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Volatile profile of basil cultivars and hybrids

[Volatile perfil de cultivares de albahaca y los híbridos]

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Abstract: The aim of this study was to evaluate the volatile profile as a result of hybridization. Data were analyzed by ANOVA, Tukey test and Principal Component Analysis. Hybridization provided the appearance of compounds in hybrids, and these compounds are absent in the parental volatile profile. The new compounds were: camphor, neral, geranial, beta-selinene, bicyclogermacrene, (E)-caryophyllene and methyl chavicol, for the hybrid 'Genovese' x 'Maria Bonita'; and camphor, for the hybrid 'Sweet Dani' x 'Genovese'.

Keywords: *Ocimum basilicum*, principal component analysis, essential oil.

Resumen: El objetivo de este estudio fue evaluar el perfil de volátiles como resultado de la hibridación. Los datos fueron analizados por ANOVA, prueba de Tukey y Análisis de Componentes Principales. La hibridación proporcionó la aparición de nuevos compuestos híbridos que no están presentes en el perfil de volátiles de los parentales, como por ejemplo el alcanfor, el neral, el geranial A, el beta-selineno, el biciclogermacrene, el (E)-cariofileno y el metil chavicol para el híbrido 'Genovese' x 'Maria Bonita', y el alcanfor para el híbrido 'Sweet Dani' x 'Genovese'.

Palabras clave: *Ocimum basilicum*, análisis de componentes principales, aceite esencial.

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INTRODUCTION

Basil (*Ocimum basilicum* L.) is an herbaceous and aromatic plant which belongs to the Lamiaceae family. According to the cultivation place, the plant may be annual or perennial. Basil is originally from Southeast Asia and Central Africa, and it is subspontaneous in Brazil (Hertwig, 1986). The plant reaches up to 1 m tall, and it has very branched stem with quadrangular and pubescent branches. Its leaves are petiolated, opposite, ovate, dentate, and green or purple-colored. They are rich in essential oils and vary from 1-4 cm in length, and 1.85 to 9.25 cm in width (Blank *et al*., 2004).

Basil has various purposes: its leaves are used in medicine and culinary, and also for essential oil production, which is a high value-added product for the pharmaceutical and cosmetic industry (Rosas *et al*., 2004). Basil essential oil is used in toiletries, perfumes, cosmetics, antiseptics and cleaning products (Liber *et al*., 2011). In the international market, sweet basil essential oil costs U$S 116.86 per kilogram (**www.essential-oil.org**).

For this reason, in many countries, the production of basil essential oil is one of the activities with major economic value (Sajjadi, 2006). Basil is grown in France, Egypt, Hungary, Indonesia, Morocco, the United States, Greece and Israel (Department: Agriculture Forestry and Fisheries, 2012). In countries such as France, Egypt and India, basil essential oil production varies between 50 and 100 tons/year (Shrinivas & Kudli, 2016), and United States are the largest basil essential oil producer and importer (Department: Agriculture Forestry and Fisheries, 2012).

Due to its use as aromatic plant, basil genotype quality is defined by the volatile compounds profile present in its essential oil (Carvalho Filho *et al*., 2006): linalool, 1,8-cineole (eucalyptol), methyl cinnamate, eugenol, citral, and methyl chavicol (estragole), which are the most important aromatic volatiles in the definition of the essential oil's quality (Department: Agriculture Forestry and Fisheries, 2012). These are potent odorous volatile of pleasant aromas described as lavender, floral (linalool), mint (1,8-cineole), strawberry (methyl cinnamate), cloves (eugenol), lemon (citral), licorice and anise (methyl chavicol) (Acree & Arn, 2016).

Different varieties within the same basil species produce essential oils with different volatile profiles, and therefore different aromas. For instance, citral (68%) is the major volatile of the cultivar 'Sweet Dani' (Morales & Simon, 1997), whereas linalool (66.40%), α-bergamotene (7.96%) and 1,8-cineole (7.23%) are the major essential oil compounds of 'Genovese' (Carovic-Stanko *et al*., 2010).

Most basil cultivars available in the market belong to common basil (*Ocimum basilicum* L.) (Liber *et al.*, 2011). One of *O. basilicum* L. most worldwide planted varieties for essential oil purposes is the European sweet basil, which is rich in linalool (40.5 to 48.2%) and methyl chavicol (28.9 to 31.6%) (Fleisher, 1981; Charles & Simon, 1990). It is also the most produced variety in France, Italy, Egypt and South Africa (Schulz *et al*., 2003). However, due to human constant demand for new flavors and aromas, researchers, through breeding programs, seek to develop new cultivars and hybrids which are more competitive in the consumer market. A recent study applied to the genetic improvement of *O. basilicum* L. generated the first Brazilian basil cultivar, 'Maria Bonita', with high essential oil yield, and rich in linalool. Its essential oil has 78% of linalool, which is superior to the European basil type (Blank *et al*., 2007).

Another method to develop different essential oils to the world market is the cross of cultivars in order to obtain hybrids. Thus, the parents choice is fundamental (Ramalho *et al*., 1998). This is because the hybrid characteristics are influenced by the parents genetic characteristics, and it is necessary to carry out numerous crosses and selection of the combinations with superior performance (Fehr, 1987). The knowledge of parents and the effect of their combinations facilitate both the selection of genotypes for future breeding programs and the development of new cultivars.

Diallelic studies stand out for providing results of high precision, especially the estimate of heterosis, and for contributing to the understanding of the genetic effects involved in the expression of the characters of interest (Ramalho *et al*., 1998). Scientists have recently shown, through diallelic studies on basil cultivars and hybrids, that variability in basil can be improved by using hybrid

combinations, and that the knowledge of the overall and specific capacity of combination and its effects facilitate the choice of genotypes (Blank *et al*., 2012). Hybridization favored the generation of compounds in the essential oil of the hybrid "Sweet Dani" × "Maria Bonita" and "Sweet Dani" × "Cinnamon", which were not present in the essential oils of the parents (Costa *et al*., 2014).

After obtaining the hybrid it becomes necessary to analyze the characteristics of interest in relation to the parents. The chemical characterization of the essential oil of the basil hybrids of this study will allow the knowledge of potentially useful chemical compounds for the market.

For these reasons, the purpose of this study was to evaluate the volatile profile in cultivars and hybrids derived from the crossing of the cultivars 'Sweet Dani' and 'Genovese', and 'Genovese' and 'Maria Bonita'.

MATERIAL AND METHODS

Hybridization

Hybridization was carried out in a greenhouse with clarite screen at the Medicinal Plants Garden of the Experimental Farm of the Rural Campus of the Federal University of Sergipe. The genotypes used were 'Dani Sweet' (*O. x citriodorum*), 'Canela', 'Genovese' and 'Maria Bonita', and the latter was characterized by Blank *et al*. (2007). Five hybrids were produced, as well as the selfing of the four genotypes (Blank *et al*., 2012). Two of these hybrids and their parents were used in this work.

Inflorescences were selected per plant, which were used as pollen recipients (female), and were marked with different color wool yarn for each male parent, and as pollen donator. To carry out the cross, it was collected the flowers from the pollen donator inflorescences, following by the emasculation of the pollen receptors inflorescences flower buds that were almost opening. The flowers collected (containing pollen) were put against the emasculated flowers stigmas. After hand pollination, the inflorescences, working as female organs, were protected with paper bags, which also protect the inflorescences from selfing (Blank *et al*., 2012).

Essential Oil Extraction

Oil extraction was carried out at the Crop Science Laboratory of the Department of Agronomic Engineering of the Federal University of Sergipe. It was used the leaves of two hybrids ('Sweet Dani' x 'Genovese', and 'Genovese' x 'Maria Bonita') and of three cultivars ('Genovese' 'Sweet Dani' and 'Maria Bonita'). After three months of cultivation in the field, leaves were collected and subjected to drying process in an oven at 40º C, for five days.

Essential oil extraction from dry basil leaves was carried out using Clevenger apparatus (Guenther, 1972) for about 2 hours and 40 minutes (Carvalho Filho *et al*., 2006).

Identification of essential oil volatiles

The volatile compounds present in the samples of the essential oils were identified at the Research Laboratory of Natural Products of the Federal University of Sergipe, using a gas chromatographer coupled to a mass spectrometer GC-MS (Shimadzu, QP 5050A model), equipped with a COA 20i injector (Shimadzu) and a fused silica capillary column (5% dephenyl 95%-dimethylpolysiloxane, 30 m x 0.25 mm i.d. x 0.25 μm film, J&W Scientific), using helium carrier gas at a flow of 1,2mL.min^{-1}. The temperature was kept at 50° C for 1.5 min, followed by an increase of 4° C/minute, until it reached 200° C. After that, it was kept at 15° C until it reached 250° C, and this temperature was kept constant for 5 min. The injector temperature was 250° C, and the detector (or interface) temperature was 280° C. It was injected a volume of 0.5 uL in ethyl acetate; the partition ratio of the volume injected was of 1:87, and the column pressure was 64.20 kPa. The mass spectrum conditions were: quadrupole ion detector operating by electronic impact, and impact energy of 70 eV; scan speed of 1,000; scan interval of 0.85 fragments/s, and fragments detected in the range of 40 to 550 Da. The quantitative analysis of chemical components was carried out by flame ionization gas chromatography (FID) using a Shimadzu GC-17A (Shimadzu Corporation, Kyoto, Japan), under the following operating conditions: capillary ZB-5MS column (5%-phenyl-arylene-95%-dimethylpolysiloxane) of fused silica (30 mx 0.25 mm i.d. x 0.25 μm film), from Phenomenex (Torrance, CA, USA), under the same conditions described for GC-MS . The quantification of each component was calculated by area normalization (%). The concentrations of the

compounds were calculated from peak areas of GC, and were arranged according to its GC elution order.

The components of the essential oil were identified by comparing their mass spectra with the spectra available in the equipment database (NIST05, NIST21 and WILEY8). This database allowed the comparison of the spectral data, and the similarity index was 80%. Furthermore, the measured retention indices were compared with the literature (Adams, 2007). The relative retention indices (RRI) were determined by using the equation of Van den Dool and Kratz (1963), in relation to a homologous series of n-alkanes (C9-C18) injected under the chromatographic conditions described above. It was calculated the area under the chromatographic peak (*count*) for each volatile compound, and this value was used as estimator of compound concentration in the sample.

Statistical analysis

The data of the chemical composition of basil essential oils were subjected to analysis of variance (ANOVA), and the means were compared with the Tukey test at a 5% probability, using the Sisvar 5.0 software. Principal components analyses (PCA) was performed using Statistica version 7.0.

RESULTS AND DISCUSSION

Table 1 shows the volatiles identified in each essential oil sample with the respective relative retention indices. The relative area was expressed as the percentage of the total chromatogram area. The sum of the areas under the identificated compounds listed in Table 1 reached values greater than 96.0%, indicating that they represent almost all of the compounds present in each sample.

Table 1 shows that linalool was the major volatile compound in the essential oils extracted from 'Maria Bonita' and 'Genovese' leaves, as well as from the hybrids 'Sweet Dani' x 'Genovese' and 'Genovese' x 'Maria Bonita'. It is observed that in the parental genotypes 'Maria Bonita' and 'Genovese' the content of linalool was respectively 75.22% and 66.51% while in the hybrids 'Sweet Dani' x 'Genovese' and 'Genovese' x 'Maria Bonita' we observed 56.24% and 53.84% of linalool. Geranial was the major compound of the cultivar 'Sweet Dani', with a content of 46.16%.

Table 1 shows that the oil of the genotypes 'Maria Bonita', 'Genovese' and 'Sweet Dani' had a volatile profile constituted by a smaller number of volatile compounds compared with the two hybrids. The essential oils of the parental genotypes 'Maria Bonita', 'Genovese' and 'Sweet Dani' contained 13, 19 and 20 volatile compounds, respectively. On the other hand, those extracted from the hybrids 'Sweet Dani' x 'Genovese' and 'Genovese' x 'Maria Bonita', respectively 24 and 27 volatile compounds.

It is observed that the hybrids have higher content of some major compounds when compared with their parents. The compound methyl chavicol was statistically higher in the hybrids (16.34 and 12.87%) when compared with their parentes (0.47 and 0.00%). The hybrid 'Genovese' x 'Maria Bonita' presented the major compounds neral (3.69%), (E) caryophellene (0.90%), α-humulene (0.19%) and β-selinene (0.38%), all absent in the parents. In turn, camphor was a new compound that appeared in the hybrid 'Sweet Dani' x 'Genovese'. This suggests that hybridization enriched volatile profile of hybrids with the emergence of new compounds.

The heterosis mentioned above suggests some hypotheses. A first possibility is the presence of co-dominance; the female parent has A1A1 allele, while the male parent presents has A2A2 allele for the same gene. However, the alleles A1 or A2 alone do not enable the formation of such compound. When hybridization occurs, it is possible that both alleles together encode an enzyme responsible for the synthesis of a new compound (Borém & Miranda, 2009). This is a possible explanation for the presence of methyl chavicol in the hybrid 'Genovese' x 'Maria Bonita' and its absence in the cultivars 'Genovese' and 'Maria Bonita'. In plants, methyl chavicol is produced by chavicol convertion by the enzyme O-methyltransferase (Gang *et al.*, 2002), which may be absent in the parental 'Genovese' and 'Maria Bonita', but present in the hybrid through the mechanism previously mentioned. There are also enzymes whose structures present two or more polypeptide chains. In this case, it is possible that A1 allele encodes one of the chains, while A2 allele encodes another. Thus, the presence of two alleles allows the synthesis of the two chains and the formation of the enzyme (Borém & Miranda, 2009).

Table 1
Content (%) of the volatile compounds identified in cultivars and hybrids of *Ocimum basilicum* L.

Compound	RRI literature	RRI exp. experiment	Content (%)				
			'Sweet Dani'	'Maria Bonita'	'Genovese'	'Genovese'x 'Maria Bonita'	'Sweet Dani' x 'Genovese'
Triciclene	921	931	0.00d	0.09c	0.29a	0.11bc	0.16b
Sabinene	969	971	0.00c	0.15b	0.28a	0.14b	0.16b
β- Pinene	974	977	0.00c	0.38b	0.68a	0.39b	0.36b
6- methylhept -5-en-2-one	981	983	0.98a	0.00b	0.00b	0.00b	0.00b
Myrcene	988	989	0.00b	0.00b	0.59a	0.47a	0.45a
Limonene	1024	1029	0.00c	0.12b	0.30a	0.14b	0.00c
1.8 Cineole	1026	1032	**0.00c**	**5.35b**	**10.80a**	**6.54b**	**5.48b**
(E) β-Ocimene	1044	1046	0.00b	0.00b	0.35a	0.37a	0.42a
Linalool	1095	1102	**0.00d**	**75.22a**	**66.51b**	**56.24c**	**53.84c**
Camphor	1141	1147	0.00c	0.00c	0.00c	0.29a	0.18b
Borneol	1170	1172	0.25a	0.00b	0.52a	0.00b	0.00b
Z-isocitral	1177	1181	0.60a	0.00b	0.00b	0.00b	0.00b
α-Terpineol	1186	1195	0.00c	0.36b	1.05a	0.38b	0.43b
Methyl chavicol	1195	1197	**0.47b**	**0.00c**	**0.00c**	**16.34a**	**12.87a**
Nerol	1222	1224	3.13a	0.00b	0.00b	0.00b	0.00b
Neral	1236	1235	**35.68a**	**0.00c**	**0.00c**	**3.69b**	**5.23b**
Geraniol	1249	1250	**0.89b**	**14.66a**	**0.00c**	**0.00c**	**1.24bc**
Geranial	1266	1267	**46.16a**	**0.00c**	**0.00c**	**5.34b**	**7.81b**
Isobornyl acetate	1283	1284	0.00c	0.00c	0.67a	0.19b	0.00c
Carvacrol	1298	1297	0.00b	0.00b	0.58a	0.00b	0.00b
Eugenol	1356	1351	0.00b	0.00b	3.91a	0.00b	0.00b
Neryl acetate	1356	1358	0.71a	0.00b	0.00b	0.00b	0.00b
α-copaene	1374	1374	0.26a	0.00b	0.00b	0.00b	0.00b
Geranyl acetate	1379	1377	0.36a	0.59a	0.00b	0.00b	0.00b
β-elemene	1389	1388	0.00b	0.00b	0.79a	0.26ab	0.56ab
(E) -Caryophyllene	1417	1419	1.65a	0.00c	0.00c	0.90b	1.48a
α -trans Bergamotene	1432	1432	0.79b	1.52b	5.11a	2.63b	1.36b
neryl propanoate	1452	1452	0.27a	0.00b	0.00b	0.22a	0.26a
α-humulene	1452	1455	0.45a	0.00c	0.00c	0.19bc	0.38ab
geranyl propanoate	1476	1483	0.00b	0.00b	0.00b	0.19a	0.00b
ϒ-muurolene	1478	1480	0.51c	0.32c	0.39c	1.48b	1.97a
β-selinene	1489	1489	1.49a	0.00d	0.00d	0.38c	0.62b
bicyclogermacrene	1500	1495	1.11a	0.00d	0.00d	0.54c	0.87b
α-bulsenene	1509	1501	0.00b	0.00b	1.04a	0.27ab	0.43ab
Germacrene A	1508	1507	0.00b	0.00b	0.00b	0.19a	0.00b
ϒ-Cadinene	1513	1513	0.00b	0.49b	1.36a	0.65ab	0.84ab
Caryophyllene oxide	1582	1581	1.21a	0.00b	0.00b	0.00b	0.00b
1.10-di-epi-Cubenol	1618	1615	0.00b	0.00b	0.51a	0.00b	0.00b
Cadinol, epi-α	1638	1643	0.00b	0.58b	3.93a	0.82b	1.37b
Total identified			96.96	99.85	99.68	99.37	98.78

Means followed by the same letters in the lines do not differ from each other by the Tukey test ($p < 0.05$)
RRI = relative retention index; Compounds using bold text are major compounds

According to principal component analysis (Figure 1), the first principal component represented 61.03% of the total variance, and was positively related to the compounds 1,8-cineole (r = 0.95), linalool (r = 0.94), and γ-cadinene (r = 0.88); and was negatively related to nerol (r = -0.95), neral (r = -0.97), and β-selinene (r = -0.95), and the second principal component represented 21.10% of the total variance, and was negatively related to the compounds methyl chavicol (r = -0.95) and γ-muurolene (r = -0.98). The two principal components represented 82.13% of the variation that exists among the essential oils of basil in this work (Figure 1).

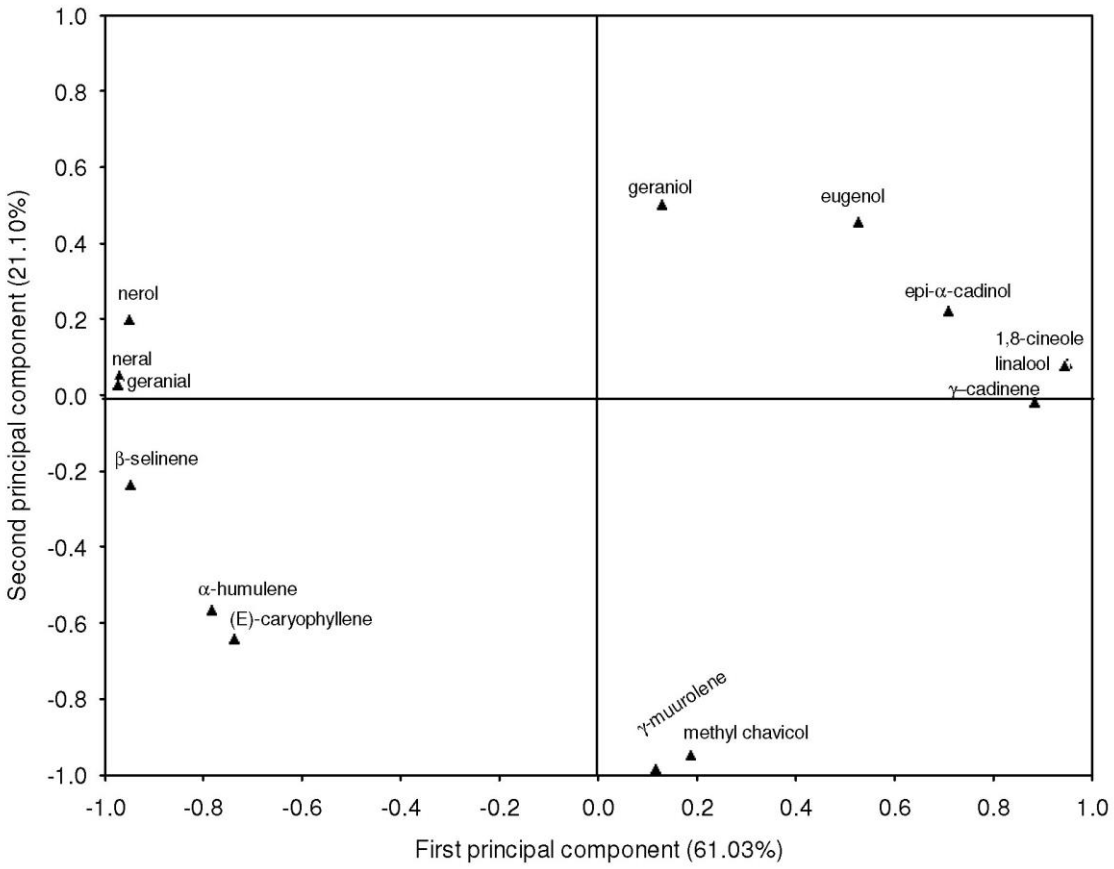

Figure 1
Distribution of the major chemical constituents of the basil essential oils in relation to the two principal components through the principal component analysis (PCA).

CONCLUSIONS

Hybridization favored the appearance of volatile compounds in the hybrids 'Genovese' x 'Maria Bonita' and 'Sweet Dani' x 'Genovese', and these compounds are absent in the parents. This study can be used as a model for the development of future basil hybrids, with different chemical profile of currently available essential oils in the consumer market.

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