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Profile of Essential Oils From the Leaves of Annona Grafted

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Abstract

Mechanical damage, during grafting, results in the formation of reactive oxygen species, which are neutralized by the enzymatic and non-enzymatic antioxidant systems which may influence the essential oil composition of grafts and rootstocks because of the formation of oxygenated terpenes, substances with higher reactivity against pathogens. Various studies have demonstrated the biological activity of Annonaceae and the important pharmacological potential of the substances produced by the genus *Annona*. Thus, we studied the essential oils of leaves collected from the graft (*Annona* × *atemoya*) and lateral budding from the rootstock (*Annona emarginata*), in the same individual, in order to characterize chemical profile. The extraction was carried out by hydrodistillation and separation, quantification and identification of the substances were performed by gas chromatography coupled to a mass spectrometer. Chemical profiles of essential oils were evaluated by principal component analysis (PCA). *Annona* × *atemoya* and *Annona emarginata* presented chemical profiles of essential oil with specific substances. Some substances are common in oils of both species. Germacrene D is predominant in *Annona* × *atemoya* and α and β-selinene, β-elemene and spathulenol occurred in *Annona emarginata*. Our results suggest that the profile of such essential oil may be another indicator for the success of the combination of these two species.

Keywords: *Annona emarginata* (Schltdl.) H. Rainer ‘araticum-de-terra-fria’, atemoya, specialized metabolism, oxygenated terpene, terpenes

1. Introduction

The *Annonaceae* family has the important genus *Annona*, mainly because of its edible fruits. Several studies have demonstrated in *Annona* biological activity of substances, which have important pharmacological potential (Boyom et al., 2011; Fontes et al., 2013; D. M. S. Ocampo & R. C. Ocampo, 2006; Siqueira et al., 2011).

Essential oils are substances produced by specialized metabolism and are characterized by the odor they produce. Essential oils are predominantly made up of terpenes (monoterpenes and sesquiterpene (Bakkali, Averbeck, Averbeck, & Idaomar, 2008).

In nature, essential oils provide important defense functions in plants, such as bactericidal, antiviral, antifungal, insecticidal, and repellent. Essential oils are also important for attracting pollen (pollinators) and seed dispersal agents (Bakkali et al., 2008; Edris, 2007; Rivoal et al., 2010) and are involved in signaling among plants (Laothawornkitkul, Taylor, Paul, & Hewitt, 2009).

Annona × *atemoya* Thompson variety presents susceptibility to stem borers (Baron, Amaro, Pina, & Ferreira, 2019), and its cultivation depends on grafting it on *Annona emarginata* (Schltdl.) H. Rainer, araticum-de-terra-fria, which has the potential of a rootstock, thereby providing required resistance.

The grafting process results in transferring molecules, such as mRNAs, between the rootstock and scion, which may interfere in the synthesis of other molecules involved in the primary (Baron et al., 2019; Baron, Bravo, Maia, Pina, & Ferreira, 2016; Kanehira et al., 2010) and specialized metabolisms, as essential oils.

The great susceptibility of atemoya to pathogens is controlled by means of grafting on *A. emarginata*, whose resistance (Baron et al., 2019) may be related, at least in part, to biosynthesized substances in the specialized metabolism, such as terpenes, which are present in essential oils.

Thus, we studied the essential oils of leaves collected from the graft (*Annona* × *atemoya*) and lateral budding of the rootstock (*Annona emarginata*), in the same individual, in order to characterize the chemical profile.

2. Materials and Methods

2.1 Plant Material

Annona × *atemoya* plants were grafted onto *Annona emarginata* (Schltdl.) H. Rainer variety of terra-fria in commercial crop. Although productivity was guaranteed, among other factors, by the removal of lateral budding of the rootstock, in this study the leaves of these shoots were collected, which allowed the study of the essential oil of the graft and rootstock in the same individual under the same conditions.

The plant material was collected on Paraizinho farm in the city of Pardinho, São Paulo, Brazil (23°5′3″ S, 48°22′38″ W; 895 m above sea level). The collection was conducted during late spring between 9:30 AM and 10:00 AM. Subsequently, the leaves were dried at 40 °C to a constant dry weight.

2.2 Oil Extraction and Analysis

After drying, 80 g dry masses of the leaves of the two species were hydro-distillized for 2 h to have essential oils extracted in a Clevenger-type apparatus. Essential oils were separated from the aqueous phase using the solvent dichloromethane (0.5 mL, Merck®). The extracted oils were stored in amber glass vials at -20 °C prior to the analysis of their chemical composition (Campos, Baron, Marques, Ferreira, & Boaro, 2014).

2.3 GC–MS Analysis

The separation, quantification, and identification of essential oils were performed using a gas chromatograph coupled to a mass spectrometer (CG-MS, Shimadzu®, QP-5000) with an electron impact (70 eV) and a capillary column of fused silica OV-5 [Ohio 'Valley Specialty Chemical®, Inc. 30.0 m × 0.25 mm × 0.25 µm; carrier gas, helium (flow rate, 1 mL min^{-1}); injection temperature, 230 °C; detector temperature, 240 °C; and split ratio, 1/20). It was injected 1 µL solution (1 µL of essential oils in 1 mL ethyl acetate, with the following temperature program: 60-240 °C, 3 °C/min (Campos et al., 2014).

The compounds were identified by comparing the obtained mass spectra with the database system GC-MS (Nist. 62 lib.), literature (Adams, 2007), and retention index (RI). In order to obtain RI of the substances, a mixture of *n*-alkanes (C_9-C_{24}; Sigma Aldrich® 99%) was employed and analyzed under the same operating conditions of samples, and the equation by Van den Dool & Kratz (Van Den Dool & Kratz 1963) were used.

2.4 Statistical Analysis

Principal component analysis (PCA) was carried out with the substances presented in the essential oil using XLSTAT program (2017).

3. Results and Discussion

Substances classified as monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, and oxygenated sesquiterpenes were identified in essential oils of both species, all of them from terpenes class (Table 1).

Sesquiterpenes were predominant in essential oils, while sesquiterpene hydrocarbons and oxygenated sesquiterpenes constituted by 90.1% and 72.9% of essential oils of atemoya and *A. emarginata*, respectively.

Monoterpenes are highly affected by temperature and luminous intensity, and thus, can be volatilized (Bakkali et al., 2008; Wang, Owen, Li, & Peñuelas, 2007). The percentage difference of sesquiterpenes observed in both species, which was higher in atemoya, may be due to the crown of atemoya and was more exposed to light and temperature. Atemoya was grafted on *A. emarginata*, whose collected lateral budding (thief branch) was more protected from light and temperature.

The multivariate analysis revealed that the rootstock species (*A. emarginata*) and graft (*Annona* × *atemoya*) presented oils with major substances, characterizing two different groups of chemical profiles. *A. emarginata* was mainly discriminated by α and β-selinene, β-elemene and spathulenol, totalizing 41.75% and *Annona* ×

atemoya by germacrene D (42.82%) (Figure 1). Biotic factors have the potential to alter the chemical composition and production of essential oils in which the molecules are formed by the interaction between plants and environment (Dudareva, Klempien, Muhlemann, & Kaplan, 2013; Holopainen & Gershenzon, 2010).

Moreover, a high percentage of oxygenated terpenes in essential oils of atemoya (12.19%) and *A. emarginata* (20.14%) was identified, which may indicate stress, because the comparison between the chemical profiles of essential oils from the ungrafted rootstock *A. emarginata,* grown in a greenhouse, demonstrated the absence of oxygenated terpenes (Campos et al., 2014).

Thus, the mechanical damage resulting from grafting (Suzuki & Mittler, 2012) may have been the cause of stress, leading to the formation of reactive oxygen species (ROS) (Suzuki & Mittler, 2012). The increased production of oxygenated terpene fractions may have contributed to ROS neutralization by non-enzymatic antioxidant system (Gill & Tuteja, 2010). Terpene oxygenates are molecules with high reactivity against pathogens (Oda, Fujinuma, Inoue, & Ohashi, 2011), which may explain their high percentage in *A. emarginata*, which is used as the rootstock for atemoya. Moreover, the pathogen resistance in *A. emarginata* may originate from the union of the species with atemoya because terpene oxygenates were observed after grafting and have not been observed in non-grafted plants (Campos et al., 2014). Furthermore, the union of plants at different ages can be an additional reason for the increased ROS production.

After grafting, the evaluation of the essential oil composition of the leaves of atemoya variety Thompson identified 28 substances, of which germacrene D was the most abundant (42.8%). In the essential oils extracted from *A. emarginata,* 36 substances were identified, with β-selinene (12.6%) and α-selinene (12.2%) forming the major components (Table 1). Germacrene D is a sesquiterpene hydrocarbon that is related to ecological roles in the interaction of plants with their predators and pollinators (Prosser et al., 2004), functions as an attraction agent, and facilitates the location of plants for food and ovipositor. Sesquiterpenes, similar to pheromones, bind to the receptors located on the antennae of insects, as demonstrated in *Heliothis virescens* (Mozuraitis, Stranden, Ramirez, Borg-Karlson, & Mustaparta, 2002; Skiri, Galizia, & Mustaparta, 2004; Stranden et al., 2003).

High percentages of germacrene D were also found in *Xylopia laevigata* collected in the cities of Santa Luzia do Itanhy (11°22′54″ S, 37°25′15″ W) and São Cristóvão (10°55′08″ S, 37°06′13″ W) in Sergipe state (60.44% and 43.62%, respectively) (Quintans et al., 2013). The similarity of the essential oils of atemoya and *X. laevigata* collected in São Cristóvão should be noted. Three predominant substances in the essential oils have the following percentages: germacrene D (42.82%-43.62%), bicyclogermacrene (14.28%-14.63%), and (*E*)-caryophyllene (8.35%-7.98%). Sesquiterpenes, identified in the essential oils extracted from the atemoya pulp (Pino & Rosado, 1999), may be indicative of the involvement of this substance in the attraction of animal seed dispersers. Study indicates possibilities for pest control from the study of sesquiterpenes (Prosser et al., 2004).

Several substances found in the essential oils of *A. emarginata* and atemoya variety Thompson (Table 1) had biological activities and were not always extracted and isolated from *Annonaceae*. Therefore, α-pinene, β-pinene (Medeiros Leite et al., 2007; Nissen, Zatta, Stefanini, Grandi, & Sgorbati, 2010), limonene (Espina, Gelaw, de Lamo-Castellví, Pagán, & García-Gonzalo, 2013), (*E*)-caryophyllene (Costa et al., 2008), and spathulenol (Limberger, Sobral, Henriques, Menut, & Bessière, 2004) showed bactericidal activity. β-elemene and spathulenol showed cytotoxic activity (Wang et al., 2005; Yao et al., 2008), β-selinene showed insecticidal and antibacterial activity (Chu et al., 2011; Souza et al., 2017), and bicyclogermacrene showed fungicidal activity (Silva et al., 2007).

These substances demonstrated biological activity, which may be related to insect–plant interactions, including the attraction of insects and protection or defense of plants against pathogens, and operated as antioxidant non-enzymatic substances against oxidative stress.

Table 1. Chemical composition (%) of the essential oils of atemoya (*Annona × atemoya*) and *Annona emarginata*

Substances	*Annona × atemoya* (graft)	*Annona emarginata* (rootstock)	RI_E	RI_L
	---------------------------- %	----------------------------		
Monoterpene hydrocarbons				
α-thujene	**-**	**0.52**	927	930
α-pinene	**2.62**	**5.55**	934	939
Camphene	**-**	**0.52**	949	954
β-pinene	**4.25**	**3.51**	978	979
Mircene	**Tr**	**1.21**	991	990
p-cymene	**-**	**0.74**	1024	1024
Limonene	**0.92**	**4.84**	1029	1029
trans-β-ocimene	**0.59**	**Tr**	1047	1050
γ-terpinene	**-**	**0.68**	1058	1059
Oxygenated monoterpene				
1,8 cineole	**-**	**2.68**	1031	1031
Linalool	**0.94**	**1.43**	1100	1096
terpinen-4-ol	**-**	**0.77**	1177	1177
α-terpineol	**-**	**2.44**	1190	1188
Sesquiterpene hydrocarbons				
δ-elemene	**1.22**	**Tr**	1339	1338
α-copaene	**0.72**	**Tr**	1378	1376
β-boubonene	**0.61**	**2.70**	1386	1388
β-cubebene	**1.49**	**-**	1392	1388
β-elemene	**2.12**	**9.66**	1394	1390
(E)-caryophyllene	**8.35**	**5.39**	1421	1419
Aromadendrene	**Tr**	**4.36**	1440	1441
α-humulene	**1.94**	**0.52**	1455	1454
β-chamigrene	**-**	**3.99**	1477	1477
germacrene D	**42.82**	**0.66**	1482	1485
β-selinene	**-**	**12.63**	1487	1490
α-selinene	**Tr**	**12.21**	1496	1498
Bicyclogermacrene	**14.28**	**-**	1496	1500
Germacrene A	**1.03**	**Tr**	1506	1509
γ-cadinene	**Tr**	**1.07**	1515	1513
δ-cadinene	**1.11**	**2.42**	1525	1523
α-cadinene	**-**	**0.74**	1539	1538
Germacrene B	**3.16**	**3.71**	1558	1561
Oxygenated sesquiterpenos				
Elemol	**1.33**	**Tr**	1550	1549
trans-nerolidol	**0.59**	**Tr**	1564	1563
Spathulenol	**2.89**	**7.25**	1578	1578
Caryophyllene oxide	**1.68**	**3.19**	1584	1583
1-epi-cupenol	**1.39**	**Tr**	1621	1628
Cubenol	**1.47**	**Tr**	1642	1646
α-cadinol	**1.90**	**2.38**	1654	1654
Monoterpene hydrocarbons	**8.38**	**17.57**		
Oxygenated Monoterpene	**0.94**	**7.32**		
Sesquiterpene hydrocarbons	**78.85**	**60.06**		
Oxygenated sesquiterpenes	**11.25**	**12.82**		
% Identification	**99.42**	**97.77**		

Note. RI_E = experimental retention index; RI_L = literature retention index (Adams 2007); (-) = absence of substance; tr = trace (tr ≤ 0.05).

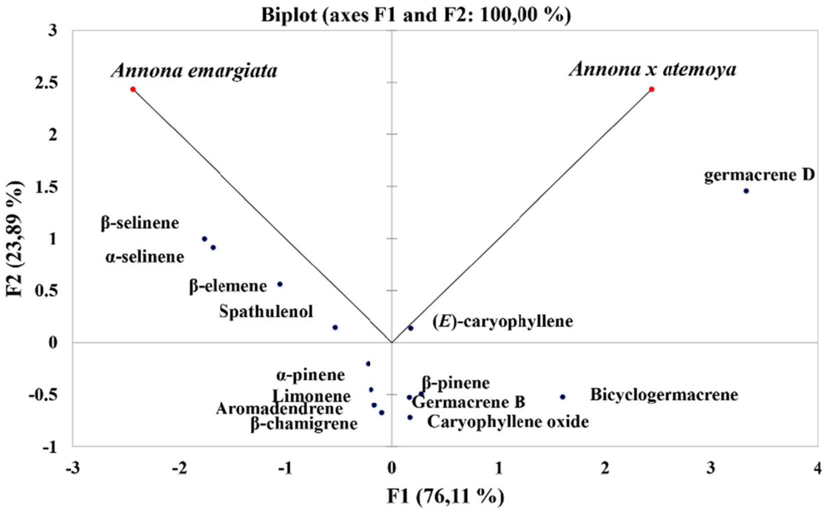

Figure 1. Principal components analysis (PCA) of the essential oil components of 14 of *Annona* × *atemoya* and *Annona emarginata* in Pardinho, São Paulo, Brazil

4. Conclusion

Annona × *atemoya* and *Annona emarginata* presented chemical profiles of essential oils with specific substances that may suggest plant-insect interaction and action on the resistance presented by the rootstock. Some substances are common in oils of both species. Our results suggest that the profile of the essential oil may be another indicator for the success of the combination of these two species.

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