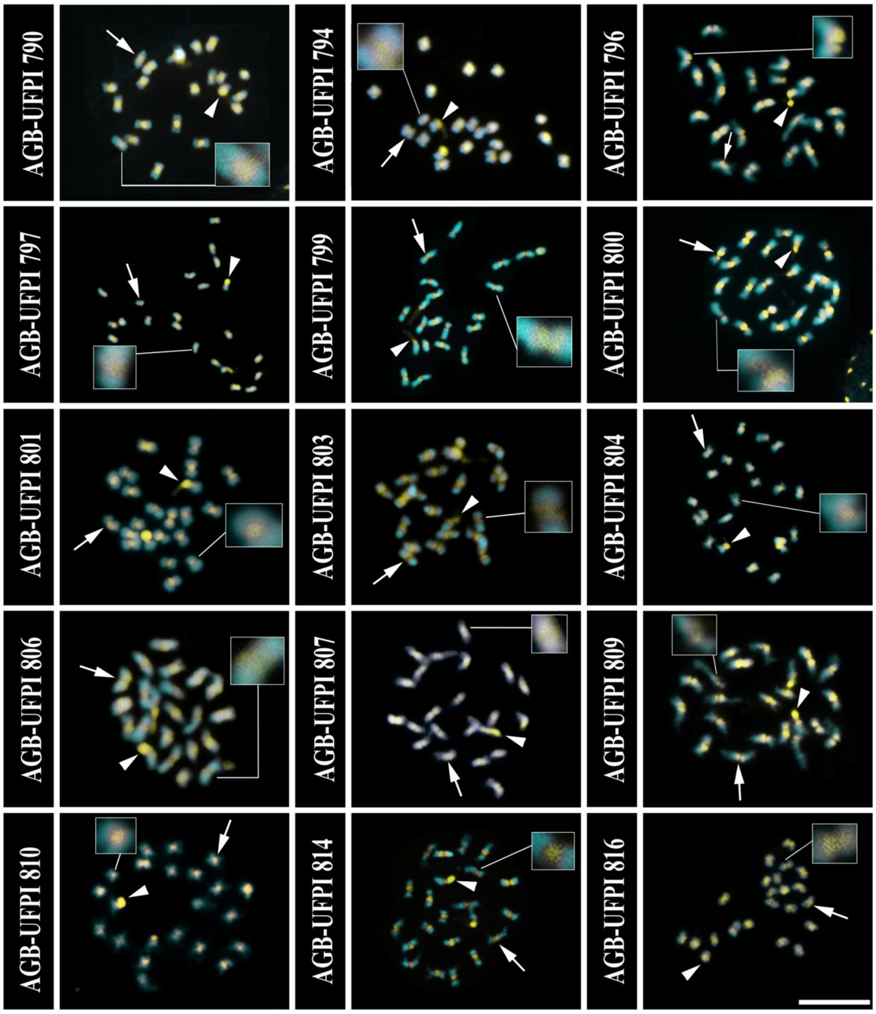

Figure 1. Double staining with CMA (yellow) and DAPI (blue) fluorochromes in some *P. lunatus* accessions (merged image). Arrows indicate CMA block in the pericentromeric chromosome regions and arrowheads indicate terminal or subterminal bands. The number of each accession is indicated at the left side of the metaphases. Inserts indicate small CMA$^+$ blocks in a region difficult to detect. Bar = 10µm.

Figure 2. Idiograms representing size, morphology, and distribution of the CMA blocks (yellow bands) located on the chromosome of the *P. lunatus* accessions analyzed. SM = Submetacentric.

other accessions had the formula 10M+1SM (PGB-UFPI 790, 795, 804, 809, and 857) and 9M+2SM (PGB-UFPI 797, 806, 815, 816, and 817) (see Table 1).

The chromosome size ranged from 0.85µm (PGB-UFPI 790) to 3.14 µm (PGB-UFPI799), corroborating previous studies (Mercado-Ruaro and Delgado-Salinas 2009). Moscone et al. (1999) showed the metaphase mitotic chromosomes ranged from 1.7µm to 3.5µm, and in Sarbhoy (1977) and others, *Phaseolus* varieties ranged from 0.70 µm to 3.00 µm. With respect to Total Chromosome Length (TCL), accessions of the *Phaseolus* Germplasm Bank had sizes ranging from 26.10 µm (PGB-UFPI 790) to 52.91 µm (PGB-UFPI 799). Similarly, Moscone et al. (1999) reported TCL of 27.38 µm for *P. lunatus*, and in fava bean species, they reported 23.32 µm (*P. xanthotrichus*), 26.0 µm (*P. maculatus*), and 29.74 µm (*P. coccineus*). However, these differences may be related to unequal degrees of chromosome condensation during cell division, to differences in the pretreatment, and/or to different classes of repetitive DNA sequences, as reported for other species (Moscone 1990, Pozzobon et al. 2006, Almeida et al. 2022).

We identified the heterochromatin blocks of the *P. lunatus* accessions using the fluorochromes chromomycin A3 (CMA) and 4,6-diamidino-2-phenylindole (DAPI). All the accessions analyzed showed GC-rich heterochromatin blocks ($CMA^+/DAPI^-$) located at the pericentromeric regions, with different sizes and intensities of the signals. We identified at least two terminal $CMA^{++}/DAPI^-$ bands in all accessions, probably corresponding to the nucleolar organizer regions (NORs) (Figure 1, Table 1). AT^+ blocks were not observed there. Idiograms of each accession are represented in Figure 2.

One of the most interesting characteristics associated with plant chromosomes is the amount of constitutive heterochromatin in the pericentromeric regions of the chromosomes. Cytogenetic studies have contributed to the characterization of the heterochromatin and repetitive sequence distribution pattern in *Phaseolus* and related genera, such as *Vigna* (Fonsêca and Pedrosa-Harand 2017, Oliveira et al. 2020, Ribeiro et al. 2020). Almeida and Pedrosa-Harand (2013) demonstrated that lima bean and common bean (*P. vulgaris* L.), both with 2*n* = 22, share common CMA sequences in the pericentromeric and in the NOR regions. Similarly, Bonifácio et al. (2012) observed a karyotype in lima bean composed of 22 predominantly metacentric chromosomes that have pericentromeric regions rich in CMA+/DAPI- heterochromatin, forming blocks of different sizes and intensities, which confirms the strong similarity and karyotypic stability of the genus. In both species, the pericentromeric region had DNA hypermethylation (5mC) and H4K5ac histone hypoacetylation, epigenetic marks associated with the formation and maintenance of constitutive heterochromatin (Fonsêca et al. 2014, Feitoza et al. 2017).

In addition to cytogenetic characterization, we also traced a morphological profile of the seeds. We observed variation regarding the length, width, and thickness of the seeds. Considering overall length, the accession PGB-UFPI 804 showed statistically significant difference from the accession PGB-UFPI 790, and both had the longest seeds, at 18.38 mm and 19.58 mm, respectively. The accessions with the shortest seeds were PGB-UFPI 817 with 11.97 mm and PGB-UFPI 816 with 11.03 mm, with no statistically significant difference between them (Table 2).

For seed width, with an overall average of 10.94 mm, the highest values were observed for PGB-UFPI 814 (12.27 mm) and PGB-UFPI 790 (12.77 mm). The smallest values were observed for PGB-UFPI 817 (9.02 mm) and PGB-UFPI 816 (8.20 mm), with flattened seeds, with statistical difference between them (Table 2).

Jesús Pires et al. (2022) investigated the genetic variability of creole varieties of fava beans from agromorphological descriptors and SSR markers and found that the average seed length ranged from 10.10 mm to 16.61 mm. In a similar study using both agromorphological and SSR analyses from the germplasm collection at the Universidade Federal do Piauí (UFPI), different values were found for length, width, and thickness, from 9.65 to 18.52 mm, 7.41 to 11.83 mm, and 5.32 to 6.90 mm, respectively (Melo et al. 2023). According to Silva et al. (2017), there is wide variation regarding the length and width of *P. lunatus* seeds, which contributes to the genetic variability of this species. The values for these traits found in this study were 8.28 to 22.53 mm and 6.54 to 14.17 mm, respectively. Thus, the results of seed morphology found here indicate there is diversity among the lima bean accessions belonging to the PGB-UFPI, showing the variability of the seeds according to the parameters analyzed (Table 2).

After measurement, the seeds were classified according to the J and H coefficients (Table 2). For the first coefficient, the accessions that had the highest values were PGB-UFPI 804 and PGB-UFPI 806, while the smallest were for PGB-UFPI 803 and PGB-UFPI 799. For the H coefficient, the highest value was obtained for the accessions PGB-UFPI 793, PGB-UFPI 816, and PGB-UFPI 817; and the shortest values were obtained for PGB-UFPI 790 and PGB-UFPI 803.

For the J coefficient, the results showed predominance of seeds with spherical form (PGB-UFPI 793, 795, 797, 799,800, 803, 810, 814, 816, 817, and 857), elliptical form (PGB-UFPI 790, 794, 796, 801, 804, 806, 807, 809, and 815), and flattened profile for all accessions (Table 2). Our results differ from Santos et al. (2002), who found different types of forms for the seeds from the analyzed accessions.

Based on molecular information, *P. lunatus* consists of two major gene pools: the Andean gene pool, which includes the landraces classified within the Big Lima cultigroup (with larger, flat shaped seeds), and the Mesoamerican gene pool (MI and MII), which includes the landraces classified within the Sieva and Potato cultigroups (with smaller, globular, flat, and kidney-shaped seeds) (Andueza-Noh et al. 2013, Martínez-Castillo et al. 2014, García et al. 2021). Regarding size and weight, seeds can be grouped as Potato (small seeds, from 35 to 50 g for 100-seed weight), Sieva (medium seeds, from 50 to 70 g), and Big Lima (big seeds, from 70 to 110 g), according to Baudet (1977) and Castiñeiras et al. (1991). The smallest value found was for PGB-UFPI 816 (36.10 g), and the largest was for PGB-UFPI 806 (91.45 g) (Table 2).

According to the evaluated traits (form, profile, and seed weight), seeds were predominantly in the Big Lima cultigroup (PGB-UFPI 790, 794, 796, 797, 801, 804, 806, 807, 809, 810, 814, and 857), specifically with flattened and elliptical seeds. However, the accession PGB-UFPI 803, also belonging to this group, has a flattened profile and spherical form. The other accessions belong to the Sieva (PGB-UFPI 793, 795, 800, and 815) and Potato (PGB-UFPI 799, 816, and 817) cultigroups (Table 2).

Previous studies morphologically characterized the lima bean seeds belonging to the PGB-UFPI. Santos et al. (2002)

Table 2. Measurement of the length, width, and thickness and classification of the shape, profile, and 100-seed weight of the lima bean seeds from the PGB-UFPI (*Phaseolus* Germplasm Bank of the Universidade Federal de Piauí)

Accession	**SL** (mm)	**SW** (mm)	**ST** (mm)	**Length/ Width** (J)	**Thickness/ Width** (H)	**Shape**	**Profile**	**Weight** (g)	**Cultigroups**
PGB-UFPI 790	19.58 b	12.77 b	5.68 b	1.53	0.44	Elliptical	Flat	90.91	Big Lima
PGB-UFPI 793	14.16 e	10.03 d	6.72 a	1.40	0.66	Spherical	Flat	63.36	Sieva
PGB-UFPI 794	17.29 d	10.82 c	6.04 a	1.59	0.55	Elliptical	Flat	71.76	Big Lima
PGB-UFPI 795	15.28 e	11.35 c	6.22 a	1.34	0.54	Spherical	Flat	65.62	Sieva
PGB-UFPI 796	15.65 e	10.44 d	6.01 a	1.46	0.57	Elliptical	Flat	70.15	Big Lima
PGB-UFPI 797	17.32 d	11.88 c	5.77 b	1.42	0.48	Spherical	Flat	85.08	Big Lima
PGB-UFPI 799	12.04 f	9.30 e	5.97 a	1.29	0.64	Spherical	Flat	41.01	Potato
PGB-UFPI 800	14.75 e	11.15 c	6.45 a	1.31	0.57	Spherical	Flat	60.21	Sieva
PGB-UFPI 801	17.18 d	11.28 c	6.03 a	1.52	0.51	Elliptical	Flat	79.58	Big Lima
PGB-UFPI 803	17.07 d	11.56 c	5.56 b	1.17	0.47	Spherical	Flat	70.59	Big Lima
PGB-UFPI 804	18.38 c	11.39 c	6.31 a	1.61	0.55	Elliptical	Flat	81.30	Big Lima
PGB-UFPI 806	18.46 c	11.54 c	6.36 a	1.60	0.55	Elliptical	Flat	91.45	Big Lima
PGB-UFPI 807	16.89 d	10.78 c	6.13 a	1.57	0.56	Elliptical	Flat	71.97	Big Lima
PGB-UFPI 809	17.45 d	11.72 c	6.35 a	1.48	0.53	Elliptical	Flat	84.50	Big Lima
PGB-UFPI 810	15.95 e	11.31 c	6.09 a	1.41	0.53	Spherical	Flat	75.90	Big Lima
PGB-UFPI 814	17.17 d	12.27 c	6.01 a	1.39	0.48	Spherical	Flat	78.30	Big Lima
PGB-UFPI 815	15.64 e	10.51 d	5.73 b	1.47	0.53	Elliptical	Flat	66.73	Sieva
PGB-UFPI 816	11.03 f	8.20 f	5.37 b	1.33	0.65	Spherical	Flat	36.10	Potato
PGB-UFPI 817	11.97 f	9.02 e	6.06 a	1.32	0.65	Spherical	Flat	40.47	Potato
PGB-UFPI 857	15.29 e	11.48 c	6.26 a	1.33	0.54	Spherical	Flat	72.10	Big Lima

Accessions followed by the same letter were grouped by the Scott-Knott test at the 5% significance level. SL (seed length), SW (seed width), ST (seed thickness). The classification was according to Baudet (1977) and Castiñeiras et al. (1991).

identified 100-seed weight ranging from 32.6 g (Branquinha and Olho-de-peixe) to 79.5 g (Orelha-de-vó). Nobre et al. (2012) found an average value of 57.37 g for 100 seeds. Sousa et al. (2015) analyzed 24 accessions from the germplasm bank and found values of 103g (PGB-UFPI 666) and 101.53 g (PGB-UFPI 622). The smallest average value was found for accession PGB-UFPI 777, with 21.1 g, corroborating considerable polymorphism regarding seed morphology in the PGB-UFPI accessions. Silva et al. (2017) observed the formation of three groups in the PGB-UFPI: Group I (composed of 74 accessions), with small, semi-flattened, and spherical seeds, and a medium overall average for length and width. Similar characteristics were found for the Mesoamerican gene pool. Group II (90% of the accessions) exhibited intercalary characteristics of both Andean and Mesoamerican pools. Group III consisted of accessions with the highest values of seed length and width, with predominantly large seeds, notable characteristics of the Andean gene pool.

In this respect, we believe that the accessions analyzed in the present study belong to both Andean (Big Lima) and Mesoamerican (Potato and Sieva) gene pools, with a predominance of the former cultigroup in the selected samples. The big, flattened seeds evaluated here suggest that these accessions belong to the Andean gene pool. These accessions are geographically restricted to Ecuador and the North of Peru and are well-adapted to dry areas (Baudoin et al. 2004). As discussed by Silva et al. (2017), in Brazil, there is a predominance of species belonging to the Mesoamerican gene pool, corroborating the assumption that this group seems to have a wider distribution than the Andean group (Andueza-Noh et al. 2013). However, Silva et al. (2019) confirmed that both the Andean and the Mesoamerican gene pools of lima bean are widely grown in Brazil, confirming the genetic diversity of the species regarding seed size and shape, which contribute to lima bean conservation in the Northeast of Brazil.

Finally, the present study validates the cytological stability for number of chromosomes, the karyotypic formula, and the CMA-rich pericentromeric heterochromatin pattern, as previously reported by other authors in the *P. lunatus* species and observed in the accessions preserved in the *Phaseolus* Active Germplasm Bank of UFPI. The seed morphological profile showed there are representatives of the Andean gene pool, represented by the Big Lima cultigroup (with larger seeds and flat shape), and the Mesoamerican gene pool, represented by the Sieva and Potato cultigroups (with small seeds, that are globular, flat, and kidney-shaped); and it corroborates that the Brazilian Northeast region could be a diversity and domestication center.

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