

**GENETIC DISSIMILARITY IN JABUTICABA (*Plinia peruviana* (Poir.)
govaerts) IN MUNICIPALITY SÃO MATEUS, NORTH OF STATE
ESPÍRITO SANTO, BRAZIL**

Vinicius de Souza Oliveira^{1*}

José Dias de Souza Neto²

Moises Zucoloto³

Taís Cristina Bastos Soares⁴

¹Universidade Federal do Espírito Santo, Centro de Ciências Agrárias e Engenharias, Alegre, Espírito Santo, Brasil.: souzaoliveiravini@gmail.com

²Instituto Federal do Espírito Santo, Campus de Alegre, Laboratório de Genética e Biologia Molecular, Espírito Santo, Brasil: jose.neto@ifes.edu.br

³Universidade Federal do Espírito Santo, Centro de Ciências Agrárias e Engenharias, Alegre, Espírito Santo, Brasil.: moises.zucoloto@ufes.br

⁴Universidade Federal do Espírito Santo, Centro de Ciências Exatas, Naturais e da Saúde, Alegre, Espírito Santo, Brasil: tais.soares@ufes.br

*Autor de correspondência

Abstract: Jabuticaba (*Plinia peruviana* (Poir.) Govaerts) is a plant rich in compounds that are very useful in industry. Its fruits are the basis for a range of food and pharmaceutical products. Due to the great potential of this species, information about genetic structure of these plants allows us to understand factors linked to quality, in addition to guiding crosses to improve this species for commercial purposes. Thus, we sought to understand the pattern of genetic diversity of jabuticaba found in the municipality São Mateus, north region of Espírito Santo State, Brazil, using ISSR markers. The results of the dissimilarity values, obtained by the Jaccard index, grouped the 19 accessions studied into five groups. The pair of accessions 10 and 19 showed the highest dissimilarity value, being indicated for further studies with specific markers, to infer the maximum heterozygosity, and possible potential for exploitation of heterosis. Accessions 16 and 18 had a lower dissimilarity value, being promising in introgression and gene association studies, due to the narrow genetic basis between the materials. Accessions 1, 4, 12, and 15 are the most divergent and can be indicated for random artificial crossings.

Keywords: Myrtaceae; molecular marker; Genetics; Atlantic Forest.

1 INTRODUCTION

The jabuticaba (*Plinia peruviana* (Poir.) Govaerts), belonging to the Myrtaceae family, is a native plant of the Atlantic Forest of the Central and Southeast regions of Brazil (DONADIO, 2000, STADNIK, 2020). Its fruits are well accepted both in the fresh market and in the food industries, in the production of jellies, alcoholic beverages, vinegar, and pharmaceuticals, due to their compounds with antioxidant properties (DANNER et al., 2008; CITADIN, et al., 2010). In addition, the plant can be used in landscape projects, as well as in civil construction and the wood industry, due to its quality for making furniture (LORENZI, 1992).

Despite the potential uses of jabuticaba, its cultivation in Brazil is still on a small scale, most often carried out by family farmers in domestic orchards on farms (CITADIN et al., 2010; DANNER et al., 2010). This fact may be associated with the scarce scientific knowledge of this culture and its genetic conservation in germplasm banks (MARTINS, 2013; SALLA, 2019). Thus, the characterization and conservation of accessions is a solution to these problems, as it can provide phenotypic and genetic information for each material (RAMALHO et al., 2000).

With information on the genetic dissimilarity of jabuticaba, it is possible to outline better conservation strategies for the species (JOLIVET; BERNASCONI, 2007; KUMAR et al., 2007), in addition to providing knowledge for better management of accessions in germplasm banks, directing the best artificial crosses and identifying the genotypes that present the most favorable agronomic characteristics (CRUZ et al., 2004a; THUL et al., 2009).

At the genetic level, among the techniques that seek to reveal diversity, the use of molecular markers Inter Simple Sequence Repeats (ISSR) stands out due to the high degree of polymorphism and the reduced costs of its application. This technique is based on the amplification, via

Polymerase Chain Reaction (PCR), of the regions between close microsatellite sequences in the DNA (SALIMATH et al., 1995). Thus, through the application of ISSR molecular markers, it is possible to generate a large amount of genetic information used both for studies of diversity, assisted selection, phylogeny, and genetic mapping (FALEIRO, 2011; VILELA et al., 2012), as well as in breeding programs of plants (SANTOS et al., 2020).

Thus, this study aimed to estimate the genetic diversity of 19 jabuticabas accessions from the municipality São Mateus, north region of Espírito Santo State, Brazil using ISSR molecular markers.

2 METHODS

Young leaves of 19 jabuticaba (*P. peruviana*) accessions were sampled in the municipality of São Mateus (18° 40' 36" S and 39° 51' 35" E), located in the municipality São Mateus, North of Espírito Santo State, Brazil. To verify the quality of the analyses, and confirm the robustness of the clusters and the bootstrap values of the bifurcations, a duplicate of accession 19 (19') was added.

DNA extraction followed the protocol described by Doyle and Doyle (1990), with modifications proposed by Abdelnoor et al. (1995). The integrity and quality of the DNA were measured by electrophoresis in 0.8% agarose gel, and also via the 260/280 and 260/230 ratios, obtained by NanoDrop 2000c®, with the concentration obtained by the latter.

The PCR conditions of the nine markers were followed according to Souza Neto et al. (2014). The products were separated by electrophoresis on a 10% polyacrylamide gel using 1X TAE buffer for three hours at 180 volts and stained with 2% silver nitrate. The images were registered with a BioRad photo documenter (Gel Doc XR+ system model).

Molecular data were analyzed using the GENES program (CRUZ, 2008) and the

dissimilarity matrix was obtained using the Jaccard index. The accessions were grouped in a dendrogram by the hierarchical Unweighted Pair Group Method using Arithmetic averages (UPGMA), from the dissimilarity matrix. The cut-off point was defined by the formula $P_c = m + ksd$ (MOJEMA, 1977), where m = the mean of the distance values of the melting levels corresponding to the stages; $k = 1.25$ (MILLIGAN; COOPER, 1985) and sd = standard deviation. To verify the efficiency of the clustering method, the cophenetic correlation coefficient (CCC) was estimated (SOKAL; ROHLF, 1962). The consistency

values of the dendrogram bifurcations were given by the bootstrap test (EFRON; TIBSHIRANI, 1993).

3 RESULTS AND DISCUSSION

The nine ISSR markers used in this study (UBC 810, 840, 855, 827, 835, 880, 812, 873, and 890) generated a total of 46 bands, with an average of 5.11 bands per marker (Table 1). The UBC 835 marker generated the greatest number of 8 bands. In contrast, the minimum number of 2 bands was found in the UBC 810 marker.

Table 1. Genetic information of nine ISSR markers used in 19 accessions of jabuticaba (*P. peruviana*) found in São Mateus, North of Espírito Santo State.

Identification (UBC)	Sequence	NPB	NMB	NGB	Polim(%)
810	(GA)8T	2	0	2	100
840	(GA)8 YT	6	0	6	100
855	(AC)8YT	6	0	6	100
827	AC)8G	4	0	4	100
835	(AG)8YC	8	0	8	100
880	(GGAGA)3	4	0	4	100
812	(GA)8 A	3	0	3	100
873	(GACA)4	6	0	6	100
890	VHV (GT)7	5	2	7	71,43
Total		44	2	46	-
Média		4,88	0,22	5,11	96,83

Sequence, number of polymorphic bands (NPB), number of monomorphic bands (NMB), number of generated bands (NGB) and percentage of polymorphism (polym(%)).

Source: Authors.

Among the 19 accessions analyzed, 96.83% of the bands were polymorphic. Only the UBC 890 marker showed monomorphic bands (Table 1). These results indicate that in the studied accessions there is a high degree of polymorphism that reflects the low genetic erosion of the species (TREUREN et al. 1991; MOURA et al., 2019). A high degree of polymorphism was also found by Cruz (2014), who estimated the genetic diversity of 35 jabuticaba accessions located in the Recôncavo region of Bahia, through 16 morphoagronomic traits and 18 ISSR molecular traits.

The analysis of the band patterns of these accesses allowed us to estimate dissimilarity values, obtained by the Jaccard index (Table 2). These values ranged from 0.07 to 0.96 between pairs, and from 0.59 to 0.78 for average accesses. The high dissimilarity value was observed between accessions 10 and 19 (0.96), suggesting that they are poorly related accessions with a broad genetic base, and that the crossing of these accessions makes it possible to generate descendants of maximum heterozygosity. However, it should be noted that further studies with specific markers, for certain characters, must be carried out to

infer the potential for exploiting heterosis for these accessions. In addition, to the possibility of building a segregating population, interesting in phenotype-genotype association approaches (CRUZ et al., 2004b).

Table 2. Matrix with dissimilarity values by the Jaccard index of 19 accessions of jaboticaba (*P. peruviana*) found in São Mateus, North of Espírito Santo State, Brazil

Acess	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	19'	Average
1	0	0.83	0.80	0.60	0.75	0.76	0.71	0.74	0.91	0.72	0.68	0.60	0.65	0.90	0.94	0.82	0.90	0.89	0.94	0.94	0.75
2		0	0.80	0.67	0.60	0.32	0.72	0.72	0.59	0.73	0.76	0.79	0.76	0.52	0.77	0.54	0.44	0.50	0.58	0.58	0.61
3			0	0.84	0.86	0.83	0.53	0.67	0.83	0.68	0.56	0.90	0.69	0.82	0.79	0.78	0.85	0.84	0.89	0.89	0.74
4				0	0.65	0.75	0.8	0.82	0.85	0.83	0.83	0.65	0.84	0.90	0.88	0.80	0.75	0.80	0.78	0.78	0.74
5					0	0.57	0.73	0.68	0.68	0.80	0.71	0.70	0.82	0.61	0.71	0.77	0.73	0.77	0.81	0.81	0.69
6						0	0.82	0.83	0.56	0.80	0.79	0.86	0.70	0.50	0.76	0.50	0.38	0.46	0.48	0.48	0.61
7							0	0.58	0.73	0.48	0.21	0.71	0.64	0.80	0.76	0.76	0.82	0.82	0.93	0.93	0.67
8								0	0.79	0.37	0.57	0.77	0.65	0.83	0.79	0.83	0.83	0.84	0.96	0.96	0.71
9									0	0.70	0.74	0.93	0.83	0.73	0.8	0.59	0.48	0.57	0.59	0.59	0.67
10										0	0.45	0.78	0.56	0.87	0.71	0.83	0.84	0.85	0.96	0.96	0.69
11											0	0.71	0.63	0.81	0.63	0.82	0.83	0.83	0.92	0.92	0.67
12												0	0.80	0.80	0.89	0.92	0.92	0.92	0.96	0.96	0.78
13													0	0.76	0.71	0.79	0.80	0.86	0.84	0.84	0.71
14														0	0.78	0.72	0.67	0.74	0.71	0.71	0.71
15															0	0.94	0.89	0.95	0.88	0.88	0.77
16																0	0.19	0.07	0.44	0.44	0.63
17																	0	0.13	0.27	0.27	0.59
18																		0	0.38	0.38	0.63
19																			0	0	0.66
19'																				0	0.66

Source: Authors.

On the other hand, accessions 16 and 18 showed lower dissimilarity (0.07), indicating that they are genetically similar materials. Due to their low dissimilarity, this pair of accessions is not recommended for hybridization, since genetically similar progenitors may share common genes, thus, these progenitors should not be crossed so that there is no restriction of genetic

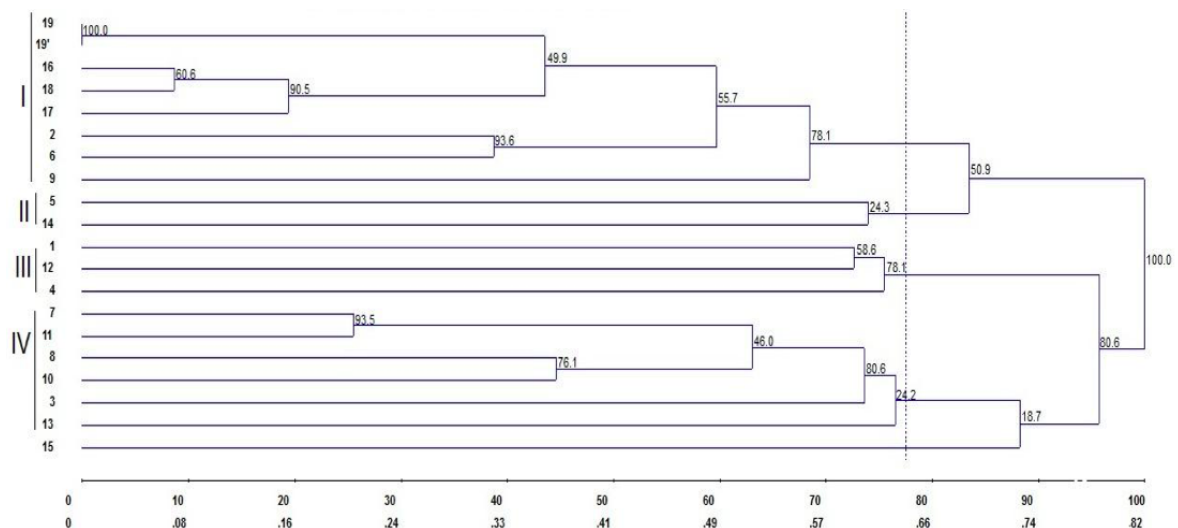
variability and gains generated by selection (ABREU et al., 1999; CRUZ et al., 2004b; TORRES et al., 2015). However, they are excellent materials whose approach is the introgression of a gene present in one of the parents and the isolation of this gene in small populations.

Based on the dissimilarity values, it was possible to group the accesses in a

dendrogram by the Unweighted Pair Group Method using Arithmetic averages (UPGMA) (Figure 1). The cut-off point for the dendrogram was 78.46%, obtained by the method of Mojema (1977), resulting in the formation of five groups. According to Melo et al. (2019), groups formed by accessions that present greater dissimilarity are

considered more favorable for breeding programs, as less dissimilarity can limit genetic variability (CRUZ; REGAZZI, 2005), making it difficult to obtain new cultivars (FARIA et al., 2012)

Figure 1. Dendrogram of 19 jabuticabas (*P. peruviana*) accessions found in the North of Espírito Santo State, Brazil, obtained based on the Jaccard index by the Unweighted Pair Group Method using Arithmetic averages (UPGMA) and bootstrap values for bifurcations, expressed in percentage (%).



Group I, formed by individuals 19, 18, 17, 16, 9, 6, and 2, comprises 36.84% of the accessions studied. It is noted that accessions belonging to group I are those with the lowest average values for dissimilarity. Group II was formed by accessions 14 and 5 (10.52% of accesses). Group III, formed by accessions 1, 4, and 12 (15.79% of accesses), presented three of the four highest average values for dissimilarity, with 0.752, 0.740, and 0.777 respectively. This suggests that the accessions of group III originated from a common or approximate material, but with a different parent since the group was formed after 70% of dissimilarity. Group IV was formed by accessions 7, 11, 8, 10, 3, 13 (31.58% of accesses). Group V is formed only by access 15 (5.26% of

accesses), which has the second-highest average (0.772) for the dissimilarity between all accesses.

Although the accessions were collected in nearby areas, mainly the accessions that makeup group III, high dissimilarity was found, which are probably not due to the effects of the environment, but were generated by genetic factors possibly resulting from sexual reproduction or natural mutation (SANTANA et al., 2011), suggesting a preference for cross-fertilization.

The value of the cophenetic correlation coefficient (CCC) for the dendrogram was 91.67%, which is considered satisfactory since according to

Rohlf and Fisher (1968) CCC values above 91% indicate the robustness of the data used in the dendrogram since there is an agreement between the matrices. In addition, the stress value for the dendrogram was 9.61%, classified between good (10%) and excellent (5%), in agreement with the values obtained for the CCC (KRUSKAI, 1964). Another important fact is that of the 20 bifurcations presented in the dendrogram, only four had values below 50% by the bootstrap test, indicating consistency of the pairs of accessions or groups formed, mainly because they are matters of sexual reproduction (EFRON; TIBSHIRANI, 1993; JESUS et al. al., 2006).

Our results show wide genetic variability in the 19 accessions studied, which according to Santos et al. (2020), indicates potential for its use in genetic improvement and conservation programs. Despite the ecological and economic importance of the Myrtaceae family, in Brazil, few studies genetically differentiate the species and aim to understand the genetic structure of populations, which would facilitate the maintenance of the germplasm banks of these species (VILELA et al., 2012). In this way, the characterization of different genetic materials allows the breeder to direct crosses to find favorable traits, such as resistance to pathogens, flower color, and amount of soluble solids, improving them according to their interest (MAROSTEGA et al., 2021). Thus, genetic variability is directly linked to the success of a plant breeding program (FARIA et al., 2012).

4 CONCLUSIONS

The molecular markers used allowed the estimation of the existing diversity among jaboticaba accessions.

Accessions 10 and 19 are the most divergent, being indicated for further studies with specific markers, to infer the maximum heterozygosity, and possible potential for exploiting heterosis.

Accessions 16 and 18 are more suitable for hybridization and gene association studies due to the narrow genetic basis between the materials.

Accessions 1, 4, 12, and 15 are the most divergent about all, being more suitable for random crosses that aim to increase the heterozygosity and genetic diversity of the species.

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