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Chemical Diversity of Volatiles From Parents, Rootstock and Atemoya Hybrid

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Abstract

Hybridization promotes the transfer of genetic material from parental species to the hybrid, which can be closely related to one of its parental species or present new features that provide them higher competitivity concerning other chemical phenotype comparing to its parents. On the other hand, grafting technique, in which occurs the combination between two species, also may lead to changes in the volatile profile of terpenes at the grafted plant. The objective of this research was to characterize the chemical profile of volatile compounds in leaves of atemoya hybrid (*Annona* × *Atemoya*) (Mobb.), its male and female parents (*Annona squamosa* Mill. and *Annona cherimola* L.), as well as its common rootstock (*Annona emarginata* (Schltdl.) H. Rainer var. “Terra-fria”). Leaf samples from atemoya, its parents and the rootstock were collected in São Bento do Sapucaí and Santa Fé do Sul, São Paulo state, Brazil. Volatile substances were obtained by microextraction solid phase (SPME). Chemical profile of volatile compounds was determined and identified by gas chromatography coupled to mass spectrometry. Atemoya hybrid presented substances also found in both parents and rootstock, with a variation on the relative percentage of compounds. Such variation allowed to form three clusters, where I was comprised by the hybrid, II comprised by *A. cherimola*, and the latter, III, grouping the rootstock and the male parent. We concluded that hybridization and grafting influence production of terpenes in atemoya hybrid (*Annona* × *Atemoya*).

Keywords: *Annona* × *atemoya*, *Annona cherimola*, *Annona squamosa*, *Annona emarginata*, terpenes

1. Introduction

Hybridization allows the transfer of genetic material from parental species to the hybrid, which can be more related to one of its parents or show features completely new. Hybrids can present variable adaptation to the local conditions where they occur, which, added to the inherited features from parents, could provide them higher competitivity comparing to the endemic and rare species (López-Caamal & Tovar-Sánchez, 2014). Though, hybrid phenotype could be typically more robust than that from its parents, resulting in a remarkable importance to agriculture (Greaves et al., 2015).

The Annonaceae family is comprised by the genus *Annona*, one of its most important, especially due to its edible fruits like those of atemoya (*Anonna* × *atemoya*), a hybrid formed by the outcrossing between *Annona cherimola* Mill. and Annona squamosa L. Atemoya is characterized by intermediate climatic adaptation in relation to its parents, like fruit quality conferred by *A. cherimola*, as well as the resistance to the climatic conditions conferred by *A. squamosa* (Tokunaga, 2005).

The theme complexity increases, even more, when hybridization is considered together with grafting process. Apparently, the processes of grafting regulation and control occur due to water and mineral nutrients absorption, hormone synthesis, and because of both protein and mRNA migration (Baron, Bravo, Maia, Pina, & Ferreira, 2016; Davis et al., 2008; Harada, 2010), added to transfer of plastidial DNA from rootstock to the graft (Stegemann & Bock, 2009). The choose of rootstock species is related to the resistance increase against pathogens, faster dissemination and production of fruits, and the ability to impact the graft phenotype, although such mechanisms are not well understood yet (Warschefsky et al., 2016).

Typically, atemoya is grafted in *Annona emarginata* (Schltdl.) H. Rainer var. 'Terra-fria' which confers diseases and pathogens resistance to hybrid, favoring its cultivation (Baron, Amaro, Pina, & Ferreira, 2019). Grafting influences the production and chemical profile of volatiles from hybrid essential oils relatively to its parents by the transfer of genetic material and, subsequently, modification in metabolic routes as well as in the specialized metabolism (Cano & Bermejo, 2011; Harada, 2010).

The production and composition of specialized metabolites could be influenced by the relationship between water availability and transpiration (Santos et al., 2015), phenophase (Herraiz-Peñalver, Ortiz De Elguea-Culebras, Sánchez-Vioque, & Santana Méridas, 2015), development (De-La-Cruz Chacón, Riley-Saldaña, & González-Esquinca, 2013), and vegetative organ from where it is extracted (Ceccarini et al., 2004).

Chemical profile of essential oils can variate between parents and hybrid, being that the latter produces higher diversity of substances, (Alzogaray, Lucia, Zerba, & Masuh, 2011) or present a similar and closer profile concerning to one of its parents (Vereecken, Cozzolino, & Schiestl, 2010; Zito, Tavella, Sajeva, Carimi, & Dötterl, 2018). Typically, hybrid shows an intermediate chemical profile from its parents (Herraiz-Peñalver et al., 2015; Ruberto & Rapisarda, 2002; Zito et al., 2018).

The expression of specialized metabolites is frequently regulated by different genes, which could explain an intermediate chemical volatile profile in hybrids and its parents (Cheng, Vrieling, & Klinkhamer, 2011; López-Caamal & Tovar-Sánchez, 2014). Variations in genetic expression of specialized metabolites could be linked to changing of its epigenome (Greaves et al., 2015), indicating an inheritance complex pattern from this trait (Cheng et al., 2011; Zito et al., 2018).

Once insects may be the main allies for several species pollination, as well as in the interaction between plants and defense against herbivores and pathogens (Alzogaray et al., 2011; Hommel et al., 2016; Wang, Liu, Wei, & Yan, 2012; Zito et al., 2018), the fluctuations in chemical profile could be important for comprehending plant survival strategies, defense and reproduction.

Essential oils present several pharmacological properties (Bakkali, Averbeck, Averbeck, & Idaomar, 2008; Nakatsu, Lupo, Chinn, & Kang, 2000), as cytotoxic and abortive effect, immunity suppressing, appetite inhibitor, antiemetic, antimicrobial, pesticide, vermicide, antimalarial and insecticidal (Garavito et al., 2006; Costa et al., 2008; da Silva Almeida et al., 2010; Costa et al., 2011; Langhasova et al., 2014; Sharkey et al., 2017; Hu et al., 2017; Fierascu et al., 2018). These essential oils confer perfumed scents due to its composition, especially, monoterpenes and sesquiterpenes, as bicyclogermacrene, (*E*)-caryophyllene, γ-muurolene, α-humulene, spathulenol, and β-elemene.

Species from Annonaceae family, including parents, rootstock and hybrids, present essential oils with different substances that, besides acting in defense strategies and pollinators attraction for reproduction and survival, also may show pharmacological properties with economic importance.

The objective of this research was to characterize and compare chemical profile composition of leaf volatiles in hybrid *Annona* × *atemoya* (Mobb.), with its parents *Annona cherimola* L., *Annona squamosa* Mill. and the rootstock *Annona emarginata* (Schltdl.) H. Rainer var. "Terra-fria".

2. Material and Methods

2.1 Study Area

Leaf samples from hybrid (*Annona* × *atemoya*), female parent (*Annona cherimola*) and the rootstock (*Annona emarginata*) were collected at Núcleo de Produção de Mudas da CATI (Coordenadoria de Assistência Técnica e Integrada) in municipality of São Bento do Sapucaí (22°41′18″ South; 45°44′11″ West, 874 m, SP, Brazil). Leaves from male parent (*Annona squamosa*), species that is usually cultivated in warmer regions, were collected in municipality Santa Fé do Sul, SP (20°12′43″ South; 50º55'33" West, 370 m, SP, Brazil). The samplings were carried out between 9:00-10:00 hours and stored in paper bags.

2.2 Capture and Analysis of Volatile Compounds

Leaves were dried in a forced air oven with a temperature held at 30 °C to achieve its constant dry mass. Volatile substances were captured by headspace-solid phase microextraction (HS-SPME). So, 50 mL of leaf dry mass grinded were stored in a 20 mL glass vial with a cover provided of a septum, and subsequently added 10 mL of distilled water. The vial glass was heated for 1 hour in a water bath at 90 °C. Afterward, the volatile compounds were captured by headspace for 15 minutes using a DVB/PDMS fiber (Manual Holder-SUPELCO). The extraction of volatile compounds was executed in triplicate for each sample.

Chemical analysis of volatile compounds was determined by gas chromatograph coupled to a mass spectrometer (GC-MS-Shimadzu, QP-5000), operating at 70 eV, equipped with a DB-5 fused silica capillary column (30 m × 0.25 mm × 0.25 µm), helium as carrier gas (1.0 mL/min), injector at 240 °C, detector at 230 °C, split: 1/20, using the temperature program of 60-240 °C, 3 °C/min.

Substances were identified by comparing its mass spectrum with data bank from CG-EM (Nist 62.lib) system; Retention indexes (IR) and literature data (Adams, 2017). Retention indexes (IR) of the substances were obtained by analysis of the mixture of n-alkanes (C_9-C_{24} Sigma Aldrich 99%), at the same operational conditions of samples, in which it was applied the equation of Van den Dool & Kratz , 1963 (Van Den Dool & Dec. Kratz, 1963).

2.3 Statistical Analysis

Principal component analysis (PCA) and Hierarchical agglomerative cluster (HAC) (algorithm UPGMA, similarity index, Pearson correlation coefficient) were carried out with relative percentages of identified substances (software XLSTAT v.2017 ADDINSOFT®, 2017).

3. Results

In this study were identified fifty volatile substances, distributed in mono and sesquiterpenes, from parents, rootstock, and hybrid. Among them, 85.93% were verified in *A. cherimola*, 94.60% in *A. squamosa*, 96.40% in *A. emarginata* and 94.02% in atemoya (*Annona* × *atemoya*) (Table 1).

Table 1. Mean chemical composition (relative percentage) from the components in atemoya hybrid (*Annona* × *atemoya*), its female parent (*Annona cherimola*), male parent (*Annona squamosa*), and rootstock (*Annona emarginata*)

Substances	IR_E	IR_L	*Annona cherimola* ♀	*Annona squamosa* ♂	*Annona emarginata (rootstock)*	*Annona × atemoya (hybrid)*
Monoterpene Hydrocarbons						
Tricyclene	924	926	t	t	0.56	0.54
α-Pinene	931	939	11.62	3.52	10.07	17.76
Camphene	946	954	2.10	9.08	3.51	0.82
Sabinene	973	975	0.01	0.24	0.37	0.63
β-Pinene	975	979	16.99	3.18	6.29	11.43
Myrcene	989	990	0.84	2.33	4.73	7.39
δ-3-Carene	1004	1011	0.66	t	t	0.88
α-Terpinene	1020	1017	0.01	t	t	0.74
ρ-Cymene	1023	1024	5.16	t	1.28	3.22
Limonene	1027	1029	2.45	2.77	13.08	15.51
(*Z*)-β-Ocimene	1035	1037	t	t	t	0.60
(*E*)-β-Ocimene	1045	1050	t	t	t	0.92
γ-Terpinene	1057	1059	0.59	t	0.45	0.96
Terpinolene	1087	1088	0.01	t	t	0.66
m-Cymenene	1087	1085	1.06	t	0.73	t
Oxygenated Monoterpenes						
1,8-Cineole	1029	1031	0.99	t	4.23	0.01
Linalool	1099	1096	9.63	t	0.43	2.23
α-Campholenal	1124	1126	1.79	t	t	t
Pinocarvone	1159	1164	3.77	t	t	t
Myrtenol	1193	1195	5.17	t	t	t
Sesquiterpene Hydrocarbons						
δ-Elemene	1333	1338	1.81	5.59	t	0.52
α-Cubebene	1345	1348	t	t	0.49	0.09
α-Copaene	1372	1376	t	0.25	0.72	0.55
β-Bourbonene	1381	1388	t	0.27	1.28	0.88
β-Elemene	1392	1390	1.88	5.54	0.59	1.37

Sesquithujene	1402	1405	t	t	0.43	t
trans-Caryophyllene	1415	1419	1.85	35.82	20.50	2.59
β-Copaene	1425	1432	t	0.25	0.67	0.09
Aromadendrene	1434	1441	t	0.17	0.90	0.09
α-Humulene	1449	1454	t	4.80	1.86	0.53
allo-Aromadendrene	1456	1460	t	0.38	0.81	0.42
cis-Muurola-4(14),5-diene	1458	1466	t	0.29	t	0.13
γ-Muurolene	1471	1479	t	0.72	0.88	0.35
γ-Gurjunene	1477	1477	4.09	4.92	2.07	9.11
ar-Curcumene	1478	1480	t	t	10.27	t
Germacrene D	1486	1481	0.50	0.40	0.01	0.26
δ-Selinene	1491	1492	0.74	6.76	4.84	8.12
α-Muurolene	1495	1500	t	0.71	0.27	0.27
β-Bisabolene	1499	1505	t	2.11	0.01	0.44
γ-Cadinene	1508	1513	t	2.35	0.56	0.44
Oxygenated Sesquiterpenes						
Cubebol	1517	1515	0.91	1.56	2.78	0.63
Spathulenol	1577	1578	0.70	t	0.28	0.45
Caryophyllene oxide	1582	1583	2.18	t	t	t
Cubenol	1640	1646	t	0.33	t	t
Other classes						
6-methyl-5-hepten-2-one	983	985	1.00	t	t	t
n-Decane	999	1000	0.21	t	t	t
2-Nonanone	1090	1090	5.53	t	t	1.99
n-Nonanal	1102	1100	1.29	t	0.30	t
2-Decanone	1190	1192	0.01	t	t	0.29
n-Decanal	1203	1201	0.21	t	t	t
Total identified			85.93	94.60	96.40	94.02
Monoterpene Hydrocarbons			41.52	21.21	41.12	62.05
Oxygenated Monoterpenes			21.35	0.05	4.69	2.27
Sesquiterpene Hydrocarbons			11.01	71.36	47.16	26.27
Oxygenated Sesquiterpenes			3.81	1.92	3.08	1.10
Other classes			8.25	0.06	0.35	2.32

Note. IR_E = retention index experimental; IR_L = retention index literature (Adams, 2007); t ≤ 0.2.

Female parent and hybrid presented major relative percentages of monoterpenes (62.86% and 64.32%; respectively). The male parent presented major relative percentages of sesquiterpenes (73.28%), and the rootstock showed similar relative percentages of mono and sesquiterpenes (45.81% and 50.24%; respectively). Among mono and sesquiterpenes, *Annona cherimola*, female parent, the predominant substances were α-pinene (11.62%), β-pinene (16.99%) and linalool (9.63%). Major substances in *A. squamosa*, male parent, were *trans*-caryophyllene (35.82%), camphene (9.08%) and δ-selinene (6.76%). Volatile compounds from rootstock, *A. emarginata*, were predominantly α-pinene (10.07%), β-pinene (6.29%), limonene (13.08%), *trans*-caryophyllene (20.50%) and *ar*-curcumene (10.27%). Major substances in atemoya hybrid were α-pinene (17.76%), β-pinene (11.43%), limonene (15.51%), γ-gurjunene (9.11%), δ-selinene (8.12%) (Table 1).

Principal component analysis with the identified substances indicates that *A. emarginata* and *A. squamosa* have chemical profile discriminated by *trans*-caryophyllene, while atemoya hybrid (*Annona* × *atemoya*) is discriminated by α and β-pinene, and at last, *A. cherimola* is discriminated by α and β-pinene, *p*-cymene, 2-nonanone, linalool and γ-gurjunene (Figure 1).

The dendrogram presents the formation of three clusters, in which cluster I is comprised by atemoya hybrid, cluster II by *A. cherimola* and, III groups the rootstock *A. emarginata* and male parent *A. squamosa* (Figure 2).

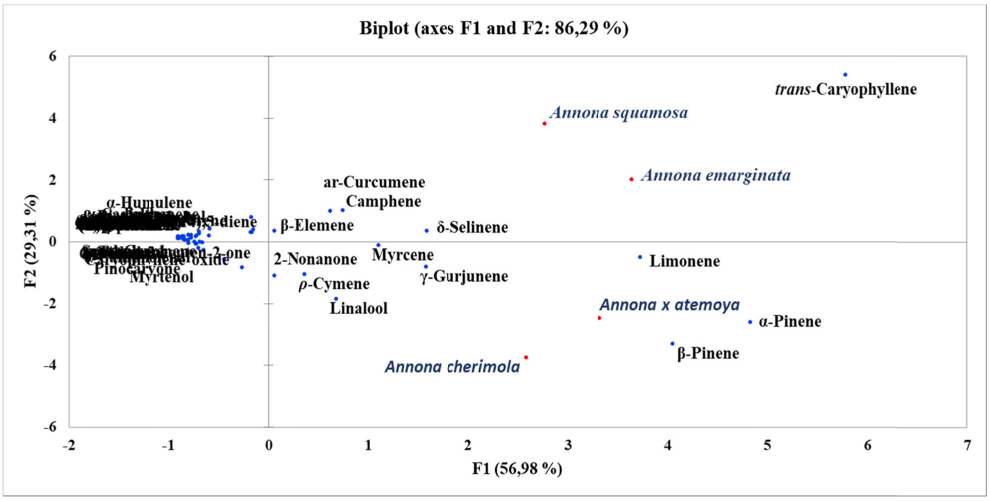

Figure 1. Principal component analysis (PCA) with chemical profile of volatiles substances from atemoya hybrid (*Annona* × *atemoya*), its female parent (*Annona cherimola*), male parent (*Annona squamosa*), and its rootstock (*Annona emarginata*)

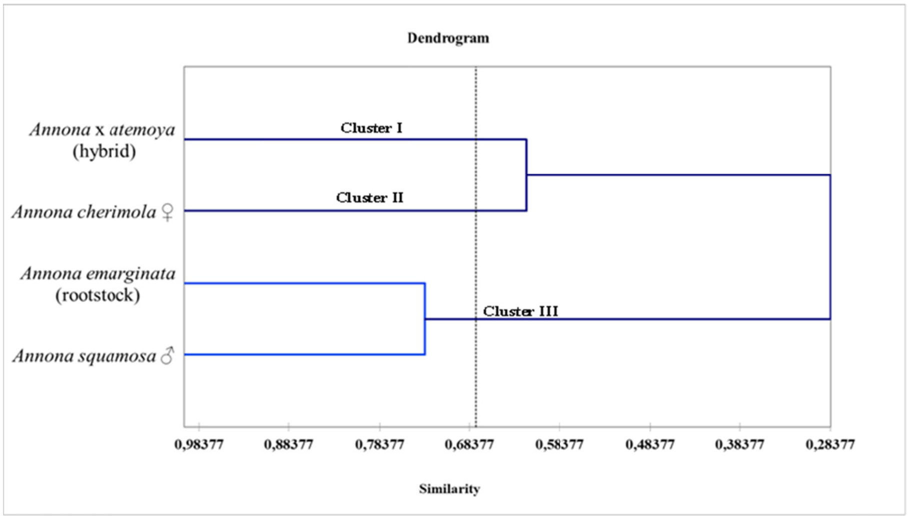

Figure 2. Dendrogram achieved by hierarchical agglomerative clustering analysis (HAC) with volatile substances from atemoya hybrid (*Annona* × *atemoya*), its female parent (*Annona cherimola*), male parent (*Annona squamosa*), and rootstock (*Annona emarginata*)

4. Discussion

Chemical profile of volatile compounds achieved demonstrated that there is a clear relationship between these substances found in female parent (*Annona cherimola*), male parent (*A. squamosa*), rootstock (*A. emarginata*) and those ones present in atemoya hybrid (*Annona* × *atemoya*).

Our results indicate that the hybrid (*Annona* × *atemoya*) have intermediate volatile substances also present in its parents, according to the also found in the literature (Cheng et al., 2011). However, this hybrid has similar substances with those that occur in the rootstock (*A. emarginata*). It must be remarked that this is the first result demonstrating this kind of relationship between hybrid (*Annona* × *atemoya*) and rootstock (*A. emarginata*) (Campos et al., 2019), which constitute part of a major study of our research group.

The dendrogram and PCA showed a similarity between rootstock (*A. emarginata*) and male parent (*A. squamosa*). In the same way, it was denoted a similarity between hybrid (*Annona* × *atemoya*) and its female parent (*Annona cherimola*). Hence, substances present in chemical profile of hybrid could be expressed as a result of genetic material transfer from both parents and rootstock, which suggest once again, that the compatibility and success in hybrid grafting frequently occur due to similar features between rootstock and at least one its parents, according to the already recorded by Baron et al. (2019), condition that may be suggested by this present work about chemical profile of volatiles.

According to the interpretation of Cheng et al. (2011), the studied chemical profiles of volatiles reveal that atemoya hybrid (*Annona* × *atemoya*) expressed intermediate phenotype in relation to its parents. However, our results reveal that atemoya also present a chemical profile intermediate to the rootstock. These results are in agreement with studies in literature, which report that the majority of specialized metabolites can be expressed in similar concentrations or even intermediate to those ones from its parents (Zito et al., 2018). Besides, in hybrid, substances may have its relative percentages raised or even suppressed (Cheng et al., 2011; López-Caamal & Tovar-Sánchez, 2014), condition that can contribute with the intermediate chemical profile.

Although it was not possible to identify and confirm the origin of substances presented in hybrid from its parents and rootstock, hybridization and grafting associated to both environment and intraspecific conditions, may contribute to variations in the expression of the volatile profile (Selmar & Kleinwächter, 2013; Zito et al., 2018). This variation can provide greater resistance to pathogens, allowing a better environment adaptation in different climatic conditions (Baron et al., 2019; Chezem & Clay, 2016).

Predominant substances responsible by discriminating the volatile profile of female parent (*Annona cherimola*), male parent (*A. squamosa*), rootstock (*A. emarginata*), and the hybrid (*Annona* × *atemoya*), suggest the expression of important volatiles for each one, which can be tightly related to plant defense (Alzogaray et al., 2011; Brophy, Forster, Goldsack, Hibbert, & Punruckvong, 2009; López-Caamal & Tovar-Sánchez, 2014; Zito et al., 2018), such as *trans*-caryophyllene, limonene, p-cymene, α and β-pinene, substances to have bactericidal activity (Marchese et al., 2017; Perigo et al., 2016; Ríos, Castrejón, Robledo, León, & Rojas, 2003) and may have contributed to the hybrid plant defense.

Despite the variation in relative percentage and substances predominance of volatile profile from both parents and rootstock, these plants show similar substances, which suggest that hybridization and grafting influence terpene production in atemoya hybrid.

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