ARTICLE

Genetic variability in subsamples of determinate growth lima bean

Mara Danielle Silva do Carmo[1], Regina Lucia Ferreira Gomes[2*], Ângela Celis de Almeida Lopes[3], Josilane Souza da Penha[2], Sulimary Oliveira Gomes[2] and José Ribamar de Assunção Filho[4]

Abstract – *Lima bean, a highly important legume, serves as a source of protein, reducing nutritional dependency on meat and other beans, especially for needy populations. This crop, with production concentrated in the Northeast of Brazil, has been the subject of very few studies, considering the evaluation of varieties predominantly with indeterminate growth habit. The characterization of material presenting determinate growth, an agronomically important characteristic, may subsidize breeding programs. We characterize subsamples of determinate growth lima bean in morpho-agronomic terms and estimate genetic divergence. We identified pairs of divergent and complementary genotypes for crosses. The genotypes were grouped in six and five groups in regard to the Tocher and UPGMA methods, respectively, for the quantitative traits. For the qualitative traits, three groups were formed. Analyses of canonical variables identified traits related to production of importance for variability. The study shows the importance of characterization of new genotypes for conservation and future utilization.*

Key words: Phaseolus lunatus*, protein source, growth habit, multivariate analysis.*

INTRODUCTION

The species *Phaseolus lunatus* L.*,* known as lima bean or butter bean, is the second most grown and consumed of the *Phaseolus* genus (Delgado-Salinas et al. 1999, Oliveira et al. 2004). Used in the most varied culinary dishes around the world due to its characteristic flavor, it assumes importance for underprivileged populations because of its protein content, substituting meat-based foods (Vieira 1992, Kee et al. 1997, Azevedo et al. 2003).

Growing of lima beans is traditionally carried out by native populations in Mexico, Peru and adjacent regions (Martinez-Castillo et al. 2004, Camarena 2005); nevertheless, the United States is the greatest producer, using even cultivars from breeding programs (Vieira 1992, Kee et al. 1997), and, in Brazil, the species assumes economic and social importance, notably in the Northeast states (Vieira 1992). In this region, the hardiness of the species allows extending the harvest period, performed in the dry period (Azevedo et al. 2003). In addition, its importance arises from

the fact of its being an alternative for food and income for the population, reducing protein dependence based almost exclusively on beans of the carioca and cow pea group (Vieira 1992, Azevedo et al. 2003, Oliveira et al. 2004).

The relevance of lima bean may be seen by means of data in regard to domestic production, which shows an increase from 2005 (13,181 t) to 2009 (20,702 t). Nevertheless, in spite of being grown in various Brazilian states, to a greater or lesser degree, and although it presents capacity for broader adaptation than common bean (Lymman 1983), it exhibits concentration in the Northeast region, which was responsible for 12,345 t in 2005 and 17,078 t in 2009 (IBGE 2010). In spite of the relative increase in production through the years, growing of lima bean is performed by family farmers and adding to this the existence of a greater tradition of common bean consumption, the particular flavor of the lima bean, which sometimes serves as an attractive and sometimes repulsive factor for consumers, and its longer cooking time, there has been reduced consumption and scarce interest in research (Santos et al. 2002, Azevedo et al. 2003).

[1] Universidade Estadual do Piauí, Campus Professor Barros Araújo, Av. Senador Helvídio Nunes s/n, 64.607-670, Picos, PI, Brazil

[2] Universidade Federal do Piauí, Centro de Ciências Agrárias, Departamento de Fitotecnia, Campus Universitário Ministro Petrônio Portella, Av. Universitária, Ininga 64.049-550, Teresina, PI, Brazil. *E-mail: rlfgomes@ufpi.edu.br

[3] Universidade Federal do Piauí, Centro de Ciências da Natureza, Departamento de Biologia, Campus Universitário Ministro Petrônio Portella, Av. Universitária, Ininga 64049-550, Teresina, PI, Brazil

[4] Escola Superior de Agricultura "Luiz de Queiroz", Av. Pádua Dias, 11, 13.418-900, Piracicaba, SP, Brazil

Thus, technologies with a view toward increasing yield have not been adopted, as for example, the use of improved cultivars and suitable mineral and organic fertilization (Azevedo et al. 2003). Furthermore, the occurrence of diseases and pests affect the quality of pods and beans, hurting yield and constituting one more limiting factor for success in effective establishment of the crop (Paula Júnior et al. 1995).

Morpho-agronomic characterization, upon describing the diverse subsamples of a germplasm collection, may be used to research the existence of variability, as well as reveal the behavior of the materials and their potential features (Zimmerman and Teixeira 1996, Nass 2007). Another aspect is allowing quantification of genetic divergence, which is an advantageous study in the process of identification of new sources of genes of interest, so as to provide information on potential parents to be used in breeding programs (Cruz et al. 2004).

Research with lima bean has been carried out through studying its genetic variability in Brazil, mainly as of data derived from morphological and agronomic characterization; nevertheless, most of these studies use only varieties with indeterminate growth habit, which are the most grown varieties in the Northeast (Santos et al. 2002, Guimarães et al. 2007). Nevertheless, genotypes with plant architecture that facilitates crop management and mechanized harvest meet current demands and trends, and are quite desirable upon evaluating genetic resources to subsidize beginning genetic breeding programs (Koinange et al. 1996).

Thus, the purpose of this study was to characterize and evaluate the genetic divergence among subsamples with a determinate growth habit derived from the Lima Bean Active Germplasm Bank of the Universidade Federal do Piauí (Federal University of Piaui), based on morphological and agronomic traits.

MATERIAL AND METHODS

The genetic material evaluated was obtained by means of collection in the state of Paraiba, Brazil, and from introduction of germplasm from the Centro Internacional de Agricultura Tropical (CIAT) (International Center of Tropical Agriculture) in Columbia, giving priority to the habit of determinate growth. The trail was conducted in an irrigated system in the second semester of 2010 in an experimental area of the Plant Science Department of the Agrarian Science Center at the Universidade Federal do Piauí in Teresina (lat 05º 05' S, long 42º 05' W and alt of 72.7 m asl), PI. The nine subsamples of lima bean (Table 1) were arranged in a randomized block experimental design, with four replications. Each plot was composed of three 3.5m length rows at a spacing of 0.6 m between rows and 0.5 m between plants, with the five center plants being evaluated.

The following quantitative and qualitative traits were evaluated per individual plant, according to crop standards published by Bioversity Internacional (IPGRI 2001): number of days for flowering (NDF); number of days to pod maturity (NDM); length of main branch (LMB); number of nodes on the main branch (NNMB); insertion height of the first pod (IHP); pod length (PL); pod width (PW); number of locules per pod (NLP); number of beans per pod (NBP); 100 seed weight (100SW); bean length (BL); bean width (BW); bean thickness (BT); pod color (PC); bean background color (BC); bean pattern color (BPC); second pattern color of the bean (SPC); bean seed coat pattern (SCP); bean shape (BS); seed testa texture (STT) and seed testa separation (STS). The growth habit score (GHS) trait, which is not listed among the descriptors available for lima bean, was checked by two evaluators, according to the scale of scores used by Menezes Júnior et al. (2008) for common bean.

Genetic divergence among the subsamples was estimated by means of Mahalanobis generalized distance (D²). With the dissimilarity matrix, the subsamples were then grouped according to the UPGMA (unweighted pair group method average) and the Tocher method for the 14 quantitative traits. In a grouping approach with the eight qualitative traits, the UPGMA and Tocher methods were used, with the mode values of each variable per subsample.

Table 1. Listing of subsamples evaluated in the field

Subsamples	Origin	Country of origin	Seed coat color
UFPI 728	Paraíba	Brazil	Cream color with brown stripes
UFPI 634	CIAT	The Philippines	Red striped with black
UFPI 639	CIAT	United States	Light brown striped with purple
UFPI 640	CIAT	Trinidad and Tobago	White
UFPI 642	CIAT	Ghana	White
UFPI 641	CIAT	United States	White
UFPI 632	CIAT	United States	White
UFPI 635	CIAT	India	White
UFPI 644	CIAT	United States	White

Identification of quantitative descriptors with greater contribution to divergence was performed in accordance with analysis of canonical variables, which also allowed identification of the traits that most contributed to variability, as well as to obtain a two-dimensional dispersion diagram of the subsamples in relation to the first and second canonical variables. Statistical-genetic analyses were performed using the Genes software.

RESULTS AND DISCUSSION

Estimates of measurements of dissimilarity among the subsamples, according to Mahalanobis generalized distance (D^2) (Table 2), indicated the subsamples UFPI 640 and UFPI 728 as the most distant between themselves, which is explained by the fact of these differing in regard to most of the traits, being similar only for traits that varied little, such as the number of nodes on the main branch and height of insertion of the first pod. Little variation for these traits was expected as the subsamples evaluated had a determinate growth habit since their vegetative development is limited up to the time in which the apex (stem and lateral branches) are transformed into a specialized structure (such as flowers and inflorescences), without the capacity for continuance as of that point (Silva 2005); therefore it is associated with lower plant height and, consequently, lower NNMB and IHP.

The subsample UFPI 728 presented greater mean values for NDF (51.55 days), NDM (100.71 days), GHS (1.59), PW (15.89 mm) and BT (4.44 mm), and lower mean values for LMB (39.09 cm), PL (54.49 mm), NLP (2.07), NLG (1.76), 100SW (20.07 g), BL (11.00 mm) and BW (7.66 mm), while UFPI 640 had contrary results for these descriptors, with lower mean values for NDF (38.50 days), NDM (75.05 days), GHS (1.24), PW (14.52 mm) and BT (4.00 mm) and greater mean values for LMB (50.93 cm), PL (61.15 mm), NLP (2.57), NBP (2.21), 100SW (28.07 g), BL (12.33 mm) and BW (8.42 mm).

The second greatest distance observed was between the subsamples UFPI 728 and UFPI 632 (800.70), which were statistically similar only for LMB, NNMB, IHP and GHS.

In a general way, it was observed that the greatest distances were related to the subsample UFPI 728, this thus being one of the most divergent in relation to the others and it may be used in crosses. In addition, this subsample showed desirable characteristics not present in the others in relation to bean size, with mean values for pod width of 15.89 mm and for bean thickness of 4.44 mm. Together with these measurements, it is observed that the subsample UFPI 728 is the only one of domestic origin, which may have presented these greater mean values through being more adapted to local conditions.

It is important to emphasize that in undertaking crosses in search of greater heterosis in the segregating population, it is recommended to choose not only divergent parents but also those with good performance *per se*, in other words, those which present greater genetic distance and at the same time which complement some characteristic of one of the parents (Cruz et al. 2004).

The subsamples UFPI 634 and UFPI 632 also presented one of the greatest genetic distances, with similarities only for IHP, GHS and 100SW, complementing each other, for example, in relation to the NLP, which has a greater mean value in UFPI 632 (2.66) and in relation to BW, with high mean value in UFPI 634 (9.17 mm). Thus, these pairs of subsamples may also be used in crosses. The most similar subsamples (UFPI 641 and UFPI 635) proved to be in agreement in regard to most of the traits, with small differences only for the traits GHS, PL, PW and BL.

The characterization of exotic material and the demonstration of genetic divergence among the subsamples reveal the importance of collection and interchange of germplasm because they allow broadening of the genetic base of a crop for later use in breeding programs (Prance 1997).

Table 2. Estimates of dissimilarity among subsamples of lima bean by Mahalanobis distance, based on quantitative traits

	UFPI634	UFPI639	UFPI640	UFPI642	UFPI641	UFPI632	UFPI635	UFPI644
UFPI 728	654.15	708.35	890.65	282.67	340.28	800.70	574.85	298.07
UFPI 634		117.24	675.44	615.63	273.74	759.77	424.42	210.29
UFPI 639			387.23	590.58	206.82	432.87	327.95	302.92
UFPI 640				385.98	241.95	85.65	160.46	525.74
UFPI 642					147.62	351.80	159.70	190.40
UFPI 641						225.02	61.15	108.71
UFPI 632							182.56	569.97
UFPI 635								179.95

Maximum distance = 890.65 (Subsamples: UFPI 728 and UFPI 640)
Minimum distance = 61.14 (Subsamples: UFPI 641 and UFPI 635)

In addition, this material with the determinate growth differential, a characteristic which provides innumerable advantages in management (Teixeira et al. 1999) in relation to most varieties grown in Brazil with indeterminate growth (Santos et al. 2002, Oliveira et al. 2004), may make a change in the profile of the crop productive system possible, proving to be favorable to business initiatives for large scale production and transforming the current economic situation of the crop, just as has occurred in other species as, for example, in cow pea (Frota et al. 2000).

The Tocher grouping showed a pattern of six main groups, maintaining the more distant subsamples according to the Mahalanobis distance (Table 2) allocated in different groups; however, the most similar did not remain in the same group.

The groups formed were not related to the location of origin, suggesting that the variability of the subsamples was quite dispersed, and it should be noted that the subsamples were obtained from a variety of countries.

Group I maintained the subsamples UFPI 632 and UFPI 644 united, which shared intermediate and statistically equal mean values for IHP, 100SW, BW and BT. The subsamples UFPI 642 and UFPI 635 were gathered in the second group, sharing statistically equal mean values for NDF, GHS, NLP, NBP, 100SW and BW. As for Group III (UFPI 640 and UFPI 641), it had 100SW, BL and BT in common. Each one of the other subsamples constituted a group; UFPI 728 was group IV, with divergences in relation to the others being observed in it for NDF, NDM, and BT (greater mean values), and for 100SW, BL and BW (lower mean values). Subsample UFPI 639 was isolated in group V, showing greater mean values for BL and BW, and subsample UFPI 634 was placed in group VI for having greater mean values for PW and BW and a lower mean value for BT.

In studies of divergence, it is common to use more than one grouping method, observing the agreement between them (Abreu et al. 2004, Karasawa et al. 2005, Sudré et al. 2005). Genetic divergence among the subsamples of lima bean estimated grouping, according to the Tocher method, proved to be partially in disagreement with the UPGMA grouping (Figure 1), this latter method generating five groups and differently allocating the subsamples.

Based on the subjective interpretation of the dendrogram (Cruz et al. 2004) group I was formed with the subsamples UFPI 641, UFPI 635 and UFPI 644, which shared equal mean values for NDM, LMB, NNMB, 100SW, BW and BT. Group II, with the subsample UFPI 642, differed from the others by presenting lower mean values for NDF, NDM, PL and BL; in group III were placed the subsamples UFPI 640 and UFPI 632, differing between themselves only for LMB and GHS, and exhibiting higher mean values for

IHP, PL, NLP, NBP, 100SW, intermediate mean values for BL and BT and low mean values for NDF, NDM. Group IV was composed of the subsamples UFPI 634 and UFPI 639, with lower mean values PL and LV and group V by the subsample UFPI 728, with greater mean values for NDF, NDM and BT.

In grouping of the subsamples based on eight qualitative traits, both according to the Tocher method and according to the UPGMA (Figure 2), three groups were formed. Most of the subsamples were allocated in group I (UFPI 640, UFPI 632, UFPI 641, UFPI 635, UFPI 644 and UFPI 642), standing out in terms of the white seed coat color, characterized by similarity for the descriptors of bean background color (BC); bean pattern color (BPC); second pattern color of the bean (SPC) and seed coat pattern (SCP). The subsamples UFPI 728 and UFPI 639 remained in group II, probably through presenting similar form and pattern of the seed coat, but differing in background color and bean pattern color. Group III contained the subsample UFPI 634, with red background color and black pattern color, the most divergent in relation to the others for these traits.

The qualitative descriptors of testa separation, testa texture and pod color did not vary among the subsamples, being absent, smooth and brown, respectively, in all subsamples.

Comparison of the results of groupings by means of the two types of traits allows one to conclude that the quantitative descriptors allow a better partition of the subsamples in groups. One example is that most of the subsamples, allocated in various groups by means of the quantitative traits, remained united in a single group (I) by means of the qualitative traits. These observations emphasize the need for evaluating the maximum number of descriptors to more completely characterize the genetic resources.

The canonical variables represent, in decreasing levels, portions of total variance; thus, the first canonical variable, through retaining a greater quantity of variation in the original data, is considered to be the most important (Cruz et al. 2004). Moreover, the descriptors with greater eigenvectors for the respective component are considered to be those of greatest importance (Iezzoni and Pritts 1991). Based on the estimates of eigenvalues of the canonical variables (Table 3 and 4), it was observed that the first two variables explained 83.28% of the variation. For the canonical variable 1, the trait that most contributed to variation was LV (0.661), which was followed by BT (0.776), in the second canonical variable (VC_2) (Table 4). It may be observed that these traits that most contributed to variation were related to production and they should be included in later studies regarding evaluations of lima bean. Those that least contributed to variability according to the eigenvectors associated with the condensed variables were NDF, with

Table 3. Eigenvalues (λ_i) of the canonical variables (VC_i) and the corresponding accumulated percentage of variation explained by them for each descriptor evaluated: number of days for flowering (NDF), number of days to pod maturity (NDM), length of main branch (LMB), number of nodes on the main branch (NNMB), height of insertion of the first pod (IHP), growth habit score (GHS) and 100 seed weight (100SW)

VC_i	Eigenvalues		Eigenvectors associated with the canonical variables						
	λ_i	% accumulated	NDF	NDM	LMB	NNMB	IHP	GHS	100SW
VC_1	87.06	46.59	0.160	0.129	-0.091	0.011	-0.010	0.046	-0.262
VC_2	68.53	83.28	0.023	-0.018	0.058	-0.018	-0.018	-0.059	0.068
VC_3	19.41	93.67	-0.158	-0.095	-0.302	-0.234	-0.105	-0.231	0.203
VC_4	4.99	96.34	-0.020	-0.109	0.640	0.019	0.330	-0.381	0.302
VC_5	3.55	98.24	-0.358	-0.442	-0.013	0.207	0.278	0.275	0.361
VC_6	2.05	99.33	0.166	-0.008	0.051	0.587	0.000	-0.125	0.135
VC_7	0.92	99.83	0.198	-0.051	-0.002	0.139	-0.523	0.116	0.316
VC_8	0.32	100	0.108	0.304	-0.208	0.383	0.554	-0.007	-0.215
VC_9	0	100	0.095	0.141	-0.006	0.114	0.018	-0.160	-0.089
VC_{10}	0	100	-0.031	-0.025	0.026	0.588	-0.376	0.010	-0.004
VC_{11}	0	100	-0.084	0.744	-0.057	-0.066	0.030	0.103	0.606
VC_{12}	0	100	0.185	0.149	0.412	-0.088	-0.203	-0.352	-0.143
VC_{13}	0	100	0.074	0.113	0.493	-0.062	0.001	**0.722**	-0.137
VC_{14}	0	100	**0.828**	-0.248	-0.155	-0.129	0.196	0.100	0.292

Table 4. Eigenvalues (λ_i) of the Canonical Variables (VC_i) and the corresponding percentage of variation explained by them for each descriptor evaluated: pod length (PL), pod width (LV), number of locules per pod (NLP), number of beans per pod (NBP), bean length (BL), bean width (BW), bean thickness (BT)

VC_i	Eigenvalues		Eigenvectors associated with the canonical variables						
	λ_i	% accumulated	PL	LV	NLP	NBP	BL	BW	BT
VC_1	87.06	46.59	-0.295	0.661	-0.469	-0.127	0.166	-0.302	-0.020
VC_2	68.53	83.28	-0.331	-0.044	0.014	0.047	-0.477	-0.209	0.776
VC_3	19.41	93.67	-0.559	-0.067	-0.218	-0.114	-0.275	0.407	-0.309
VC_4	4.99	96.34	-0.209	-0.053	-0.062	-0.048	0.038	-0.338	-0.252
VC_5	3.55	98.24	0.149	0.504	-0.007	0.131	-0.131	0.189	0.057
VC_6	2.05	99.33	-0.207	-0.148	-0.202	-0.061	0.474	0.412	0.297
VC_7	0.92	99.83	-0.150	0.018	0.000	0.670	0.027	-0.218	-0.180
VC_8	0.32	100	-0.048	-0.127	-0.097	0.399	-0.352	0.004	-0.206
VC_9	0	100	-0.330	0.379	0.806	-0.022	0.063	0.118	-0.062
VC_{10}	0	100	0.119	-0.009	0.016	-0.481	-0.428	-0.175	-0.226
VC_{11}	0	100	0.123	0.110	-0.027	-0.147	0.008	0.001	0.052
VC_{12}	0	100	0.327	0.308	-0.163	0.188	-0.296	0.476	0.040
VC_{13}	0	100	-0.316	-0.101	-0.007	-0.070	-0.101	0.244	-0.104
VC_{14}	0	100	0.113	0.027	0.040	-0.210	-0.130	0.036	-0.044

a high magnitude value (0.828) in the VC_{14} (Table 3) and low value associated with VC_1 (0.160), followed by GHS (0.722) in VC_{13} (Table 3), which is not surprising because in bean plants it is observed that early cycles may be associated with determinate growth (Dalla Corte et al. 2002).

According to Cruz et al. (2004), traits dispensable in divergence studies are those that show little relative variation among the cultivars studied and/or are redundant through being correlated with other traits. In this study, the results shown by analysis of canonical variables were consistent with the mean values presented because the traits with less contribution (NDF and GHS) had little variability among the subsamples evaluated. Although having low contribu-

tion, these traits need to be studied later because they are agronomically relevant. In addition, these observations show the need for increasing the variability for such traits to the extent there is interest.

According to the dispersion diagram in relation to the first and second canonical variables, in two-dimensional space (Figure 1), the subsamples could be joined in five groups. The subsamples UFPI 728 and UFPI 642, situated in the upper right part, showed the most extreme values in regard to the canonical variables 1 and 2, which may be explained by their greater mean values for LV and for BT, such that they form individual groups. As for subsamples UFPI 634 and UFPI 639, located in the lower right part,
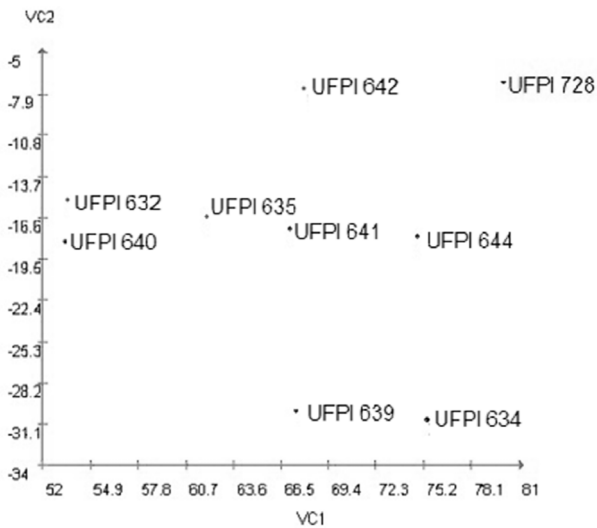
Figure 1. Dispersion diagram of nine subsamples of lima bean in relation to the first and second canonical variables (VC$_1$ and VC$_2$) for 14 quantitative traits.

they presented high mean values for the trait with greatest contribution over VC$_1$ (LV) and reduced mean values in regard to BT (trait that most contributed in VC$_2$), in relation to the other subsamples.

With intermediary and high mean values for LV (VC$_1$) and low mean values for BT (VC$_2$), subsamples UFPI 632 and UFPI 640 are located in the middle to left position of the dispersion diagram. The other subsamples (UFPI 635, UFPI 641 and UFPI 644), situated approximately in the center region, present intermediate mean values for BT

(VC$_2$), and for LV (VC$_1$) increasing mean values, from left to right, according to their placement on the diagram.

The groupings obtained by means of the dispersion diagram in regard to the two canonical variables were partially in agreement with those obtained by the groupings by the Tocher method and by the UPGMA, and allowed recognition, just as the others, of more dissimilar genotypes, for example, UFPI 728. Thus, this data may also be used in breeding programs that involve crosses through identification of highly divergent parents in a suitable manner.

Considering that many lima bean germplasm collections are conserved *ex situ* in the form of seed banks (Knudsen 2000, Silva et al. 2001, Camarena 2005), and that studies with the crop are scarce, it is interesting to maintain, at least initially, the maximum degree of variability represented in these banks so that characterization studies may be deepened by means of other approaches, such as molecular markers. In addition, the introduction of these subsamples, restricted to determinate growth varieties, represents a pioneering initiative in the country, which may permit their later utilization by mean of hybridizations, making the generation of more variability for improvement programs possible.

ACKNOWLEDGEMENTS

Variabilidade genética em subamostras de feijão-fava de crescimento determinado

Resumo – *Feijão-fava, leguminosa de expressiva importância, atua como fonte protéica, diminuindo a dependência alimentar da carne e outros feijões, especialmente para populações carentes. A cultura, com produção concentrada no Nordeste do Brasil, tem escassos estudos contemplando a avaliação de variedades predominantemente de hábito de crescimento indeterminado. A caracterização de material apresentando crescimento determinado, característica importante agronomicamente, pode subsidiar programas de melhoramento. O presente trabalho objetivou caracterizar morfo-agronomicamente subamostras de feijão-fava de crescimento determinado e estimar a divergência genética. Os resultados identificaram pares de genótipos divergentes e complementares para cruzamentos. Os genótipos foram agrupados em seis e cinco grupos, respectivamente quanto às metodologias de Tocher e UPGMA para os caracteres quantitativos, parcialmente concordantes. Para os caracteres qualitativos, formaram-se três grupos. As análises de variáveis canônicas identificaram caracteres relacionados à produção de importância para a variabilidade. O estudo revela a importância da caracterização de novos genótipos para conservação e utilização futura.*
Palavras-chave: *Phaseolus lunatus, fonte protéica, hábito de crescimento, análise multivariada.*

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