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Genetic diversity and promising crosses indication in lima bean (*Phaseolus lunatus*) accessions

Diversidade genética e indicação de cruzamentos promissores entre acessos de feijão-fava (*Phaseolus lunatus*)

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Abstract

Lima bean is a source of income for the rural population of Northeast Brazil because people purchase and consume the immature and mature beans. In this region, all of the germplasms used by farmers come from their own crops or they trade between rural communities. Understanding the genetic diversity of the lima bean germplasm provides important information for both managing germplasm banks and genetic conservation. In this study, we aimed to estimate the genetic diversity between 24 lima bean accessions from the Active Germplasm Bank (AGB) at the Universidade Federal do Piauí (UFPI) based on morphoagronomic descriptors. The experiment was conducted under field conditions in lattice design with four replications and ten plants per plot, from February to August 2009. Seven qualitative and six quantitative descriptors were used. Four groups were created for quantitative traits using the Tocher and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) methods, while qualitative traits were divided into three groups using the Tocher method and into six groups using UPGMA. The trait that contributed the most to genetic diversity (35.23%) was pod width. UFPI-220 produced a large number of pods per plant, an important trait for identifying potentially productive accessions. We expect that beneficial combinations can be made between UFPI-220 x UFPI-468 due to both the high level of dissimilarity and the average performance of these accessions.

Key words: Morphoagronomic characterization, descriptors, parental selection

Resumo

O feijão-fava é uma alternativa de renda para a população rural do Nordeste do Brasil, que consome seus grãos verdes ou maduros. Nessa região, todo germoplasma utilizado pelos agricultores é originário de seus próprios cultivos havendo intercâmbio de sementes entre comunidades rurais. O conhecimento sobre a diversidade genética tem proporcionado importantes contribuições no gerenciamento de bancos de germoplasmas e na conservação de recursos genéticos. Neste trabalho, objetivou-se estimar a diversidade genética entre vinte quatro acessos de feijão-fava do Banco Ativo de Germoplasma (BAG) da Universidade Federal do Piauí (UFPI), com base em descritores morfoagronômicos. O experimento foi conduzido em condições de campo, em látice com quatro repetições e dez plantas por parcela, no período de fevereiro a agosto de 2009, sendo utilizados sete descritores qualitativos e seis quantitativos. Para os caracteres quantitativos foram formados quatro grupos pelos métodos de Tocher e UPGMA, enquanto que para os qualitativos formaram-se três grupos pelo método de Tocher e seis pelo UPGMA.

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O caráter que mais contribuiu para divergência genética (35,23%) foi largura da vagem. UFPI-220 produziu grande número de vagens por planta, característica importante na identificação de acessos potencialmente produtivos. Há expectativa de combinação promissora entre UFPI-220 x UFPI-468, em virtude da maior dissimilaridade observada e do melhor desempenho médio desses acessos.

Palavras-chave: Caracterização morfoagronômica, descritores, seleção de genitores

Introduction

Lima bean (*Phaseolus lunatus* L.) is a subsistence crop in the humid tropics of America and an important source of protein for rural populations in South America and Africa. Significant efforts to collect, evaluate genetic diversity and domestication of *P. lunatus* have been made in their natural environment (MARTÍNEZ-CASTILLO et al., 2004, 2006, 2007, 2008, 2011; MOTTA-ALDANA et al., 2010; ANDUEZA-NOH et al., 2013). The existence of an adequate collection of germplasm is an essential requirement to maintain levels of genetic diversity in any specie, as well as to determine its center of domestication. These aspects, in turn, are essential for the development of strategies for genetic breeding and for *in situ* and *ex situ* seed conservation.

In the Northeast region of Brazil, the lima bean germplasm used by farmers originate from their own crops and from trade between rural communities. These are genotypes have been cultivated for a long time and can be considered landraces. Although the genotypes maintain several undesirable traits, including pest and disease susceptibility, indeterminate growth, long life cycle, and high hydrocyanic acid content, among others, they also have good local adaptation and a large amount of genetic variability for most agronomic traits (SANTOS et al., 2002).

Characterizing and evaluating the germplasm is essential for better use of the available genotypes and, consequently, for the continued development of more yielding cultivars with improved quality and disease resistance (LOPES, 1998). In addition, understanding genetic diversity provides important information for creating germplasm banks and conserving genetic resources.

Measuring genetic diversity using multivariate analysis, with which several traits can be evaluated simultaneously, is highly advantageous. Among the multivariate techniques, clustering method is particularly useful to separate a group of observations into subgroups in a way that maximizes homogeneity within and heterogeneity between subgroups. Of these methods, hierarchical and optimization techniques are widely used for plant improvement. The Tocher clustering method assumes that the average dissimilarity within each group should be less than the average distance between groups and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) hierarchical method uses arithmetic means of the dissimilarity measurements to avoid characterizing dissimilarity by the extreme values of the included genotypes (CRUZ; CARNEIRO, 2003).

To estimate the genetic diversity of the Active Germplasm Bank at the Universidade Federal do Piauí (AGB/UFPI), we characterized and evaluated the agromorphological traits of the lima bean accessions.

Materials and Methods

The 24 lima bean accessions in the BAG/UFPI were selected by their distinct coloration patterns and locations of origin (Table 1). The lima beans were characterized between February and August 2009 in the experimental area of the UFPI, Plant Science Department in the Center for Agricultural Sciences in the municipality of Teresina - Piauí State, Brazil, located at 05°05'05" S latitude and 42°05' W longitude, and altitude of 72.7 m. The region's climate is Aw' based on the Köppen classification system and has annual averages of 27°C, 74%

relative humidity, and 1,500 mm of annual rainfall (ANDRADE JÚNIOR et al., 2005). The soil in the area is Quartzipsamment with a sandy texture. A 5 x

5 lattice design with four replicates was used. Each plot contained 10 plants in four rows spaced five meters of 1.0 x 1.0 m.

Table 1. Identification, common name, seed tegument color and procedence of 24 lima bean accessions from the Active Germplasm Bank at the Universidade Federal do Piauí. Teresina, Piauí State, Brazil, 2010.

Accession	Common Name	Seed Tegument Color	Procedence
UFPI-032	-	Brown	Várzea Grande-PI
UFPI-121	-	Yellow	Bom Jesus-PI
UFPI-123	'Fava-branca'	White	Dermeval -Lobão-PI
UFPI-177	-	Brick Red	Fortaleza-CE
UFPI-220	-	Brown	Ipameri-GO
UFPI-222	-	Brown	Ipameri-GO
UFPI-228	-	Cream with brown hilum	São Pedro-PI
UFPI-230	-	Brown	Porto Firme-MG
UFPI-243	-	Orange with black spots	Rio Casca-MG
UFPI-251	-	Light grey	Cáceres-MG
UFPI-274	'Branca'	White	Cajazeiras-PB
UFPI-275	'Rajada preta'	Black striped	Cajazeiras-PB
UFPI-276	'Pintada roxa'	Painted purple	Cajazeiras-PB
UFPI-278	'Coquinho'	Yellow	Cajazeiras-PB
UFPI-463	'Fava preta'	Black	Arraial-PI
UFPI-465	-	Cream	Mirador-MA
UFPI-468	-	Cream	Barra do Corda-MA
UFPI-470	-	Cream	CAS ¹ -Teresina-PI
UFPI-483	-	Black	Várzea Grande-PI
UFPI-494	-	Light brown	Açailândia-MA
UFPI-500	-	Cream with brown hilum	Guaramirangá-CE
UFPI-515	-	White with brown hilum	CAS ¹ -Teresina-PI
UFPI-579	-	Light beige	Campina Grande-PB
UFPI-582	'Boca-de-moça'	Cream with a differentiated hilum	Novo Oriente-PI

¹ Colégio Família Agrícola do Soinho.

Source: Elaboration of the authors.

Agromorphological characterization and evaluation of the accessions included the following traits: number of days to flowering (NDF) (determined when 50% of the flowers are open); number of days to maturation (NDM) (the number of days from emergence to stage when 90% of plants have mature pods); number of pods per plant (NPP) (the total number of fruit per plant); pod length (PL) and pod width (PW) (measured in cm and recorded for 10 randomly selected mature pods) and number of beans per pod (NBP) (measured from 10 randomly selected mature pods). The bean

seeds traits were base bean color (BBC); bean color pattern (BCP); secondary color pattern (SCP); seed tegument pattern (STP); seed shape (SS); testa separation (TS) and testa texture (TT). The traits were evaluated in 10 randomly selected seeds for each accession.

Quantitative traits were analyzed to verify the existence of variability among accessions. NPP measurements were transformed to $\sqrt{x} + 0.5$ to meet the assumptions of this analysis. Means were clustered according to Scott-Knott test ($P < 0.05$).

Identification of the traits importance was based on the method proposed by Singh (1981), and the divergence among accessions was determined by clustering using the Tocher optimization and UPGMA hierarchical methods using the Mahalanobis distance as a measure of dissimilarity.

Qualitative descriptor analysis included the calculation of a dissimilarity matrix with multicategorical traits. This matrix was constructed using the mode of each variable for each accession without repetition. Clustering analysis using the Tocher and UPGMA methods was then performed. Genetic statistical analysis were performed using the softwares SAS (1989) and GENES (CRUZ, 2013).

Results and Discussion

The lima bean accessions differed from each other for most of the evaluated traits, with the exception of NDF, NDM, and NBP, indicating genetic variability (Table 2). NPP average was 8.70 and ranged from four (UFPI-582) to 16 (UFPI-220) pods per plant. According to Guimarães et al. (2007), this variability is important for identifying potentially productive *P. lunatus* accessions. The accessions had an average PL of 69.50 mm, and the largest averages were recorded for UFPI-276 (99.85 mm), UFPI-468 (86.20 mm), and UFPI-032 (82.98 mm). The overall average for PW was 15.05 mm, and 12 accessions had particularly wide pods ranging from 15.29 mm (UFPI-515) to 17.62 mm (UFPI-468). The accessions with the narrowest pods were UFPI-251 (12.20 mm), UFPI-230 (12.40 mm), and UFPI-220 (13.28 mm). Santos et al. (2002) studied the yield of eight lima-bean varieties and determined that the 'Orelha-de-vó' (89.9 mm and 18.6 mm) and 'Raio-de-sol' (83.9 mm and 19.8 mm) varieties had the largest average lengths and

widths, respectively. The 276-UFPI access stood out on the length and width of the lima bean pods. Their pods were wider than the 'Orelha-de-vó' variety, showing up promising to improve these characteristics that are related to the larger size of the seed.

The smallest coefficient of variation (CV) values were observed for the traits NDF (5.71%), PL (7.79%), NDM (11.50%), and NBP (11.37%), and the highest values were observed for PL (16.04%) and NPP (33.14%). Oliveira, Torres e Bebedito (2011) analyzed seven morphological traits and the agronomic performance of eight lima bean accessions in the edaphoclimatic conditions in Mossoró, Rio Grande do Norte State, Brazil.. The authors observed a larger CV for pod length (23.18%) and a smaller CV for yield (0.69%). The CV obtained from the analysis of variance of an experimental test demonstrates its degree of precision (PIMENTEL-GOMES, 2009).

The genetic dissimilarity, estimated by the generalized Mahalanobis distance (D^2), among the 24 accessions demonstrated that UFPI-468 and UFPI-220 ($D^2 = 35.89$) were the most divergent and that UFPI-251 and UFPI-230 ($D^2 = 0.60$) were least divergent. Quantifying dissimilarity is important to obtain transgressive segregants and populations with a large amount of genetic variability (CRUZ; CARNEIRO, 2003). This aids the plant breeder in selecting combinations that are both more promising and favorable for crossing. In this study, we expect that beneficial combination can be made between UFPI-220 x UFPI-468 due to both the high level of dissimilarity and the average performance of these accessions. UFPI-220 had the largest average NPP and UFPI-468 is one of the accessions with the largest average PL and PW, which are important characteristics for identifying potentially productive accessions.

Table 2. Means¹ for 24 lima bean accessions considering number of days to flowering (NDF), number of days to maturation (NDM), number of pods per plants (NPP), pod length (PL), pod width (PW), and number of beans per pod (NBP). Teresina, Piauí State, Brazil, 2010.

Accessions	NDF	NDM	NPP	PL	PW	NBP
UFPI-032	89.50	150.82	6.72 c	82.98 a	16.50 a	2.55
UFPI-121	92.02	143.07	12.30 a	65.40 c	14.25 b	2.30
UFPI-123	91.87	144.15	6.00 c	66.57 c	14.65 b	2.77
UFPI-177	98.90	155.47	9.54 b	70.92 b	16.41 a	2.72
UFPI-220	91.80	152.87	16.05 a	49.48 c	13.28 c	2.80
UFPI-222	94.02	149.77	12.25 a	60.80 c	14.27 b	2.95
UFPI-228	92.85	157.07	7.30 c	56.34 c	14.05 b	2.95
UFPI-230	89.40	156.67	12.34 a	56.61 c	12.40 c	2.50
UFPI-243	94.47	157.62	14.26 a	61.56 c	13.72 b	2.82
UFPI-251	91.12	155.85	11.31 b	59.49 c	12.20 c	2.62
UFPI-274	93.92	154.10	4.31 c	70.83 b	14.93 b	2.55
UFPI-275	92.50	154.64	8.70 b	77.90 b	17.30 a	2.70
UFPI-276	95.60	130.87	9.34 b	99.85 a	15.67 a	2.55
UFPI-278	90.97	151.30	10.53 b	67.83 c	15.35 a	2.80
UFPI-463	98.05	145.10	8.05 c	78.15 b	15.60 a	2.52
UFPI-465	91.02	150.97	8.88 b	62.21 c	14.27 b	2.58
UFPI-468	92.77	136.18	5.40 c	86.20 a	17.62 a	2.17
UFPI-470	92.40	143.07	6.03 c	74.35 b	16.32 a	2.60
UFPI-483	91.80	159.14	6.03 c	71.74 b	14.18 b	2.60
UFPI-494	93.55	143.22	4.39 c	65.51 c	16.40 a	2.95
UFPI-500	93.27	150.55	9.43 b	73.58 b	16.00 a	2.52
UFPI-515	90.92	162.25	6.25 c	72.18 b	15.29 a	2.37
UFPI-579	91.85	149.25	9.05 b	58.88 c	13.87 b	2.50
UFPI-582	91.22	146.10	4.26 c	78.69 b	16.61 a	2.62
Overall mean	92.74	150.0	8.7	69.5	15.05	2.60
CV (%)	5.71	11.05	33.14	16.04	7.79	11.37

¹Means followed by the same letter do not differ by the Scott-Knott test ($P < 0.05$).

Source: Elaboration of the authors.

The Tocher method allowed us to group the 24 lima bean accessions into four groups based on three quantitative descriptors (Table 3). Creating these groups was important for choosing parents, as the novel hybrids to be created should be chosen using the magnitude of their dissimilarities and the potential of the parental *per se*. Placement of genotypes in groups that are relatively far apart indicates that they are dissimilar and are thus promising combinations for artificial crosses. However, in addition to their dissimilarity, the genotypes must have high average values and variability for the traits being selected for improvement (ABREU et al., 2004). Group I contained the accessions with the lowest PL

averages: UFPI-032, UFPI-121, UFPI-123, UFPI-177, UFPI-222, UFPI-228, UFPI-230, UFPI-243, UFPI-251, UFPI-274, UFPI-278, UFPI-463, UFPI-465, UFPI-470, UFPI-483, UFPI-500, UFPI-515, UFPI-579, and UFPI-582. Group II contained UFPI-275, UFPI-468, and UFPI-494, which had the largest average PL. Group III contained UFPI-276 and had the largest average PL and PW. Group IV consisted of UFPI-220, which had the smallest PL and PW and the largest average NPP, which is an important characteristic for identifying potentially productive accessions. Santos et al. (2010) concluded that the Tocher optimization method was effective for evaluating the genetic divergence of 27 lima bean

accessions from the lima bean AGB/UFPI because it formed 10 groups based on physical and chemical seed characteristics.

The criteria adopted by the UPGMA hierarchical clustering method dictates that a cluster of similar genotypes must be formed first and then the distances of the others must be calculated relative to this first group (CRUZ; CARNEIRO, 2003). Groupings are made subjectively by finding points representing large changes in the dendrogram (ARRIEL et al., 2006). Using this method on quantitative traits, four groups were created (Figure 1). Group I contained accessions UFPI-121, UFPI-220, UFPI-222, UFPI-230, UFPI-243, UFPI-251, and UFPI-465, which collectively had the highest average NPP and the lowest average PW. Group II included accessions UFPI-123, UFPI-228, UFPI-274, UFPI-483, UFPI-515, and UFPI-579, which had the highest average PW and the lowest average NPP and PL. Group III had the highest average PW and contained the following 10 accessions: UFPI-032, UFPI-177, UFPI-275, UFPI-278, UFPI-463, UFPI-468, UFPI-470, UFPI-494, UFPI-500, and UFPI-582.

Accession UFPI-276, one of the accessions with the largest average PL and PW and the lowest NPP, was placed in Group IV. According to Salehi, Faramarzi e Mohebalipour (2010), the number of pods per plant was the only effective trait on seed yield of common bean. Therefore, the accessions of Group I have the potential to be more yielding.

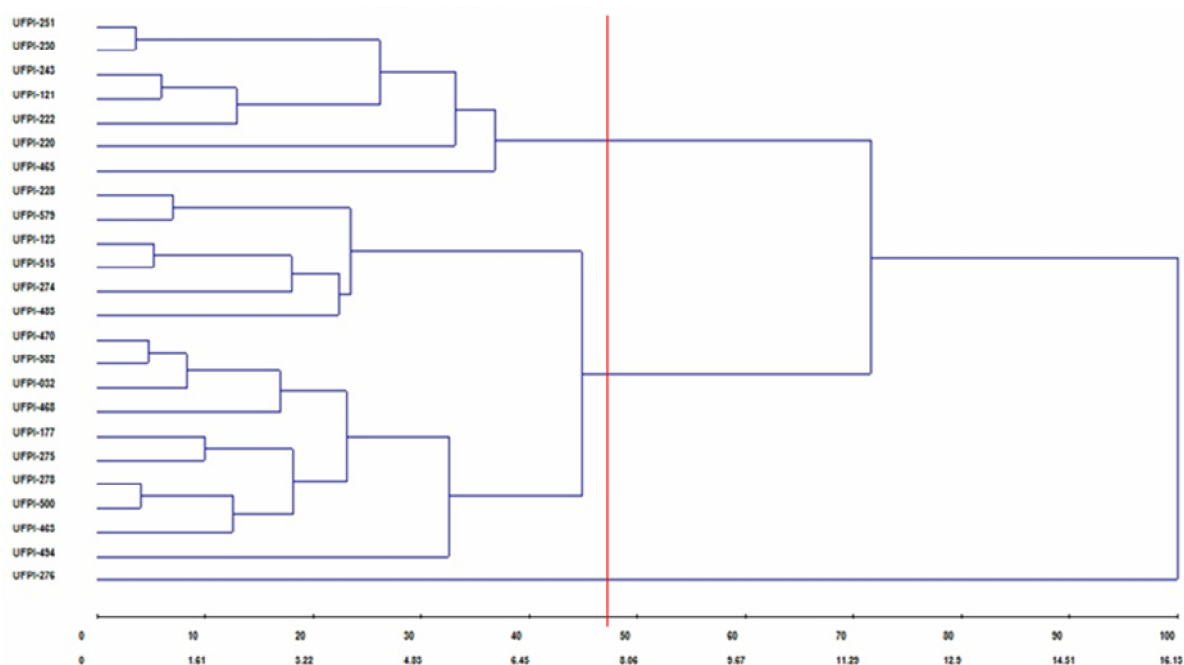
The use of the Tocher clustering method based on a dissimilarity matrix containing the Mahalanobis distances (D^2) calculated from five qualitative bean seeds characteristics enabled the accessions to be divided into three groups (Table 4). The testa separation and testa texture traits were not variable among these accessions. Group I contained 87.5% of the accessions whose second color pattern was absent. Group II consisted of accessions UFPI-251 and UFPI-582 and had a pale brown color pattern with a distinct halo and few markings on the seed. Group III contained UFPI-243 and had a grey base color and a red pattern. The color and seed size have been used to explain lima bean diversity and both are the main characteristics considered by consumers when they choose the product in the market (VARGAS et al., 2003).

Table 3. Clustering of 24 lima bean accessions by the Tocher method based on quantitative traits. Teresina, Piauí State, Brazil, 2010.

Clusters	Accessions						
I	UFPI-032	UFPI-121	UFPI-123	UFPI-177	UFPI-222	UFPI-228	UFPI-230
	UFPI-243	UFPI-251	UFPI-274	UFPI-278	UFPI-463	UFPI-465	UFPI-470
	UFPI-483	UFPI-500	UFPI-515	UFPI-579	UFPI-582		
II	UFPI-275	UFPI-468	UFPI-494				
III	UFPI-276						
IV	UFPI-220						

Source: Elaboration of the authors.

Figure 1. Dendrogram of the genetic dissimilarity between 24 lima bean (*Phaseolus lunatus*) accessions in the Active Germplasm Bank at UFPI (BAG/UFPI) created by the UPGMA method and quantitative traits. Teresina, Piauí State, Brazil, 2010.



Source: Elaboration of the authors.

Table 4. Clustering of 24 lima bean accessions by the Tocher method based on seven qualitative seed traits to build a dissimilarity matrix. Teresina, Piauí State, Brazil, 2010.

Groups	Accessions
I	UFPI-032 UFPI-121 UFPI-123 UFPI-177 UFPI-220 UFPI-222 UFPI-228 UFPI-230 UFPI-274 UFPI-275 UFPI-276 UFPI-278 UFPI-463 UFPI-465 UFPI-468 UFPI-470 UFPI-483 UFPI-494 UFPI-500 UFPI-515 UFPI-579
II	UFPI-251 UFPI-582
III	UFPI-243

Source: Elaboration of the authors.

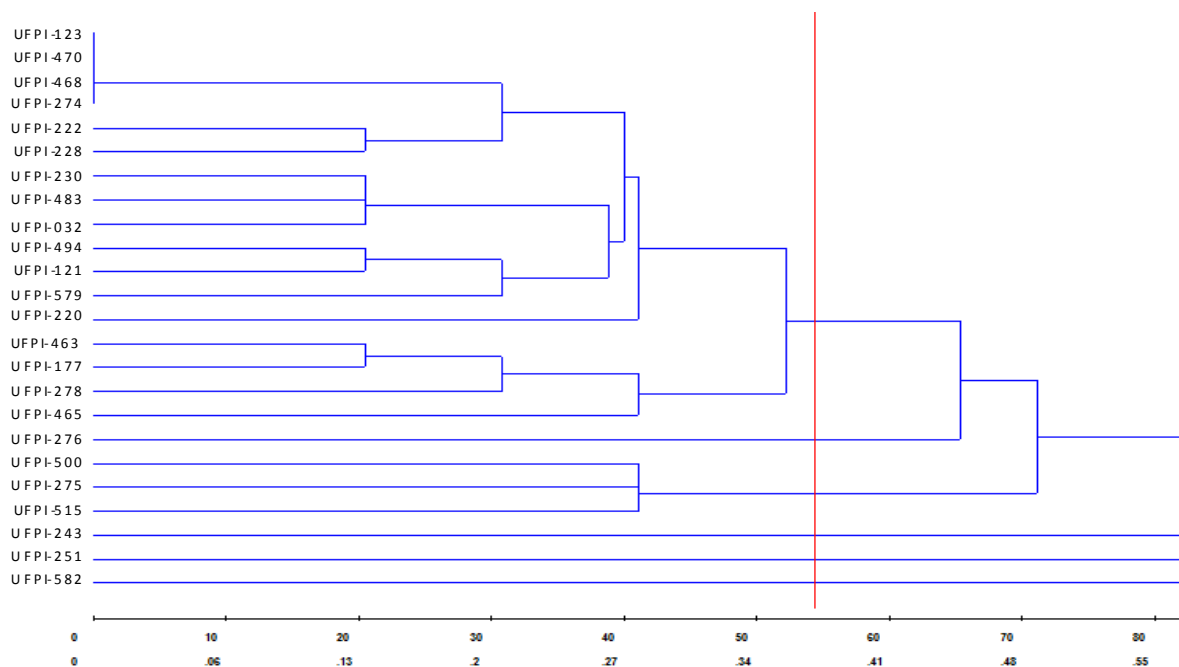
Analysis of seven qualitative seed traits using the UPGMA clustering method created six groups (Figure 2). The lima beans in Group I lacked a color pattern, a secondary color pattern, and a tegument pattern, and this group consisted of the following accessions: UFPI-032, UFPI-121, UFPI-123, UFPI-177, UFPI-220, UFPI-222, UFPI-228, UFPI-230, UFPI-274, UFPI-278, UFPI-463, UFPI-465, UFPI-468, UFPI-470, UFPI-483, UFPI-494, and UFPI-579. Group II consisted of UFPI-276, which

had a gray base color and a red pattern. Group III contained UFPI-275, UFPI-500, and UFPI-515 and had a white base color, no secondary color pattern, and the same seed shape. Group IV contained only UFPI-243 and had a brick red base color, a red pattern, and a tegument pattern with spots. Group V was made up of UFPI-251 and had a red base color, a light brown color pattern, a dark-red secondary color pattern, and a tegument pattern containing few spots. Group VI consisted of accession 582 and had

a white base color, light brown color pattern, black secondary color pattern, and a tegument pattern containing few marks. Elias et al. (2007) evaluated the genetic diversity among 45 cultivars of black beans using UPGMA based on 11 morphoagronomic and nutritional traits and found two groups. These

researchers stated that this method results in a better fit between the original and estimated distances. According to Santos et al. (2010), the color patterns, seed shapes, and tegument colors of lima beans vary widely, which represents a large increment of the aesthetic grains among beans used for food.

Figure 2. Dendrogram of the genetic dissimilarities between 24 lima bean (*Phaseolus lunatus*) accessions in the Active Germplasm Bank at UFPI using the UPGMA method and based on qualitative descriptors. Teresina, Piauí State, Brazil, 2010.



Source: Elaboration of the authors.

Accessions from the same location were generally not placed in the same group, indicating that there is more variation within a location than among locations. Genetic drift and selection in various environments can cause more divergence than geographic distance. Laurentin and Karlovsky (2006) studied a sesame collection that was genetically very variable and did not show an association between geographical origin and AFLP patterns. According to the authors, the traditional assumption that selecting genotypes of different geographical origin will maximize the diversity

available to a breeding project does not hold in sesame.

Of the traits included in this analysis, the ones with the largest contribution to genetic divergence were PW (33.45%), NPP (25.69%), PL (20.27%), and NBP (10.73%) (Table 5). According to Correa e Gonçalves (2012), the trait that best explains the genetic divergence among 13 bean genotypes was the mass of 100 seeds, representing 65.83% of the total dissimilarity, and indicated that this trait should be prioritized in the selection of parents for breeding programs, especially considering seed size

is important for the consumer market. Likewise, the width of the pod with the highest contribution to the

genetic divergence in this study, which is positively correlated with seed size, should be prioritized.

Table 5. Relative contribution of morphoagronomic traits to the genetic divergence of 24 lima bean accessions according to Singh (1981). Teresina, Piauí State, Brazil, 2010.

Trait	Value (%)
Pod width	33.45
Number of pods per plant	25.69
Pod length	20.27
Number of beans per pod	10.73
Number of days to maturation	5.41
Number of days to flowering	4.43

Source: Elaboration of the authors.

Conclusion

The 24 lima bean accessions in the Active Germplasm Bank at UFPI demonstrate the genetic diversity of both quantitative and qualitative traits. Pod width was the trait that most contributed to the genetic divergence. Combination can be made between UFPI-220 x UFPI-468 due to both the high level of dissimilarity and the average performance of these accessions.

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