Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Intraspecific C-value variation and the outcomes in *Psidium cattleyanum* Sabine essential oil

Variação do valor C intraespecífico e os efeitos no óleo essencial de *Psidium cattleyanum* Sabine

M. S. Spadeto[a], L. C. Vasconcelos[a], L. Menini[b], W. R. Clarindo[c], J. H. S. Guilhen[d], M. F. S. Ferreira[d] and M. M. Praça-Fontes[a]*

[a]Universidade Federal do Espírito Santo – UFES, Centro de Ciências Exatas, Naturais e da Saúde, Departamento de Biologia, Programa de Pós-graduação em Genética e Melhoramento, Laboratório de Citogenética e Cultura de Tecidos Vegetais, Grupo de Pesquisa Agroquímicos e Análise de Toxicidade em Bioensaios, Alegre, ES, Brasil

[b]Instituto Federal de Educação, Ciência e Tecnologia do Espírito Santo – IFES, Laboratório de Química Aplicada, Alegre, ES, Brasil

[c]Universidade Federal de Viçosa – UFV, Departamento de Biologia Geral, Programa de Pós-graduação em Genética e Melhoramento – PPGGM, Laboratório de Citogenética e Citometria, Grupo de Pesquisa Agroquímicos e Análise de Toxicidade em Bioensaios, Viçosa, MG, Brasil

[d]Universidade Federal do Espírito Santo – UFES, Centro de Ciências Agrárias e Engenharias, Departamento de Agronomia, Programa de Pós-graduação em Genética e Melhoramento, Laboratório de Genética Vegetal, Grupo de Pesquisa Agroquímicos e Análise de Toxicidade em Bioensaios, Alegre, ES, Brasil

Abstract

Polyploidy, a numerical alteration of the karyotype, is one of the most important mechanisms in plant speciation and diversification, but could also be detected among populations, the cytotypes. For example, *Psidium cattleyanum,* a polyploid complex, has chromosome numbers ranging from 2n=3x=33 to 2n=12x=132. Polyploidization causes an increase in DNA content, and both modifications may cause alteration in plant growth, physiology, and epigenetics. Based on this possibility, here we aim to verify the influence of the polyploidization on the production of *P. cattleyanum* essential oil chemotypes. Differences in the DNA contents, as a proxy to different ploidies, were observed and three distinct chemotypes were identified through the chromatographic profile analysis. The *Psidium cattleyanum* DNA content and qualitative and quantitative characteristics of the essential oils presented a positive relationship. Plants with higher DNA contents presented higher levels of oil production, which was mostly composed of hydrogenated sesquiterpenes, while plants with lower DNA contents produced lower amount of oil, which was mostly composed of hydrogenated monoterpenes. Based on the importance of essential oils, polyploid plants, which present higher DNA content, are recommended as possible matrices for the propagation of new plants with the potential to produce major compounds of agronomic and pharmacological interest.

Keywords: DNA content, C-value, volatile compounds, *Psidium*.

Resumo

A poliploidia, uma alteração numérica do cariótipo, é um dos mecanismos mais importantes na especiação e diversificação das plantas, mas também pode ser detectada entre populações, os citótipos. Por exemplo, *Psidium cattleyanum*, um complexo poliplóide, tem números de cromossomos que variam de 2n=3x=33 a 2n=12x=132. A poliploidização causa um aumento no conteúdo de DNA, e ambas as modificações podem alterar o crescimento, a fisiologia e a epigenética da planta. Com base nessa possibilidade, objetivamos verificar a influência da poliploidização na produção de quimiotipos de óleo essencial de *P. cattleyanum*. Diferenças nos conteúdos de DNA, representando diferentes ploidias, foram observadas e três quimiotipos distintos foram identificados através da análise do perfil cromatográfico. O conteúdo de DNA de *Psidium cattleyanum* e as características qualitativas e quantitativas dos óleos essenciais apresentaram correlação positiva. Plantas com maiores conteúdos de DNA apresentaram maiores rendimentos na produção de óleo, que era majoritariamente composto por sesquiterpenos hidrogenados, enquanto plantas com menores conteúdos de DNA produziram menores quantidade de óleo, que era majoritariamente composto por monoterpenos hidrogenados. Com base na importância dos óleos essenciais, plantas poliplóides, que apresentam maiores conteúdos de DNA, são recomendadas como possíveis matrizes para a propagação de novas plantas com potencial para produzir compostos importantes de interesse agronômico e farmacológico.

Palavras-chave: conteúdo de DNA, valor C, compostos voláteis, *Psidium*.

*e-mail: milenemiranda@yahoo.com.br
Received: January 27, 2022 – Accepted: September 14, 2022

1. Introduction

Polyploidy is defined as the presence of more than two complete sets of chromosomes per cell nucleus. A polyploid individual arising within one or between populations of a single species is denominated autopolyploid, while the term allopolyploid refers to individuals of hybrid origin (Stebbins, 1950; Comai, 2005; Beest et al., 2012; Machado et al., 2022). Polyploidy is a frequent phenomenon in plants, especially in angiosperms (Jiao et al., 2011)

For the identification of polyploid individuals, direct and indirect methods are used. While the direct method is chromosome counting, indirect methods involve physiological and/or morphological traits, and nuclear genome size measurement by flow cytometry (Sattler et al., 2016). Chromosome counting has been considered the most accurate method to detect polyploid variants. However, cytogenetic techniques are often laborious, requiring highly specific protocols for each species (Doležel et al., 2007). Alternatively, flow cytometry is a rapid, reliable, and simple method that based on the DNA content estimation can be used as a proxy to infer the ploidy level of target plants (Roy et al., 2001). Since the increments in chromosome number always cause increments in DNA content, the DNA content of an exemplar with a known ploidy level can be used as a reference standard to determine the DNA ploidy level of an unknown sample (Doležel et al., 2007).

Psidium L. presents polyploid species, with the basic number defined as x=11 (Atchison, 1947; Costa and Forni-Martins, 2006). Polyploidy has played an important role in the evolution and diversification of the genus (Marques et al., 2016; Tuler et al., 2019; Machado et al., 2021), in which records of diploid, triploid, tetraploid, hexaploid, and octaploid species are common (Costa and Forni-Martins, 2006; Tuler et al., 2015, 2019; Machado et al., 2021). The species *Psidium cattleyanum* Sabine also present a polyploid serie with different chromosome numbers reported, such as 2n=33, 44, 55, 66, 77, 88, 99, 100, 110, and 132 (Atchison, 1947; Costa and Forni-Martins, 2006; Hirano and Nakasone, 1969; Medina, 2014; Souza et al., 2015; Machado et al., 2021, 2022). Souza et al. (2015) reported cytotypes that are not multiples of the basic number of the family (x=11), such as 2n=46, 48, 58, 82.

The determination of the DNA content may complement the information about the genomic variations of species (Souza et al., 2015) but, in accordance with Costa et al. (2008), just a few Myrtaceae species have their 2C value estimated. Souza et al. (2015) estimated the DNA content for two guava (*P. guajava*) and sixteen araça (*P. cattleyanum*) accessions, detecting a variation from 0.99 picograms in the former to 5.47 pg in the last. Concerning just the *P. cattleyanum*, seven different ploidies were identified with DNA contents ranging from 1.99 to 5.47 pg (2n=44: 1.99 pg; 2n=46: 2.20 pg, 2.54 pg, 2.70 pg; 2n=55: 2.88 pg; 2n=66: 3.01 pg, 3.11 pg, 5.32 pg; 2n=82: 5.47 pg; Souza et al., 2015).

Polyploid plants show more vigor caused by heterosis and a buffer effect in deleterious mutations (Comai, 2005). They usually present Giga-effect by showing greater plant structures, as larger fruits and in higher quantities. They can also be resistant to disease or stressful environmental conditions (Baniaga et al., 2020; Moura et al., 2021). The inter-and intraspecific genetic variability of polyploid species creates differences in the production of secondary metabolites and consequently in the chemical composition of essential oils (Iannicelli et al., 2016; Kulheim et al., 2015; Souza et al., 2017).

Essential oils present environmental importance, helping to protect plants against pathogen attacks and to attract pollinators (Sholberg and Gaunce, 1995; Negrini et al., 2019). It also presents economic interest due to its herbal action, which is widely marketed in the pharmaceutical industry (Almeida et al., 2014). Variations in the chemical composition of essential oils can be inter and intraspecific and be caused by different factors, including genetic factors, as ploidy level and C-value (Kulheim et al., 2015; Souza et al., 2017). Thus, given the *P. cattleyanum* ploidy variation and considering the DNA content as a proxy to ploidy inference, the present study aims to analyze the relationship between the ploidy/DNA content of *P. cattleyanum* with the production of essential oil and its chemical composition.

2. Material and Methods

2.1. General experimental procedures

The studied cytotypes are part of the field collection from the Center for Agricultural Sciences and Engineering at the Federal University of Espírito Santo (CCAE/UFES). Flow cytometry analyzes were performed at the cytogenetics laboratory of the Federal University of Viçosa (UFV, Viçosa, MG). The extraction of essential oils was carried out in the Laboratory of Vegetal Sample Preparation at CCAE/UFES and the chemical composition analyzes of the oils were conducted in the Applied Chemistry laboratory of the Federal Institute of Espírito Santo (IFES, Alegre, ES).

2.2. Collection of plant material

Leaves of *P. cattleyanum* accessions were collected at 8 a.m., all specimens on the same day. The collection was carried out at breast height (1.6m) and around the crown diameter. About 500g of fully developed leaves were collected to obtain the oil and chemical characterization. The material was packed in paper bags, identified, and transported to the Plant Sample Preparation laboratory at CCAE-UFES. The leaves were dried at room temperature and kept in plastic bags, stored in a freezer at -20 °C until the essential oil extraction.

2.3. Flow cytometry

Solanum lycopersicum L 'Stupické' (2C = 2.0 pg) was used as an internal standard (Praça-Fontes et al., 2011) to determine the genome size of *P. cattleyanum* accessions. Leaf fragments (2 cm^2) from the standard and sample were dissociated into a Petri dish containing 500 µL of OTTO-I nuclear extraction buffer (0.1 M citric acid, 0.5% Tween 20, 2 mM dithiothreitol and 50 µg/ml RNAse) (Otto, 1990) supplemented with polyethylene glycol (PEG). To the dissociated material obtained, 500 µL of the same buffer was added, which was filtered with a 30 µm nylon mesh

(Partec®), placed in a microtube and centrifuged at 100 g for 5 minutes.

The precipitate obtained was resuspended in OTTO-I buffer and kept there for 10 minutes (Praça-Fontes et al., 2011). Subsequently, 1.5 ml of OTTO-I staining buffer was added: OTTO-II (400 mM $Na_2HPO_4.H_2O$, 2 mM dithiothreitol, 50 µg/ml propidium iodide and 50 µg/ml RNAse, 1: 2, v:v) (Otto, 1990). The nucleus suspensions were kept in the dark for 30 minutes in the staining solution and then filtered through a 20 µm nylon mesh (Partec®).

The samples were analyzed in a Partec PAS II/III flow cytometer (Partec GmbH, Munster, Germany). Each sample was analysed in triplicate. To assess the quality of the sample, a coefficient of variation of less than 5% was used as a reference (Doležel and Bartoš, 2005). The genome size was calculated through the multiplication of the genome size of *S. lycopersicum* (internal standard) by the fluorescent intensity ratio of sample/standard G_1 peaks.

2.4. Extraction and yield of essential oils

Essential oils were extracted by hydrodistillation, in a Clevenger apparatus, for four hours according to the methodology recommended by the Brazilian Pharmacopeia (ANVISA, 2010). These extractions were done in duplicate for each sample. For the extractions, approximately 300g of leaves were placed in 1,000 mL of reverse osmosis water, in a 2,000 mL round-bottom flask. The water and oil vapors were mixed and, after cooling, the molecules condensed and were separated by differences in solubility and density. The mixture of oil and water was placed in a 1.5 mL tube and centrifuged. Afterward, the oil was removed with a micropipette and stored in the freezer at -20 °C, protected from light.

Yields from the essential oil extractions were determined to check whether the different DNA contents influence the oil production. The dry mass ratio of the plant regarding the extracted oil mass ($m.m^{-1}$) was used, in duplicate. An analytical balance (Shimadzu AUY220) with four decimal places was used for weighing the samples.

2.5. Chromatographic profile of essential oils

Samples of the essential oils were analyzed by Gas Chromatography with Flame Ionization Detector (GC-FID) (Shimadzu GC-2010 Plus) and Gas Chromatography coupled with Mass Spectrometry (GC-MS) (Shimadzu GCMS-QP2010 SE). The following conditions were used: carrier gas - He (for the two detectors), with the flow and linear velocity of 2.80 $mL.min^{-1}$ and 50.8 $cm.sec^{-1}$ (GC-FID) and 1.98 $mL.min^{-1}$ and 50.9 $cm.sec^{-1}$ (GC-MS), respectively; the injector temperature was of 220 °C in a 1:30 split ratio; fused silica capillary column (30 m x 0.25 mm) was used; stationary phase Rtx®-5MS (0.25 µm film thickness). Oven temperature programming: initial temperature of 40 °C, which remained for 3 minutes; temperature gradually increased to 3 $°C.min^{-1}$ until reaching 180 °C, remaining for 10 minutes, with a total analysis time of 1 hour; the temperatures used in the FID and MS detectors were of 240 and 200 °C, respectively (Souza et al., 2017). The samples were removed from the vials in a volume of 1 µL of a 3% solution of essential oil dissolved in hexane with 0.1 $mol.L^{-1}$ DMA (external standard for reproducibility control).

The GC-MS analyzes were performed in an electronic impact device with an impact energy of 70 eV; 1000 sweep speed; scan interval from 0.50 $fragments.sec^{-1}$ and detected fragments from 29 to 400 (m/z). The GC-FID analyzes were performed by a flame formed by H_2 and atmospheric air at a temperature of 300 °C. Flows of 40 $mL.min^{-1}$ and 400 $mL.min^{-1}$ were used for H_2 and air, respectively.

The identification of essential oil components was performed by comparing the mass spectra obtained with those available in the spectrotheque database (Wiley 7, NIST 05, and NIST 05s) and by the LTPRI retention indices (RI). A mixture of saturated alkanes C7C40 (Supelco-USA) and the adjusted retention time of each compound, obtained through GC-FID, were used to calculate the RI. Then, the calculated values for each compound were compared with those in the literature (Adams, 2007; Lemmon et al., 2011).

The relative percentage of each compound from the essential oil was calculated through the ratio between the integral area of the peaks and the total area of all the sample compounds, data obtained by the GC-FID analyzes. Compounds with the relative area above 1% were identified and above 10% were considered the majority. The extraction and analysis of the chemical composition of the oils were carried out in duplicate, the first extraction was carried out in February 2018 and the second in June 2019.

2.6. Correlation between DNA content and essential oil yield

The relationship between DNA content and essential oil yield was examined using Pearson's correlation coefficient ($p < 0.05$). Statistical analysis was performed using the R program, version 4.1.0 (R Core Team 2021).

3. Results

According to the flow cytometry analysis, the seven *Psidium cattleyanum* accessions presented DNA contents ranging from 2C = 3.2 to 6.03 pg and there was an increase in the yield of essential oils in plants with higher DNA contents (Table 1). A Pearson's correlation analysis was carried out to quantify the relationship between DNA

Table 1. Correlation (r) between DNA content (pg) and yield (%) of oils from seven accessions of *Psidium cattleyanum*.

Accession	DNA content (2C value) (pg)	Yield (%)
CAT1	3.95	0.75
CAT2	5.81	0.90
CAT3	6.03	0.95
CAT4	3.23	0.70
CAT5	3.80	0.73
CAT6	3.20	0.70
CAT8	4.71	0.80

$r_{2C \times Yield} = 0.99$; *P value* = 1.74^{e-05}.

content and yield of essential oils from leaves of the accessions. The DNA content was positively correlated with the yield of essential oils (r = 0.9903633; *P value* = 1.74^{e-05}) (Table 1). In addition to the quantitative variations, the plants also showed qualitative variations regarding the chemical composition of their essential oils (Table 2). Considering that two extractions were carried out at different periods, there was variation only CAT6 in the pattern of the compounds produced (Tables 2 and S1).

Fifteen compounds were identified in the essential oils of the seven accessions of *P. cattleyanum*, through chromatographic analysis at different periods. The oils of each accession presented 100% of the compounds with a relative area above 2%, which were classified as hydrogenated (HM) and oxygenated (OM) monoterpenes and hydrogenated (HS) and oxygenated (OS) sesquiterpenes (Figure 1). There was a predominance of hydrogenated compounds in all the essential oils analyzed, with a greater representation of the class of sesquiterpenes. However, the CAT4, CAT5 (Table 2) and CAT6 (Table S1) accessions presented a greater amount of both oxygenated and hydrogenated monoterpenes.

Seven monoterpenes (five hydrogenated and two oxygenated) and eight sesquiterpenes (seven hydrogenated and one oxygenated) were identified. Trans-caryophyllene (hydrogenated sesquiterpene) was the major compound present in all the accessions, followed by alpha-pinene (HM), copaene (HS), and alpha-humulene (HS) and eucalyptol (OM). The five major compounds (relative area > 10%) presented in the essential oils were identified in Figure 2.

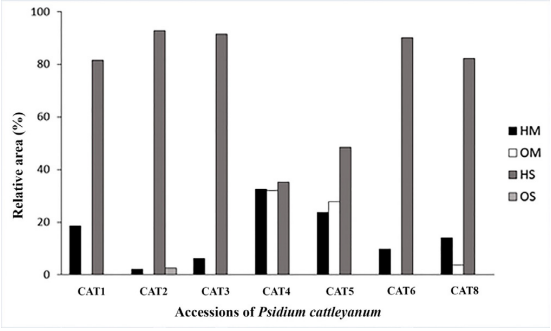

Figure 1. Relative area (%) of terpenic classes in relation to accessions of *Psidium cattleyanum* (CAT1, CAT2, CAT3, CAT4, CAT5, CAT6 and CAT8). Hydrogenated monoterpenes (HM), oxygenated monoterpenes (OM), hydrogenated sesquiterpenes (HS) and oxygenated sesquiterpenes (OS).

Table 2. Chemical composition, in percentage, of the relative area of essential oils from *Psidium cattleyanum* accessions.

Compounds[a]	Retention time (min)[b]	Retention index[c]	Relative area[d]							Terpene classes[e]
			CAT1	CAT2	CAT3	CAT4	CAT5	CAT6	CAT8	
alpha-Pinene	8.795	933	**10.35**	2.03	6.06	**23.80**	**19.56**	5.70	4.88	HM
beta-Pinene	10.725	976				3.62				HM
Myrcene	11.520	990	8.16					2.01		HM
Limonene	12.925	1025						2.05	9.17	HM
Eucalyptol	13.280	1033				**27.70**	**24.34**		3.80	OM
Beta-ocimene	13.735	1041				5.20	4.17			HM
alpha-Terpineol	20.805	1192				4.38	3.45			OM
Copaene	29.030	1374		9.88	9.90			**10.20**	3.68	HS
trans-Caryophyllene	30.820	1417	**72.16**	**62.27**	**58.45**	**35.30**	**41.80**	**57.69**	**57.05**	HS
alpha-Humulene	32.270	1453	6.68	**13.93**	**13.38**		3.11	**13.06**	**11.68**	HS
alpha-Amorphene	33.255	1476	2.65				3.57			HS
beta.-Selinene	34.279	1490						6.39	2.96	HS
alpha.-selinene	34.672	1498						2.90	2.72	HS
delta-Cadinene	35.175	1523		6.78	6.34				4.06	HS
NI[f]	37.455	-			3.44					
Viridiflorol	37.485	1580		2.61						OS
NI	39.220	-		2.50	2.43					
DNA content (pg)			3.95	5.81	6.03	3.23	3.80	3.20	4.71	
Yield (%)			0.75	0.90	0.95	0.70	0.73	0.70	0.80	

[a]Compounds were identified by LTPRI Index (GC/FID) and Mass Spectrometry (GC/MS) using the Rtx ®-5MS column; [b]Tabulated Retention Index (Adams, 2007; Lemmon et al., 2011); [c]Retention index calculated from data obtained by sampling saturated n-alkanes (C7-C40); [d]Compounds with relative areas >2% have been identified; and [e]Terpene classes (HM: hydrogenated monoterpenes; OM: oxygenated monoterpenes; HS: hydrogenated sesquiterpenes); [f]Unidentified compounds.

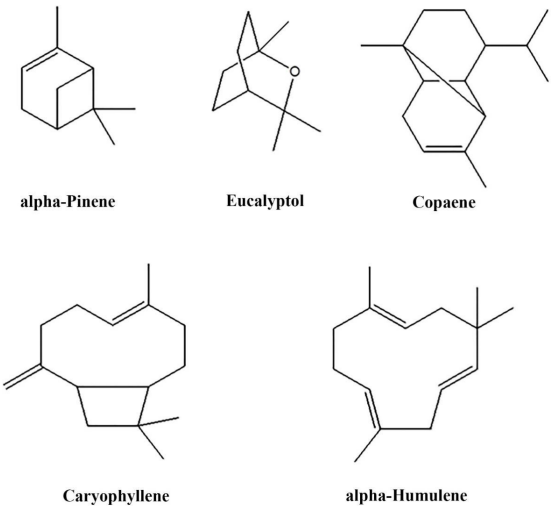

Figure 2. Chemical structure of the major compounds found in accessions of *Psidium cattleyanum*.

4. Discussion

Ploidy intraspecific variation was identified in *P. cattleyanum* accessions according to the flow cytometry analysis. This technique is a safe tool to detect polyploidy indirectly, according to Medina (2014), which reported that the 2C value would have a direct relationship with the ploidy level determined for cytotypes 2n=77 and 2n=88 of *P. cattleyanum* in Uruguay. Souza et al. (2015) also report that the variation of nuclear DNA content presented a coherent and proportional relationship with the ploidy level of the investigated *P. cattleyanum* accessions in their study. Furthermore, flow cytometry is considered a fast, reliable, and simple method to measure the level of ploidy and confirm polyploidy (Roy et al., 2001). According to Doležel et al. (2007), the ploidy level is indirectly inferred by its correlation with the relative or absolute DNA content (DNA ploidy level).

Polyploidy was already described in other species from the genus *Psidium*, such as *P. acutangulum* Mart. ex DC, *P. friedrichstalianum* (O. Berg) Nied, *P. guajava* L., *P. guinense* Sw., and others (Atchison, 1947; Hirano and Nakasone, 1969; Kumar and Ranade, 1952). Different ploidy levels were reported for the first time for *P. cattleyanum*, by Costa and Forni-Martins (2006), in which individuals with yellow and red fruits presented different chromosome numbers. However, the correlation of DNA content with the qualitative and quantitative characteristics of oil production in this species is described for the first time in this study.

The oil production of *P. cattleyanum* is expressive when compared to other *Psidium* species. Here, the oil production ranged from 0.70 to 0.95% (Table 1), while Souza et al. (2017) obtained a yield ranging from 0.17 to 0.56% in 22 genotypes of *P. guajava*. In addition to the "gigas" effect, which increases cell size and consequently the number of gene copies, the type of biosynthetic pathway present in the species can explain the high yield in *P. cattleyanum*. Some studies indicate that the terpene biosynthetic pathway is the main source of essential oil production (Külheim et al., 2015; Padovan et al., 2014; Webb et al., 2013; Webb et al., 2014). The essential oils of the analyzed plants are mostly compounded by terpenes, thus, plants with a larger genome present more terpene genes and consequently produce more oil.

In addition to the observation of three groups of plants with similar DNA contents and yields, can also be inferred that the profile of the compounds produced by them is similar (Figure 2). Thus, the presence of three different chemotypes in the samples is observed. The number of molecules of sesquiterpenes and monoterpenes produced by plants is close, however, monoterpenes were produced almost exclusively by individuals with lower DNA content (CAT 1, 4, 5, 6 and 8), while sesquiterpenes were produced in greater quantities and with greater variability in individuals with higher DNA content (CAT2 and CAT3).

The relative proportion of secondary metabolites occurs at different levels (seasonal and circadian-daily; intraplant, inter and intraspecific) and results from the cell specialization in which their manifestations occur according to the differential expression of genes (Souza et al., 2017). The pattern found in *P. cattleyanum* may occur due to the terpene synthase (TPS) gene family, which is a class of specialized enzymes for terpene biosynthesis. This family comprises three classes and seven subfamilies. Class I consists of TPS-c (copalyl diphosphate and ent-kaurene), TPS-e/f (ent-kaurene and other diterpenes, as well as some mono and sesquiterpenes) and TPS-h (specific to Selaginella). Class II consists of TPS-d (specific to gymnosperms) and class III presents TPS-a (sesquiterpenes), TPS-b (cyclic monoterpenes and hemiterpenes) and TPS-g (acyclic monoterpenes) (Külheim et al., 2015). *Psidium cattleyanum* plants, analyzed in this study, are supposed to present a TPS class III pattern (TPS-a and TPS-b).

Psidium cattleyanum presented a small variation in the types of terpenes found, which indicates the presence of identical TPS genes, which were possibly duplicated in these autopolyploids. For all the individuals, trans-caryophyllene, alpha-pinene, copaene, alpha-humulene and eucalyptol were the compounds that presented the highest averages in the relative proportions (Table 2 and Table S1). Such compounds have proven biological activities for medicinal purposes (Scur et al., 2016). Trans-caryophillene presents antileishmaniasis, antischistosomiasis and antifungal activity. Alpha-Humulene is anti-inflammatory. Alpha-pinene has antibacterial and antifungal effects (Hong et al., 2004). Eucalyptol has been widely used in the pharmaceutical industry for its antibacterial, antifungal, sedative, anticonvulsant (Monforte et al., 2011), and analgesic properties (Takaishi et al., 2012).

The two collection times showed little influence on the chemical composition of the analyzed plants, according to the chromatographic profile of the essential oils in the two extractions carried out, with the exception of the CAT6 accession (Table 2 and S1). Thus, the feasibility of exploiting the essential oil for pharmacological purposes is evidenced, since the production of the major compounds of interest was noticed in the two collection times.

5. Conclusion

The DNA content in *P. cattleyanum* directly influences the quantitative and qualitative characteristics of its essential oils, and plants with higher DNA content (CAT2 and CAT3) are recommended as possible matrices for the propagation of new plants with the potential to produce major compounds of agronomic and pharmacological interest such as trans-caryophyllene and alpha-humulene.

Acknowledgements

M.F.S. FERREIRA, M.M. Praça-Fontes and W.R. CLARINDO thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the research grant "Bolsa de Produtividade em Pesquisa". The authors are also greatful to Fundação de Amparo à Pesquisa e Inovação do Espírito Santo (FAPES) for "Universal" grant (#484/2021, 2021-ZK8PJ), and "Cooperação CAPES/FAPES – PDPG" grant. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior -Brasil (CAPES) - Finance Code 001.

References

ADAMS, R.P., 2007. *Identification of essential oil components by gas chromatography/mass spectroscopy*. 4th ed. Carol Stream: Allured Publishing Corporation.

AGÊNCIA DE VIGILÂNCIA SANITÁRIA – ANVISA, 2010. *Farmacopeia brasileira*. 5ª ed. Brasília: ANVISA, vol. 1.

ALMEIDA, L.F.R., PORTELLA, R.O., FACANALI, R., MARQUES, M.O.M. and FREI, F., 2014. Dry and wet seasons set the phytochemical profile of the *Copaifera langsdorffii* Desf. essential oils. *The Journal of Essential Oil Research*, vol. 26, no. 1, pp. 292-300. http://dx.doi.org/10.1080/10412905.2014.889050.

ATCHISON, E., 1947. Chromosome numbers in the Myrtaceae. *American Journal of Botany*, vol. 34, no. 3, pp. 159-164. http://dx.doi.org/10.1002/j.1537-2197.1947.tb12970.x. PMid:20295180.

BANIAGA, A.E., MARX, H.E., ARRIGO, N. and BARKER, M.S., 2020. Polyploid plants have faster rates of multivariate niche differentiation than their diploid relatives. *Ecology Letters*, vol. 23, no. 1, pp. 68-78. http://dx.doi.org/10.1111/ele.13402. PMid:31637845.

BEEST, M., LE ROUX, J.J., RICHARDSON, D.M., BRYSTING, A.K., SUDA, J., KUBESOVÁ, M. and PYSEK, P., 2012. The more the better? The role of polyploidy in facilitating plant invasions. *Annals of Botany*, vol. 109, no. 1, pp. 19-45. http://dx.doi.org/10.1093/aob/mcr277. PMid:22040744.

COMAI, L., 2005. The advantages and disadvantages of being polyploid. *Nature Reviews. Genetics*, vol. 6, no. 11, pp. 836-846. http://dx.doi.org/10.1038/nrg1711. PMid:16304599.

COSTA, I.R. and FORNI-MARTINS, E.R., 2006. Chromosome studies in Brazilian species of *Campomanesia* Ruiz & Pávon and *Psidium* L. (*Myrtaceae Juss.*). *Caryologia*, vol. 59, no. 1, pp. 7-13. http://dx.doi.org/10.1080/00087114.2006.10797891.

COSTA, I.R., DORNELAS, M.C. and FORNI-MARTINS, E.R., 2008. Evolution of nuclear DNA amounts in Neotropical Myrtaceae (fleshy-fruited Myrteae). *Plant Systematics and Evolution*, vol. 276, no. 3-4, pp. 209-217. http://dx.doi.org/10.1007/s00606-008-0088-x.

DOLEŽEL, J. and BARTOŠ, J., 2005. Plant DNA flow cytometry and estimation of nuclear genome size. *Annals of Botany*, vol. 95, no. 1, pp. 99-110. http://dx.doi.org/10.1093/aob/mci005. PMid:15596459.

DOLEŽEL, J., GREILHUBER, J. and SUDA, J., 2007. *Flow cytometry with plant cells: analysis of genes, chromosomes and genomes*. Weinheim: Wiley-VCH. http://dx.doi.org/10.1002/9783527610921.

HIRANO, R.T. and NAKASONE, H.Y., 1969. Chromosome numbers of ten species and clones in the genus *Psidium*. *Journal of the American Society for Horticultural Science*, vol. 94, no. 2, pp. 83-86. http://dx.doi.org/10.21273/JASHS.94.2.83.

HONG, E.J., NA, K.J., CHOI, I.G., CHOI, K.C. and JEUNG, E.B., 2004. Antibacterial and antifungal effects of essential oils from coniferous trees. *Biological & Pharmaceutical Bulletin*, vol. 27, no. 6, pp. 863-866. http://dx.doi.org/10.1248/bpb.27.863. PMid:15187434.

IANNICELLI, M.A., ELECHOSA, M.A., JUÁREZ, A., MARTINEZ, V., BUGALLO, A.L., BANDONI, A.S., ESCANDÓN, C.M. and VAN BAREN, C.M., 2016. Effect of polyploidization in the production of essential oils in *Lippia integrifolia*. *Industrial Crops and Products*, vol. 81, no. 1, pp. 20-29. http://dx.doi.org/10.1016/j.indcrop.2015.11.053.

JIAO, Y., WICKETT, N.J., AYYAMPALAYAM, S., CHANDERBALI, A.S., LANDHERR, L., RALPH, E.P., TOMSHO, L.P., HU, Y., LIANG, H., SOLTIS, P.S., SOLTIS, D.E., CLIFTON, S.W., SCHLARBAUM, S.E., SCHUSTER, S.C., MA, H., LEEBENS-MACK, J. and PAMPHILIS, C.W., 2011. Ancestral polyploidy in seed plants and angiosperms. *Nature*, vol. 473, no. 7345, pp. 97-100. http://dx.doi.org/10.1038/nature09916. PMid:21478875.

KÜLHEIM, C.S.H., PADOVAN, A., HEFER, C., KRAUSE, S.T., KOLLNER, T.G., MYBURG, A.A., DEGENHARDT, J. and FOLEY, W.J., 2015. The *Eucalyptus* terpene synthase gene Family. *BMC Genomics*, vol. 16, no. 1, pp. 450. http://dx.doi.org/10.1186/s12864-015-1598-x. PMid:26062733.

KUMAR, L.S.S. and RANADE, S.G., 1952. Autotriploidy in guava (*Psidium guajava* Linn.). *Current Science*, vol. 21, pp. 75-76.

LEMMON, E.W., MCLINDEN, M.O., FRIEND, D.G., LINSTROM, P. and MALLARD, W., 2011. *NIST standard reference database 69: NIST chemistry WebBook*. Gaithersburg: NIST.

MACHADO, R.M., OLIVEIRA, F.A., ALVES, F.M., SOUZA, A.P. and FORNI-MARTINS, E.R., 2021. Population genetics of polyploid complex *Psidium cattleyanum* Sabine (Myrtaceae): preliminary analyses based on new species-specific microsatellite loci and extension to other species of the genus. *Biochemical Genetics*, vol. 59, no. 1, pp. 219-234. http://dx.doi.org/10.1007/s10528-020-10002-1. PMid:32980958.

MACHADO, R.M., OLIVEIRA, F.O., CASTELLO, A.C.D., ALVES, F.M., SOUZA, A.P. and FORNI-MARTINS, E.R., 2022. Population structure and intraspecifc ecological niche diferentiation point to lineage divergence promoted by polyploidization in *Psidium cattleyanum* (Myrtaceae). *Tree Genetics & Genomes*, vol. 18, no. 3, pp. 1-13. http://dx.doi.org/10.1007/s11295-022-01551-0.

MARQUES, A., TULER, A.C., CARVALHO, C.R., CARRIJO, T., SILVA FERREIRA, M.F. and CLARINDO, W.R., 2016. Refinement of the karyological aspects of *Psidium guineense* Swartz, 1788: a comparison with *Psidium guajava* Linnaeus, 1753. *Comparative Cytogenetics*, vol. 10, no. 1, pp. 117-128. http://dx.doi.org/10.3897/CompCytogen.v10i1.6462. PMid:27186342.

MEDINA, S.N.V., 2014. *Psidium cattleyanum Sabine y Acca sellowiana (Berg.) Burret (Myrtaceae): caracterización cromossómica y cariotípica em poblaciones silvestre y genótipos selecionados em programas nacionales de mejoramiento*. Uruguai: Faculdade de Agronomia, Universidade da República, 96 p. Monografia em Ciências Biológicas.

MONFORTE, M.T., TZAKOU, O., NOSTRO, A., ZIMBALATTI, V. and GALATI, E.M., 2011. Chemical Composition and biological activities of *Calamintha officinalis* Moench essential oil. *Journal of Medicinal Food*, vol. 14, no. 3, pp. 297-303. http://dx.doi.org/10.1089/jmf.2009.0191. PMid:21142949.

MOURA, R.F., QUEIROGA, D., VILELA, E. and MORAES, A.P., 2021. Polyploidy and high environmental tolerance increase the invasive success of plants. *Journal of Plant Research*, vol. 134, no. 1, pp. 105-114. http://dx.doi.org/10.1007/s10265-020-01236-6. PMid:33155178.

NEGRINI, M., FIDELIS, E.G., SCHURT, D.A., SILVA, F.S., PEREIRA, R.S. and BIZZO, H.R., 2019. Insecticidal activity of essential oils in controlling fall armyworm, Spodoptera frugiperda. *Arquivos do Instituto Biológico*, vol. 86, e1112018. http://dx.doi.org/10.1590/1808-1657001112018.

OTTO, F., 1990. DAPI staining of fixed cells for high-resolution flow cytometry of nuclear DNA. *Methods in Cell Biology*, vol. 33, pp. 105-110. http://dx.doi.org/10.1016/S0091-679X(08)60516-6. PMid:1707478.

PADOVAN, A., KESZEI, A., KÜLHEIM, C. and FOLEY, W.J., 2014. The evolution of foliar terpene diversity in Myrtaceae. *Phytochemistry Reviews*, vol. 13, no. 3, pp. 695-716. http://dx.doi.org/10.1007/s11101-013-9331-3.

PRAÇA-FONTES, M.M., CARVALHO, C.R., CLARINDO, W.R. and CRUZ, C.D., 2011. Revisiting the DNA C-values of the genome size-standards used in plant flow cytometry to choose th "best primary standards. *Plant Cell Reports*, vol. 30, no. 7, pp. 1183-1191. http://dx.doi.org/10.1007/s00299-011-1026-x. PMid:21318354.

R CORE TEAM, 2021 [viewed 27 January 2022]. *R: a language and environment for statistical computing* [online]. Vienna: R Foundation for Statistical Computing. Available from: https://www.R-project.org/

ROY, A.T., LEGGETT, G. and KOUTOULIS, A., 2001. In vitro tetraploid induction and generation of tetraploids from mixoploids in hop (*Humulus lupulus* L.). *Plant Cell Reports*, vol. 20, no. 6, pp. 489-495. http://dx.doi.org/10.1007/s002990100364.

SATTLER, M.C., CARVALHO, C.R. and CLARINDO, W.R., 2016. The polyploidy and its key role in plant breeding. *Planta*, vol. 243, no. 2, pp. 281-296. http://dx.doi.org/10.1007/s00425-015-2450-x. PMid:26715561.

SCUR, M.C., PINTO, F.G.S., PANDINI, J.A., COSTA, W.F., LEITE, C.W. and TEMPONI, L.G., 2016. Antimicrobial and antioxidant activity of essential oil and different plant extracts of *Psidium cattleianum* Sabine. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 76, no. 1, pp. 101-108. http://dx.doi.org/10.1590/1519-6984.13714. PMid:26871744.

SHOLBERG, P.L. and GAUNCE, A.P., 1995. Fumigation of fruit with acetic acid to prevent postharvest decay. *Horticultural Science*, vol. 30, no. 1, pp. 1271-1275.

SOUZA, A.G.D., RESENDE, L.V., LIMA, I.P.D., MARTINS, L.S.S. and TECHIO, V.H., 2015. Chromosome number and nuclear DNA amount in *Psidium* app. resistant and susceptible to *Meloidogyne enterolobii* and its relation with compatibility between rootstocks and commercial varieties of guava tree. *Plant Systematics and Evolution*, vol. 301, no. 1, pp. 231-237. http://dx.doi.org/10.1007/s00606-014-1068-y.

SOUZA, T.S., FERREIRA, M.F.S., MENINI, L., SOUZA, J.R.C.L., PARREIRA, L.A., CECON, P.R. and FERREIRA, A., 2017. Essential oil of *Psidium guajava*: influence of genotypes and environment. *Scientia Horticulturae*, vol. 216, pp. 38-44. http://dx.doi.org/10.1016/j.scienta.2016.12.026.

STEBBINS, G.L., 1950. *Variation and evolution in plants*. New York: Columbia University Press. http://dx.doi.org/10.7312/steb94536.

TAKAISHI, M., FUJITA, F., UCHIDA, K., YAMAMOTO, S., SAWADA, M., HATAI, C., SHIMIZU, M. and TOMINAGA, M., 2012. 1, 8-cineole, a TRPM8 agonist, is a novel natural antagonist of human TRPA1. *Molecular Pain*, vol. 8, no. 1, pp. 1744-8069. http://dx.doi.org/10.1186/1744-8069-8-86. PMid:23192000.

TULER, A.C., CARRIJO, T.T., NÓIA, L.R., FERREIRA, A., PEIXOTO, A.L. and SILVA FERREIRA, M.F., 2015. SSR markers: a tool for species identifcation in *Psidium* Myrtaceae. *Molecular Biology Reports*, vol. 42, no. 11, pp. 1501-1513. http://dx.doi.org/10.1007/s11033-015-3927-1. PMid:26476530.

TULER, A.C., CARRIJO, T.T., PEIXOTO, A.L., GARBIN, M.L., FERREIRA, M.F.S., CARVALHO, C.R., SPADETO, M.S. and CLARINDO, W.R., 2019. Diversifcation and geo- graphical distribution of *Psidium* Myrtaceae species with distinct ploidy levels. *Trees*, vol. 33, no. 4, pp. 1101-1110. http://dx.doi.org/10.1007/s00468-019-01845-2.

WEBB, H., FOLEY, W.J. and KÜLHEIM, C., 2014. The genetic basis of foliar terpene yield: implications for breeding and profitability of Australian essential oil crops. *Plant Biotechnology*, vol. 31, no. 5, pp. 363-376. http://dx.doi.org/10.5511/plantbiotechnology.14.1009a.

WEBB, H., LANFEAR, R., HAMILL, J., FOLEY, W.J. and KÜLHEIM, C., 2013. The yield of essential oils in *Melaleuca alternifolia* (Myrtaceae) is regulated through transcript abundance of genes in the MEP pathway. *PLoS One*, vol. 8, no. 3, e60631. http://dx.doi.org/10.1371/journal.pone.0060631. PMid:23544156.

Supplementary Material

Table S1. Identification of essential oil compounds from six accessions of *Psidium cattleyanum* extracted from leaves collected in February 2018.

Compounds[a]	Retention time (min)[b]	Retention index[c]	Relative area[d]							Terpene classes[e]
			CAT1	CAT2	CAT3	CAT4	CAT5	CAT6	CAT8	
alpha-Pinene	8.795	933	10.35	2.03	6.06	**23.80**	**19.56**	**24.46**	4.88	HM
beta-Pinene	10.725	976				3.62				HM
Myrcene	11.520	990	8.16					2.26		HM
Limonene	12.925	1025						3.11	9.17	HM
Eucalyptol	13.280	1033				**27.70**	**24.34**	**25.48**	3.80	OM
Beta-ocimene	13.735	1041				5.20	4.17			HM
alpha-Terpineol	20.805	1192				4.38	3.45			OM
Copaene	29.030	1374		**9.88**	**9.90**			6.25	3.68	HS
trans-Caryophyllene	30.820	1417	**72.16**	**62.27**	**58.45**	**35.30**	**41.80**	**21.86**	**57.05**	HS
alpha-Humulene	32.270	1453	6.68	**13.93**	**13.38**		3.11	**10.50**	**11.68**	HS
alpha-Amorphene	33.255	1476	2.65				3.57			HS
beta.-Selinene	34.279	1490							2.96	HS
alpha.-selinene	34.672	1498							2.72	HS
delta-Cadinene	35.175	1523		6.78	6.34			6.08	4.06	HS
NI[f]	37.455	-			3.44					
Viridiflorol	37.485	1580		2.61						OS
NI	39.220	-		2.50	2.43					

[a]Compounds were identified by LTPRI Index (GC/FID) and Mass Spectrometry (GC/MS) using the Rtx ®-5MS column; [b]Tabulated Retention Index (Adams, 2007; Lemmon et al., 2011); [c]Retention index calculated from data obtained by sampling saturated n-alkanes (C7-C40); [d]Compounds with relative areas >2% have been identified; and [e]Terpene classes (HM: hydrogenated monoterpenes; OM: oxygenated monoterpenes; HS: hydrogenated sesquiterpenes); [f]Unidentified compounds.

This material is available as part of the online article from 10.1590/1519-6984.260455