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Investigation of the genetic diversity of common bean (*Phaseolus vulgaris*.) cultivars using molecular markers

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ABSTRACT. The common bean (*Phaseolus vulgaris*) is a widespread crop in Brazil of dietary and economic importance, and it is cultivated primarily through family farming. Knowledge of genetic variability in landraces and improved bean cultivars is essential to explore the existing diversity, identify superior genotypes adapted to the climatic conditions of specific regions, and support genetic improvement strategies. Estimates of genetic diversity can be obtained using DNA molecular markers, and ISSR markers are widely used. We evaluated the genetic diversity of 57 common bean genotypes, including accessions provided by the Brazilian Agricultural Research Corporation (EMBRAPA - Wheat), local genotypes of the Fortaleza community (Muqui-Espírito Santo) and commercial cultivars, using ISSR markers. A total of 11 primers were used, generating 51 fragments, of which 76%were polymorphic. The polymorphic information content ranged from 0.19 to 0.48, with a mean of 0.36. There was an unequal distribution between genetic distances, ranging from 0.00 to 1.0, and a mean of 0.44, evidencing wide genetic variability. The Pérola cultivar stood out as it showed the highest mean dissimilarity (0.76). Cluster analysis revealed the formation of 11 groups, with a tendency to cluster genotypes by the region of origin and growth habit. There was wide genetic diversity among the genotypes of the

Fortaleza community and a narrower diversity for the EMBRAPA and commercial cultivars. ISSR markers were efficient in quantifying the genetic diversity of the genotypes; the most divergent markers will help select candidates for conservation in germplasm banks.

Key words: Bean plant; Molecular marker ISSR; Variability; Genetic breeding

INTRODUCTION

The common bean (*Phaseolus vulgaris*, 2n=22) is an annual legume that is predominantly autogamous and was domesticated over 7000 years ago in two main centers of origin: Mesoamerica (Mexico and Central America) and the Andean region (Geptset al. 1986; Koenig and Gepts 1989; Kwak and Gepts, 2009; Bitocchi et al., 2013; Fisseha et al., 2016), as well as several secondary domestication centers (Kwak and Gepts, 2009). This history contributed to great genetic and phenotypic diversity, resulting in an enormous variety of colors, textures and grain sizes (Guidolin, 2003).

The various types of beans are cultivated from sea level to over three thousand meters altitude, mainly by small farmers, with relatively simple technology (Schoonhoven and Voysest, 1991). Beans are a traditional food in the diet of the Brazilian population. The grains of this legume provide high levels of energy and protein, as well as other nutrients, such as iron, calcium, vitamins and fiber (Anderson, et al., 1999; Resende et al., 2008).

Brazilis one of the world's largest bean producers and is a major consumer market for this crop (FAO, 2018). In the state of Espírito Santo, beans are the fourth most economically important agricultural product (IBGE, 2016); they are grown by family farmers who mostly use landraces (Fonseca, 2007).

Family farming, also called subsistence farming, has played a key role in the conservation of the genetic variability of this crop in Brazil because as it is cultivated in small properties, the genotypes most adapted to the local agro-morphological and socioeconomic conditions were selected, contributing to *a posteriori* improvement (Cordeiro and Marcatto, 1994). However, the genetic variability that has been preserved by family farms is currently being lost due to the substitution of local cultivars with commercial varieties (Rodrigues et al., 2002). Consequently, knowledge of the genetic diversity among landraces and improved cultivars is important to support plant breeding programs, so that breeders can exploit the existing genotypes adapted to the climatic conditions of specific regions (Loarce et al., 1996; Franco et al., 2001).

Genetic diversity studies are of great importance in breeding programs because they allow for the identification of divergent genotypes, the choice of suitable selection methods, the identification of duplicates, and the reduction of maintenance costs of germplasm banks (Carvalho et al., 2008; Singh, 2001). In addition, in the process of choosing the most appropriate breeding strategy, knowledge of germplasm diversity is of vital importance. Such diversity can only be efficiently used if it is duly evaluated and quantified (Vanderborght, 1988).

Among the tools used to estimate genetic diversity in a set of genotypes, molecular markers enable direct estimation of genetic diversity at the DNA level, reducing the interference of environmental variation, and they are not influenced by the environment (Ferreira and Grattapaglia, 1998).DNA molecular markers include inter simple sequence repeat (ISSR) markers, which are widely used in genetic diversity studies because they are universal and highly polymorphic, require single primers, have a low cost of development and use, and have high reproducibility of results (Silva et al., 2016). González et al. (2005) and Svetleva et al.

(2006) reported the high efficiency of ISSR markers in the quantification of genetic diversity among bean genotypes, even though they were closely related.

The objective of this work was to evaluate the genetic diversity and identify possible duplicates between landraces and commercial bean cultivars using ISSR molecular markers.

MATERIAL AND METHODS

Genetic material

The genetic material consisted of 57 bean accessions (Table 1), with 20 accessions provided by the Brazilian Agricultural Research Corporation (EMBRAPA - Wheat), 31 local genotypes belonging to the Fortaleza community of the municipality of Muqui - Espírito Santo state (ES), and six commercial cultivars: Carioca, Serrano, IAPAR 31, IAPAR 44, IAPAR 81 and Pérola.

Table 1. Identification of genotypes regarding origin, growth habit (GH), commercial group (CG) and 100-seed weight in grams (100SW).

[1]Ident	[2]Origin	[3]HC	[4]GC	P100	Ident	Origin	HC	GC	100SW
Pérola	C	lll	C	23.93	F33	L	l	R	36.38
F2	L	ll	M	21.68	F34	L	l	J	46.02
F3	L	ll	M	18.48	F35	L	ll	P	15.74
F5	L	ll	M	17.13	F36	L	ll	O	38.99
F6	L	ll	P	17.03	F37	L	ll	P	18.2
F7	L	lll	R	19.77	F38	L	ll	P	15.07
F8	L	lll	J	41.37	E 01	E	ll	P	16.68
F9	L	l	O	36.37	E 02	E	lll	C	22.87
F10	L	lll	P	17.81	E 03	E	ll	P	19.15
F11	L	ll	P	18.5	E 04	E	ll	P	19.24
F13	L	ll	M	18.06	Iapar 31	C	ll	O	23.23
F14	L	ll	P	17.62	E 06	E	ll	P	22.27
F15	L	ll	R	14.24	E 07	E	ll	P	21.59
F16	L	ll	C	15.77	E 08	E	ll	P	20.13
F17	L	ll	O	17.76	E 09	E	ll	P	16.89
F18	L	ll	R	14.52	E 10	E	ll	P	26.39
F19	L	ll	M	18.21	E 11	E	ll	P	21.06
F20	L	ll	O	18.16	E 12	E	ll	C	22.36
F21	L	ll	M	18.84	E 13	E	ll	P	20.63
F23	L	ll	P	15.28	E 14	E	ll	P	20.21
F24	L	ll	P	17.42	E 15	E	ll	P	19.61
F25	L	ll	R	17.73	E 16	E	ll	P	21.39
F26	L	ll	P	21.97	E 17	E	ll	P	21.23
Iapar 81	C	ll	C	20.92	E 18	E	ll	P	20.78
F28	L	ll	M	16.97	E 19	E	lll	C	21.17
Carioca	C	lll	C	22.1	Iapar 44	C	ll	P	19.25
Serrano	C	ll	P	16.04	E 21	E	ll	P	28.09
F31	L	l	J	36.2	E 22	E	l	O	36.38
F32	L	l	P	30.96					

[1]Ident: identification of genotypes; [2] Source: L= local, E= EMBRAPA e C= commercial; HC: l= tipo l; ll= tipo ll e lll= tipo lll; [4]GC: C= carioca, M= mulatinho, P= preto, R= rosinha, O= others. Source: Cabral et al. (2011).

DNA extraction

Leaf samples from 57 genotypes were used for the extraction and purification of genomic DNA using the CTAB (cetyltrimethylammonium bromide) method of Doyle and Doyle (1990) with the modifications proposed by Abdelnoor et al. (1995).

The DNA concentration was estimated using a 0.8% agarose gel to compare the patterns produced by the samples and the molecular weight marker (phage lambda DNA) at concentrations of 25, 50 and 100 ng/μL. DNA samples were diluted to a final concentration of 10 ng/μL.

ISSR analysis

The DNA samples were amplified using ISSR markers. Initially were tested 43 ISSR primers developed by the University of Britsh Columbia (UBC), Vancouver, Canada, on DNA samples from five individuals. A total of 20 primers were selected for the common bean samples, because they presented better amplification profile, distinct and distinct bands. Amplification reactions were performed in a final volume of 25μLcontaining $MgCl_2$ (2.4 mM), Tris/KCl pH 8.3 (0.25 mM), dNTPs (0.25 mM of each nucleotide), 0.2 μM of primer, 1 U of Taq polymerase and 30 ng of DNA.

The amplifications were performed in a Techne thermal cycler (TC 412) under the following conditions: 94°C for 15 minutes, followed by 30 cycles, each cycle consisting of three steps: a) 94°C for 30 seconds, b) 52°C for 30 seconds and c) 72°C for one minute, and a final step of 72°C for seven minutes. The molecular data consisted of the polymorphic bands of the cultivars detected using a 2.5% agarose gel.

Statistical analyses

From the analysis of the gels, a matrix of binary values was obtained by considering the presence (1) and absence (0) of the amplified fragments.

The pairs of genotypes were compared using the genetic dissimilarity indexes based on the arithmetic complement of the Jaccard index, which was used to obtain a dissimilarity matrix. Based on this matrix, the number of clusters was determined by the unweighted pair-group method average (UPGMA), and the results were represented in the form of a dendrogram. The clustering consistency was verified by the cophenetic correlation coefficient (CCC) between the matrix of genetic dissimilarities and the matrix of cophenetic values. These analyses were performed with the help of the GENES software (Cruz, 2016).

The polymorphic information content (PIC) for each ISSR primer was estimated as proposed by Roldan-Ruiz et al. (2000) using the formula $PIC_i=2f_i(1-f_i)$, where f_i is the frequency of the amplified fragments (presence of band) and $1-fi$ is the frequency of the absent fragments (absence of band).

RESULTS AND DISCUSSION

Of the 20 ISSR primers selected to assess genetic diversity in the 57 bean genotypes, 15 showed good definition of the amplified fragments, with clear and well-defined bands. Of these 15 primers, 11 were polymorphic. A total of 51 fragments were produced, of which 39 were polymorphic (76.4% polymorphism). The number of polymorphic bands per primer varied from two (UBC 880 and 890) to six (UBC 843), with a mean of 3.5 polymorphic bands/primer (Table 2).

Table 2. ISSR primers, sequences of each primer, annealing temperature (^{o}Tm), number of polymorphic bands (NB), polymorphic information content (PIC) analyzed in common bean cultivars.

Primer	Sequence (5'-3')*	^{o}Tm	NB	PIC
UBC 841	GAGAGAGAGAGAGAGAYC	52	3	0.39
UBC 843	CTCTCTCTCTCTCTCTRA	52	3	0.48
UBC 854	TCTCTCTCTCTCTCTCRG	52	4	0.39
UBC 855	ACACACACACACACACYT	52	4	0.31
UBC 857	ACACACACACACACACYG	52	3	0.38
UBC 859	TGTGTGTGTGTGTGTGRC	52	3	0.34
UBC 880	GGAGAGGAGAGGAGA	52	2	0.46
UBC 808	AGAGAGAGAGAGAGAGC	52	5	0.34
UBC 810	GAGAGAGAGAGAGAGAT	52	4	0.27
UBC 834	AGAGAGAGAGAGAGAGYT	52	6	0.41
UBC 890	VHVGTGTGTGTGTGTGT	52	2	0.19
Mean			**3.5**	**0.36**

* A= Adenine; T= Thymine; C= Cytosine; G= Guanine; H = (A, T or C); R = (A or G); V = (A, C ou G) e Y = (C or T). UBC: primers ISSR, developed by the University of British Columbia, Vancouver, Canada.

Cabral et al. (2011) studying the same genotypes found 81% polymorphism. The authors used markers that are codominant in nature and are multi-allelic, which explains the higher percentage of polymorphism in relation to the same genotypes. However, the 76.4% found with dominant markers revealed the efficiency of these markers for detecting polymorphisms in the common bean. The results are similar to those found by other authors, although there is variation in the polymorphism percentage due to the discard of weak-intensity bands that were not analyzed. Dias et al. (2015), when analyzing the genetic variability of cowpeas, used nine ISSR primers and obtained 47 polymorphic bands and 75.81% polymorphism, values very close to those found in this study. In contrast, Asfaw et al. (2018) obtained higher polymorphism values. When analyzing the genetic diversity of beans with 11 primers, they obtained 107 polymorphic bands and 90% polymorphism.

The polymorphic information content ranged from 0.19 (UBC 890) to 0.48 (UBC843), with a mean of 0.36. The PIC reveals the quality of the marker and its ability to detect variability in genetic studies (Botstein et al., 1980). Dominant markers, such as ISSR, tend to have lower PIC values due to their biallelic nature (Roldan-Ruiz et al., 2000). The maximum expected value for dominant markers is 0.5, and the most informative primers will present PICs ranging from 0.4 to 0.5 (Tatikonda et al., 2009). Thus, the primers UBC 834, 843 and 880 were the most informative in this study.

Genetic distances among the 57 genotypes presented a fairly uneven distribution, ranging from 0.00 to 1.0 and with a mean of 0.44, indicating wide genetic variability. The

range of 0.2 to 0.69 contained more than 86% of the total dissimilarity observed, and a higher frequency of dissimilarity (23.37%) was observed in the range of 0.5 to 0.59 (Figure 1). Alzate-Marin et al. (2003) reported a concentration of 17 of the 21 common bean elite cultivars studied between the distances of 0.03 to 0.33 dissimilarity. The detection of genetic variability in a species is the basis for breeding and selection of cultivars in breeding programs (Loarce et al., 1996). Knowledge of the diversity among genotypes enables identifying those that are different and complementary to be used as parents, increasing the probability of selection of superior characteristics in segregating generations (Cruz and Regazzi, 2001).

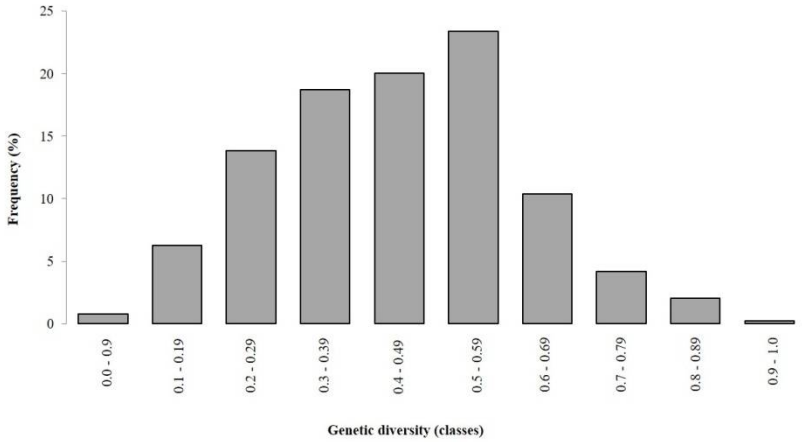

Figure 1. Frequency distribution of genetic dissimilarity obtained by ISSR markers among the 57 common bean genotypes in the 10 classes.

The lowest genetic dissimilarity (0.0) was observed between the genotype pairs E22-F07, F16-E19 and F28-F38; this low dissimilarity may indicate genotypes with a common origin, or they may be duplicates. The largest genetic distance (1.0) was found between F16 and the other three cultivars, Pérola, F08 and F13. The Pérola cultivar obtained the highest mean dissimilarity (0.76), demonstrating that this cultivar has a high divergence relative to the other genotypes studied and was the most divergent among the commercial genotypes. The wide variation in dissimilarity found in this study suggests the existence of very divergent genotypes in the southern region of Espírito Santo, and those with greater genetic distances have the potential for future conservation in germplasm banks.

In the cluster analysis (Figure 2), 11 groups were formed based on the cut-off point of approximately 50%. These results differ from Cabral et al. (2011) who found only 4 groups. The difference can be attributed to the nature of the markers and the number of primers used. The distribution of genotypes in different groups shows heterogeneity among individuals. The cophenetic correlation coefficient was 0.81, revealing a good fit between the cophenetic and original distance matrices. CCC values above 0.8 indicate good representativeness between distances (Bussad et al., 1990).

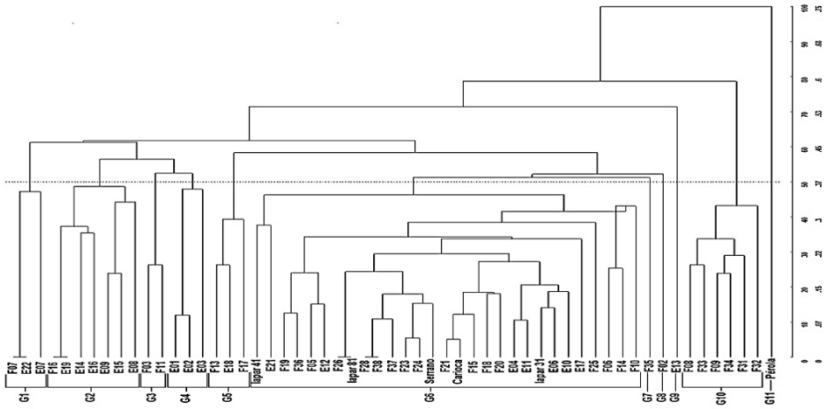

Figure 2. Dendrogram representative of genetic dissimilarity among the 57 genotypes of common beans obtained by the UPGMA method, forming 11 groups (G1 to G11). Cut-off point of approximately 50% (dotted line).

Local genotypes from the Fortaleza community were widely distributed in different groups, showing wide genetic variability in relation to the other genotypes. This reveals the great genetic diversity maintained by small farmers in the southern region of Espírito Santo. Similar results were found by Souza et al. (2009), who worked with some of these genotypes and found significant variability. Ogliari et al. (2007) emphasized the importance of maintaining local genotypes by small farmers as sources of desirable traits, containing alleles for adaptation, resistance and tolerance to the diverse local edaphoclimatic conditions.

Additionally, the cluster analysis revealed the distribution of the genotypes in relation to their region of origin. Morphological, biochemical (Gepts et al., 1986, Chacón et al., 2005; Pereira et al., 2009) and molecular (Chacón et al., 2000; Carvalho et al., 2008; Buso et al., 2008) differences have already evidenced the two different regions of origin of the bean crop:Andean (large and heavy seeds, with predominance of T-typephaseolin) and Mesoamerican (small, light seeds, with predominance of S-type phaseolin) (Singh et al. 1991).

In group 1 (F07, E22 and E07), a genotype of Andean origin (E22) was found, clustering with two genotypes of Mesoamerican origin (F07 and E07). Genotype F36 did not cluster with the other Andean genotypes. One possibility of this separation may be the fact that these genotypes (E22 and F36) present a low percentage of Andean genes despite the weight and size of their seeds (Alzate-Marin et al., 2003). Similar results were found by Cabral et al (2011), who evaluated the diversity of the same bean plants using SSR markers, observing that the genotypes F36 and F31, considered to be of Andean origin, were not clustered with the Andean genotypes. In contrast, the G10 group (Figure 2) was only formed by the genotypes F08, F33, F09, F34, F31 and F32,which are considered to originate from the same region (Andean), demonstrating a tendency of ISSR markers to group individuals by region of origin.

No clustering tendency was observed among genotypes with the same seed coat color. This disagrees with the results of González et al. (2005), who worked with 329 bean genotypes from four varieties (Bege, Negro Brilhante, Negro Opaco and Roxo) and reported

that ISSR markers were able to separate the four groups. In contrast, a tendency to cluster genotypes according to growth habit (GH) was observed (Table 1). Among the genotypes of type 1 GH, characterized by determinate, bush and erect growth(F9, F31, F32, F33, F34 and E22), only E22 was not clustered in the G10 group.

Most commercial cultivars (Iapar 41, Iapar 81, Serrano, Carioca, Iapar 31) were distributed in a single group (G6), a result that may be related to the narrow genetic base in which they were generated. According to Singh (2001), the narrow genetic base found among commercial cultivars is a result of strict commercial requirements, conservative breeding strategies and restricted use of exotic germplasm. The cultivar Pérola, in turn, formed an isolated group (G11), indicating its greater divergence relative to the other commercial genotypes.

Genetic dissimilarity among commercial genotypes ranged from 0.18 to 0.76, with the lowest dissimilarity being observed between Iapar 31 and Serrano (Table 3). Among the cultivars Iapar 81 x Carioca, Carioca x Serrano and Iapar 31 x Serrano, a very marked similarity was observed. A high degree of similarity between the commercial bean genotypes was also found by Carvalho et al. (2008) and Emygdio et al. (2003). The Pérola cultivar was the most divergent relative to the other commercial cultivars, with a dissimilarity of 0.73. These data indicate that the Pérola cultivar has genetic characteristics that can contribute to heterosis in breeding programs.

Table 3. Matrix of dissimilarity among the commercial common bean cultivars, obtained by the Jaccard similarity index using ISSR markers.

	Pérola	**Iapar 81**	**Carioca**	**Serrano**	**Iapar 31**
Iapar 81	0.72				
Carioca	0.72	0.19			
Serrano	0.76	0.29	0.19		
Iapar 31	0.72	0.24	0.21	0.18	
Iapar 44	0.74	0.29	0.31	0.54	0.35
Mean	**0.73**	**0.35**	**0.32**	**0.39**	**0.34**

Progress in the breeding of common bean cultivars has been a slow process worldwide, probably due to the limited variability used in the original crosses. The parents used were selected from the same set of genes, i.e., the Mesoamerican genetic base (Alzate-Marin et al., 2003). This selection of genotypes from the Mesoamerican center of origin was a result of consumers' demands for size, shape and color of the seed (CONAFE 2005), which explains the proximity in the clustering of commercial cultivars. Thus, introgression of genotypes of Andean origin into bean breeding programs is important for increasing genetic diversity and reducing vulnerability among improved cultivars (Pereira et al., 2009).

Previous analyzes with molecular markers SSR were performed by Cabral et al. (2011) using the same common bean cultivars. The SSR markers have a codominant character and are multi-allelic, and can reveal a wide diversity, since, besides providing the presence or absence of a certain allele variable, it reveals the different allelic forms of a given locus (Laborda et al., 2005). Codominant markers are developed specifically for the species of interest, which raises the cost of their use, since the success of transferability between genotypes is still limited (Souza, 2015).ISSR markers despite the dominant and biallelic character are also able to discriminate the divergence between genotypes as a presented in this study. These markers are highly reproducible, do not require prior knowledge of the target genome, are low cost and have high transferability and accessibility (Ng and Tan, 2015). Thus, the current study reveals the importance of ISSR markers as a low cost tool, high information and great value for analysis of genetic diversity in bean accesses.

CONCLUSIONS

The bean genotypes of the Fortaleza community have a wide genetic diversity compared to commercial genotypes and those from EMBRAPA, whose diversity is narrower. The most divergent cultivars may be recommended for storage in germplasm banks, which can provide support for bean breeding programs.

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