

NOTE

Characterization of genetic variability among common bean genotypes by morphological descriptors

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Abstract – *The purpose of this study was to characterize the genetic variability in 100 genotypes of the Active Germplasm Bank of common bean of the Federal University of Viçosa, by morphological descriptors, classify them in groups of genetic similarity and to identify the degree of relevance of descriptors of genetic divergence. The genotypes were evaluated based on 22 quantitative and qualitative morphological descriptors. The high-yielding genotypes V 7936, Gold Gate, LM 95103904, 1829 S 349 Venezuela, and PF 9029975, CNFC 9454 and Fe 732015, with upright growth, have potential for use as parents in common bean breeding programs. By genetic divergence analysis, the genotypes were clustered in eight groups of genetic dissimilarity. By methods of principal components, 9 of the 22 descriptors were eliminated, for being redundant or little variable, suggesting that 10-20 morphological descriptors can be used in studies of characterization of genetic variation.*

Key words: *Phaseolus vulgaris, germplasm, descriptors, genetic diversity.*

INTRODUCTION

Brazil is the world's largest producer of common bean, which is the main protein source in the average diet of the population (Costa et al. 2011). Besides the role it plays in the Brazilian diet, common bean is one of the agricultural products with greatest socio-economic importance (Machado et al. 2008), mainly due to the manpower required during the crop cycle.

The species *Phaseolus vulgaris* L. has two centers of origin, a Mesoamerican and an Andean (Singh et al.

1991). Much of the genetic variability of this species in the world has been maintained and conserved ex situ, outside the centers of origin, in genebanks (Borda 2011). The maintenance of this diversity in collections is fundamental to develop and support breeding programs. However, the lack of information about these genetic resources is a major cause of its low exploitation by breeders (Valls 2007).

For a practical use and exploitation of the germplasm conserved in genebanks, its characterization

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is essential (Faleiro and Junqueira 2010). Several characters can be used to characterize genetic resources, particularly morphological and agronomic (Singh 2001), and biochemical and molecular traits (Beebe et al. 2000). In the preliminary characterization of the genotypes, morphological and agronomic traits of the plant are preferred, for being cheaper and easier to assess.

The morpho-agronomic description provides information underlying conclusions on the genetic variability of the genotypes of the bank, identification of accessions maintained in duplicate (Valls 2007), improvement of the data of identification and classification of accessions (Chiorato et al. 2007) and support the regeneration and maintenance of the genetic integrity of genotypes. Among other information that can be obtained from the characterization of germplasm, the determination of the relative importance of the traits used to describe the genetic diversity (Rodrigues et al. 2002, Chiorato et al. 2005) is noteworthy because, in case of limited financial and/or human resources, the least relevant traits can be eliminated.

To study genetic diversity, multivariate analysis procedures are used. Among these, the principal component analysis, canonical variables and cluster analysis are particularly appropriate (Cruz and Carneiro 2006). Basically, the clustering methods can be separated in hierarchical and optimization techniques. Among the first, the nearest neighbor method and the UPGMA (Unweighted Pair-Group Method using Arithmetic Averages) are promising, in which clusters are identified in dendrograms. Of the optimization methods, the most commonly used is the Tocher algorithm. The goal is to partition the genotypes in exclusive groupings according to their genetic distance; in this case, the intragroup distance must be smaller than any intergroup distance.

All these methods of diversity analysis are based on a dissimilarity matrix obtained from defined measures, according to the type of variable, which can be qualitative, quantitative or multicategorical (Cruz 2008). The Euclidean distance, Mahalanobis' distance and the coincidence index (Cruz and Carneiro 2006) are used as dissimilarity measures. To assess the genetic variability of common bean, some authors have used multivariate analysis methods (Machado et al. 2002, Rodrigues et al. 2002, Chiorato et al. 2005, Ceolin et al. 2007, Elias et al. 2007).

The objectives of this study were to characterize the genetic variability of 100 common bean genotypes

from the Active Germplasm Bank of the Federal University of Viçosa (UFV), using morphological descriptors to cluster them in groups of genetic dissimilarity, rank their usefulness in breeding programs and identify the descriptors according to the irrelevance in the assessment of genetic diversity.

MATERIAL AND METHODS

The experiment was conducted at the Experimental Station of the Department of Plant Science of UFV (Federal University of Viçosa), in Coimbra, Minas Gerais, Brazil, (lat 20° 50' 30" S, long 42° 48' 30" W, and alt 720 m asl). At the station with tropical altitude climate, summers are rainy, the average annual temperatures around 19 °C, with variations between 14 °C (mean minimum) and 26 °C (mean maximum) and annual rainfall 1220 mm.

Of the germplasm bank of common bean of the UFV, 100 genotypes were evaluated (Table 1), using the following cultivars as control: Pérola, BRSMG Talismã, BRSMG Majestosos (carioca), Ouro Negro, Meia Noite, BRS Valente, BRS Supremo (black) and Ouro Vermelho (red grain). The trial was arranged in a 10 x 10 square lattice design with three replications, in plots containing two 2-m rows m each, spaced 0.5 m apart. The plants were sown on March 7, 2007, in the soil prepared with plow and disk, fertilized with 350 kg ha⁻¹ of 8-28-16 NPK (N-P₂O₅-K₂O) and 25 days after emergence, 100 kg ha⁻¹ urea was side dressed. The experimental area was sprinkle-irrigated and cultural practices were performed as recommended for the crop.

Morphological and agronomic traits were used to characterize the genotypes. The morphological characters were chosen from a minimum list of descriptors for the registration of common bean in the National Register of Cultivars (RNC), as stated in Decree 2366/1997 (Brazil 1997). The traits of plant architecture and grain yield were included in view of their importance for common bean breeding programs. Eighteen quality (Silva 2005) and four quantitative traits were evaluated. The quantitative included: presence of anthocyanin in the cotyledons and hypocotyl (in V1 - seedling stage), plant growth habit, presence of anthocyanin in the stem, leaf roughness, flower color (in R6 - flowering), primary and secondary pod color, profile and shape of the pod apex, shape and position of the pod apex (at R9 - harvest). Seeds were evaluated for uniformity, gloss, presence of halo, grain color, shape ($J = L/W$ where L

Table 1. Descriptors of 100 common bean genotypes evaluated for morpho-agronomic traits

Registration number ^a	Name of the genotype	Grain color	Origin	Grain size ^b	Registration number ^a	Name of the genotype	Grain color	Origin	Grain size ^b
1	EMP-117	Carioca	Embrapa	Median (26.8)	29	3272	Light brown	CIAT, Colômbia	Medium (35.4)
2	AN 910518	Carioca	Embrapa	Small (22.9)	30	LM 96107901	Brown	Embrapa	Median (26.5)
3	FEB 171	Carioca	CIAT, Colômbia	Median (27.0)	31	Vinagre	Brown	Unknown	Small (23.6)
4	PF 902975	Carioca	ESAL/Embrapa	Small (21.4)	32	1843 55 G	Yellow	Estação Experimental Patos	Median 34.4
5	LR 720982 CP	Carioca	Embrapa	Median (27.8)	33	1860 Sacavem 63	Yellow	Estação Experimental Patos	Large (42.2)
6	38 F	Carioca	Unknown	Small (23.7)	34	1852 TaquariSarges	Yellowish	Estação Experimental Patos	Median (32.1)
7	Raça D	Carioca	Unknown	Median (29.2)	35	S-856-B	Light brown	Costa Rica	Median (27.7)
8	CNFC 9444	Carioca	Embrapa	Small (24.9)	36	Golden Gate	Light brown	Beltsville, Maryland	Median (25.9)
9	CNFC 8006	Carioca	Embrapa	Median (27.8)	37	P. White 6301	Black	University of California	Median (25.8)
10	CNFC 9466	Carioca	Embrapa	Median (26.6)	38	1829 S 349	Black	Estação Experimental Patos	Small (23.1)
11	CNFC 9455	Carioca	Embrapa	Median (29.4)	39	1831 S 353	Black	Estação Experimental Patos	Small (21.4)
12	CNFC 9454	Carioca	Embrapa	Median (29.2)	40	1836 S 464	Black	Estação Experimental Patos	Median (26.6)
13	CNFC 9458	Carioca	Embrapa	Median (29.5)	41	1840 4 PS	Black	Estação Experimental Patos	Small (23.8)
14	FEB 199	Carioca	CIAT, Colômbia	Small (23.8)	42	1844 74 G	Black	Estação Experimental Patos	Small (22.8)
15	Carioca 1030	Carioca	IAC	Median (27.4)	43	1867 Sacavem 1031	Black	Estação Experimental Patos	Small (22.4)
16	LM 96108664	Carioca	Embrapa	Small (23.8)	44	1869 Sacavem 1084	Black	Estação Experimental Patos	Small (22.1)
17	LM 95102682	Carioca	Embrapa	Median (26.1)	45	Costa Rica	Black	Perambuco	Small (23.3)
18	LM 96107218	Carioca	Embrapa	Median (28.9)	46	Cornell 49-242	Black	Austrália	Small (22.2)
19	BR-JPA-11 (Brigida)	Carioca	Embrapa/IPA	Small (24.6)	47	P-16 Trujillo 4	Black	CIAT, Colômbia	Median (26.0)
20	Pérola	Carioca	Embrapa	Median (29.7)	48	P 501 (Puebla 199)	Black	CIAT, Colômbia	Median (26.7)
21	BRSMG Talismã	Carioca	UFV/UFPA/UFV/UFPA/Epamig/Embrapa	Median (28.6)	49	P 326 (PI 310.740)	Black	CIAT, Colômbia	Small (24.9)
22	Majestoso	Carioca	Epamig/Embrapa	Median (31.2)	50	BAT 65	Black	CIAT, Colômbia	Small (20.0)
23	Fosco 11	Manteiço	UFV	Large (57.5)	51	BAT 304	Black	CIAT, Colômbia	Median (26.3)
24	DRK 18	Manteiço	Unknown	Large (64.0)	52	V 7936	Black	Desconhecido	Median (29.9)

To be continued

Table 1. Cont.

Registration number	Name of the genotype	Grain color	Origin	Grain sizeb	Registration numbera	Name of the genotype	Grain color	Origin	Grain sizeb
25	1835 S 459 Venezuela	Brown	Estação Experimental Patos	Small (24.3)	53	GF 2570	Black	CIAT, Colômbia	Median (27.8)
26	1862 Sacavem 538	Brown	Estação Experimental Patos	Median (27.2)	54	LM 21135	Black	Embrapa	Median (26.6)
27	1864 Sacavem 860	Brown	Estação Experimental Patos	Median (27.6)	55	Fe 732015	Black	Embrapa	Small (24.2)
28	1868 Sacavem 1061	Brown	Estação Experimental Patos	Median (27.1)	56	Fe 731998	Black	Embrapa	Small (24.3)
57	AN 911120	Black	Embrapa	Median (27.3)	79	Rico 1735	Black	UFV/EPAMIG	Median (25.1)
58	AN 911104	Black	Embrapa	Median (26.4)	80	BR-2-Large Rio	Black	Embrapa/PESAGRO	Median (27.9)
59	SC 9029935	Black	Embrapa	Median (29.7)	81	BRS Supremo	Black	Embrapa	Median (27.3)
60	51051	Black	Unknown	Small (24.8)	82	BRS Valente	Black	Embrapa	Median (29.6)
61	Ouro Negro	Black	UFV/EPAMIG	Median (30.6)	83	CNFRJ 10301	Others	Embrapa	Median (30.5)
62	Meia Noite	Black	EPAMIG	Median (25.2)	84	HI 822510	Pinkish	Embrapa	Median (26.8)
63	BRS Valente	Black	Embrapa	Median (25.5)	85	LM 30013	Pinkish	Embrapa	Small (24.7)
64	CB 733782	Black	Embrapa	Median (28.9)	86	Rosinha G2	Pinkish	IAC	Median (27.6)
65	ICA Pijão	Black	CIAT, Colômbia	Median (28.3)	87	Rosinha precoce	Pinkish	Produtor	Median (27.6)
66	IAPAR 44	Black	IAPAR	Small (24.2)	88	P-36	Purplish	Embrapa	Median (27.1)
67	Porrillo 70	Black	CIAT, Colômbia	Median 27.5	89	FEB (desc.)	Purplish	Unknown	Median (27.3)
68	ARC-1	Black	CIAT, Colômbia	Median 26.5	90	AN 910522	Carioca	Embrapa	Median (27.8)
69	LM 95103904	Black	Embrapa	Median 31.0	91	1845 77 G	Red	Estação Experimental Patos	Median (33.5)
70	CB 733760	Black	Embrapa	Median 27.6	92	1849 Flor. 13041	Red	Estação Experimental Patos	Median (32.8)
71	LM 95103786	Black	Embrapa	Median 25.4	93	1861 Sacavem 486	Red	Estação Experimental Patos	Median (31.8)
72	POT 51	Black	CIAT, Colômbia	Small (23.4)	94	Field grown 49-242	Red	Corwell Univ.	Median (28.4)
73	LM 95103856	Black	Embrapa	Median (29.0)	95	CNFC 5552	Brown	Esal	Median (25.2)
74	2970196	Black	UFV	Median (25.8)	96	Vi-16-3-3	Red	UFV	Median (27.8)
75	2970149	Black	UFV	Median (32.7)	97	Red	Red	Unknown	Large (46.2)
76	2970168	Black	UFV	Median (30.3)	98	Ouro Red	Red	UFV/UFVLA/Epamig/Embrapa	Median (29.6)
77	2970264	Black	UFV	Median (25.2)	99	Vermelhinho	Red	Viçosa-MG	Median (28.4)
78	Serrano	Black	Emcape	Small (24.7)	100	Red 2157	Red	CIAT, Colômbia	Median (29.6)

^a Registration number as indexed in the Active Germplasm bank of common bean of the UFV; ^b Grain size according to Voysest (1983); 100-grain weight in gram.

indicates grain length and W the width) and the degree of flattening ($H = E/W$, where E stands for the grain thickness and W for the width). The four variables were days from planting to flowering, plant architecture, 100-grain weight and grain yield.

Plant architecture was assessed on a 1-5 scale as similarly proposed by Collicchio et al. (1997), where grade 1 indicates upright plants, with a stem and high insertion of the first pods; 2- upright plants with some branching; 3 - upright plants with many ramifications and tendency to lodge; 4 - semi-erect and moderately lodged plants, and 5 - plants with long internodes and very prostrate. The data of grain yield, days from planting to flowering, plant architecture and 100-grain weight were subjected to analysis of variance.

The assessment of genetic diversity of 100 genotypes was simultaneously based on quantitative and qualitative variables. For this analysis, the phenotypes of each qualitative trait were indexed according to the proposed minimum list of descriptors (Silva 2005). Subsequently, for these traits the most frequent values (mode) of the three replications for each genotype were used. For the quantitative variables, the means of the three replications per genotype were grouped into classes based on the frequency distribution (Ramalho et al. 2005). The number of classes was determined by the expression $K=(Rn^{1/3}/3.49s)$, where R is the total range of variation, s the standard deviation and n the sample number. Each mean was indexed with the number of the class it belonged to. Then, the qualitative and quantitative data together were subjected to combined analysis of genetic diversity by the procedure multicategorical classification (Cruz 2008). By this methodology a dissimilarity index is calculated, based on the arithmetic complement of the simple matching coefficient ($1 - c$), where c is given by $c = \frac{C}{C+D}$, where c is the simple coincidence rate, C the number of coincidence of classes and D the number of discrepancies of classes of between the pairs of genotypes.

To group the genotypes, the Tocher method was used. The variables that contributed least to the genetic divergence among the genotypes were identified by principal component analysis (Cruz et al. 2004), considering the higher weighting coefficient (element of the eigenvector) in the component with lowest eigenvalue. The dispensable variables were determined

by the estimate of the cophenetic correlation coefficient between the elements of the dissimilarity matrix with all variables and the matrix without the least important variable. This procedure was repeated until the estimated correlation coefficient was reduced to values below 80 %. The Mantel test was applied to test the significance of the correlation coefficients, using software Genes (Cruz 2008) for all analyses.

RESULTS AND DISCUSSION

Since the relative efficiency of the lattice design compared with randomized blocks was low (less than 110 %), it was decided to use analysis of variance with randomized blocks (Table 2). The effect of genotypes for days from planting to flowering, plant architecture, yield and 100-grain weight was significant (Table 2). The existence of genetic variability among genotypes was confirmed by the frequency distribution of the means of these traits (Figure 1).

Considering days from planting to flowering (Figure 1a), classes with between 34.4 and 43.3 days were formed. The average grade of plant architecture ranged from 1.3 to 4.8 and resulted in five classes (Figure 1b). The grades of the genotypes PF 9029975, CNFC 9454 and Fe 732015 were lower than 2.0 and were in the same class as the control BRS Supremo. This trait has also received attention in bean breeding programmes, because upright plants and pods higher above the ground have advantages, e.g., ease of cultivation, possibility of mechanical harvest and reduction of disease incidence, especially of white mold (Collicchio et al. 1997). Another advantage of more upright plants is the reduction of grain quality loss, if the harvest period coincides with prolonged rainfall, since the pods of more upright plants do not touch the moist soil.

For grain yield, the cultivars were grouped in seven classes, with yields from 906 to 5272 kg ha⁻¹ (Figure 1c). Genotypes V 7936 (5222 kg ha⁻¹), Gold Gate (5100 kg ha⁻¹), 95103904 LM (4803 kg ha⁻¹), 1829 S 349 Venezuela (4847 kg ha⁻¹) and 1831 S 353 Venezuela (4678 kg ha⁻¹) and the control Ouro Vermelho (4845 kg ha⁻¹) were in the most productive class, with an average yield of 4898 kg ha⁻¹. Genotypes with lowest yields were Rosinha Precoce (1443kg ha⁻¹), 1868 Sacavem 1061 (1896 kg ha⁻¹) and Vermelho (2404 kg ha⁻¹). For 100 grain-weight, eight classes with between 20.0 and 64.0 g for 100 grains were formed (Figure 1d). According to

Table 2. Summary of analysis of variance for four traits of 100 common bean genotypes

Source of variation	df	Mean square			
		Days from planting to flowering	Plant architecture	Yield	100-grain weight
Blocks	2	43.030	1.082	4981682.837	0.160
Genotypes	99	6.141***	1.657***	1545048.226**	110.301**
Error	198	1.040	0.160	823574.697	0.222
Mean	-	39.0	3.4	3575.0	28.0
CV(%)	-	2.6	11.9	25.4	1.7
Efficiency of the lattice design	-	101.3	109.0	104.1	-

*** and **significant at 0.1 and 1 % probability, respectively, by the F test.

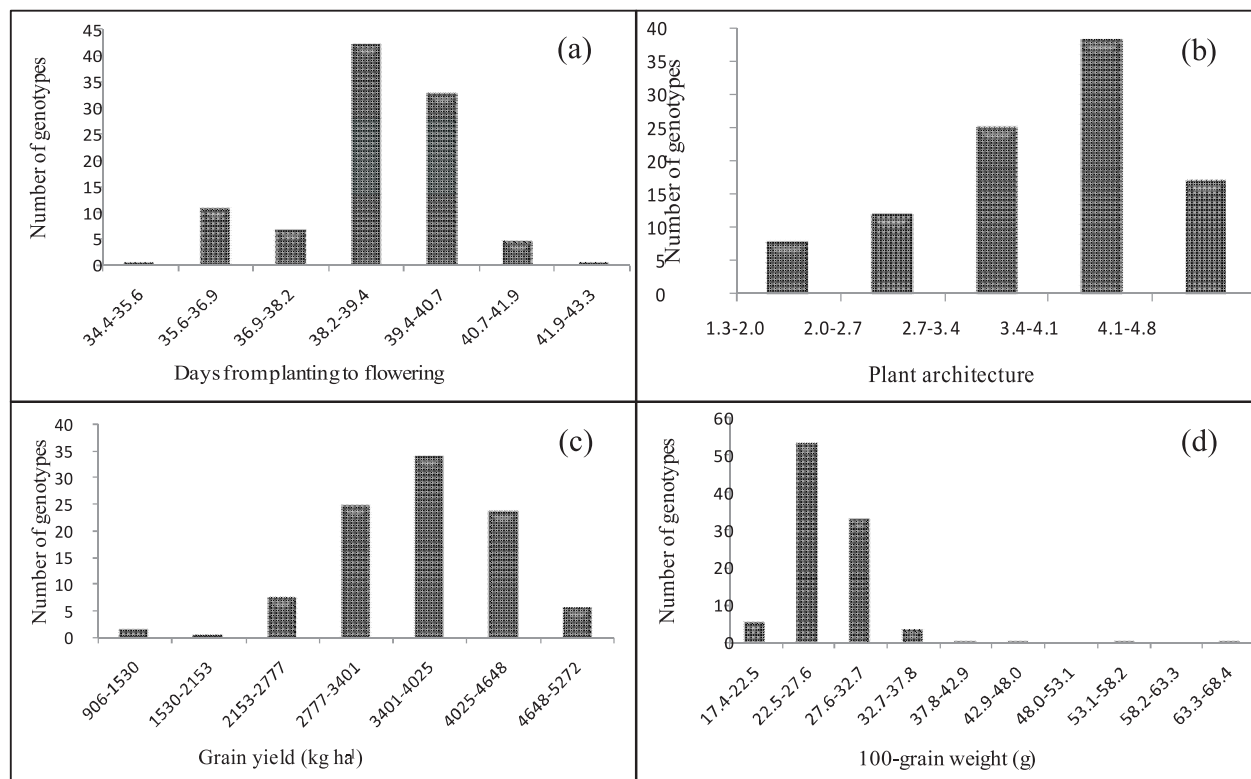


Figure 1. Frequency distribution of four traits of 100 common bean genotypes.

Voysest (1983) and Singh (1989), the grain size can vary from less than 15 to 90 g per 100 grains and be grouped into small (<25 g 100 grains⁻¹), median (25-40 g 100 grains⁻¹) and large (> 40 g 100 grains⁻¹). Based on this study, the grain size of most genotypes was median. The genotypes BAT 65, PF 9029975, 1831 S 353 Venezuela, 1867 Sacavem 1031, 1869 Sacavem 108, and Cornell 49-242 have small

grains, and 1860 Sacavem 63, Manteigão Fôsc0 11, DRK 18, and Vermelho have large grains. Results related to grain size were also reported by Elias et al. (2007).

By the Tocher grouping method (Table 3), the similarity of genotypes in general, was related to the grain color. All bean genotypes with black skin were clustered in group I. The classification of all genotypes

Table 3. Clustering of 100 common bean genotypes

Group	Genotypes ^a
I	47, 54, 48, 56, 67, 79, 50, Ouro Negro ^b , 82, 53, 42, BRS Valente ^b , 72, 43, 74, 68, 80, 64, 51, 60, 55, 78, 75, 30, 59, 45, 49, 58, 71, 38, 52, 41, 46, 69, 57, 77, 73, 65, 66, 76, 39, 70, 44, 35, BRS Supremo ^b , Meia Noite ^b , 36, 37, 27, 1, 26, 28, 90, 96, 99, 32, 92, 31, 19, 88, 2, 86, 91, 15, 25, 100, 87, 95, 33, 84
II	5, Pérola ^b , BRSMG Talismã ^b , BRSMG Majestoso ^b , 11, 9, 18, 7, 10, 12, 16, 4, 17, 14, 3, 13, 8, 6, 85, 89
III	93, OuroVermelho ^b , 34, 97
IV	23, 24
V	83
VI	94
VII	40
VIII	29

^a The genotype number indicates the index in BAGF-UFV; ^b Cultivars/lines used as control

with black grain in the same group was also reported by Rodrigues et al. (2002). Most carioca genotypes formed group II. However, the group was not restricted to grain color. The genotypes of Andean origin, Manteigão fosco 11 and DRK 18 formed group IV. Genotype Vermelho was part of group III. It is worth mentioning that this genotype has large, but more rounded grains than the group Manteigão and is also among the least productive.

By principal component analysis, it was found that the first two components explained only 34 % of the total variation and to explain 80 % of the total variation, the first 10 components were needed. Low variation in the first two components was also observed by Machado et al. (2002), Rodrigues et al. (2002) and Chiorato et al. (2005) in studies of common bean. Cruz and Carneiro (2006) argue that, when at least 80 % of the variation is not absorbed by the first two components, a two-dimensional graph impairs the visualization of the dispersion, and the technique of principal components is ineffective to show the genetic diversity. However, even under these conditions, this analysis allows the identification of more and less important variables for genotype discrimination, with high or low variance and/or redundant.

Based on this criterion, the nine most important variables in the eigenvectors associated to the last eigenvalues were discarded. Notably, the estimate of cophenetic correlation between the elements of the dissimilarity matrix based on 22 variables and the matrix

without the nine least important was 0.889. This coefficient was significant by the Mantel test, at 1 % probability, with 500 simulations. When the 10th variable was eliminated from the diversity analysis, the estimate of the cophenetic correlation coefficient was 0.018, indicating the importance of this variable in the discrimination of genotypes. The most important of the 22 descriptors were therefore ranked: presence of anthocyanin in the stem, grain color, secondary pod color, pod profile, yield, position of the pod apex, pod profile, seed flattening, and plant architecture. On the other hand, the nine least important and dispensable traits were ranked as follows: seed gloss, seed uniformity, presence of anthocyanin in the cotyledons, primary color of dry pod, growth habit, 100-grain weight, shape of pod apex, days from planting to flowering and presence of anthocyanin in the hypocotyl. Although the trait 100-grain weight is considered very important in the reduction of grain size (Singh et al. 1991), it was ranked among the descriptors of lower contribution for the study of genetic diversity among genotypes. The reason was probably because 96 % of the genotypes belong to the Mesoamerican gene pool (small and Median grain). The least important descriptors detected here were the same as reported in previous studies (Rodrigues et al. 2002, Chiorato et al. 2005), except for growth habit.

In general, our results show that characterization studies of genetic variability can be based on 10-20 morphological bean descriptors with the primary descriptors: presence of anthocyanin in the stem, grain

color, secondary pod color, pod profile, yield etc, since a higher number would be unnecessary and costly. Traits such as seed gloss, seed uniformity, growth habit etc may be important for genetic improvement and consumer preference, but are less relevant to characterize the genetic diversity of genotypes.

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Caracterização da variabilidade genética entre genótipos de feijoeiro comum por meio de descritores morfoagronômicos

Resumo - Os objetivos deste trabalho foram caracterizar a variabilidade genética entre 100 genótipos do Banco Ativo de Germoplasma de Feijão da Universidade Federal de Viçosa, por meio de descritores morfoagronômicos; reuni-los em grupos de dissimilaridade genética; e identificar os descritores de maior e menor importância na avaliação da divergência genética. Os genótipos foram avaliados quanto a 22 descritores morfoagronômicos quantitativos e qualitativos. Os genótipos V 7936, Gold Gate, LM 95103904, 1829 S 349 Venezuela, de alta produtividade, e PF 9029975, CNFC 9454 e Fe 732015, de porte ereto, apresentam potencial para uso como genitores nos programas de melhoramento do feijoeiro. A análise de divergência genética possibilitou reunir os genótipos em oito grupos de dissimilaridade genética. Métodos de componentes principais permitiram o descarte de nove descritores, dentre os 22 avaliados, por serem redundantes ou pouco variáveis, sugerindo que estudos de caracterização da variabilidade genética podem ser realizados com 10 a 20 descritores morfoagronômicos.

Palavras-chave: *Phaseolus vulgaris*, germoplasma, descritores, diversidade genética.

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