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Jaboticaba genotypes: analysis of fruits, seeds, and artisanal wine

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Abstract

Jaboticaba is a fruit species native to Brazil that is appreciated for its sweet fruits, which are rich in chemical compounds. Despite the remarkable potential of this species, only few studies have explored fruit trees native to Brazil. This study aimed to evaluate the physical and chemical characteristics of fruits, the physiological quality of seeds, the production of artisanal fermented beverages, and the molecular characteristics of eight jaboticaba genotypes. The experiment was carried out in a completely randomized design and the following features were analyzed: the physical and chemical characteristics of fruits using four replicates of 25 ripe fruits; the physiological quality of seeds using four replicates of 50 seeds, with four replicates of 25 seedlings employed for growth evaluation; the production and quality of the artisanal fermented jabuticaba, including an analysis of the alcohol content (WAC - %), pH, and total acidity of the fermented jabuticaba (WTA - meq L-1), and molecular diversity, with 50 SSRs evaluated for transferability in eight jabuticaba genotypes. Of these, 12 SSR were selected to produce clear bands that indicated specificity. Genotype 4 presented one of the largest values for fruit mass (15.25 g) and SS/TA ratio (51.92), which are ideal values for *in natura* consumption and are associated with higher contents of antioxidant compounds, such as anthocyanins and total phenolics. The genotypes had no statistical difference in seedling emergence, with a mean of 97.6%, despite genotype 5 presenting the highest emergence speed (2.75) and the lowest mean emergence time (18.10 days). Genotype 5 was found to be more vigorous. Further, genotypes 2 and 4 were considered ideal for *in natura* consumption, with fruits presenting high pulp yield, balanced SS/TA ratio, and high contents of antioxidant compounds. Genotypes 6, 3, and 8 displayed great potential for use in industrial processes. The groups of genotypes reflect the detected genotypes with different purposes and may guide divergent crosses for traits of interest in breeding programs.

Keywords: chemical characterization; emergence; fermented beverages; genetic transferability; *Plinia* sp.

Introduction

Jaboticaba (*Plinia* sp.), which belongs to the family, Myrtaceae, is a fruit species of Brazilian origin that is found in the central and southeast regions (Mattos, 1983) in domestic orchards and backyards. Jaboticaba has great economic potential owing to its organoleptic characteristics. In fact, its fruits are rich in vitamin C and minerals, such as iron, calcium, and potassium; is consumed *in natura*; and is employed in the food industry and in the production of liqueurs and wines (Silva et al., 2008). Jaboticaba has economic, historical, and cultural importance. Sabará, a city in the metropolitan region of Belo Horizonte in Minas Gerais, Brazil, is considered "The Land of Jaboticaba," producing several derivatives, such as liqueurs, jellies, jams, sauces, dessert toppings, and one of its latest releases, the *jabuçaí*, which has açaí consistency and a sweet jaboticaba flavor (Emater, 2019).

Jaboticaba fruits possess nutrients that have garnered interest for their consumption either *in natura* or as pulps and juices, which are highlighted in the prevention of several diseases (Wu et al., 2012). Salla et al. (2015) verified that the relationship between the anthocyanins in the peels, which are responsible for the antioxidant effect of the fruits, and the soluble solids of the pulp is caused by the gene that expresses the chalcone synthase enzyme, which demands

sugars to activate the synthesis reaction of anthocyanins. This reaction is important for the content of anthocyanins in the pulp and peel of jaboticaba fruits. Phenolic compounds also have anticarcinogenic and antimutagenic properties, which are important antioxidants for human health (Leite-Legatti et al., 2012).

Several dark-peel exotic fruit species have been employed as alternatives for the production of juices, liqueurs, and fermented liquids (Wu et al., 2012). As a result, Brazil has innovated and has stood out in viniculture, producing good quality products (Silva et al., 2008).

As exotic species, such as jaboticaba, have garnered interest for consumption and commercial use owing to their numerous characteristics, potential jaboticaba genotypes should be selected for *in natura* fruit consumption and use by the industry, ultimately enabling the identification of possible genetic materials for breeding programs, propagation, and later commercial use. Therefore, the fruit should be subjected to evaluations that characterize the genotype, enable the selection of superior individuals, are easy to measure, are correlated with others that are of great importance, and are difficult to obtain (Salla et al., 2015).

Molecular markers assist in the identification of genetic divergence in superior genotypes of commercial interest. In particular, microsatellite markers, are beneficial as they are codominant, multiallelic, of high polymorphism, and are used in genetic diversity analyses (Padmakar et al., 2015). Several microsatellites are available for the jaboticaba genotype owing to transferability studies with the family, Myrtaceae (Nogueira et al., 2015, Bernardes et al., 2018).

In seed propagation, seed viability and vigor are important factors for the formation of vigorous seedlings. Jaboticaba seeds do not tolerate desiccation at a moisture level of approximately 10%, are considered recalcitrant, and thus must be extracted from ripe fruits and sown immediately after harvest (Danner et al., 2011b; Silva et al., 2019). By assessing the viability of jaboticaba seeds, Danner et al. (2011a) verified that the seeds extracted and sown immediately after fruit harvest, with a water content of 41.2%, had an emergence rate of 100%.

This study aimed to evaluate the physical and chemical characteristics of fruits, the physiological quality of seeds, the production of artisanal fermented liquid, and the genetic divergence by microsatellite markers of jaboticaba genotypes.

Results

Physical and chemical characterization of fruits

The highest fruit mass values were recorded for genotypes 3 (16.18 g), 4 (15.26 g), 5 (15.01 g), and 8 (15.33 g) (Table 1). Further, genotype 3 presented the largest longitudinal and equatorial fruit diameter (30.15 and 30.20 mm, respectively), which is justified by this genotype displaying the highest number of seeds (2.98) and consequently, the lowest pulp yield (54%) (Table 1). The lowest peel thickness value was found for genotype 8, which had one of the highest pulp yield values, despite no statistical difference in the peel thickness trait compared with genotype 5.

The highest pulp yield values were found for genotypes 2 (63.57%) and 6 (62.09%), with no direct relationship found for fruit mass and pulp yield, as genotype 2 had the lowest fruit mass value (10.48 g) (Table 1). Nonetheless, the fruits from this genotype had higher contents of soluble solids (10.32 ^{o}Brix) and higher SS/TA ratio (44.39) (Table 1), leading to lower total acidity (0.225%). Such finding suggests that the fruits were sweeter. A similar behavior was observed for genotype 4, which had one of the highest values for SS/TA ratio (51.92) and soluble solids (10.35 ^{o}Brix), and the lowest total acidity (0.211%) (Table 1). The fruits of genotypes 3 (0.361%) and 8 (0.395%) (Table 1) had the highest titratable acidity while fruits of the genotypes that presented a higher SS/TA ratio (Table 1) had the lowest titratable acidity.

The highest 1000-seed mass values were found for genotypes 5 and 8 (2,218.0 and 2,538.4 g); such result may be associated with the smaller peel thickness (0.764 and 0.647 mm), higher fruit mass (15.01 and 15.33 g), and higher number of seeds (2.81 and 2.67), respectively (Table 1).

The antioxidant potential, characterized by the high content of anthocyanins (14.15 and 13.94 mg 100 g^{-1}) and phenolic compounds in the pulp (209.30 and 198.92 mg AG 100 g^{-1}), was found to be higher for genotypes 1 and 4, respectively (Table 1), relative to the other genotypes.

Physiological quality of jaboticaba seeds

The emergence values were high, varying from 95.5% to 100%, and were not statistically different between the studied genotypes (Table 1). However, genotype 5 stood out in seedling production due to its high emergence percentage (99.5%), speed (2.750), and lower mean emergence time (18.10 days) (Table 1).

The highest shoot growth values were found for genotypes 1 (10.6 cm), 2 (11.9 cm), 3 (12.0 cm), 4 (11.9 cm), 5 (11.5 cm), and 7 (11.4 cm), whereas the highest values of shoot and root dry mass were observed in genotypes 3 (3.327 and 1.052 g), 5 (3.464 and 1.035 g), and 7 (3.510 and 1.215 g) (Table 1).

Monoembryony was more prevalent in genotypes 1 (42%) and 7 (42.5%); however, such prevalence was not found in genotypes 2, 3, 4, and 5. Polyembryony was not significantly different between the genotypes. Polystems were more frequent in genotypes 6 (36.5%) and 8 (38.5%); however, such frequency was not found in genotypes 2, 3, 4, 5, and 7 (Table 1).

Quality of the artisanal fermented jaboticaba and molecular diversity of the genotypes

The WTA in all genotypes showed no statistical difference (Table 1). The wine total acidity was higher for genotype 3 (128.66 meq L^{-1}) and lower for genotypes 1 (75.24 meq·L^{-1}) and 7 (72.75 meq·L^{-1}) relative to the other genotypes. The values for wine pH were below 4. Further, the jaboticaba wine produced from genotype 2 had the highest pH (3.74) (Table 1).

The clustering formed by the UPGMA method using the generalized distance of Mahalanobis (D2) (Figure 2(a)) allowed the formation of two groups of similar genotypes (Figure 2(a)). Group 1 consisted of genotypes 2, 8, 7, and 6, while group 2 consisted of genotypes 1, 5, 4, and 3. Group 2, except for genotype 3, comprised genotypes that presented the highest contents of anthocyanins, phenolic compounds, soluble solids, and SS/TA ratio. As a result, the genotypes were found to be grouped according to the production of sweeter fruits, better flavor, and high antioxidant power.

Genotypes 6 and 8, which were within the same cluster (Figure 2(a)), had the highest polystem percentages, which contributed the most to genetic divergence (86.04%) (Table 2). Polyembryony (12.58%), was the second trait that contributed the most to genetic divergence, and along with polystems, this variable explained 98.62% of the genetic divergence in the jaboticaba genotypes by Singh's criterion (1981).

Based on microsatellite genotyping, 10 polymorphic alleles were detected at 12 *loci*. The maximum similarity was 0.83, which was between genotypes 3 and 4, while the minimum was 0.42, between genotypes 1 and 7. In the UPGMA analyses, the genotypes were divided into two groups: the first comprised genotypes 1, 2, 3, 4, 5, and 6, while the second comprised genotypes 7 and 8 (Figure 2b). Some of the genotypes in these groups were found to share common chemical and physical characteristics.

Association between the physical and chemical characteristics of fruits, physiological quality of seeds, and quality of artisanal fermented jabuticaba

Based on the perceptual map, the first and second components explained 30.78% and 23.34% of the data variability, respectively (Figure 3). These two components were identified to explain a large part of the data variability (54.12%), adapting to the analysis of the relationships between the variables (Figure 3).

Positive correlations were responsible for the discrimination of genotypes located to the right of CP1 while negative correlations were responsible for the discrimination of genotypes to the left of CP1. The positively correlated variables were responsible for discriminating the genotypes located in the upper part of CP2, while the negatively correlated variables were responsible for discriminating the genotypes located in the lower part of CP2 (Figure 3).

Treatment G1 had a high value for PC1 and a low value for PC2, revealing a high correlation with the variable root length and low correlation with mean time to emergence, showing one of the highest averages (Figure 3). Treatments G3 and G8 led to low values for CP1 and high values for CP2, revealing a high correlation with the longitudinal length of the fruits and total acidity of the fermented jabuticaba. Accordingly, the highest values of these variables were found in the corresponding treatment with the highest means (Figure 3). Treatments G4 and G5 had high values for CP1 and CP2, revealing high means for the emergence, germination speed index, and shoot length, highlighting G5, which had the lowest average emergence time (Figure 3; Table 1).

Genotypes 2, 6, and 7, with the other variables, were located close to the origin; thus, intermediate values were obtained and justified by the low statistical variation of the means (Figure 3; Table 1).

Discussion

Physical and chemical characterization of fruits

Several studies on the selection of jaboticaba genotypes with better agronomical conditions have been performed to identify more promising genetic materials. As a result, of the eight genotypes, genotype 2 stood out as it had sweet fruits with higher pulp yield, which is ideal for *in natura* consumption. However, the same genotype presented one of the highest values of peel thickness, in addition to genotypes 4 and 7 (Table 1). Further, their fruits can be used in the industry to produce jams, jellies, creams, and liqueurs. According to Danner et al. (2011a), among the 36 jaboticaba genotypes studied in five accessions from the southwest of the Paraná state, Brazil, genotype CV5 presented a high content of soluble solids (17.3 °Brix), whereas the genotypes of accessions, CH and CL, had higher fruit peel percentages. These data suggest that jaboticaba has desirable organoleptic characteristics for marketing. Further, studies on such traits are demonstrated to be essential.

Owing to the great diversity of native and wild fruit species, an abundance of anthocyanins is found in some genetic materials, which act as important antioxidants for human health. The highest contents of anthocyanin were present in the fruit pulps of genotypes 1 and 4 (14.15 and 13.94 mg 100 g^{-1}, respectively) (Table 1). However, few studies have assessed the content of these compounds in the fruit pulp. According to Abe et al. (2011), the content of anthocyanins increases with the ripening of the jaboticaba fruits, despite the identification of anthocyanin in jaboticaba peel alone. Danner et al. (2011a) verified that the anthocyanin content in whole jaboticaba fruits vary from to 367-1420 mg 100 g^{-1}. Further, Leite-Legatti et al. (2012) reported 2,598.32 mg 100 g^{-1} of total anthocyanins in lyophilized jaboticaba peels, demonstrating their antioxidant capacity. These high contents of anthocyanins, which are mainly present in the fruit peels, are responsible for the dark color of jaboticaba byproducts.

Physiological quality of jaboticaba seeds

To identify productive genotypes for development studies, jaboticaba cultivation must be expanded and the inherent characteristics of its propagation must be understood. In the present study, emergence was found to be uniform and began at 10 days, revealing its quickness. Furthermore, vigorous seedlings could be obtained with rapid growth, indicating that the eight genotypes presented quality seeds for crop cultivation in the field.

Polystems and polyembryony were found to have a greater relative contribution (86.04 and 12.589%, respectively) than other variables (Table 2). The disadvantages associated with the production of jaboticaba seedlings via seeds include the difficulty associated with generating clones, which is attributed to the remarkable genetic variability, and the delay in fruit production due to the long juvenile period (approximately 15 years); this is despite the occurrence of polyembryony in jaboticaba seeds by apomixis, in which some of the seedlings are considered clones of the genotype plant, large-scale identification is expensive, and the juvenile period remains extensive (Danner et al. 2011b, Silva et al. 2019). However, genetic variability is an important factor in the selection of superior genotypes. Costa et al. (2012) reported that genetic variability plays an important role in several selection stages that aim to ensure the selection of fruits with high quality and yield.

Quality of the artisanal fermented jaboticaba and molecular diversity of the genotypes

There are several possibilities for adding value to agricultural products, including fruits. Therefore, jaboticaba, which initially had a restricted market, is currently used to fabricate several products, such as wine, which is an alcoholic beverage consumed worldwide (Silva et al., 2008).

Jaboticaba is among the fruit species that present desirable sensory characteristics for the production of alternative "wines." Thus, to produce quality wine, these products must fit the standards of the legislation. According to the legislation that classifies fermented beverages according to their alcohol content, the limits established for table wines range from 8.6-14% (v/v) (Brasil, 2019). Genotypes 2, 4, 7, and 8 were found to be suitable for the production of artisanal jaboticaba wine as they presented alcohol contents within the proper limits (8.65, 10.65, 8.60, and 9.82%, respectively) (Table 1).

Another important characteristic for wine production is total acidity, whose minimum and maximum limits, according to

Table 1 Biometry of fruits and seeds (FM. Fruit mass, g; EFD. Equatorial fruit diameter, mm; LFD. Longitudinal fruit diameter, mm; FPT. Fruit peel thickness, mm; NS. Number of seeds; 1000SM. 1000-seed mass, g), emergence, vigor, and growth of seedlings (E. Emergence, %; ESI. Emergence speed index; MET. Mean emergence time, days; ME Monoembryony, %; PE. Polyembryony, %; PS. Polystems, %; SL. Shoot length, cm; RL. Root length, cm; SDM. Shoot dry mass, g; RDM. Root dry mass, g), and chemical characterization of fruits and wine (PY. Pulp yield, %; SS. Soluble solids, °Brix; TA. Titratable acidity, %; *RATIO*. SS/TA ratio; TAN. Total Anthocyanin, mg 100 g^{-1}; TP. Total phenolics, mg AG 100 g^{-1}; WpH. Wine Hydrogen potential; WAC. Wine alcohol content, %; WTA. Wine total acidity, meq·L^{-1}) of jaboticaba genotypes.

Genotypes	FM	EFD	LFD	FPT	NS
1	12.04 cd[1]	27.12 cde	26.03 d	0.933 ab	2.35 d
2	10.48 d	29.63 ab	29.12 abc	1.084 a	2.94 ab
3	16.18 a	30.15 a	30.20 a	0.939 ab	2.98 a
4	15.25 a	28.60 bc	29.51 ab	1.027 a	2.95 ab
5	15.01 ab	27.05 de	27.81 bcd	0.764 bc	2.81 abc
6	12.96 bc	26.97 de	27.24 cd	1.018 a	2.50 cd
7	12.98 bc	28.82 e	27.08 cd	0.902 ab	2.58 bcd
8	15.33 a	28.50 bcd	28.26 abc	0.647 c	2.67 abcd
Genotypes	1000SM	E	ESI	MET	ME
1	2.070,0 b	97.0 a	2.200 cd	22.75 a	42.0 a
2	2.048,8 b	98.5 a	2.375 bc	21.45 a	34.0 ab
3	2.204,0 b	98.5 a	2.475 b	21.13 a	37.0 ab
4	2.334,5 b	100.0 a	2.475 b	21.12 a	36.5 ab
5	2.218,0 ab	99.5 a	2.750 a	18.10 b	31.0 ab
6	2.090,0 b	96.0 a	2.325 bc	21.53 a	28.0 b
7	2.160,0 b	95.5 a	2.200 cd	22.47 a	42.5 a
8	2.538,4 a	96.0 a	2.100 d	23.20 a	24.5 b
Genotypes	PE	PS	SL	RL	SDM
1	33.5 a	24.5 b	10.6 abc	11.8 a	2.610 c
2	35.5 a	30.5 ab	11.9 a	11.3 a	2.790 c
3	33.0 a	30.0 ab	12.0 a	10.9 a	3.327 ab
4	30.5 a	33.0 ab	11.9 a	10.9 a	2.886 bc
5	35.5 a	33.5 ab	11.5 ab	11.4 a	3.464 a
6	35.5 a	36.5 a	10.1 bc	11.1 a	2.882 bc
7	23.0 a	34.5 ab	11.4 ab	11.4 a	3.510 a
8	37.0 a	38.5 a	9.6 c	10.6 a	2.748 c
Genotypes	RDM	PY	SS	TA	*RATIO*
1	0.936 b	52.52 d	10.32 a	0.231 b	45.00 a
2	0.984 b	63.57 a	10.32 a	0.225 b	44.39 a
3	1.052 ab	54.00 d	8.52 ab	0.361 a	22.98 b
4	0.947 b	57.23 abcd	10.35 a	0.211 b	51.92 a
5	1.035 ab	55.90 bcd	10.35 a	0.216 b	47.59 a
6	0.894 b	62.09 ab	9.25 ab	0.221 b	45.82 a
7	1.215 a	55.53 cd	8.95 ab	0.246 b	42.51 a
8	0.947 b	61.68 abc	7.60 b	0.395 a	17.24 b
Genotypes	TAN	TP	WpH	WAC	WTA
1	14.15 a	209.30 a	3.60 b	7.15 a	75.24 d
2	11.25 b	177.95 bc	3.74 a	8.65 a	95.12 bc
3	11.96 ab	161.92 c	3.55 b	8.02 a	128.66 a
4	13.94 a	198.92 ab	3.58 b	10.65 a	91.95 c
5	6.58 c	227.30 a	3.52 b	8.50 a	107.49 b
6	9.62 b	153.17 cd	3.55 b	8.20 a	92.61 c
7	6.38 c	159.22 c	3.35 c	8.60 a	72.75 d
8	4.63 c	127.90 d	3.51 b	9.82 a	94.59 bc

[1]Means followed by the same letter in the column do not differ from each other by Tukey's test.

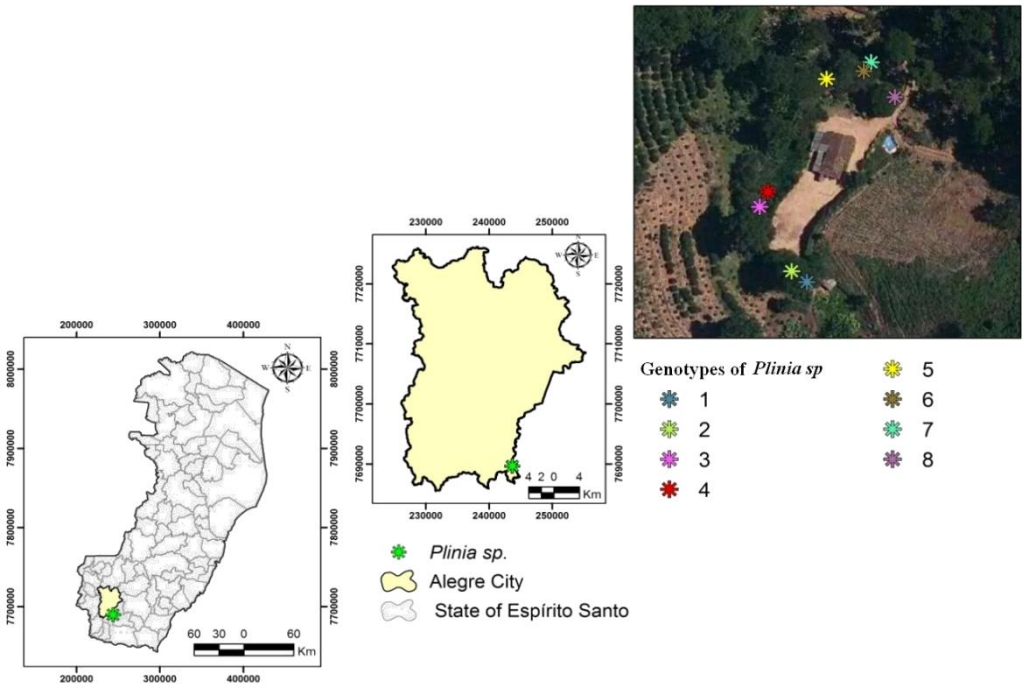

Fig 1. Location of the genotypes in the region of Lagoa Seca, Alegre - Espírito Santo.

Table 2. Relative contribution (%) of fruit, seed, and artisanal wine characteristics for the genetic divergence between jaboticaba genotypes by the Singh's method (1981).

Characteristics	S.j	Relative contribution (%)
Polystems (%)	7939761.08	86.04
Polyembryony (%)	1161598.85	12.58
Emergence speed index	65772.37	0.712
Soluble solids (°Brix)	19712.16	0.213
Titratable acidity (%)	8871.15	0.096
1000-seed mass (g)	7386.54	0.080
Wine pH	6472.04	0.070
Fruit mass (g)	5777.02	0.062
Root length (cm)	3202.64	0.034
Shoot length (cm)	3028.62	0.032
Number of seeds	2651.24	0.028
Longitudinal fruit diameter (mm)	1732.95	0.018
Fruit peel thickness (mm)	657.31	0.007
Equatorial fruit diameter (mm)	472.82	0.005
Emergence (%)	0.0	0.0
Mean emergence time (dias)	0.0	0.0
Monoembryony(%)	0.0	0.0
Shoot dry mass (g)	0.0	0.0
Root dry mass (g)	0.0	0.0
Pulp yield (%)	0.0	0.0
RATIO: SS/AT	0.0	0.0
Total anthocyanin (mg 100 g^{-1})	0.0	0.0
Total phenolics (mg AG $100g^{-1}$)	0.0	0.0
Wine alcohol content	0.0	0.0
Wine total acidity (meq L^{-1})	0.0	0.0

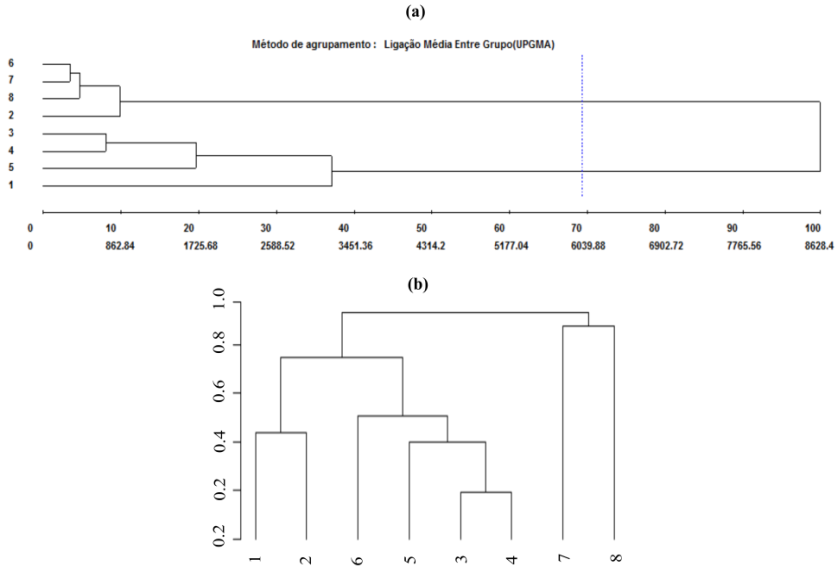

Fig 2. Dendrograms obtained by the UPGMA clustering method, using the generalized distance of Mahalanobis (D2) based on twenty-five physical-chemical traits of the jaboticaba genotypes. Cophenetic correlation coefficient = 0.78 (a); from the products of the amplification with the SSR primers. Cophenetic correlation coefficient = 0.94 (b).

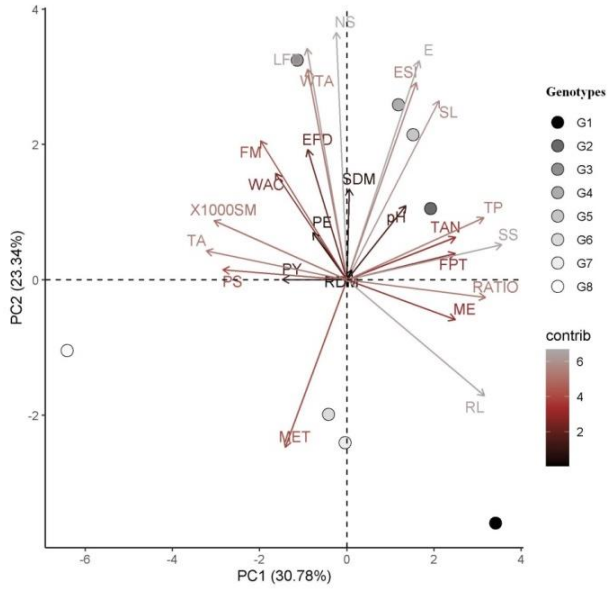

Fig 3. Principal component analysis biplot showing the importance of variables for genotype dispersion.

the legislation for light and/or table wines, vary from 40 meq·L^{-1} to 130 meq·L^{-1}, respectively (Brasil, 2019). Genotypes 2, 4, 7, and 8 were in accordance with the legislation, presenting 95.12, 91.95, 72.75, and 94.59 meq·L^{-1} of total acidity, respectively. Genotypes 1, 3, 5, and 6 were also in accordance with the limits established for total acidity (75.24, 128.66, 107.49, and 92.61 meq·L^{-1}); however, they were not ideal for wine marketing as they were not within the limits established for the alcohol content (Table 1). There are growing studies on fermented jaboticaba beverages that reveal their importance for alternative wines due to their quality and characteristic flavor, and aim to identify genotypes and protocols that better adapt to the legislation. By determining the chemical composition of fermented alcoholic jaboticaba beverages from different cropping seasons, Silva et al. (2008) recorded total acidity values above the limit established by MAPA in the cropping seasons from 2002 to 2005. In fact, in the 2005 cropping season, more than 90% of the samples had total acidity values above 185 meq·L^{-1}. However, for the alcohol content of the 2002, 2003, and 2006 seasons, the values were within the legislation for light and/or table wines (Mapa, 1998).

The wine pH values interfere with the quality of the final product, and wines with lower pH should be obtained to avoid contamination by fungi. Therefore, fruits of genotype 4 were found to behave as a good alternative for wine production as they presented low pH (3.58), total acidity within the allowed margin (91.95 meq L^{-1}), and high contents of anthocyanin and phenolic compounds (13.94 mg 100 g^{-1} and 198.92 mg AG 100 g^{-1}, respectively). Such finding indicates that jaboticaba is a source of anthocyanins and phenolic compounds, in accordance with Wu et al. (2013) and Pereira et al. (2016), respectively. The presence of these compounds influences the nutritional value and sensory quality, conferring attributes such as color, texture, bitterness, and astringency (Rocha et al. 2011). The alcohol

content was found to be within the desirable range (10.35%) (Brasil, 2019); these characteristics also conferred a pleasant taste, with 10.35 °Brix and SS/TA ratio of 51.52 (Table 1).

The association between the characteristics, such as those of agricultural production and organoleptic nature, as demonstrated by the molecular and physical-chemical analysis, highlights the relevance of identifying potential genotypes for use in the market, which is valuable for the cultivation of exotic species.

The association mentioned above can be observed through genotypes 7 and 8, which remained grouped for both phenotypic (Figure 2(a)) and molecular dissimilarity (Figure 2(b)). These two genotypes differ from each other in terms of biometric characteristics, such as peel thickness and seed mass, although they are similar in terms of chemical characteristics, such as the anthocyanin and phenolic content (Table 1).

The clustering of genotypes 3, 4, and 5 in both dendrograms (Figure 2(a-b)) depicted the molecular, morphological, and chemical proximity between these genotypes, ultimately indicating that the higher the fruit mass, the higher the content of soluble solids and the lower the pH values, leading to the production of sweeter and tastier fruits (Table 1). Similar results were found for *Psidium guajava,* in which the genotypes of the Cortibel variety presented similar morphological and molecular fruit characteristics (Coser et al. 2012).

Association between the physical and chemical characteristics of fruits, physiological quality of seeds, and the quality of artisanal fermented jabuticaba

The variables that contributed the most to data variation are presented in Table 2, where the variables with the greatest relative contribution are displayed, justifying the association between the groups of variables and the genotypes in Figure 3. In PCA, the accumulation of genotypes in an area of the plot indicates the similarity between them, highlighting superior genotypes based on ideal values for each analyzed characteristic and commercial potential.

Most of the emergence, vigor, and seedling growth traits are on the right in CP1, and may have higher means for the G1, G4, and G5 genotypes (Figure 3). In addition to these variables, the chemical characteristics of fruits such as soluble solids, RATIO, anthocyanins, and total phenolics are on the right in CP1, indicating that these genotypes have appreciable fruits for fresh consumption. Jabuticaba is among the wide variety of exotic and native species found in Brazil and is widely consumed fresh in small regions; this is because it has tasty fruits and nutraceutical characteristics that are beneficial to health. However, its market introduction is still restricted owing to its low viability and short shelf life. In addition, cultural treatments are difficult because of the characteristics of the plant, in which flowering and fruiting occur throughout the stem, making mechanized harvesting difficult (Clerici; Carvalho-Silva, 2011).

The biometric characteristics of fruits and seeds, and chemical characteristics of the fermented jabuticaba are on the left of CP1, and could have higher averages for the G3, G6, and G8 genotypes, indicating that these genotypes have adequate characteristics for the industrialization process. Several by-products are obtained from jabuticaba, such as juices, fermented products, and jellies. Another alternative is the use of husks and residues that can be used for the manufacture of flour, ultimately contributing to the prevention of chronic diseases; the industrialization process it also increases the shelf life of perishable fruits, with availability products throughout the year, not only in the production station (Resende et al., 2020).

Materials and methods

Jaboticaba fruits (*Plinia* sp.) were collected from eight genotype plants in the region of Lagoa Seca, Alegre-ES at the following geographic coordinates: 1. 20º52’32.9” S and 41º27’50.6” W; 2. 20º52’32.8” S and 41º27’50.8”; 3. 20º52’32.2” S and 41º27’51.1” W; 4. 20º52’32.0” S and 41º27’59.0” W; 5. 20º50’30.9” S and 41º27’50.4” W; 6. 20º52’30.7” S and 41º27’50.0” W; 7. 20º52’30.8” S and 41º27’50.0” W; and 8. 20º52’31.1” S and 41º27’49.7” W (Figure 1).

Physical and chemical characterization of fruits

The analyses were performed in the Laboratory of Forest Seeds at the Department of Forest and Wood Sciences of the Center of Agricultural Sciences and Engineering of the Federal University of Espírito Santo, municipality of Jerônimo Monteiro-ES. Four replications of 25 ripe fruits were used for each of the eight genotypes to analyze the following variables: fruit mass (FM - g), 1000-seed weight (1000SW - g) determined with an electronic balance (0.01 g); equatorial fruit diameter and polar fruit diameter (EFD and PFD - cm), determined with a digital pachymeter; fruit peel thickness (FPT - mm), measured using fruits sectioned in half and a digital pachymeter; gross pulp mass (GPM - g); pulp yield (PY - %), determined by the ratio between the gross pulp mass and the total fruit mass; number of seeds per fruit (NS); soluble solids (SS - °Brix), using a handheld analog refractometer, with temperature compensation; titratable acidity (TA - % of citric acid), determined by titration with 0.1 N sodium hydroxide (NaOH) using 5 g of pulp diluted in 50 mL of distilled water and the phenolphthalein indicator, with acidity values expressed as a percentage of citric acid; *ratio*, SS/TA ratio of the fruit pulp; total anthocyanins (TAN - mg 100 g^{-1}) and total phenolics (TP - mg AG 100 g^{-1}) in the fruit pulp. Anthocyanins were analyzed according to Francis (1982), with adaptations. The analyses were performed in triplicate, with four replications per stock; each replicate contained 0.150 g of *in natura* fruit pulp, as weighed on an analytical balance (0.0001 g). Thereafter, 4 mL of the 95% ethanol extracting solution was addedto 1.5 mol·L^{-1} HCl (85:15 v/v). The mixture was homogenized and transferred to a 4 mL test tube, which was refrigerated for 24 h in the dark. Subsequently, the reading was performed on an AGILENT CARY60 UV-VIS spectrophotometer at a wavelength of 535 nm. The data were converted to mg of anthocyanins 100 g^{-1} of the wet sample using the following equation:

$$TA\ (mg\ 100g^{-1}) = \frac{A_{(535)} \times V_{Tube} \times 10^5}{982 \times L \times M_{sample}}$$

Where; TA (mg 100 g^{-1}) = content of anthocyanin; $A_{(535)}$ = absorbance at 535 nm; $V_{(tube)}$ = tube volume (mL); L = optical path (1 cm); $M_{(sample)}$ = mass of the sample (g).

Total phenolic content was determined by spectrophotometry using the Folin-Ciocalteau reagent, as described by Singleton et al. (1999). To perform the extraction, 0.25 g of wet biomass was weighed, and 5 mL of 2 N HCl was added prior to heating for 30 min in a water bath at 95 ºC. Subsequently, the mixture was kept at ambient temperature until cool. A 50-µL aliquot was then removed and transferred to a test tube, in which 2.5 mL of

the Folin-Ciocalteu reagent was added at 1:10 (v v^{-1}). After 5 min of rest, 2.0 mL of Na_2CO_3 4% (m v^{-1}) was added, and the solutions were kept in a dark place at ambient temperature for 2 h. Thereafter, the reading was performed on a spectrophotometer at 740 nm. The calibration curve was prepared with gallic acid (0.1, 1 mg mL^{-1}), resulting in the linear equation $\hat{Y} = 0.04193 + 0.76973x$, with $R^2 = 0.999$. Further, the total phenolic content was calculated; the result is expressed in mg of gallic acid 100 g^{-1} of the sample.

Physiological quality of the seeds

The seeds were manually removed from the ripe fruits and processed. Mucilage was then extracted using the quicklime method, and sown in 290 cm^3 tubes containing the Bioplant® organic substrate.

The following characteristics were analyzed: emergence percentage (Brasil, 2009); emergence speed index (ESI) (Maguire, 1962); mean emergence time (MET, day) (Labouriau, 1983), monoembryony (%), polyembryony (%), polystems (%), shoot and root length (cm) using a millimeter ruler; shoot and root dry matter of the seedlings (g) using an analytical balance (0.0001 g; these variable were measured after the material was placed in kraft paper bags and kept in a forced-air oven at 72 °C for 72 h).

The experimental design was completely randomized, with four replications of 50 seeds. Seedling growth was evaluated using 100 seedlings divided into four replications at 90 days after sowing.

Production and quality of the artisanal fermented jaboticaba

Ripe fruits from each genotype were selected, processed, and stored in containers with water to eliminate floating fruits. The remaining fruits were disinfected in a 2% sodium chloride solution. Thereafter, 5 L of fruit was transferred to a 20 L container consisting of sugar (0.5 kg) and allowed to ferment for 40 days at 25 °C. Thereafter, the samples were removed for the analysis of the fermented alcohol content (WAC - %), determined in triplicate, with four replications per genotype. Quantification of the alcohol content was performed by the densimetric method using a pycnometer (Brasil, 2005); pH was measured using a digital pH meter; fermented total acidity (WTA - meq L^{-1}), determined by titration, according to the method described by the Ministry of Agriculture, Live genotype, and Supply (Mapa, 2012).

Molecular diversity

The genomic DNA of the eight genotypes was isolated from young leaves using the CTAB method (Doyle and Doyle, 1987), modified with the addition of 1% polyvinylpyrrolidone (PVP) in the extraction buffer.

Fifty microsatellite markers were selected and tested based on transferability studies of these markers originating from *Psidium guajava* for several Myrtaceae species, including jaboticaba species (Bernardes et al., 2018). Twelve markers were selected to produce clear bands: mPgCIR18, mPgCIR21, mPgCIR97, mPgCIR108, mPgCIR174, mPgCIR187, mPgCIR198, mPgCIR 209, mPgCIR225, mPgCIR347, mPgCIR378, and mPgCIR446. The primer sequences for each marker are available in Supplementary Material (Table 1).

Polymerase chain reaction (PCR) mixtures were prepared according to the original protocol for Taq DNA polymerase (Genbiotech) using the respective annealing temperatures for each marker (Supplementary Material, Table 1). PCR amplification was confirmed by polyacrylamide (10%) gel electrophoresis, stained with GelRed™ (Biotium), and visualized under UV light in a Chemic-DocTM imaging system (BIO-RAD).

Statistical analysis

Data on the physical and chemical characteristics of the fruits from the eight jaboticaba genotypes were analyzed using univariate and multivariate statistics. For the univariate statistics, the data were subjected to analysis of variance and the F-test at a 5% level of probability. The means of the treatments, when significant, were grouped by Tukey's test at 1% and 5% probability.

To estimate the genetic divergence between the eight genotypes based on the standardized means of the 25 characteristics studied, the generalized distance of Mahalanobis (D^2) was used as a dissimilarity measure to determine the relative contribution by Singh's method (1981). Based on the dissimilarity genotype, a dendrogram was generated using the unweighted pair group method using arithmetic averages (UPGMA), where the cut-off point in the dendrogram was determined according to the method of Mojena (1977) using the relative levels of fusion (distances) in the dendrogram. Statistical analyses were performed using the Gene computer software (Cruz, 2016). A multivariate analysis of principal components was applied to these data, adopting the eight genotypes as individuals and the morphoagronomic characteristics as vectors, using R software (R Core Team, 2021).

The products generated by the amplification of the multiallelic markers were analyzed and used in the R software to calculate the genetic dissimilarity genotype for the accessions. The analyses were performed using the UPGMA clustering method, followed by hierarchical clustering.

Conclusions

Genotype 5 was found to be more vigorous than the ither genotypes. Genotypes 2 and 4 were recognized to be ideal for *in natura* consumption, with fruits presenting high pulp yield, a balanced SS/TA ratio, and high contents of antioxidant compounds. Genotypes 6, 3, and 8 presented great potential for use in industrial processes. The groups of genotypes reflect the detected genotypes with different purposes and may guide divergent crosses for traits of interest in breeding programs.

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