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Biology-Genetics

Development and validation of new IRAP markers for common bean

Desenvolvimento e validação de novos marcadores moleculares IRAP para feijão comum

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ABSTRACT

Molecular markers are powerful tools for detecting genetic variation at the DNA level, offering a direct, reliable, and efficient approach for the characterization, conservation, management, and utilization of plant germplasm. Among these, Inter-Retrotransposon Amplified Polymorphism (IRAP) markers target the genomic regions between retrotransposon insertions, revealing polymorphisms based on insertion patterns. This study aimed to develop and validate new IRAP markers for the genetic characterization of common bean (*Phaseolus vulgaris*) genotypes. Twelve IRAP primers were designed based on LTR retrotransposon sequences identified in the *P. vulgaris* genome database and tested in a panel of 22 genotypes, including 11 landraces, 10 commercial cultivars, and one F_2 individual derived from a cross between cultivars IPR Andorinha and BRS Estilo. Genomic DNA was extracted from young leaves using a modified CTAB protocol, and PCR amplifications were performed using the newly designed primers. The average polymorphism rate among the markers was 90.67%, and the mean genetic similarity among the genotypes was 0.557. The polymorphic information content (PIC) values ranged from 0.086 (Pv_IRAP_8) to 0.500 (Pv_IRAP_10), with the most informative primers being Pv_IRAP_4, Pv_IRAP_6, Pv_IRAP_7, Pv_IRAP_9, and Pv_IRAP_10. High genetic similarity was observed between some landraces and commercial cultivars, suggesting redundancy within the germplasm collection and highlighting the importance of molecular tools in optimizing genetic resource management in common bean breeding programs.

Keywords: *Phaseolus vulgaris* L; Genetic variability; PIC

RESUMO

Marcadores moleculares são ferramentas poderosas para detectar variações genéticas no nível do DNA, oferecendo uma abordagem direta, confiável e eficiente para a caracterização, conservação, manejo e uso de recursos genéticos vegetais. Dentre esses marcadores, os de Amplificação Inter-Retrotransposon (IRAP) detectam polimorfismos em regiões genômicas localizadas entre inserções de retrotransposons, revelando padrões de inserção específicos. Este estudo teve como objetivo desenvolver e validar novos marcadores IRAP para a caracterização genética de genótipos de feijão-comum (*Phaseolus vulgaris*). Foram desenhados doze primers IRAP com base em sequências de retrotransposons LTR identificadas no banco de dados genômico de *P. vulgaris* e testados em um painel composto por 22 genótipos, incluindo 11 variedades crioulas, 10 cultivares comerciais e um indivíduo F_2 derivado do cruzamento entre as cultivares IPR Andorinha e BRS Estilo. O DNA genômico foi extraído de folhas jovens utilizando um protocolo CTAB modificado, e as amplificações por PCR foram realizadas com os primers desenvolvidos. A taxa média de polimorfismo observada entre os marcadores foi de 90,67%, e a similaridade genética média entre os genótipos foi de 0,557. Os valores de conteúdo de informação polimórfica (PIC) variaram de 0,086 (Pv_IRAP_8) a 0,500 (Pv_IRAP_10), sendo os primers mais informativos Pv_IRAP_4, Pv_IRAP_6, Pv_IRAP_7, Pv_IRAP_9 e Pv_IRAP_10. Observou-se alta similaridade genética entre algumas variedades crioulas e cultivares comerciais, indicando redundância na coleção de germoplasma e destacando a importância do uso de ferramentas moleculares para otimizar o manejo dos recursos genéticos em programas de melhoramento do feijão-comum.

Palavras-chave: *Phaseolus vulgaris* L; Variabilidade genética; PIC

1 INTRODUCTION

Genetic variability of common bean (*Phaseolus vulgaris* L.) present mainly in landraces varieties is essential as a source of genes and alleles for plant breeding. Unlike high-yielding varieties, the landraces varieties maintained by farmers are endowed with high genetic variability because they are not subjected to subtle selection over a long period (Kanwar et al., 2020). Landraces varieties can be used in breeding programs to obtain cultivars that are more productive and resistant or tolerant to biotic and abiotic stresses, and they are able to exploit the existing variability already adapted to the climatic conditions of specific regions (Gonçalves et al., 2016).

Genetic variation and relationships between common bean varieties are evaluated by biotechnological techniques such as molecular (or DNA) markers. These markers may reveal differences between genotypes at the DNA level, providing a more direct, reliable, and efficient tool for germplasm characterization,

conservation, and management (Luo et al., 2019). Among available DNA-based markers, retrotransposon markers are useful for gene mapping, phylogenetic studies, and genetic variability (Holiloglu et al., 2022; Naeem et al., 2021).

Retrotransposons produce considerable insertion activity, and they have conserved sites that serve to design primers to generate DNA footprints (Kalendar et al., 2011). Retrotransposons contribute to the structure, variation, and diversity of the genome. In addition, they can affect gene function (Kuhn et al., 2016). These characteristics make them an excellent tool for many studies of genetic variability, and the most frequently used retrotransposon-based molecular marker method is the inter-retrotransposon amplified polymorphism (IRAP) (Kalendar and Schulman 2021).

IRAP markers generate polymorphic products from retrotransposons using a single polymerase chain reaction (PCR) facing outward primer (Kalendar, Shustov & Schulman, 2021). IRAP markers have high reproducibility, abundant polymorphism, easy viewing in a single gel run, and wide genome coverage (Silva et al., 2012). Moreover, this method serves to establish pedigrees of lines and understand the evolutionary history and phylogeny of species (Alsayied et al., 2016). IRAP markers have been successfully used to molecularly characterize species like as *Vigna unguiculata* L. (Walp) (Otwe et al., 2017), *Olea europaea* L. (Kaya and Yilmaz-Gokdogan, 2016), *Vicia faba* L. (Tomás et al., 2016), and *Hordeum vulgare* (Singh et al., 2017).

Thus, the selection of appropriate primers for the genetic analysis of common bean varieties is an essential tool for obtaining reliable data. In addition, the genetic variability of landraces varieties could be an important source of genetic resistance for plant breeders, including alleles for local adaptation, disease resistance, and tolerance to climate adversities.

Therefore, the objective of this study was the assessment of the genetic variability of common bean varieties using IRAP markers. In this study, 12 IRAP primers were designed and used for similarity analysis that provided information

about the genetic variability in a group of 22 genotypes of *P. vulgaris,* including landraces and commercial varieties.

2 MATERIAL AND METHODS

Young and healthy leaves of 22 common bean genotypes were used. This group comprised 11 landraces and 10 commercial cultivars, in addition to an F2 variety resulting from the cross between the cultivars IPR Andorinha and BRS Estilo (Table 1).

Table 1 – Description of seed morphological traits and commercial group of commercial and local common bean (*Phaseolus vulgaris*) genotypes used for genotyping with IRAP markers

(Continue)

Genotype	Group	Seeds		Additional Information
		Tegument	Size	
ANfc 9	Commercial	Carioca	Small	
ANfp 110	Commercial	Black	Small	
IPR Andorinha	Commercial	Carioca	Small	IPR Andorinha cultivar probably originated from the natural cross between the SEL 37-20 line, sib of the normal cycle cultivar IPR 139, and the early cycle IPR Colibri cultivar.
BRS Estilo	Commercial	Carioca	Small	Cultivar BRS Estilo originated from the crossing EMP 250/4/ A 769 /// A 429 / XAN 252 // V 8025 / PINTO VI 114, carried out in 1991 at the International Center for Tropical Agriculture (CIAT), located in Cali, Colombia.
F2	Crossing	Carioca	Small	Second generation of the crossing between IPR Andorinha and BRS Estilo cultivars.
Land1	Landrace	Beige with purple stripes	Large	
Land2	Landrace	Yellow	Small	
Land3	Landrace	Red	Medium	
Land 4		Red with black spots	Medium	

Table 1 – Description of seed morphological traits and commercial group of commercial and local common bean (*Phaseolus vulgaris*) genotypes used for genotyping with IRAP markers (Conclusion)

Genotype	Group	Seeds		Additional Information
		Tegument	Size	
Land5	Landrace	Gray/purple with black stripes	Medium	
Land6	Landrace	Beige	Small	Seed originated from the Land7 genotype, which has a black tegument.
Land7	Landrace	Black	Small	
Land8	Landrace	Gray with black stripes	Medium	Seed originated from the Land7 genotype, which has a black tegument.
Land9	Landrace	Bicolor - black and white	Small	
Land10	Landrace	Beige with black stripes	Large	
Land11	Landrace	Brown with beige stripes	Small	
IAC Imperador	Commercial	Carioca	Small	Originated from multiple crossings between IAC Carioca Eté and Carioca early cultivars, backcrossing the F1 with IAC Carioca Eté.
Pérola	Commercial	Carioca	Small	Pérola cultivar (line LR 720982 CPL53) comes from the selection of pure lines within the Aporé cultivar.
Rajado Comercial	Commercial grain	Beige brown brindle	Large	Grain for food. It was acquired to be similar in size to the Land1 and Land10 genotypes.
Bolinha	Commercial grain	Yellow	Small	Seeds morphologically identical to the Land2 genotype.
IPR Siriri	Commercial	Carioca	Small	A commercial cultivar developed by IAPAR in 1995 from the “carioca” group. This cultivar is a result of the crossing between IAPAR 31 and IAC Akitã.
IPR Tangará	Commercial	Carioca	Small	Originated from the improved LP95-92 line, developed by IAPAR, descendant from IAPAR 31 and Pérola cultivars.

Source: the authors, 2022

The genomic DNA of each genotype was isolated according to the protocol of Doyle and Doyle (1987), with modifications proposed by Lodhi et al. (1994) and Lefort and Douglas (1999). To the tube containing ground leaves, 600 µL of extraction buffer (20 mM EDTA, 50 mM Tris–HCl pH 8.0, 1.1 M NaCl, 0.4 M LiCl, 1 % CTAB, 2% PVP40, 0.5% Tween 20, 0.2 % β-mercaptoethanol) was added and thoroughly mixed. The resulting emulsion was incubated in a water bath at 60 °C for 25 min, and the DNA was isolated by precipitation and successive washes with alcohol.

For the design of the IRAP primers, the LTR (Long Terminal Repeat)-type retrotransposon sequences were located in the Phytozome database (Goodstein et al., 2012), which contains the *P. vulgaris* DNA sequences (Schmutz et al., 2014). The sequences containing mobile elements and repetitive DNA located in the intergenic regions were found. The default primer-designing parameters used in the RJPrimer program (You et al., 2010) were 20 base pairs, CG content ranging from 40 to 55%, and an annealing temperature of 60 °C. Twelve primers were designed, and all were used for the molecular analysis (Table 2).

PCR amplification of target DNA for each genotype was conducted in a final volume of 12.5 µL, containing: 75 ng of DNA, 12.5 mM Tris-HCL (pH 8.3), 62.5 mM KCl, 2.5 mM MgCl 2.75 µM of each of the dNTP, 0.4 µM of each primer pair and 1 U Taq DNA polymerase. For amplification, the thermal cycler was programmed for initial denaturation at 94 ºC for 5 min, then 30 cycles of 94 ºC for 1 min, the annealing temperature of the primers for 1 min, and 72 ºC for 1 min. At the end of the PCR program, a final step at 72 °C for 10 min was included for the final extension of the fragments.

PCR amplification products were resolved by 2% agarose gel electrophoresis at a constant voltage of 80 V for four hours. The result of the electrophoresis was visualized in UV light and photo documented. To determine the size of the amplified fragments, the molecular weight marker DNA Ladder 100 bp was used.

Amplification products were rated visually for the presence (1) or absence (0) of the fragments being analyzed. The percentage of polymorphism of each IRAP

primer was calculated by dividing the number of polymorphic fragments by the total number of amplified fragments.

For each IRAP primer, the PIC (Polymorphic information content) calculation was performed to quantify the genetic polymorphism of the loci under analysis. The PIC was calculated as PICi = 2fi(1-fi) where fi is the frequency of fragments present in the marker at the same locus and 1-fi is the frequency of fragments missing (Roldán-Ruiz et al., 2000).

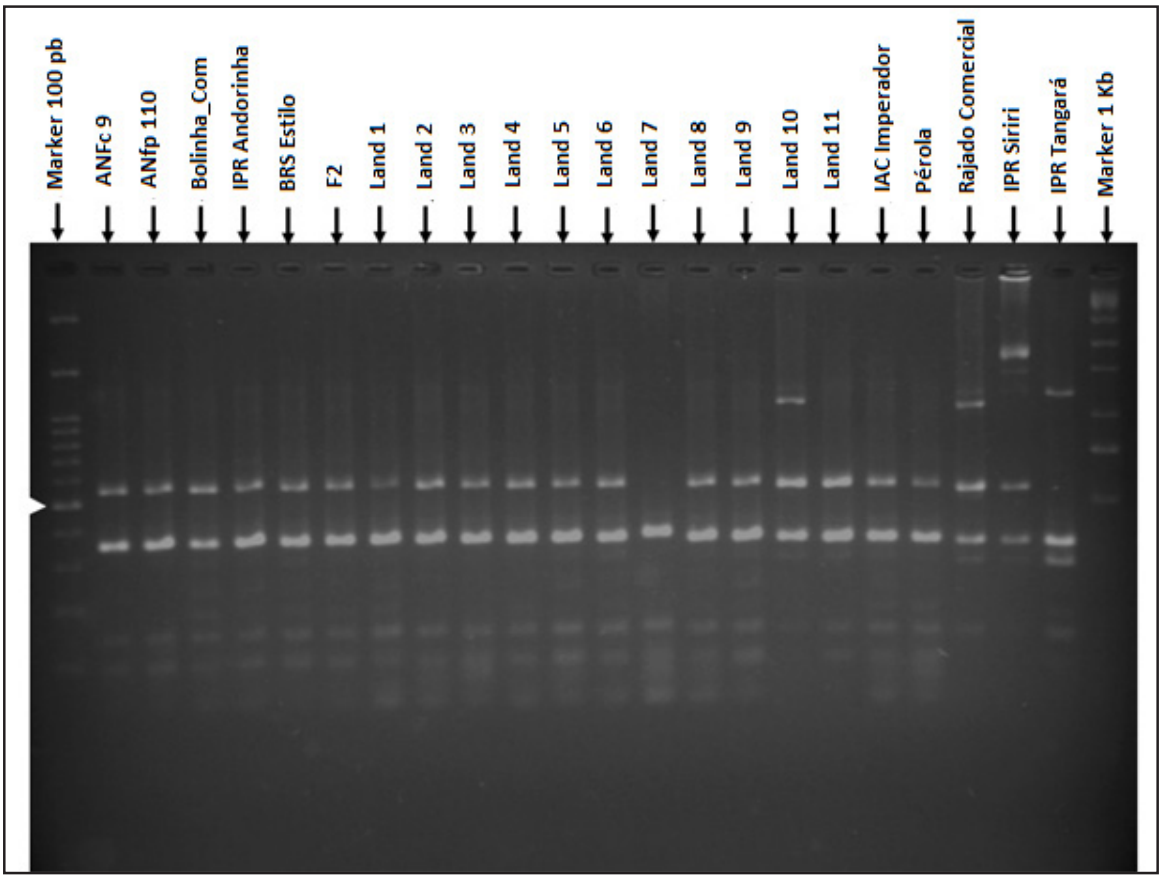
The similarity matrix among the genotypes was established using the Dice coefficient (Dice 1945). Based on this matrix, the dendrogram was generated from UPGMA (Unweighted Pair Group Method with Arithmetic Means) cluster analysis using the NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System for personal computers, Version 2.1, Applied Biostatistics, Inc.) software (Rohlf, 2000). To verify the fit of the matrix and the respective dendrogram, the cophenetic correlation coefficient (r) (Sokal and Rohlf 1962) was estimated. The Mantel test (Mantel, 1967) with 1000 permutations was performed to estimate the degree of correlation of the similarity matrix obtained using the NTSYS-PC (Rohlf, 2000).

3 RESULTS AND DISCUSSION

All 12 IRAP primers showed good amplification profiles for similarity analysis of the 22 *P. vulgaris* genotypes. PCR products amplified using the IRAP-targeting primer Pv_IRAP_2 are shown in Figure 1.

The total number of fragments amplified by the 12 markers was 458, with a mean of 38.16 fragments per primer. Of the 458 fragments, 371 were polymorphic. The mean percentage of polymorphism among the primers was 90.67%. The number of fragments amplified and the percentage of polymorphism of each primer are shown in Table 2. The largest number of amplified fragments was eight, obtained with the primer Pv_IRAP_4, and the smallest was one with Pv_IRAP_1 and Pv_IRAP_9. IRAP primers generally amplified fragments with individual sizes from 20 to 1600 bp (Table 2).

Figure 1 – Amplification pattern of primer IRAP Pv_IRAP_2 for the 22 common bean genotypes. The left arrow indicates a fragment of 500 bp—molecular weight marker (100 bp DNA Ladder)



Source: the authors, 2022

Although the primers were designed for sequences of each of the bean chromosomes, it was observed that most IRAP markers could amplify different regions of the genome. This feature was expected since the IRAP markers are based on retrotransposons and can be replicated and inserted into any part of the genome.

Retrotransposons are dispersed in the genome, but they can be found in clusters, which makes it possible to use the IRAP technique and detects insertion polymorphisms by amplifying DNA fragments between two retrotransposons (Kalendar et al., 2020).

Table 2 – DNA sequences of the 12 primers used with their respective sequences and variables in *Phaseolus vulgaris*. ROC - Region of origin of the DNA fragment on the chromosome; AT (ºC) - annealing temperature; RF (pb) – Range of fragments; % P - Percentage of polymorphism; PIC - Polymorphic information content

Primer	ROC	Sequence	AT (o C)	RF (pb)	% P	PIC value
Pv_IRAP_1_F	Chr09:8260000..8282999	TATCGAACGTTACAAGGCCC	59.96	500	100	0.351
Pv_IRAP_1_R	Chr09:8260000..8282999	CCATTGCCTAGAAGCCTGTT	59.34			
Pv_IRAP_2_F	Chr05:10103000..10187999	CCAAGCCTTCAACATCCTTC	59.67	100-1600	85.71	0.184
Pv_IRAP_2_R	Chr05:10103000..10187999	TGGGTTTTCAGGAACAAGCT	59.71			
Pv_IRAP_3_F	Chr09:8260000..8282999	TATCGAACGTTACAAGGCCC	59.96	150-320	100	0.232
Pv_IRAP_3_R	Chr09:8260000..8282999	CCATTGCCTAGAAGCCTGTT	59.34			
Pv_IRAP_4_F	Chr08:46878000..47009999	GCCAGAAGGATGCAAAAGAG	59.96	250-800	100	0.393
Pv_IRAP_4_R	Chr08:46878000..47009999	TGGAACAAGAGATGCTGAACA	59.43			
Pv_IRAP_5_F	Chr10:37197000..37229999	CCGTGAAGAAAGGCATTATTG	59.59	120-800	100	0.240
Pv_IRAP_5_R	Chr10:37197000..37229999	GGTGGTGGCAAGTGCTCTAT	60.14			
Pv_IRAP_6_F	Chr11:12718000..12787999	GACAATTGGGTGAAAATGGG	60.03	20-500	66.66	0.425
Pv_IRAP_6_R	Chr11:12718000..12787999	TGTCAATCTCAACTTGGCTCTT	58.98			
Pv_IRAP_7_F	Chr06:8169000..8308999	CTTGGAGCTTGCTTCAGTCC	60.13	200-750	100	0.427
Pv_IRAP7_R	Chr06:8169000..8308999	GTTGCAAGGTTGGAGGTTGT	60.01			
Pv_IRAP8_F	Chr04:10902000..10947999	CGCCTTTCTGAAACACCATT	60.11	300	100	0.086
Pv_IRAP8_R	Chr04:10902000..10947999	CCTCACATCATAATGTTGGCACT	61.16			
Pv_IRAP9_F	Chr05:10103000..10187999	TGGGTTTTCAGGAACAAGCT	59.71	300-350	100	0.473
Pv_IRAP9_R	Chr05:10103000..10187999	CTTGGAGCTTGCTTCAGTCC	60.13			
Pv_IRAP10_F	Chr05:10103000..10187999	TGGATGTCAAAAGTGCCTTCT	59.73	150-500	50	0.500
Pv_IRAP10_R	Chr05:10103000..10187999	TAACAGCTTGATGGCATTGG	59.69			
Pv_IRAP11_F	Chr05:10103000..10187999	TTGTGGCATAAACGTTTGAGTC	60.04	50-300	85.71	0.346
Pv_IRAP11_R	Chr05:10103000..10187999	CCAAGCCTTCAACATCCTTC	59.67			
Pv_IRAP12_F	Chr06:14516000..14621999	ACACCCCAACAAAATGGTGT	59.99	100-300	100	0.338
Pv_IRAP12_R	Chr06:14516000..14621999	TTGCTGCTATCCAGTTGCTG	60.16			

Source: the authors, 2022

Fatmawati et al. (2021) analyzed the genetic variability among one hundred interspecific F2 hybrids generated from the cross between mung bean and common bean, where IRAP markers successfully discriminated 100% of the hybrids. The IRAP marker showed high heterozygosity and moderate polymorphism. Carvalho et al. (2010) analyzed the genetic variability among 48 wheat cultivars with IRAP and REMAP markers, and they observed that the mean polymorphism using IRAP markers was

91.15%. Alikhani et al. (2014) studied the genetic variability of *Quercus brantii* using 10 IRAP primers, and they verified that 97.65% of the fragments found were polymorphic. These studies corroborate the results found in our study. When working with IRAP markers, the percentage of polymorphism can be used as an indicator of genetic variability among genotypes of the same species.

In this study, PIC values ranged from 0.086 (Pv_IRAP_8) to 0.500 (Pv_IRAP_10) (Table 2). The primers that obtained the best values were Pv_IRAP_4, Pv_IRAP_6, Pv_IRAP_7, Pv_IRAP_9, and Pv_IRAP_10, with 0.393, 0.425, 0.427, 0.473, and 0.500, respectively. PIC measures the ability of a marker to detect polymorphisms and therefore has enormous importance in selecting markers for genetic studies; furthermore, the PIC value of each marker represents the probability of the absence and presence of this marker in two random genotypes between the population and ranges from zero to 0.5 monomorphic markers for markers present in 50% of the genotypes (Serrote et al.,2020; Roldán-Ruiz et al., 2000).

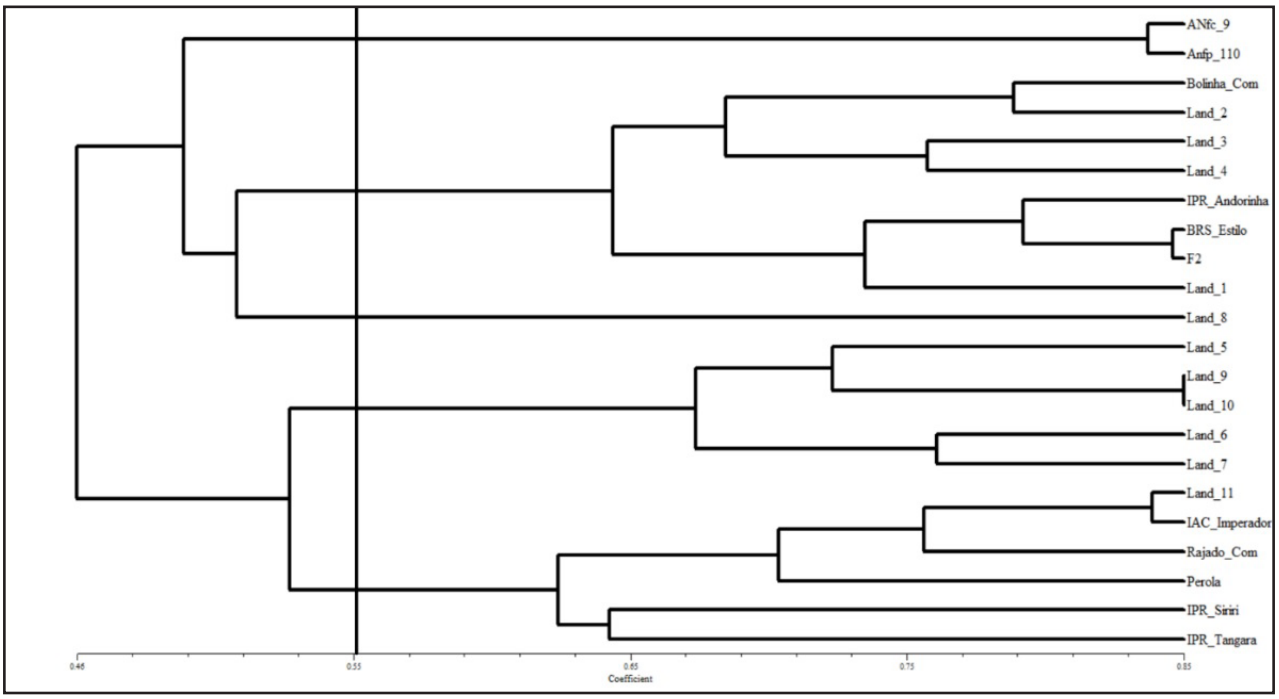
PIC is an important primer feature that indicates its potential to differentiate several genotypes (Zargar et al., 2016). Yuan et al. (2012) analyzed the genetic differences among 43 genotypes of *Diospyros kaki* from nine IRAP primers and observed that the mean value of PIC was 0.26. Alikhani et al. (2014) found that PIC values ranged from 0.26 to 0.42, with an average of 0.32, in *Quercus brantii* populations.

The study of genetic variability in common bean genotypes using molecular markers and PIC values to select primers or combinations of these was verified by Langarica et al. (2014). The researchers studied the molecular characterization of common bean cultivars in the Mexican semiarid plateau using combinations of AFLP markers, and they found the total value of PIC (0.32). They conclude that combining E-ACA + M-AGA primers is recommended to analyze the genetic variability of *P. vulgaris* landraces varieties.

However, IRAP primers amplify different fragment sizes that can be used as markers to detect polymorphism of genotypes and to measure the variability or reconstruct phylogeny (Boronnikova and Kalendar 2010).

Regarding genetic variability, data from all 371 polymorphic fragments generated by the 12 primers were used to generate the genetic similarity matrix and to calculate the genetic distance between the genotypes. The cophenetic correlation coefficient was 0.69447, and the mean similarity was 0.557. In the generated dendrogram (Figure 2), it was possible to observe five groups. In the first, the genotypes ANfc 9 and ANfp 110 were grouped with a similarity of 0.837.

Figure 2 – Dendrogram of the 22 common bean genotypes obtained using 12 IRAP primers



Source: the authors, 2022

The cultivars BRS Estilo and IPR Andorinha were crossed, originating F2, which in the second group was more similar to BRS Estilo with 0.846 of similarity. The third and fourth groups were composed only of local genotypes, with the most similar ones, Land9 and Land10, being in the fourth group. The latter group comprises most of the commercial bean genotypes used in the experiment. The cultivars IPR Siriri and IPR Tangará were grouped, as both have as their parent the cultivar IPR 31. The genotypes Land11 and IAC Imperador presented 0.838 of similarity, considered the most similar of this group (Figure 2).

Concerning the similarity matrix, the lowest and the highest genetic similarity were 0.193 and 0.850, respectively. Nasri et al. (2013) studied the genetic variability among 101 wheat cultivars and improved lines through IRAP markers and observed that the lowest similarity was 0.68 and the highest 0.98, indicating low genetic variability. Comparing the study by Nasri et al. (2013) with the present study, we can observe high variability among the bean genotypes studied. The Land8 genotype was dissimilar, this genotype is a descendant of the Land7, but it originated seeds with gray tegument and black stripes quite distinct from their maternal line, which has a black tegument. Chediak et al. (2007) found a similar case, where the markers detected genetic variation and visual by the phenotypic pattern of the seed, indicating the occurrence of natural crossing in the process of obtaining genetic seed. This corroborates the hypothesis that there was a natural crossing at the time of production of seeds of the Land7 genotype originating seeds with distinct patterns of their maternal progenitor.

Accurate molecular characterization demands markers that produce reproducible data and polymorphism appropriate for the identification and differentiation of taxa. In our study, the IRAP markers revealed high genetic similarity among the common bean genotypes examined, indicating that the commercial and landrace common bean varieties are very similar. In addition, the developed IRAP markers can be used both for selecting parents in a breeding program and for the characterization of conserved germplasm collections.

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