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GENETIC DISSIMILARITIES BETWEEN FAVA BEAN ACCESSIONS USING MORPHOAGRONOMIC CHARACTERS[1]

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ABSTRACT - Fava bean (*Phaseolus lunatus* L.) has great potential for the supply of vegetable protein, but its cultivation is still of little relevance when compared to common bean (*Phaseolus vulgaris* L.), being, in most cases, intercropped with other crops. The preservation of its genetic variability in germplasm banks, as well as the study of its genetic dissimilarity, has been helping in strategic design for improvement of the crop. The objective of this work was to characterize the genetic variability of morphoagronomic characters and to quantify the genetic dissimilarity among Fava bean accessions by multivariate analysis. Eight accessions from the Active Fava Bean Germplasm Bank of the Federal University of Piauí were evaluated through a randomized complete block design, with four replications, based on the number of days to maturation (NDM), mean pod length (CV), mean pod width (LV), mean pod thickness (EV), number of seeds per pod (NSV), weight of one hundred seeds (P100S) and productivity (PROD). Among the traits evaluated, only CV showed no genetic variability among the accessions. The analysis of divergence showed accessions UFPI 799 and UFPI 817 as the most divergent in relation to the others. The grouping method of Tocher and UPGMA allowed the formation of three groups. Singh's method indicated that LV, PROD and P100S contributed the most to the genetic divergence between the evaluated accessions. The accessions UFPI 817 and UFPI 797 can be used in crossings, as they are genetically distant and complementary in their characteristics.

Keywords: *Phaseolus lunatus* L. Multivariate analysis. Genetic variability. Germplasm.

DISSIMILARIDADES GENÉTICA ENTRE ACESSOS DE FEIJÃO-FAVA UTILIZANDO CARACTERES MORFOAGRONÔMICOS

RESUMO - O feijão fava (*Phaseolus lunatus* L.) apresenta um grande potencial para o fornecimento de proteína vegetal, mas, o seu cultivo ainda tem pouca relevância se comparado ao do feijão comum (*Phaseolus vulgaris* L.), sendo, na maioria das vezes, consorciada com outras culturas. A preservação da sua variabilidade genética em bancos de germoplasma assim como o estudo da sua dissimilaridade genética, vem auxiliando no delineamento estratégico para o melhoramento da cultura. O objetivo desse trabalho foi caracterizar a variabilidade genética de caracteres morfoagronômicos e quantificar a dissimilaridade genética entre acessos de feijão-fava por meio de análise multivariada. Foram avaliados oito acessos do Banco Ativo de Germoplasma de Feijão-Fava da Universidade Federal do Piauí, em delineamento de blocos completos ao acaso, com quatro repetições, com base no número de dias para maturação (NDM), comprimento médio da vagem (CV), largura média da vagem (LV), espessura média da vagem (EV), número de sementes por vagem (NSV), peso de cem sementes (P100S) e produtividade (PROD). Dentre os caracteres avaliados, apenas CV, não apresentou variabilidade genética entre os acessos. A análise de divergência mostrou os acessos UFPI 799 e UFPI 817 como os mais divergentes em relação aos demais. O método de agrupamento de Tocher e UPGMA possibilitou a formação de três grupos. O método de Singh indicou que LV, PROD e P100S foram os que mais contribuíram para a divergência genética entre os acessos. Os acessos UFPI 817 e UFPI 797 podem ser utilizados em cruzamentos, pois são distantes geneticamente e complementares em suas características.

Palavras-chave: *Phaseolus lunatus* L. Análise multivariada. Variabilidade genética. Germoplasma.

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INTRODUCTION

The Fava bean (*Phaseolus lunatus* L.), also known as Fava or lima beans is cultivated in North America, South America, Europe, East and West Africa and Southeast Asia (BAUDOIN, 1988). It is the second most important legume of the genus *Phaseolus*, presenting great potential for the supply of vegetable protein, decreasing the almost exclusive dependence on common bean (*Phaseolus vulgaris* L.) (SANTOS et al., 2008). In Brazil, its cultivation still has little relevance when compared to common beans, despite its rusticity and broader adaptability. The main reasons for the relatively limited cultivation of this legume in the country are the tradition of consumption of common beans by the population; its bitter taste, long cooking time; and the lack of varieties adapted to the producing regions (SANTOS et al., 2002; BARREIRO NETO et al., 2015; ASSUNÇÃO NETO et al., 2018).

Many researches on Fava beans still need to be done, especially in the area of genetics and plant breeding, as the lack of information makes it difficult to strategically plan for crop improvement.

In this sense, the conservation and maintenance of the genetic variability *ex situ*, through germplasm banks, is of paramount importance for plant breeding, since it constitutes a source of genes of certain characteristics that can be used to improve commercial cultivars (RANGEL et al., 2013). In germplasm banks, various activities are performed, such as prospecting, collection, introduction, exchange, quarantine, characterization, conservation, inspection, multiplication and regeneration of germplasm (RAMALHO et al., 2012).

The characterization of the conserved accessions in the germplasm banks and those that are introduced after a collecting activity, allows the breeders to know the characteristics available in the germplasm of the species that will be conserved. The characterization aims to evaluate existing genetic variability, whether between accessions of a germplasm bank, as in natural populations or in commercial plantations. For breeding programs, the identification of contrasting genotypes allows the exploration of heterosis or hybrid vigor resulting from hybridization between them (GRIGOLO et al., 2018). Among the types of Fava bean characterization, we have mainly used agromorphological and molecular descriptors (GUIMARÃES et al., 2007; SILVA et al., 2015; SILVA et al., 2017; ASSUNÇÃO NETO et al., 2018).

The success of breeding programs depends on the careful choice of the parents who will be used to perform the hybridization, and these need to be genetically divergent, but complementary in their characteristics (BORÉM; MIRANDA, 2013; TAVARES et al., 2018). The study of diversity has as consequence the estimation and increase of genetic variability, which is regarded as the raw material of plant breeding programs and essential for the evolution of species (GONÇALVES et al., 2016).

Measurement of genetic diversity can be performed using multivariate techniques, which consists in the simultaneous evaluation of several characters, allowing the establishment of a genetic distance coefficient between the evaluated genotypes. Among the multivariate methods, discriminant analysis, principal components, principal coordinate analysis and clustering methods are used, such as the Tocher clustering method (RAO, 1952) and UPGMA *(Unweighted Pair Group Method with Arithmetic Mean)* (CRUZ; REGAZZI;CARNEIRO, 2012).

Thus, the objective was to study the genetic dissimilarity among eight accessions of Fava bean (*Phaseolus lunatus* L.) from UFPI's Active Germplasm Bank, based on agromorphological characterization.

MATERIALS AND METHODS

The experiment was conducted from February to August 2015, in the experimental area of the Department of Phytotechnics of the Center for Agricultural Sciences of the Federal University of Piauí, located at latitude 05º 05' S, longitude 42º 48' W, with a mean altitude of 72 m. The climate of the region is of type Aw'' (tropical subhumid hot) according to Köppen climatic classification.

The experimental design was a randomized block design with four replications and eight treatments, chosen due to their different places of origin (Table 1). The plot consisted of four rows of 5.0 m, spaced 0.80 m x 0.70 m, using three seeds per pit, leaving one plant per pit after thinning. The cultivation of accessions was intercropped with corn (AL Piratininga), which offerred support to the legume.

Two weeks before the maize was sown, plowing was done, followed by harrowing. In the fertilization of maize foundation, the following dosages were used at planting: 30 $kg.ha^{-1}$ urea, 270 $kg.ha^{-1}$ single superphosphate and 85 $kg.ha^{-1}$ potassium chloride. As cover fertilization, 180 $kg.ha^{-1}$ urea and 90 $kg.ha^{-1}$ potassium chloride were used. This fertilization was divided into three times, the first was performed when the maize had 4 to 6 leaves, representing 40%; the second with 8 to 10 leaves, 40% and the third with 12 leaves, 20% of the total. Maize was sown by a three-row planter, distributing six seeds per linear meter.

Table 1. Identification of eight Fava bean (*Phaseolus lunatus* L.) accessions from the Active Germplasm Bank of the Federal University of Piauí, characterized in Teresina, PI, Brazil, 2016.

Accessions (BGA code)	Name	Origin	Seed Color
UFPI 791	Fava Branca	Pedra Branca - Ceará	White
UFPI 797	Fava Branca	Riachão - Maranhão	White
UFPI 798	Fava Branca	Riachão - Maranhão	White
UFPI 799	Fava Branca	Nova Colina - Maranhão	White
UFPI 806	Boca de Moça	Palmeirais - Piauí	White and Brown
UFPI 815	Fava Branca	Picuí - Piauí	White
UFPI 817	Cara Larga	Remígio - Paraíba	White and red
UFPI 832	Rosinha	Remígio - Paraíba	Pink

Fava bean sowing was carried out in pits shortly after maize emergence. Mineral fertilization was calculated according to the recommendations of Lopes et al. (2010), generating the following dosages: 20 kg.ha^{-1} urea, 40 kg.ha^{-1} simple superphosphate and 30 kg.ha^{-1} potassium chloride, applied only at planting. The control of invasive plants was performed through manual weeding during the crop cycle. At the time of the maturation of the pods, the harvest was carried out in two stages, per plot and, for the purposes of statistical analysis, the total sum of the harvests carried out was subsequently calculated.

The characters evaluated in the field were the number of days until maturity (NDM) (number of days from emergence until 50% of the plants in the plot had mature pods); mean pod length (CV), mean pod width (LV) and mean pod thickness (EV), all measured using a digital caliper, in mm, of 10 mature pods taken at random in the plot; number of seeds per pod (NSV), determined in 10 mature pods taken at random in the plot; weight of one hundred seeds (P100S), in grams (g), of one hundred seeds taken at random in the plot; and grain yield (PROD), expressed in kilograms per hectare (Kg.ha^{-1}), after weighing in grams of the total grain of the parcel.

To verify the genetic variability among the accessions in relation to the seven variables mentioned above, univariate analysis of variance was used, and then the means of the characters were grouped by the Tukey test ($P < 0.05$). The study of genetic dissimilarity between accessions was performed through the analysis of canonical variables and the Tocher optimization grouping methods (RAO, 1952) and the UPGMA hierarchical method, based on Mahalannobis generalized distance. The identification of the importance of descriptors was made based on the method proposed by Singh (1981).

Statistical-genetic analyses were performed with the aid of the computer program GENES (CRUZ, 2013).

RESULTS AND DISCUSSION

There were significant differences at 1% probability between accessions for the evaluated characteristics, except for pod length (Table 2). This indicates the existence of genetic variability, so that we can select genotypes that have desirable characteristics for crop genetic improvement. The coefficient of variation (CV) for the seven characters ranged from 1.57% to 19.28% for number of days for pod maturation and yield, respectively (Table 2). According to the Pimentel-Gomes (2009) classification, the CV was classified from medium to low, indicating good experimental precision.

Table 2. Analysis of variance of seven characters of Fava bean (*Phaseolus lunatus* L.) from the Active Germplasm Bank of the Federal University of Piauí, characterized in Teresina, PI, Brazil, 2016.

Sources of variation	GL	Mean squares						
		NDM	CV	LV	EV	NSV	P100S	PROD
Block	3	2.16	689.46	0.22	0.51	0.03	5.89	49348.21
Accession	7	90.21**	44.92^{ns}	13.79**	4.28**	0.18**	1097.36**	745076.74**
Residue	21	4.64	76.6	0.14	0.13	0.013	13.33	10858.99
Mean	-	136.62	75.8	16.05	10.25	2.61	62.28	540.40
CV (%)	-	1.57	11.54	2.37	3.52	4.47	5.86	19.28
Minimum Value	-	126.00	57.21	13.26	8.21	2.20	36.64	17.00
Maximum Value	-	148.00	92.00	19.46	12.20	3.00	90.91	1281.19

*, **, ns, significant at 5% and 1% probability and not significant, respectively, by the F test.

The number of days for pod maturation averaged 136.62 days, with UFPI 799 (146 days) being a = the latest and UFPI 815 (130 days) the earliest (Table 3). According to Trani et al. (2015), Fava bean maturation cycle, under dry season, lasts on average 120 days. Early Fava bean genotypes are less susceptible to pest and disease attack, lowering production costs and performing well in crop rotation (SILVA et al., 2017).

The mean values for pod width ranged from 13.71 mm to 18.65 mm for the UFPI 799 and UFPI 815 accessions, respectively, with an overall mean of 16.05 mm. Barreiro Neto et al. (2015), studying the morphological and productive characteristics in ten Fava bean accessions in the state of Paraíba, found values similar to this one, with a mean of 17.9 mm for pod width. There is a positive correlation between pod size and number of seeds per pod, and this is a desirable feature for hand-harvested crops such as Fava beans (SILVA; NEVES, 2011).

The number of seeds obtained per pod averaged 2.61 seeds, with the accession UFPI 791 (2.92) standing out for the highest number of seeds, not differing from UFPI 798, UFPI 815 and UFPI 817. A variation of 2 to 4 seeds per pod was observed by Guimarães et al. (2007) when evaluating the genetic divergence between 22 Fava bean accessions.

Table 3. Mean[1] of seven Fava bean (*Phaseolus lunatus* L.) characters from the Active Germplasm Bank of the Federal University of Piauí, characterized in Teresina, PI, Brazil, 2016.

Accession	NDM (days)	CV (mm)	LV (mm)	EV (mm)	NSV	P100S	PROD
UFPI 791	137 bc	75.05 a	16.33 c	10.35 b	2.92 a	66.29 cd	774.09 b
UFPI 797	133 cd	74.32 a	13.82 d	9.32 cd	2.43 cd	39.60 f	87.64 cd
UFPI 798	137 bc	78.20 a	17.50 b	11.60 a	2.82 ab	73.97 bc	932.65 b
UFPI 799	146 a	69.93 a	13.71 d	9.47 cd	2.44 cd	40.69 f	62.82 d
UFPI 806	139 b	74.26 a	14.40 d	10.01 bc	2.59 bc	52.51 e	327.25 c
UFPI 815	130 d	80.67 a	18.65 a	10.48 b	2.74 ab	76.49 ab	787.03 b
UFPI 817	137 bc	75.12 a	17.25 b	11.81 a	2.60 abc	84.31 a	1185.45 a
UFPI 832	134 bcd	78.83 a	16.79 bc	8.97 d	2.30 d	64.41 d	166.25 cd
Mean	136.63	75.80	16.05	10.25	2.61	62.28	540.39

[1]Means followed by the same letter vertically do not differ from each other by the Tukey test at 5% probability. NDM: number of days for pod maturation; CV: pod length; LV: pod width; EV: pod thickness; NSV: number of seeds per pod; P100S: weight of one hundred seeds; PROD: productivity.

Regarding the weight of one hundred seeds, we highlight accession UFPI 817 with 84.31 g. According to Guimarães et al. (2007), Fava bean with 100 seeds weighing over 60 g are considered large seeds, an important characteristic for the crop, since the consumer market has a preference for larger seeds (SILVA et al., 2017). For grain yield, with an average of 540.40 kg, UFPI 817 accession was the most productive (1185.45 $kg.ha^{-1}$) and UFPI 799 accession recorded the lowest grain yield (62.82 $kg.ha^{-1}$).

The genetic divergence among the accessions, estimated based on the generalized Mahalanobis distance (D^2), indicated that the accessions UFPI 799 and UFPI 815 ($D^2 = 292.27$) were the most distant, and UFPI 798 and UFPI 817 ($D^2 = 18.44$) are the closest (Table 4). The greater genetic distance between UFPI 799 and UFPI 815 can be explained by the fact that accessions are contrasting with most characters, excluding only pod length (Table 3).

The accessions UFPI 817 and UFPI 797, with $D^2 = 286.53$, had one of the largest genetic distances and are complementary in some characteristics, for example, in relation to the number of days for pod maturation, the accession UFPI 797 (133 days) was earlier than accession 817 (137 days). In contrast, the accession UFPI 817 had higher averages for LV, EV, PROD, and P100S, given the preference of the Fava bean market consumer for larger seeds. Thus, it is expected that the crossing between accessions UFPI 817 and UFPI 797 can generate potentially productive earlier progenies, that meet the needs of consumers of Fava beans.

Utilization of the Tocher Optimization method allowed the distribution of the accessions studied in three distinct groups (Table 5). Using the UPGMA method, grouping is performed subjectively from the point where major changes in the dendrogram occur (ARRIEL et al., 2006). By analyzing the Dendrogram (Figure 1), we observed a clustering agreement between this method and the Tocher optimization method. Studying the genetic divergence among 24 Fava bean accessions, Silva et al. (2015) achieved the formation of four groups by the same methods.

Table 4. Estimates of dissimilarity among eight accessions of Fava bean (*Phaseolus lunatus* L.) by the Mahalanobis distance, coming from the Active Germplasm Bank of the Federal University of Piauí, characterized in Teresina, PI, Brazil, 2016.

	UFPI 797	UFPI 798	UFPI 799	UFPI 806	UFPI 815	UFPI 817	UFPI 832
UFPI 791	128.67	28.99	160.29	57.16	45.57	57.11	80.65
UFPI 797		219.75	50.09	28.66	236.37	286.53	95.65
UFPI 798			230.45	116.45	29.55	18.44	115.26
UFPI 799				33.74	292.27	286.29	105.66
UFPI 806					156.66	157.36	60.44
UFPI 815						64.34	91.21
UFPI 817							164.01

Maximum Distance = 292.27 (Accessions: UFPI 799 and UFPI 815)
Minimum Distance = 18.44 (Accessions: UFPI 798 and UFPI 817)

In both methods, Group I was formed by the accessions UFPI 798, UFPI 817, UFPI 791 and UFPI 815, which share the highest averages for PROD, P100S, NSV and EV. The accessions UFPI 797, UFPI 806 and UFPI 799 were allocated to the second group, sharing intermediate averages of EV and NSV and the lowest averages for P100S and LV. The third group was formed by the accession UFPI 832, which had an intermediate mean for P100S and LV and the lowest mean for EV and NSV. The accessions of Group I presented desirable characteristics for the improvement of Fava beans, such as higher grain yield and larger seeds, and can be used as parents in the crossing blocks.

Grouping methods are important for the selection of parents to be inserted in the crossings blocks, even if their genetic distances are known (SIMON; KAMADA; MOITEIRO, 2012). Genotypes which are genetically distant and complementary in their characteristics can be recommended for hybridization, aiming to maximize the hybrid combinations with greater heterotic effect and increase the possibility of recovery of transgressive genotypes, essential for autogamous breeding programs (CRUZ; REGAZZI; CARNEIRO, 2012).

Table 5. Grouping of eight Fava bean (Phaseolus lunatus L.) accessions by the Tocher method, from the Germplasm Active Bank of the Federal University of Piauí, characterized in Teresina, PI, Brazil, 2016.

Group	Accessions
I	UFPI 798, UFPI 817, UFPI 791, UFPI 815
II	UFPI 797, UFPI 806, UFPI 799
III	UFPI 832

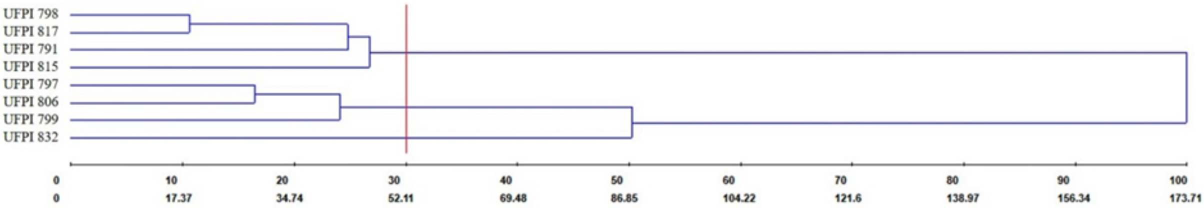

Figure 1. Dendrogram of genetic dissimilarity among eight accessions of Fava bean (*Phaseolus lunatus* L.) by the UPGMA method from the Active Germplasm Bank of the Federal University of Piauí, characterized in Teresina, PI, Brazil, 2016.

Singh's method (1981), used to estimate the relative contribution of each character in the expression of genetic variability among the evaluated accessions, indicated that pod width, yield, and weight of one hundred seeds were the main contributors to genetic divergence with 30.08%, 22.64% and 21.21%, respectively, totaling 73.93% of the variability (Table 6). These traits were more efficient in explaining the dissimilarity between genotypes and could prioritize those with the greatest contribution to genetic variability for the selection of parents for breeding purposes.

The character that least contributed to the genetic divergence was the length of the pod, with 0.03%. Unrepresentative characteristics can be discarded, saving time, labor and personal and financial costs for conducting the experiments. Different results were found by Silva et al. (2015) evaluating 24 Fava bean accessions in Teresina, Piauí. The authors observed that pod length made a major contribution to genetic divergence between accessions.

Table 6. Relative contribution of the seven characters of Fava bean (Phaseolus lunatus L.), by the method proposed by Singh (1981), coming from the Germplasm Active Bank of the Federal University of Piauí, characterized in Teresina, PI, Brazil, 2016.

Variable	% of Contribution
Pod width	30.08
Productivity	22.64
Weight of 100 seeds	21.21
Pod thickness	9.67
Number of days for pod maturation	8.93
Number of seeds per pod	7.40
Pod length	0.03

Based on the variances (eigenvalues) and the accumulated percentage variances of the canonical variables, related to the eight Fava bean accessions and the seven evaluated characters, it appears that the first two variables were sufficient to explain 88.19% of the variation, demonstrating that the two-dimensional graph is suitable for interpretation of results (Table 7). The first canonical variable accounted for 73.87% of the total variance, and the second variable for 14.32%. Costa et al. (2018), evaluating six soybean genotypes, found values higher than these, with the first two canonical variables explaining 91% of the total variation.

According to Cruz, Regazzi and Carneiro (2012), the first canonical variable, because it has the largest amount of variation of the original data, is the most important. The authors also stated that characters having the largest eigenvectors are considered of greater importance within their component. Also, the characters considered of minor importance for the genetic divergence among the accessions studied are those whose eigenvectors are of greater magnitude in the last canonical variables.

Therefore, it was observed that in the first component (VC1), the character that contributed the most to the variation was pod width (0.664), followed by number of days for maturation (0.517) in the second component (VC2). The character that least contributed to variation was pod length (0.929) in VC7.

Table 7. Variances, percent and cumulative variances of the canonical variables obtained from the seven characters of Fava bean (*Phaseolus lunatus* L.), from the active Germplasm Bank of the Federal University of Piauí, characterized in Teresina, PI, Brazil, 2016.

VCi	Eigenvalues			Eigenvectors associated with canonical variables						
	λi	%	% Cumulative	NDM	CV	LV	EV	NSV	P100S	PROD
VC1	44.82	73.87	73.87	-0.145	-0.005	0.664	0.414	0.170	0.413	0.407
VC2	8.68	14.32	88.19	0.517	0.067	-0.484	0.459	-0.131	0.044	0.516
VC3	4.67	7.7	95.90	-0.469	-0.131	-0.339	0.048	0.686	-0.267	0.319
VC4	1.34	2.21	98.11	0.644	0.178	0.337	-0.126	0.546	-0.345	-0.065
VC5	1.04	1.71	99.83	0.173	-0.002	-0.216	-0.55	0.276	0.726	0.122
VC6	0.10	0.16	99.99	0.021	-0.286	0.219	-0.518	-0.314	-0.320	0.631
VC7	0	0	100	-0.221	0.929	-0.006	-0.16	-0.093	-0.069	0.217

In this study, the results exposed by the canonical variables analysis were partially consistent with the observed averages and the relative contribution value of the characters to the divergence by the method proposed by Singh (1981) (Tables 3 and 6). The pod length character did not present statistically significant differences between the accessions studied (Table 2), thus explaining its low contribution to genetic divergence.

According to the two-dimensional scatter plot, in relation to the first and second canonical variables, it was observed that the eight accessions were grouped into four groups (Figure 2).

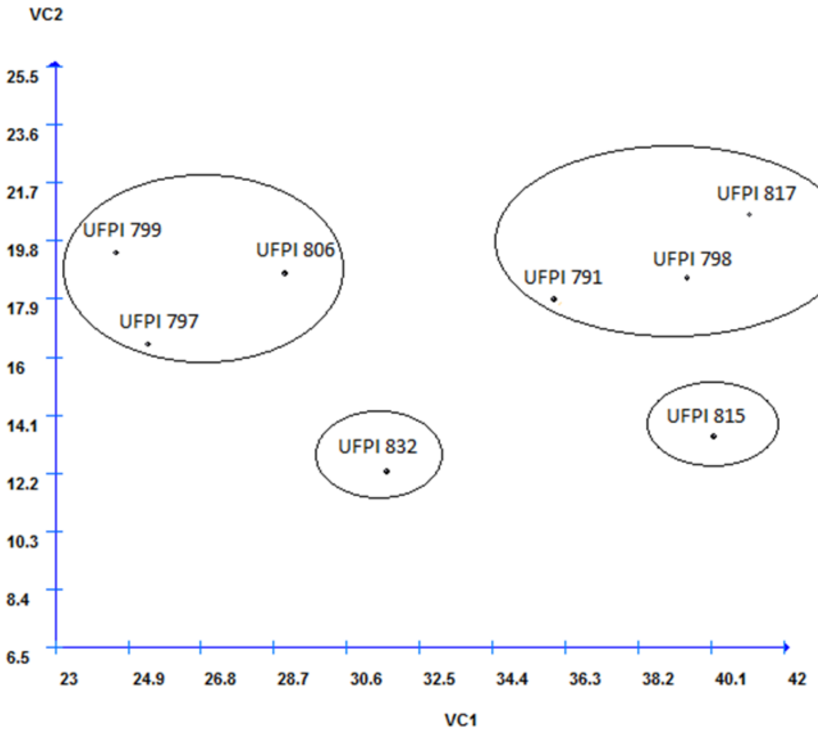

Figure 2. Scatter plot of eight Fava bean accessions in relation to the first and second canonical variables for the seven characters evaluated in Teresina, PI, Brazil, 2016.

The accessions UFPI 799, UFPI 806 and UFPI 797, located in the upper left, presented the lowest means for pod width (VC1) and intermediate and high values for number of days to maturation (VC2). Already the accessions UFPI 791, UFPI 798 and UFPI 817, located in the upper right, had the highest averages for pod width (VC1) as well as intermediate and high values for number of days to maturation (VC2).

The accessions UFPI 832 and UFPI 815 formed individual groups. UFPI 832 presented intermediate mean for pod width (VC1) and low mean for number of days for maturation (VC2). While UFPI 815 had the highest pod width (VC1), and a lower number of days for maturation (VC2).

The clusters obtained by the Scatter plot in relation to the two canonical variables were partially in agreement with those obtained by the Tocher and UPGMA method. Therefore, these results can also be used in breeding programs that use crossbreeding, as they allowed the identification of genetically divergent accessions.

CONCLUSION

The Fava bean accessions evaluated had genetic divergence among them, with UFPI 799 and UFPI 815 being the most divergent. The accession pairs UFPI 817 and UFPI 797 can be used in crossings, as they are genetically distant and complementary in their characteristics.

The characters that contributed most to the genetic divergence among the accessions evaluated by the Singh method were pod width, yield and weight of one hundred seeds. The canonical variables highlight the pod width and the number of days for maturation.

The grouping of accessions was partially concordant between the Tocher method, UPGMA and the canonical variables method.

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