



## Interrelationship between morphological, agronomic and molecular characteristics in the analysis of common bean genetic diversity

David Teixeira Guidoti<sup>1\*</sup>, Adriana Gonela<sup>1</sup>, Maria Celeste Gonçalves Vidigal<sup>1</sup>, Thiago Vincenzi Conrado<sup>2</sup> and Isaac Romani<sup>3</sup>

<sup>1</sup>Departamento de Agronomia, Universidade Estadual de Maringá, Av Colombo, 5790, 87020-900, Maringá, Paraná, Brazil. <sup>2</sup>Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil. <sup>3</sup>Departamento de Medicina Veterinária, Centro Universitário Ingá, Maringá, Paraná, Brazil. \*Author for correspondence. E-mail: davidguidoti@live.com

**ABSTRACT.** The present study aimed to analyze, through 12 morpho-agronomic traits and 18 micro satellite loci, the genetic diversity in 17 common bean accessions from the Bean Germplasm Bank of the Center for Applied Agricultural Research of the State University of Maringá (BGF/Nupagri/UEM), in Paraná State, Brazil. Genetic diversity was assessed by joint analysis of phenotypic and genotypic characteristics using the Genetics platform of SAS software. To that end, a dissimilarity matrix was constructed based on the Jaccard index. This was used to generate a dendrogram via UPGMA hierarchical clustering, validated by multidimensional scaling and nonorthogonal principal components analysis. Based on genetic diversity analysis, the accessions were clustered into two large groups: one consisting of 11 accessions of Andean origin and the other containing six Mesoamerican accessions. The 17 accessions from the BGF/Nupagri/UEM were found to be an important source of genetic variability for inclusion in common bean breeding programs, contributing to the development of cultivars with desirable agronomic characteristics.

**Keywords:** *Phaseolus vulgaris* L., microsatellite marker, germplasm bank.

### Interrelação entre características morfológicas, agrônômicas e moleculares na análise de diversidade genética em feijão comum

**RESUMO.** O presente trabalho teve como objetivo analisar, por meio de 12 características morfoagronômicas e 18 loci microssatélites, a diversidade genética existente entre 17 acessos tradicionais de feijão comum, pertencentes ao Núcleo de Pesquisa Aplicada à Agricultura (Nupagri), Universidade Estadual de Maringá (UEM) - BGF/Nupagri/UEM, Paraná, Brasil. A análise da diversidade genética foi obtida através da análise conjunta de características fenotípicas e genotípicas, utilizando a plataforma Genetics do programa SAS. Para tanto, uma matriz de dissimilaridade foi elaborada, com base no coeficiente de Jaccard, usado para gerar um dendrograma, estabelecido pelo método UPGMA e validado pela análise multidimensional escalar e de componentes principais não ortogonais. De acordo com a análise de diversidade genética realizada, os acessos foram agrupados em dois grandes grupos, sendo um composto por 11 acessos de origem Andina e o outro grupo constituído por seis acessos Mesoamericanos. Os 17 acessos pertencentes ao BGF/Nupagri/UEM mostrou ser uma importante fonte de variabilidade genética que poderá ser inserida nos programas de melhoramento do feijão comum, contribuindo para o desenvolvimento de cultivares com características agrônômicas de interesse.

**Palavras-chave:** *Phaseolus vulgaris* L., marcador microssatélite, banco de germoplasma.

### Introduction

The common bean (*Phaseolus vulgaris* L.) is one of the most important Fabaceae plants in human nutrition, especially in Latin American and African populations, due to its nutritional properties, including high protein (16 to 33%) and fiber content, complex carbohydrates, and other dietary supplements such as folic acid (source of B-complex vitamins), iron, zinc, magnesium, and potassium (Broughton et al., 2003; Gepts et al., 2008; CIAT, 2016). This legume is grown in different regions of the

world, particularly Latin America, which is home to the world's main common bean producing areas. Brazil is the third largest producer of this crop, at approximately 2.8 million metric tons in 2013 (FAO, 2016).

The germplasm of the common bean is divided into two main gene pools, namely, the Mesoamerican and Andean (Toro, Tohme, & Debouck, 1990), which diverged from a common ancestor approximately 100,000 years ago (Mamidi et al., 2013). These two gene pools are characterized by partial reproductive isolation (Gepts & Bliss, 1985; Koinange & Gepts,

1992), with both domesticated and wild landraces. The Mesoamerican gene pool extends from the Southeastern United States to Panama, with the main characteristics being its small seeds (< 40 g 100 seeds<sup>-1</sup>) and predominantly S-type phaseolin. On the other hand, the Andean gene pool is distributed from Columbia to Northern Argentina, with large, broad seeds (> 40 g 100 seeds<sup>-1</sup>) and primarily T-type phaseolin (Gepts & Bliss, 1986; Gepts, Osborn, Rashka, & Bliss, 1986). Beans from both gene pools can be found in Brazil, demonstrating three possible routes of introduction (Gepts, Kmiecik, Pereira, & Bliss, 1988); however, Mesoamerican beans are the most commercially cultivated, including Carioca and Preto (black) varieties.

Breeding programs focus on obtaining high yield cultivars that are disease and pest resistant and tolerant of drought and low-fertility soils (Kelly & Vallejo, 2004; Miklas, Kelly, Beebe, & Blair, 2006). To that end, sources of genetic variability are available to these programs in the form of common bean germplasm banks, such as the International Center of Tropical Agriculture, with approximately 36,000 *Phaseolus* accessions (CIAT, 2016), the USDA-ARW Western Regional Plant Introduction Station, Pullman, WA, USA, containing approximately 17,000 accessions (USDA-ARS, 2016), Embrapa Rice and Beans, consisting of approximately 14,350 registered accessions (Cenargen, 2016), the Agronomic Institute of Campinas (IAC), with 2,139 registered accessions (APTA, 2016), and the Center for Applied Agricultural Research (Nupagri) of the State University of Maringá (UEM) - BGF/Nupagri/UEM in Paraná State, Brazil, with a collection of approximately 217 bean accessions, 181 of which correspond to *Phaseolus vulgaris* L. and are undergoing agronomic, morphological and molecular characterization (Gonçalves-Vidigal, Lacanallo, & Vidigal, 2008; Gonçalves-Vidigal, Vidigal Filho, Medeiros, & Pastor-Corrales, 2009; Gonçalves et al., 2010; Gonela et al., 2010; Barelli et al., 2011; Gonçalves-Vidigal et al., 2012).

Knowledge of and access to genetic diversity preserved in germplasm banks or used by small farmers is essential for expanding the genetic basis in common bean breeding programs, primarily for the selection of divergent parental lineages for obtaining superior genotypes (Kumar et al., 2008). To that end, diversity should be quantified or predictively quantified, the latter based on morphological and molecular differences quantified using a dissimilarity measurement capable of expressing the degree of diversity between parent plants (Rosales-Serna, Hernandez-Delgado, Gonzalez-Paz, Acosta-

Gallegos, & Mayek-Perez, 2005; Blair et al., 2006; Galvan, Menéndez-Sevillano, De Ron, Santalla, & Balatti, 2006).

The present study aimed to analyze the genetic diversity of 17 common bean accessions from the BGF/Nupagri/UEM through joint analysis of morpho-agronomic and molecular data, and assess their reaction to the *Colletotrichum lindemuthianum* pathogen.

## Material and methods

### Plant material

Genetic diversity was analyzed in 17 common bean accessions from the Bean Germplasm Bank of the Center for Applied Agricultural Research of the State University of Maringá (BGF/Nupagri/UEM). The accessions studied were from the city of Toledo (24° 42' 50" S, 53° 44' 34" W, altitude 560 m), in Paraná State, Brazil. Seeds from each accession were planted in pots containing substrate and kept in a greenhouse in order to obtain pure lineages. This procedure was repeated for two cycles. The morphological, agronomic, and molecular characteristics of the accessions were assessed.

### Morphological characteristic evaluation

A total of 11 characteristics were analyzed: seed size; seed coat color (primary/secondary); predominant distribution of the seed's secondary color; brightness/opacity of the seed coat; hilum color; hypocotyl color; flower color; mature pod color (primary/secondary); and growth habit. The seed flatness index and seed shape were determined by the H (thickness/width) and J (length/width) coefficients (Puerta Romero, 1961). The accessions were grouped into their respective gene pools according to seed size and genotyping using the RAPD marker OPG19 (5'-GTCAGGGCAA-3') (Gonçalves-Vidigal, Costa, Vidigal Filho, Gonela, & Sansigolo, 2007). Accessions that exhibited a 1,790 bp band were classified as Mesoamerican and those with a 1,400 bp band as Andean. The accessions were also grouped into market classes.

### Sample Preparation for DNA extraction

Ten seeds from each accession were placed in plastic trays containing peat moss and vermiculite and kept in a greenhouse until the first trifoliate leaf emerged. Next, a young leaf was collected from each seedling and placed in 1.5 mL plastic microtubes, frozen in liquid nitrogen, and stored in a freezer (-20°C) for future DNA extraction. The trays were then transferred to a chamber with controlled temperature (22 ± 2°C) and subsequently

inoculated with a spore suspension of *Colletotrichum lindemuthianum* races 73 and 2047.

#### **Anthracnose reaction of the accessions to *Colletotrichum lindemuthianum* races 73 and 2047**

The inoculum was prepared according to the methodology proposed by Cárdenas, Adams, and Andersen (1964), which consists of multiplying spores of each *C. lindemuthianum* pathotype in test tubes containing sterilized pods partially immersed in water agar (WA) culture medium. After inoculation, the test tubes were incubated for 14 days at  $20 \pm 2^\circ\text{C}$ . Next, pods from each test tube were removed using tweezers and transferred to a beaker containing sterile distilled water. Double gauze was used to filter the suspensions obtained for each pathotype, and the spore suspension was adjusted to  $1.2 \times 10^6$  spores  $\text{mL}^{-1}$  by diluting it with sterile distilled water. Plants in the mist chamber were inoculated using a brush moistened with spore suspension and kept in the chamber for 72 h at a temperature of  $20 \pm 2^\circ\text{C}$ , with controlled light (12h of light at 680 lux / 12h darkness) and 100% humidity. After 72 hours, the plants were moved to a room with a temperature of  $22 \pm 2^\circ\text{C}$  under artificial light until symptom assessment. Symptoms were visually evaluated approximately 10 days after inoculation using the severity scale system (Van Schoonhoven & Pastor-Corrales, 1987), with values ranging from 1 to 9. Plants that scored from 1 to 3 were considered resistant, and those from 4 to 9 were considered susceptible.

#### **Genomic DNA extraction and analysis using SSR markers**

DNA extraction was carried out according to the methodology proposed by Afanador, Haley, and Kelly (1993). A total of 18 Simple Sequence Repeats (SSR) loci were analyzed, of which 17 (*Bmd-2*, *Bmd-9*, *Bmd-10*, *Bmd-12*, *Bmd-15*, *Bmd-16*, *Bmd-17*, *Bmd-25*, *Bmd-26*, *Bmd-33*, *Bmd-36*, *Bmd-40*, *Bmd-42*, *Bmd-45*, *Bmd-46*, *Bmd-52*, and *Bmd-53*) were identified by Blair et al. (2003) and one (PVBR128) by Grisi et al. (2007). Amplification reactions were conducted in a TC-412 thermal cycler with a total volume of 25  $\mu\text{L}$  each. The PCR products were separated in 1.2% MetaPhor agarose gels prepared with 0.5X TBE buffer (0.89 M tris-acetate, 0.89 M boric acid, and 0.02 M EDTA) containing 0.02% ethidium bromide. The DNA bands were visualized under UV light using Endurance software and a Canon 7.1 (Powershot A620) digital camera. The 50 bp DNA ladder was used as a control. When a locus appeared to be monomorphic, analyses were conducted in a 10% polyacrylamide gel stained with silver nitrate according to the protocol developed by Sanguinetti, Dias-Neto, and Simpson (1994).

#### **Statistical analyses**

Genetic analysis is generally performed using statistical tools to analyze data on genetic markers (morphological, biochemical and/or molecular) and group genotypes according to their similarities based on the patterns identified. However, if the available allows for it, a joint analysis of characteristics can be carried out, which gives a more accurate view of the level of divergence between the assessed genotypes. Thus, genetic diversity among the 17 common bean accessions was determined by joint analysis of the characteristics studied, using SAS software (Statistical Analysis System - SAS Institute, 1989) with the SAS/Genetics (Allele and CaseControl Procedures), SAS/IML and SAS/Stat (Cluster, Distance, MDS, Tree and VarCluster Procedures) packages. First, a Jaccard distance matrix was constructed (Jaccard, 1908) using Proc Distance and disregarding double zeros (species absence) as a similarity (to avoid inflating values based on RAPD data). This matrix was used to generate the dendrogram (UPGMA hierarchical clustering) via Proc Cluster and Proc Tree, with the cutoff point defined by 5,000 bootstrap interactions. The dendrogram was validated by two methods: (a) Multidimensional Scaling (MDS), using Proc MDS with 12 interactions, and (b) Non orthogonal Principal Component Analysis (NOPCA), using VarCluster. Allele frequency and polymorphism information content (PIC) values for each locus analyzed that were calculated using SAS software.

#### **Results and discussion**

Clustering of the 17 accessions based on morphological, agronomic (Table 1) and molecular characteristics, validated by multidimensional scaling (MDS), indicated the formation of two large groups (Figure 1), which exhibited a dissimilarity value of 0.81.

The first group (I) contained 35.3% of the accessions studied, all of Mesoamerican origin. The second group (II) was composed of 64.7% of the accessions assessed, all from the Andean line.

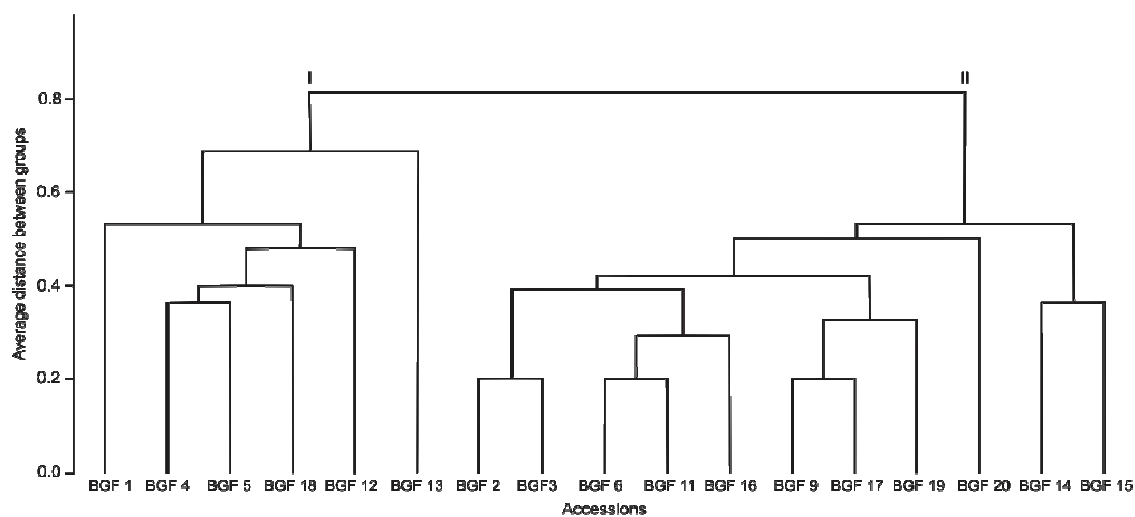
Thus, joint analysis of data on morpho-agronomic and molecular origin grouped the accessions according to primary genotypes (Andean or Mesoamerican).

Beans from both primary genotypes are found in Brazil, which, according to Gepts et al. (1988), suggests three possible routes of introduction: the first involving genotypes from Central America, the second involving genotypes from the Andes, and the third via European immigrants, especially Italians, in the states of Santa Catarina (SC) and Rio Grande do Sul (RS).

**Table 1.** Classification of the 17 common bean accessions analyzed according to their respective gene pool, market class, brightness/opacity of the seed coat (BS), hilum color (HC), seed flatness index (H), and shape (J), hypocotyl color (HY), flower color (FC), mature pod color (PC), growth habit (GH) and incompatibility reaction to *Colletotrichum lindemuthianum* races 73 and 2047.

Assession code	Gene pool	Market class	Seed			HY	FC	PC	GH	Reaction to <i>Colletotrichum lindemuthianum</i>	
			BS	HC	H/J					73	2047
BGF 1	M	Rosinha	1	2	2/2	1	1	1	I	S	R
BGF 2	A	Preto	3	5	2/2	2	3	4	I	S	S
BGF 3	A	Preto	2	5	2/2	2	2	1	I	S	R
BGF 4	M	Preto	1	5	1/2	1	2	2	IV	R	S
BGF 5	M	Rosinha	1	3	3/3	1	1	1	I	R	R
BGF 6	A	Manteigão	3	4	3/4	1	2	5	I	R	R
BGF 9	A	Cores	1	5	3/1	1	2	6	IV	S	R
BGF 11	A	Manteigão	3	1	2/3	1	2	3	I	R	R
BGF 12	M	Roxinho	1	4	2/1	2	1	1	I	R	S
BGF 13	M	Cores	1	5	2/1	1	2	6	IV	R	S
BGF 14	A	Cores	3	5	3/1	2	2	4	I	S	S
BGF 15	A	Manteigão	2	4	2/2	1	2	5	IV	R	R
BGF 16	A	Manteigão	2	1	2/2	1	2	5	I	R	S
BGF 17	A	Manteigão	3	2	2/3	1	2	6	I	R	S
BGF 18	M	Cores	1	5	2/1	1	1	1	I	S	R
BGF 19	A	Roxinho	3	4	2/1	1	2	4	I	R	S
BGF 20	A	Manteigão	3	2	2/1	1	2	4	I	R	R

Gene Pool: A = Andean; M = Mesoamerican. BS: 1 = opaque; 2 = average; 3 = brightness. HC: 1 = orange; 2 = beige; 3 = pink; 4 = violet; 5 = black. H: 1 = flat (< 0.69); 2 = semi-full (0.70 to 0.79); 3 = full (> 0.80). J: 1 = elliptical (1.43 to 1.65); 2 = oblong/short-reniform (1.66 to 1.85); 3 = oblong/medium-reniform (1.86 to 2.00); 4 = oblong/long-reniform (> 2.00). HY: 1 = green; 2 = purple. FC: 1 = white; 2 = pink; 3 = violet. PC: 1 = pink; 2 = pink with red stripes; 3 = red with dark red stripes; 4 = green; 5 = green with red stripes; 6 = green with violet stripes; 7 = violet. GH: 1 = determined bushy; IV = undetermined climbing. Incompatibility reaction to *Colletotrichum lindemuthianum* races 73 and 2047: R = resistant; S = susceptible.



**Figure 1.** Dendrogram illustrating the dissimilarity pattern among the 17 common bean accessions via UPGMA hierarchical clustering, based on a Jaccard distance matrix and validated by multidimensional scaling (MDS). Joint analysis of phenotypic and genotypic characteristics was assessed using the Genetics platform of SAS software.

It is important to note that the municipality of Toledo was primarily settled by Italian immigrants from Caxias do Sul (RS), which may have contributed to the introduction of their preferred ‘Carnaval’ bean variety to the region.

Following MDS analysis, the dendrogram was validated by non orthogonal principal component analysis (NOPCA). Clustering obtained via NOPCA converged to 0.8863 of the MDS value (Table 2).

The only differences observed between clustering by NOPCA and MDS were for accessions BGF2 and BGF3. In the case of MDS, accessions BGF2 and BGF3 were designated to the same group as BGF6, BGF9, BGF11, BGF16, BGF17, and

BGF19 (G01), while BGF12 was isolated in group G04. In turn, validation by NOPCA designated BGF2 and BGF3 to group G04. This difference can be attributed to the way in which clustering is performed in the two methods, that is, NOPCA divides the groups so that the resulting clusters satisfactorily explain the behavior of accessions, whereas three-dimensional MDS is a spatial model that attempts to fit the dissimilarity matrix data.

The multidimensional scaling coordinates used to validate the dendrogram, obtained via construction of a Jaccard distance matrix, enabled a three-dimensional graph to be plotted (Figure 2).

Group G01 was the most representative, containing eight accessions (BGF 2, BGF 3, BGF 6, BGF 9, BGF 11, BGF 16, BGF 17, and BGF 19), group G02 comprised 17.6% of the accessions studied (BGF 4, BGF 5, and BGF 18), and group G03 contained 11.8% (BGF 14 and BGF 15). The remaining 23.5% of the accessions analyzed were distributed among the other four groups (G04, G05, G06, and G07), with one accession each.

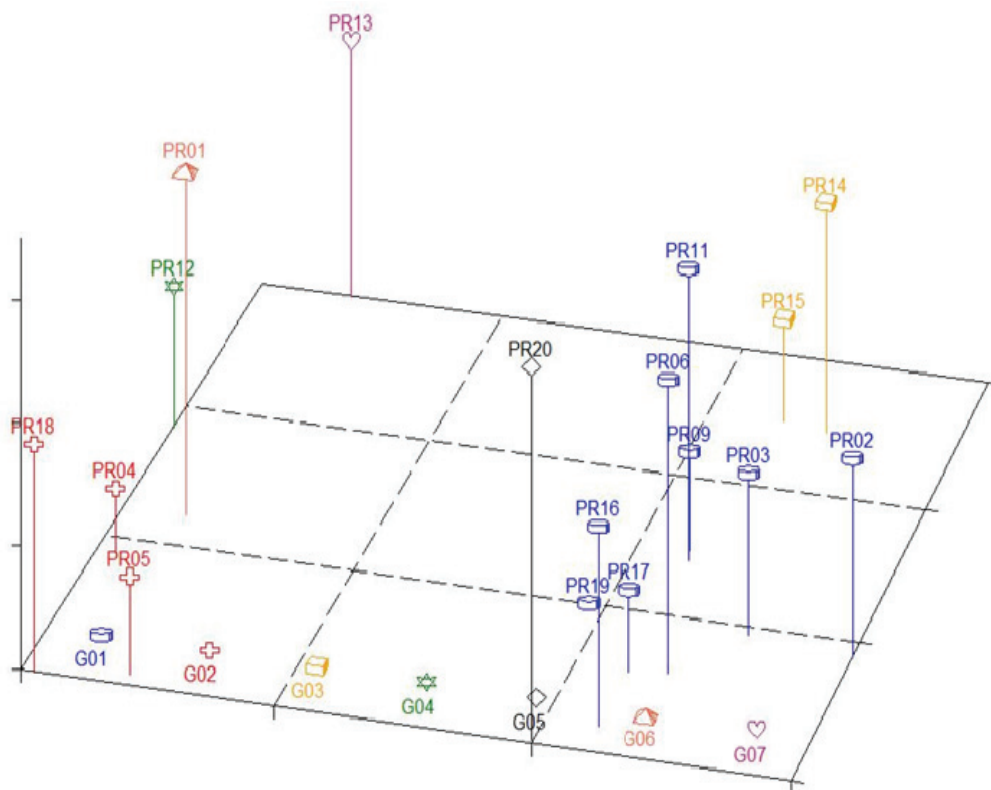
**Table 2.** Comparison between groupings obtained by multidimensional scaling (MDS) and non orthogonal principal component analysis (NOPCA)

Accession code	Grouping		Accession code	Grouping	
	MDS	PCA		MDS	PCA
BGF 1	G06	G06	BGF 13	G07	G07
BGF 2	G01	G04	BGF 14	G03	G03
BGF 3	G01	G04	BGF 15	G03	G03
BGF 4	G02	G02	BGF 16	G01	G01
BGF 5	G02	G02	BGF 17	G01	G01
BGF 6	G01	G01	BGF 18	G02	G02
BGF 9	G01	G01	BGF 19	G01	G01
BGF 11	G01	G01	BGF 20	G05	G05
BGF 12	G04	G04			

The Jaccard index was used to determine the genetic dissimilarity between the 17 accessions (Table 3). The most similar accessions were BGF 2 x BGF3, BGF 6 x BGF 11, and BGF 9 x BGF 17,

with a root mean square deviation (RMSD) value of 0.20. Root mean square deviation is a measurement of distance, whereby the lower the value the more similar the individuals. However, the formation of a tie must be considered since its presence indicates the need to randomly cluster individuals, given that several grouping possibilities exhibited the same similarity. As such, the mere existence of a tie indicates that inconsistent clustering is possible at the level in question, which may become consistent at a superior hierarchical level.

With respect to the most dissimilar accessions, the greatest dissimilarity (RMSD = 0.81) was obtained between the clusters CL2 (group II) and CL3 (group I), as previously mentioned. The second highest measurement (RMSD = 0.69) was obtained between cluster CL4 (BGF 1, BGF 4, BGF 5, BGF 12, and BGF 18) and BGF 13, all of Mesoamerican origin. The third largest measurement (RMSD = 0.53) recorded was between clusters CL5 (BGF 14 and BGF 15) and CL10 (BGF 2, BGF 3, BGF 6, BGF 11, BGF 16, BGF 9, BGF 17, BGF 19, and BGF 20), all of Andean origin, and between BGF1 and cluster CL6 (BGF 4, BGF 5, BGF 18, and BGF 12). Clusters were formed considering the mean distance between accessions.



**Figure 2.** Three-dimensional clustering of the 17 common bean accessions obtained after validation by MDS, where PR replaces the abbreviation BGF.

**Table 3.** Dissimilarity between the 17 common bean accessions using the Jaccard index, in accordance with UPGMA hierarchical clustering.

CLN	Clusters		FREQ	RMSD	Tie
16	BGF 2	BGF 3	2	0.20	T
15	BGF 6	BGF 11	2	0.20	T
14	BGF 9	BGF 17	2	0.20	T
13	CL15	BGF 16	3	0.29	
12	CL14	BGF 19	3	0.33	
11	BGF 4	BGF 5	2	0.36	T
10	BGF 14	BGF 15	2	0.36	T
9	CL16	CL13	5	0.39	
8	CL11	BGF 18	3	0.40	
7	CL9	CL12	8	0.42	
6	CL8	BGF 12	4	0.48	
5	CL7	BGF 20	9	0.50	
4	BGF 1	CL6	5	0.53	
3	CL5	CL10	11	0.53	
2	CL4	BGF 13	6	0.69	
1	CL2	CL3	17	0.81	

CLN = cluster number; FREQ = frequency; RMSD = root mean square standard deviation.

Knowledge of genetic diversity through dissimilarity analysis allows the selection of parent plants for recombination, according to breeding program objectives (Singh, 2001).

Depending on the goal of the breeder, the selected crossover is either that which exhibits the greatest dissimilarity on the genetic pyramid that confers characteristics of agronomic interest, thereby increasing genetic variability, or that which exhibits the greatest similarity. In this respect, with a view to generating genetic variability, it can be inferred that crossovers between accession BGF13 and those belonging to cluster CL4 are the most recommended since they have the highest dissimilarity value. Moreover, it is important to underscore that crossovers between clusters CL5 and CL10 and between BGF1 and CL6 are also recommended, since these combinations exhibit high genetic dissimilarity, allowing new superior breeding lines to be obtained.

Analysis of the 17 accessions demonstrated significant genetic diversity. The accessions were obtained in western Paraná State, specifically the municipality of Toledo, where they are grown by small farmers as a source of food and income. Important breeding characteristics were observed among the 17 accessions, including opaque seed coat (BGF1, BGF 4, BGF 5, BGF 9, BGF 12, BGF 13, and BGF 18); type I growth habit (BGF 1, BGF 2, BGF 3, BGF 5, BGF 6, BGF 11, BGF 12, BGF 14, BGF 16, BGF 17, BGF 18, BGF 19, and BGF 20) and small seeds.

The morphological characteristics exhibited by the 17 accessions studied deserve attention in breeding programs because they directly reflect acceptance of the product by the consumer market (Gepts et al., 2008).

Brazilian consumers generally accept small (Mesoamerican origin) seeds. With respect to commercial varieties, Carioca and Preto are the most accepted cultivars (MAPA, 2016). In addition to ideal size, favorable characteristics for Carioca cultivars include light coloring with few inconspicuous brown streaks, while those from the Preto group should provide a good quality broth, minimal discoloration after cooking and a tough seed coat.

In regard to brightness, beans with a shiny seed coat absorb water more slowly and therefore take longer to cook than those with an opaque seed coat (Konzen and Tsai, 2014); therefore, the former are disregarded in the cultivar selection process.

Growth habit is another important factor evaluated by breeders. Plants with a more compact architecture are used in crossovers to select for erectness and precocity, since they exhibit lower production potential compared to prostrate plants. In turn, cultivars with an undetermined growth habit display higher yields than those with a defined habit because vegetative development progresses through the production of new buds, which generate flowers and improve the yield potential (Dawo Sanders & Pilbeam, 2007). However, the ideal plants for mechanical harvesting are those with type I and II growth habits (Miklas & Singh, 2007).

Hilum color is another important characteristic, particularly in Carioca cultivars, since these can exhibit a yellow or orange color indicating an undesirable phenotype, meaning breeders should select seeds without this trait (Tomaz, Moda-Cirino, Fonseca Junior, & Ruas, 2007).

The BGF/Nupagri/UEM has a collection of 181 *Phaseolus vulgaris* L. accessions primarily from the states of Mato Grosso do Sul, Paraná and Santa Catarina, of which only 17 (9.4%) were analyzed in the present study. Nevertheless, significant genetic variability was observed, particularly among accessions belonging to the same gene pool.

The main contribution of the BGF/Nupagri/UEM is related to the identification of anthracnose-resistant genes. In the 17 accessions studied, two such genes have been identified: one in BGF 9 (Corinthiano), *Co-15*, which provides resistance to races 8, 89, and 2047 (Gonçalves et al., 2010), and one in BGF 15 (Pitanga), *Co-14*, conferring resistance to races 23, 64, 65, 73, and 2047 (Gonçalves-Vidigal et al., 2012).

Other accessions also stand out as important sources of resistance to *Colletotrichum lindemuthianum*. In group I, containing the Mesoamerican accessions (Figure 1), BGF 4, BGF 12, and BGF 13 showed resistance to race 73, while BGF 1 and BGF 18 were

resistant to race 2047, and BGF 5 was resistant to both races. In group II (Andean), accessions BGF 16, BGF 17, and BGF 19 were resistant to race 73, BGF 3 and BGF 9 to race 2047, and BGF 16, BGF 11, BGF 15, and BGF 20 to both races. Therefore, of the 17 accessions assessed, 15 were resistant to at least one of the races tested. This highlights the importance of this germplasm bank in breeding programs for the species, particularly in terms of obtaining anthracnose-resistant cultivars.

## Conclusion

Joint analysis of morphological, agronomic and molecular traits demonstrated significant genetic diversity among the 17 accessions studied, identifying them as an important gene source in genetic improvement programs for the species.

## Acknowledgements

To CAPES - Coordination of Improvement of Higher Education Personnel for the grant awarded to D. T. Guidoti. To the Agronomic Engineer Rodrigo Garcia (*in memoriam*) for donating the seeds of the accessions analyzed in the present study to BGF/Nupagri/UEM.

## References

- Afanador, L. K., Haley, S. D., & Kelly, J. D. (1993). Adoption of a 'mini-prep' DNA extraction protocol for RAPD marker analysis in common bean (*Phaseolus vulgaris* L.). *Annual Report of Bean Improvement Cooperative*, 36(1), 10-11.
- Agência Paulista de Tecnologia dos Agronegócios. [APTA]. (2016). *Banco Ativo de Germoplasma de Feijão do Instituto Agrônomo de Campinas*. Retrieved on Aug. 9, 2016 from <[http://www.apta.sp.gov.br/curadoria/ver\\_colectao.php?id\\_colectao=13](http://www.apta.sp.gov.br/curadoria/ver_colectao.php?id_colectao=13)>.
- Barelli, M. A. A., Poletine, J. P., Thomazella, C., Vidigal Filho, P. S., Souto, E. R., Pacheco, C. M. N. A., ... Gonçalves-Vidigal, M. C. (2011). Evaluation of genetic divergence among traditional accessions of common bean by using RAPD molecular markers. *International Journal of Food, Agriculture and Environment*, 9(2), 195-199.
- Blair, M. W., Pedraza, F., Buendia, H. F., Gaitán-Solís, E., Beebe, S. E., Gepts, P., & Tohme, J. (2003). Development of a genome-wide anchored microsatellite map for common bean (*Phaseolus vulgaris* L.). *Theoretical and Applied Genetics*, 107(8), 1362-1374. doi: 10.1007/s00122-003-1398-6
- Blair, M. W., Giraldo, M. C., Buendía, H. F., Tovar, E., Duque, M. C., & Beebe, S. E. (2006). Microsatellite marker diversity in common bean (*Phaseolus vulgaris* L.). *Theoretical Applied Genetics*, 113(1), 100-109. doi:10.1007/s00122-006-0276-4
- Botstein, D., White, R. L., Skolnick, M., & Davis, R. W. (1980). Construction of genetic linkage map in man using restriction fragment length polymorphism. *American Journal of Human Genetics*, 32(3), 314-331.
- Broughton, W. J., Hernandez, G., Blair, M., Beebe, S., Gepts, P., & Vanderleyden, J. (2003). Beans (*Phaseolus* spp.) model food legumes. *Plant Soil*, 252(1), 55-128. doi:10.1023/A:1024146710611
- Cárdenas, F., Adams, M. W., & Andersen, A. (1964). The genetic system for reaction of field beans (*Phaseolus vulgaris* L.) to infection by three physiologic races of *Colletotrichum lindemuthianum*. *Euphytica*, 13(2), 178-186. doi:10.1007/BF00033307
- Embrapa Recursos Genéticos. Cenargem Rede Nacional de Recursos Genéticos Vegetais. (2016). PA4 - Banco ativo de germoplasma de feijão (*Phaseolus vulgaris*). Retrieved on Aug. 9, 2016 From <<http://plataformarg.cenargem.embrapa.br/rede-vegetal/projetos-componentes/pc3-bancos-ativos-de-germoplasma-de-especies-leguminosas-oleaginosas-e-fibrosas/planos-de-acao/pa4-banco-ativo-de-germoplasma-de-feijao-phaseolus-vulgaris>>.
- International Center for Tropical Agriculture [CIAT]. (2016). *About Bean Research*. Retrieved on Aug. 9, 2016 from <<https://ciat.cgiar.org/bean-research>>.
- Dawo, M. I., Sanders, F. E., & Pilbeam, D. J. (2007). Yield, yield components and plant architecture in the F3 generation of common bean (*Phaseolus vulgaris* L.) derived from a cross between the determinate cultivar 'Prelude' and an indeterminate landrace. *Euphytica*, 156(1), 77-87. doi:10.1007/s10681-007-9354-1
- Food and Agriculture Organization [FAO]. (2016). *Faostat database gateway*. Retrieved on Aug. 9, 2016 from <[http://faostat3.fao.org/browse/rankings/countries\\_by\\_commodity/E](http://faostat3.fao.org/browse/rankings/countries_by_commodity/E)>.
- Galvan, M. Z., Menéndez-Sevillano, M. C., De Ron, A. M., Santalla, M., & Balatti, P.A. (2006). Genetic diversity among wild common beans from northwestern Argentina based on morpho agronomic and RAPD data. *Genetic Resources and Crop Evolution*, 53(5), 891-900. doi:10.1007/s10722-004-0981-2
- Gepts, P., & Bliss, F. A. (1985). F1 hybrid weakness in the common bean: differential geographic origin suggests two gene pools in cultivated bean germplasm. *Journal of Heredity*, 76(1), 447-450.
- Gepts, P., & Bliss, F. A. (1986). Phaseolin variability among wild and cultivated common beans (*Phaseolus vulgaris*) from Colombia. *Economic Botany*, 40(4), 469-478.
- Gepts, P., Kmicik, K., Pereira, P., & Bliss, F. A. (1988). Dissemination pathways of the common beans (*Phaseolus vulgaris*, Fabaceae) deduced from phaseolin electrophoretic variability. I. The Americas. *Economic Botany*, 42(1), 73-85.
- Gepts, P., Osborn, T. C., Rashka, K., & Bliss, F. A. (1986). Phaseolin-protein variability in wild forms and landraces of the common beans (*Phaseolus vulgaris*): evidence for multiple centers of domestication. *Economy Botany*, 40(4), 451-468.

- Gepts, P., Aragão, F. J. L., Barros, E., Blair, M. W., Brondani, R., Broughton, W., ... Yu, K. (2008). Genomics of *Phaseolus* beans, a major source of dietary protein and micronutrients in the tropics. In: P. H. Moore & R. Ming (Eds.), *Genomics of Tropical Crop Plants* (p.113-142). New York, NY: Springer.
- Gonçalves, A. M. O., Gonçalves-Vidigal, M. C., Vidigal Filho, P. S., Poletine, J. P., Lacanallo, G. F., & Coimbra, G. K. (2010). Characterization of the anthracnose resistance gene in Andean common bean Corinthiano cultivar. *Annual Report of the Bean Improvement Cooperative*, 53(53), 220-221.
- Gonçalves-Vidigal, M. C., Costa, M. R., Vidigal Filho, P. S., Gonela, A., & Sansigolo, A. (2007). Molecular characterization of common bean cultivars by phaseolin and RAPD markers. *Annual Report of the Bean Improvement*, 50(50), 71-72.
- Gonçalves-Vidigal, M. C., Lacanallo, G. F., & Vidigal, P. S. (2008). A new Andean gene conferring resistance to anthracnose in common bean (*Phaseolus vulgaris* L.) cultivar Jalo Vermelho. *Plant Breeding*, 127(6), 592-596. doi: 10.1111/j.1439-0523.2008.01530.x
- Gonçalves-Vidigal, M. C., Meirelles, A. C., Poletine, J. P., Sousa, L. L., Cruz, A. S., Nunes, M.P., ... & Vidigal Filho, P. S. (2012). Genetic analysis of anthracnose resistance in 'Pitanga' dry bean cultivar. *Plant Breeding*, 131(3), 423-429. doi:10.1111/j.1439-0523.2011.01939.x
- Gonçalves-Vidigal, M. C., Vidigal Filho, P. S., Medeiros, A. F., & Pastor-Corrales, M. A. (2009). Common bean landrace Jalo Listras Pretas is the source of a new Andean anthracnose resistance gene. *Crop Science*, 49(1), 133-138. doi: 10.2135/cropsci2008.01.0004
- Gonela, A., Romani, I., Gonçalves-Vidigal, M. C., Vidigal Filho, P. S., Lacanallo, G. F., Reche, D., ... Guidoti, D. T. (2010). Genetic diversity in common bean germplasm from Brazil using microsatellite markers. *Annual Report of the Bean Improvement Cooperative*, 53(53), 188-189.
- Grisi, M. C. M., Blair, M. W., Gepts, P., Brondani, C., Pereira, P. A. A., & Brondani, R. P. V. (2007). Genetic mapping of a new set of microsatellite markers in a reference common bean (*Phaseolus vulgaris*) population BAT93 x Jalo EET558. *Genetic and Molecular Resource*, 6(3), 691-706.
- Jaccard, P. (1908). Nouvelles recherches sur la distribution florale. *Bulletin de la Société Vaudoise des Sciences Naturelles*, 44(163), 223-270. doi: 10.5169/seals-268384
- Kelly, J. D., & Vallejo, V. A. (2004). A comprehensive review of the major genes conditioning resistance to anthracnose in common bean. *HortScience*, 39(6), 1196-1207.
- Koinange, E. M. K., & Gepts, P. (1992). Hybrid weakness in wild *Phaseolus vulgaris* L. *Journal of Heredity*, 83(2), 135-139.
- Konzen, E. R., & Tsai, S. M. (2014). Seed coat shininess in *Phaseolus vulgaris*: rescuing a neglected trait by its screening on commercial lines and landraces. *Journal of Agricultural Science*, 6(8), 113-130. doi:10.5539/jas.v6n8p113
- Kumar, V., Sharma, S., Kero, S., Sharma, S., Sharma, A. K., Kumar, M., & Bhat, K. V. (2008). Assessment of genetic diversity in common bean (*Phaseolus vulgaris* L.) germplasm using amplified fragment length polymorphism (AFLP). *Scientia Horticulturae*, 116(2), 138-143. doi:10.1016/j.scienta.2007.12.001
- Mamidi, S., Rossi, M., Moghaddam, S. M., Annam, D., Lee, R., Papa, R., MacClean, P. E. (2013). Demographic factors shaped diversity in the two gene pools of wild common bean *Phaseolus vulgaris* L. *Heredity*, 110(3), 267-276.
- Ministério da Agricultura [MAPA]. (2016). Perfil do feijão no Brasil. Retrived on Dec. 1, 2016 from <<http://www.agricultura.gov.br/vegetal/culturas/feijao/saiba-mais>>.
- Miklas, P. N., Kelly, J. D., Beebe, S. E., & Blair, M. W. (2006). Common bean breeding for resistance against biotic and abiotic stresses: from classical to MAS breeding. *Euphytica*, 147(1), 105-131. doi:10.1007/s10681-006-4600-5
- Miklas, P. N., & Singh, S. P. (2007). Common Bean. In C. Kole (Ed.), *Genome mapping and molecular breeding in plants pulses, sugar and tuber crops* (p. 1-31). Heidelberg, Germany: Springer.
- Puerta Romero, J. (1961). *Varietades de judias cultivada em Espanha*. Madrid, Spain: Publicaciones del Ministerio de Agricultura, Subdirección de Capacitación Agraria.
- Rosales-Serna, R., Hernandez-Delgado, S., Gonzalez-Paz, M., Acosta-Gallegos, J. A., & Mayek-Perez, N. (2005). Genetic relationships and diversity revealed by AFLP markers in Mexican common bean bred cultivars. *Crop Science*, 45(5), 1951-1957. doi:10.2135/cropsci2004.0582
- Sanguinetti, C., Dias-Neto, E., & Simpson, A. J. G. (1994). RAPD silver staining and recovery of PCR products separated on polyacrylamide gels. *Biotechniques*, 17(5), 914-921.
- Satistical Analysis System [SAS]. (1989). *SAS/STAT user's guide, version 6* (4th ed.) Cary, NC: SAS Institute Inc.
- Singh, S. P. (2001). Broadening the genetic base of common bean cultivars: a review. *Crop Science*, 41(6), 1659-1675. doi:10.2135/cropsci2001.1659
- Tomaz, J. P., Moda-Cirino, V., Fonseca Junior, N. S., & Ruas, P. M. (2007). Genetic control of orange hilum corona of carioca beans (*Phaseolus vulgaris*). *Genetics and Molecular Biology*, 30(3), 594-598. doi:10.1590/S1415-47572007000400016
- Toro, O., Tohme, J., & Debouck, D. G. (1990). *Wild bean (Phaseolus vulgaris L.): description and distribution*. Cali:



Colombia: International Board for Plant Genetic Resources (IBPGR), Centro Internacional de Agricultura Tropical.

United States Department of Agriculture - Agricultural Research Service [USDA-ARS]. (2016). Retrieved on Feb. 3, 2016 from <https://www.ars.usda.gov/pacific-west-area/pullman-wa/plant-germplasm-introduction-and-testing-research/#>

Van Schoonhoven, A., & Pastor-Corrales, M. A. (1987).

Standart system for the evaluation of bean germplasm. Cali, Colômbia: CIAT.

*Received on August 9, 2016.*

*Accepted on December 30, 2016.*

License information: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.